DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

3

ROLE OF CHOLECYSTOKININ RECEPTORS IN THE REGULATION OF BEHAVIOUR AND IN THE ACTION OF HALOPERIDOL AND DIAZEPAM

by

Eero Vasar

TARTU 1992

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

3

ROLE OF CHOLECYSTOKININ RECEPTORS IN THE REGULATION OF BEHAVIOUR AND IN THE ACTION OF HALOPERIDOL AND DIAZEPAM

by

Eero Vasar

TARTU 1992

Department of Physiology, University of Tartu, Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Medical Sciences on October 30th, 1991 by the Council of the Faculty of Medicine of Tartu University

Official opponents:

Professor Pekka T. Männistö, M.D., Helsinki
Professor Vija Klusha, M.D., Riga
Professor Aleksandr Zarkovsky, M.D., Tartu

Commencement: February 5th, 1992

Department of Physiology, University of Tartu, Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Medical Sciences on October 30th, 1991 by the Council of the Faculty of Medicine of Tartu University

Official opponents:

Professor Pekka T. Männistö, M.D., Helsinki
Professor Vija Klusha, M.D., Riga
Professor Aleksandr Zarkovsky, M.D., Tartu

Commencement: February 5th, 1992

CONTENTS

KOLETSÜSTOKINIINI RETSEPTORITE TÄHENDUS KÄITUMISE REGULATSIOONIS NING HALOPERIDOOLI JA DIASEPAAMI TOIMES

Kokkuvõte

Käesoleva töö üheks eesmärgiks oli selgitada, milline on koletsüstokiniini (CCK) retseptori alatüüpide tähtsus CCK agonistide (tseruleiini, CCK-8, pentagastriini ja CCK-4) käitumuslikes efektides. Uuriti järgmiseid CCK agonistide käitumuslikke toimeid: sedatiivset, amfetamiinivastast, anksiogeenset, agressiivsusevastast ja krambivastast toimet. Jälgiti, millisel määral üks või teine CCK agonistide toime on kõrvaldatav valikuliste koletsüstokiniini antagonistide poolt. Eksperimentides kasutati CCK_A ('perifeersete') retseptorite antagonisti devasepiidi ja CCK_B ('tsentraalsete') retseptorite antagonisti L-365,260. Devasepiidi ja L-365,260 mõju katseloomade käitumisele ja [^{3}H]pCCK-8 sidumisele ajus uuriti nende ainete ühekordse ja kestva manustamise järgselt. Selgitamaks CCK-ergiliste mehhanismide osa neuroleptilise ravimi haloperidooli efektides, võrreldi haloperidooli ja CCK agonisti tseruleiini pikaajalise (14-päevase) manustamise toimet hiirte käitumisele ja erinevate [^{3}H]-radioligandite sidumisele ajus. Töö teiseks eesmärgiks oli selgitada, millisel määral on CCK retseptori alatüübid seotud neuroleptikumide ja anksiolüütiliste preparaatide toimega. Selleks uuriti muutusi CCK retseptoritel haloperidooli ja diasepaami kestva manustamise järgselt.

Teostatud farmakoloogilise analüüsi alusel võib väita, et ainult mittevalikulised CCK agonistid tseruleiin ja CCK-8 pärsivad katseloomade motoorset aktiivsust, selektiivsetel CCK_B agonistidel (pentagastriin ja CCK-4) antud toime puudub. Kuid valikuliste CCK antagonistide (devasepiid ja L-365,260) vastupidine mõju tseruleiini ja CCK-8 liikumisaktiivsust pärssivale toimele annab alust arvata, et CCK_A ja CCK_B retseptorid omavad vastupidist rolli loomade motoorse aktiivsuse regulatsioonis. CCK agonistide liikumisaktiivsust vähendav toime realiseerub eelkõige CCK_A retseptorite vahendusel. Tseruleiin ja CCK-8, mitte aga CCK_B/gastriini retseptorite agonist pentagastriin, kõrvaldasid amfetamiinist tingitud hüperlokomotsiooni hiirtel. Devasepiidi manustamine väikestes annustes (1-100 µg/kg), mis toimivad ainult perifeerset tüüpi CCK retseptoritele, kõrvaldas täielikult tseruleiini amfetamiinivastase toime. Devasepiidi suur annus (1 mg/kg), mis avaldab toimet ka CCK_B retseptoritele, oli aga ise võimeline kõrvaldama amfetamiini motoorikat stimuleerivat efekti. Need tulemused viitavad eelkõige CCK_A ja CCK_B retseptorite antagonistlikule interaktsioonile dopamiinergiliste neuronite aktiivsuse regulatsioonis katseloomade ajus.

CCK agonistid (tseruleiin, CCK-8, pentagastriin, CCK-4) vähendasid märkimisväärselt rottide uurimisaktiivsust pluss-puuris. CCK agonistide 'anksiogeenne' toime korrelleerus nende afiinsusega CCK_B retseptorite suhtes ajukoores, kuid mitte CCK_A retseptorite suhtes pankreases. Valikuline CCK_B retseptorite blokaator L-365,260 oli tugevam CCK-4 'anksiogeense' toime antagonist kui devasepiid. Erinevalt tseruleiinist põhjustas CCK_B agonisti CCK-4 ajusisene või süsteemne manustamine agressiivse käitumise tunduvat suurenemist isastel rottidel. Järelikult etendavad CCK_B retseptorid väga olulist osa katseloomade emotsionaalse käitumise kontrollis.

Tseruleiini ja CCK-8, kuid mitte pentagastriini, manustamine antagoniseeris pikrotoksiini ja pilokarpiini poolt esile kutsutud krampe hiirtel. Erinevate konvulsiivsete ainete (pikrotoksiin, pilokarpiin ja N-metüül-D-aspartaat) krampe põhjustav toime korrelleerus CCK retseptorite tiheduse vähenemisega katseloomade ajus. L-365,260 ja devasepiidi ühesugune annus (1 mg/kg) blokeeris täielikult CCK-8 mõju pilokarpiinist tingitud 'limbilistele' krampidele, mis viitab CCK retseptori mõlema alatüübi osalusele CCK agonistide krambivastases toimes.

CCK antagonistide L-365,260 ja devasepiidi kestev manustamine põhjustas erinevaid muutusi loomade käitumises ja [^{3}H]pCCK-8 sidumises hiirte eesajus. Devasepiidi mõjul vähenes tseruleiini motoorikat pärssiv toime ja tugevnes amfetamiinist tingitud hüperlokomotsioon. Samal ajal L-365,260 suurendas märkimisväärselt CCK retseptorite tihedust hiire ajus, avaldamata aga olulist mõju loomade käitumisele. Järelikult etendavad CCK_A retseptorid uuritavates käitumisavaldustes suuremat tähendust kui CCK_B retseptorid.

Haloperidooli ja CCK agonisti tseruleiini pikaajaline kasutamine põhjustas sarnaseid nihkeid loomade käitumises ja erinevate [^{3}H]-radioligandite sidumises ajus. Tolerantsus kujunes tseruleiini, mustsimooli ja flumaseniili motoorsete efektide suhtes, kuid amfetamiinist tingitud hüperlokomotsioon on oluliselt suurenenud 14-päevase haloperidooli ja tseruleiini manustamise järgselt. Paralleelselt käitumuslike nihetega suurenes hiire ajus opioid ja dopamiin$_2$-retseptorite tihedus, kuid vähenes $GABA_A$, bensodiasepiini ja CCK retseptorite arv. Antud tulemused viitavad CCK_A retseptorite olulisele osale haloperidooli pikaajalise manustamise toimes.

Kestev haloperidooli ja diasepaami manustamine põhjustas tolerantsust

tseruleiini käitumist pärssivate efektide (sedatiivne, amfetamiinivastane, krambivastane ja antiagressiivne toime) suhtes. Haloperidooli ja diasepaami pikaajalise kasutamise järgselt tuli ilmsiks CCK agonisti tugev proagressiivne toime. Seejuures on oluline rõhutada, et haloperidooli ja diasepaami kroonilise süstimise mõjul suurenes CCK retseptorite afiinsus ajukoores. Järelikult tekib haloperidooli ja diasepaami 14-päevase manustamise vältel alatundlikkus CCK_A ja ülitundlikkus CCK_B retseptoritel.

Läbiviidud farmakoloogilise analüüsi alusel võib väita, et CCK_A ja CCK_B retseptorite vahel eksisteerib funktsionaalne antagonism mitmesuguste käitumisavalduste regulatsioonis. Haloperidooli ja diasepaami pikaajalisel manustamisel leiavad aset vastupidised nihked CCK_A ja CCK_B retseptorite tundlikkuses, mis on ilmselt seotud organismi adapteerumisega nende ravimite suhtes.

LIST OF ORIGINAL PUBLICATIONS

I. *Vasar E., Otter M. and Rägo L.* (1982) Intraventricular administration of cholecystokinin decreases the activity of dopamine- and serotoninergic systems in the brain. Sechenov Physiol. J. of the USSR, 68: 1218-1222 (in Russian).

II. *Vasar E., Maimets M., Nurk A. and Allikmets L.* (1984) Caerulein stimulates [^{3}H]-spiperone binding in vivo after long-term haloperidol administration. Psychopharmacol. Bulletin, 20: 691-692.

III. *Vasar E., Maimets M. and Allikmets L.* (1984) The role of serotonin$_2$-receptors in the regulation of aggressive behaviour. J.Higher Nervous Activity, 34:283-289 (in Russian).

IV. *Vasar E., Nurk A., Maimets M. and Allikmets L.* (1985) Stimulation with caerulein, an analog of cholecystokinin octapeptide, of ^{3}H-spiroperidol binding after prolonged administration of neuroleptics. Bull. Exp. Biol. Med., 99: 72- 74 (in Russian).

V. *Vasar E., Rägo L., Soosaar A., Nurk A. and Maimets M.* (1985) Modulatory effect of caerulein on benzodiazepine receptors. Bull. Exp. Biol. Med., 100: 711-713 (in Russian).

VI. *Vasar E., Maimets M., Nurk A., Soosaar A. and Allikmets L.* (1986) Comparison of motor depressant effects of caerulein and N-propylnorapomorphine in mice. Pharmacol. Biochem. Behav., 24: 469-478.

VII. *Vasar E., Soosaar A., Maimets M. and Allikmets L.* (1986) Reduced sensitivity of the brain cholecystokinin receptors under the effect of haloperidol prolonged administration. Bull. Exp. Biol. Med., 102: 583-585 (in Russian).

VIII. *Vasar E., Allikmets L., Soosaar A. and Lang A.* (1987) Change of behavioural and biochemical effects of caerulein, an analogue of cholecystokinin octapeptide (CCK-8), following long-term administration of haloperidol. J.Higher Nervous Activity, 37: 696-702 (in Russian).

IX. *Vasar E., Allikmets L. and Soosaar A.* (1988) Caerulein, an agonist of CCK-8 receptors, antagonizes the behavioural effects of ketamine in mice and rats. Bull. Exp. Biol. Med., 105: 43-45 (in Russian).

X. *Vasar E., Allikmets L., Ryzhov I., Prakhye I., Soosaar A. and Mirzaev S.* (1988) Interspecies differences in the behavioural effects of caerulein, an agonist of CCK-8 receptors, in mice and rats. Bull. Exp. Biol. Med., 105: 168- 170 (in Russian).

XI. *Vasar E., Soosaar, A. and Lang A.* (1988) The involvement of cholecystokinin receptors in the realization of behavioral and biochemical effects of long-term haloperidol administration. Proc. Acad. Sci. of the Estonian SSR, 37: 131- 139 (in

Russian).

XII. *Allikmets L. and Vasar E.* (1990) Adaptational changes in GABA, benzodiazepine and cholecystokinin receptors elicited by long-term haloperidol administration. Sov.Med.Rev.G.Neuropharm., 1: 101-126.

XIII. *Vasar E., Allikmets L., Soosaar A. and Lang A.* (1990) Similar behavioral and biochemical effects of long-term haloperidol and caerulein treatment in albino mice. Pharmacol. Biochem. Behav. 35: 855-859.

XIV. *Vasar E., Harro J., Soosaar A. and Lang A.* (1990) The changes at cholecystokinin receptors after long-term treatment with diazepam. In: Psychosomatic Disorders. (V.Vasar, ed.). Acta et Commentationes Universitatis Tartuensis. 891: 215-223.

XV. *Vasar E., Harro J., Lang A., Põld A. and Soosaar A.* (1991) Differential involvement of CCK-A and CCK-B receptors in the regulation of locomotor activity in the mouse. Psychopharmacol, 105: 393-399.

XVI. *Vasar E., Stephenson J.D and Meldrum B.S.* (1991) Changes in motor activity and forebrain [^{3}H]pCCK-8 binding in mice after repeated administration of drugs affecting cholecystokinin receptors. Eur.J.Pharmacol, 202: 385-390.

XVII. *Vasar E., Harro J., Lang A. and Soosaar A.* (1991) Pilocarpine-induced limbic seizures - an involvement of CCK receptors. In: Molecular Pharmacology of Receptors IV. (L.Allikmets, ed.). Acta et Commentationes Universitatis Tartuensis. 929: 34-41.

XVIII. *Vasar E., Harro J., Põld A. and Lang A.* (1991) CCK-8 receptors and anxiety in rats. In: Multiple cholecystokinin receptors in the CNS (S.T.Cooper and C.D.Dourish, eds.), Oxford University Press (in press).

ABBREVATIONS

CCK	cholecystokinin
CCK-8	octapeptide of cholecystokinin
CCK_A receptor	'peripheral' subtype of cholecystokinin receptors
CCK_B receptor	'central' subtype of cholecystokinin receptors
[^{3}H]pCCK-8	[propionyl-^{3}H]-propionylated-cholecystokinin octapeptide
GABA	γ-aminobutyric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
icv	intracerebroventricular administration
ip	intraperitoneal administration
L-365,260	selective antagonist at 'central' cholecystokinin receptors. 3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H- 1,4-benzodiazepin-3yl)-N'-(3-methyl-phenyl)urea
MK-329	devazepide, selective antagonist at 'peripheral' cholecystokinin receptors, 1-methyl-3-(2-indoloyl)amino-5- phenyl-3H-1,4-benzodiazepin-2-one
NMDA	N-methyl-D-aspartate
NPA	N-propylnorapomorphine
sc	subcutaneous administration
Tris	Tris(hydroxymethyl)aminomethan

1. INTRODUCTION

Cholecystokinin (CCK) is an important intestinal hormone with a major role in regulating the control of digestive processes (pancreatic secretion and gall bladder contraction) and in inhibiting feeding behaviors (Morley, 1987). Vanderhaeghen et al. (1975) discovered gastrin-like immunoreactivity in the mammalian brain. Several years later this immunoreactive substance was identified as the sulfated C-terminal octapeptide of cholecystokinin (CCK-8) (Eng et al., 1982). CCK-8 is probably the most widely distributed neuropeptide in the mammalian brain, which fulfills many of the criteria for a neurotransmitter (Beinfeld, 1988). CCK-8 is localized in high concentrations in the cerebral cortex, hippocampus and other limbic structures, midbrain and spinal cord neurons (Beinfeld, 1983). CCK is released from the rat cerebral cortex synaptosomes, from the rat striatum tissue slices, and from the rat nucleus accumbens tissue slices, after calcium-, potassium, and veratridine-induced depolarization (Meyer, Krauss, 1983; Voight et al., 1986; Vickroy, Bianchi, 1989). Specific high-affinity binding sites for [^{125}I]-CCK-8 have been identified, with the anatomical localization of terminals containing CCK-8 (Innis, Snyder, 1980; Saito et al., 1981; Beinfeld, 1983). Over the past decade major advances have occurred in our understanding of CCK receptors. There are at least 2 types of CCK-8 receptor designated CCK_A ('peripheral') and CCK_B ('central') (Dourish, Hill, 1987). Neurophysiological studies of CCK-8 indicate its function as an excitatory transmitter throughout the central nervous system (Skirboll et al., 1981; White, Wang, 1984). Behavioural studies have suggested that high doses of CCK-8 administered systemically have analgesic, sedative or neuroleptic-like activity (Zetler, 1980; Kadar et al., 1985), while centrally administered CCK-8 may have opposite functional effects (Faris et al., 1983; Crawley et al., 1985). The discovery that CCK coexists with dopamine and GABA in certain neurons of the rat brain (Hökfelt et al., 1980; Somogyi et al., 1984) aroused great interest in the role of CCK-8 in biochemical and behavioral processes which might be relevant to the action of anxiolytic and neuroleptic drugs.

In the present work an attempt to learn more about the role of CCK receptors in the regulation of behaviour was made. The selective CCK antagonists (devazepide and L-365,260) were used throughout the study to reveal the relevance of the CCK receptor subtypes in the action of CCK agonists. In the second part of this work the involvement of CCK receptors in the action of long-term treatment with a neuroleptic drug haloperidol and an anxiolytic compound diazepam was studied.

2. REVIEW OF LITERATURE

2.1. Behavioural effects of CCK agonists

Caerulein and CCK-8, the unselective agonists at CCK receptors, are shown to induce many behavioural effects after systemic or intracerebral administration. However, in the present study main attention is directed to the interaction of CCK agonists with motor activity, amphetamine-induced hyperlocomotion, seizures induced by picrotoxin, pilocarpine and quinolinate, and emotional behaviour ('anxiogenic-like' effect in an elevated plus-maze, antiaggressive action). These behavioural effects are believed to be related to the interaction of CCK with dopamine and GABA (Zetler, 1985; Harro, Vasar, 1991b) and may be affected by benzodiazepine tranquillizers, exerting their effect through the facilitation of GABA-ergic neurotransmission (Haefely et al., 1985), and neuroleptic drugs, the potent antagonists at dopamine$_2$- receptors (Seeman, 1980).

2.1.1. Motor depressant and antiamphetamine effect of CCK agonists

The motor depressant effect of caerulein and CCK-8 in mice and rats manifested itself as a reduction both in motility and in frequency of rearings, and also as a potentiation of central depressant drugs (barbiturates, ethanol). The systemic and intracerebroventricular administration of CCK-8 and caerulein, but not of CCK-4, induced the hypolocomotion and blocked amphetamine-induced hyperlocomotion in the mouse (Zetler, 1985; Moroji et al., 1987; Hagino, Moroji, 1989). The pretreatment of mice with a selective CCK_A receptor antagonist devazepide antagonized the sedative effect of systemically and intracerebroventricularily administered CCK-8, reflecting the involvement of the CCK_A receptor subtype in the action of CCK agonist (Khosla, Crawley, 1988; O'Neill et al., 1991). On the other hand, motor depression occurred in the rat not only after intracerebroventricular administration, but also after microinjection of a few ng into the periaquaductal grey and ventromedial thalamus (Jurna, Zetler, 1981; Katsuura, Itoh, 1982; Matsushita, Itoh, 1982). The rearing inhibiting potency of caerulein in mice was many times greater than that of reference drugs as clonazepam, diazepam, haloperidol and clonidine (Zetler, 1980; 1983; 1984). It is thought that the motor depressant effect of CCK-8 and the suppression of dopaminergic activity by large doses of CCK agonists are of peripheral origin.

since they could be abolished by abdominal vagotomy in rats (Crawley, Kiss, 1985; Hamamura et al., 1989). Nevertheless, not all authors have been able to reproduce the finding that vagotomy can reverse the behavioural effects of CCK agonists in rodents. Moroji and Hagino (1987) have demonstrated that bilateral subdiaphragmatic vagotomy does not prevent the behavioural effects of systematically administered caerulein in mice. The suppression of electrical self-stimulation by caerulein is completely insensitive to vagotomy in rats (De Witte et al., 1986). Altar and Boyar (1989) have shown that peripherally injected CCK-8 interacts through CCK_B receptors with central dopaminergic mechanisms.

2.1.2. Interaction of CCK agonists with emotional behaviour in rodents

The systemic administration of CCK agonists (caerulein, pentagastrin, CCK-4) at very low doses inhibited the exploratory activity of mice and rodents in an elevated plus-maze (Harro et al., 1988, 1989, 1990a). The pretreatment of animals with proglumide, an unselective CCK antagonist, attenuated the anxiogenic-like effect of CCK agonists (Harro et al., 1989). Moreover, proglumide was able to antagonize the antiexploratory effect of GABA-negative drugs DMCM and pentetrazole in the plus-maze test (Harro et al., 1989). There it was possible to select the rats according to their behaviour in the elevated plus-maze. The animals with "anxious" behaviour had evidently higher density of CCK receptors in the cerebral cortex as compared with "non-anxious" animals (Harro et al., 1990). Evidence exists that the CCK_B receptor antagonist CI-988 reversed the anxiogenic-like effect induced by the cessation of long-term diazepam treatment in the mouse (Hughes et al., 1990). Rataud et al. (1991) have shown that the treatment of mice with the CCK_B receptor antagonist L-365,260, but not with the CCK_A receptor antagonist devazepide, causes the anxiolytic-like effect in the elevated plus-maze. The intracerebroventricular administration of pentagastrin signif.:antly reduced the exploratory activity of rats in the elevated plus-maze and this effect was reversed by pretreatment with CI-988 (Singh et al., 1991).

Several times higher doses of caerulein antagonized foot- shock- and isolatio'-induced aggressiveness in mice (Zetler, Baumann, 1986; Vasar et al., 1987). The antiaggressive effect of caerulein was blocked by pretreatment with proglumide and naloxone, an antagonist at opioid receptors (Vasar et al., 1987). Probably the antiaggressive effect of caerulein at high doses is related to its antinociceptive action (Zetler, 1985; Barber et al., 1989).

2.1.3. Anticonvulsant action of CCK agonists

Caerulein and CCK-8 delayed or prevented convulsions induced by picrotoxin, harman, thiosemicarbazide and isoniazid, whereas they were only weak antagonists or inactive against other convulsants such as bicuculline, pentetrazol and strychnine (Kadar et al., 1983; 1984; Zetler, 1980, 1981, 1985). The inactivity of caerulein and CCK-8 against convulsants, bicuculline and pentetrazol, and the resistance of the antiharman effect of caerulein against the benzodiazepine antagonist, flumazenil, separates the anticonvulsant action of caerulein and CCK-8 from that of diazepam (Zetler, 1985). The tonic-clonic convulsions induced by maximal electroshock were not prevented by caerulein and CCK-8, but latency to the onset of clonic seizures and the duration of postictal motor inactivity were prolonged (Zetler, 1985). The anticonvulsant effect of caerulein against picrotoxin induced seizures was reversed by pretreatment with an unselective CCK antagonist proglumide (Vasar et al., 1987). The benzodiazepine antagonist CGS 8216, but not flumazenil, also blocked the anti-picrotoxin effect of caerulein (Vasar et al., 1987).

2.2. Multiple CCK receptors in the brain and the selective antagonists at CCK receptors

Two CCK receptor subtypes have been differentiated according to their affinity for CCK fragments and analogues (Innis, Snyder, 1980; Moran et al., 1986; Dourish, Hill, 1987). 'Peripheral' CCK receptors (CCK_A) located in organs such as the gallbladder and pancreas (Sankaran et al., 1980), but also in several discrete brain regions such as the area postrema, interpeduncular nucleus, nucleus tractus solitarius, nucleus accumbens and the dorsal raphe (Moran et al., 1986; Hill et al., 1987; Barrett et al., 1989; Vickroy, Bianchi, 1989; Pinnock et al., 1990). CCK_A receptors exhibit a high affinity for the sulphated octapeptide fragment and a lower affinity for the desulphated octapeptide, gastrin and cholecystokinin tetrapeptide (CCK-4). Conversely, 'central' CCK sites (CCK_B) display a high affinity for all these CCK fragments and gastrin (Innis, Snyder, 1980). The vast majority of CCK receptors in the brain are of the CCK_B subtype and these receptors are ubiquitous in the mammalian brain (van Dijk et al., 1984; Hill et al., 1987). The careful analysis of dissociation curves also revealed the presence of two subtypes binding sites for [^{3}H]-pCCK-8 and [^{125}I]-CCK-8 in the rodents' brain (Wennogle et al., 1985; Sekiguchi, Moroji, 1986). There was only the 2-4-fold difference between the affinities of these binding sites in the different species (Sekiguchi, Moroji, 1986).

The preincubation of brain membranes at 37°C converted all the binding sites for [^{3}H]pCCK-8 into the low- affinity state (Soosaar et al., 1988). The relation of these binding sites of CCK to CCK_A and CCK_B receptors remains to be established.

In recent years very specific and highly potent non-peptide CCK antagonists have been developed, including some that are highly selective for CCK receptor subtypes and have good brain penetrability. These include the CCK_A receptor antagonists MK-329 [devazepide] (Chang, Lotti, 1986), A65186 (Kerwin et al., 1989) and lorglumide (Rovati etal., 1987), and the CCK_B receptor antagonists L-365,260 (Lotti, Chang, 1989), CI-988 (Hughes et al., 1990) and LY-262684 (Howbert et al., 1991). MK-329 (devazepide) is shown to antagonize the decreased feeding induced by systemic injection of CCK-8 (Dourish et al., 1989). Behavioural studies showed that both MK-329 (devazepide) and L-365,260 increased food intake and postponed the onset of satiety, however, the CCK_B receptor antagonist was 100 times more potent than MK-329 (Dourish et al., 1989). In contrast in the rat tail flick test, L-365,260 was only 5 times more potent than devazepide in enhancing of morphine analgesia (Dourish et al., 1990). L-365,260 and CI-988, but not devazepide, exhibited anxiolytic-like properties in several behavioural tests in rodents (Hughes et al., 1990; Singh et al., 1991; Rataud et al., 1991).

2.3. Interaction of neuroleptics and anxiolytic drugs with CCK-ergic neurotransmission

CCK-8 is shown to colocalize with dopamine in the mesencephalic dopaminergic neurons (Hökfelt et al., 1980) and with the major inhibitory transmitter GABA in the cerebral cortex and hippocampus (Somogyi et al., 1984; Hendry et al., 1984). Therefore it is not surprising that the administration of dopaminergic drugs, but also compounds affecting the GABA-ergic neurotransmission, is changing the CCK-ergic activity in the brain. Repeated administration, but not acute treatment, of different dopamine antagonists (clozapine, chlorpromazine and haloperidol) evidently increased the amount of CCK-8 in the striatum and mesolimbic structures (Frey et al., 1983). Chang et al. (1983) have shown that long-term treatment with haloperidol increases the density of CCK receptors in the cortical and limbic structures of mice and guinea pigs. By contrast, chronic treatment with an indirect dopamine agonist metamphetamine decreased the number of CCK-8 receptors in the rat cerebral cortex (Suzuki,

Moroji, 1989). In addition, the repeated, but not acute, administration of dopamine antagonists (haloperidol, chlorpromazine, clozapine etc.) induced, through the CCK-8 sensitive mechanisms, depolarization and subsequent inactivation of dopaminergic neurons in the rat midbrain (Chiodo, Bunney, 1983; Bunney et al., 1985). The CCK_A receptor subtype is shown to be involved in the mediation of this effect of neuroleptic drugs (Jiang et al., 1988; Zhang et al., 1991; Minabe et al., 1991).

Benzodiazepine tranquillizers (lorazepam, diazepam), exerting their action through the facilitation of GABA-ergic neurotransmission in the brain (Haefely et al., 1985), selectively depressed the CCK-8-induced excitation of rat hippocampal pyramidal cells (Bradwejn, De Montigny, 1984). The blockade of CCK-8 receptors by a selective CCK_A receptor antagonist lorglumide is shown to augment the action of diazepam in the rotarod motor performance test (Panerai et al., 1987). The withdrawal of long-term treatment with diazepam was demonstrated to increase the density of CCK receptors in the cerebral cortex and hippocampus of rats (Harro et al., 1990). The CCK_B receptor antagonist CI-988 has been shown to antagonize the behavioural signs of benzodiazepine withdrawal (Hughes et al., 1990).

3. AIMS OF THE PRESENT STUDY

The general purpose of the present work was to study the role of CCK receptors in the regulation of behaviour, but also in the action of haloperidol (a 'classical' neuroleptic drug) and diazepam (a widely used anxiolytic compound). In detail the aims of the present study were:

1. To examine the role of CCK_A and CCK_B receptors in the different behavioural effects of CCK agonists (motor depressant, antiamphetamine, modulation of emotional behaviour, anticonvulsant effect).

2. To analyze the interaction of CCK antagonists (devazepide and L-365,260) with the behavioural effects of CCK agonists.

3. To investigate the effects of long-term treatment with CCK antagonists (devazepide and L-365,260) on mice behaviour and [^{3}H]pCCK-8 binding in the mouse brain.

4. To compare the effects of long-term administration of caerulein and haloperidol on mice behaviour and on the parameters of $dopamine_2$-, opioid, CCK-8 and $GABA_A$-benzodiazepine receptors in the mouse brain.

5. To examine the changes at CCK receptors and in behavioural effects of caerulein after long-term treatment with diazepam and haloperidol in rodents.

4. MATERIALS AND METHODS

4.1. *Animals*

Male and female albino mice, weighing 20-25 g, and rats, weighing 150-300 g, were used throughout the study. Mice and rats were maintained at 20±3°C with food and water ad lib. Every experimental group consisted of 8-16 animals.

4.2. *Behavioural experiments*

4.2.1. *Exploratory activity in an elevated plus-maze.*

The method suggested initially by Handley and Mithani (1984) for measuring exploratory activity was used in rats with our modifications (Harro et al., 1990). The apparatus consisted of two opposite open arms (50x10 cm) without side walls and two enclosed arms (50x10x40 cm) with side walls and an end wall, extending from a central area (10x10 cm). To determine the exploratory activity in the open-part of the plus-maze, the maze (together with the central open square) was divided by lines into 7 equal squares. The maze was elevated to the height of 50 cm, and placed in a room exposed to daylight. During a 4-min test session the following measures were taken by an observer: (1) the latency period of the first open part entry, (2) the number of lines crossed in the open part, (3) the total time spent in the open part of plus-maze, and (4) the total number of closed and open part entries. At the beginning of the experiment the rat was placed at the centre of the plus-maze, facing usually the right closed arm. The rats clearly preferred the closed arms. An entry was counted only when all four limbs of the rat were within a given arm.

4.2.2. *Locomotor activity in an open-field test.*

After testing in the plus-maze the mice and the rats were placed singly into an open field (for mice 30x30x18 cm; for rats 100x100x40 cm, divided by lines into 16 equal squares) and observed during 3 min. The number of line crossings and rearings was counted.

4.2.3. *Measurement of motor activity in the photocell cages.*

Locomotor activity and (+)-amphetamine-induced hyperlocomotion in the mice were also measured in individual photocell cages. The cage for the registration of motor activity was a cylinder with an inner diameter of 40 cm and two photocells (located in the walls) for detection of motor activity. The motor depressant effect of

caerulein was measured between 0 and 30 min after subcutaneous administration of CCK agonist (15 μg/kg). The antiamphetamine action of caerulein (100 μg/kg, sc) was determined between 15 and 45 min after intraperitoneal injection (+)-amphetamine (an indirect dopamine agonist, 5 mg/kg). Caerulein was given 5 min after the administration of amphetamine.

4.2.4 *Foot-shock-induced aggressive behaviour.*

The interaction of caerulein with aggressive behaviour of the mice was detected by using the foot-shock-induced aggressive behaviour. A pair of mice was placed into a special box (15x15x15 cm) with a grid floor where during 2 min they received 30 foot-shocks with an intensity 1.5 mA. The number of aggressive contacts (bitings, boxings etc.) was counted during this period. Caerulein (40 μg/kg) was given subcutaneously 15 min before the experiment. The animals were used only once.

4.2.5 *Interaction of CCK agonists with seizures.*

The interaction of caerulein with picrotoxin and pilocarpine-induced seizures was detected in the individual observation boxes. The animals were placed there 15 min before the start of the experiment (20x20x20 cm). After this habituation period each animal was treated with caerulein (20-250 μg/kg sc) or saline. Picrotoxin (10 mg/kg ip), a potent antagonist at chloride channel, and the muscarinic agonist pilocarpine (380 mg/kg ip) were given 10 min later. After that the mice were observed for 60 min and the latencies to onset of clonic seizures, tonic extension and death were registered. In one part of the experiments the interaction of caerulein with quinolinate (5 μg icv) and N-methyl-D-aspartate (0.1 μg icv) induced seizures was studied. Caerulein was injected (1-50 ng icv or 100-500 μg/kg sc) 5 min before intraventricular administration of NMDA agonists. The unselective CCK antagonist proglumide (25-100 mg/kg ip) was injected 10 min before treatment with convulsants. The behaviour of the mice was observed for 10 min and the number of mice with clonic seizures was registered.

4.3. *Preparation of brain membranes for radioligand binding experiments*

Following decapitation the whole brain was rapidly removed from the skull. The different brain regions (cerebral cortex, striata, mesolimbic structures [nucleus accumbens and tuberculum olfactorium] and brainstem) were dissected on ice. Freehand method was used for dissection of the brainstem, whereas the other struc-

tures were dissected according to the method of Glowinski and Iversen (1966). Brain regions from 5-8 mice were pooled and homogenized in 10 volumes of ice-cold 50 mM TrisHCl, pH 7.4 at 20°C, using motor-driven Teflon-glass homogenizer for 12 strokes. The homogenate was centrifuged at 40000 x g for 15 min, resuspended in the same volume of buffer and again centrifuged for 15 min. The membrane preparation for all radioligands was the same, except for [^{3}H]-etorphine binding. In this case the homogenate of the mesolimbic structures was incubated for 45 min at 37°C between two centrifugations (for elimination of endogenous opioid peptides). In the case of [^{3}H]-muscimol binding the membranes were washed (centrifuged) 7 times at 40000 x g for 15 min.

4.4. *Radioligand binding experiments*

Different incubation mixtures were used for the radioligand binding experiments. The binding of [^{3}H]-etorphine (36 Ci/mmole, Amersham International), [^{3}H]-flunitrazepam (81 Ci/mmole, Amersham International) and [^{3}H]-muscimol (19 Ci/mmole, Amersham International) were performed in 50 mM TrisHCl (pH 7.4 at 20°C). [^{3}H]-spiroperidol (77 Ci/mmole, Amersham International) binding was determined in an incubation buffer consisting of the following: 50 mM TrisHCl (pH 7.4 at 20°C), 120 mM NaCl, 5 mM KCl, 2mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $EDTANa_2$, 50 µM pargyline and 0.1 % ascorbic acid. [^{3}H]-pentagastrin (81 Ci/mmole, NEN-Dupont) and [propionyl-^{3}H]propionylated-CCK-8 ([^{3}H]pCCK-8, 60-81 Ci/mmole, Amersham International) binding was studied in the following incubation medium: 10 mM HEPES-KOH (pH 6.8 at 20°C), 130 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM sodium, 0.2 % bovine serum albumine.

For the binding experiments each polypropylene tube (1.5 ml) received 50 µl of [^{3}H]-ligand, 50 µl of incubation medium or displacing compound and 400 µl of brain membrane homogenate (1-4 mg of original tissue wet weight). [^{3}H]-flunitrazepam was added in concentrations from 0.6 to 16 nM. The nonspecific binding was determined by using 1 µm flunitrazepam. The membranes of the cerebral cortex and brainstem were incubated at 0°C for 60 min. [^{3}H]-muscimol was used in concentrations from 1 to 80 nM. The nonspecific binding was measured by 100 µM muscimol. The membranes of the cerebral cortex and brainstem were incubated for 10 min at 0°C. [^{3}H]-etorphine was added in concentrations from 0.05 to 3 nM, the nonspecific binding was detected by adding naloxone (10 µM). The incubation of the mesolimbic membranes performed at 25°C for 45 min. [^{3}H]-spiroperidol was

used in concentrations from 0.1 to 2 nM and the nonspecific binding was measured by adding 1 μM spiroperidol. The membranes of murine striata were incubated for 30 min at 37°C. [^{3}H]-pentagastrin was added to the incubation medium in concentrations from 0.1 to 20 nM, nonspecific binding was detected with 1 μM caerulein. Incubation of [^{3}H]-pentagastrin was performed for 75 min at 25 °C. In the case of [^{3}H]pCCK-8 binding the brain membranes were preincubated for 25 min at 23°C with or without 200 nM CCK-8, after which the radioligand was added and the samples were carefully mixed. The membranes were incubated in the presence of radioligand for 120 min at 23°C.

In all cases the incubation was terminated by rapid centrifugation in a Beckman microfuge (11000 x g) for 3 min at room temperature. The supernatants were carefully aspirated and the pellets washed three times with 250 μl of ice-cold incubation buffer before transfer to scintillation vials. Radioactivity of samples was counted after stabilization in scintillation cocktail within 24 hours, using a Beckman LS 6800 (counting efficacy 50-54%). The binding experiments were repeated at least three times. The specific binding of [^{3}H]- radioligand was defined as the difference between the degree of binding in the absence and presence of excess of unlabelled ligand. Saturation curves were analyzed using a non-linear, regression program (ENZFITTER, Leatherbarrow, 1987).

4.5. [^{3}H]-spiroperidol binding "in vivo"

[^{3}H]-spiroperidol (5 μg/kg, 17 Ci/mmole, Amersham International) was injected subcutaneously into the dorsal part of the mouse's neck. N-propylnorapomorphine (NPA, 5 and 50 μg/kg) and caerulein (20-250 μg/kg) were used to inhibit [^{3}H]-spiroperidol binding. Two doses of NPA with different action on rodent behaviour were selected because two sites with different affinity for dopamine and its agonists existed on dopamine$_2$-receptors (Creese, Leff, 1982; Grigoriadis, Seeman, 1984). Five μg/kg NPA is ED_{50} for suppression of exploratory activity in mice, whereas 50 μg/kg is ED_{50} for motor excitation in rodents (Bradbury et al., 1983). NPA and caerulein were administered 15 min before [^{3}H]-spiroperidol. The animals (6 mice per group) were sacrificed 20 min after [^{3}H]-spiroperidol treatment by cervical dislocation. The brains were rapidly removed and the dorsal cortex and subcortical forebrain structures (striata and limbic structures) were dissected on ice. The dissected brain areas of each group were pooled and homogenized using a glass-teflon homogenizer by hand during 1 min. The homogenization procedure was performed in ice-cold Tris-HCl buffer (50 mM, pH 7.4 at 20°c) in the volume of 40 mg tissue

per ml. After homogenization 0.5 ml (20 mg tissue) of suspension was pipetted into 6 polypropylene tubes (1.5 ml) and centrifuged during 10 min at 9000g. The supernatant was carefully discarded and the remaining pellet was washed and cut into vials. Radioactivity of the samples was counted after stabilization in the Bray cocktail within 12 hours in Beckman LS 6800 with the counting of efficacy 43 %. The binding experiments were repeated at least three times and the data were analyzed by using Student's t-test.

4.6. *Drugs and their administration*

The drugs used in the present investigation are caerulein (*Bachem; Farmitalia Carlo Erba*), CCK-8 (*Bachem; Bristol-Myers & Squibb*)), pentagastrin (*Sanitas*), CCK-4 (*Bachem*), proglumide (*Rotta Pharmaceutici*), devazepide, L-365,260 (*Merck Sharp & Dohme*), haloperidol (*Gedeon Richter*), spiroperidol (*Janssen Pharmaceutica*), naloxone (*Dupont*), flunitrazepam, flumazenil (*Hoffmann-La Roche*), diazepam (*Gedeon Richter*), muscimol (*Serva*), pilocarpine (*Sigma*), picrotoxin (*Sigma*), quinolinic acid (*Sigma*), N-methyl-D-aspartate (*Sigma*), apomorphine (*Sigma*), N-propylnorapomorphine (*NPA, Sterling-Winthrop*) and (+)-amphetamine (*Sigma*). CCK agonists, proglumide, dopamine agonists (apomorphine, NPA and amphetamine), muscimol, naloxone and commercial solutions of haloperidol, diazepam and pentagastrin were prepared in saline (0.9 % NaCl solution w/v). Devazepide (MK-329, 1-methyl-3-(2-indoloyl)amino-5- phenyl-3H-1,4-benzodiazepin-2-one), L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3yl)-N'-(3-methyl-phenyl)urea and flumazenil were suspended in saline with 1-2 drops of Tween-85. Each injection was done in a volume 0.1 ml/10 g body weight.

Devazepide (2 mg/kg twice daily) and L-365,260 (2 mg/kg twice daily) were administered for 10 days. The effects of caerulein and amphetamine on motor activity were studied 24 h after the last injection of CCK-8 antagonists. [^{3}H]pCCK-8 binding experiments were carried out also 24 h after the last injection of drugs. Haloperidol (0.5 mg/kg) and caerulein (0.1 mg/kg) were injected once daily for 15 days. The doses of haloperidol and caeruelin were chosen according to the previous behavioural experiments. Acute administration of haloperidol (0.5 mg/kg) and caerulein (0.1 mg/kg) caused significant neuroleptic effects in the mice (catalepsy, motor depression and the reversal of the behavioural effects of dopamine agonists). The behavioural and radioligand experiments were performed 72 hours after the

cessation of haloperidol and caerulein treatment.

Haloperidol (1 mg/kg), diazepam (5 mg/kg) and saline were injected once daily for 14 days. The doses of caerulein, haloperidol and diazepam were chosen according to our previous studies (Vasar et al., 1990; Harro et al., 1990a). The behavioural and radioligand experiments were done 72 hours after the last injection of haloperidol and diazepam treatment. This withdrawal period was chosen in order to be sure that most haloperidol and diazepam had been washed out from the mouse's body, because large doses of diazepam and haloperidol may substantially mask the behavioural actions of caerulein in the mouse (Harro et al., 1990a; our unpublished data).

4.7. *Statistical analysis*

The behavioural data were analyzed by using a one-way analysis of variance (ANOVA). Post hoc comparisons between the groups were made by using the Newman-Keuls test. The Mann-Whitney U-test was also used to analyze the behavioural experiments. The results of radioligand binding studies were evaluated by Student's t-test.

5. RESULTS AND DISCUSSION

5.1. Motor depressant and antiamphetamine effect of CCK agonists

Subcutaneous treatment with caerulein and CCK-8 (1-50 μg/kg) in a dose-dependent manner depressed the locomotory activity of mice (Table 1). Five μg/kg caerulein caused a significant reduction of motor activity. The administration of 10 μg/kg CCK-8 did induce the same effect on the locomotory activity of the mice. The CCK_B receptor agonists pentagastrin and CCK-4 did not change the locomotor activity up to the dose of 100 μg/kg. The pretreatment of the mice with an unselective CCK antagonist proglumide (1-50 mg/kg) failed to affect the motor depressant action of caerulein (Table 2). The pretreatment of the animals with a selective CCK_A receptor antagonist devazepide (0.1-10 μg/kg) only partially antagonized the sedative effect of the CCK agonist. By contrast, a selective CCK_B receptor antagonist L-365,260 (0.1- 1000 μg/kg) significantly enhanced the motor depressant effect of caerulein. A dopamine agonist apomorphine in a low dose (0.1 mg/kg) also reduced the motor activity of the mice. Pretreatment with L-365,260 (1-10 μg/kg) significantly enhanced the sedative effect of apomorphine in the mouse (Table 2). Small doses of devazepide (1-10 μg/kg) only partially attenuated the sedative effect of apomorphine, whereas high doses (100 and 1000 μg/kg) enhanced the action of the dopamine agonist. The pretreatment with caerulein (15 μg/kg) significantly potentiated apomorphine-induced hypolocomotion in the mouse. The co-administration of apomorphine and caerulein caused nearly complete loss of motor activity in the mice. Neither devazepide, nor L-365,260 could antagonize the effect of concomitant treatment with apomorphine and caerulein. According to the existing data the motor depressant effect of apomorphine and caerulein are thought to be related to the decreased activity of dopaminergic cells in the mesencephalon (Strömbom, 1977; Zetler, 1985). The behavioural effects of CCK antagonists probably reflect the distinct role of CCK_A and CCK_B receptors in the regulation of presynaptic dopaminergic activity in the mouse's brain. The blockade of CCK_B receptors by L-365,260 decreases the dopaminergic activity, whereas the interaction of devazepide with CCK_A receptors increases it in the mouse's brain. It seems probable that the CCK_A receptors at which caerulein and CCK-8 act to reduce locomotor activity are in the periphery and are associated in some way through the vagal afferent pathway with dopaminergic neurons (Crawley, Schwaber, 1984; Crawley, Kiss, 1985; Hommer et al., 1985). However, it is important to stress that in our study devazepide, in contrast to the investigation of Khosla and Crawley (1988), only moderately

Table 1

THE BEHAVIOURAL EFFECTS OF CCK AGONISTS IN RODENTS

Behavioural effect	Caerulein	CCK-8	Pentagastrin	CCK-4
Motor depression	+	+	0	0
Inhibition of exploratory activity	+	+	+	+
Antiaggressive effect	+		0	
Antiamphetamine effect	+	+	0	
Anticonvulsant effect	+	+	+/0	

+ - strong effect; +/0 - moderate effect; 0 - ineffective

Table 2

THE INTERACTION OF CCK ANTAGONISTS WITH THE BEHAVIOURAL EFFECTS OF CCK AND DOPAMINE AGONISTS

Behavioural effect	Proglumide	Devazepide	L-365,260
CCK-8-induced hypo-locomotion	ineffective	antagonism	potentiation
Antiamphetamine effect of caerulein	ineffective	antagonism	moderate antagonism
Antiexploratory effect of CCK-4	antagonism at low dose	antagonism at high dose	antagonism at low dose
Anticonvulsant effect of CCK agonists	antagonism at high dose	antagonism at high dose	antagonism at high dose
Apomorphine-induced hypolocomotion	ineffective	antagonism	potentiation
Amphetamine-induced hyperlocomotion	antagonism at high dose	antagonism at high dose	ineffective

antagonized the motor depressant effect of caerulein. This seems to support the idea that not only the CCK_A receptor subtype is involved in mediating the effect of caerulein. The concomitant treatment with a low dose of apomorphine and caerulein causes nearly complete loss of motor activity in the mice. Devazepide and L-365,260 were completely ineffective against the motor depression induced by simultaneous administration of caerulein and apomorphine. According to the studies of Hommer et al. (1986) and Crawley (1989) the CCK receptors that mediate the potentiation of dopamine-induced hypolocomotion and suppression of the electrical activity of dopamine neurons in the rat mesencephalon by CCK belong to the CCK_B subtype. Altar and Boyar (1989) have found that the antagonistic effect of centrally or peripherally administered CCK agonists on the amphetamine-evoked dopamine release in the mouse is also related to the CCK_B receptor subtype. Nevertheless, it is not clear whether CCK_B receptors are involved in the potentiation of apomorphine-induced hypolocomotion induced by caerulein in the mouse and it remains to be established.

An indirect dopamine agonist (+)-amphetamine (5 mg/kg) caused a three-fold increase in the number of motor activity counts. Pretreatment with caerulein (25-125 μg/kg) induced a dose-dependent inhibition of (+)-amphetamine-induced hyperlocomotion (Table 1). CCK-8 significantly antagonized the behavioural effect of (+)-amphetamine at a dose 200 μg/kg, whereas pentagastrin was completely ineffective up to 1 mg/kg. It is worth noting that intraventricular administration of CCK-33 (1 Ivy Unit) also blocked the behavioural effects of (+)-amphetamine (stereotyped behaviour and motor stimulation). Taking into account that the selective CCK_A receptor antagonist devazepide (10 μg/kg) also completely antagonized the behavioural effects of dopamine agonist, it is possible that CCK-33 interacted with (+)-amphetamine-induced behavioural effects through the CCK_B receptor subtype. This finding is in accordance with the study of Crawley et al. (1985) where administration of CCK-8 into the anteriolateral part of the nucleus accumbens reversed (+)-amphetamine-induced hyperlocomotion by interacting with the CCK_B receptor subtype. L-365,260 had no effect on (+)-amphetamine induced hyperactivity, whereas devazepide in high doses (above 1 mg/kg) suppressed spontaneous motor activity and completely antagonized the motor stimulation induced by (+)-amphetamine in the mice. The unselective CCK antagonist proglumide induced a similar antiamphetamine effect at doses 5-15 mg/kg. The pretreatment of mice with devazepide over a wide dose range (1-100 μg/kg) completely blocked the antiamphetamine effect of caerulein (Table 2). The administration of L-365,260 at a low dose (1 μg/kg) also did counteract the

antiamphetamine effect of the CCK agonist. Proglumide (1-100 mg/kg) could not affect the antiamphetamine effect of caerulein. The interaction of CCK antagonists with amphetamine-induced hyperlocomotion and the antiamphetamine effect of caerulein is somewhat different from their action on caerulein- and apomorphine-elicited hypolocomotion. It is suggested that the different pharmacology of CCK-8 against dopamine-induced hypolocomotion and hyperlocomotion is related to the involvement of distinct brain regions in the development of two opposite behavioural effects of dopamine in the rat (Crawley, 1989). The $CCK\text{-}_B$/gastrin antagonist L-365,260 did not significantly change amphetamine-induced hyperlocomotion, but paradoxically it reversed (at a low dose) the antiamphetamine effect of caerulein. Devazepide antagonized the antiamphetamine effect of caerulein at low doses, where it probably interacts selectively with CCK_A receptors. However, at a high dose (1 mg/kg), which also interacts with CCK_B receptors (Dourish et al., 1989), devazepide *per se* reverses the motor excitation induced by (+)-amphetamine. It is noteworthy that proglumide, which failed to interact with the sedative and antiamphetamine effect of caerulin, substantially antagonized the motor excitation induced by (+)-amphetamine (Table 2). According to the studies of Moroji and Hagino (1987) the antiamphetamine effect of caerulein in mice is completely resistant to vagotomy. Accordingly, it seems very probable that the CCK_A receptors involved in the antiamphetamine effect of caerulein are distinct from the CCK_A receptors related to caerulein- and apomorphine-induced hypolocomotion. The idea that these CCK_A receptors are located in the mouse's brain is supported by the study of Hagino et al. (1989), in which the intraventricular administration of CCK-8 and caerulein, but not desulfated CCK- 8 and CCK-4, antagonizes amphetamine-induced motor excitation in the mouse. The possible mediation of the antiamphetamine effect of caerulein through the CCK_A receptors in the mouse's brain may reflect the substantial difference between CCK_A receptors in the mouse and rat brains. Crawley et al. (1985; Crawley, 1989) have shown that CCK-8 by interacting with CCK_A receptors facilitates dopamine-induced hyperlocomotion in the posteriomedial part of the nucleus accumbens of the rat. The different pharmacology of CCK_A receptors in the mouse and the rat brains seems to account for the interspecies differences in the behavioural effects of caerulein in the mouse and the rat. Namely, systemic treatment with caerulein reversed the behavioural effects of amphetamine in the mouse, but not in the rat. On the other hand, devazepide at a moderate dose (10 μg/kg) completely reversed amphetamine-induced stereotypy and motor excitation in the rat, whereas in the mouse it potently antagonized the antiamphetamine effect of caerulein.

In conclusion, the results of the present study reflect the apparently distinct role of CCK_A and CCK_B receptors in the regulation of motor activity. The opposite effect of devazepide and L-365,260 on caerulein- and apomorphine-induced hypolocomotion is probably related to the antagonistic role of CCK_A and CCK_B receptor subtypes in the regulation of dopaminergic cells. The CCK_A receptors, at which caerulein acts to reduced locomotor activity, are possibly in the periphery and are associated in some way through the vagal afferent pathways with dopaminergic neurons in the mesencephalon (Crawley and Schwaber, 1984; Crawley and Kiss, 1985). The antiamphetamine effect of caerulein seems to be linked to the stimulation of CCK_A receptors in the mouse's brain, whereas probably the blockade of both subtypes of the CCK-8 receptor is involved in the antiamphetamine effect of devazepide.

5.2. Interaction of CCK agonists and antagonists with emotional behaviour

The lowest dose of caerulein to cause the anxiogenic- like effect on the exploratory behaviour of the rat in the elevated plus-maze was 100 ng/kg (Table 3) pentagastrin had a similar effect after administration of 500 ng/kg. The subcutaneous treatment with 10 µg/kg CCK-4 in some experiments also significantly decreased the exploratory activity of the rats. The maximal reduction of the animals' behaviour was seen after injection of 25 and 50 µg/kg of CCK-4. The anxiogenic-like effect of CCK agonists in the elevated plus-maze was in good accordance with their potency to inhibit [^{3}H]pCCK-8 (0.3 nM) binding in the cerebral cortex, but not in the pancreas (Table 3). According to these results it is very likely that CCK_B receptors have a significance in the anxiogenic-like action of CCK-8 agonists on the rat. The interaction of different CCK antagonists (proglumide, devazepide and L-365,260) with the anxiogenic-like effect of CCK-4 (50 µg/kg) was also studied (Table 2). The pretreatment with 1 µg/kg L-365.260, the selective antagonist at CCK_B receptors, moderately reduced the anti-exploratory effect of CCK-4. But only 10 µg/kg L-365,260 caused statistically evident antagonism with the anxiogenic-like action of CCK-4. The CCK_B receptor antagonist L-365,260 was nearly 100-fold more effective than the selective CCK_A receptor antagonist devazepide (1 mg/kg) and the unselective CCK antagonist proglumide (1 mg/kg). Consequently, the experiments with the selective CCK antagonists obviously support the idea that the anxiogenic-like effect of peripherally administered CCK agonists is related to the CCK_B receptor subtype in the rat.

Table 3

THE CORRELATION BETWEEN ANXIOGENIC-LIKE EFFECT OF CCK AGONISTS AND THEIR AFFINITY AT CCK RECEPTORS IN THE RAT CEREBRAL CORTEX AND PANCREAS

CCK agonist	Anti-exploratory effect in plus-maze (pmol/kg)	IC_{50} values against [^{3}H]-pCCK-8	
		cerebral cortex (nM)	pancreas (nM)
Caerulein	0.074	1.1	0.6
Pentagastrin	0.670	10	6200
CCK-4	17.3	411	>10000
Pearson's γ		0.9999	0.808
		p=0.008	p>0.4

The systemic administration of caerulein (2-100 µg/kg), but not that of pentagastrin, induced a dose-dependent reduction of foot-shock aggressiveness in the male mice. The failure of pentagastrin, the agonist at CCK_B/gastrin receptors, to reduce the aggressive behaviour, probably supports the opinion that the CCK_A receptor subtype is mediating the antiaggressive effect of caerulein in the mouse. The pretreatment of mice with the unselective CCK antagonist proglumide (5-25 mg/kg) antagonized the antiaggressive effect of caerulein (40 µg/kg). The opioid antagonist naloxone (0.5- 2.5 mg/kg) also blocked the antiaggressive effect of CCK agonist. Concomitant repeated administration of caerulein (100 µg/kg twice daily, for 14 days), but not of pentagastrin (250 µg/kg twice daily), with apomorphine (1 mg/kg twice daily) potently antagonized the development of apomorphine aggressiveness in the male rat. These data seem to support the above mentioned idea that the antiaggressive effect of caerulein in rodents is linked to the CCK_A receptor subtype. Moreover, intraventricular injection of 200 ng of CCK-4, but not of caerulein, induced an appreciable enhancement of the foot-shock-induced aggressiveness. The animals receiving CCK-4, differently from the control animals, inflicted injuries on one another. When the dose of CCK-4 was further increased, at first the aggressive reactions were reduced (1000 ng), but then (5000 ng) they again

exceeded the control level. Pirenperone, the selective antagonist at serotonin$_2$-receptors, in comparison with the dopamine$_2$-receptor antagonist haloperidol, had a more pronounced effect on the CCK-4-enhance foot-shock aggressiveness. A low dose of haloperidol (0.01 mg/kg) potentiated the action of CCK-4, and only the administration of 0.2 mg/kg haloperidol significantly suppressed aggressive behaviour. Pirenperone, in contrast to haloperidol, significantly lowered the intensity of aggressive behaviour in a dose of only 0.01 mg/kg. When the dose was further increased, the antiaggressive action of pirenperone became more profound. The increase of aggressive behaviour, although not so significant, was also established after systemic treatment with a nearly 60-fold higher dose of CCK-4 (50 µg/kg, 12.5 µg per rat). Accordingly, there is considerable evidence that CCK-4 potentiates the foot-shock aggressiveness through the CCK_B receptor subtype. The strong antagonism of pirenperone against the proaggressive action of CCK-4 seems to support the role of serotoninergic mechanisms in the action of the CCK_B agonist.

In conclusion, the above described results reflect the apparently distinct action of CCK agonists on the emotional behavior in rodents. At very low doses caerulein, CCK-8, pentagastrin and CCK-4 induced anxiogenic-like effect on the exploratory behaviour in the rats. There is good correlation between the minimal effective doses of CCK agonists and their affinity at CCK_B receptors in the rat's cerebral cortex, but not at CCK_A receptors in the pancreas. L-365,260 was a stronger antagonist of the anxiogenic-like effect of CCK-4 as compared with devazepide. Accordingly, the anxiogenic-like effect of CCK agonists is related to the CCK_B receptor subtype in the rat. On the other hand, the unselective CCK_B/CCK_A agonist caerulein at high doses inhibited through the naloxone-sensitive mechanisms the aggressive behaviour in the mice. Probably, the antiaggressive effect of caerulein is related to CCK_A receptors. By contrast, the selective CCK_B agonist CCK-4 increased the foot-shock-induced aggressive behaviour in the rat after systemic, as well as after intracerebroventricular administration. The comparison of doses of CCK-4 affecting the aggressive behavior after the intracerebral and systemic administration left little doubt that this action of CCK-4 is related to the CCK_B receptor subtype in the brain. However, it seems possible that CCK_A and CCK_B receptors have a distinct role in the regulation of negative emotions and this may explain why CCK-4 and pentagastrin, but not CCK-8, induce generalized anxiety and panic attacks in man (De Montigny, 1989; Abelson, Nesse, 1990; Bradwejn et al., 1990).

5.3. Anticonvulsant effect of CCK agonists

The administration of picrotoxin (10 mg/kg) induced fatal seizures in all the tested animals. Pretreatment of control animals with caerulein (20-500 µg/kg) obviously delayed the onset of clonic seizures, tonic seizures, and death (Table 1). Moreover, caerulein (125 µg/kg) was able to protect 60 % of mice against the fatal action of picrotoxin. The concomitant administration of the CCK antagonist proglumide (50 mg/kg) with caerulein evidently antagonized the anticonvulsant action of CCK agonist (Table 2).

Systemic treatment with muscarinic agonist pilocarpine (380 mg/kg) evoked fatal seizures in all injected male mice. The pretreatment of mice with CCK-8 (25-200 µg/kg) significantly antagonized the effect of 380 mg/kg pilocarpine (Table 1). 50 µg/kg CCK-8 obviously reversed the effect of muscarinic agonist, the further increase of CCK-8 dose did not enhance the effect of neuropeptide. 13 mice from the 39 tested survived pilocarpine-induced seizures after administration of 200 µg/kg CCK-8. The CCK_B/gastrin agonist pentagastrin only moderately reduced the convulsant action of pilocarpine (Table 1). The CCK_A receptor antagonist devazepide at a high dose (1 mg/kg) evidently antagonized the anticonvulsant effect of CCK- 8 (Table 2). The CCK_B receptor antagonist L-365,260 also after the administration of a high dose (1 mg/kg) reversed the anticonvulsant action of CCK.

Intracerebroventricular administration (1-50 ng), but not systemic injection (100-500 µg/kg sc), of caerulein completely blocked the seizures induced by quinolinc acid (5 µg icv) and N-methyl-D-aspartate (NMDA, 0.2 µg icv) in the mice. The antagonist at CCK receptors proglumide (50 mg/kg) attenuated the anticonvulsant effect of caerulein. The coadministration of proglumide (25 mg/kg) with a subthreshold dose of quinolinic acid (2.5 µg icv) induced fatal seizures in all the tested animals.

Picrotoxin, pilocarpine and NMDA up to 1 mM did not interact with [^{3}H]pCCK-8 binding in the radioligand studies in '*in vitro*'. The anxiogenic dose of picrotoxin (0.5 mg/kg) increased the density of CCK receptors in the cerebral cortex and hippocampus (FIGURE 1). The administration of picrotoxin at the convulsant doses (1 and 2.5 mg/kg) increased the affinity, but decreased the density of [^{3}H]pCCK-8 binding sites in the rat's forebrain. The injection of a high dose of pilocarpine (380 mg/kg) changed the parameters of [^{3}H]pCCK-8 binding sites in several for brain structures of the rat. Pilocarpine also reduced significantly the

number of [^{3}H]pCCK-8 binding sites in the striatum, frontal cortex and hippocampus. Simultaneously, their affinity was increased in the striatum and hippocampus. The anxiogenic dose of NMDA increased also the density [^{3}H]pCCK-8 binding sites in the mouse's forebrain. By contrast, the administration of NMDA at convulsant doses (100-200 mg/kg) increased the affinity of CCK receptors, but decreased their density in the mouse's brain.

The above presented data reflect a significant role of CCK-8 receptors in the modulation of the epileptogenic effect of picrotxin, pilocarpine and NMDA agonists. CCK-8 and caerulein potently antagonized the seizures induced by chemoconvulsants. The selective CCK antagonists devazepide and L-365,260 reversed the anticonvulsant effect of caerulein and CCK-8 against the pilocarpine-induced seizures. However, this happened after the administration of a very high dose (1 mg/kg) of CCK antagonists. It is noteworthy that the effect of L-365,260 was somewhat stronger as compared with devazepide. Indeed, L-365,260, in a wide dose range (10-1000 µg/kg), blocked also the effect of CCK-8 on pilocarpine-induced lethality. Nevertheless, both subtypes of CCK-8 (CCK_A and CCK_B) seem to be involved in the anticonvulsant effect of CCK-8. However, the site of the anticonvulsant action of CCK agonists remains to be ambiguous. The administration of picrotoxin, NMDA and pilocarpine at convulsant doses significantly decreased the density of CCK receptors in the different forebrain structures of the rodents. The administration of picrotoxin and kainic acid was shown to reduce the concentrations of CCK-8-like immunoreactivity in the rat's limbic structures (Kato et al., 1988, Gall, 1988). Therefore, it is possible that the CCK_B receptor subtype is, at least partially, involved in the modulation of seizures induced by different chemoconvulsants (pilocarpine, picrotoxin, NMDA etc.). Last not least, the unselective CCK agonists (caerulein and CCK-8) seem to be the unique anxiogenic-like compounds. They possess, differently from the other anxiogenic drugs, the anticonvulsant action.

5.4. Effect of repeated administration of devazepide and L-365,260 on motor activity and [3H]pCCK-8 binding in mice

A single injection of the CCK_A antagonist, devazepide (2 mg/kg) increased the frequencies of rearings and line-crossings, whereas the CCK_B antagonist, L-365,260 (2 mg/kg) only increased the number of rearings. Tolerance developed to the locomotor effects of the antagonists after their repeated administration (for 10

days, twice daily). However, tolerance to devazepide was not seen in all the mice, about 20 % becoming aggressive with repeated treatment. The mice attempted to bite the backs of other mice in the cage. Administration of a moderate dose of caerulein (20 µg/kg) 24 h after the last injection of CCK antagonists reduced motor activity in control animals pretreated with vehicle and in mice treated with L-365,260. The sedative effect of caerulein (20 µg/kg) was significantly reduced in mice pretreated with devazepide. Administration of (+)-amphetamine produced a 54% increase in the number of line crossings and this effect was not altered by 10 days pretreatment with L-365,260. Injection of (+)-amphetamine in animals pretreated with devazepide increased motor activity more than in animals pretreated with vehicle. This increase was more marked when compared with the saline + vehicle group. Repeated treatment with devazepide and L-365,260 altered [^{3}H]pCCK-8 binding in the mouse's forebrain. Treatment with devazepide slightly increased the affinity of [^{3}H]pCCK-8 binding sites whereas after L-365,260 there was no significant change. Devazepide and L-365,260 increased the number of [^{3}H]pCCK-8 binding sites, but only an 83 % increase after l-365,260 was significant.

Repeated treatment with devazepide significantly affected the locomotor activity of mice and their behavioural responses to caerulein and (+)-amphetamine. The results are interpreted in the light of recent suggestions that CCK_A and CCK_B receptors have opposite effects on dopamine-mediated behaviours (Crawley, 1989; Koshikawa et al., 1990). Chronic treatment with proglumide increased the activity of dopaminergic cells in the mesolimbic system (Chiodo et al., 1987). A similar effect occurring after repeated treatment with devazepide might explain the reduced sedative effect of caerulein. The increased motor stimulant effect of (+)-amphetamine is most likely due to increased sensitivity of striatal and mesolimbic $dopamine_2$-receptors because long-term administration of CCK antagonists (proglumide, devazepide) increased the number of $dopamine_2$-receptors in the basal ganglia of the rodents (Csernansky et al., 1987; our unpublished data). Increased $dopamine_2$-receptors sensitivity would also account for the increased aggressiveness seen in some mice during repeated treatment with devazepide. However, it is difficult to explain the discrepancy between the increase in the density of CCK-8 receptors and the lack of any changes in behaviour after 10-day treatment with L-365,260. It may be that the behaviours studied are more dependent on CCK_A receptors and that L-365,260 does not affect these receptors even at high doses.

5.5. *Comparison of the effects of long-term haloperidol and caerulein treatment on mice behaviour and [^{3}H]-radioligand binding in the mouse brain*

According to our preliminary experiments the cessation of long-term administration of haloperidol and caerulein did not cause significant signs of withdrawal. The basal motor activity of mice was unaltered 72 hours after the last injection of repeated treatment with saline and haloperidol as well as caerulein. In addition, we found that quinolinic-acid- and picrotoxin-induced seizures were identical after the withdrawal of long-term saline, haloperidol or caerulein treatment. Moreover, there were no significant differences in the binding values of [^{3}H]-spiroperidol, [^{3}H]-flunitrazepam and [^{3}H]-pentagastrin if the tissues were obtained 2 or 72 hours after the last injection of haloperidol and caerulein. Therefore the changes in mice behaviour and radioligand binding described below were not caused by the withdrawal of haloperidol and caerulein, but were rather induced by the repeated administration of both drugs.

Seventy-two hours after the cessation of 15 days of haloperidol (0.5 mg/kg daily) and caerulein (0.1 mg/kg) treatment the effects of different drugs on mice motor activity were changed. The motor excitation induced by amphetamine (3 mg/kg) was evidently increased after haloperidol and caerulein treatment (Table 4). However, tolerance developed to the action of muscimol (1 mg/kg), caerulein (15 µg/kg) and flumazenil (10 mg/kg). Muscimol and caerulein were not able to suppress the motor activity of the mice after haloperidol or caerulein administration. Flumazenil, which increased the motor activity in saline-treated animals, failed to affect the activity after 15 days of haloperidol or caerulein treatment.

The prolonged haloperidol and caerulein treatment also affected the binding of different radioligands to washed brain membranes in a similar way. The density of [^{3}H]-spiroperidol binding sites in striatum (mainly dopamine$_2$-receptors) was significantly increased after the administration of both drugs (Table 4). Similar increase of [^{3}H]-etorphine (labelling mu-, delta- and kappa-opioid receptors) binding sites was detected in the mesolimbic structures. Accordingly, our data suggest that both compounds increase the number of dopamine$_2$-receptors in the striatum and opioid receptors in the mesolimbic structures. The increased sensitivity of the mice to the motor stimulating effect of amphetamine, a compound that increases the release of dopamine, probably reflects the enhancement of

dopamine$_2$-receptors density after caerulein or haloperidol treatment. Some authors have demonstrated that opioid receptors play an important role in the regulation of dopamine receptors' sensitivity (Matsubara, Matsushita, 1986; Stinus et al., 1986). It seems probable that the increased sensitivity of opioid receptors is obligatory for the development of hypersensitivity at dopamine receptors in the mesolimbic area.

Table 4
THE COMPARISON OF LONG-TERM EFFECTS OF HALOPERIDOL AND CAERULEIN

	Haloperidol	Caerulein
Behavioural effects		
Caerulein-induced hypolocomotion	tolerance	tolerance
Muscimol-induced hypolocomtion	tolerance	tolerance
Flumazenil-induced motor stimulation	tolerance	tolerance
Amphetamine-induced motor excitation	increase	increase
Radioligand binding studies		
Dopamine$_2$-receptors in striatum	increased density	increased density
Opioid receptors in mesolimbic structures	increased density	increased density
CCK-8 receptors in cerebral cortex	decreased density*	decreased density*
$GABA_A$-benzodiazepine receptors		
in cerebral cortex	decreased density	decreased density
in brainstem	increased density	increased density

* - increased affinity (decrease of K_d values)

Differently from [^{3}H]-spiroperidol and [^{3}H]-etorphine binding the number of [^{3}H]-pentagastrin (a ligand interacting with CCK_H/gastrin receptors) binding sites was evidently decreased, but their affinity was increased in the mouse cerebral

cortex (Table 4). The significant reduction of motor depressant effect of caerulein after haloperidol or caerulein treatment is probably related to the decrease of the CCK-8 receptor number in the brain. Many behavioural studies now support the idea that CCK-8 acts as a functional antagonist of dopamine and endogenous opioid peptides in the brain (Faris et al., 1983; Zetler, 1985; Matsubara, Matsushita, 1986). Accordingly, the subsensitivity of CCK-8 receptors seems to be necessary for the development of hypersensitivity at dopamine and opioid receptors. The changes in [^{3}H]-flunitrazepam and [^{3}H]-muscimol binding were dependent on the brain region studied. In the cerebral cortex their number was reduced, whereas in the brainstem the density of [^{3}H]-flunitrazepam and [^{3}H]-muscimol binding sites was increased after 15-day treatment of haloperidol and caerulein. The similar alteration of CCK-8 and benzodiazepine-$GABA_A$ receptors may be linked to the finding that CCK-8 and GABA are comediators in the same neurons of the cerebral cortex and hippocampus (Somogyi et al., 1984). The molecular changes at benzodiazepine and $GABA_A$ receptors are probably associated with tolerance of behavioural effects of $GABA_A$ agonist muscimol and benzodiazepine antagonist flumazenil. Muscimol did not suppress and flumazenil did not increase the motor activity of the mice after long-term treatment of haloperidol and caerulein.

In conclusion, the similar actions of haloperidol and caerulein after long-term treatment seem to be related to the fact that the effects of haloperidol are mediated not only through dopaminergic, but also via CCK-8-ergic mechanisms. The effect of CCK-8 seems to be related to the modulation (probably through CCK_A receptors) of the sensitivity of different neurotransmitter receptors (dopamine, endogenous opioid peptides and GABA).

5.6. Changes at CCK receptors after long-term treatment with haloperidol and diazepam

Administration of caerulein (15 μg/kg) to the saline pretreated mice produced an evident inhibition of the locomotor activity in the animals. After diazepam withdrawal the motor depressant effect of caerulein was somewhat reduced, as compared with the saline + diazepam treated mice. But, it was still statistically significant in comparison with the saline + saline treated group. By contrast, caerulein was unable to decrease the motor activity of the mice after the long-term administration of haloperidol (Table 5). The administration of (+)-amphetamine (5 mg/kg) induced a nearly 4-fold increase in the motor activity of the mice as

compared with the saline-treated control mice. The co-administration of caerulein (100 μg/kg) with (+)-amphetamine potently antagonized the action of dopamine agonist. Long-term treatment with haloperidol induced a complete tolerance to the antagonistic action of caerulein against (+)-amphetamine- induced motor excitation. Two-weeks administration of diazepam did not change the antiamphetamine effect of caerulein. There is considerable evidence that the motor depressant and antiamphetamine effect of CCK agonists (caerulein, CCK-8) are related to their interaction with CCK_A receptors in the mouse (Khosla, Crawley, 1988; Hagino et al., 1989; Crawley, 1989; O'Neill et al., 1991). The intraventricular administration of caerulein (5-50 ng) also decreased the locomotor activity in the rat. However, after the long-term treatment with haloperidol caerulein (50 ng icv) significantly increased the motor activity of the animals in the open-field test. On the other hand, repeated treatment with haloperidol potentiated the long-term antiamphetamine effect of caerulein and CCK_B/gastrin agonist pentagastrin in the rat. Thus, it is probable that the long-term antiamphetamine effect of CCK agonists in the rat is related to their interaction with the CCK_B receptor subtype. Accordingly, it is most likely that long-term treatment with haloperidol causes the subsensitivity at CCK_A receptors modulating the activity of dopaminergic neurons, whereas the CCK_B receptor subtype became more sensitized to the action of CCK agonists. By contrast, repeated administration of diazepam seems to have only a weak influence on CCK_A receptors affecting the activity of dopaminergic neurons. There exists evidence that long-term haloperidol administration induced through the indirect interaction with CCK_A receptors the depolarization inactivation of dopamine neurons in the midbrain (Bunney et al., 1985; Zhang et al., 1991; Minabe et al., 1991). This inactivation of dopaminergic neurons may explain to some extent the development of subsensitivity at CCK_A receptors after repeated haloperidol treatment. The injection of caerulein at doses (20-250 μg/kg), decreasing the motor activity and blocking (+)-amphetamine-induced motor excitation, inhibited [^{3}H]-spiroperidol binding in '*in vivo*' studies in the mouse forebrain. The CCK antagonist proglumide (25-50 mg/kg) counteracted the effect of caerulein. On the other hand, the CCK_B/gastrin agonist pentagastrin (100-2500 μg/kg) was unable to influence [^{3}H]-spiroperidol binding performed in "*in vivo*" conditions in the mouse forebrain. It is noteworthy that after long-term treatment with haloperidol caerulein caused an opposite effect: it stimulated the binding of [^{3}H]-spiroperidol in the mouse forebrain. It seems probable that these changes in [^{3}H]-spiroperidol binding after repeated treatment with haloperidol also reflect the development of subsensitivity at CCK_A receptors.

Table 5

COMPARISON OF LONG-TERM EFFECTS OF DIAZEPAM AND HALOPERIDOL ON THE BEHAVIOURAL EFFECTS OF CAERULEIN AND ON [^{3}H]pCCK-8 BINDING IN THE MOUSE BRAIN

	Diazepam	Haloperidol
Behavioural effects of caerulein		
Motor depression	Moderate decrease	Complete tolerance
Antiaggressive effect	Increased aggressiveness	Increased aggressiveness
Anticonvulsant effect	Complete tolerance	Moderate decrease
Antiamphetamine effect	Unchanged	Complete tolerance
[3H]pCCK-8 binding in the mouse forebrain		
Affinity	Increased	Increased
Density	Decreased	Decreased

The injection of 40 µg/kg caerulein to the saline-treated control animals induced the statistically significant reduction of aggressive behaviour. On the contrary, after long-term treatment with haloperidol and diazepam, caerulein markedly increased the intensity of aggressive behaviour, especially the number of biting attacks was increased (Table 5). The antiaggressive effect of caerulein was reversed after 14 days administration of haloperidol and diazepam. The increased aggressiveness induced by caerulein is in agreement with the above described studies where the intraventricular administration of a CCK_B agonist CCK-4 (0.2 µg per animal) induced very dramatic potentiation of foot-shock-induced aggressiveness in the male rat. Moreover, the subcutaneous administration of CCK-4 (25-50 µg/kg) induced the anxiogenic-like interaction with the exploratory behaviour in the rat, and this effect had clearly the CCK_B receptor subtype pharmacology (Harro, Vasar, 1991). Some evidence exists that CCK_B receptors are involved in the mediation of anxiety-like states in the mouse (Hughes et al., 1990; Rataud et al., 1991). Therefore, it is likely that the increased aggressiveness induced by caerulein, after two-weeks administration of haloperidol and diazepam, could be explained by the increased sensitivity of the CCK_B receptor subtype.

The systemic treatment with caerulein is shown to antagonize the seizures induced by picrotoxin, an antagonist at chloride channel, in the mouse (Zetler, 1985). Two-weeks treatment with haloperidol moderately reduced the anticonvulsant effect of caerulein, especially the latency to death was shorter, as compared with the saline-treated control mice (Table 5). After two-weeks administration of diazepam caerulein was unable to affect the development of picrotoxin- induced seizures in the mice. The site of caerulein's interaction with picrotoxin-induced seizures is still unclear. However, the development of tolerance to the antipicrotoxin effect of caerulein after repeated administration of diazepam may support the involvement of GABA-ergic mechanisms in the action of CCK agonist. Harro et al. (1990a) have established that two-weeks administration of diazepam induced tolerance to the anxiogenic-like effect of caerulein. These behavioural data are in accordance with the electrophysiological study of Bouthillier and De Montigny (1988), showing that long-term treatment with diazepam significantly reduced the responsiveness of hippocampal pyramidal neurons to the application of CCK. There is a possibility that the anti- picrotoxin effect of CCK agonists is related to the CCK_B receptor subtype. Indeed, the nearly similar doses of sulfated CCK-8 and unsulfated CCK-8, the selective agonist at CCK_B receptors, antagonized picrotoxin-induced seizures in the mouse (Kadar et al., 1985). It is possible that CCK receptors, functionally linked to the GABA-ergic system, are involved in the anti-picrotoxin action of caerulein. Probably, long-term treatment with diazepam induces the subsensitivity at these CCK receptors.

Haloperidol and diazepam (up to 1 mM) failed to inhibit [^{3}H]pCCK-8 binding in "*in vitro*" studies. Acute administration of haloperidol (1 mg/kg) and diazepam (5 mg/kg) did not influence [^{3}H]pCCK-8 binding in the mouse forebrain. However, after the cessation of long-term treatment with diazepam and haloperidol the parameters of [^{3}H]pCCK-8 binding sites were significantly changed (Table 5). Namely, the affinity of [^{3}H]pCCK-8 binding sites was increased (K_d values were decreased), but their number was evidently decreased in the mouse forebrain. Long-term treatment with haloperidol as well as with diazepam is shown to increase the density of CCK receptors in the forebrain structures of rodents (Chang et al., 1983; Harro et al., 1990b). In the light of these findings the data of the present study are really unexpected. Indeed, two-weeks haloperidol and diazepam treatment significantly decreased the density of [^{3}H]pCCK-8 binding sites, but simultaneously increased their affinity. The careful analysis of dissociation curves for [^{3}H]pCCK-8 and [^{125}I]CCK-8 revealed the existence of two distinct binding sites for CCK in the different brain structures of the rodents (Wennogle et al.,

1985; Sekiguchi, Moroji, 1986). It might be possible that long-term diazepam and haloperidol treatment differently alters these subtypes of the CCK receptor. Thus, one could speculate that the high-affinity binding sites for [^{3}H]pCCK-8 started to prevail over the low-affinity sites after long-term treatment with haloperidol and diazepam.

Long-term treatment with diazepam and haloperidol reduced or induced tolerance to the inhibiting effects of caerulein on the mouse behaviour. Simultaneously, a clear proaggressive action of CCK agonist became evident after the cessation of haloperidol and diazepam administration. These behavioural changes seem to be in favour of the opinion that the subsensitivity developes at one subtype of CCK receptors, whereas the other subtype of CCK receptors becomes more sensitized to the action of caerulein. There is evidence (the development of tolerance to the sedative and antiamphetamine effect, but also to the inhibiting effect of caerulein on [^{3}H]-spiroperidol binding in '*in vivo*') that the sensitivity of CCK_A receptors is decreased after two-weeks haloperidol treatment. By contrast, the changes occurring at the CCK_B receptor subtype during long-term haloperidol and diazepam treatment are still less clear. Nonetheless, the possibility remains that the hypersensitivity develops at one part of CCK_B receptors (behaviourally related to the regulation of aggressive behaviour). This idea is supported by the increased affinity of CCK_B receptors in the mouse forebrain after long-term administration of haloperidol and diazepam.

6. CONCLUSIONS

1. The unselective CCK_A/CCK_B agonists caerulein and CCK-8, but not the CCK_B agonists pentagastrin and CCK-4, dose-dependently inhibited the locomotor activity of rodents. A selective CCK_A receptor antagonist devazepide attenuated the motor depressant effect of CCK agonists, whereas a selective CCK_B receptor antagonist L-365,260 potentiated their action. Therefore, CCK_A and CCK_B receptors have the opposite role in the regulation of motor activity in the rodents.

2. Caerulein, but not pentagastrin, antagonized (+)-amphetamine-induced hyperlocomotion in the mice. Pretreatment with devazepide at low doses (1-100 μg/kg), interacting with CCK_A receptors, blocked the antiamphetamine effect of caerulein. Accordingly, the antiamphetamine effect of caerulein is related to the stimulation the CCK_A receptor subtype in the brain. The blockade of both subtypes of the CCK receptor by a high dose (1 mg/kg) of devazepide completely blocked the locomotor stimulation induced by (+)-amphetamine.

3. CCK agonists (caerulein, CCK-8, pentagastrin, CCK-4) reduced the exploratory activity of the rodents in the elevated plus-maze. The anxiogenic-like effect of CCK agonists correlated with their affinity at CCK_B receptors in the cerebral cortex, but not at CCK_A receptors in the pancreas. L-365,260 was a stronger antagonist of anti-exploratory action of CCK-4 as compared with devazepide. Thus, the CCK_B receptor subtype is involved in the anti-exploratory effect of CCK agonists.

4. Systemic treatment with the unselective CCK_A/CCK_B agonist caerulein, but not with the CCK_B/gastrin agonist pentagastrin, blocked the foot-shock-elicited aggressiveness in the mouse and antagonized the development of apomorphine aggressiveness in the rat. Intraventricular and subcutaneous administration of the CCK_B agonist CCK-4, differently from caerulein, increased the intensity of foot-shock-induced aggressiveness in male rats. Consequently, the CCK_A and CCK_B receptor subtypes are play an opposite role in the regulation of aggressive behaviour in the rodents.

5. Systemic treatment with caerulein and CCK-8, but not with pentagastrin, significantly antagonized picrotoxin- and pilocarpine-induced seizures in the mouse. Intraventricular, but not subcutaneous, administration of caerulein potently blocked quinolinate- and NMDA-induced seizures. Proglumide attenuated the

anticonvulsant effect of caerulein against picrotoxin- and quinolinate-induced convulsions. The selective CCK antagonists L-365,260 and devazepide at a high dose (1 mg/kg) blocked the anticonvulsant effect of CCK-8 against pilocarpine seizures. Accordingly, both subtypes of CCK receptors are related to the anticonvulsant action of CCK agonists.

6. Long-term treatment with devazepide and L365,260 induced different changes in the behaviour and in the [^{3}H]pCCK-8 binding in the mouse forebrain. Repeated administration of devazepide reduced the motor depressant effect of caerulein and increased (+)-amphetamine-induced hyperlocomotion in the mice. L-365,260 failed to change the behavioural effects of caerulein and (+)-amphetamine, but significantly increased the density of CCK receptors. These data obviously support the involvement of the CCK_A receptor subtype in the sedative and antiamphetamine action of caerulein.

7. Long-term treatment with haloperidol and caerulein caused very similar changes in the mice's behaviour and [^{3}H]-radioligand binding in the mouse forebrain. Tolerance developed to the locomotor effects of caerulein, muscimol and flumazenil, whereas amphetamine-induced hyperlocomotion was increased after 15-days haloperidol and caerulein treatment. Simultaneously, the number of opioid and $dopamine_2$-receptors was increased, however, the density of $GABA_A$-benzodiazepine and CCK-8 receptors was reduced in the mouse forebrain. It is most likely that CCK_A receptors play a role in the long-term effects of haloperidol.

8. Long-term diazepam and haloperidol treatment decreased or induced tolerance to the inhibiting effects (motor depressant, antiamphetamine, anticonvulsant and antiaggressive) of caerulein on the mouse behaviour. Simultaneously, the proaggressive action of caerulein became evident. The number of [^{3}H]pCCK-8 binding sites was decreased, whereas their affinity was increased after the withdrawal of long-term haloperidol and diazepam treatment. Therefore, long-term administration of diazepam and haloperidol induced the subsensitivity at one subtype of the CCK receptor (mainly the CCK_A subtype), whereas the others (mainly the CCK_B subtype) became more sensitized to the action of CCK agonists.

ACKNOWLEDGEMENTS

This study was performed at *the Psychopharmacology Laboratory, Institute of General & Molecular Pathology of Tartu University.*

I wish to express my deepest gratitude and respect to all colleagues and friends who made this study possible:

My teacher, Professor Lembit Allikmets for his constant guidance and support throughout this work.

My very close co-workers, Doctors Jaanus Harro, Aavo Lang, Andres Soosaar, Anu Põld and Tiina Ööpik for a very inspiring and enthusiastic collaboration, and for valuable help in every way.

Professor Brian S. Meldrum and John D. Stephenson, for a stimulating collaboration and for providing possibility to work at the Department of Neurology, Institute of Psychiatry, University of London.

Doctor Susan D. Iversen and Doctor Colin T. Dourish (*Merck Sharp & Dohme*), for the generous gift of CCK antagonists.

The financial support from the British Council is gratefully acknowledged.

Finally, my warmest and most sincere thanks go to my family and my parents, for interest, encouragement and support.

REFERENCES

1. Abelson, J.L. and Nesse, R.M. (1990) Cholecystokinin-4 and panic. Arch Gen.Psychiatry, 47: 395.

2. Altar, C.A. and Boyar W.C. (1989) Brain CCK-B receptors mediate the suppression of dopamine release by cholecystokinin. Brain Res. 483: 321-326.

3. Barber, N.S., Dourish, C.T. and Hill, D.R. (1989) The role of CCK, caerulein and CCK antagonists in nociception. Pain, 39: 307-328.

4. Barrett, R.W., Steffey, W.E. and Wolfram, C.A.W. (1989) Type-A CCK binding sites in cow brain: characterization using (-)-3H-L-364,718 membrane binding assays. Mol.Pharmacol., 36: 285-290.

5. Beinfeld, M.C. (1983) Cholecystokinin in the central nervous system: a minireview. Neuropeptides, 3: 411-427.

Beinfeld, M.C. (1988) Cholecystokinin and gastrin: chemistry, distribution, release and activity. In: Negro-Vilar, A. and Conn, P.M. (eds.), Peptide Hormones: Effects and Mechanisms of Action. CRC Press. Boca Raton, Florida, pp. 41-77.

6. Bouthillier, A. and DeMontigny, C. (1988) Long-term benzodiazepine treatment reduces neuronal responsiveness to cholecystokinin: an electrophysiological study in the rat. Eur.J.Pharmacol., 151: 135-138.

7. Bradbury A.J., Costall, B., Naylor, R.J. and Neumeyer J.L. (1983) Motor inhibition induced by apomorphine derivatives in the mouse. J.Pharm.Pharmacol. 35: 494-499.

8. Bradwejn, J. and DeMontigny,C. (1984) Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurones. Nature 312: 363-364.

9. Bradwejn, J., Koszycki, D. and Meterissian G. (1990) Cholecystokinin tetrapeptide induces panic attacks in patients with panic disorder. Can.J.Psychiatry, 35: 83-85.

10. Bunney, B.S., Chiodo, L.A. and Freeman, A.S. (1985) Further studies on the specificity of proglumide as a selective cholecystokinin antagonist in the central nervous system. Ann.N.Y.Acad.Sci. 448:345-351.

11. Chang, R.S.L., Lotti, V.S., Martin, G.E. and Chen, T.B. (1983) Increase in brain [125I]-cholecystokinin (CCK) receptor binding following chronic haloperidol treatment, intracisternal 6-

hydroxydopamine and ventral tegmental lesions. Life Sci., 32: 871-878.

12. Chang, R.S.L. and Lotti, V.J. (1986) Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc.Natl.Acad.Sci. USA, 83: 4923-4926.

13. Chiodo, L.A. and Bunney B.S. (1983) Typical and atypical neuroleptics: differential effects of chronic administration on the activity A9 and A10 midbrain dopaminergic neurons. J.Neurosci., 3:1607-1609.

14. Chiodo, L.A. and Bunney, B.S. (1987) Population response of midbrain dopaminergic neurons to neuroleptics: further studies on time course and nondopaminergic neuronal influences. J.Neurosci. 7: 629-635.

15. Crawley, J. (1989) Microinjection of cholecystokinin into the rat ventral tegmental area potentiates dopamine- induced hypolocomotion. Synapse, 3:346-355.

16. Crawley, J.N. and Kiss, J.Z. (1985) Tracing the sensory pathway from the gut to brain regions mediating the actions of cholecystokinin on feeding and exploration. Ann.N.Y.Acad.Sci. 448: 586-588.

17. Crawley, J.N. and Schwaber, J.S. (1984) Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of parvocellular subdivision of the nucleus tractus solitarius. Brain Res. 295: 289-299.

18. Crawley, J.N., Stivers, J.A., Blumstein, L.K. and Paul, S.M. (1985) Cholecystokinin potentiates dopamine-mediated behaviors: evidence for modulation specific to a site of coexistence. J.Neurosci., 5:1972-1983.

19. Creese, I. and Leff S.E. (1982) Dopamine receptors: a classification. J.Clin.Psychopharmacol. 2: 329-335.

20. Csernansky, J.G., Glick, S. and Mellentin S. (1987) Differential effects of progiumide on mesolimbic and nigrostriatal dopamine function. Psychopharmacology, 91: 440- 446.

21. De Montigny, C. (1989) Cholecystokini tetrapeptide induces panic-like attacks in healthy volunteers. Arch.Gen.Psychiatry, 46: 511-517.

22. De Witte, P., Goldman, S., Gewiss, M., Poels, J.F., Van Boxez, P., Van Der Veken, E. and Vanderhaeghen, J.J. (1986) Similar effects of caerulein on intracranial self-stimulation in vagotomized and non-vagotomized rats. Neurochem.Int. 8: 339-343.

23. Dourish, C.T. and Hill D.R. (1987) Classification and function of CCK receptors. TIPS, 8: 207-208.

24. Dourish, C.T., Ruckert, A.C., Tattersall, F.D. and Iversen, S.D. (1989a) Evidence that decreased feeding induced by systemic injection of cholecystokinin is mediated by CCK-A receptors. Eur.J.Pharmacol., 173: 233-234.

25. Dourish, C.T., Rycroft, N. and Iversen, S.D. (1989b) Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. Science, 245: 1509-1511.

26. Dourish, C.T., O'Neill, M.F., Coughlan, J., Kitchener, S.J., Hawley, D. and Iversen S.D. (1990) The selective CCK-B antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat: evidence for mediation of CCK/opioid interactions by CCK-B receptor. Eur.J.Pharmacol. 176: 35-44.

27. Eng, J., Shiina, Y., Straus, E. and Yalow, R.S. (1982) Post-translational processing of cholecystokinin in pig brain and gut. Proc.Natl.Acad.Sci. USA 79: 6060-6064.

28. Faris, P.L., Komisaruk, B.R., Watkins, L.R. and Mayer D.J. (1983) Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science, 219: 310-312.

29. Frey, P. (1983) Cholecystokinin octapeptide levels in rat brain are changed after subchronic neur oleptic treatment. Eur.J.Pharmacol., 95:87-92.

30. Gall, C. (1988) Seizure induce dramatic and distinctly different changes in enkephalin, dynorphin and CCK immunoreactivities in mouse hippocampal mossy fibers. J.Neurosci., 8: 1852-1862.

31. Grigoriadis, D. and Seeman, P. (1984) The dopamine/neuroleptic receptor. Can.J.Neurol.Sci. 11: 108-113.

32. Haefely, W., Kyburz, E., Gerecke, M. and Möhler, H. (1985) Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists. Adv.Drug Res. 14: 165-322.

33. Hagino, Y., Moroji, T. and Iizuka R. (1989) A behavioural pharmacological study of intracerebroventricularly administered CCK-8 related peptides in mice. Neuropeptides, 13: 107-113.

34. Hamamura, T., Kazahaya, Y. and Otsuki, S. (1989) Ceruletide suppresses endogenous dopamine release via vagal afferent system studied by in vivo intracerebral dialysis. Brain Res., 483: 78-83.

35. Handley, S.L. and Mithani, S. (1984) Effects of alpha- adrenoreceptor agonists and antagonists in a maze-exploration model of "fear"-motivated behaviour. Naunyn-Schmiedeberg's Arch.Pharmacol., 327: 1-5.

36. Harro, J., Vasar, E. and Allikmets, L. (1988) Role of CCK-8-ergic mechanisms in the development of anxiety states in mice. In: "Neuromediators and Behaviour". Novosibirsk, pp. 113-114.

37. Harro, J., Pôld, M., Vasar, E. and Allikmets L. (1989) Involvement of CCK-8-ergic mechanisms in the regulation of emotional behaviour in rodents. J.Higher Nervous Activity, 39: 877-883.

38. Harro, J., Pôld, M. and Vasar, E. (1990a) Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: influence of acute and subchronic diazepam treatment. Naunyn-Schmiedeberg's Arch. Pharmacol., 341: 62-67.

39. Harro, J., Lang, A. and Vasar, E. (1990b) Long-term diazepam treatment produces changes in cholecystokinin receptor binding in rat brain. Eur. J. Pharmacol., 180: 77-83.

40. Harro, J. and Vasar, E. (1991a) Evidence that CCK_B receptors mediate the regulation of exploratory behaviour in the rat. Eur.J.Pharmacology, 193: 379-381.

41. Harro, J. and Vasar, E. (1991b) Cholecystokinin-induced anxiety: how is it reflected in studies on exploratory behaviour? Neuroscience & Biobehavioral Reviews, 15: (in press)

42. Hendry, S.H.C., Jones E.G., DeFilipe, J., Schmechel D., Brandon, C. and Emson P.C. (1984) Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. Proc.Natl.Acad.Sci. USA, 81: 6526-6530.

43. Hill, D.R., Campbell, N.J., Shaw, T.M. and Woodruff, C.N. (1987) Autoradiographic localization and characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J.Neurosci., 7: 2967-2976.

44. Hökfelt, T., Rehfeld, J.F., Skirboll, L., Ivemark, B., Goldstein, M. and Markey K. (1980) Evidence for coexistence of dopamine and CCK in mesolimbic neurones. Nature, 285: 476-478.

45. Hommer, D.W., Palkovits, M., Crawley, J.N., Paul, S.M. and Skirboll, L.R. (1985) Cholecystokinin-induced excitation in the substantia nigra: evidence for peripheral and central components. J.Neurosci. 5: 1387-1397.

46. Hommer, D.W., Stoner, G., Crawley, J.N., Paul, S.M. and Skirboll, L.R. (1986) Cholecystokinin-dopamine coexistence: electrophysiological actions corresponding to

cholecystokinin receptor subtype. J.Neurosci. 6:3039-3043.

47. Howbert, J.J., Lobb, K.L., Brown, R.F., Reel, J.K., Neel, D.A. et al. (1991) A novel series of non-peptide CCK and gastrin antagonists: medicinal chemistry and electrophysiological demonstration of antagonism. In: Multiple Cholecystokinin Receptors in the CNS (Dourish, C.T. and Cooper, S.J., eds.) Oxford University Press (in press)

48. Hughes, J., Boden, P., Costall, B., Domeney, A., Kelly, E., Horwell, D.C., Hunter, J.C., Pinnock, R.D. and Woodruff G.N. (1990) Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. Proc.Natl.Acad.Sci. USA, 87: 6728-6732.

49. Innis, R.B. and Snyder, S.H. (1980) Distinct cholecystokinin receptors in brain and pancreas. Proc.Natl.Acad.Sci. USA, 77: 6917-6921.

50. Jiang, L.H., Kasser, R.J. and Wang R.Y. (1988) Cholecystokinin antagonist lorglumide reverses chronic haloperidol-induced effects on dopamine neurons. Brain Res., 473: 165-168.

51. Juma, I. and Zetler, G. (1981) Antiniociceptive effect of centrally administered caerulein and cholecystokinin octapeptide (CCK-8). Eur.J.Pharmacol. 73: 323-331.

52. Kadar, T., Pesti, A., Penke, B., Toth, G., Zarandi, M. and Telegdy, G. (1983) Structure-activity and dose-effect relationships of the antagonism of picrotoxin-induced seizures by cholecystokinin, fragments and analogues of cholecystokinin in mice. Neuropharmacol., 22: 1223-1229.

53. Kadar, T., Pesti, A., Penke, B. and Telegdy, G. (1984) Inhibition of seizures induced by picrotoxin and electroshock by cholecystokinin octapeptide and their fragments in rats after intracerebroventricular administration. Neuropharmacol., 23: 955-961

54. Kadar, T., Borda, I., Penke, B., Kovacs, K. and Telegdy, G. (1985a) Cataleptic and anticataleptic activity produced by cholecystokinin octapeptides in mice.

55. Kadar, T., Penke, B., Pesti, A. and Telegdy, G. (1985b) Multiple treatment potentiates the anticonvulsive activity of cholecystopkinin octapeptides. Peptides, 6: 1009-1014.

56. Kato, T., Izmi, Y., Takita, M. and Kushima Y. (1988) Time dependent changes in the concentrations of immunoreactive cholecystokinin octapeptide sulfate in rat brain regions after systemic injection of picrotoxin. Neurochem.Int., 12. 493-497.

57. Katsuura, G. and Itoh, S. (1982) Sedative action of cholecystokinin octapeptide on behavioral excitation by thyrotropin releasing hormone and metamphetamine in the rat. Jpn.J.Physiol. 42. 83-91.

58. Kerwin, J.F., Nazdan, A.M., Kopecka, H., Lin, C.W., Wittle T.M.D. et al. (1989) Hybrid cholecystokinin (CCK) anatgonists: new implications in the design and modification of CCK antagonists. J.Med.Chem., 32: 739-742.

59. Khosla, S. and Crawley, J. (1988) Potency of L-364,718 as an antagonist of the behavioural effects of peripherally administered cholecystokinin. Life Sci., 42: 153-159.

60. Koshikawa, N., Kikuchi de Beltran, K., Kobayashi, M. and Stephenson J.D. (1990) Facilitatory effects of CCK-8 in the dorsomedial nucleus accumbens on apomorphine-induced jaw movements in rats. Eur.J.Pharmacol., 183: 1118-1119.

61. Leatherbarrow R.J. (1987) Enzfitter, a non-linear regression data analysis program for the IBM PC. Elsevier Science Publisher, Amsterdam, The Netherlands.

62. Lotti, V. and Chang, R.S.L. (1989) A potent and selective non-peptide gastrin antagonist and brain (CCK-B) receptor ligand: L-365,260. Eur.J.Pharmacol., 32: 13-16

63. Matsubara, K. and Matsushita A. (1986) β-Endorphin involvement in the antidopaminergic effect of caerulein. Jpn.J.Pharmacol. 40: 417-422.

64. Matsushita, A. and Itoh, S. (1982) Pharmacological properties of caerulein, a CCK-8 analogous peptide. In: World Psychiatric Association Regional Symposium. (Ohashi, H., Nakayama, K., Saito, M. and Saletu, B., eds.). Tokyo: The Japanese Society of Psychiatry and Neurology. pp. 170-173.

65. Meyer, D.K. and Krauss, J. (1983) Dopamine modulates cholecystokinin release in neostriatum. Nature, 301: 338-340.

66. Minabe, Y., Ashby, C.R. and Wang R.Y. (1991) The CCK-A receptor antagonist devazepide but not the CCK-B receptor antagonist L-365,260 reverses the effects of chronic clozapine and haloperidol on midbrain dopamine neurons. Brain Res., 549: 151-154.

67. Moran, T.H., Robinson, P.H., Goldrich, M. and McHugh, P.R. (1986) Two brain CCK receptors: implications for behavioral actions. Brain Res., 362: 175-179.

68. Morley, J.E. (1987) Behavioral effects of peripherally administered cholecystokinin. ISI Atlas Pharmacol., 1: 49-51.

69. Moroji, T. and Hagino, Y. (1987) Bilateral subdiaphragmatic vagotomy does not prevent the behavioural effects of systematically administered ceruletide in mice. Neuropeptides, 9: 217-224.

70. O'Neill, M.F., Dourish, C.T. and Iversen S.D. (1991) Hypolocomotion induced by peripheral

or central injection of CCK in the mouse is blocked by the CCK_A receptor antagonist devazepide but not by the CCK_B receptor antagonist L-365,260. Eur.J.Pharmacol. 193: 203-208.

71. Panerai A.E., L.C.Rovati, M.Bianchi, S.R.Bareggi and P.Mantegazza (1987) Effect of a cholecystokinin antagonist on some effects of diazepam. Neuropharmacology 26: 1285-1287. 72. Pinnock, R.D., Woodruff, G.N. and Boden P.R. (1990) Cholecystokinin excites dorsal raphe neurones via a CCK-A receptor. Br.J.Pharmacol. (Proc.Suppl.) 100:349P.

73. Rataud, J., Darche, F., Piot, O., Stutzmann, J.-M., Böhme, G.A. and Blanchard, J.-C. (1991) "Anxiolytic" effect of CCK antagonists on plus-maze behavior in mice. Brain Res., 548: 315-317.

74. Rovati, L.C., Bani, M., Makovec, F., Revel, L. and Setnikar, I. (1987) Lorglumide and loxiglumide: two potent and specific antagonists of peripheral CCK. In: Gastrin and cholecystokinin. Chemistry, Physiology and Pharmacology. (Bali, J.-P. and Martinez, J., eds.). Amsterdam: Elsevier, pp. 45-48.

75. Sankaran, H., Goldfine, I.D., Deveney, C.W., Wong, K.-Y. and Williams, J.A. (1980) Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. J.Biol.Chem., 255: 1849-1853.

76. Saito, A., Sankaran, H., Goldfine, I.D. and Williams J.A. (1980) Cholecystokinin receptors in the brain: characterization and distribution. Science, 208: 1155-1156.

77. Seeman, P. (1980) Brain dopamine receptors. Pharmacol Rev., 32: 229-313.

78. Sekiguchi, R. and Moroji, T. (1986) A comparative study on characterization and distribution of cholecystokinin binding sites among the rat, mouse and guinea-pig brain. Brain Res., 399: 271-281.

79. Singh, L., Lewis, A.S., Field, M.J., Hughes, J. and Woodruff, G.N. (1991) Evidence for involvement of the brain cholecystokinin B receptor in anxiety. Proc.Natl.Acad.Sci. USA 88: 1130-1133.

80. Skirboll, L., Grace, A.A., Hommer, D.W., Rehfeld, J., Goldstein, M., Hökfelt, T. and Bunney, B.S. (1981) Peptide-monoamine coexistence: studies of the actions of cholecystokinin-like peptide on the electrical activity of midbrain dopamine neurons. Neuroscience, 6: 2111-2114.

81. Somogyi, P., Hodgson, A.J., Smith, A.D., Nunzi, M.G., Gorio, A. and Wu, J.-Y. (1984) Different populations of GABAergic neurons in visual cortex and hippocampus of cat contain somatostatin or cholecystokinin-immunoreactive material. Neuroscience, 14: 2590-2603.

82. Soosaar, A., Vasar, E. and Lang E. (1988) The parameters of cholecystokinin receptors in

different brain structures of the rat. Acta et Commentationes Universitatis Tartuensis, 839: 95-108.

83. Stinus, L., Nadaud, D., Jauregui, J. and Kelley A.E. (1986) Chronic treatment with five different neuroleptics elicits behavioural supersensitivity to opiate infusion into nucleus accumbens. Biol.Psychiatry 21;34-48.

84. Strömbom, U. (1977) Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine. J.Neural Transm. 40: 191-194.

85. Suzuki, T. and Moroji, T. (1989) Cholecystokinin binding sites in the rat forebrain: effects of acute and chronic metamphetamine administration. J.Neural Transm., 77:181-195.

86. Vanderhaeghen, J.J., Signeau, J.C. and Gepts, W. (1975) New peptide in vertebrate CNS reacting with antigastrin antibodies. Nature, 257: 604-605.

87. Van Dijk, A., Richards, J.G., Treciak, A., Gillessen, D. and Möhler, H. (1984) Cholecystokinin receptors: biochemical demonstration and autoradiographic localization in rat brain and pancreas using [^{3}H]-cholecystokinin as radioligand. J.Neurosci. 4:1021-1033.

88. Vasar, E., Soosaar, A. and Lang, A. (1987) The involvement of dopamine, opiate and benzodiazepine receptors in the action of caerulein, an analogue of cholecystokinin octapeptide. In: Molecular mechanism of action of psychotropic drugs. (Allikmets, L., ed.). Tartu: Tartu University Press. pp.16-29.

89. Vickroy, T.W. and Bianchi, B.R. (1989) Pharmacological and mechanistic studies of cholecystokinin-facilitated [^{3}H]- dopamine efflux from rat nucleus accumbens. Neuropeptides, 13:43-50.

90. Voight, M., Wang, R.Y. and Westfall, T.C. (1986) Cholecystokinin octapeptides alter the release of endogenous dopamine from the rat nucleus accumbens in vitro. J.Pharmacol.Exp.Ther. 237: 147-153.

91. Wennogle, L., Steel, D.J. and Petrack B. (1985) Characterization of central cholecystokinin receptors using a radioiodinated octapeptide probe. Life Sci., 36: 1485-1492.

92. White, F.J. and Wang R.Y. (1984) Interactions of cholecystokinin octapeptide and dopamine on nucleus accumbens neurons. Brain Res., 300: 161-166.

93. Zetler, G. (1980a) Analgesia and ptosis caused by caerulein and cholecystokinin octapeptide (CCK-8). Neuropharmacol., 19: 415-422.

94. Zetler, G. (1980b) Anticonvulsant effects of caerulein and cholecystokinin octapeptide,

compared with those of diazeapm. Eur.J.Pharmacol., 65: 297-300.

95. Zetler, G. (1981) Anticonvulsant effects of caerulein, cholecystokinin octapeptide (CCK-8) and diazepam against seizures produced in mice by harman, thiosemicarbazide and isoniazid. Neurosci.Lett. 24: 175-180.

96. Zetler, G. (1983) Cholecystokinin octapeptide (CCK-8), ceruletide and analogues of ceruletide: effects of tremor induced by oxotremorine, harmine and iboganine. A comparison with prolyl-leucyl-glycine amide (MIF0, anti-parkinsonian drugs and clonazepam. Neuropharmacol., 22: 757-766.

97. Zetler, G. (1984) Clonidine and yohimbine separate sedation and ptosis by cholecystokinin octapeptide and ceruletide. Eur.J.Pharmacol. 102: 333-340.

98. Zetler, G. (1985) Caerulein and its analogues: neuropharmacological properties. Peptides 6 (Suppl. 3): 33-46.

99. Zetler, G. and Baumann, G.H. (1986) The cholecystokinin-like peptide, ceruletide, reduces isolation-induced aggression in mice. Neuropharmacology, 25: 831-832.

100. Zhang, J., Chiodo, L.A. and Freeman, A.S. (1991) Effects of the CCK-A receptor antagonist CR 1409 on the activity of rat midbrain dopamine neurons. Peptides, 12:339- 343.

PUBLICATIONS

ФИЗИОЛОГИЧЕСКИЙ ЖУРНАЛ СССР им. И. М. СЕЧЕНОВА

SECHENOV PHYSIOLOGICAL JOURNAL OF THE USSR
LXVIII · № 9 · 1982

УДК 612.821.1

ИНТРАЦЕРЕБРОВЕНТРИКУЛЯРНОЕ ВВЕДЕНИЕ ХОЛЕЦИСТОКИНИНА УГНЕТАЕТ АКТИВНОСТЬ ДОФАМИН- И СЕРОТОНИНЕРГИЧЕСКОЙ СИСТЕМ МОЗГА

Э. Э. Васар, М. Я. Оттер и Л. К. Ряго

Кафедры физиологии (зав. Э. Ф. Васар) и фармакологии (зав. Л. Х. Алликметс) Государственного университета, Тарту

В опытах на крысах-самцах линии Вистар исследовали поведенческие и биохимические эффекты интрацеребровентрикулярного введения холецистокинина. Холецистокинин вызывал специфические, зависящие от дозы, изменения в поведении животных. При малых его дозах превалировало угнетающее влияние на поведение, при больших дозах наблюдались стереотипное поведение, встряхивания головой и повышенная реактивность на болевые раздражители. Холецистокинин заметно угнетал по сравнению с физиологическим раствором кругооборот серотонина и дофамина в мозговых структурах. Введение холецистокинина на фоне фенамина и 5-окситриптофана на короткое время полностью устраняло вызываемые этими веществами поведенческие эффекты. На основе полученных данных можно предполагать, что холецистокинин является эндогенным модулятором активности моноаминергических систем мозга.

Ключевые слова: холецистокинин, стереотипное поведение, кругооборот моноаминов, фенамин, 5-окситриптофан.

В последние годы установлено [5, 10, 12], что холецистокинин, кроме желудочно-кишечного тракта, в значительных количествах содержится в разных структурах головного мозга. Особенно высокие концентрации этого пептида и его фрагментов выявлены в коре больших полушарий, лимбических структурах, гипоталамусе и стриатуме [13], т. е. в структурах, получающих обильную афферентную иннервацию от моноаминергических систем мозга. Fuxe и соавт. [9] нашли, что холецистокинин в малых дозах угнетает кругооборот дофамина в передних ядрах стриатума и прилегающем ядре. О функциональной роли холецистокинина в центральной нервной системе пока известно относительно мало. Некоторые авторы связывают его функцию с регуляцией пищевого поведения [4], причем, по их мнению, его действие опосредуется через серотонинергические механизмы.

Основываясь на вышеизложенных фактах, в настоящей работе поставлена задача исследовать поведенческие эффекты интрацеребровентрикулярного введения холецистокинина, а также его влияние на центральные серотонин- и дофаминергические процессы.

МЕТОДИКА

Опыты проводились на крысах самцах линии Вистар (массой 250—300 г). Под эфирным наркозом по координатам атласа Фифковой и Маршала [1] в черепе просверливались отверстия и спустя 4—5 ч после этого животные брались в опыт. Холецистокинин (фирма «Boots», Англия) разводили в физиологическом растворе. Через билатеральные отверстия пептид в общем объеме 10 мкл вводили в течение 60 с в боковые желудочки мозга. Контролем служили инъекции физиологического раствора в том же объеме. Холецистокинин вводился в разных дозах — от 0.1 до 8 Ед по Ivy. Сразу после внутримозговых инъекций животных помещали в клетку размерами 60×60×35 см и наблюдали за их спонтанным поведением и реакцией на внешние раздражители. Спустя 10 мин после введения холецистокинина в дозе 0.1, 1, 4 Ед проводились также биохимические исследования. По методике Earley и Leonard [6] определяли изменения в содержании дофамина и его главных метаболитов —

гомованилиновой кислоты и 3,4-диоксифенилуксусной кислоты в стриатуме и лимбических структурах. Содержание серотонина и его метаболита 5-оксииндолуксусной кислоты определялись в черном веществе и стволе мозга по методике Curzon, Green [3]. Все эти определения проводились с помощью флуоресцентного спектрофотометра МПФ-2А фирмы «Хитачи».

В отдельной серии опытов исследовалось влияние средней дозы холецистокинина (1 Ед) на поведенческие эффекты фенамина (2.5 мг/кг) и 5-окситриптофана (150 мг/кг), влияющих на активность дофамин- и серотонинергической системы через пресинаптические механизмы [14, 15]. Внутрижелудочковые введения физиологического раствора или холецистокинина проводили через 30 мин после подкожной инъекции фенамина. Стереотипию оценивали по методике, предложенной Costall, Naylor [2], перед внутримозговым введением и спустя 7 мин после введения. В опытах с 5-окситриптофаном холецистокинин и физиологический раствор вводились через 45 мин после предшественника серотонина. Число встряхиваний головой подсчитывалось в период от 3 до 10 мин после внутрижелудочковых инъекций. Все экспериментальные данные подвергались статистической обработке с использованием t-теста Стьюдента.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЯ

Интрацеребровентрикулярное введение холецистокинина вызывало определенные изменения в поведении животных, находившихся в тесной корреляции с вводимой дозой. Доза 0.1 Ед в отличие от физиологического раствора вызывала полное прекращение двигательной активности и заметную сонливость у некоторых животных. С повышением дозы через 3—4 мин после введения появлялись повороты тела в сторону первой инъекции, а затем стереотипные движения в виде принюхивания и жевания в течение 3—4 мин. После

Влияние интрацеребровентрикулярного введения холецистокинина на содержание дофамина, гомованилиновой кислоты, 3,4-диоксифенилуксусной кислоты в стриатуме и лимбических структурах; серотонина и 5-оксииндолуксусной кислоты в черном веществе и стволе мозга крысы (приведены средние данные в мкг на 1 г ткани мозга и достоверные границы к ним)

	Вещество, доза			
	Дофамин		Гомованилиновая кислота	
	стриатум	лимбические структуры	стриатум	лимбические структуры
Интактные животные	6.64 ±0.38	2.98 ±0.28	0.40 ±0.06	0.55 ±0.06
Контроль	9.13 ±0.42	2.27 ±0.26	1.44 ±0.12	1.08 ±0.12
Холецистокинин в дозе:				
0.1 Ед	7.36 ±0.36*	2.30 ±0.30	1.53 ±0.12	0.85 ±0.09
1 Ед	6.77 ±0.31*	2.72 ±0.18	0.99 ±0.11 *	0.90 ±0.12
4 Ед	6.03 ±0.32*	2.89 ±0.31	1.00 ±0.08 *	0.54 ±0.04*

Продолжение

	Вещество, доза					
	3,4-диоксифенилуксусная кислота		Серотонин		5-оксииндолуксусная кислота	
	стриатум	лимбические структуры	черное вещество	ствол мозга	черное вещество	ствол мозга
Интактные животные	0.78 ±0.05	0.56 ±0.05	1.81 ±0.18	0.70 ±0.04	0.86 ±0.07	0.54 ±0.04
Контроль	1.10 ±0.11	1.09 ±0.09	5.12 ±0.45	0.77 ±0.07	1.70 ±0.16	0.74 ±0.08
Холецистокинин в дозе:						
0.1 Ед	1.28 ±0.10	0.66 ±0.07*	2.24 ±0.22*	0.77 ±0.04	1.51 ±0.19	0.61 ±0.06
1 Ед	0.79 ±0.09*	0.69 ±0.08*	2.09 ±0.25*	0.88 ±0.08	1.39 ±0.15	0.54 ±0.04*
4 Ед	0.67 ±0.07	0.47 ±0.06*	2.44 ±0.22*	0.81 ±0.07	0.78 ±0.04*	0.52 ±0.03*

Примечание. Контроль — интрацеребровентрикулярное введение физиологического раствора (10 мкл); * — достоверное различие с контролем при $p < 0.05$.

этого двигательные реакции полностью прекращались и у части крыс развивалась сонливость. В дозах 4 и 8 Ед холецистокинин вызывал перед появлением указанных стереотипных реакций кратковременные встряхивания головой. Реакция животных на болевые раздражения находилась в прямой корреляции с вводимой дозой пептида. В малых дозах она не отличалась от таковой у животных контрольной группы, однако была ниже по сравнению с интактными крысами. После внутримозгового введения холецистокинина в дозе 4 или 8 Ед в течение 1 ч наблюдалась бурная реакция на болевые раздражения, при этом между животными легко провоцировались драки.

Введение физиологического раствора (см. таблицу) в значительной степени повышало по сравнению с интактными животными синтез и кругооборот

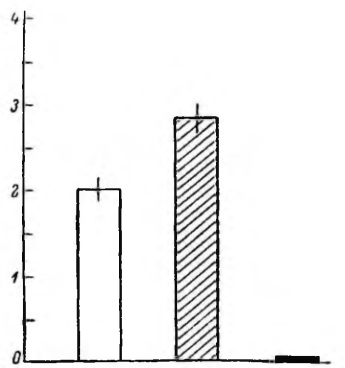


Рис. 1. Интенсивность реакции на введение фенамина (2.5 мг/кг) у крыс после интрацеребровентрикулярного введения физиологического раствора и холецистокинина (1 Ед).

По оси ординат — интенсивность стереотипного поведения, в баллах. *Столбики*: *белые* — до, *заштрихованные* — после введения физиологического раствора ($p < 0.05$), *черные* — после введения холецистокинина ($p < 0.01$).

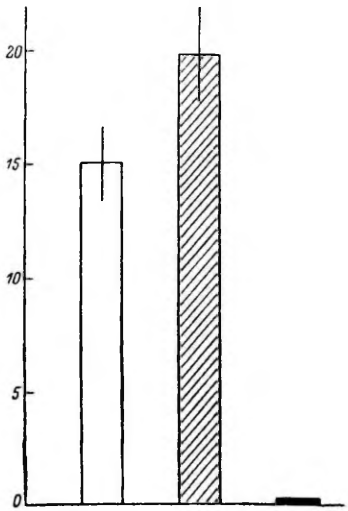


Рис. 2. Число встряхиваний головой, вызванных 5-окситриптофаном (150 мг/кг) у интактных животных и после интрацеребровентрикулярного введения физиологического раствора и холецистокинина (1 Ед).

По оси ординат — число встряхиваний головой. *Столбики*: *белые* — интактные животные, *заштрихованные* — после введения физиологического раствора, *черные* — после введения холецистокинина ($p < 0.01$).

моноаминов во всех нами исследованных мозговых структурах. Холецистокинин в зависимости от дозы угнетал синтез и оборот моноаминов, причем в дозе 4 Ед отмечалось снижение содержания моноаминов и их метаболитов до уровня, наблюдаемого у интактных животных. Особенно заметными были эффекты холецистокинина в лимбических структурах (кругооборот дофамина) и черном веществе (кругооборот серотонина), причем в этих структурах действие пептида проявлялось уже в дозе 0.1 Ед.

При интрацеребровентрикулярном введении физиологического раствора (рис. 1) через 30 мин после инъекции фенамина (2.5 мг/кг) вначале стереотипные двигательные реакции прекращались, а затем в течение 8—10 мин наблюдалось достоверное усиление их интенсивности. Холецистокинин (1 Ед) на 10—12 мин полностью устранял все признаки действия фенамина у всех подопытных животных. Введение физиологического раствора (рис. 2) через 45 мин после инъекции 5-окситриптофана (150 мг/кг) вначале также несколько уменьшало число встряхиваний головой и интенсивность тремора, однако по-

том эти проявления становились более интенсивными по сравнению с интактными животными. Холецистокинин в дозе 1 Ед на 10—12 мин полностью блокировал встряхивания головой у всех животных.

ОБСУЖДЕНИЕ РЕЗУЛЬТАТОВ

Полученные результаты показывают, что холецистокинин вызывает специфические сдвиги в поведении животных, и эти сдвиги находятся в тесной корреляции с вводимой дозой. После введения малых доз наблюдается седативное действие, а более высокие дозы вызывают усиление двигательных реакций (повороты тела в сторону первой инъекции и стереотипные движения). При дозах 4 и 8 Ед перед стереотипными реакциями возникают встряхивания головой. Zetler [16] установил, что холецистокинин обладает болеутоляющим действием, причем данный эффект устраняется введением налоксона — опиатного антагониста в очень малых дозах. Однако наши результаты, напротив, показывают, что после введения холецистокинина в больших дозах болевые раздражения могут вызвать бурные реакции и даже агрессивность — эффекты, во многом напоминающие действие дофаминомиметиков. Холецистокинин угнетает по сравнению с физиологическим раствором метаболизм дофамина и серотонина, что особенно выражено в лимбических структурах и черном веществе. В тесной корреляции с биохимическими данными находится влияние холецистокинина на поведенческие эффекты фенамина и 5-окситриптофана, повышающих активность дофамин- и серотонинергической систем через пресинаптические механизмы [14, 15]. Холецистокинин в отличие от физиологического раствора на 10—12 мин устраняет действие обоих веществ. Все эти данные свидетельствуют о том, что под влиянием холецистокинина резко понижается активность пресинаптических механизмов дофамин- и серотонинергической систем. Однако наряду со снижением активности пресинаптических механизмов наблюдается повышение чувствительности постсинаптических рецепторов этих нейромедиаторных систем, о чем свидетельствуют поведенческие эффекты после интрацеребровентрикулярного введения холецистокинина (встряхивания головой, повороты тела, стереотипные движения и повышенная реактивность на болевые раздражители). В пользу этого мнения указывают и наши предыдущие исследования, где холецистокинин заметно потенцировал интенсивность апоморфиновой стереотипии у крыс, т. е. повышал чувствительность постсинаптических дофаминовых рецепторов. Аналогичные результаты получили Kovacs и соавт. [11], которые нашли, что холецистокинин значительно усиливает синдром «лазанья», вызываемого апоморфином у мышей.

Исследованиями последних лет установлено [6, 7], что в передних мозговых структурах холецистокинин содержится в форме COOH-концевого октапептида. Вполне возможно, что наблюдаемые нами модулирующие влияния на дофамин- и серотонинергические процессы реализуются именно через рецепторные механизмы, связанные с холецистокинином.

ЛИТЕРАТУРА

[1] *Bures J., Petran M., Zachar I.* Electrophysiological methods in biological research. Prague, 1967.

[2] *Costall B., Naylor R.* Stereotyped and circling behavior induced by dopaminergic agonists after lesions of the midbrain raphe nuclei. Eur. J. Pharmacol., 1974, *29*, 206—222.

[3] *Curzon G., Green A. R.* Rapid method for the determination of 5-hydroxy-tryptamine and 5-hydroxyindolacetic acid in small regions of rat brain. Brit. J. Pharmacol., 1970, *39*, 653—655.

[4] *Dafny N., Jacobson E. D.* Cholecystokinin and central nervous regulation of appetite. In: Gastrointestinal Hormones. A Symposium. Austin—London, Univ. Texas Press, 1975, 643—649.

[5] *Dockray G. J.* Immunochemical evidence of cholecystokinin-like peptides in brain. Nature, 1976, *264*, 568—570.

[6] *Dockray G. J., Gregory R. A., Hutchison J. B., Harris J. I., Runswick M. J.* Isolation, structure and biological activity of two cholecystokinin octapeptides from sheep brain. Nature, 1978, *274*, 711—713.

[7] *Dockray G. J.* Cholecystokinins in rat cerebral cortex: identification, purification and characterization by immunochemical methods. Brain Res., 1980, *188*, 155—165.

[8] *Earley C. J., Leonard B. E.* Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine and several metabolites from brain tissue using disposable Bio-Rad columns packed with Sephadex G-10. J. Pharmacol. Methods, 1978, *1*, 67—79.

[9] *Fuxe K., Andersson K., Locatelli V., Agnati L. F., Hökfelt T., Skirboll L., Mutt V.* Cholecystokinin peptides produce marked reduction of dopamine turnover in discrete areas in rat brain following intraventricular injection. Eur. J. Pharmacol., 1980, *67*, 329—331.

[10] *Innis R. B., Correa F. M., Uhl G. R., Schneider B., Snyder S.* Cholecystokinin octapeptide-like immunoreactivity histochemical localization in rat brain. Proc. Nat. Acad. Sci. USA, 1979, *76*, 521—525.

[11] *Kovacs G. L., Szabo G., Penke B., Telegdy G.* Effects of cholecystokinin octapeptide on striatal dopamine metabolism and on apomorphine-induced stereotyped cage-climbing in mice. Eur. J. Pharmacol., 1981, *69*, 313—320.

[12] *Rehfeld J. F.* Cholecystokinins and gastrins in brain and gut. Acta Pharm. Toxicol., 1977, *41*, 24.

[13] *Saito A., Sankaran H., Goldfine I., Williams J. A.* Cholecystokinin receptors in the brain: characterization and distribution. Science, 1980, *208*, 1155—1156.

[14] *Sloviter R. S., Drust E. G., Connor J. D.* Serotonin agonist actions of p-chlorophenylalanine. Neuropharmacol., 1978, *17*, 1029—1033.

[15] *Taylor R., Snyder S.* Differential effects of d- and l-amphetamine on behavior and on catecholamine disposition in dopamine and norepinephrine containing neurons of rat brain. Brain Res., 1971, *28*, 259—309.

[16] *Zetler G.* Analgesia and ptosis caused by caerulein and cholecystokinin octapeptide (CCK-8). Neuropharmacol., 1980, *19*, 415—422.

Поступило 13 VII 1981

INTRAVENTRICULAR ADMINISTRATION OF CHOLECYSTOKININ DECREASES THE ACTIVITY OF DOPAMINE- AND SEROTONINERGIC SYSTEMS IN THE BRAIN

E. E. Vasar, M. Ya. Otter and *L. K. Rägo*

State University, Tartu

In male Wistar rats, intraventricular administration of cholecystokinin caused specific dose-dependent behavioral changes: low doses (0.1—0.25 U) depressed the exploratory activity whereas higher doses (0.5—8.0 U) caused head twitches, stereotyped gnawing and hyperreactivity. Cholecystokinin suppressing markedly dopamine and serotonin turnover in various brain structures, completely blocked the behavioral effects of amphetamine (2.5 mg/kg) and 5-hydroxytryptophan (150 mg/kg). The data obtained suggest that cholecystokinin suppresses presynaptic dopamine- and serotoninergic mechanisms but ehances the sensitivity of postsynaptic receptors of these systems.

Caerulein Stimulates [^{3}H]-Spiperone Binding in vivo After Long-Term Haloperidol Administration

Eero Vasar, M.D.,[1] Matti Maimets, M.D.,[1] Ants Nurk, M.D.,[1] and Lembit Allikmets, D.Sc.

Recently the in vitro modulation of affinity and density of neuroleptic binding sites in striatum by cholecystokinin octapeptide (CCK-8) was described (Agnati et al., 1983). CCK-8 increased the affinity of neuroleptics to its binding sites but reduced their density. Concomitant use of different CCK-related peptides with neuroleptic drugs in neuroleptic-resistant schizophrenic patients was reported to reduce the intensity of disease symptomatology (Moroji et al., 1982; Nair et al., 1982). According to the proposed hypothesis, CCK-8 and related compounds may be acting by potentiating the effect of neuroleptic drugs in neuroleptic-resistant patients (Bloom et al., 1983). In the present work an attempt to clarify the possible mechanism of interaction between caerulein, an analogue of CCK-8, and neuroleptic drugs, with the help of in vivo [^{3}H]-spiperone (^{3}H-SPI) binding after long-term haloperidol treatment was done.

According to the studies of Battaglia and Titeler (1982), there are two existing binding sites (with low and high affinity) for N-propylnorapomorphine (NPA) on dopamine$_2$-receptors, whereas neuroleptic drugs possess high affinity for both. To study the possible changes in dopamine$_2$- and serotonin$_2$-receptor subpopulations, NPA as a displacer in different doses was used.

Male mice weighing 25-27 g were used. Intraperitoneal haloperidol (Gedeon Richter, Hungary) 0.25 mg/kg twice daily or saline injections were given for 14 days. Seventy-two hours after withdrawal, in vivo ^{3}H-SPI (Amersham International, U.K., 17 Ci/mmole) binding studies were performed. NPA (5 and 50 μg/kg, Sterling-Winthrop, USA) and caerulein (200 μg/kg, gift from Prof. R. De Gastiglione, Farmitalia, Italy) were injected subcutaneously. Five μg/kg NPA caused suppression of motor activity whereas 50 μg/kg induced stereotyped behavior in control animals. ^{3}H-SPI (5 μg/kg SC) followed 15 min after NPA and caerulein. The animals (6 per group) were sacrificed 20 min after ^{3}H-SPI. The brains were rapidly removed, and frontal cortex and forebrain subcortical structures (limbic structures and striata) dissected on ice. The dissected brain areas of each group were pooled and homogenized in glass-teflon homogenizer by hand in ice-cold Tris HCl (50 mM, pH 7.4, 20°C) within 1 min in the volume of 40 mg tissue per ml. After homogenization 0.5 ml of suspension was pipetted into six polypropylene tubes (1.5 ml) and centrifuged at 9000 r.p.m. during 10 min. The supernatant was discarded and pellet washed four times with 1 ml ice-cold buffer and cut into vials. Radioactivity was counted after stabilization in Bray scintillation cocktail 12 hours in Beckman LS 6800 with counting efficacy 43%. The experiment was repeated three times and the data analyzed using Student's *t*-test.

In the figure, inhibition of ^{3}H-SPI binding by NPA 5μg/kg was expressed as 100%, showing the amount of high affinity sites for NPA. As the higher dose of NPA could inhibit ^{3}H-SPI binding to both high and low affinity sites, the difference between 50 and 5 μg/kg NPA shows the number of low affinity sites. After 2 weeks' saline treatment the ^{3}H-SPI displacing potency of 5 μg/kg was lower than the action of 50 μg/kg NPA. Caerulein inhibited ^{3}H-SPI binding in subcortex and in frontal cortex. After chronic haloperidol 50 μg/kg NPA more readily inhibited ^{3}H-SPI binding, whereas the displacing potency of 5 μg/kg NPA was significantly reduced. Caerulein, in contrast to the inhibition of ^{3}H-SPI binding in chronic saline group, stimulated it after chronic haloperidol.

It was concluded that long-term haloperidol treatment increased the affinity of ^{3}H-SPI to

[1]Institute of General and Molecular Pathology, Tartu State University, Tartu, Estonia, U.S.S.R.

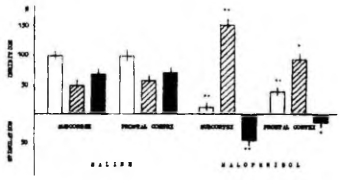


FIGURE. The effect of N-propylnorapomorphine and caerulein on ^{3}H-spiperone binding after chronic saline and haloperidol. Each column is the mean of three independent experiments, the vertical bars represent SEM. The NPA 5 μg/kg displaceable part of total binding is 100% (18140 ± 1250 DPM/g tissue in subcortex and 15350 ± 1100 DPM/g tissue in frontal cortex) in the chronic saline group. Significant differences between saline and haloperidol pretreated animals: * = $p<0.05$, ** = $p<0.01$. Light columns show the effect of NPA 5 μg/kg, striped columns the difference between the effect of NPA 50 μg/kg and 5 μg/kg; the dark is for caerulein.

high-affinity binding sites for NPA on dopamine$_2$- and serotonin$_2$-receptors but decreased it towards low-affinity sites bound by high concentrations of NPA. Considering the stimulating action of caerulein on ^{3}H-SPI binding, it seems possible that endogenous CCK-8 increased the interaction of ^{3}H-SPI with high-affinity binding sites for NPA on dopamine$_2$- and serotonin$_2$-receptors. The number of CCK binding sites has been shown to increase twice after chronic neuroleptic treatment (Chang et al., 1983). The present work supports the study of Agnati et al. (1983) showing the ability of CCK-8 to increase the affinity of neuroleptics to its binding sites. It seems that the same mechanism may be involved in the beneficial action of CCK-related peptides in neuroleptic-resistant schizophrenic patients.

References

Agnati, L.F., Fuxe, K., Benfenati, F. et al. Differential modulation by CCK-8 and CCK-4 of ^{3}H-spiperone binding sites linked to dopamine and 5-hydroxytryptamine receptors in the brain of the rat. *Neurosci. Lett.*, 35:179-183, 1983.

Battaglia, G., and Titeller, M. ^{3}H-N-propylnorapomorphine and ^{3}H-spiperone binding in brain indicate two states of D$_2$-dopamine receptor. *Eur. J. Pharmacol.*, 81:493-498, 1982.

Bloom, D.M., Nair, N.P.V., and Schwartz, G. CCK-8 in the treatment of chronic schizophrenia. *Psychopharmacol. Bull.*, 19(3):361-363, 1983.

Chang, R.S.L., Lotti, V.J., Martin, G.E., and Chen, T.B. Increase of brain ^{125}I-cholecystokinin (CCK) receptor binding following chronic haloperidol treatment, intracisternal 6-hydroxy-dopamine, or ventral tegmental lesions. *Life Sci.*, 32:871-878, 1983.

ЖУРНАЛ ВЫСШЕЙ НЕРВНОЙ ДЕЯТЕЛЬНОСТИ
ТОМ XXXIV 1984 ВЫП. 2

УДК 612.82/.83+615.78+612.821

РОЛЬ СЕРОТОНИН$_2$-РЕЦЕПТОРОВ В РЕГУЛЯЦИИ АГРЕССИВНОГО ПОВЕДЕНИЯ

ВАСАР Э. Э., МАЙМЕТС М. О., АЛЛИКМЕТС Л. Х.

Лаборатория психофармакологии и кафедра фармакологии Тартуского государственного университета

С. Пероутка и С. Снайдер [11], основываясь на экспериментах со связыванием меченых лигандов, разделяли серотониновые рецепторы на два подтипа. По их классификации существуют серотонин$_1$-рецепторы, связанные с действием серотонина, и сертонин$_2$-рецепторы, опосредующие эффекты галлюциногенных серотониномиметиков. В поведенческих исследованиях установлено, что крысы очень быстро обучаются по методике самовведения отличать введение галлюциногенных серотониномиметиков от введения других веществ [5], причем только пиренперон — избирательный антагонист серотониновых рецепторов, блокировал в низких дозах данный эффект. С другой стороны, из работы [13] видно, что мескалин — стимулятор серотонин$_2$-рецепторов, вызывает патологическую агрессивность у крыс-самцов. Авторы [14] нашли, что введение высоких доз апоморфина вызывает у кошек поведение, не отличающееся от поведения при действии другого стимулятора серотонин$_2$-рецепторов — диэтиламида лизергиновой кислоты. Имеются данные, что высокие дозы опиатного антагониста налоксона усиливают поведенческие и электрофизиологические эффекты галлюциногенных серотониномиметиков [6]. В настоящем исследовании приводятся данные, показывающие, что в ЦНС существуют два подтипа серотонин$_2$-рецепторов, противоположно влияющие на агрессивное поведение. Вероятным модулятором активности серотониновых рецепторов, связанных с агрессивным поведением, является тетрапептид холецистокинина.

МЕТОДИКА

Опыты проведены на 300 крысах-самцах линии Вистар, разделенных на 29 групп, по 10—12 животных в каждой. Влияние квипазина (фирма «Майлз Лабс», Англия) и пиренперона (фирма «Янссен», Бельгия) на связывание ^{3}H-спироперидола (фирма «Амершам Интернейшонал». Англия) изучалось в стриатуме и фронтальной коре, где места связывания нейролептиков соответственно относятся к дофамин$_2$- и серотонин$_2$-рецепторам [9]. Связывание изучали по методике [4]. Действие спироперидола и пиренперона на связывание ^{3}H-спироперидола (0,5 нмоль, удельная активность 21 Ки/ммоль) определялось в пределах концентраций 1 нмоль — 10 мкмоль, влияние стимулятора серотониновых рецепторов квипазина — 10 нмоль — 50 мкмоль. Содержание белка в суспензии определяли по методике [10]. Результаты опытов связывания обрабатывали с помощью анализа Скетчарда.

Во второй части исследования сравнивали влияние системного введения галоперидола (0,01—0,2 мг/кг внутрибрюшинно) и пиренперона (0,07—0,3 мг/кг внутрибрюшинно) на поведенческие эффекты апоморфина (0,5 мг/кг подкожно). Галоперидол и пиренперон всегда вводили за 1 ч до введения апоморфина. Все исследуемые вещества вводили в течение 10 дней, 2 раза в день. Поведенческие тесты проводили на 1, 3, 7-й и 10-й день длительного введения. Интенсивность стереотипии определяли по методике Б. Косталл и соавт. [7], агрессивного поведения по методике, разработанной Л. Х. Алликметсом и соавт. [3].

В третьей части исследования изучали влияние разных доз налоксона (0,5—15,0 мг/кг подкожно, фирма «Эндо Лабс», США) на развитие

апоморфиновой (0,5 мг/кг подкожно) агрессивности. Развитие спонтанной агрессивности оценивали у сгруппированных крыс (по 10—12 животных в группе). Апоморфин и налоксон вводили в течение 10 дней 2 раза в день. Через 48 ч после их отмены определяли поведенческие эффекты квипазина (2,5 мг/кг) — число встряхиваний головой в течение 40 мин и спонтанную агрессивность.

В четвертой части исследования изучали взаимодействие тетрапептида холецистокинина (ХЦК-4, И. К. И., Англия), который, по нашим предварительным данным, из всех пептидов холецистокининового ряда обладает самым выраженным усиливающим влиянием на агрессивное поведение с серотонин$_2$-рецепторами. ХЦК-4 вводили в боковые желудочки мозга по методике [1]. ХЦК-4 применяли в разных дозах (200—5000 нг) через 10 мин после внутрибрюшинного введения квипазина (2,5 мг/кг). Число встряхиваний головой подсчитывали с 5-й по 10-ю минуту после введения ХЦК-4. Параллельно исследовали влияние этих же доз ХЦК-4 на агрессивность, вызванную электроболевым раздражением. Спустя 5 мин после введения ХЦК-4, двух крыс подвергали электроболевым раздражениям (96 включений тока в течение 2 мин, напряжением 35 В). Интенсивность агрессивного поведения оценивали по числу агрессивных контактов между животными. Определяли также влияние разных доз пиренперона (0,01—0,1 мг/кг) и галоперидола (0,01—0,2 мг/кг) на усиленную ХЦК-4 (200 нг) электроболевую агрессивность. Оба вещества вводили за 60 мин до введения ХЦК-4. В отдельной серии опытов исследовали влияние ХЦК-4 (200—5000 нг) на электроболевую агрессивность после 10-дневного предварительного введения апоморфина (0,5 мг/кг, 2 раза в день). ХЦК-4 вводили спустя 48 ч после заключительной инъекции апоморфина. Все результаты поведенческих исследований подвергали статистической обработке с использованием t-теста Стьюдента.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЙ

Пиренперон и квипазин в разной степени вытесняли ^{3}H-спироперидол из мест связывания в стриатуме и фронтальной коре (табл. 1). В стриатуме вытеснение меченого спироперидола достигало только 30%, в то время как во фронтальной коре этот показатель был около 90%. Однако сродство квипазина к местам связывания спироперидола было значительно ниже по сравнению с пиренпероном. Этот факт объясняется существованием неодинаковых мест связывания для агонистов и антагонистов серотонин$_2$-рецепторов.

Галоперидол и пиренперон по-разному влияли на поведенческие эффекты апоморфина (табл. 2). Малая доза галоперидола (0,01 мг/кг), не блокирующая стереотипного поведения, потенцировала развитие апоморфиновой агрессивности. Только 0,2 мг/кг галоперидола, которое полностью подавляло все признаки стереотипного поведения, устраняло раз-

Таблица 1

Влияние спироперидола (1 нмоль — 10 мкмоль), пиренперона (1 нмоль — 10 мкмоль) и квипазина (10 нмоль — 50 мкмоль) на связывание ^{3}H-спироперидола с мембранами фронтальной коры и стриатума

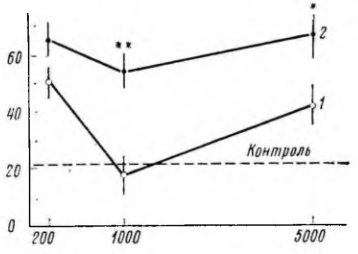
Вещество	Стриатум		Фронтальная кора	
	$K_д$	$Св_{макс}$	$K_д$	$Св_{макс}$
Спироперидол	2,1	285±21	1,5	370±25
Пиренперон	4,8	80±10*	4,0	330±23
Квипазин	78,0	88±7*	83,0	315±28

Примечание. Приведены средние величины трех независимых исследований. $K_д$ — константа диссоциации, нмоль. $Св_{макс}$ — максимальное число мест связывания, из которых исследуемые вещества вытесняли меченый спироперидол (фмоль/мг белка).

* $p<0,05$.

витие апоморфиновой агрессивности. 0,07 мг/кг пирепперона также заметно ускоряло развитие апоморфиновой агрессивности, в то время как более высокие дозы оказывали доза-зависимое снижение агрессивности. При этом пирепперон существенно не изменял параметров апоморфиновой стереотипии, т. е. оказывал в отличие от галоперидола избирательное влияние на апоморфиновую агрессивность.

Одновременное введение 15 мг/кг налоксона с апоморфином резко ускоряло развитие апоморфиновой агрессивности. После отмены этой комбинации отмечалось достоверное уменьшение числа встряхиваний головой, вызванных квипазином, по сравнению с группой, получавшей только апоморфин (табл. 3). Следует отметить, что квипазин мог вызывать у таких крыс спонтанную агрессивность, которая не отличалась от апоморфиновой. Малая доза налоксона (0,5 мг/кг) оказывала аналогичное с высокой дозой (15 мг/кг) действие, хотя и более слабое. Средняя доза налоксона (5 мг/кг) при одновременном введении с апоморфином несколько тормозила развитие апоморфиновой агрессивности. После отмены этой комбинации наблюдалось значительное увеличение числа встряхиваний головой, вызванных квипазином, однако не отмечались агрессивные реакции.



Влияние длительного предварительного введения апоморфина (0,5 мг/кг в течение 10 дней, 2 раза в день) на усиленную тетрапептидом холецистокинина (ХЦК-4) электроболевую агрессивность. ХЦК-4 вводили внутрижелудочково через 48 ч после отмены апоморфина. По оси ординат—число агрессивных контактов в течение 2 мин: по оси абсцисс — доза ХЦК-4, нг. Контроль — внутрижелудочковое введение физиологического раствора. *1* — после длительного введения физиологического раствора и *2* — апоморфина. * — $p<0,05$; ** — $p<0,02$

Таблица 2

Влияние длительного (в течение 10 дней, 2 раза в день) одновременного введения пирепперона (0,07—0,3 мг/кг) либо галоперидола (0,01—0,2 мг/кг) с апоморфином (0,5 мг/кг) на интенсивность апоморфиновой стереотипии и агрессивности. Приведены средние величины (баллы) для всех групп и стандартные ошибки

Вещество, доза (мг/кг)	Стереотипия	Агрессивность	Стереотипия	Агрессивность
	1-й день		3-й день	
Апоморфин 0,5 + физиологический раствор	3,1±0,18	0	2,7±0,19	0,3±0,22
Апоморфин 0,5 + пирепперон 0,07	2,8±0,21	0	2,6±0,23	2,9±0,25*
Апоморфин 0,5 + пирепперон 0,15	2,7±0,26	0	2,5±0,18	0
Апоморфин 0,5 + пирепперон 0,30	2,4±0,34	0	2,4±0,22	0
Апоморфин 0,5 + галоперидол 0,01	3,0±0,22	0	3,2±0,20	2,2±0,32*
Апоморфин 0,5 + галоперидол 0,10	0*	0	0*	0
Апоморфин 0,5 + галоперидол 0,20	0*	0	0*	0

Вещество, доза (мг/кг)	Стереотипия	Агрессивность	Стереотипия	Агрессивность
	7-й день		10-й день	
Апоморфин 0,5 + физиологический раствор	2,2±0,19	2,3±0,18	2,0±0,17	3,2±0,22
Апоморфин 0,5 + пирепперон 0,07	2,7±0,22	3,6±0,15	—	—
Апоморфин 0,5 + пирепперон 0,15	2,3±0,22	0,8±0,42*	2,2±0,24	1,0±0,45*
Апоморфин 0,5 + пирепперон 0,30	2,2±0,17	0,3±0,22*	1,7±0,19	0,3±0,22*
Апоморфин 0,5 + галоперидол 0,01	3,0±0,19	3,5±0,23*	3,0±0,24*	3,9±0,10
Апоморфин 0,5 + галоперидол 0,10	1,4±0,16	0,6±0,32*	2,4±0,17	2,3±0,25
Апоморфин 0,5 + галоперидол 0,20	0*	0*	0*	0*

* $p<0,05$.

Таблица 3

Корреляция между развитием апоморфиновой агрессивности и поведенческими эффектами квипазина (2,5 мг/кг) после отмены длительного (в течение 10 дней, 2 раза в день) одновременного введения апоморфина (0,5 мг/кг) и налоксона (0,5—15,0 мг/кг)

Вещество, доза (мг/кг)	Квипазин		Апоморфиновая агрессивность
	число встряхиваний головой	агрессивность	
Физиологический раствор	32±3,4	—	(—)
Апоморфин 0,5 + физиологический раствор	55±4,2*	—	(8-й день) *
Апоморфин 0,5 + налоксон 0,5	31±5,5	+	↑(5-й день)
Апоморфин 0,5 + налоксон 5,0	88±5,8**	—	↓(10-й день)
Апоморфин 0,5 + налоксон 15,0	19±2,7*	++	↑↑(2-й день)

Примечание. «+», «—» — наличие или отсутствие спонтанной агрессивности после введения квипазина. ↑, ↓ — усиление или ослабление апоморфиновой агрессивности под влиянием налоксона. * — в скобках приведен день длительного введения, когда все животные в группе становились агрессивными.

* $p<0,05$.
** $p<0,01$.

Таблица 4

Влияние внутрижелудочкового введения тетрапептида холецистокинина (ХЦК-4) на электроболевую агрессивность и встряхивания головой, вызванные квипазином (2,5 мг/кг). Действие пиренперона (0,01—0,1 мг/кг) и галоперидола (0,01—0,2 мг/кг) на усиленную ХЦК-4 электроболевую агрессивность

Вещество, доза	Число встряхиваний головой, с 5-й по 10-ю минуту после введения ХЦК-4	Электроболевая агрессивность, число агрессивных контактов в течение 2 мин
Физиологический раствор	8±1,5	22±2,5
ХЦК-4		
200 нг	5±0,9	50±4,2**
1000 »	8±1,7	19±2,6
5000 »	4±1,2	40±3,9*
ХЦК-4 200 нг + галоперидол 0,01 мг/кг	—	62±4,5*
0,05 »	—	33±4,0
0,2 »	—	15±2,6
ХЦК-4 200 нг + пиренперон 0,01 мг/кг	—	31±2,8
0,05 »	—	2±0,8**
0,1 »	—	0**

* $p<0,05$.
** $p<0,02$.

Внутрижелудочковое введение 200 нг ХЦК-4 вызывало заметное усиление электроболевой агрессивности, причем животные наносили повреждения друг другу. При дальнейшем повышении дозы ХЦК-4 вначале агрессивные реакции ослаблялись (1000 нг), а потом (5000 нг) опять превышали контрольный уровень (табл. 4). Следует отметить, что эти дозы ХЦК-4 оказывали также разное влияние на число встряхиваний головой, вызванных квипазином (2,5 мг/кг). Введение 200 и 5000 нг ХЦК-4 угнетало, в то время как 1000 нг не изменяло поведенческого эффекта квипазина (табл. 4). Пиренперон обладал по сравнению с галоперидолом более выраженным влияние на усиленную ХЦК-4 электроболевую агрессивность. Малая доза галоперидола (0,01 мг/кг) потенцировала действие ХЦК-4 и лишь введение 0,2 мг/кг галоперидола значительно подавляло агрессивное поведение. Пиренперон в отличие от галоперидола уже в дозе 0,01 мг/кг достоверно понижал интенсивность агрессивного поведения (табл. 4). При дальнейшем повышении дозы антиагрессивное действие пиренперона углублялось. После отмены длительного введения апоморфина проагрессивное действие ХЦК-4 усиливалось (рисунок), причем такое изменение было особенно очевидно после введения 1000 нг ХЦК-4.

ОБСУЖДЕНИЕ РЕЗУЛЬТАТОВ

Анализ связывания меченого спироперидола свидетельствует, что определенная часть мест связывания нейролептиков имеет отношение к серотонинергическим механизмам переднего мозга. Наши данные согласуются с мнением автора [12], что в стриатуме места связывания нейролептиков в первую очередь относятся к дофаминергической, в то время как во фронтальной коре — к серотонинергической системе. Существование неоднородных мест связывания для нейролептиков согласуется и с тем, что пиренперон и галоперидол по-разному изменяют поведенческие эффекты апоморфина. Антиагрессивное действие галоперидола коррелирует с подавлением апоморфиновой стереотипии, в то время как пиренперон в дозах, подавляющих апоморфиновую агрессивность, не изменяет интенсивности стереотипного поведения. Известно, что снижение стереотипного поведения под влиянием нейролептиков в первую очередь реализуется через блокаду дофаминовых рецепторов в полосатом теле [4]. Пиренперон не взаимодействует в использованных нами дозах с этими рецепторами и поэтому не влияет на интенсивность стереотипного поведения. За антиагрессивное действие пиренперона ответственны другие структуры переднего мозга, где места связывания нейролептиков имеют тесную связь с серотониновыми рецепторами — в лимбической системе и фронтальной коре. Полученные нами данные свидетельствуют также о том, что серотонинергическим механизмам принадлежит более существенная роль в развитии апоморфиновой агрессивности по сравнению с дофаминергическими. Это мнение подтверждается и фактом, что после длительного совместного введения апоморфина с определенными дозами налоксона квипазин, стимулятор серотонин$_2$-рецепторов, вызывает спонтанную агрессивность, причем агрессивное поведение не отличается от апоморфиновой агрессивности. С другой стороны, эти результаты дают нам возможность полагать, что среди серотонин$_2$-рецепторов существуют функционально неоднородные подтипы рецепторов. Одновременное введение определенных доз налоксона и апоморфина повышает чувствительность серотониновых рецепторов, включающих агрессивное поведение, однако параллельно развивается состояние пониженной чувствительности к рецепторам с противоположным влиянием на агрессивное поведение.

В предыдущих наших исследованиях установлено, что пептиды холецистокининового ряда обладают апоморфин-подобным действием [2]. Описанные выше данные показывают, что ХЦК-4 участвует в регуляции чувствительности серотониновых рецепторов. ХЦК-4, как и одновременное введение определенных доз налоксона и апоморфина, неодинаково изменяет чувствительность разных подтипов серотонин$_2$-рецепторов и в связи с этим ведет к усилению оборонительных реакций организма.

Таким образом, в результате проведенных исследований можно сделать заключение, что в мозге имеется два подтипа серотонин$_2$-рецепторов, опосредующих противоположные влияния на агрессивное поведение. Одновременное введение определенных доз налоксона с апоморфином вызывает сдвиг в сторону активации рецепторов, включающих агрессивные реакции. Результаты настоящего исследования свидетельствуют, что аналогичным действием обладает также ХЦК-4. Известно, что ХЦК-4 содержится в структурах переднего мозга в значительных концентрациях [8]. В связи с этим можно полагать, что ХЦК-4 является эндогенным модулятором чувствительности серотониновых рецепторов, связанных с регуляцией агрессивного поведения.

ВЫВОДЫ

1. Пиренперон, антагонист серотонин$_2$-рецепторов в отличие от галоперидола является избирательным антагонистом апоморфиновой агрессивности. Длительное совместное введение определенных доз налоксона с апоморфином выявляет разные подтипы серотонин$_2$-рецепторов, что вы-

ражается в угнетении встряхиваний головой и появлении спонтанной агрессивности после введения квипазина, стимулятора серотонин$_2$-рецепторов.

2. Тетрапептид холецистокинина значительно усиливает электроболевую агрессивность и в тех же дозах уменьшает число встряхиваний головой, вызванных квипазином. Пиренперон является избирательным блокатором действия тетрапептида холецистокинина.

3. Тетрапептид холецистокинина, возможно, является эндогенным модулятором чувствительности серотониновых рецепторов. Высказывается предположение, что его функциональная роль заключается в активации серотонин$_2$-рецепторов, включающих оборонительные агрессивные реакции.

Литература

1. *Алликметс Л. Х.* Влияние адрен- и серотонинергических веществ на поведение амигдалэктомированных крыс и на агрессивность, вызванную интраамигдалярным введением ацетилхолина.— Ж. высш. нервн. деят., 1975, т. 25, № 1, с. 164.
2. *Васар Э. Э., Оттер М. Я., Рягo Л. К.* Интрацеребровентрикулярное введение холецистокинина угнетает активность дофамин- и серотонинергической систем мозга.— Физиол. ж. СССР, 1982, т. 68, № 9, с. 1218.
3. *Allikmets L. H., Stanley M., Gershon S.* The effect of lithium on chronic haloperidol enhanced apomorphine aggression in rats.— Life Sci., 1979, v. 25, p. 165.
4. *Bürki H. R.* Correlation between ^{3}H-haloperidol binding in the striatum and brain amine metabolism in the rat after treatment with neuroleptics.— Life Sci., 1978, v. 23, p. 437.
5. *Colpaert F. C., Niemegeers C. J. E., Janssen P. A. J.* A drug discrimination analysis of lysergic acid diethylamide: in vivo agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, an LSD antagonist.— J. Pharmacol. and Exptl Therap., 1982, v. 221, p. 206.
6. *Contreras C. M., Guzman-Florez C., Dorantes M. E., Ervin F. R., Palmour R.* Naloxone and phencyclidine: interacting effects of the limbic system and behavior.— Physiol. Behav., 1981, v. 27, p. 1019.
7. *Costall B., Naylor R. J.* Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphe nuclei.— Europ. J. Pharmacol., 1974, v. 29, p. 206.
8. *Gottermann N. R., Stengaard-Pedersen K., Rehfeld J. F., Christensen N. J.* Newly synthesized cholecystokinin in subcellular fractions of the rat brain.— J. Neurochem., 1981, v. 36, p. 959.
9. *Leysen J. E., Gommeren W., Laduron P. M.* Spiperone: a ligand of choice for neuroleptic receptors. I. Kinetics and characteristics of in vitro binding.— Biochem. Pharmacol., 1978, v. 27, p. 307.
10. *Lowry O. H., Rosenbrough N. J., Farr A. L., Randall R. J.* Protein measurement with the Folin reagent.— J. Biol. Chem., 1951, v. 193, p. 265.
11. *Peroutka S. J., Snyder S. H.* Multiple serotonin receptors: differential binding of ^{3}H-5-hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol.— Mol. Pharmacol., 1979, v. 16, p. 687.
12. *Seeman P.* Brain dopamine receptors.— Pharmacol. Rev., 1981, v. 32, p. 229.
13. *Sbordone R. J., Gorelick D. A., Elliott M. L.* An ethological analysis of drug-induced pathological aggression.— In: Multidisciplinary approaches to aggression research/ /Eds. P. F. Brain, D. Benton. Elsevier North-Holland Biomedical Press, 1981, p. 369.
14. *Trulson M. E., Crisp T.* Behavioral and neurochemical effects of apomorphine in the cat.— Europ. J. Pharmacol., 1982, v. 80, p. 295.

Поступила в редакцию
28.VI.1983

THE ROLE OF SEROTONIN$_2$-RECEPTORS IN THE REGULATION OF AGGRESSIVE BEHAVIOUR

VASAR E. E., MAIMETS M. O., ALLIKMETS L. H.

Chair of Pharmacology, Tartu University, Tartu

Quipazine and pirenperone, the drugs interacting with serotonin$_2$-receptors, more readily displaced ^{3}H-spiroperidol from its binding sites in the frontal cortex than in the striatum. Pirenperone (0,07—0,3 mg/kg), antagonist of serotonin$_2$-receptors, selectively decreased the intensity of apomorphine aggresiveness. The antiaggressive action of haloperidol (0,01—0.2 mg/kg) was in correlation with its antistereotypic activity. Long-term

administration of naloxone (0,5; 15.0 mg/kg), together with apomorphine (0,5 mg/kg) reduced the number of head-twitches caused by quipazine (2,5 mg/kg). The administration of quipazine 48 hours after the last injection of naloxone and apomorphine caused spontaneous aggressiveness that did not differ from apomorphine aggressiveness. Intracerebroventricular injection of cholecystokinin tetrapeptide (CCK-4) markedly enhanced the foot-shock aggression. The same dose of CCK-4 also decreased the intensity of quipazine (2,5 mg/kg) head-twitches. Compared to haloperidol, pirenperone was a more selective antagonist of CCK-4. After long-term apomorphine treatment (0,5 mg/kg during 10 days, twice daily), the effect of CCK-4 on aggressive behaviour was markedly enhanced. It is possible that two subtypes of serotonin$_2$-receptors exist in the brain and have opposite action on the aggressive behaviour. CCK-4 may play the role of an endogenous modulator of sensitivity of serotonin$_2$-receptors involved in the control of aggressiveness.

ISSN 0365-9615

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

Бюл. эксперим. биологии и медицины, 1985, XCIX, № 1, 1–128

УДК 615.355:577.175.734.015.4:612.822.1

Ключевые слова: церулеин, Н-пропилморфин, апоморфин; ^{3}Н-спироперидол, дофаминовые и серотониновые рецепторы.

Э. Э. Васар, А. М. Нурк, М. О. Майметс, Л. Х. Алликметс

СТИМУЛЯЦИЯ ЦЕРУЛЕИНОМ — АНАЛОГОМ ОКТАПЕПТИДА ХОЛЕЦИСТОКИНИНА — СВЯЗЫВАНИЯ ^{3}Н-СПИРОПЕРИДОЛА ПОСЛЕ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ НЕЙРОЛЕПТИКОВ

Лаборатория психофармакологии НИИ общей и молекулярной патологии, кафедра фармакологии Тартуского университета

Представлена акад. АМН СССР А. В. Вальдманом

Имеются данные о том, что пептиды холецистокининового ряда в низких концентрациях модулируют взаимодействие спироперидола с дофамин$_2$- и серотонин$_2$-рецепторами [2]. В исследованиях поведенческих реакций выявлено, что холецистокинин и его аналоги при внутримозговом и периферическом введении оказывают действие, подобное влиянию как нейролептиков [4, 15], так и апоморфина [1]. Параллельно поведенческим сдвигам холецистокинин и его аналоги вызывают подавление метаболизма дофамина и серотонина в структурах переднего мозга [1, 7]. В настоящее время появились данные, свидетельствующие о том, что холецистокинин и его аналог церулеин оказывают на больных шизофренией, резистентных к нейролептикам, значительное антипсихотическое действие [10, 11].

В связи с этим представляло практический интерес изучение влияния церулеина — высокоаффинного аналога октапептида холецистокинина [15] на связывание ^{3}Н-спироперидола в опытах in vivo. Учитывая апоморфиноподобное действие церулеина, данный биохимический анализ проводили в сравнении с Н-пропилнорапоморфином (НПА) — высокоаффинным аналогом апоморфина.

Методика исследования. Опыты проводили на белых беспородных мышах-самцах массой 20—25 г. В течение 2 нед 2 раза в день животным вводили галоперидол (0,25 мг/кг, фирма «Gedeon Richter», Венгрия), пипенперон (0,25 мг/кг, фирма «Janssen Pharmaceutica», Бельгия) или физиологический раствор. Спустя 72 ч после отмены длительного введения ставили опыты по связыванию in vivo: 6 животным из каждой группы (физиологический раствор, галоперидол и пипенперон) вводили только ^{3}Н-спироперидол в дозе 5 мкг/кг подкожно (уд. радиоактивность 17 Ки/ммоль, фирма «Amersham», Англия) и спустя 20 мин их декапитировали. Остальным мышам из тех же групп (по 6 животных) перед меченым спироперидолом вводили вытесняющие вещества. Галоперидол в дозе 2,5 мг/кг был введен внутрибрюшинно за 40 мин до введения меченого лиганда, а церулеин в дозе 0,4 мг/кг (подкожно, фирма «Farmitalia», Италия) и (НПА в дозах 5 и 50 мкг/кг (фирма «Research Biochemicals Inc.», США) вводили за 15 мин перед ^{3}Н-спироперидолом. После декапитации животных на льду быстро извлекали мозг и препарировали подкорковые образования переднего мозга (лимбическая система и стриатум) и фронтальную кору. Выделенные структуры гомогенизировали в 25 объемах трис-HCl-буфера (50 мМ pH 7,4 при 20 °С). Затем пробы центрифугировали при 9000 об. в течение 10 мин. Супернатант выливали и осадок осторожно промывали несколько раз с помощью холодного трис-HCl-буфера. Радиоактивность проб (5 параллелей) определяли в сцинтилляторе Брея на счетчике β-частиц «Ультро-Бета 1210» (фирма LKB, Швеция). Опыты повторяли 3 раза.

Результаты исследования. Вытесняющее действие высокой дозы галоперидола (2,5 мг/кг) существенно не изменялось после длительного введения галоперидола (0,25 мг/кг) и пипенперона (0,25 мг/кг) по сравнению с показателем в группе животных, получавших фи-

Влияние галоперидола (2,5 мг/кг), НПА (5 и 50 мкг/кг) и церулеина (0,4 мг/кг) на связывание ^{3}Н-спироперидола (5 мкг/кг) в опытах in vivo после длительного введения галоперидола (0,25 мг/кг) и пипенперона (0,25 мг/кг)

Вещество	Физиологический раствор		Галоперидол		Пипенперон	
	подкорковые структуры	фронтальная кора	подкорковые структуры	фронтальная кора	подкорковые структуры	фронтальная кора
Галоперидол, 2,5 мг/кг	9 050 ± 840	11 100 ± 860	9 700 ± 780	11 200 ± 740	9 400 ± 850	5 500 ± 890*
НПА						
50 мкг/кг	8 600 ± 760	14 400 ± 890	12 400 ± 800*	11 800 ± 1 020*	12 300 ± 790*	10 750 ± 1 010
5 мкг/кг	7 300 ± 670	12 250 ± 1 010	1 100 ± 400**	3 750 ± 520**	2 700 ± 600**	3 350 ± 650**
Церулеин, 0,4 мг/кг	1 150 ± 300	7 300 ± 610	3 600 ± 400*	4 100 ± 250*	1 100 ± 180**	2 450 ± 300**

Примечание. Приведены средние различия (число импульсов на 1 регистрацию) между группами, получавшими только ^{3}Н-спироперидол или ^{3}Н-спироперидол на фоне вытесняющих веществ. Знак «плюс» — стимулирующее влияние на связывание ^{3}Н-спироперидола. Одна звездочка — $P \leq 0{,}05$, две — $P \leq 0{,}02$ по сравнению с контролем (физиологический раствор).

знологический раствор (см. таблицу). Только после многократного введения серотонин$_2$-антагониста пипенперона уменьшалось его вытесняющее влияние во фронтальной коре. В отличие от действия галоперидола влияние НПА заметно изменялось после длительного введения галоперидола и пипенперона. В дозе 50 мкг/кг НПА значительно сильнее вытеснял ^{3}H-спироперидол в подкорковых структурах после длительного введения как галоперидола, так и пипенперона. Во фронтальной коре вытесняющий эффект НПА в этой дозе даже несколько ослаблялся после длительного введения нейролептиков. Такое различие в действии НПА в двух регионах мозга, по-видимому, объясняется его более слабым агонистическим действием на серотонин$_2$-рецепторы по сравнению с дофаминовыми. Установлено, что только высокие дозы апоморфина вызывают подобные галлюциногенным серотониномиметикам поведенческие эффекты [14] и значительные концентрации апоморфина вытесняют ^{3}H-кетансерин — антагонист серотонин$_2$-рецепторов — из мест связывания в префронтальной коре [8]. К вытесняющему действию малой дозы НПА (5 мкг/кг) развивалась, однако, толерантность после длительного введения галоперидола и пипенперона (см. таблицу). Этот факт свидетельствует о том, что на определенных местах связывания ^{3}H-спироперидол сильнее взаимодействует с дофаминовыми и серотониновыми рецепторами после длительного введения нейролептиков. Результаты настоящего исследования во многом согласуются с данными литературы [5], согласно которым нейролептики и апоморфин неодинаково взаимодействуют с дофамин$_2$-рецепторами. Показано, что взаимодействие апоморфина с ^{3}H-спироперидолом на дофамин$_2$-рецепторах осуществляется через низко- и высокоаффинные места связывания, в то время как нейролептики имеют на этих рецепторах только высокоаффинные места связывания [5]. Выявлено, что константы диссоциации этих двух мест связывания для апоморфина отличаются приблизительно в 10 раз. Учитывая различное действие разных доз НПА после длительного введения галоперидола и пипенперона, можно полагать, что аффинность нейролептиков к этим двум местам связывания для апоморфина изменяется неодинаково. По-видимому, понижается аффинность ^{3}H-спироперидола к низкоаффинным местам связывания для апоморфина, в то время как на высокоаффинных местах связывание ^{3}H-спироперидола существенным образом увеличивается. Вероятно, что изменение чувствительности низкоаффинных мест связывания отражает развитие гиперчувствительности к дофамино- и серотониномиметикам и ослабление разных эффектов нейролептиков, в то время как повышение аффинности ^{3}H-спироперидола к высокоаффинным местам связывания для апоморфина, по всей вероятности, связано с развитием антипсихотического действия в процессе длительного введения нейролептиков.

После длительного введения нейролептиков не изменяется только действие НПА, а также церулеина — высокоаффинного аналога октапептида холецистокинина [15]. Существует мнение, что ряд эффектов холецистокинина и его аналогов реализуется при периферическом введении через афферентные механизмы блуждающего нерва [9, 13]. Однако наши исследования свидетельствуют о том, что церулеин проникал в мозг и вытеснял у контрольных животных ^{3}H-спироперидол из мест связывания (см. таблицу). После 2-недельного введения галоперидола и пипенперона действие церулеина, однако, стало противоположным. Церулеин не вытеснял, а стимулировал связывание спироперидола в обоих нами исследованных регионах переднего мозга. Следует отметить, что после длительного введения антагониста серотонин$_2$-рецепторов пипенперона стимулирующее действие церулеина было более выраженным во фронтальной коре (см. таблицу), а после галоперидола, взаимодействующего преимущественно с дофаминовыми рецепторами, это действие церулеина было более значимым в подкорковых структурах. Эти данные согласуются с исследованиями [12], показавшими, что во фронтальной коре места связывания для нейролептиков в основном относятся к серотонин$_2$-рецепторам, в то время как в подкорковых структурах превалирует взаимодействие с дофаминовыми рецепторами. Если учитывать, что длительное введение галоперидола (2—3 мг/кг) повышает плотность холецистокининовых рецепторов почти в 2 раза [3], можно полагать, что увеличение связывания ^{3}H-спироперидола на высокоаффинных местах связывания для апоморфина обусловлено именно усиленным действием эндогенного октапептида холецистокинина после длительного введения нейролептиков.

Таким образом, полученные данные свидетельствуют о том, что после длительного введения нейролептиков усиливается их взаимодействие с высокоаффинными местами связывания для апоморфина на дофамин$_2$- и серотонин$_2$-рецепторах. Этот механизм, по-видимому, лежит в основе антипсихотического действия нейролептиков. Однако, как показывают экспериментальные [6] и клинические исследования [10], это действие нейролептиков реализуется только на фоне достаточных концентраций октапептида холецистокинина. Установлено, что у больных шизофренией, резистентных к нейролептикам, после смерти выявляется низкое содержание холецистокинина в лимбических структурах [6].

ЛИТЕРАТУРА

1. *Висар Э. Э., Оттер М. Я., Ряго Л. К.* — Физиол. ж. СССР, 1982, № 9, с. 1218—1222.
2. *Agnati L. F., Fuxe K., Benfenati F.* et al. Neurosci. Lett., 1983, v. 35, p. 179—183.

3. *Chang R. S. L., Lotti V. J., Martin G. E.* et al. — Life Sci., 1983, v. 32, p. 871—878.
4. *Cohen S. L., Knight M., Tamminga C. A.* et al. — Europ. J. Pharmacol., 1982, v. 83, p. 213—222.
5. *Creese I., Leff S. A.* — J. clin. Psychopharmacol., 1982, v. 2, p. 329—335.
6. *Ferrier N., Roberts G. W., Crow T. J.* et al. — Life Sci., 1983, v. 33, p. 475—482.
7. *Fuxe K., Andersson K., Locatelli V.* et al. — Europ. J. Pharmacol., 1980, v. 67, p. 329—331.
8. *Leysen J. E., Niemegeers C. J. E., Van Nueten J. M.* et al. — Molec. Pharmacol., 1982, v. 21, p. 301—304.
9. *Lorenz D. N., Goldman S. A.* — Physiol. Behav., 1982, v. 29, p. 599—604.
10. *Nair N. P. V., Bloom D. M., Nestoros J. N.* — Progr. Neuro-Psychopharmacol. Biol. Psychiat., 1982, v. 6, p. 808—812.
11. *Moroji T., Watanabe M., Aoki N.* et al. — Arch. gen. Psychiat., 1982, v. 39, p. 485—486.
12. *Seeman P.* — Pharmacol. Rev., 1981, v. 32, p. 229—313.
13. *Smith G. P., Jerome C., Cushin B. J.* et al. — Science, 1981, v. 231, p. 1036—1037.
14. *Trulson M. E., Crisp T.* — Europ. J. Pharmacol., 1982, v. 80, p. 295—309.
15. *Zetler G.* — Neuropharmacology, 1981, v. 20, p. 277—283.

Поступила 12.01.84

STIMULATION WITH CERULEIN, AN ANALOG OF CHOLECYSTOKININ OCTAPEPTIDE, OF ^{3}H-SPIROPERIDOL BINDING AFTER PROLONGED ADMINISTRATION OF NEUROLEPTICS

E. E. Vasar, A. M. Nurk, M. O. Maimets, L. Kh. Allikmets

Research Institute of General and Molecular Pathology, Tartu University

It has been established in experiments on white male rats that prolonged administration (twice a day for 14 days) of haloperidol (0.25 mg/kg) and pyreneperone (0.25 mg/kg) resulted in the reduced interaction between ^{3}H-spiroperidol and low affinity binding sites for apomorphine in subcortical structures, whereas ^{3}H-spiroperidol binding with high affinity binding sites for apomorphine increased both in the frontal cortex and subcortical structures of the forebrain. After prolonged administration of neuroleptics the displacing effect of cerulein, an analog of cholecystokinin octapeptide, was replaced by the stimulant action on ^{3}H-spiroperidol binding. It is assumed that increased interaction between ^{3}H-spiroperidol and high affinity binding sites for apomorphine on dopamine$_2$- and serotonin$_1$-receptors underlies the antipsychotic action of neuroleptics after their prolonged administration. Cholecystokinin octapeptide is a necessary factor for realization of this action of neuroleptics.

УДК 618.281-07:616-008.949.4:615.281.8+615.281.8033:616.2

Ключевые слова: *беременность, ремантадин*

Н. Ф. Правдина, В. М. Шобухов, И. Г. Петрова, С. Г. Тулькес, Г. А. Галегов

КИНЕТИКА НАКОПЛЕНИЯ И ВЫВЕДЕНИЯ ^{3}H-РЕМАНТАДИНА В ТКАНЯХ БЕРЕМЕННЫХ МЫШЕЙ И ПЛОДАХ

Институт вирусологии им. Д. И. Ивановского АМН СССР, Москва НИИ по биологическим испытаниям химических соединений

Представлена акад. АМН СССР В. В. Закусовым

Аминопроизводные адамантана, в частности ремантадин (α-метил-1-адамантанметиламин), применяются в качестве средств профилактики и лечения гриппа [2, 4, 7]. Одна из важных фармакокинетических характеристик химиопрепаратов, используемых для широких контингентов населения, а к ним, безусловно, относится ремантадин, является проницаемость через плаценту и динамика накопления и особенно выведения их из плодов. В литературе подобные данные относительно ремантадина отсутствуют.

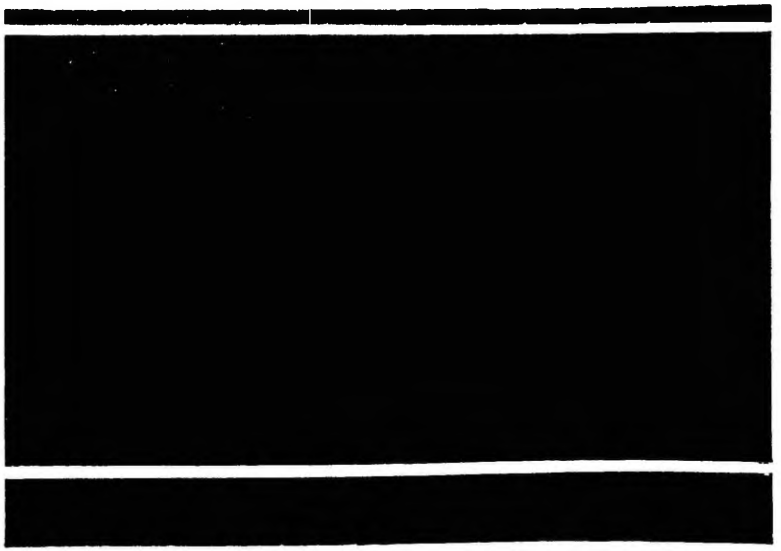
В связи с этим задачей настоящего исследования явилось изучение кинетики накопления и элиминации ^{3}H-ремантадина в плодах и тканях беременных мышей.

Методика исследования. В работе использован ^{3}H-ремантадин, полученный нами по ранее описанной методике [5]. Удельная радиоактивность препарата 30 мКи/ммоль. Опыты ставили на беспородных белых мышах-самках массой 30 г на 15—16-е дни беременности, а также на беспородных белых мышах массой 10—12 г. ^{3}H-ремантадин вводили в 0,3 мл физиологического раствора перорально (2,8 мг/кг). В установленные сроки (15 и 30 мин, 1, 2, 6 и 12 ч после введения препарата) мышей декапитировали и извлеченные органы помещали в 5 н. NaOH (7 мл — на плоды, 5 мл — на печень и по 3 мл — на почки и селезенку). Ткани гомогенизировали и к гомогенату добавляли равный объем бензола для экстракции меченого ремантадина. После интенсивного встряхивания в течение 10 мин суспензию центрифугировали 15 мин при 4000 об/мин для отделения бензола от водной фазы. От каждой пробы отбирали аликвоты (0,2 мл — для печени и по 1 мл — для плодов, селезенки и почек) и определяли радиоактивность в 10 мл толуолового сцинтиллятора на жидкостном сцинтилляционном спектрометре SL-30 фирмы «Intertechnique» (Франция).

Результаты исследования. Данные, представленные в табл. 1, отражают распределение ^{3}H-ремантадина в плодах, печени, почках и селезенке беременных мышей в течение 12 ч

ISSN 0365-9615

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ



Бюл. эксперим. биологии и медицины 1985,
№ 12, 641—768

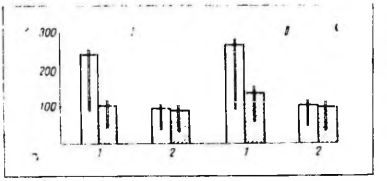


Рис. 2. Влияние никотина в дозах 0,05 мг/кг (*I*) и 0,1 мг/кг (*II*) на длительность иммобилизации крыс.

По оси ординат — время (в с). *1* — низкоактивные крысы, *2* — высокоактивные крысы. Светлые столбики — фон, заштрихованные — опыт.

(контроль) вводили 0,15 мл физиологического раствора. При этом введение 0,05 мг/кг никотина вызывало значительное повышение ЭПС «подчиненной» крысы и в большинстве случаев (в 6 из 8) происходила смена доминирующей особи (рис. 3). Введение 0,1 мг/кг никотина «подчиненной» особи также повышало ее ЭПС, однако смена доминирования произошла только в 4 из 8 случаев.

При изучении влияния никотина в широком диапазоне доз на двигательную активность мышей какого-либо значимого эффекта не наблюдалось.

Таким образом, можно сделать вывод, что никотин оказывает специфически активирующее действие на животных с низким адаптивным потенциалом, отличное от действия психостимуляторов, чем, по-видимому, и обусловлено их выраженное влечение к никотину.

Проведенные эксперименты свидетельствуют о наличии у части особей в популяции самцов беспородных белых крыс выраженной предрасположенности к развитию никотиновой токсикомании. Представляет интерес тот факт, что данная патология развивается у животных с низким адаптивным потенциалом, занимающих в зоосоциальной иерархии низшие ранги. Подобные особи обычно характеризуются высокой чувствительностью к стрессорным факторам и выраженной склонностью к развитию экспериментального алкоголизма, основанной на нормализующем влиянии, которое этанол оказывает на эмоциональную сферу и поведение этих особей [1, 6]. Следовательно, полученные нами данные свидетельствуют об общности причин, вызывающих влечение как к никотину, так и к алкоголю.

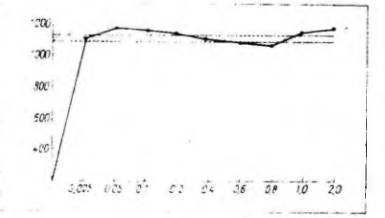


Рис. 3. Влияние никотина в разных дозах на двигательную активность мышей.

По оси абсцисс — дозы никотина (в мг/кг); по оси ординат — двигательная активность мышей.
Горизонтальная полоса — **контроль**.

ЛИТЕРАТУРА

1. *Борисенко С. А., Кампов-Полевой А. Б.* — Журн. высш. нервн. деят., 1980, № 3, с. 513.
2. *Буров Ю. В.* — Вестн. АМН СССР, 1982, № 5, с. 72—77.
3. *Буров Ю. В., Кампов-Полевой А. Б., Каминка Л. И.* — Журн. высш. нервн. деят., 1983, № 5, с. 941.
4. *Кампов-Полевой А. Б.* — Бюл. экспер. биол., 1978, № 9, с. 306.
5. *Кампов-Полевой А. Б.* — Депонировано в ВИНИТИ, 1979, № 825—79.
6. *Кампов-Полевой А. Б.* — В кн.: Фармакология экспериментального алкоголизма. М., 1982, с. 130—135.
7. *Плохинский Н. А.* Биометрия. М., 1970.
8. *Meddis R.* — Brit. J. Psychol., 1975, vol. 66, p. 225.

Поступила 28.02.85

THE FOLE OF NICOTINE PSYCHOTROPIC EFFECTS IN INDUCING INCLINATION TO INCOTINE IN RATS

T. Khodzhageldyev

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow

The experiments have proved some male noninbred while rats to of inclined to toxicomania development. It is of interest that such pathology develops in animals with low adaptive potential occupying the lowest rank in zoosocial hierarchy. These animals are usually characterized by high sensitivity to stress factors and pronounced inclination to the development of experimental alcoholism. Thus, it suggests the existence of common reasons causing the inclination to both nicotine and alcohol consumption.

УДК 615.357.34:577.175.734].017:615.213.015.2:615.214.22:547.891.2

Ключевые слова: *церулеин, прогумид, пикротоксиновые судороги, бензодиазепиновые рецепторы, ионофор хлора.*

Э. Э. Васар, Л. К. Ряго, А. Х. Соосаар, А. М. Нурк, М. О. Майметс

МОДУЛИРУЮЩЕЕ ВЛИЯНИЕ ЦЕРУЛЕИНА НА БЕНЗОДИАЗЕПИНОВЫЕ РЕЦЕПТОРЫ

Лаборатория психофармакологии НИИ общей и молекулярной патологии Тартуского университета

Представлена акад. АМН СССР А. В. Вальдманом

Установлено, что октапептид холецистокинина (ХЦК-8) и его аналог более сильного действия церулеин вызывают при системном введении эффекты, подобные эффектам транквилизаторов

бензодиазепинового ряда [6, 8]. Относительно высокие дозы церулеина и ХЦК-8 удлиняют латентные периоды проявления судорог, вызванных тиосемикарбазидом и гарманом, и повышают также пороговую дозу пикротоксина, необходимую для вызывания судорог у мышей [6, 8], причем церулеин по своему противосудорожному действию превосходит диазепам. Аналогичное противосудорожное действие наблюдается при внутрижелудочковом введении низких доз ХЦК-8 (1 и 100 нг) [4]. В то же время ХЦК-8 и церулеин оказывают действие, существенно отличающееся от влияния транквилизаторов бензодиазепинового ряда. Церулеин в отличие от диазепама потенцирует судороги, вызванные антагонистом ГАМК-рецепторов бикукуллином [6]. Введение Ro 15-1788 — антагониста бензодиазепинов — не устраняет седативное и противосудорожное действие церулеина и ХЦК-8 [8].

В настоящей работе исследовали участие бензодиазепиновых рецепторов в противосудорожном действии церулеина.

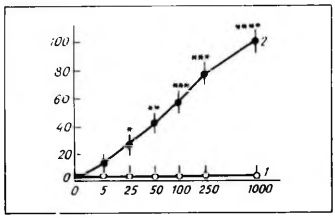
Методика исследования. Опыты проводили на мышах-самцах массой 25—30 г. При исследовании пикротоксиновых судорог церулеин (фирма «Farmitalia — Carlo Erba», Италия) вводили в разных дозах (20—500 мкг/кг) подкожно за 10 мин до внутрибрюшинного введения пикротоксина в дозе 8 мг/кг (фирма «Serva», ФРГ). В I серии экспериментов определяли влияние церулеина (5—1000 нМ) на связывание ^{3}H-флунитразепама (1 нМ) в среде 50 мМ трис-HCl-буферного раствора. Во II серии опытов к этой среде добавляли 120 мМ KCl. В каждой серии было по 16—20 животных. Определяли 3 различных параметра пикротоксиновых судорог: латентные периоды клонических судорог, латентные периоды тонических судорог и продолжительность жизни мышей после введения 8 мг/кг пикротоксина. Реакцию мышей на введение пикротоксина наблюдали в течение 30 мин. Если в течение этого времени у животных не развивались судороги или животные не погибали, то реакция мышей по всем исследованным параметрам соответствовала 30 мин. Антагонист ХЦК-8 проглумид (фирма «Rotta Farmaceutici», Италия) [3] вводили внутрибрюшинно в дозах 5 и 25 мг/кг за 5 мин до введения церулеина. Параллельно с изучением поведенческих реакций исследовали влияние церулеина на связывание ^{3}H-флунитразепама в опытах in vitro и in vivo. Проглумид (5 и 25 мг/кг) и церулеин (20—500 мкг/кг) вводили за 5 мин до подкожной инъекции меченого флунитразепама в дозе 0,3 мкг/кг (удельная радиоактивность 84 Ки/ммоль, фирма «Amersham», Англия). Животных (по 6 мышей из каждой серии) декапитировали через 30 мин после введения изотопа. Передний мозг животных одной группы объединили в один пул и гомогенизировали с помощью гомогенизатора Поттера в 40 объемах трис-HCl-буферного раствора (50 мМ pH 7,4) при 20 °C. Специфическое связывание флунитразепама определяли при добавлении 10 мкМ немеченого флунитразепама к гомогенатам мозга. Разница между показателями радиоактивности проб без лиганда и с немеченым лигандом характеризовала специфическое связывание флунитразепама. Инкубацию проводили при 0 °C в течение 60 мин. После инкубации пробы фильтровали через фильтры ГФ/Б (фирма «Whatman», Англия), которые затем промывали дважды 5 мл буфера. Радиоактивность фильтров определяли в сцинтилляторе Брея на счетчике β-частиц ЛС-7500 (фирмы «Beckman», США). Опыты по связыванию ^{3}H-флунитразепама in vitro в переднем мозге мышей проводили по описанной ранее методике [1].

Результаты исследования. Предварительное подкожное введение относительно высоких доз церулеина (более 100 мкг/кг) замедляло развитие пикротоксиновых судорог (см. таблицу), при этом удлинялись латентные периоды клонических и тонических судорог, а также увеличивалась продолжительность жизни мышей. Церулеин в дозе 250 мкг/кг оказывал наиболее выраженное

Влияние церулеина и проглумида на пикротоксиновые судороги и связывание ^{3}H-флунитразепама в опытах in vivo ($M \pm m$, $n = 3$)

Препараты	Доза	Специфическое связывание ^{3}H-флунитразепама в переднем мозге, имп. на 1 г ткани	Латентные периоды пикротоксиновых судорог		
			клонических, с	тонических, мин	продолжительность жизни мышей, мин
Физиологический раствор	—	14 970±829	417±23	13,4±1,4	13,8±1,4
Церулеин	20	14 840±850	428±36	13,5±1,8	14,0±1,7
	50	14 020±790	486±42	15,6±1,5	16,2±1,8
	100	12 200±680	593±41*	19,3±2,3*	21,3±2,5*
	250	6 145±420***	674±58**	19,8±1,5**	21,6±2,0*
	500	5 720±380***	573±62*	20,4±2,7*	21,0±2,8*
Проглумид	5	15 030±790	432±32	13,6±1,5	14,2±1,5
	25	15 840±760	406±25	12,8±1,7	13,1±1,8
Проглумид + церулеин	5+100	10 820±860**	644±48**	20,8±1,5**	23,4±1,9**
Проглумид + церулеин	25+100	15 620±670	504±36	17,4±1,9	18,6±2,0
Проглумид + церулеин	25+250	11 640±870*	—	—	—

Примечание. Одна звездочка — $P < 0,05$, две — $P < 0,01$, три — $P < 0,001$.



Влияние церулеина (5—1000 нМ) на связывание ^{3}H-флунитразепама (1 нМ) в опытах in vitro.

По оси абсцисс — концентрация церулеина (в нМ), по оси ординат — процент угнетения церулеином связывания ^{3}H-флунитразепама. За 100 % принято ингибирующее действие 1 мкМ немеченого флунитразепама. *1* — действие церулеина в среде 50 мМ трис-HCl. *2* — в среде 5 мМ KCl и 120 мМ NaCl в 50 мМ трис-HCl. Одна звездочка — $P<0,05$, две — $P<0,01$, три $P<0,001$, четыре — $P<0,0001$ (по критерию *t* Стьюдента). Представлены данные 3 независимых опытов.

влияние на пикротоксиновые судороги, дальнейшее увеличение дозы церулеина не приводило к усилению его противосудорожного действия. В дозах, угнетающих пикротоксиновые судороги, церулеин достоверно ингибировал связывание ^{3}H-флунитразепама в опытах in vivo. В дозах 250 и 500 мкг/кг церулеин вызывал более чем 50 % уменьшение специфического связывания флунитразепама в переднем мозге. Антагонист ХЦК-8 проглумид в исследованных дозах существенно не изменял пикротоксиновых судорог и лишь в дозе 25 мкг/кг незначительно повышал специфическое связывание ^{3}H-флунитразепама (см. таблицу). В дозе 5 мг/кг проглумид потенцировал угнетающее влияние церулеина как на пикротоксиновые судороги, так и на специфическое связывание флунитразепама. В дозе 25 мг/кг проглумид оказал противоположное действие: уменьшал антипикротоксиновый эффект 100 мкг/кг церулеина и устранял ингибирующее влияние церулеина (250 мкг/кг) на специфическое связывание флунитразепама. В опытах in vitro церулеин (5—1000 нМ) противодействовал связыванию ^{3}H-флунитразепама только в присутствии 5 мМ KCl и 120 мМ NaCl (см. рисунок). В этих условиях параллельно повышению концентрации церулеина наблюдалось его ингибирующее влияние на связывание меченого флунитразепама.

Результаты настоящего исследования свидетельствуют о модулирующем влиянии церулеина на бензодиазепиновые рецепторы. Начиная с дозы 100 мкг/кг церулеин угнетал как пикротоксиновые судороги, так и специфическое связывание ^{3}H-флунитразепама в переднем мозге мышей в опытах in vivo. Эти данные показывают, что ингибирующее влияние церулеина на пикротоксиновые судороги и на связывание ^{3}H-флунитразепама реализуются через одни и те же механизмы. О вовлеченности холецистокининовых рецепторов в эти эффекты церулеина свидетельствует усиление последних под влиянием низкой дозы (5 мг/кг) проглумида, а также их ослабление после введения более высокой дозы (25 мкг/кг) проглумида. Известно, что низкие дозы церулеина (27 мкг/кг) отчетливо удлиняют гексобарбиталовый сон [8]. По существующим представлениям, действие пикротоксина и барбитуратов на поведение животных реализуется через их непосредственное влияние на хлорный канал [2, 5]. В опытах in vitro церулеин ингибировал связывание ^{3}H-флунитразепама только в присутствии существенных концентраций аниона хлора, что свидетельствует также в пользу взаимодействия церулеина с ионофором хлора. На основании этого можно полагать, что именно через ионофор хлора реализуется противосудорожное действие ХЦК-8 и церулеина, а также модулирующее влияние церулеина на связывание ^{3}H-флунитразепама.

ЛИТЕРАТУРА

1. *Васар Э. Э., Рэго Л. К., Алликметс Л. Х.* — Журн. высш. нервн. деят., 1983, т. 33, с. 864.
2. *Braestrup C., Nielsen M.* — In: Handbook of Psychopharmacology. New York, 1983, vol. 17, p. 285.
3. *Hahne W. F., Janssn R. T., Lemp G. F., Gardner J. D.* — Proc. nat. Acad. Sci. USA, 1981, vol. 78, p. 6304.
4. *Kadar T., Pesti A., Penke B. Telegdy G.* — Neuropharmacology, 1984, vol. 23, p. 955.
5. *Polc P., Bonetti E. P., Schaffner R., Haefely W.* — Naunyn-Schmiedeberg's Arch. Pharmakol., 1982, Bd 321, S. 260.
6. *Zetler G.* — Neuropharmacology, 1981, vol. 20, p. 277.
7. *Zetler G.* — Neurosci. Lett., 1982, vol. 25, p. 287.
8. *Zetler G.* — Psychopharmacol. Bull., 1983, vol. 19, p. 347.

Поступила 21.03.85

MODULATORY EFFECT OF CAERULEIN ON BENZODIAZEPINE RECEPTORS

E. E. Vasar, L. K. Rägo, A. Kh. Soosaar, A. N. Nurk, M. O. Maimets

Institute of General and Molecular Pathology, Tartu State University, Tartu

Subcutaneous administration of caerulein (100—500 μg/kg) significantly reduced the development of picrotoxin (8 mg/kg) seizures in male mice. The same doses of caerulein inhibited ^{3}H-flunitrazepam binding in *in vivo* experiments. Proglumide, an antagonist of cholecystokinin receptors, in low dose (5 mg/kg) potentiated the effects of caerulein (100 μg/kg), whereas the administration of proglumide in high dose (25 mg/kg) reduced the action of caerulein on ^{3}H-flunitrazepam binding and picrotoxin seizures. Caerulein (5-1000 nM) decreased ^{3}H-flunitrazepam binding in *in vitro* experiments only after supplementation of the binding medium with 120 mM NaCl and 5mM KCl. The results suggest the possible interaction of caerulein with chloride ionophor. It seems probable that the direct interaction of caerulein with chloride ionophor in involved in the inhibitory effect of caerulein on picrotoxin seizures and ^{3}H-flunitrazepam binding.

Comparison of Motor Depressant Effects of Caerulein and N-Propylnorapomorphine in Mice

EERO VASAR, MATTI MAIMETS, ANTS NURK, ANDRES SOOSAAR AND LEMBIT ALLIKMETS

Laboratory of Psychopharmacology, Institute of General and Molecular Pathology, Tartu State University, 34 Burdenko Street, 202 400 Tartu, Estonia, U.S.S.R.

Received 18 April 1985

VASAR, E., M. MAIMETS, A. NURK, A. SOOSAAR AND L. ALLIKMETS. *Comparison of motor depressant effects of caerulein and N-propylnorapomorphine in mice.* PHARMACOL BIOCHEM BEHAV 24(3) 469–478, 1986.—The motor depressant effects of caerulein and N-propylnorapomorphine (NPA) were compared in male mice. Caerulein (1–50 μg/kg SC) in a dose dependent manner depressed the exploratory activity, whereas NPA in lower doses (0.5–10 μg/kg SC) decreased the motor activity, but in higher doses (over 50 μg/kg) had stimulating effect on the exploratory behavior. In mice selected according to their motor response after administration of 100 μg/kg NPA to weak and strong responders, the low dose of NPA (1 μg/kg) similarly suppressed motor activity in both selected groups, while the effect of caerulein (2 μg/kg) was apparently higher in weak responders. Destruction of catecholaminergic terminals by 6-hydroxydopamine (60 μg ICV) reversed completely the motor depressant effect of NPA, whereas degeneration of serotoninergic terminals (5,7-dihydroxytryptamine 60 μg ICV or p-chloroamphetamine 2×15 mg/kg IP) enhanced the sedative effect of NPA. The motor depressant effect of caerulein remained unchanged after lesions of monoaminergic terminals in forebrain. Subchronic haloperidol (0.25 mg/kg IP, twice daily during 14 days) treatment, reducing significantly the density of high-affinity dopamine$_2$- and serotonin$_2$-receptors, decreased the motor depressant action of caerulein. It is possible that motor depressant effect of caerulein, differently from the action of NPA, is mediated through the high-affinity dopamine$_2$-receptors and in lesser extent through the high-affinity serotonin$_2$-receptors.

Exploratory activity Caerulein N-propylnorapomorphine Dopamine$_2$-receptors Serotonin$_2$-receptors

THE suppression of spontaneous locomotor activity by low doses of apomorphine in rodents is a widely studied behavioral phenomenon. It is generally accepted that the sedative action of apomorphine and its more powerful analog N-propylnorapomorphine (NPA) is mediated through the stimulation of dopamine "autoreceptors," inhibiting the dopaminergic neurons activity [9, 10, 38, 45]. This opinion is supported by various investigations. The subcutaneous administration of apomorphine in low doses inhibited the firing rate of dopaminergic neurons in mesencephalon [2], decreased dopamine release and suppressed dopamine turnover in forebrain structures [32,44]. Lesion of dopaminergic terminals by 6-hydroxydopamine and administration of different neuroleptic drugs in low doses reversed the inhibiting action of apomorphine on behavior and dopaminergic neurons activity [3, 42, 46]. However, some recent investigations demonstrated a more complicated nature of apomorphine's action in low and moderate doses. It was found [16] that haloperidol and sulpiride reversed the sedative effect of moderate dose (150 μg/kg) of apomorphine, whereas the action of low dose (25 μg/kg) of apomorphine was resistant to the antagonizing action of neuroleptic drugs. The complicated nature of apomorphine's action in low doses was described also in chronic schizophrenic patients, evidently resistant to neuroleptic medication. The reduction of schizophrenic symptomatology was demonstrated in approximately 50% of patients, suffering mainly from paranoid schizophrenia [47,48]. It was quite surprising that apomorphine possessed its beneficial activity when coadministered with neuroleptic drugs, but not alone [1, 21, 37].

Obviously similar suppression of animals' spontaneous behavior was found after systemic administration of cholecystokinin octapeptide (CCK-8) and caerulein in mice [56, 57, 58]. CCK-8 and caerulein significantly potentiated apomorphine-induced inhibition of dopaminergic neurons in mesencephalon [30]. There is strict evidence that CCK and dopamine coexist in some mesencephalic cells innervating forebrain limbic and cortical regions [31]. In addition, CCK has been reported to decrease dopamine turnover in the discrete regions of caudate-putamen [24]. However, CCK also decreased serotonin turnover [51], whereas apomorphine had the opposite effect on serotonin metabolism [26]. Recently the rapid and long-lasting reduction of psychotic symptoms, mainly negative, in schizophrenic patients after administration of different CCK-related peptides was demonstrated [7, 39, 40].

The main task of the present investigation was to compare the mechanisms of inhibiting action of apomorphine and CCK on the animals' behavior. The attention was drawn to the study of interaction of caerulein and NPA with dopamine- and

serotoninergic mechanisms. Caerulein and NPA were selected for the present investigation as the most effective compounds among, respectively, CCK-8 and apomorphine analogs [8, 55, 56].

GENERAL METHOD

Animals

Male albino mice weighing 25±3 g were used. Mice were maintained at 20±2°C and on 12 hr light, between 8 a.m. and 8 p.m., with food and water allowed ad lib.

Measurement of Spontaneous Locomotor Activity

Spontaneous locomotor activity was measured in grouped albino mice, 10 animals in each group, between 10 a.m. and 4 p.m. Immediately after systemic administration of drugs a group of mice was placed in the middle of an open-field cage. The open-field consisted of a 1×1 m area surrounded by a 40 cm high wall. The locomotor activity of animals was counted by 5 independent photocells located in walls. Interruptions of the light beams were recorded electromechanically and the level of locomotor activity was expressed in counts per 15 or 30 min period. The experiment was repeated with each drug combination at least three times on different days and the data analyzed using Student's *t*-test.

Selection of Mice According to Their Motor Response to Administration of NPA

There exists the possibility of selecting rats according to their motor response after 50 μg/kg NPA treatment [15]. A similar attempt was made for selection of mice. In the present study the selection was performed with subcutaneous administration of 100 μg/kg NPA in 400 male mice. The experiment was carried out in individual cages. The cage for measuring individual locomotor activity was a cylinder with an inner diameter 40 cm and 2 photocells for detection of locomotor activity. Locomotor activity was counted between 15 and 30 min after subcutaneous NPA (100 μg/kg) treatment.

Lesions of Brain Monoaminergic Terminals

Monoaminergic neurotoxins 6-hydroxydopamine (6-OHDA) and 5,7-dihydroxytryptamine (5,7-DHT) were dissolved in 0.1% solution of ascorbic acid. 6-OHDA (60 μg in 5 μl) and 5,7-DHT (60 μg in 5 μl) were injected into the right lateral ventricle of mice under the ether anesthesia. The behavioral and binding experiments were carried out 8 days after the injection of neurotoxins. Finally, the injection sites were confirmed histologically to be located within the right lateral ventricle. p-Chloroamphetamine in neurotoxic dose (2×15 mg/kg 8 and 7 days before the experiment) was also used for lesioning of serotoninergic terminals [5]. The effect of neurotoxins on the content of monamines and their major metabolites in brain structures was assessed biochemically using fluorimetric assay [20].

In Vivo ^{3}H-Spiperone Binding

^{3}H-spiperone (5 μg/kg, 17 Ci/mmole, Amersham International, U.K.) was injected subcutaneously into the dorsal part of mouse neck. NPA (5 and 50 μg/kg) and caerulein (20–250 μg/kg) were used to inhibit ^{3}H-spiperone binding. Two doses of NPA with different action on rodent behavior were selected because two sites with different affinity for dopamine and its agonists existed on dopamine$_2$-receptors [18,27]. Five μg/kg NPA is ED_{50} for suppression of exploratory activity in mice, whereas 50 μg/kg NPA is ED_{50} for motor excitation in rodents [8]. NPA and caerulein were administered 15 min before ^{3}H-spiperone. The animals (6 mice per group) were sacrificed 20 min after ^{3}H-spiperone treatment by cervical dislocation. The brains were rapidly

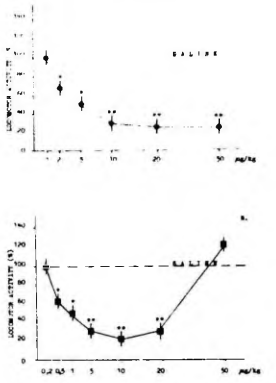


FIG. 1. The effect of different doses of N-propylnorapomorphine and caerulein on exploratory behavior in mice. Each point in the figure represents mean value of three independent studies in grouped mice (10 animals in group). Abscissa—the dose of NPA or caerulein in μg/kg. Caerulein—A, NPA—B. The mean value for saline treated group was 1182±170 counts during 30 min. Statistically evident differences from saline treated mice: *$p<0.05$; **$p<0.01$ (Student's *t*-test).

TABLE 1

THE ACTION OF CONCOMITANT ADMINISTRATION OF CAERULEIN AND NPA ON MICE SPONTANEOUS LOCOMOTOR ACTIVITY

Drug dose	Spontaneous locomotor activity of mice: Counts during 15 min	%	Counts during 30 min	%
Saline	608 ± 58	100	1230 ± 162	100
NPA 0.5 μg/kg	380 ± 42*	63	780 ± 68*	63
Caerulein 2 μg/kg	352 ± 38*	58	746 ± 65*	61
NPA + Caerulein	170 ± 16†	28	276 ± 24†	22
NPA 10 μg/kg	158 ± 12†	26	240 ± 32†	20
Caerulein 1 μg/kg	560 ± 57	92	1080 ± 182	88
NPA + Caerulein	33 ± 4‡	5	61 ± 8‡	5

The mean values of four independent experiments on grouped mice (10 animals in group) are presented. *$p<0.05$; †$p<0.01$; ‡$p<0.001$ (Student's paired *t*-test, in relation to saline treated animals).

TABLE 2

THE EFFECT OF CAERULEIN AND NPA ON EXPLORATORY ACTIVITY AND ^{3}H-SPIPERONE IN VIVO BINDING IN MICE SELECTED WITH 100 μg/kg NPA

	Inhibition of locomotor activity to 100 μg/kg NPA			
	Weak Responders		Strong Responders	
	Motor activity counts during 30 min			
Drug/dose		%		%
Saline	1168 ± 98	100	1224 ± 115	100
NPA 1 μg/kg	550 ± 58	47	630 ± 52	52
Caerulein 2 μg/kg	292 ± 34*	25	690 ± 68	56

	Inhibition of ^{3}H-spiperone binding to 100 μg/kg NPA			
	Weak Responders		Strong Responders	
	cpm per gram tissue			
	Subcortex	Dorsal cortex	Subcortex	Dorsal cortex
NPA 5 μg/kg	+1600 ± 280†	+750 ± 200†	9900 ± 1020	10950 ± 1200
NPA (50–5) μg/kg	5180 ± 380*	3750 ± 280*	10200 ± 980	6900 ± 520
Caerulein 100 μg/kg	+1800 ± 360†	+1200 ± 300†	11840 ± 930	11150 ± 1060

The experiments were carried out 10–12 days after mice selection.

The mean values of three independent experiments are advanced in table. +—Stimulation of ^{3}H-spiperone binding. *p<0.05; †p<0.01 (Student's paired t-test, compared to strong responding mice).

removed and dorsal cortex and subcortical forebrain structures (striata and limbic structures) were dissected on ice. The dissected brain areas of each group were pooled and homogenized using a glass-teflon homogenizer by hand during 1 min. The homogenization procedure was performed in ice-cold Tris-HCl buffer (50 mM, pH 7.4, 20°C) in the volume of 40 mg tissue per ml. After homogenization 0.5 ml (20 mg tissue) of suspension was pippeted into 6 polypropylene tubes (1.5 ml) and centrifuged during 10 min at 9000×g. The supernatant was carefully discarded and remaining pellet was washed and cut into vials. Radioactivity of samples was counted after stabilization in Bray scintillation cocktail within 12 hours in Beckman LS 6800 with counting efficacy 43%. The binding experiments were repeated at least three times and the data analyzed using Student's t-test.

Drugs

Drugs used in the present investigation were caerulein (Ceruletide, Farmitalia Carlo Erba, Italy), haloperidol (Gedeon Richter, Hungary), N-propylnorapomorphine (Sterling-Winthrop, USA), p-chloroamphetamine, 6-hydroxydopamine, 5,7-dihydroxytryptamine (Sigma, USA). Caerulein, commercial solution of haloperidol and p-chloroamphetamine were dissolved in saline. The injection solution of NPA was prepared in 0.001 N HCl. Each injection was done in a volume of 0.1 ml/10 g body weight.

EXPERIMENT 1: THE INVOLVEMENT OF DOPAMINERGIC MECHANISMS IN THE MOTOR DEPRESSANT ACTION OF CAERULEIN AND N-PROPYLNORAPOMORPHINE

The aim of experiment 1 was to study the role of dopaminergic mechanisms in the sedative effects of caerulein and NPA. The problems under examination were: (1) the action of different doses of caerulein and NPA on exploratory activity in mice; (2) the effect of concomitant use of caerulein and NPA on locomotor activity in mice; (3) the action of caerulein and NPA on exploratory activity and ^{3}H-

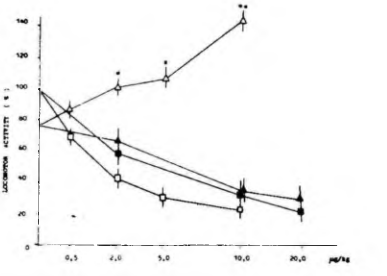


FIG. 2. The changes in motor depressant effect of caerulein and N-propylnorapomorphine after intraventricular administration of 6-hydroxydopamine. White signs—the action of NPA; black signs—caerulein. Triangles—after administration of 6-OHDA; squares—after intraventricular injection of 0.1% ascorbic acid. Abscissa—the dose of NPA or caerulein in μg/kg. The mean value for saline medicated mouse was 1098±156 counts during 30 min in the case of 0.1% ascorbic acid and 780±78 in case of 6-OHDA. Statistically evident differences from ascorbic acid pretreated group: *p<0.05; **p<0.01 (Student's t-test).

TABLE 3

THE CHANGES IN ^{3}H-SPIPERONE BINDING AFTER INTRACEREBROVENTRICULAR ADMINISTRATION OF 6-HYDROXYDOPAMINE AND LONG-TERM ADMINISTRATION OF HALOPERIDOL AND P-CHLOROAMPHETAMINE

	Inhibition of ^{3}H-spiperone binding cpm per gram tissue					
	NPA 5 μg/kg		NPA (50–5) μg/kg		Caerulein 50 μg/kg	
Drug/dose	Subcortex	Dorsal cortex	Subcortex	Dorsal cortex	Subcortex	Dorsal cortex
Saline	7800 ± 580	6950 ± 620	5200 ± 640	4000 ± 480	5250 ± 420	4750 ± 390
6-OHDA 60 μg	14400 ± 930†	11800 ± 1060*	1020 ± 200†	2040 ± 240*	3000 ± 470*	2600 ± 320
PCA 2×15 mg/kg	4240 ± 560*	3320 ± 310†	5800 ± 670	3900 ± 350	3500 ± 430	3600 ± 410
Haloperidol 0.25 mg/kg	1100 ± 120†	2150 ± 380*	10400 ± 980†	6700 ± 530*	+3600 ± 320‡	+400 ± 120†

The binding of ^{3}H-spiperone after intraventricular administration of 0.1% ascorbic acid did not differ from the binding after long-term saline treatment. +—Stimulation of ^{3}H-spiperone binding. *$p<0.05$; †$p<0.01$; ‡$p<0.001$, compared to saline treated mice (Student's t-test).

spiperone binding parameters in pharmacologically selected mice. The animals were selected according to their motor response after administration of 100 μg/kg NPA into two groups—weak and strong responders. The uneven motor reaction after NPA administration reflected the different density of postsynaptic dopamine$_2$-receptors in rodents [14, 15, 29]; (4) the effects of caerulein and NPA on locomotor activity in mice and ^{3}H-spiperone binding parameters after destruction of presynaptic dopaminergic terminals by 6-hydroxydopamine.

METHOD

The group of mice was placed into the center of an open-field cage immediately after subcutaneous injection of caerulein (1–50 μg/kg) or NPA (0.2–50 μg/kg). After selection of appropriate doses, giving marked suppression of spontaneous locomotor activity, the effect of concomitant use of caerulein and NPA was studied. The action of NPA (1 μg/kg) and caerulein (2 μg/kg) was also examined in mice selected according to their motor response to the administration of NPA in a high dose (100 μg/kg). The groups of weak and strong responders to 100 μg/kg NPA were selected among 400 mice. The motor activity was assessed in individual cages from 15 to 30 min after 100 μg/kg NPA injection. The mean value of motor activity for the first group (weak responders) was 36±3.8 counts during 15 min and 216±15.2 for the second (strong responders). The response of these two groups to saline administration did not differ markedly. It was 1168±98 counts during 30 min for weak responders and 1224±115 counts for strong responders. Simultaneously with behavioral investigations ^{3}H-spiperone in vivo binding studies were performed. NPA (5 and 50 μg/kg) and caerulein (100 μg/kg) were used as displacing drugs. Two doses of NPA were administered to demonstrate two distinct binding sites for NPA on dopamine$_2$- and serotonin$_2$-receptors. Inhibition of ^{3}H-spiperone binding by 5 μg/kg NPA expressed the amount of high-affinity sites for NPA, whereas the difference between the inhibiting action of 50 and 5 μg/kg NPA demonstrated the number of low-affinity sites. Catecholaminergic neurotoxin 6-OHDA (60 μg) was injected into the right lateral cerebral ventricle in a volume of 5 μl during 3 min under ether anesthesia. Seven days were allowed for recovery from intraventricular intervention. After completion of behavioral experiments the site of microinjection was detected histologically.

RESULTS

Effect of Caerulein and NPA on Exploratory Motor Activity

Caerulein in a dose dependent manner depressed the exploratory activity in male mice (Fig. 1A). Two μg/kg caerulein caused the minimal significant reduction of motor activity and 20–50 μg/kg the maximal effect. Low doses of NPA also reduced the animals' spontaneous locomotor activity. 0.5 μg/kg NPA caused remarkable and 10 μg/kg NPA induced the maximal reduction of mice exploratory behavior (Fig. 1B). The further elevation of NPA dose did not enhance the sedative action, but on the contrary 50 μg/kg NPA had a mild stimulating effect on motor activity of mice. After coadministration of NPA and caerulein the reduction of motor activity was obviously higher compared to the treatment of both drugs alone (Table 1). One μg/kg caerulein, which did not significantly affect the mice behavior, potentiated the motor depressant effect of NPA (10 μg/kg). This combination of drugs caused nearly complete suppression of locomotor activity. In mice, selected according to their motor response after administration of 100 μg/kg NPA, 1 μg/kg NPA in a similar manner suppressed exploratory activity in strong as well as in weak responders (Table 2). However, the sedative effect of caerulein (2 μg/kg) was dependent on the mice sensitivity to 100 μg/kg NPA. In strong responders the sedative effect of caerulein was lower. Significant differences were found also in ^{3}H-spiperone binding performed in "in vivo" conditions (Table 2). In weak responders caerulein (100 μg/kg) stimulated ^{3}H-spiperone binding in both brain regions studied, whereas in strong responders it had the opposite effect, inhibiting ^{3}H-spiperone binding (Table 2). Five μg/kg NPA also increased ^{3}H-spiperone binding in weak responders, while the displacing potency of 50 μg/kg NPA in weak responders was lower than the effect of 5 μg/kg NPA in strong responders.

Effect of 6-OHDA on Locomotor Effects of Caerulein and NPA, and ^{3}H-Spiperone Binding

Intraventricular administration of 6-OHDA (60 μg) induced more than 60% reduction of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) levels in striatal slices (dorsal cortex, striata and mesolimbic structures) of mice brain without changing markedly serotonin levels. Simultaneously the reduction of spontaneous locomotor activity was seen in mice after 6-OHDA treatment (Fig.

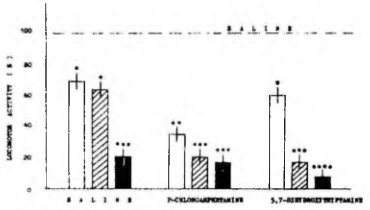


FIG. 3. The influence of p-chloroamphetamine and 5,7-dihydroxytryptamine pretreatment on motor depressant effect of caerulein and N-propylnorapomorphine. White bars—caerulein 2 μg/kg, striped bars—NPA 0.5 μg/kg and black bars—caerulein+NPA. The mean value of motor activity for saline treated group was 1180±122 in case of long-term saline administration, 1020±140 in case of 5,7-DHT and 1270±178 counts during 30 min in case of PCA. *$p<0.05$; **$p<0.02$; ***$p<0.01$; ****$p<0.001$, compared to saline pretreatment (Student's t-test).

TABLE 4

THE EFFECT OF HALOPERIDOL AND CAERULEIN ON MICE EXPLORATORY ACTIVITY AFTER 14 DAYS HALOPERIDOL TREATMENT

	Saline		Haloperidol	
	Motor activity counts during 30 min			
Drug/dose		%		%
Saline	1180 ± 188	100	1054 ± 143	100
Caerulein 2 μg/kg	680 ± 78*	58	920 ± 89	90
Haloperidol 50 μg/kg	880 ± 96	75	1280 ± 160	122
Caerulein + Haloperidol	620 ± 64*	53	520 ± 56†	49

The investigation was performed 72 hours after cessation of haloperidol or saline treatment. The mean values of three independent studies are advanced. *$p<0.05$; †$p<0.01$, compared to saline treated animals (Student's t-test).

2). NPA completely lost its sedative action and stimulated the mice exploratory activity after administration of 6-OHDA, while the action of caerulein remained unchanged (Fig. 2). In binding experiments 6-OHDA caused a significant increase in displacing action of 5 μg/kg NPA, but reduced the potency of 50 μg/kg (Table 3). The inhibiting action of caerulein (50 μg/kg) on ^{3}H-spiperone binding was also somewhat lower after 6-OHDA treatment. Administration of 6-OHDA altered ^{3}H-spiperone binding more relevantly in subcortical structures than in dorsal cortex.

DISCUSSION

Caerulein and NPA in low doses caused similar suppression of exploratory activity of mice. Coadministration of NPA and caerulein evidently potentiated their depressive action on behavior. There is clear evidence for coexistence of dopamine and CCK-8 in the same mesencephalic dopaminergic neurons [31]. It was demonstrated that CCK-8 and caerulein potentiated apomorphine-induced inhibition of dopaminergic neurons in mesencephalon [30]. Lesion of presynaptic dopaminergic terminals by 6-OHDA completely reversed the motor depressant action of NPA, demonstrating the prevalent role of presynaptic mechanisms in the action of NPA. The motor depressant effect of caerulein was resistant to the administration of 6-OHDA. The different action of caerulein in selected mice according to their response to 100 μg/kg NPA revealed that the sedative effect of caerulein was more probably related to postsynaptic dopamine receptors. The sedative effect of caerulein was higher in weak NPA responders, which evidently had lower density of dopamine$_2$- and serotonin$_1$-receptors in forebrain structures. It was established that dopamine$_2$-receptors had one high-affinity site for neuroleptic drugs, but two sites—low- and high-affinity—for dopamine, apomorphine and NPA [18,27]. In weak responders caerulein and 5 μg/kg NPA stimulated ^{3}H-spiperone binding, but inhibited it in strong responders. NPA had similar sedative action in both groups of selected mice, revealing that dopamine "autoreceptors" were not related to high-affinity dopamine$_2$-receptors [27]. Costall [14] has found that in strong responding rats to NPA (50 μg/kg) the content of dopamine (in nucleus accumbens) was approximately twice higher than in weak responders. It appears that displacing potency of caerulein against ^{3}H-spiperone binding is dependent on dopamine content in brain structures and caerulein only modulates the interaction of endogenous dopamine with dopamine$_2$-receptors. It is quite possible that these differences in the action of caerulein on ^{3}H-spiperone binding in two selected groups of mice are linked to the different sedative effects of caerulein in these animals.

In conclusion, experiment 1 evidences that the sedative effect of caerulein is related, differently from NPA action, to postsynaptic dopamine receptors. Caerulein seems to act as a functional antagonist of behavior stimulating effect of dopamine.

EXPERIMENT 2: THE EFFECT OF SEROTONINERGIC LESIONS AND LONG-TERM HALOPERIDOL TREATMENT ON MOTOR DEPRESSANT AND ^{3}H-SPIPERONE BINDING INHIBITING EFFECTS OF CAERULEIN AND N-PROPYLNORAPOMORPHINE

Experiment 1 suggested differences in the mechanism of sedative action of caerulein and NPA. The aim of experiment 2 was to study further the mechanisms of action of caerulein and NPA using serotoninergic lesions and long-term administration of haloperidol.

METHOD

Serotoninergic neurotoxin 5,7-DHT (60 μg) was injected into the right lateral ventricle in a volume of 5 μl during 3 min under ether anesthesia. Seven days were allowed for recovery from intraventricular intervention. After completion of behavioral experiments the site of microinjection was detected histologically. According to some authors [5, 28, 35], administration of p-chloroamphetamine (PCA) in high doses causes degeneration of serotoninergic terminals in forebrain structures. PCA was injected twice in a dose of 15 mg/kg, 8 and 7 days before the behavioral and binding experiments. The action of NPA and caerulein was also studied after 14 days

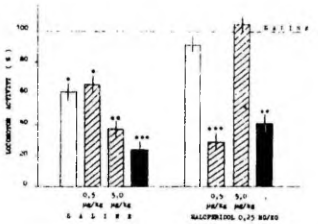


FIG. 4. The changes in motor inhibiting action of N-propylnorapomorphine and caerulein after cessation of 14 days haloperidol medication. White bars—caerulein 2 μg/kg, striped—NPA 0.5 and 5.0 μg/kg, black—the combination of caerulein and 0.5 μg/kg NPA. The mean value of motor activity for saline treated group was 1180±147 counts during 30 min. *$p<0.05$; **$p<0.02$; ***$p<0.01$ (Student's t-test).

administration of haloperidol (0.25 mg/kg, twice daily), increasing the sensitivity of pre- and postsynaptic dopamine receptors [11,53]. Seventy-two hours after cessation of two weeks haloperidol treatment the behavioral experiment with appropriate doses of caerulein, NPA and haloperidol was performed. Simultaneously with the behavioral experiment the in vivo ^{3}H-spiperone binding studies were carried out after long-term administration of PCA and haloperidol. After lesioning of serotoninergic terminals of brain by PCA and 5,7-DHT the spectrofluorimetric method was used for detection of dopamine, serotonin and their major metabolites [20].

RESULTS

Effect of PCA and 5,7-DHT on Locomotor Effect of Caerulein and NPA

The pretreatment with PCA and 5,7-DHT decreased obviously (50–60%) the levels of serotonin and its major metabolite 5-hydroxyindoleacetic acid in striatal slices, without changing dopamine concentrations. The administration of both serotoninergic neurotoxins evidently potentiated the motor inhibiting effect of NPA. The action of simultaneous administration of NPA and caerulein was also augmented, whereas the sedative effect of caerulein in grouped mice was somewhat enhanced only after administration of PCA (Fig. 3). The pretreatment with PCA (2×15 mg/kg) inhibited the displacing potency of 5 μg/kg NPA and 50 μg/kg caerulein (Table 3), while the part of ^{3}H-spiperone binding displacable only by 50 μg/kg NPA remained unchanged.

Effect of NPA and Caerulein on Locomotor Activity and ^{3}H-Spiperone Binding After Long-Term Haloperidol Treatment

The mild sedative effect of 50 μg/kg haloperidol was reversed to stimulation of exploratory activity after cessation of long-term haloperidol (0.25 mg/kg twice daily during two weeks) treatment (Table 4). Tolerance developed also to the motor depressant action of 2 μg/kg caerulein. In saline pretreated mice the sedative action of simultaneous treatment of

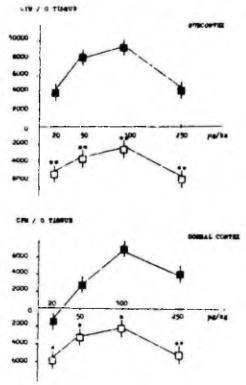


FIG. 5. The action of caerulein on ^{3}H-spiperone binding after cessation of 14 days haloperidol treatment. Black squares—the action of caerulein after saline pretreatment, white squares—after two weeks haloperidol administration. Abscissa: the dose of caerulein in μg/kg, ordinate: radioactivity counts per gram tissue. −—inhibition, and +—stimulation of ^{3}H-spiperone binding. *$p<0.05$; **$p<0.01$ vs. saline pretreated animals (Student's t-test).

haloperidol and caerulein did not differ from the action of caerulein alone. However, after withdrawal of long-term administration of haloperidol the concomitant treatment of caerulein and haloperidol completely reversed the tolerance to the action of both drugs (Table 4). The changes in motor depressant action of NPA were dependent on the dose of NPA. 0.5 μg/kg NPA had more pronounced inhibiting effect after two weeks haloperidol medication (Fig. 4), while the action of 5 μg/kg NPA was significantly reduced. Two weeks haloperidol treatment also reduced the interaction between NPA and caerulein (Fig. 4). Some animals became hyperexcitable after simultaneous administration of NPA and caerulein to haloperidol pretreated mice. The diminution of 5 μg/kg NPA inhibiting action on ^{3}H-spiperone binding was seen after 14 days haloperidol medication (Table 3), whereas the action of 50 μg/kg NPA was evidently increased. The inhibiting action of 50 μg/kg caerulein was turned to stimulation of ^{3}H-spiperone binding after cessation of long-term neuroleptic treatment (Table 3). More detailed analysis of caerulein inhibiting action revealed (Fig. 5) the more pronounced effect of caerulein on ^{3}H-spiperone binding in subcortical structures, with maximal inhibition after administration of 100 μg/kg caerulein. After cessation of two weeks haloperidol treatment the inhibition curve of caerulein was shifted to stimulation of ^{3}H-spiperone binding (Fig. 5).

DISCUSSION

Experiment 2 evidently supports our opinion that the sedative effects of caerulein and NPA are mediated through dissimilar mechanisms. Lesions of serotoninergic terminals

by PCA and 5,7-DHT demonstrate the involvement of serotoninergic mechanisms in the inhibitory action of NPA. This opinion was supported by our previous investigation [52], where the potentiation of apomorphine sedative effect by low dose of pirenperone, a selective antagonist of serotonin$_2$-receptors, was shown. The sedative effect of caerulein was influenced only by pretreatment with PCA, but not by microinjection of 5,7-DHT. The possible explanation for these differences may be the dissimilar action of 5,7-DHT and PCA on postsynaptic serotonin$_2$-receptors sensitivity. It was found that 5,7-DHT caused behavioral hypersensitivity on serotonin receptors [6, 49, 50], while PCA induced subsensitivity to serotonin agonists [5]. These findings may support the involvement of postsynaptic serotonin$_2$-receptors in the action of caerulein, but to a lesser extent than dopamine$_2$-receptors. This opinion is in agreement with binding studies where higher doses of caerulein were needed for inhibition of ^{3}H-spiperone binding to serotonin$_2$ receptors in dorsal cortex than to dopamine$_2$-receptors in subcortical structures.

Investigations performed after cessation of two weeks haloperidol treatment support the hypothesis of Protais [42] that the sedative effect of moderate doses of NPA is related to other types of dopamine receptors than the action of low doses. Long-term haloperidol medication induced tolerance to the sedative effect of 5 μg/kg NPA, but increased the action of 0.5 μg/kg NPA. In the binding experiments the reduction of displacing potency of 5 μg/kg NPA after withdrawal of 14 days haloperidol was also seen. It is probable that NPA in moderate doses interacts with postsynaptic dopamine$_2$-receptors having high-affinity for dopamine agonists and not only with so-called dopamine "autoreceptors." Two weeks haloperidol treatment caused tolerance to both effects of caerulein—sedative and inhibition of ^{3}H-spiperone binding. The interaction between NPA and caerulein was also decreased after 14 days neuroleptic administration, while coadministration of haloperidol and caerulein in low doses reversed the tolerance to the sedative effects of both drugs. It is probable that the stimulation of ^{3}H-spiperone binding to dopamine$_2$- and serotonin$_2$-receptors after long-term neuroleptic medication plays a role in the antipsychotic action of neuroleptic drugs. There was described the substantial dose dependent increase of CCK-8 content in subcortical forebrain structures after two weeks administration of different neuroleptic drugs (haloperidol, chlorpromazine, clozapine) [23]. The density of CCK binding sites was also elevated after long-term neuroleptic medication [12].

In conclusion, experiment 2 supports the idea about the involvement of postsynaptic dopamine$_2$-receptors and to a lesser extent serotonin$_2$-receptors in the action of caerulein. It is probable that the action of caerulein on animals behavior and ^{3}H-spiperone binding is related to the functional activity of dopamine$_2$- and serotonin$_2$-receptors, but also to the levels of endogenous neurotransmitters.

GENERAL DISCUSSION

There are two opposite concepts existing about the site of action of CCK-8 and caerulein after systemic administration. The first group of investigators [17,34] has demonstrated the relation of sedative effects of CCK-8 and caerulein to the afferent system of nervus vagus. Vagotomy [34] or lesions of nucleus tractus solitarius [17], the central termination of vagal sensory fibers, abolished the depression of somatic function induced by CCK-8 or caeurlein. However, the pharmacological experiments described by Zetler [56, 57, 58] suggest that CCK-like peptides possess marked effects in animal behavior models known to reliably reflect the efficacy of well-known centrally active drugs such as analgesics, neuroleptics and tranquilizers.

The present investigation reveals that at least partly the central monoaminergic mechanisms are involved in the depressive action of caerulein on mice behavior. This idea is supported by the following findings: (1) Caerulein inhibits in vivo ^{3}H-spiperone binding in the brain, in lower doses to dopamine$_2$-receptors in subcortex and in somewnat higher doses to serotonin$_2$-receptors in dorsal cortex. This finding is in agreement with the in vitro investigations [4] showing that 10 nM CCK-8 significantly modulates ^{3}H-spiperone binding to dopamine$_2$-receptors in striatum and moderatley to serotonin$_2$-receptors in dorsal cortex; (2) The sedative effect of caerulein was in negative correlation with reaction of mice to motor stimulating action of NPA (100 μg/kg) and density of ^{3}H-spiperone binding sites in forebrain structures; (3) Two weeks haloperidol administration induced the tolerance to the motor depressant effect of caerulein and reversed the inhibiting action of caerulein into stimulation of ^{3}H-spiperone binding.

The potentiation of apomorphine-induced inhibition of dopamine neurons by CCK-8 and caerulein was demonstrated in mesencephalon [30]. But, the present investigation indicates the differences in the mechanism of motor depressant action of NPA and caerulein. It appears that NPA releases its inhibiting action of mice behavior through the presynaptic dopamine receptors, while caerulein mainly interacts with postsynaptic dopamine$_2$-receptors. Intraventricular administration of 6-OHDA, destructing presynaptic dopaminergic terminals, shifted the sedative effect of NPA into stimulation of mice exploratory activity, whereas the action of caerulein remained unchanged. In fact, the sedative effect of caerulein was in negative relation with the postsynaptic effect of NPA—to stimulation of locomotor activity. Similar correlation was found between the behavioral effect of caerulein and density of ^{3}H-spiperone binding sites in forebrain. These findings are in agreement with investigations [4,25] showing that CCK-8 more readily interacted with ^{3}H-spiperone than ^{4}H-NPA binding in "in vitro" conditions. There was described [18,27] the existence of two binding sites for dopamine agonists on dopamine$_2$-receptors (high- and low-affinity) and only high-affinity site for neuroleptic drugs. It was found [43] that these two sites for dopamine agonists had different localization in striatum—high-affinity sites were located predominantly on intrinsic neurons and low-affinity sites on corticostriatal fibers. The high-affinity sites were regulated by guanine nucleotides: GTP or its analogs significantly reduced the interaction of dopamine agonists with dopamine$_2$-receptors [27,43]. It seems that caerulein more probably interacts with high-affinity binding sites for dopamine agonists on dopamine$_2$-receptors, antagonizing the stimulating action of dopamine and its analogs on animals' behavior. Caerulein (75 μg/kg and higher doses) effectively reversed the motor stimulating action of dl-amphetamine (5 mg/kg), but did not affect quipazine (5 mg/kg), serotonin$_2$-receptors agonist, head-twitches (our unpublished data) and cage climbing behavior induced by higher doses of apomorphine in mice [57]. The selection of mice according to their response after administration of 100 μg/kg NPA also support the involvement of high-affinity dopamine$_2$-receptors in the action of caerulein. The clearcut

positive correlation between the content of dopamine in nucleus accumbens and the response to motor stimulating effect of NPA was discovered in rats [14]. In strong responders the concentration of dopamine in nucleus accumbens was approximately two times higher compared to weak responders [14]. In the present study, caerulein and 5 μg/kg NPA stimulated ^{3}H-spiperone binding in weak responding mice, while in strong responders both drugs had the opposite effect. It appears that the action of caerulein on ^{3}H-spiperone binding is dependent on the levels of dopamine and affinity of dopamine$_2$-receptors to dopamine. The long-term infusion of dopamine into nucleus accumbens caused the opposite changes in dopamine$_2$-receptors sensitivity in selected rats [15]. In weak responders dopamine demonstrated dopamine receptor antagonist like properties, increasing the sensitivity of dopamine$_2$-receptors, while in strong responders it had the opposite effect, decreasing the affinity of dopamine receptors. It is probable that NPA, similar to dopamine, has dopamine antagonist properties in weak responders in moderate dose (stimulation of ^{3}H-spiperone binding) and in high responders it acts as a receptor agonist (inhibition of ^{3}H-spiperone binding). The mixed agonist-antagonist properties of apomorphine and NPA seem to have the clinical relevance, because apomorphine reduces the psychotic symptomatology only in one subgroup of schizophrenic patients, suffering mainly from the paranoid schizophrenia, receiving neuroleptic medication, but not without neuroleptic drugs [1, 19, 21, 37, 47]. Probably, this action of apomorphine is different from the sedative action of apomorphine, which was antagonized by neuroleptic drugs [13]. It is possible that in these patients apomorphine caused the short-lasting stimulation of neuroleptics binding to dopamine$_2$- and serotonin$_2$-receptors.

The differences in the action of NPA and caerulein also involve the serotoninergic mechanisms. It seems that the inhibiting action of caerulein on mice behavior is mainly dependent on dopaminergic mechanisms, while NPA also interacts with serotonin receptors. There was demonstrated the displacement of ^{3}H-ketanserin from serotonin$_2$-receptors by apomorphine [36]. In the present study NPA inhibited similarly ^{3}H-spiperone binding in dorsal cortex (mainly serotonin$_2$-receptors) as well as in subcortical forebrain structures (prevailing dopamine$_2$-receptors). Caerulein in lower doses interacted with dopamine$_2$-receptors, whereas the higher doses were needed for interaction with serotonin$_2$-receptors. It was found that to suppress dopamine turnover lower concentrations of CCK were needed than to inhibit serotonin turnover [51]. Destruction of serotoninergic terminals by PCA and 5,7-DHT significantly increased the motor depressant effect of NPA, while only PCA, decreasing also serotonin$_2$-receptors sensitivity [5], moderately potentiated the action of caerulein. The involvement of serotoninergic mechanisms in the behavioral effects of apomorphine was also stated by other authors. The administration of different serotonin agonists into median raphe nuclei, innervating mesolimbic area, potentiated in rats the motor stimulation induced by apomorphine [22]. Apomorphine in high dose (over 4 mg/kg) induced in cats behavioral effects similar to LSD, an agonist of serotonin$_2$-receptors [50]. In the clinical studies [33], it was established that apomorphine had pronounced sedative action only in patients with enlarged cerebral ventricles. In this subgroup of schizophrenic patients the decreased content of 5-hydroxyindoleacetic acid, the major metabolite of serotonin, in cerebrospinal fluid was described [41]. These clinical observations are in agreement with our study showing the increased sedative effect of apomorphine and NPA in the case of deficiency of central serotoninergic mechanisms.

Special attention was drawn to the interaction between haloperidol, the classical neuroleptic drug, and caerulein. In the pharmacological experiments similarities were found in the behavioral effects of caerulein and haloperidol, but a positive interaction between these drugs was not found [55, 57, 58]. Similar absence of interaction in intact animals was established in the present study. The interaction between caerulein and haloperidol became evident after two weeks haloperidol administration. Caerulein reversed the tolerance to the sedative effect of haloperidol and increased ^{3}H-spiperone binding after long-term neuroleptic medication. The increased number of CCK binding sites was demonstrated after long-term haloperidol treatment [12]. Different neuroleptic drugs (haloperidol, chlorpromazine, clozapine) induced dose dependent elevation of CCK-8 content in forebrain subcortical structures after two weeks administration [23]. It is possible the mechanisms described above are involved in the beneficial action of CCK-like peptides in neuroleptic-resistant schizophrenic patients [39,40].

In conclusion, it is probable that apomorphine and NPA have at least three distinct levels of action: (1) The stimulation of dopamine "autoreceptors" causes the sedative effect in animals and humans [37]; (2) The interaction with high-affinity dopamine$_2$- and serotonin$_2$-receptors induces the stimulation of ^{3}H-spiperone binding in animals responding weakly to motor stimulant action of NPA. The beneficial clinical effect of apomorphine and NPA [47,48] might be related to these monoaminergic mechanisms; (3) Through the stimulation of low affinity dopamine$_2$- and serotonin$_2$-receptors are mediated the typical behavioral effects of apomorphine and NPA in higher doses (stereotyped behavior, cage climbing behavior, aggressiveness, etc.).

Caerulein, after systemic administration, more probably interacts with high-affinity dopamine$_2$-receptors and to a lesser extent with high-affinity serotonin$_2$-receptors, inhibiting the stimulating effect of dopamine and its analogs on animals' behavior.

ACKNOWLEDGEMENTS

We would like to thank Farmitalia Carlo Erba (Italy) and Sterling-Winthrop (USA) for the generous gifts of drugs.

REFERENCES

1. Aadamsoo, A. M. and E. Vasar. Effect of apomorphine in schizophrenic patients. In: *Acta et Commentationes Universitatis Tartuensis, 600*. Tartu: University of Tartu Press, 1982, pp. 65–72.

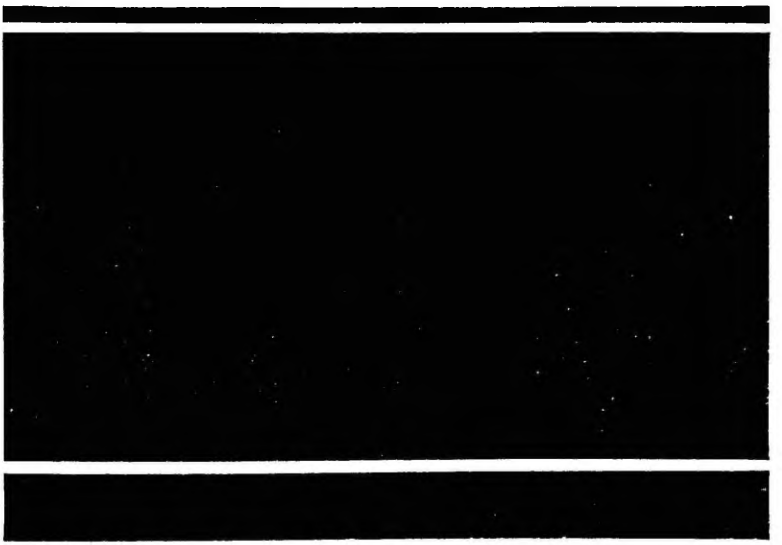
2. Aghajanian, G. K. and B. S. Bunney. Central dopaminergic neurons: neurophysiological identification and response to drugs. In: *Frontiers in Catecholamine Research*, edited by S. H. Snyder and E. Usdin. New York: Pergamon Press, 1973, pp. 643–648.

3. Aghajanian, G. K. and B. S. Bunney. Pre- and postsynaptic feedback mechanisms in central dopaminergic neurons. In: *Frontiers of Neurology and Neuroscience Research*, edited by P. Seeman and G. M. Brown. Toronto: University of Toronto Press, 1974, pp. 4–11.
4. Agnati, L. F., K. Fuxe, F. Benfenati, M. F. Celani, V. Battistini, V. Mutt, L. Cavicchioli, G. Galli and T. Hökfelt. Differential modulation by CCK-8 and CCK-4 of ^{3}H-spiperone binding sites linked to dopamine and 5-hydroxytryptamine receptors in the brain of rat. *Neurosci Lett* **35**: 179–183, 1983.
5. Archer, T., S.-O. Ögren and S. B. Ross. Serotonin involvement in aversive conditioning: reversal of the fear retention by long-term p-chloroamphetamine but not p-chlorophenylalanine. *Neurosci Lett* **34**: 75–82, 1982.
6. Bednarczyk, B. and J. Vetulani. Antagonism of clonidine to shaking behavior in abstinence syndrome and to head twitches produced by serotoninergic agents in the rat. *Pol J Pharmacol Pharm* **30**: 307–322, 1978.
7. Bloom, D. M., N. P. V. Nair and G. Schwartz. CCK-8 in the treatment of chronic schizophrenia. *Psychopharmacol Bull* **19**: 361–363, 1983.
8. Bradbury, A. J., B. Costall, R. J. Naylor and J. L. Neumeyer. Motor inhibition induced by apomorphine derivatives in the mouse. *J Pharm Pharmacol* **35**: 494–499, 1983.
9. Carlsson, A. Dopaminergic autoreceptors. In: *Chemical Tools in Catecholamine Research*, vol 2, edited by O. Almgren, A. Carlsson and L. Engel. Amsterdam: North-Holland Publishing Co., 1975, pp. 219–234.
10. Carlsson, A. Receptor mediated control of dopamine metabolism. In: *Pre- and Postsynaptic Receptors*, edited by E. Usdin and W. E. Bunney, Jr. New York: Marcel Dekker, 1975, pp. 49–65.
11. Casu, M., G. Biggio, G. Serra, and G. L. Gessa. Distinct receptors controlling dopamine synthesis and tyrosine hydroxylase in striatum. In: *Neuroactive Drugs in Endocrinology*, edited by E. E. Müller. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980, pp. 89–97.
12. Chang, R. S. L., V. S. Lotti, G. E. Martin and T. B. Chen. Increase in brain ^{125}I-cholecystokinin (CCK) receptor binding following chronic haloperidol treatment, intracisternal 6-hydroxydopamine or ventral tegmental lesions. *Life Sci* **32**: 871–878, 1983.
13. Corsini, G. U., G. R. Pitzalis, F. Bernardi, A. Bocchetta and M. Del Zompo. The use of dopamine agonists in the treatment of schizophrenia. *Neuropharmacology* **20**: 1309–1313, 1981.
14. Costall, B., S.-C. G. Hui and R. J. Naylor. Denervation in the dopaminergic mesolimbic system: functional changes followed using (−)-N-n-propylnorapomorphine depend on the basal activity levels of rats. *Neuropharmacology* **19**: 1039–1048, 1980.
15. Costall, B., A. M. Domeney and R. J. Naylor. A comparison of the behavioral consequences of chronic stimulation of dopamine receptors in the nucleus accumbens of rat brain effected by a continuous infusion or by single daily injections. *Naunyn Schmiedebergs Arch Pharmacol* **324**: 27–33, 1983.
16. Costentin, J., I. Dubuc and P. Protais. Behavioral data suggesting the plurality of central dopamine receptors. In: *CNS Receptors—From Molecular Pharmacology to Behavior*, edited by P. Mandel and F. V. De Feudis. New York: Raven Press, 1983, pp. 289–298.
17. Crawley, J. N. and J. S. Schwaber. Nucleus tractus solitarius lesions block the behavioral actions of cholecystokinin. *Peptides* **4**: 743–747, 1983.
18. Creese, I. and S. E. Leff. Dopamine receptors: a classification. *J Clin Psychopharmacol* **2**: 329–335, 1982.
19. Cutler, N. R., D. V. Jeste, F. Karoum and R. J. Wyatt. Low dose apomorphine reduces serum homovanillic acid concentration in schizophrenic patients. *Life Sci* **30**: 753–756, 1982.
20. Earley, C. J. and B. E. Leonard. Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine and several metabolites from brain tissue using disposable Bio-Rad columns packed with Sephadex G-10. *J Pharmacol Methods* **1**: 67–79, 1978.
21. Ferrier, I. N., E. C. Johnstone and T. J. Crow. Clinical effects of apomorphine in schizophrenia. *Br J Psychiatry* **144**: 341–348, 1984.
22. Fink, H. and W. Oelssner. LSD, mescaline and serotonin injected into medial raphe nucleus potentiate apomorphine hypermotility. *Eur J Pharmacol* **75**: 289–296, 1981.
23. Frey, P. Cholecystokinin octapeptide levels in rat brain are changed after subchronic neuroleptic treatment. *Eur J Pharmacol* **95**: 87–92, 1983.
24. Fuxe, K., K. Andersson, V. Locatelli, L. F. Agnati, T. Hökfelt, L. Skirboll and V. Mutt. Cholecystokinin peptides produce marked reduction of dopamine turnover in discrete areas in the rat brain following intraventricular injection. *Eur J Pharmacol* **67**: 329–332, 1980.
25. Fuxe, K., L. F. Agnati, C. Köhler, D. Kuonen, S.-O. Ögren, K. Anderson and T. Hökfelt. Characterization of normal and supersensitive dopamine receptors: effects of ergot drugs and neuropeptides. *J Neural Transm* **51**: 3–37, 1981.
26. Grabowska, M. Influence of apomorphine on serotonin turnover rate. *Pharmacol Biochem Behav* **3**: 589–591, 1975.
27. Grigoriadis, D. and P. Seeman. The dopamine/neuroleptic receptor. *Can J Neurol Sci* **11**: 108–113, 1984.
28. Harvey, J. A. Neurotoxic action of halogenated amphetamines. *Ann NY Acad Sci* **305**: 289–304, 1978.
29. Helmeste, D. M. Spontaneous and apomorphine-induced locomotor changes parallel dopamine receptor differences in two rat strains. *Pharmacol Biochem Behav* **19**: 153–155, 1983.
30. Hommer, D. W. and L. R. Skirboll. Cholecystokinin-like peptides potentiate apomorphine-induced inhibition of dopamine neurons. *Eur J Pharmacol* **91**: 151–152, 1983.
31. Hökfelt, T., L. Skirboll, J. F. Rehfeld, M. Goldstein, K. Markey and O. Dann. A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence for immunochemistry combined with retrograde tracing. *Neuroscience* **5**: 2093–2124, 1980.
32. Iversen, L. L., M. A. Koganski and R. J. Miller. Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic receptors in the rat striatum. *Mol Pharmacol* **12**: 251–262, 1976.
33. Jeste, D. V., S. Zalcman, D. K. Weinberger, N. R. Cutler, L. B. Bigelow, J. E. Kleinman, A. Rogol and R. J. Wyatt. Apomorphine response and subtyping of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* **7**: 83–88, 1983.
34. Kawasaki, K., M. Kodama and A. Matsushita. Caerulein, a cholecystokinin-related peptide, depresses somatic function via the vagal afferent system. *Life Sci* **33**: 1045–1050, 1983.
35. Köhler, C., S. B. Ross, B. Srebro and S.-O. Ögren. Long-term biochemical and behavioral effects of p-chloroamphetamine in the rat. *Ann NY Acad Sci* **305**: 645–663, 1978.
36. Leysen, J. E., C. J. E. Niemegeers, J. M. VanNueten and P. M. Laduron. ^{3}H-ketanserin (R 41468), a selective ^{3}H-ligand for serotonin$_2$-receptors binding sites. Binding properties, brain distribution and functional role. *Mol Pharmacol* **21**: 301–315, 1982.
37. Meltzer, H. Y. Relevance of dopamine autoreceptors for psychiatry. Preclinical and clinical studies. *Schizophrenia Bull* **3**: 456–467, 1980.
38. Mereu, G., A. Argiolas, M. R. Melis and G. L. Gessa. Inhibition of nigral dopaminergic firing by N-n-propylnorapomorphine: behavioral and biochemical correlates. *Ann Ist Super Sanita* **18**: 57–62, 1982.
39. Moroji, T., N. Watanabe, N. Aoki and S. Itoh. Antipsychotic effects of caerulein, a decapeptide chemically related to cholecystokinin octapeptide, on schizophrenia. *Int Pharmacopsychiatry* **17**: 255–273, 1982.
40. Nair, N. P. V., D. M. Bloom and J. N. Nestoros. Cholecystokinin appears to have antipsychotic properties. *Prog Neuropsychopharmacol Biol Psychiatry* **6**: 509–512, 1982.
41. Potkin, S. G., D. R. Weinberger, M. Linnoila and R. Wyatt. Low CSF 5-hydroxyindoleacetic acid in schizophrenic patients with enlarged cerebral ventricles. *Am J Psychiatry* **140**: 21–25, 1983.

42. Protais, P., J. J. Bonnet and J. Costentin. Pharmacological characterization of the receptors involved in the apomorphine-induced polyphasic modification of locomotor activity in mice. *Psychopharmacology* **81**: 126–134, 1983.
43. Severson, J. A. and P. K. Randall. Localization of ^{3}H-propylnorapomorphine binding in mouse striatum. *Brain Res* **279**: 295–298, 1983.
44. Starke, K., N. Reimann, A. Zumstein and G. Hetting. Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus in vitro. *Naunyn Schmiedebergs Arch Pharmacol* **305**: 27–36, 1978.
45. Strömbom, U. Effects of low doses of catecholamine receptor agonists on exploration in mice. *J Neural Transm* **37**: 229–235, 1975.
46. Strömbom, U. Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine. *J Neural Transm* **40**: 191–194, 1977.
47. Tamminga, C. A., M. H. Schaffer, R. C. Smith and J. M. Davis. Schizophrenic symptoms improve with apomorphine. *Science* **200**: 567–568, 1978.
48. Tamminga, C. A., E. G. DeFraites, M. D. Gotts and T. N. Chase. Apomorphine and N-n-propylnorapomorphine in the treatment of schizophrenia. In: *Apomorphine and Other Dopaminomimetics: Clinical Pharmacology*, edited by G. U. Corsini and G. L. Gessa. New York: Raven Press, 1981, pp. 49–55.
49. Trulson, M. E., E. E. Eubanks and B. L. Jacobs. Behavioral evidence for supersensitivity following destruction of central serotoninergic nerve terminals by 5,7-dihydroxytryptamine. *J Pharmacol Exp Ther* **198**: 23–32, 1976.
50. Trulson, M. E. and T. Crisp. Behavioral and neurochemical effects of apomorphine in the cat. *Eur J Pharmacol* **80**: 295–309, 1982.
51. Vasar, E., M. Otter and L. Rägo. Intraventricular administration of cholecystokinin decreases the activity of dopamine- and serotoninergic system in the brain. *Physiol J USSR* **68**: 1218–1223, 1982.
52. Vasar, E., M. Maimets, L. Rägo, A. Nurk and L. Allikmets. Influence of imidazobenzodiazepine (Ro 15-1788) on aggressive behavior of rat. *Bull Exp Biol Med* **48**: 441–443, 1984.
53. Verimer, T., D. B. Goodale, J. P. Long and J. R. Flynn. Lithium effects on haloperidol-induced pre- and postsynaptic dopamine receptors supersensitivity. *J Pharm Pharmacol* **32**: 665–666, 1980.
54. Yamamoto, I. and S. Ueki. The role of central serotoninergic mechanisms on head-twitch and backward locomotion induced by hallucinogenic drugs. *Pharmacol Biochem Behav* **14**: 89–95, 1981.
55. Zetler, G. Differential cataleptogenic and antistereotypic effects of caerulein and haloperidol. *Neuropharmacology* **20**: 681–686, 1981.
56. Zetler, G. Central depressant effects of caerulein and cholecystokinin octapeptide (CCK-8) differ from those of diazepam and haloperidol. *Neuropharmacology* **20**: 277–283, 1981.
57. Zetler, G. Neuroleptic-like effects of ceruletide and cholecystokinin octapeptide: interactions with apomorphine, methylphenidate and picrotoxin. *Eur J Pharmacol* **94**: 261–270, 1983.
58. Zetler, G. Behavioral pharmacology of CCK and analogs. *Psychopharmacol Bull* **19**: 347–351, 1983.

ISSN 0365-9615

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ



Бюл. эксперим. биологии и медицины, 1986, CII, 11, 513–640

revealed peripheral activity of atropine-protein conjugates. There was a correlation between the pharmacological activity of atropine-protein conjugates (25–50 mg/kg) and the activity of atropine (5 mg/kg), however the pharmacological effect of atropine-protein conjugates was somewhat longer. The results suggest that atropine-protein conjugates are both immunologically and pharmacologically active compounds.

УДК 615.214.22:547.879].045.4:612.822.1.014.46:577.175.731

Ключевые слова: *холецистокининовые рецепторы, галоперидол, церулеин.*

Э. Э. Васар, А. Х. Соосаар, М. О. Майметс, Л. Х. Алликметс

ПОНИЖЕНИЕ ЧУВСТВИТЕЛЬНОСТИ ХОЛЕЦИСТОКИНИНОВЫХ РЕЦЕПТОРОВ В МОЗГЕ ПОД ВЛИЯНИЕМ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ ГАЛОПЕРИДОЛА

Лаборатория психофармакологии НИИ общей и молекулярной патологии Тартуского университета

Представлена акад. АМН СССР А. В. Вальдманом

По имеющимся данным, длительное применение нейролептиков оказывает существенное влияние на холецистокинин-8-(ХЦК-8)-ергические процессы мозга. Установлено, что 2-недельное введение галоперидола или резерпина повышает плотность ХЦК-8-рецепторов в переднем мозге мышей [2]. Применение разных нейролептиков (клозапина, хлорпромазина и галоперидола) увеличивает содержание ХЦК-8 в подкорковых лимбических структурах и хвостатом ядре [5]. В наших предыдущих исследованиях было показано, что длительное введение галоперидола вызывает извращение ингибирующего влияния церулеина, агониста ХЦК-8, на связывание ^{3}H-спироперидола в опытах in vivo [7]. Церулеин стимулировал связывание ^{3}H-спироперидола с дофамин$_2$- и серотонин$_2$-рецепторами после 15-дневного применения галоперидола [7]. Существует мнение, что снижение содержания ХЦК-8 в некоторых структурах переднего мозга может лежать в основе резистентности к нейролептикам у психических больных [3]. В настоящем исследовании изучено влияние длительного введения типичного нейролептика галоперидола на связывание ^{3}H-холецистокинина в переднем мозге мышей и на поведенческие эффекты церулеина, агониста ХЦК-8-рецепторов

Методика исследования. Опыты проведены на белых мышах-самцах массой 25—30 г. Физиологический раствор или галоперидол (0,25 мг/кг, «Gedeon Richter», ВНР) вводили 2 раза в день на протяжении 15 дней. Поведенческие опыты и опыты по радиолигандному связыванию ставили через 48—72 ч после последней инъекции галоперидола. В опытах по связыванию ^{3}H-ХЦК-8 (86 Ки/ммоль, «Amersham», Англия) в переднем мозге мышей использовали модифицированную методику [4]. Мозговую ткань гомогенизировали в 10 объемах холодного трис-HCl буферного раствора (pH 7,4) при 20 °C с помощью гомогенизатора Поттера С. Гомогенизированную ткань центрифугировали при 48 000 g в течение 15 мин, после чего полученный осадок снова гомогенизировали в 10 объемах трис-HCl-буферного раствора и гомогенат центрифугировали при 48 000 g в течение 15 мин. Окончательный осадок гомогенизировали в 100 объемах инкубационного буфера, состоящего из 10 мМ HEPES, 130 мМ хлорида натрия, 5 мМ хлорида калия, 5 мМ хлорида магния и 1 мМ ЭДТА (pH доводили до 7,4 с помощью гидроксида калия). ^{3}H-ХЦК-8 добавили в инкубационную смесь в разных концентрациях от 50 пМ до 3 нМ. Неспецифическое связывание определяли добавлением 1 мкМ церулеина («Farmitalia», Италия). Пробы инкубировали при 24 °C в течение 90 мин. После инкубации пробы центрифугировали при 12 00 g в течение 2½ мин. Супернатант выливали и осадок осторожно промывали несколько раз с помощью холодного инкубационного буфера. Радиоактивность проб (4 параллелей) определяли в сцинтилляторе Брея на счетчике β-частиц «ЛС-6800» («Beckman», США). Опыты повторяли 3—4 раза. Полученные данные обрабатывали с помощью анализа Скетчарда.

Параллельно с опытами по радиолигандному связыванию проводили исследование изменений в поведенческих эффектах агониста ХЦК-8-рецепторов церулеина. Влияние церулеина (10 мкг/кг подкожно) на ориентировочно-исследовательскую активность мышей регистрировали с помощью фотоэлектрического актометра. Сразу после введения церулеина или физиологического раствора животных помещали в индивидуальные клетки актометра и определяли их двигательную активность в течение 30 мин. По методике электроболевого раздражения изучали действие 40 мкг/кг церулеина на агрессивное поведение мышей после 2-недельной инъекции галоперидола. Через 20 мин после введения церулеина 2 животных помещали в камеру электроболевого раздражения, где они на протяжении 2 мин получили 48 электрических ударов напряжением 40 В. Параметром интенсивности агрессивного поведения служило число агрессивных контактов между животными. Противосудорожное действие церулеина (125 мкг/кг подкожно) исследовали на модели пикротоксиновых судорог. Установлено, что ХЦК-8 и его аналоги в малых дозах антагонизируют пикротоксиновым судорогам как при внутрижелудочковом, так и при системном введении [6]. Церулеин применяли за 10 мин до введения 10 мг/кг пикротоксина. Определяли 3 основных параметра пикротоксиновых судорог: латентные периоды клонических судорог, латентные периоды тони-

ческих судорог и продолжительность жизни мышей. Все полученные в поведенческих опытах данные обработаны статистически с помощью критерия *t* Стьюдента.

Результаты исследования. Применение галоперидола в течение 15 дней (0,25 мг/кг 2 раза в день) в наших опытах повышало плотность высокоаффинных мест связывания ХЦК-8 (табл. 1). Число низкоаффинных мест связывания уменьшалось, однако их аффинность к ХЦК-8 заметно повышалась. Параллельно изменениям на местах связывания ХЦК-8 наблюдалось ослабление или извращение поведенческих эффектов церулеина, агониста ХЦК-8. Церулеин (10 мкг/кг) у мышей, получавших предварительно в течение 15 дней галоперидол, в значительной степени утратил свою способность подавлять ориентировочно-исследовательскую реакцию, в то время как антиагрессивное действие церулеина извращалось (табл. 2). Церулеин в дозе 40 мкг/кг значительно повышал число агрессивных контактов между мышами (см. табл. 2). Длительное предварительное введение галоперидола достоверно ослабляло антагонистическое влияние церулеина (125 мкг/кг) на пикротоксиновые судороги (табл. 3).

Таким образом, результаты настоящего исследования свидетельствуют о том, что длительное применение типичного нейролептика галоперидола, несмотря на повышение числа высокоаффинных и увеличение аффинности низкоаффинных мест связывания ХЦК-8, вызывает ослабление или извращение поведенческих эффектов церулеина. О пониженной чувствительности ХЦК-8-рецепторов свидетельствуют также данные наших предыдущих исследований [1, 7], в которых было установлено, что длительное введение галоперидола возвращает ингибирующее влияние церулеина на связывание ^{3}H-спироперидола в опытах in vivo. В то же время длительное применение галоперидола существенным образом снижает число высокоаффинных дофамин$_2$- и серотонин$_2$-рецепторов [1, 7]. Понижение плотности этих моноаминергических рецепторов, по-видимому, и определяет гипочувствительность ХЦК-8-рецепторов. Учитывая тесную морфофункциональную связь между ХЦК-8, дофамином и серотонином, а также значительные адаптационные изменения на ХЦК-8-рецепторах при длительном введении типичного нейролептика галоперидола, можно полагать, что ХЦК-8 имеет значение для реализации как антипсихотического [3], так и побочных эффектов нейролептиков.

Таблица 1

Влияние длительного введения галоперидола на связывание ^{3}H-холецистокинина в переднем мозге мышей

Вводимое вещество	Высокоаффинные места связывания		Низкоаффинные места связывания	
	$K_д$	$Св_{макс}$	$K_д$	$Св_{макс}$
Физиологический раствор	0,45±0,05	7,3±0,8	2,66±0,25	31,5±2,5
Галоперидол	0,45±0,05	11,7±1,0*	1,14±0,12**	21,4±2,0*

Примечание. $K_д$ — константа диссоциации (в нМ), $Св_{макс}$ — максимальная плотность мест связывания (в фмоль на 1 мг белка). Здесь и в табл. 2 и 3 одна звездочка — $P<0,05$, две — $P<0,01$.

Таблица 2

Влияние длительного применения галоперидола на седативное и антиагрессивное действие церулеина

Условия опыта	Длительное введение			
	физиологического раствора		галоперидола	
	Ориентировочно-исследовательская активность мышей число импульсов в течение			
	15 мин	30 мин	15 мин	30 мин
Физиологический раствор	187±17	325±39	160±12	276±17
Церулеин (10 мкг/кг)	115±19	198±23	142±23	252±27
	Электроболевая агрессивность мышей число агрессивных контактов в течение 2 мин			
Физиологический раствор	13,5±0,95		8,6±0,93	
Церулеин 40 мкг/кг	5,8±0,82		21,8±2,99	

Таблица 3

Изменение противосудорожного действия церулеина после длительного введения галоперидола

Параметры пикротоксиновых судорог	Пикротоксин (10 мг/кг)+ физиологический раствор	Пикротоксин (10 мг/кг)+ церулеин (125 мкг/кг)
Длительное введение физиологического раствора		
Латентное время клонических судорог, с	441±25	785±72**
Латентное время тонических судорог, мин	16±1,5	24,9±2,0**
Продолжительность жизни, мин	17,4±1,7	26,1±1,6**
Длительное введение галоперидола		
Латентное время клонических судорог, с	427±32	776±65**
Латентное время тонических судорог, мин	15,8±1,4	20,5±1,5*
Продолжительность жизни, мин	18,1±1,5	21,5±1,5

ЛИТЕРАТУРА

1. *Висор Э. Э., Нурк А. М., Майметс М. О., Аллик-метс Л. Х.* // Бюл. экспер. биол. — 1985. — № 1. — С. 72—74.
2. *Chung R. S. L., Lotti V. J., Martin G. E.* et al. // Life Sci. — 1983. — Vol. 32. — P. 871—878.

3. *Ferrier I. N., Roberts G. W., Grow T. J.* et al. // Ibid. — Vol. 33. — P. 475—482.
4. *Finkelstein J. A., Stegless A. W., Martinez P. A., Praissman M.* // Peptides. — 1984. — Vol. 5. — P. 11—14.
5. *Frey P.* // Europ. J. Pharmacol. — 1983. — Vol. 95. — P. 87—92.
6. *Kadar T., Pesti A., Penke B.* et al. // Neuropharmacology. — 1984. — Vol. 23. — P. 955—961.
7. *Vasar E., Maimets M., Nurk A., Allikmets L.* // Psychopharmacol. Bull. — 1984. — Vol. 20. — P. 691—692.

Поступила 12.12.85

REDUCED SENSITIVITY OF THE BRAIN CHOLECYSTOKININ RECEPTORS UNDER THE EFFECT OF HALOPERIDOL PROLONGED ADMINISTRATION

E. E. Vasar, A. Kh. Soosaar, M. O. Maimets, L. K. Allikmets

Tartu University, Tartu

The authors have used behavioural and radioreceptor methods of investigation, that helped to find correlates of the behavioural phenomena on the receptor level.

УДК 616.127-005.4-085.23-036.8

Ключевые слова: *натрия оксибутират; пирацетам; пиридоксинил-глиоксилат; креатинфосфокиназа; инфаркт миокарда.*

И. Б. Цорин, Г. Г. Чичканов

ДЕЙСТВИЕ ПРЕПАРАТОВ С АНТИГИПОКСИЧЕСКИМИ СВОЙСТВАМИ НА ИШЕМИЧЕСКОЕ ПОВРЕЖДЕНИЕ МИОКАРДА

Лаборатория фармакологии антиангинальных средств (зав. — доктор мед. наук Г. Г. Чичканов) Института фармакологии АМН СССР, Москва

Представлена акад. АМН СССР А. В. Вальдманом

В последние годы широко обсуждается вопрос о возможности ограничения размеров инфаркта миокарда (ИМ) с помощью фармакологических веществ. Высказываются противоположные точки зрения даже в отношении таких традиционных «антиинфарктных» средств, как β-адреноблокаторы [8, 9, 13].

В настоящем исследовании изучено влияние препаратов с антигипоксическим действием — пиридоксинил-глиоксилата (глио-6), натрия оксибутирата и пирацетама — на развитие ишемического повреждения и конечный размер ИМ в условиях окклюзии коронарной артерии.

Методика исследования. Для суждения о влиянии препаратов на развитие ишемического повреждения в сердце при окклюзии коронарной артерии эксперименты проводили на кошках массой 3—4 кг, анестезированных этаминалом натрия (40 мг/кг внутривенно), в условиях искусственного дыхания. У животных перевязывали переднюю нисходящую ветвь левой коронарной артерии в средней ее трети. Внутривенно вводили гепарин в дозе 1000 ЕД/кг. Пробы крови брали из коронарного синуса перед окклюзией венечного сосуда, а также через 20 и 60 мин после нее. Проведены 4 серии экспериментов. Препараты вводили сразу после окклюзии коронарной артерии внутривенно: натрия оксибутират — 200 мг/кг, пирацетам — 400 мг/кг, глио-6 — 100 мг/кг. В контрольной серии животным вводили эквивалентный объем физиологического раствора. Активность креатинфосфокиназы (КФК) в плазме крови определяли по методу [10]. Полученные данные обрабатывали статистически. Коэффициенты линейных регрессий определяли с помощью непараметрического критерия Тейла. Достоверность различий между углами наклона регрессионных прямых определяли с помощью одностороннего непараметрического критерия Холлендера.

Для изучения влияния препаратов на размеры ИМ проводили опыты на беспородных белых крысах-самцах массой 180—200 г. Всего в экспериментах использованы 44 крысы (5 серий, по 8—10 животных в каждой). ИМ воспроизводили по методу [11]. У животных регистрировали ЭКГ в 3 стандартных отведениях до окклюзии венечной артерии, через 5 мин после нее и перед забоем животных. Крыс забивали через 72 ч после операции. Сердца извлекали и готовили в криостате срезы. Делали 5 срезов толщиной 25 мкм через каждые 2 мм, начиная от верхушки левого желудочка. Срезы окрашивали с помощью нитротетразолиевого синего, выявляя, таким образом, активность сукцинатдегидрогеназы. Для определения размеров ИМ использовали математическую модель [4]. Размер ИМ рассчитывали по объему некротической массы, выраженному в процентах от объема миокарда всего левого желудочка. Препараты вводили внутрибрюшинно в разовых дозах: натрия оксибутират — 200 мг/кг, пирацетам — 400 мг/кг, глио-6 — 100 мг/кг. В качестве препарата сравнения использовали пропранолол в разовой дозе 1 мг/кг. Схема введения препаратов была следующей: 1-е введение — за 15 мин до окклюзии коронарной артерии, 2-е — через 2 ч после окклюзии, затем по 2 введения ежедневно в течение следующих 2 сут. В контрольной серии животным вводили эквивалентный объем физиологического раствора. Полученные результаты обрабатывали статистически. Достоверность различий определяли с помощью критерия *t* Стьюдента.

Результаты исследования. Хорошо известно, что степень увеличения активности КФК в крови является важным показателем тяжести ишемического повреждения [6, 12]. По динамике нарастания активности КФК и условиях острой ишемии миокарда можно судить о скорости перехода обратимых ишемических повреждений в необратимые. Установлено, что при окклюзии коронарной артерии препараты с противоишемическим действием снижают актив

ЖУРНАЛ ВЫСШЕЙ НЕРВНОЙ ДЕЯТЕЛЬНОСТИ
ТОМ XXXVII 1987 ВЫП. 4

УДК 612.821.6+612.8.015

ИЗМЕНЕНИЕ ПОВЕДЕНЧЕСКИХ И БИОХИМИЧЕСКИХ ЭФФЕКТОВ ЦЕРУЛЕИНА, АНАЛОГА ОКТАПЕПТИДА ХОЛЕЦИСТОКИНИНА, ПОСЛЕ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ ГАЛОПЕРИДОЛА

ВАСАР Э. Э., АЛЛИКМЕТС Л. Х., СООСААР А. Х., ЛАНГ А. Э.

Лаборатория психофармакологии Тартуского государственного университета

По существующим представлениям октапептид холецистокинина (ХЦК-8) имеет тесные морфофункциональные связи с дофаминергическими системами переднего мозга [13, 21]. ХЦК-8, являясь комедиатором дофамина в нейронах центральной части покрышки, влияет на высвобождение и метаболизм дофамина [10, 20] и изменяет аффинность и плотность дофамин$_2$-рецепторов [11]. Имеются данные, что дофаминергические механизмы участвуют в реализации некоторых поведенческих эффектов ХЦК-8 и его аналога церулеина: седативного действия, подавления фенаминового возбуждения, антиагрессивного действия и т. д. [15, 22, 23]. С другой стороны, установлено модулирующее влияние дофамина на холецистокининергические механизмы. Длительная блокада дофаминовых рецепторов нейролептиками приводит к повышению содержания ХЦК-8 и количества его рецепторов в переднем мозге подопытных животных [5, 9].

Однако функциональное значение влияния дофаминергических механизмов на холецистокининергические процессы изучено в меньшей степени. В связи с этим целью настоящей работы было изучение влияния длительной блокады дофаминовых рецепторов галоперидолом на поведенческие эффекты церулеина, агониста ХЦК-8-рецепторов, а также выявление возможных механизмов изменения поведенческих эффектов церулеина под влиянием галоперидола.

МЕТОДИКА

Опыты проведены на 320 мышах-самцах массой 20—25 г и 250 крысах-самцах массой 200—250 г, разделенных на группы по 10—12 животных в каждой. Галоперидол, как правило, вводили в течение 15 дней в дозе 0,25 мг/кг 2 раза в день. Через 72 ч после последней инъекции галоперидола определяли поведенческие и биохимические эффекты церулеина (производство «Фармиталия—Карло Эрба», Италия). Седативное действие церулеина у мышей исследовали с помощью фотоэлектрического актометра. Животных помещали сразу после подкожного введения церулеина (15 мкг/кг) в актометр, где в течение 30 мин определяли двигательную активность. Противосудорожное действие церулеина исследовали в модели пикротоксиновых судорог. Церулеин (125 мкг/кг) вводили за 10 мин до внутрибрюшинного введения пикротоксина (8 мг/кг). Определяли три параметра пикротоксиновых судорог: латентные периоды клонических и тонических судорог, а также продолжительность жизни мышей после введения пикротоксина. При исследовании седативного и антипикротоксинового действия церулеина у крыс им под эфирным наркозом за 7—8 дней до опыта имплантировали унилатеральные канюли для микроинъекций в латеральный желудочек мозга по координатам атласа мозга крысы [4]. Канюли прикрепляли к черепу зубным цементом. Церулеин разводили в стерильном физиологическом растворе и в течение 30 с вводили с помощью микроинъектора в правый латеральный

желудочек мозга в объеме 5 мкл (в дозах 5—500 нг). Контролем служили микроинъекции физиологического раствора в том же объеме. На 60-й секунде после введения церулеина или физиологического раствора крыс помещали в центр открытого поля или им вводили пикротоксин (4 мг/кг). В открытом поле (размером 100×100×40 см) в течение 5 мин с помощью пяти независимых фотоэлектрических каналов определяли двигательную активность крыс, а также число вставаний на задние лапы и число обнюхиваний гнезд. После введения пикротоксина изучали латентные периоды возникновения тремора и клонических судорог, измеряли продолжительность жизни животных. В поведенческих исследованиях установлено, что церулеин при однократном введении с галоперидолом вызывает у крыс двухнедельное подавление возбуждающего действия фенамина [15]. В настоящем исследовании оценивали влияние хронического введения галоперидола на такой длительный эффект церулеина. Для этого через 72 ч после последней инъекции физиологического раствора или галоперидола части крыс вводили подкожно церулеин (40 мкг/кг). Контролем служило подкожное введение физиологического раствора в том же объеме. Опыты с d-фенамином (2,5 мг/кг) проводили трижды: через 1 сут, на 5-й и 12-й день после однократного введения церулеина. На 30-й минуте после введения фенамина определяли интенсивность стереотипного поведения [6] и через 45 мин в течение 5 мин изучали действие фенамина на двигательную активность крыс в модели открытого поля. Исследовали также связывание ^{3}H-спироперидола и ^{3}H-ХЦК-8. В опытах *in vivo* наблюдали влияние разных доз церулеина (20—250 мкг/кг) на связывание ^{3}H-спироперидола (5 мкг/кг, 17 Ки/ммоль) в переднем мозге мышей. Церулеин вводили за 15 мин до введения меченого нейролептика, а через 20 мин после введения ^{3}H-спироперидола животных декапитировали. Опыты связывания проводили по методике, описанной нами ранее [2, 19]. Опыты связывания ^{3}H-ХЦК-8 (86 Ки/ммоль) проводили в переднем мозге крыс и мышей по модифицированной методике [317]. Мозговую ткань гомогенизировали в 10 объемах холодного трис-HCl-буферного раствора (pH 7,4 при 20° С) с помощью гомогенизатора Поттера-С («Браун Мелсунген» ФРГ). Гомогенизированную ткань центрифугировали при 48 000 *g* в течение 15 мин, после чего полученный осадок снова гомогенизировали в 10 объемах трис-HCl-буферного раствора и гомогенат центрифугировали при 48000 *g* в течение 15 мин. Окончательный осадок гомогенизировали в 100 объемах инкубационного буфера, состоящего из 10 мМ ХЕПЕС (N-2-гидрокси-этилпиперазин-N′-2-этансулфоновая кислота; «Сигма», США), 120 мМ хлорида натрия, 5 мМ хлорида калия, 5 мМ хлорида магния и 1 мМ этилендиаминтетрауксусной кислоты (pH было доведено до 7,0 с помощью гидроксида калия). ^{3}H-ХЦК-8 добавляли в инкубационную среду в разных концентрациях — от 50 пМ до 3 нМ. Неспецифическое связывание определяли добавлением 1 мкМ церулеина. Пробы были инкубированы при 24° С в течение 90 мин. После инкубации пробы центрифугировали при 12000 *g* в течение 2 мин. Супернатант выливали и осадок осторожно промывали с помощью холодного инкубационного буфера. Радиоактивность проб (четыре параллели) определяли в сцинтилляторе Брея на счетчике бета-частиц ЛС-6800 («Бекман», США). Опыты повторяли 3—4 раза. Полученные данные подвергали анализу Скэтчарда.

РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЙ

У контрольных мышей церулеин в дозе 15 мкг/кг вызывал торможение двигательной активности (рис. 1). Однако после длительного применения галоперидола (0,25 мг/кг 2 раза в день в течение 15 дней) церулеин не изменял моторную активность подопытных животных, тормозящее действие исчезало. После галоперидола ослаблялось также антагонистическое действие церулеина (125 мкг/кг) на пикротоксиновые (8 мг/кг) судороги у мышей (табл. 1). У животных, получавших предварительно галоперидол, церулеин значительно слабее удлинял латент-

Таблица 1

Влияние длительного введения галоперидола на противосудорожное действие церулеина у мышей

Параметры пикротоксиновых судорог	Пикротоксин 8 мг/кг + физиологический раствор	Пикротоксин 8 мг/кг + церулеин 125 мкг/кг
Длительное введение физиологического раствора		
Латентное время клонических судорог, с	440±25	780±72**
Латентное время тонических судорог, мин	16±1,5	25±2,0**
Продолжительность жизни, мин	17±1,7	26±1,6**
Длительное введение галоперидола		
Латентное время клонических судорог, с	430±32	780±75**
Латентное время тонических судорог, мин	16±1,4	20±1,5*
Продолжительность жизни, мин	18±1,5	21±1,5

Примечание. * — $p<0,05$; ** — $p<0,01$ по сравнению с введением физиологического раствора + пикротоксина (по t-тесту Стьюдента; то же для табл. 3).

ный период тонических судорог и продолжительность жизни по сравнению с контрольной группой. Сходное ослабление или извращение действия церулеина наблюдалось также у крыс при внутрижелудочковом его введении. Если у контрольных крыс малые дозы церулеина (5 и 50 нг) угнетали ориентировочно-исследовательскую активность, то у животных, получавших галоперидол, церулеин не подавлял (5 нг) или даже усиливал (50 нг) активность животных. Введение 50 нг церулеина в латеральный желудочек мозга не только усиливало двигательную активность животных, но повышало и число вставаний на задние лапы и обнюхиваний гнезд (табл. 2). После галоперидола развивалась также толерантность к противосудорожному действию церулеина у крыс (табл. 3). После длительного применения галоперидола церулеин (5 нг) не вызывал достоверного ослабления пикротоксиновых (4 мг/кг) судорог. Единственным поведенческим эффектом церулеина, который существенным образом усиливался после длительного введения галоперидола, было длительное угнетение возбуждающего действия d-фенамина (2,5 мг/кг), причем интенсивность фенаминовой стереотипии при этом у крыс не изменялась. У контрольных крыс церулеин достоверно угнетал эффект фенамина только на 5-й день после однократного введения 40 мкг/кг церулеина (рис. 2), в то время как у животных, получивших галоперидол, такое действие церулеина было заметно уже через 24 ч после его однократного введения. Угнетающее влияние церулеина на возбуждающее действие фенамина было заметно на 15—20-й день после однократного введения церулеина.

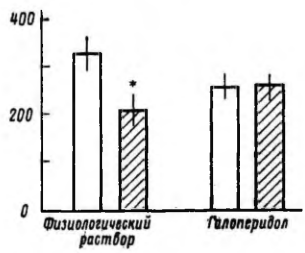


Рис. 1. Влияние длительного введения галоперидола на седативное действие церулеина у мышей. По оси ординат—число импульсов в течение 30 мин. Светлые столбики — эффект введения физиологического раствора; заштрихованные—церулеина (15 мкг/кг). * — $p<0,05$ (по U-тесту Манна — Уитни)

У контрольных мышей церулеин достоверно угнетал специфическое связывание ^{3}H-спироперидола в опытах *in vivo*, однако при длительном введении галоперидола очевидным стал противоположный эффект церулеина — стимуляция связывания ^{3}H-спироперидола (рис. 3). В опытах

Таблица 2

Влияние внутрижелудочкового введения церулеина на ориентировочно-исследовательскую активность крыс, получавших предварительно на протяжении 15 дней галоперидол

Вещество и доза	Число импульсов		Число вставаний		Число обнюхиваний гнезд	
	физиологический раствор	галоперидол	физиологический раствор	галоперидол	физиологический раствор	галоперидол
Физиологический раствор	40±3,1	58±7,5	2,2±0,38	4,2±1,07	5,9±0,92	4,8±0,77
Церулеин, 5 нг	29±4,4*	45±10,9	2,7±0,70	4,5±0,87	4,8±0,69	8,2±1,45*
Церулеин, 50 нг	27±3,6*	93±9,2*	0,8±0,26*	12±2,86*	4,2±1,03	11,3±1,83**
Церулеин, 500 нг	52±6,6	53±5,6	3,0±1,39	3,7±0,55	6,2±1,10	5,0±0,61

Примечание: * — $p<0,05$; ** — $p<0,01$ по сравнению с внутрижелудочковым введением физиологического раствора (по U-тесту Манна — Уитни).

Таблица 3

Влияние длительного введения галоперидола на противосудорожное действие церулеина у крыс

Параметры пикротоксиновых судорог	Пикротоксин, 4 мг кг+физиологический раствор	Пикротоксин, 4 мг/кг+церулеин 5 нг
Длительное введение физиологического раствора		
Латентное время тремора, мин	9,7±0,35	12,1±0,55**
Латентное время клонических судорог, мин	13,1±0,46	15,2±1,04*
Продолжительность жизни, мин	34,5±1,35	40,6±1,78*
Длительное введение галоперидола		
Латентное время тремора, мин	10,9±0,63	11,8±1,80
Латентное время клонических судорог, мин	15,3±1,05	15,3±1,54
Продолжительность жизни, мин	37,0±1,63	39,2±2,38

Таблица 4

Влияние длительного введения галоперидола на связывание ^{3}H-холецистокинина в переднем мозге

Вещество	Высокоаффинные места связывания		Низкоаффинные	
	$K_д$	$Св_{макс}$	$K_д$	$Св_{макс}$
Крысы				
Физиологический раствор	0,19±0,04	11,7±1,88	0,66±0,08	21±2,08
Галоперидол	0,26±0,06	13,1±2,08	0,40±0,04	15±1,22*
Мыши				
Физиологический раствор	0,35±0,05	7,3±0,80	2,66±0,25	31±2,5
Галоперидол	0,45±0,05	11,7±1,00*	1,14±0,12	21±2,0*

Примечание. $K_д$ — константа диссоциации, нмоль; $Св_{макс}$ — плотность мест связывания, фмоль/мг белка; * — $p<0,05$ (по t-тесту Стьюдента).

связывания *in vitro* выяснилось, что ХЦК-8 имеет два места связывания в переднем мозге как у мышей, так и у крыс. Разница в аффинности этих двух мест связывания была более значительной в переднем мозге мышей, чем крыс (табл. 4). Длительное введение галоперидола несколько уменьшало аффинность и повышало плотность высокоаффинных мест связывания ХЦК-8, в то время как аффинность низкоаффинных мест повышалась с одновременным понижением их плотности. Указанные изменения были одинаковыми в переднем мозге крыс и мышей.

ОБСУЖДЕНИЕ РЕЗУЛЬТАТОВ

Проведенный анализ поведения свидетельствует о том, что длительное применение галоперидола изменяет все основные эффекты церулеина, агониста ХЦК-8-рецепторов. В основном эффекты церулеина ослабляются или извращаются, только антифенаминовое действие церулеина усиливается после 15-дневного применения галоперидола. Ослабление или извращение поведенческих эффектов церулеина одинаково как при подкожном введении мышам, так и при внутрижелудочковом введении крысам. Полученные данные свидетельствуют в пользу мнения, что седативное и противосудорожное действия церулеина в основном реализуются через центральные механизмы, а не только через афферентную систему блуждающего нерва, как утверждают некоторые авторы [7, 14]. Длительное введение галоперидола изменяет не только состояние моноаминергических (дофамин, серотонин и ГАМК) процессов мозга

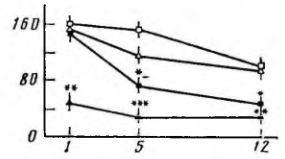


Рис. 2.

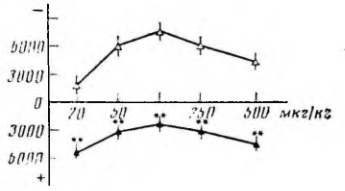


Рис. 3.

Рис. 2. Длительное угнетение церулеином возбуждающего действия фенамина у крыс, получивших предварительно галоперидол. По оси ординат — число импульсов в течение 5 мин; по оси абсцисс — дни после однократного введения церулеина (40 мкг/кг) или физиологического раствора. Светлые квадраты — действие фенамина (2,5 мг/кг) у крыс, получивших физиологический раствор + физиологический раствор; темные — физиологический раствор + церулеин; светлые треугольники — галоперидол + физиологический раствор; темные — галоперидол + церулеин. * — $p<0,05$; ** — $p<0,01$; *** — $p<0,005$ по сравнению с группой физиологический раствор + физиологический раствор (по U-тесту Манна — Уитни)

Рис. 3. Влияние церулеина на связывание ^{3}H-спироперидола в опытах *in vivo* в переднем мозге мышей, получивших предварительно галоперидол. По оси ординат — изменение специфического связывания ^{3}H-спироперидола (5 мкг/кг) под влиянием церулеина (20—500 мкг/кг), число распадов на 1 г ткани. «—» — угнетение связывания ^{3}H-спироперидола; «+» — стимулирование связывания. По оси абсцисс — доза церулеина, мкг/кг. Светлые треугольники — эффект церулеина после введения физиологического раствора; темные — галоперидола. ** — $p<0,01$ (по t-тесту Стьюдента)

[1], но вмешивается и в регуляцию центральных холецистокининергических механизмов. Однако, вероятно, такое действие галоперидола не является непосредственным, так как галоперидол не взаимодействует с ХЦК-8-рецепторами, его влияние опосредуется через те дофаминергические механизмы, комедиатором которых ХЦК-8 является [13]. По существующим представлениям, угнетающее влияние церулеина на дофаминергические процессы реализуется через два разных механизма [21]. Во-первых, церулеин, подобно ХЦК-8, устраняет ингибирующее влияние дофамина на постсинаптических мембранах в прилегающем ядре и, во-вторых, как и ХЦК-8, подавляет вызванное ионами калия кальций-зависимое высвобождение дофамина [21]. В постсинаптическом действии церулеина, по-видимому, ведущее значение имеет его взаимодействие с высокоаффинными дофамин$_2$-рецепторами на вставочных нейронах в переднем мозге [12, 18]. Внутримозговое введение каиновой кислоты разрушает именно ХЦК-8-рецепторы и высокоаффинные дофамин$_2$-рецепторы [8, 18]. Аналогичное снижение высокоаффинных дофамин$_2$-рецепторов [2, 3, 19] и ХЦК-8-рецепторов наблюдается после длительного введения галоперидола, и в связи с этим в опытах *in vivo* церулеин не ингибирует связывание ^{3}H-спироперидола, а, наоборот, даже стимулирует связывание ^{3}H-нейролептика. На основе приведенных данных можно

полагать, что длительное введение галоперидола приводит к функциональному выключению определенной части вставочных нейронов в переднем мозге, на которых и взаимодействуют дофамин и ХЦК-8. Именно функциональным выключением части вставочных нейронов и объясняется ослабление или извращение поведенческих и биохимических эффектов церулеина.

В отличие от других эффектов церулеина длительное антифенаминовое действие, вызванное однократным введением, значительно усиливается после длительного введения галоперидола. Этот факт свидетельствует о том, что антифенаминовое действие церулеина реализуется через другие механизмы по сравнению с изложенными выше. Существует мнение, что указанный эффект церулеина реализуется через пресинаптические дофаминергические механизмы в прилегающем ядре [15], церулеин подавляет высвобождение дофамина, вызванное фенамином, причем промежуточным звеном здесь является бета-эндорфин [16]. О различиях между постсинаптическим и пресинаптическим действием ХЦК-8 и церулеина на уровне дофаминергической системы говорит и факт, что антагонист ХЦК-8-рецепторов проглумид устраняет только постсинаптическое действие церулеина и ХЦК-8, не влияя при этом на их действия на высвобождение дофамина [21].

Итак, длительное введение галоперидола оказывает неодинаковое влияние на поведенческие эффекты церулеина, сильного агониста ХЦК-8-рецепторов. В основном поведенческие и биохимические эффекты церулеина ослабляются или извращаются, усиливается только длительный антагонизм с фенаминовым возбуждением у крыс. Ведущим во многих эффектах церулеина является его влияние на высокоаффинные дофамин$_2$-рецепторы, находящиеся на вставочных нейронах хвостатого ядра и лимбических структур. Длительное введение галоперидола вызывает функциональное выключение этих нейронов, чем обусловлено и ослабление или извращение эффектов церулеина. Длительное антифенаминовое действие церулеина реализуется через пресинаптические дофаминергические механизмы, причем бета-эндорфины выполняют здесь роль промежуточного звена между ХЦК-8- и дофамином [16].

ВЫВОДЫ

1. Длительное введение галоперидола в основном ослабляет или извращает поведенческие эффекты церулеина, агониста ХЦК-8-рецепторов. Только длительный антагонизм церулеина с возбуждающим действием фенамина у крыс усиливается под влиянием галоперидола.

2. В основе ослабления или извращения поведенческих эффектов церулеина после отмены длительного введения галоперидола лежит функциональное выключение части вставочных нейронов в подкорковых структурах переднего мозга, что выражается в понижении числа высокоаффинных дофамин$_2$-рецепторов и низкоаффинных ХЦК-8-рецепторов.

3. Усиление антагонизма церулеина с возбуждающим действием фенамина у крыс обусловлено более выраженным угнетающим влиянием церулеина на высвобождение дофамина из пресинаптических дофаминергических терминалей после длительного введения галоперидола.

Литература

1. *Алликметс Л. Х., Жарковский А. М., Нурк А. М. и др.* Влияние длительного введения нейролептиков на пластичность рецепторов ЦНС//Вестн. АМН СССР. 1984. т. 16. № 11. С. 37.
2. *Васар Э. Э., Нурк А. М., Майметс М. О., Алликметс Л. Х.* Стимуляция церулеином — аналогом октапептида холецистокинина — связывания ^{3}H-спироперидола после длительного введения нейролептиков//Бюл. эксперим. биологии и медицины. 1985. Т. 99. № 1. С. 72.
3. *Васар Э. Э., Алликметс Л. Х., Соосаар А. Х.* Адаптационные изменения на холецистокининовых рецепторах при длительном применении галоперидола//Актуальные вопросы фармакологии и токсикологии нейротропных средств. Рига: Мед. ин-т, 1986. С. 23.
4. *Bureš J., Petran M., Zachar J.* Electrophysiological methods in biological research. Prague. Publ. House Czechosl. Acad. Sci., 1960. 515 p.
5. *Chang R. S. L., Lotti V. J., Martin G. E., Chen T. B.* Increase in brain ^{125}I-cholecystokinin (CCK) receptor binding following chronic haloperidol treatment, intracisternal

6-hydroxydopamine or ventral tegmental lesions//Life Sci. 1983. V. 32. No. 3. P. 871.
6. *Costall B., Naylor R. J.* Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphe nuclei//Europ. J. Pharmacol. 1974. V. 29. No. 5. P. 206.
7. *Crawley J. N., Schwaber J. S.* Nucleus tractus solitarius lesions block the behavioral actions of cholecystokinin//Peptides. 1983. V. 4. No. 1. P. 743.
8. *Emson P. C., Sandberg B. E. B.* Cholecystokinin and substance P in the central nervous system//Ann. Rep. Med. Chem. 1983. V. 18. No. 1. P. 31.
9. *Frey P.* Cholecystokinin octapeptide levels in rat brain are changed after subchronic neuroleptic treatment//Europ. J. Pharmacol. 1983. V. 95. No. 12. P. 87.
10. *Fuxe K., Andersson K., Locatelli V. et al.* Cholecystokinin peptides produce marked reduction of dopamine turnover in discrete areas of rat brain following intraventricular injection//Europ. J. Pharmacol. 1980. V. 67. No. 2. P. 329.
11. *Fuxe K., Agnati L. F., Benfenati F. et al.* Modulation by cholecystokinins of ^{3}H-spiroperidol binding in rat striatum: evidence for increased affinity and reduction in the number of binding sites//Acta physiol. scand. 1981. V. 113, No. 4. P. 567.
12. *Grigoriadis D., Seeman P.* The dopamine/neuroleptic receptor//Canad. J. Neurol. Sci. 1984. V. 11. No. 2. P. 108.
13. *Hökfelt T., Skirboll L., Rehfeld J. F. et al.* A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunochemistry combined with retrograde tracing//Neuroscience. 1980. V. 5. No. 12. P. 2093.
14. *Kawasaki K., Kodama M., Matsushita A.* Caerulein, a cholecystokinin-related peptide, depresses somatic function via the vagal afferent system//Life Sci. 1983. V. 33. No. 3. P. 1045.
15. *Matsubara K., Matsushita A.* Long-lasting reduction of amphetamine-induced hyperactivity in rats after combined administration of caerulein with haloperidol//Europ. J. Pharmacol. 1984. V. 101. No. 2. P. 157.
16. *Matsubara K., Matsushita A.* β-endorphin involvement in the anti-dopaminergic effect of caerulein//Japan J. Pharmacol. 1986. V. 40. No. 3. P. 417.
17. *Praismann M., Martinez P. A., Saladino C. F. et al.* Characterization of cholecystokinin binding sites in rat cerebral cortex using a ^{125}I-CCK-8 probe resistant to degradation//J. Neurochem. 1983. V. 40. No. 6. P. 1406.
18. *Severson J. A., Randall P. K.* Localization of ^{3}H-N-propylnorapomorphine binding sites in mouse striatum//Brain Res. 1983. V. 279. No. 3. P. 295.
19. *Vasar E., Maimets M., Nurk A., Allikmets L.* Caerulein stimulates ^{3}H-spiperone binding in vivo after long-term haloperidol administration//Psychopharmacol. Bull. 1984. V. 20. No. 4. P. 691.
20. *Voigt M. M., Wang R. X.* In vivo release of dopamine in the nucleus accumbens of the rat: modulation by cholecystokinin//Brain Res. 1984. V. 296. No. 2. P. 189.
21. *Wang R. X., White F. J., Voigt M. M.* Interactions of cholecystokinin and dopamine in the nucleus accumbens//Ann. N. Y. Acad. Sci. 1985. V. 448. No. 7. P. 352.
22. *Zetler G.* Central depressant effects of caerulein and cholecystokinin octapeptide (CCK-8) differ from those of diazepam and haloperidol//Neuropharmacology. 1981. V. 20. No. 4. P. 277.
23. *Zetler G.* Behavioral pharmacology of CCK and analogs//Psychopharmacol. Bull. 1983. V. 19. No. 3. P. 347.

Поступила в редакцию
30.VI.1986

CHANGE OF BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF CAERULEIN, AN ANALOGUE OF CHOLECYSTOKININ OCTAPEPTIDE (CCK-8), FOLLOWING LONG-TERM ADMINISTRATION OF HALOPERIDOL

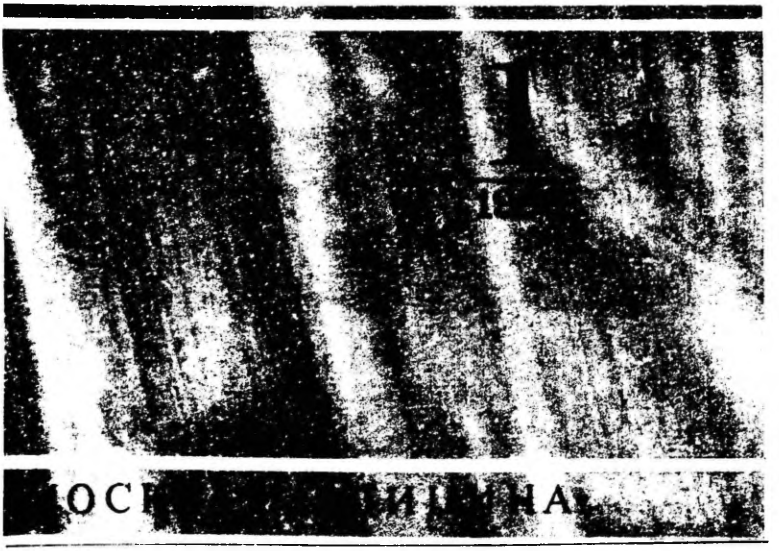
VASAR E., ALLIKMETS L., SOOSAAR A., LANG A.

University, Tartu

In experiments on male mice and rats, long-term haloperidol administration (0,25 mg/kg twice a day during 15 days) significantly changed behavioural effects of caerulein, an agonist of CCK-8 receptors. As a rule, the effects of caerulein were reduced or inverted; only long-term antagonism with amphetamine motor excitation in rats increased after the cessation of haloperidol administration. The decrease or inversion of caerulein's effects was connected with reduction of high-affinity dopamine$_2$- and low-affinity CCK-8 receptors' density, reflecting the inhibition of some interneurons' activity in subcortical forebrain structures after haloperidol treatment. A more pronounced inhibition of dopamine's release by caerulein was the reason for the increased antiamphetamine action after long-term haloperidol treatment. It seems possible that both above mechanisms are involved in the antipsychotic action of haloperidol.

ISSN 0365-9615

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ



Бюлл. эксперим. биологии и медицины, 1988, CV, 1, 1–128

ваннии значительных различий в свойствах серотониновых C_{1A}-рецепторов в мозге человека по сравнению с мозгом крысы и быка (например, об их гетерогенности по сродству к буспирону), либо о выраженных возрастных изменениях этих рецепторов, приводящих к снижению сродства для буспирона. Возможность таких возрастных изменений в свойствах серотониновых рецепторов подтверждается данными литературы [9].

В заключение можно сделать вывод, что анксиолитик буспирон взаимодействует в микромолярных концентрациях с серотониновыми C_1- и C_2-рецепторами мозга человека.

ЛИТЕРАТУРА

1. *Cascio C. S., Keller K. J.* // Europ. J. Pharmacol. — 1983. — Vol. 95. — P. 31—39.
2. *Engel G., Muller-Schweinitzer B., Palacios J. M.* // Naunyn Schmiedeberg's Arch. Pharmakol. — 1984. — Bd 325. — S. 328—331.
3. *Hoyer D., Pazos A., Probst A., Palacios J. M.* // Brain Res. — 1986. — Vol. 337. — P. 85—96.
4. *Mohler H., Ocada T.* // Life Sci. — 1977. — Vol. 28. — P. 2101—2105.
5. *Peroutka S. J., Snyder S. H.* // Molec. Pharmacol. — 1979. — Vol. 16. — P. 687—691.
6. *Peroutka S. J.* // Brain Res. — 1985. — Vol. 344. — P. 167—171.
7. *Raisman R., Briley M.* // Europ. J. Pharmacol. — 1980. — Vol. 61. — P. 373—378.
8. *Riblet L. A., Taylor D. P., Eison M. S., Stanton H. C.* // J. clin. Psychiat. — 1982. — Vol. 43. — P. 11—16.
9. *Schin J., Young H.* // Life Sci. — 1978. — Vol. 23. — P. 1441—1448.
10. *Skolnic P., Paul S.* // J. clin. Psychiat. — 1982. — Vol. 43. — P. 40—42.
11. *Temple D., Yevich J., New J.* // Ibid. — P. 4—9.
12. *Young A. B., Snyder S. H.* // Proc. nat. Acad. Sci. USA. — 1974. — Vol. 71. — P. 4002—4007.

Поступила 02.09.86

INTERACTION OF AN ANXIOLYTIC AGENT BUSPIRON, WITH HUMAN BRAIN SEROTONIN AND SOME OTHER RECEPTORS

A. Ya. Korneev, M. I. Factor, Chan Tkhi Bin An

USSR Research Centre for Mental Health, Academy of Medical Sciences of the USSR, Moscow

Buspiron and Mj 138-05 (up to 0.1 mM) did not displace specifically bound (^{3}H) triptamine, (^{3}H) strychnine, (^{3}H) flunitrazepam and (^{3}H) imipramine in human cortical and hippocampal membrane preparations. At the same time both compounds displayed similar to serotonin affinity (IC_{50} in the range of 2-6 μM) for (^{125}I)-LSD specific binding sites in the human cortex and hippocamp. IC_{50} of serotonin and buspiron and Mi 138-05 for (^{3}H) LSD (2 nM) specific binding sites in the hippocamp was determined as 0.14 μM, 2.3 μM and 6.1 μM, respectively; and for (^{3}H) serotonin specific binding sites in the hippocamp as 0.005 μM, 3.8 μM and 21 μM, respectively. The affinity for human cortex (^{3}H) LSD binding sites was 10-fold lower in case of serotonin and 4-fold lower in case of buspiron and Mj 138-05 than in the hippocamp. However, the affinity for (^{3}H) serotonin binding sites in the cortex was the same as in the hippocamp in case of serotonin and 12-15-fold lower than in the hippocamp in case of buspiron and Mj 138-05. It is concluded that in human brain buspiron and Mj 138-05 interact with micromolar affinity with 5 HT_2 and are capable of binding to a subpopulation of 5 HT_1 receptors in the hippocamp.

УДК 615.212.7:547.57].015.4:612.821.1/.3.014.46:615.213

Ключевые слова: *церулеин; кетамин; стереотипное поведение; двигательное возбуждение; атаксия; амнезия*

Э. Э. Васар, Л. Х. Алликметс, А. Х. Соосаар

АНТАГОНИЗМ ЦЕРУЛЕИНА, АГОНИСТА ХЦК-8 РЕЦЕПТОРОВ К ПОВЕДЕНЧЕСКИМ ЭФФЕКТАМ КЕТАМИНА У МЫШЕЙ И КРЫС

Лаборатория психофармакологии Тартуского университета

Представлена акад. АМН СССР А. В. Вальдманом

Фенциклидин и другие арилциклогексиламины в субамнестических дозах оказывают психотомиметическое действие на человека [1]. Применение фенциклидина или его более слабого аналога кетамина сопровождается амнезией у человека [2, 6]. У крыс и мышей фенциклидин и кетамин вызывают усиление двигательной активности и стереотипное поведение — поведенческие эффекты, напоминающие во многом действие фенамина и других дофаминомиметиков у этих животных [9]. Активация моторики при введении фенциклидина или кетамина сопровождается атаксией [14]. Показано, что введение фенциклидина мышам полностью подавляет выработку защитного рефлекса по методике пассивного избегания [11]. Существует мнение, что стереотипное поведение, вызванное фенциклидином, обусловлено его взаимодействием с серотонин$_2$-рецепторами [9], в то время как амнестическое действие фенциклидина реализуется через опиоидные рецепторы [11]. Октапептид холецистокинина (ХЦК-8) и его близкий аналог церулеин оказывают антидофаминергическое действие, антагонизируют возбуждающему действию фенамина на поведение крыс и мышей [15]. Появились также данные, что ХЦК-8 вызывает у крыс выраженный антиамнестический эффект [7].

Целью настоящего исследования было изучение влияния церуленна, агониста ХЦК-8 рецепторов, на поведенческие эффекты кетамина у крыс и мышей, при этом специальное внимание обращали на изменения в опиоид- и дофаминергической системах.

Методика исследования. Все поведенческие опыты были проведены на мышах-самцах массой 25—30 г и на крысах-самцах массой 220—270 г. В экспериментах на мышах изучали влияние церуленна на основные поведенческие эффекты кетамина — усиление двигательной активности, стереотипное поведение и атаксию. Кетамин "Gedeon Richter", Венгрия) в дозе 15—30 мг/кг вводили мышам подкожно за 5 мин до подкожного введения церуленна в дозе 75—375 мкг/кг ("Farmitalia-Carlo Erba", Италия). Галоперидол (0,1—1,5 мг/мкг внутрибрюшинно, "Gedeon Richter", Венгрия), антагонист дофа-

мин$_2$-рецепторов, вводили за 30 мин до введения кетамина. Интенсивность стереотипного поведения исследовали по методике [4] на 10-й минуте после введения кетамина. Интенсивность атаксии оценивали также на 10-й минуте после введения по условно выработанной шкале [3]. С 10-й по 15-ю минуты после введения кетамина исследовали его влияние на ориентировочно-исследовательскую активность мышей в открытом поле. Открытое поле (30×30×15 см) разделяли линиями на 16 секторов (в центре каждого было гнездо). В течение 5 мин определяли число секторов, пройденных мышами, число вставаний на задние лапы и число обследованных гнезд. У крыс изучали влияние церулеина на амнестическое действие кетамина по методике пассивного избегания в челночной камере. В 1-й день исследования животных адаптировали к обстановке опыта. На 2-й день проводили обучение животных. После перехода крысы в темный отсек дверь между двумя частями челночной камеры закрывали и животное получало 4 электрических удара через пол камеры напряжением 40 В. Интервал между электрическими ударами 45 с. Для опыта отбирали только тех животных, которые в течение 20 с переходили из светлого отсека камеры в темный. Сразу после обучения животным вводили кетамин (7,5—30 мг/кг подкожно), церулеин (10 мкг/кг подкожно) и налоксон (5 мг/кг подкожно, "Endo Labs", США), а также церулеин и налоксон в сочетании с кетамином. Через 24 ч после обучения определяли латентное время перехода животного из светлой в темную часть челночной камеры, а также время пребывания крысы в темном отсеке. За поведением каждого животного наблюдали в течение 3 мин. Параллельно с поведенческими опытами исследовали влияние кетамина на связывание ^{3}H-спироперидола во фронтальной коре крыс в присутствии 5 мкМ сульпирида ("Ravizza", Италия), избирательного антагониста дофамин$_2$-рецепторов, и в хвостатом ядре в присутствии 1 мкМ пиренперона ("Janssen Pharmaceutica", Бельгия), антагониста серотонин$_2$-рецепторов. Связывание ^{3}H-спироперидола (16 Ки/ммоль, "Amersham International", Англия) исследовали по методике [5]. Влияние кетамина на связывание ^{3}H-эторфина (36 Ки/ммоль, "Amersham International" изучали в переднем мозге крыс по методике [12].

Результаты исследования. Подкожное введение кетамина в дозах 15 и 30 мг/кг вызывало отчетливое усиление двигательной активности — двигательное возбуждение у мышей в открытом поле, у животных отмечались интенсивные стереотипные принюхивания, а также атаксия (табл. 1). Из-за атаксии у мышей, получавших кетамин, отсутствовали вставания на задние лапы. Церулеин в дозе 75 мкг/кг антагонизировал двигательному возбуждению, вызванному кетамином (30 мг/кг), и лишь в дозе 360 мкг/кг полностью подавлял кетаминовую стереотипию. При этом церулеин слабо влиял на кетаминовую атаксию (см. табл. 1). Галоперидол в дозе 0,5 мг/кг достоверно ослаблял двигательное возбуждение и стереотипное поведение, вызванные кетамином (30 мг/кг). На крыс кетамин оказывал амнестическое действие (табл. 2). В дозах 15 и 30 мг/кг препарат достоверно нарушал обучение крыс. В дозе 10 мкг/кг церулеин не влиял на обучение крыс в челночной камере, однако полностью устранял амнестическое действие кетамина. Аналогичное действие оказывал антагонист опиоидных рецепторов налоксон в дозе 5 мг/кг (см. табл. 2). В опытах по изучению радиолигандного связывания кетамин даже в концентрации 100 мкМ не влиял на связывание ^{3}H-

Таблица 1

Влияние церулеина и галоперидола на поведенческие эффекты кетамина у мышей

Вещество	Доза	Стереотипное поведение, баллы	Атаксия, баллы	Ориентировочно-исследовательская активность в течение 5 мин		
				число пройденных секторов	число обследованных гнезд	число вставаний на задние лапы
Физиологический раствор		0±0	0±0	40±3,2	9±1,2	7±1,6
Кетамин	15 мг/кг	1,75±0,15	1,20±0,20	60±5,8	8±1,8	0±0
То же	30 мг/кг	1,92±0,12	1,83±0,25	85±6,6	8±0,9	0±0
Кетамин + церулеин	30 мг/кг+75 мкг/кг	1,75±0,20	1,75±0,15	48±4,2*	6±0,8	0±0
Кетамин + церулеин	30 мг/кг+150 мкг/кг	1,20±0,18*	1,50±0,20	35±4,0**	6±0,9	0±0
Кетамин + церулеин	30 мг/кг+225 мкг/кг	0,83±0,12*	1,40±0,20	24±5,2**	4±0,5*	0±0
Кетамин + церулеин	30 мг/кг+375 мкг/кг	0,33±0,15**	1,32±0,15	11±1,6***	3±0,6**	0±0
Кетамин + галоперидол	30+0,1 мг/кг	1,50±0,20	1,42±0,20	58±5,2	6±0,9	0±0
Кетамин + галоперидол	30+0,5 мг/кг	1,20±0,16*	1,17±0,25	21±4,2**	4±0,5*	0±0
Кетамин + галоперидол	30+1,5 мг/кг	0,33±0,15**	1,83±0,20	4±0,2***	0±0***	0±0

Примечание. Звездочки — достоверность различия У-тест Манна-Уитни показателей по сравнению с введением кетамина: одна — $p<0,05$, две — $p<0,01$, три — $p<0,001$.

Таблица 2

Влияние церулеина и налоксона на амнестическое действие кетамина у крыс

Вещество	Доза	Латентный период перехода в темную камеру, с		Суммарное время пребывания в темной камере, с
		до обучения	после обучения	
Физиологический раствор		11±1,2	105±20	58±10
Кетамин	7,5 мг/кг	14±2,0	96±25	59±15
Кетамин	15 мг/кг	8±1,4	66±20	95±17
Кетамин	30 мг/кг	13±1,2	33±12	135±26
Кетамин+церулеин	15 мг/кг+ 10 мкг/кг	11±1,8	150±0**	0±0**
Кетамин+церулеин	30 мг/кг+ 10 мкг/кг	9±2,0	158±15*	3±3**
Церулеин	10 мкг/кг	12±2,0	120±15	47±12
Кетамин+налоксон	30+5 мг/кг	10±2,2	180±0**	0±0**
Налоксон	5 мг/кг	14±1,8	110±25	45±15

Примечание. Звездочки — достоверность различия (*t*-тест Стьюдента) показателей по сравнению с введением кетамина: одна — $p<0.05$, две — $p<0.01$.

спироперидола (0,25 нМ) в хвостатом ядре (дофамин$_2$-рецепторы) и во фронтальной коре (серотонин$_2$-рецепторы). Кетамин вызывал полуингибирование связывания ^{3}H-эторфина (0,25 нМ) при концентрации 30 мкМ. При дальнейшем повышении концентрации кетамина его влияние на связывание ^{3}H-эторфина не изменялось.

Таким образом, полученные данные свидетельствуют о том, что агонист ХЦК-8 рецепторов церулеин способен антагонизировать определенным поведенческим эффектам кетамина — стимулятора фенциклидиновых рецепторов. Высказано предположение, что двигательное возбуждение, вызванное фенциклидином, у крыс и мышей реализуется через серотонин$_2$-рецепторы [9]. Однако по данным настоящего исследования, кетамин такое действие не оказывал. Кетамин не взаимодействовал с серотонин$_2$-рецепторами и не вызывал поведенческих эффектов, характерных для серотониномиметиков (встряхивание головой, встряхивания «мокрой собаки» и т. д.). По всей вероятности, стереотипное поведение и двигательное возбуждение, наблюдаемые после введения кетамина, обусловлены, как и в случае фенамина, усилением высвобождения дофамина из пресинаптических терминалей в хвостатом ядре и мезолимбических структурах. Как известно, церулеин оказывает антидофаминергическое действие [13, 15]. С антидофаминергическим действием церулеина связан, вероятно, и антагонизм церулеина со стереотипным поведением и двигательным возбуждением, вызванных кетамином. В пользу этого предположения свидетельствует и факт, что галоперидол, преимущественный антагонист дофамин$_2$-рецепторов, оказывает аналогичное с церулеином угнетающее влияние на поведенческие эффекты кетамина у мышей. По данным Contreras и соавт. [3], атаксия, наблюдаемая после введения кетамина и фенциклидина, реализуется по сравнению со стереотипным поведением через другие механизмы. Этим обстоятельством, по-видимому, обусловлено и слабое влияние церулеина на кетаминовую атаксию. В амнестическом действии кетамина взаимодействие препарата с опиоидными рецепторами имеет ведущее значение, как и в случае с фенциклидином [11]. Опиоидный антагонист налоксон является эффективным антагонистом амнестического действия кетамина. Установлено, что церулеин в дозе 10 мкг/кг и меньше блокирует аналгезию, вызванную морфином у крыс [8]. Можно полагать, что функциональный антагонизм с опиоидными рецепторами находится в основе антагонизма церулеина с амнестическим действием кетамина.

ЛИТЕРАТУРА

1. *Allen R. M., Young S. J.* // Amer. J. Psychiat. — 1978. — Vol. 135. — P. 1081—1089.
2. *Burns R. S., Lerner S. E.* // Clin. Toxicol. — 1976. — Vol. 9. — P. 477—501.
3. *Contreras P. C., Rice K. C., Jacobson A. E., O'Donohue T. L.* // Europ. J. Pharmacol. — 1986. — Vol. 121. — P. 9—18.
4. *Costall B., Naylor R. J.* // Ibid. — 1974. — Vol. 29. — P. 206—222.
5. *Creese I., Schneider R., Snyder S. H.* // Ibid. — 1977. — Vol. 45. — P. 377—381.
6. *Ghoneim M. M., Hinrichs J. V., Mewaldt S. P., Petersen R. C.* // J. clin. Psychopharmacol. — 1985. — Vol. 5. — P. 70—77.
7. *Katsuura G., Itoh S.* // Drug Dev. Res. — 1986. — Vol. 7. — P. 269—276.
8. *Magnani M., Mantovani P., Pepeu G.* // Neuropharmacology. — 1984. — Vol. 23. — P. 1305—1309.
9. *Nabeshima T., Yamaguchi K., Yamada K.* et al. // Europ. J. Pharmacol. — 1983. — Vol. 93. — P. 229—234.
10. *Nabeshima T., Noda Y., Matsuno K.* et al. // Res. Commun. Subst. Abuse. — 1984. — Vol. 5. — P. 81—87.
11. *Nabeshima T., Kozawa T., Furukawa H., Kameyama T.* // Psychopharmacology. — 1986. — Vol. 89. — P. 334—337.
12. *Owen F., Bourne R. C., Poulter M.* et al. // Brit. J. Psychiat. — 1985. — Vol. 146. — P. 507—509.
13. *Vasar E., Maimets M., Nurk A.* et al. // Pharm. Biochem. Behav. — 1986. — Vol. 24. — P. 469—478.
14. *Vincent J. P., Kartalovski B., Geneste P.* et al. // Proc. nat. Acad. Sci. USA. — 1979. — Vol. 76. — P. 4678—4682.
15. *Zetler G.* // Peptides. — 1985. — Vol. 6. — P. 33—46.

Поступила 20.06.87

CAERULEIN, AN AGONIST OF CCK-8 RECEPTORS, ANTAGONIZES THE BEHAVIOURAL EFFECTS OF KETAMINE IN MICE AND RATS

E. E. Vasar, L. Kh. Allikmets, A. Kh. Soosaar

Tartu State University

It has been established in experiments on male mice and rats that caerulein antagonized the behavioural effects of ketamine, an agonist of phencyclidine receptors. Caerulein (75-375 μg/kg) and haloperidol (0.1-1.5 mg/kg) suppressed the stereotyped behaviour and motor excitation induced by ketamine (30 mg/kg) in mice. Caerulein and haloperidol failed to affect ketamine-induced ataxia. Caerulein (10 μg/kg) and the opioid antagonist naloxone (5 mg/kg) completely blocked the amnestic action of ketamine (30 mg/kg) in passive avoidance experiments on rats. It seems likely that the suppression of the behavioural effects of ketamine by caerulein is related to its functional antagonism with dopamine and opioid receptors.

ISSN 0365-9615

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

МОСКВА • МЕДИЦИНА •

Бюл. эксперим. биологии и медицины. 1988. СV. 2. 129–253

15. *Vernes A. // Lancet.* 1980. Vol. 2. N 8207. P. 1297—1298.

Поступила 14.01.87

NBT-TEST FOR THE EVALUATION OF THE FUNCTIONAL STATE OF LEUKOCYTES IN MICE INFECTED WITH MALARIA

E. Gilpin Jackson, B. F. Shadrin

E. I. Martsinovsky Institute of Medical Parasitology and Tropical Medicine, Ministry of Health of the USSR, Moscow

NBT-test for circulating neutrophils and monocytes in the blood of mice inoculated with *Plasmodium berghei*, strain N or LNK 65, have been performed. Within the first 24 h of the infection, before the onset of the registerable parasitemia or in the course of the subsequent six days (depending of the strain used for inoculation) a 50—100% reduction in NBT positive cells was observed. This demonstrates the ability of malaria parasite to suppress the oxygen dependent enzyme system in circulating phagocytes, neutrophils and monocytes of the host blood. The results of NBT test could be utilized for the investigation of immunological disorders and also for the differential diagnosis of malarial infection.

ФАРМАКОЛОГИЯ

УДК 612.821.014.46:615.357.34:577.175.734

Ключевые слова: *церулеин, фенамин, хинолиновая кислота, двигательное возбуждение, судороги*

Э. Э. Васар, Л. Х. Алликметс, И. В. Рыжов, И. Б. Прахье, А. Х. Соосаар, С. Мирзаев

ВИДОВЫЕ РАЗЛИЧИЯ В ПОВЕДЕНЧЕСКИХ ЭФФЕКТАХ ЦЕРУЛЕИНА — АГОНИСТА РЕЦЕПТОРОВ ОКТАПЕПТИДА ХОЛЕЦИСТОКИНИНА — У БЕЛЫХ МЫШЕЙ И КРЫС

Лаборатория психофармакологии НИИ общей и молекулярной патологии Тартуского университета, лаборатория психофармакологии Ленинградского научно-исследовательского психоневрологического института им. В. М. Бехтерева

Представлена акад. АМН СССР А. В. Вальдманом

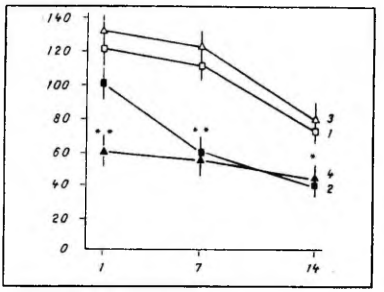
Октапептид холецистокинина (ХЦК-8) и его близкий аналог церулеин обладают широким спектром фармакологического действия [15]. Они угнетают спонтанную двигательную активность, противодействуют фенаминовому двигательному возбуждению, блокируют стереотипное поведение, вызванное дофаминомиметиками, оказывают противосудорожное действие и т. д. [3, 8, 14, 15]. Однако не всегда исследователям удавалось в своих опытах воспроизвести результаты, полученные другими авторами. Так, в одних исследованиях церулеин и ХЦК-8 угнетали поведенческие эффекты апоморфина [12, 15], в других наблюдалось противоположное действие — усиление эффектов этого дофаминомиметика [2, 13].

В настоящем исследовании была поставлена цель выяснить причину такой разноречивости данных. В связи с этим мы изучили видовые различия в действии церулеина — агониста рецепторов ХЦК-8. В опытах на мышах-самцах и крысах-самцах в сравнительном аспекте были изучены длительное антифенаминовое действие церулеина [7] и антагонизм церулеина с эндогенным конвульсантом хинолиновой кислотой (ХИК) [1, 11].

Методика исследования. В опытах использовали белых беспородных мышей-самцов массой 18—24 г и беспородных крыс-самцов массой 180—220 г из питомника «Раппполово» АМН СССР (Ленинградская область) в весенне-летний период.

Антагонизм с фенаминовым двигательным возбуждением изучали по следующей схеме: в первый день опыта одной группе крыс или мышей внутрибрюшинно вводили физиологический раствор, другой — подкожно церулеин (крысам — 40 мкг/кг, мышам — 50 и 100 мкг/кг), третьей — внутрибрюшинно галоперидол (0,25 мг/кг), четвертой — галоперидол совместно с церулеином. Опыты с фенамином были проведены трижды: через 1 сут, на 7-е и 14-е сутки после однократного введения галоперидола («Gedeon Richter», ВНР) и церулеина («Farmitalia — Carlo Erba», Италия). Возбуждающее действие фенамина (3 мг/кг) на моторику мышей определяли с помощью фотоэлектрического актометра. Животных помещали в актометр через 15 мин после внутрибрюшинного введения фенамина и в течение 30 мин определяли двигательную активность животных. Влияние фенамина (2 мг/кг) на двигательную активность крыс определяли через 45 мин после внутрибрюшинного введения в течение 5 мин в тесте открытого поля (регистрация фотоэлектрическим способом с помощью 5 независимых каналов). В отдельной серии опытов определяли действие совместного введения проглумида (50 мг/кг, «Rotta Research Labs», Италия) — антагониста рецепторов ХЦК-8 — с галоперидолом и церулеином на длительное антифенаминовое действие церулеина у крыс.

Влияние церулеина на эффекты различных конвульсантов у мышей определяли следующим об-



Влияние однократного предварительного подкожного введения церулеина (40 мкг/кг) и внутрибрюшинного введения галоперидола (0,25 мг/кг) на двигательное возбуждение, вызванное фенамином (2 мг/кг) у крыс.

По оси абсцисс — срок после однократного введения церулеина или галоперидола (в сут), по оси ординат — число импульсов в течение 5 мин. *1* — контроль (действие фенамина у крыс, получивших предварительно физиологический раствор); *2* — церулеин; *3* — галоперидол; *4* — галоперидол + церулеин. Одна звездочка — $p < 0.05$, две — $p < 0.01$ по сравнению с контрольными животными, получавшими физиологический раствор (U-тест Манна — Уитни).

разом: ХИК (5 мкг), L-каиновую кислоту (0,2 мкг), L-кинуренина сульфат (50 мкг) и Н-метил-D-аспартат (0,1 мкг; все — фирмы «Sigma», США) вводили в латеральный желудочек мозга с помощью полуавтоматического аппарата по ранее описанной методике [5] в судорожных дозах ($ЭД_{100}$) в постоянном объеме 2 мкл. Проглумид вводили мышам внутрибрюшинно за 5 мин до внутрижелудочкового введения церулеина или за 10 мин до конвульсанта. Церулеин (1—50 нг в желудочек мозга и 100—500 мкг/кг подкожно) вводили за 5 мин до введения конвульсантов. Длительность наблюдения после введения конвульсанта составляла 10 мин.

В опытах на крысах изучали судороги, вызванные ХИК, и церулеин вводили подкожно в дозе 200 мкг/кг или в желудочек мозга в дозах 2—20 нг за 5 мин до ХИК (30 и 60 мкг в желудочек мозга). Вживление канюли в левый боковой желудочек крыс проводили под нембуталовым наркозом (40—50 мг/кг). Детально метод вживления описан ранее [6]. В эксперимент животных брали через 4—5 сут после операции. Растворы препаратов вводили с помощью шприца Гамильтона и полиэтиленовой трубочки. За поведением крыс наблюдали в течение 90 мин после введения конвульсанта. Во всех группах определяли 4 показателя: латентный период наступления клонических судорог, частоту клонических и тонических экстензий и летальность в группе.

Результаты исследования. У крыс однократное совместное введение церулеина и галоперидола и в меньшей степени введение одного церулеина оказывало длительное ингибирующее влияние на возбуждающее действие 2 мг/кг фенамина (см. рисунок). Уже через 1 сут после совместного введения церулеина и галоперидола четко проявилось их антифенаминовое действие. Существует мнение, что длительное ингибирующее влияние совместного введения галоперидола и церулеина на фенаминовое возбуждение моторики реализуется через β-эндорфин в прилегающем ядре [7]. Наши данные указывают на то, что влияние церулеина на β-эндорфинергические процессы опосредуется через рецепторы ХЦК-8. В пользу этого предположения свидетельствует тот факт, что антифенаминовое действие развивается и после введения одного церулеина. Однако проглумид (50 мг/кг), известный антагонист ХЦК-8, не устранял эффекта, вызванного совместным введением галоперидола и церулеина. Это позволяет полагать, что проглумид не взаимодействует с рецепторами ХЦК-8. Роль галоперидола заключается в повышении чувствительности опиоидных рецепторов к β-эндорфину в мезолимбических структурах [10]. В опытах на мышах церулеин и галоперидол такого антифенаминового действия не оказывали. Предварительное введение церулеина (50 и 100 мкг/кг) и совместное введение церулеина и галоперидола даже усиливало эффект фенамина, т. е. наблюдалась гиперчувствительность к возбуждающему действию фенамина у мышей (см. таблицу).

Влияние однократного предварительного подкожного введения церулеина (50—100 мкг/кг) и внутрибрюшинного введения галоперидола (0,25 мг/кг) на двигательное возбуждение, вызванное фенамином (3 мг/кг) у мышей ($M \pm m$)

Вещество	Число импульсов в течение 30 мин	
	1-й день	7-й день
Физиологический раствор + физиологический раствор	314±36	356±34
Физиологический раствор + фенамин (3 мг/кг)	589±69	496±57
Церулеин (50 мг/кг) + фенамин (3 мг/кг)	540±61	615±62
Церулеин (100 мг/кг + фенамин (3 мг/кг)	854±98*	563±70
Галоперидол (0,25 мг/кг + фенамин (3 мг/кг)	717±58	578±69
Галоперидол (0,25 мг/кг) + церулеин (50 мг/кг) + фенамин (3 мг/кг)	815±80*	799±80*
Галоперидол (0,25 мг/кг) + церулеин (100 мг/кг) + фенамин (3 мг/кг)	814±76*	747±76*

Примечание. Звездочка — $p < 0,05$ (тест *U* Манна—Уитни) по сравнению с группой мышей, получивших физиологический раствор + фенамин (3 мг/кг).

Введение церулеина (1 нг) в желудочек мозга предупреждало у мышей судороги, вызванные ХИК. Проглумид (50 мг/кг) полностью устранял защитный эффект церулеина. В меньшей дозе (25 мг/кг) проглумид потенцировал судорожный эффект подпороговой дозы ХИК (2,5 мкг) уве-

личивал число животных с судорогами от 0 до 5 в группе из 6 мышей. Следует отметить, что противосудорожное действие церулеина в данной модели имеет, по-видимому, довольно избирательный характер. Церулеин предупреждал вызванные только ХИК и Н-метил-D-аспартатом судороги. Это подтверждает предположение о том, что данные вещества действуют на один общий Н-метил-D-аспартатный рецептор [11]. Церулеин был неактивен против каиновой кислоты и кинуренина. При подкожном введении мышам церулеин в большом диапазоне доз (100—500 мкг/кг) слабо влиял на ХИК-судороги, удлинив лишь латентный период их наступления. Предварительное подкожное (200 мкг/кг) или внутрижелудочковое введение (2—20 нг) церулеина не препятствовало развитию ХИК-судорог у крыс, не изменялись ни количество судорожных приступов, ни латентные периоды клонических и тонических судорог. Продолжительность жизни животных была даже короче в опытной группе по сравнению с контролем (соответственно 52 и 92 мин).

Таким образом, полученные данные свидетельствуют о значительных различиях в действии церулеина на крыс и мышей. Неодинаковое влияние церулеина на возбуждающее действие фенамина, по-видимому, обусловлено различиями во взаимодействии между ХЦК-8 и дофаминергическими системами у крыс и мышей. Если в опытах на мышах церулеин при подкожном введении в дозе 75 мкг/кг и более устранял повышенную двигательную активность, вызванную фенамином [13], то у крыс доза 40 мкг/кг церулеина (при подкожном введении) не изменяла эффект фенамина [7]. На основании этих данных можно полагать, что у мышей церулеин при системном введении оказывает непосредственное угнетающее влияние на дофаминергические процессы в лимбических структурах, что обусловливает повышенную чувствительность мышей к возбуждающему действию фенамина после однократного введения церулеина. У крыс взаимодействие между дофамином и ХЦК-8 является более сложным и, возможно, опосредуется через усиление высвобождения β-эндорфина в прилегающем ядре [7], что в конечном счете приводит к длительному понижению чувствительности крыс к возбуждающему действию фенамина.

В опытах на мышах церулеин при внутрижелудочковом (но не при системном) введении был сильным и избирательным антагонистом эндогенных конвульсантов — ХИК и Н-метил-D-аспартата. Следует отметить, что у травяной лягушки (Rana temporaria), в коже которой обнаружено большое количество церулеина, его предварительное введение (1—5 нг) также предупреждало ХИК-судороги, а проглумид (50—100 мг/кг) снимал этот эффект. В опытах на крысах церулеин такого действия не оказывал. Кроме того, у крыс при внутрижелудочковом его введении ХИК-судороги развивались значительно медленнее, чем у мышей. Вероятным объяснением этих различий у крыс и мышей является неодинаковое пространственное расположение латеральных желудочка и гиппокампа в мозге [4, 6, 9], что обусловливает неодинаковое проникновение исследуемых веществ в гиппокамп при их внутрижелудочковом введении.

ЛИТЕРАТУРА

1. *Лапин И. П., Ремеев И. В.* // Фармакол. и токсикол. — 1985. — № 6. — С. 13—17.
2. *Crawley J. N.* // Psychopharmacol. Bull. — 1985. — Vol. 21. — P. 523—527.
3. *Ellinwood E. H., Rockwell W. J. K., Wagoner N.* // Pharmacol. Biochem. Behav. — 1983. — Vol. 19. — P. 969—971.
4. *König J. F. R., Klippel R. A.* The rat brain: A stereotaxic atlas of the Forebrain and Lower Part of the Brain Stem. — Baltimore, 1963.
5. *Lapin I. P.* // J. Neural Transm. — 1978. — Vol. 42.— P. 37—43.
6. *Lapin I. P., Prukhie I. B., Kiseleva I. P.* // J. Neural Transm. — 1982. — Vol. 54. — P. 229—238.
7. *Matsubara K., Matsushita A.* // Jap. J. Pharmacol. — 1986. — Vol. 40. — P. 417—422.
8. *Matsushita A., Ueki S.* // World Psychiatric Accociation Regional Symposium / Eds. H. Ohashi et al. — Tokyo, 1982. — P. 170—173.
9. *Slotnick B. M., Leonard C. M.* A Stereotaxic Atlas of the Albino Mouse fore Brain. — Rockville, 1975.
10. *Stinus L., Nadaud D., Jauregui J., Kelley A. E.* // Biol. Psychiat. — 1986. — Vol. 21. — P. 34—38.
11. *Stone T. W., Connick J. H.* // Neurosci. — 1985. — Vol. 15. — P. 597—617.
12. *Van Ree J. M., Gaffori O., De Wied D.* // Europ. J. Pharmacol. — 1983. — Vol. 93. — P. 63—78.
13. *Vasar E., Maimets M., Nurk A. et al.* // Pharmacol. Biochem. Behav. — 1986. — Vol. 24. — P. 469—478.
14. *Zetler G.* // Neuropharmacology. — 1981. — Vol. 20.
15. *Zetler G.* // Peptides. — 1985. — Vol. 6, Suppl. 3. — P. 33—46.

Поступила 25.11.86

INTERSPECIES DIFFERENCES IN THE BEHAVIOURAL EFFECTS OF CAERULEIN, AN AGONIST OF CCK-8 RECEPTORS, IN MICE AND RATS

E. E. Vasar, L. Kh. Allikmets, I. V. Ryzhov, I. B. Prakhye, A. Kh. Sorsar, S. Mirzaev

Tarty State University; V. M. Bekhterev Institute of Psychoneurology, Leningrad

It has been shown in the behavioural experiments that combined pretreatment with haloperidol (0,25 mg/kg) and caerulein (40 μg/kg), and to a lesser extent pretreatment with cerulein alone caused long-term reversal of amphetamine (2 mg/kg) induced hyperexcitability in rats. Administration of proglumide (50 mg/kg), an antagonist of CCK-8 receptors, did not reverse long-term antiamphetamine effect of caerulein. In mice pretreatment with caerulein (50 and 100 μg/kg) alone or in combination with haloperidol (0.25 mg/kg) caused hypersensitivity to the behavioural effect of amphetamine (3 mg/kg). Intraventricular (1 ng), but not systemic (100—500 μg/kg) administration of caerulein selectively antagonized seizures in mice induced by intraventricular administration of quinolinic acid (5 μg) and N-methyl-D-aspartate (0,2 μg). Pretreatment with proglumide (50 mg/kg) reversed the anticonvulsive effect of caerulein in mice. In rats, caerulein failed to affect the seizures caused by intraventricular administration of quinolinic acid.

EESTI NSV TEADUSTE AKADEEMIA TOIMETISED. BIOLOOGIA
ИЗВЕСТИЯ АКАДЕМИИ НАУК ЭСТОНСКОЙ ССР. БИОЛОГИЯ
PROCEEDINGS OF THE ACADEMY OF SCIENCES OF THE ESTONIAN SSR. BIOLOGY

1988, 37, 2

УДК 615.767.22

Ээро ВАСАР, Андрес СООСААР, Аавo ЛАНГ

УЧАСТИЕ ХОЛЕЦИСТОКИНИНОВЫХ РЕЦЕПТОРОВ В РЕАЛИЗАЦИИ ПОВЕДЕНЧЕСКИХ И БИОХИМИЧЕСКИХ ЭФФЕКТОВ ДЛИТЕЛЬНОГО ВВЕДЕНИЯ ГАЛОПЕРИДОЛА

Длительное введение нейролептиков, эффективных и распространенных антипсихопатических веществ, вызывает весьма разнонаправленные изменения в активности нейромедиаторных систем мозга (Алликметс и др., 1984). Среди этих изменений наиболее значимыми являются сдвиги в плотности нейрональных рецепторов центральной нервной системы (Алликметс и др., 1984, 1986). Установлено, что длительное введение нейролептиков вызывает увеличение числа дофамин$_2$- и глутаматных рецепторов в переднем мозге (Жарковский, Алликметс, 1986), в то время как плотность ГАМК$_A$- и бензодиазепиновых рецепторов понижается (Алликметс и др., 1986). Отражением этих изменений на молекулярном уровне является гиперчувствительность подопытных животных к поведенческим эффектам дофаминомиметиков (Жарковский, Алликметс, 1986), поведенческие же эффекты агониста ГАМК$_A$-рецепторов мусцимола и бензодиазепинового антагониста Ro 15-1788 извращаются (Васар и др., 1986). В последние годы появились данные, что длительное применение различных по химической структуре нейролептиков (галоперидола, хлорпромазина и клозапина) вызывает заметное увеличение содержания октапептида холецистокинина (ХЦК-8) в подкорковых структурах мозга (Frey, 1983). Установлено, что длительное введение типичного нейролептика галоперидола понижает плотность ХЦК-8 рецепторов в переднем мозге (Васар и др., 1986) и ослабляет поведенческие эффекты агониста ХЦК-8 рецепторов церулеина, вызывая к ним гипочувствительность. Показано, что ХЦК-8 является сомедиатором дофамина в мезолимбических структурах (Hökfelt и др., 1980), а ГАМК — в гиппокампе и корковых структурах больших полушарий (Kosaka и др., 1985). Целью настоящего исследования было изучение роли ХЦК-8 рецепторов в тех поведенческих и нейрохимических изменениях, которые наблюдаются после длительного введения галоперидола.

Методика

Опыты проводили на крысах (самцы массой 220—270 г) и мышах (самцы массой 20—25 г). На крысах провели два исследования: первый в октябре, второй — в декабре 1986 г. Следует отметить, что второй опыт был завершен непосредственно перед резким похолоданием. В течение 15 дней крысам вводили внутрибрюшинно галоперидол (0,5 мг/кг в день, «Gedeon Richter», Венгрия) или физиологический раствор. Через 72 ч после отмены галоперидола животных разделили на две группы: одни были использованы для поведенческих исследований, другие — для опытов радиолигандного связывания. Перед началом поведенческих опытов половине крыс подкожно ввели церулеин (40 мкг/кг, «Farmitalia-Carlo Erba», Италия), остальным — физиологи-

ческий раствор. После введения дважды — через 24 ч и через 7 дней — определяли основные поведенческие эффекты непрямого дофаминомиметика фенамина (2 мг/кг): стереотипное поведение по условной шкале (Costall, Naylor, 1974) и усиление ориентировочно-исследовательской активности. Первое исследовали через 30 мин после введения фенамина, второе — через 45 мин. Ориентировочно-исследовательскую активность оценивали на открытом поле (100×100×40 см), где в течение 5 мин с помощью независимых фотоэлектрических каналов определяли двигательную активность крыс.

Радиолигандное связывание определяли на основе параметров связывания ^{3}H-пентагастрина (уд. активность 81 Ки/ммоль, NEN, США) и ^{3}H-флунитразепама (уд. активность 81 Ки/ммоль, «Amersham International», Англия) в коре больших полушарий, и ^{3}H-спироперидола (уд. активность 17 Ки/ммоль, «Amersham International», Англия) в хвостатом ядре. Связывание ^{3}H-пентагастрина, лиганда центральных ХЦК-8 рецепторов, проводили по методике М. Преизманна (Praissman и др., 1983), связывание ^{3}H-флунитразепама и ^{3}H-спироперидола — по методике, описанной нами ранее (Нурк и др., 1984). Данные опытов обрабатывали с помощью анализа Скетчарда.

В отдельной серии опытов на мышах-самцах в течение 15 дней определяли влияние длительного введения галоперидола (0,5 мг/кг в день) и церулеина (0,1 мг/кг в день) на поведенческие эффекты фенамина, мусцимола («Serva», ФРГ) и Ro 15-1788 («Hoffmann-La Roche», Швейцария). Фенамин (3 мг/кг), мусцимол (1 мг/кг) и Ro 15-1788 (10 мг/кг) вводили за 15 мин до помещения мышей в фотоэлектрический актометр (диаметр 40 см). Параллельно с поведенческими опытами исследовали связывание ^{3}H-спироперидола, ^{3}H-пентагастрина, ^{3}H-флунитразепама и ^{3}H-эторфина (уд. активность 36 Ки/ммоль, «Amersham International», Англия) по методике Owen и др., 1985.

Результаты исследования и обсуждение

Результаты двух независимых исследований существенным образом различались (табл. 1). Если в октябре фенамин у контрольных крыс вызывал характерное усиление двигательной активности, то в декабре, как ни парадоксально, он таким действием не обладал. В октябре длительное введение галоперидола усиливало поведенческие эффекты фенамина: двигательную активность больше, стереотипную — меньше. В декабре действие фенамина ослабилось (табл. 1). Неодинаковым было в этих двух опытах и действие однократно введенного церулеина: в октябре он устранял повышенную чувствительность к фенамину, вызванную длительным введением галоперидола, в декабре, наоборот, восстанавливал (табл. 1).

Заметно отличалось и действие длительного введения галоперидола на связывание различных радиолигандов (табл. 2). В октябре отмечали достоверное увеличение плотности мест связывания ^{3}H-спироперидола в хвостатом ядре (дофамин$_2$-рецепторы) и понижение ^{3}H-флунитразепама и ^{3}H-пентагастрина (ХЦК-8 рецепторы) в коре больших полушарий. Эти изменения характерны длительному введению галоперидола (Bacar и др., 1986). В декабре установили лишь умеренное увеличение числа дофамин$_2$-рецепторов, в то время как плотность бензодиазепиновых и ХЦК-8 рецепторов имела даже тенденцию к повышению (табл. 2).

В опытах на мышах длительное введение галоперидола и церулеина оказывало весьма сходное влияние на поведенческие эффекты фенамина, мусцимола и Ro 15-1788 (табл. 3). После их длительного введе-

Таблица 1

Влияние длительного введения галоперидола (0,5 мг/кг в день, в течение 15 дней) на поведенческие эффекты фенамина (2 мг/кг) и церулеина (40 мкг/кг)

	I опыт (октябрь)					II опыт (декабрь)				
	Физиологический раствор + физиологический раствор	Физиологический раствор + фенамин	Физиологический раствор + церулеин + фенамин	Галоперидол + фенамин	Галоперидол + церулеин + фенамин	Физиологический раствор + физиологический раствор	Физиологический раствор + фенамин	Физиологический раствор + церулеин + фенамин	Галоперидол + фенамин	Галоперидол + церулеин + фенамин
1-й день										
Стереотипная активность, баллы	0	1,28±0,12	1,13±0,14	1,57±0,15	1,20±0,13*	0	1,16±0,15	0,84±0,14	1,38±0,12	1,44±0,20
Двигательная активность, имп / 5 мин	38±3,6	106±7,2	105±6,4	138±8,2	75±6,3*	44±5,2	57±4,8	55±5,3	39±4,0	81±5,6*
7-й день										
Стереотипная активность, баллы	0	1,52±0,15	1,09±0,17*	1,53±0,18	1,11±0,12*	0	1,28±0,13	1,21±0,15	1,43±0,12	1,63±0,15
Двигательная активность, имп / 5 мин	32±3,8	104±8,2	71±6,9*	108±7,9	54±5,6*	38±4,6	46±4,5	40±3,8	34±3,0	91±8,0*

* $p<0.05$ (по u-тесту Манна—Уйтни).

Таблица 2

Влияние длительного введения галоперидола (0,5 мг/кг, в течение 15 дней) на дофамин$_2$-, бензодиазепиновые и ХЦК-8 рецепторы в мозге крыс

	I опыт (октябрь)			II опыт (декабрь)		
	Физиологический раствор	Галоперидол	%	Физиологический раствор	Галоперидол	%
^{3}H-спироперидол						
$K_д$, нМ	0,47±0,06	0,46±0,07	98	0,60±0,07	0,58±0,05	97
$Св_{макс}$, фмолей/мг белка	352±25	460±28*	131	382±24	425±20	111
^{3}H-флунитразепам						
$K_д$, нМ	2,56±0,20	3,09±0,25	121	1,84±0,25	1,52±0,15	83
$Св_{макс}$, фмолей/мг белка	2930±280	2380±270*	81	1535±180	1690±150	110
^{3}H-нентагастрин						
$K_д$, нМ	1,07±0,10	1,01±0,12	94	0,80±0,06	0,69±0,07	86
$Св_{макс}$, фмолей/мг белка	40,2±2,0	33,2±2,5*	83	39,2±2,5	42,0±2,7	107

* $p<0,05$ (t — тест Стьюдента). $Св_{макс}$ — число мест связывания; $K_д$ — константа диссоциации.

Таблица 3

Влияние длительного введения (15 дней) галоперидола (0,5 мг/кг в день) и церулеина (0,1 мг/кг в день) на действие фенамина, мусцимола и Ro 15—1788 при изучении двигательной активности мышей, имп/30 мин

Вещество, доза	Физиологический раствор	Галоперидол	Церулеин
Физиологический раствор	171±15	188±14	184±18
Фенамин (3 мг/кг)	409±30	598±45*	704±62*
Мусцимол (1 мг/кг)	89±10	203±36*	170±28*
Ro (15—1788 (10 мг/кг)	261±17	162±15*	193±16*

* $p<0,05$ (y-тест Манна—Уйтни, по сравнению с длительным введением физиологического раствора).

ния фенамин (3 мг/кг) еще сильнее стимулировал двигательную активность мышей, в то время как к эффектам мусцимола (1 мг/кг) и Ro 15-1788 (10 мг/кг) развивалась толерантность. Мусцимол не был способен угнетать двигательную активность, а Ro 15-1788 больше не оказывал стимулирующего влияния на поведение мышей (табл. 3). Сходным было и влияние длительного введения галоперидола и церулеина на плотность разных рецепторов в головном мозге мышей (табл. 4). Под их влиянием повысилось число дофамин$_2$-рецепторов в хвостатом ядре и опиоидных рецепторов в лимбических структурах. Число ХЦК-8 рецепторов уменьшилось в коре больших полушарий как после длительного применения галоперидола, так и церулеина (табл. 4). Плотность бензодиазепиновых рецепторов изменялась в зависимости от исследованных структур. Если в переднем мозге галоперидол и церулеин уменьшали их число, то в стволе мозга наблюдалось достоверное их повышение.

Сравнение данных двух независимых исследований, проведенных в октябре и декабре, дает нам основание полагать, что чувствительность дофаминовых рецепторов во многом зависит от функционального состояния ХЦК-8 и бензодиазепиновых рецепторов в переднем мозге. Гиперчувствительность на дофаминовых рецепторах в хвостатом ядре и мезолимбических структурах развивается только при существенном понижении числа бензодиазепиновых и ХЦК-8 рецепторов в коре больших полушарий при длительном введении галоперидола. На фоне некоторого увеличения их числа в декабре наблюдается понижение чувствительности дофаминовых рецепторов на двигательную активность крыс, о чем свидетельствует ослабление стимулирующего влияния индиректного дофаминомиметика фенамина. Следует отметить, что поведенческие и биохимические изменения, вызванные галоперидолом в октябре, типичны, в то время как в декабре галоперидол оказывал парадоксальное действие. По всей вероятности сдвиги последнего можно связывать с метеорологическими условиями, а именно, с быстрым и резким похолоданием. От изменения числа ХЦК-8 и бензодиазепиновых рецепторов зависит и действие церулеина после длительного введения галоперидола. Однако в любом случае церулеин изменял чувствительность дофаминовых рецепторов, вызванную галоперидолом. При развитии гиперчувствительности дофаминовых рецепторов однократное введение церулеина полностью устраняло усиление поведенческих эффектов фенамина, вызванное галоперидолом, а при пониженной чувствительности — восстанавливало. Уменьшение чувствительности дофаминовых рецепторов под влиянием церулеина хорошо согласуется с клиниче-

Таблица 4

Влияние длительного введения (15 дней) галоперидола (0,5 мг/кг в день) и церулеина (0,1 мг/кг в день) на связывание дофамин$_2$-, бензодиазепиновых, опиоидных и ХЦК-8 рецепторов в мозге мышей

Радиолиганды	Физиологический раствор		Галоперидол		Церулеин	
	$K_д$	$Св_{макс}$	$K_д$	$Св_{макс}$	$K_д$	$Св_{макс}$
^{3}H-спироперидол в стриатуме	0,47±0,05	348±30	0,62±0,05	450±25*	0,63±0,05	492±32**
^{3}H-флунитразепам в коре больших полушарий	1,70±0,25	1980±120	1,60±0,25	1440±150*	1,50±0,18	1380±140*
^{3}H-флунитразепам в стволе мозга	2,42±0,20	1030±80	1,92±0,18	1250±120	2,62±0,17	1420±160*
^{3}H-эторфин в лимбических структурах	0,62±0,05	328±24	0,61±0,05	420±25*	0,77±0,05	460±32*
^{3}H-пентагастрин в коре больших полушарий	3,50±0,40	50±5	3,20±0,30	35±3*	3,20±0,32	32±3*

* $p<0,05$; ** $p<0,01$ (t-тест Стьюдента по сравнению с длительным введением физиологического раствора). $K_д$ — константа диссоциации, нМ; $Св_{макс}$ — число мест связывания, фмолей/мг белка.

скими наблюдениями, в которых церулеин оказывает благоприятное влияние на симптоматику побочного эффекта нейролептического лечения — позднюю дискинезию (Nishikawa и др., 1986). Влияние церулеина на чувствительность дофаминовых рецепторов хорошо коррелирует с данными наших предыдущих исследований, где на мышах, отселектированных с помощью Н-пропилнорапоморфина, установлено противоположное влияние церулеина на связывание ^{3}Н-спироперидола в опытах *in vivo* (Vasar и др., 1986). У мышей, реагирующих на введение 100 мкг/кг Н-пропилнорапоморфина сильным усилением двигательной активности, церулеин значительно понижал связывание ^{3}Н-спироперидола, в то время как у мышей, реагирующих уменьшением двигательной активности, достоверно повышал. Можно полагать, что именно эти изменения в плотности дофаминовых рецепторов находятся в основе модулирующего влияния церулеина на поведенческие эффекты фенамина после длительного применения галоперидола. О существовании двух подтипов ХЦК-8 рецепторов, оказывающих противоположное влияние на дофаминергические процессы, свидетельствуют и данные других авторов (Voigt и др., 1986; Hommer и др., 1986). В прилегающем ядре выявлены два подтипа ХЦК-8 рецепторов, оказывающих противоположное влияние на высвобождение дофамина из пресинаптических терминалей (Voigt и др., 1986). В черном веществе существует также два подтипа ХЦК-8 рецепторов, противоположно влияющих на электрофизиологические параметры дофаминовых нейронов (Hommer и др., 1986).

О существовании заметного модулирующего влияния со стороны ХЦК-8 на эффекты длительного введения галоперидола свидетельствует и сравнительное изучение эффектов длительного введения галоперидола и церулеина. Их введение вызвало гиперчувствительность дофаминовых рецепторов, о чем свидетельствует усиление фенаминового двигательного возбуждения и повышение числа дофамин$_2$-рецепторов в хвостатом ядре. Под влиянием церулеина и галоперидола повышалось число опиоидных рецепторов в лимбических структурах, понижалось число бензодиазепиновых рецепторов в переднем мозге и увеличивалось в стволе мозга. Отражением этих молекулярных преобразований является полное исчезновение поведенческих эффектов мусцимола и Ro 15-1788. Длительное введение как церулеина, так и галоперидола вызывает значительное понижение числа ХЦК-8 рецепторов в коре больших полушарий. Недавно была выдвинута гипотеза, что длительное введение нейролептиков вызывает деполяризационную блокаду дофаминовых нейронов (Chiodo, Bunney, 1983). Фармакологический и электрофизиологический анализы показали, что ХЦК-8 оказывает подобное нейролептикам действие, в то время как антагонист ХЦК-8 рецепторов проглумид полностью устраняет деполяризационную блокаду, вызванную длительным введением нейролептиков (Bunney и др., 1985). Можно полагать, что одинаковое влияние длительного введения галоперидола и церулеина на поведение животных и на разные нейрональные рецепторы отражает деполяризационную блокаду дофаминовых нейронов. По всей вероятности, часть эффектов длительного введения нейролептиков реализуется именно через ХЦК-8 ергические механизмы.

ЛИТЕРАТУРА

Алликметс Л. Х., Жарковский А. М., Нурк А. М., Васар Э. Э., Майметс М. О., Ряго Л. К. Влияние длительного введения нейролептиков на пластичность рецепторов ЦНС. Вестник АМН СССР, 1984, № 11, 37–42

Алликметс Л. Х., Васар Э. Э., Нурк Л. М., Майметс М. О. Адаптационные изменения на ГАМК- и бензодиазепиновых рецепторах после длительного введения галоперидола. — В сб.: Фармакологическая регуляция состояний дезадаптации. М., 1986, 36—43.

Васар Э. Э., Нурк А. М., Майметс М. О., Соосаар А. Х., Алликметс Л. Х. Неодинаковое влияние длительного введения галоперидола на $ГАМК_А$- и бензодиазепиновые рецепторы в разных отделах мозга. — Бюл. эксп. биол. мед., 1986, № 4, 433—436.

Васар Э. Э., Соосаар А. Х., Майметс М. О., Алликметс Л. Х. Понижение чувствительности холецистокининовых рецепторов в мозге под влиянием длительного введения галоперидола. — Бюл. эксп. биол., мед., 1986, № 11, 583—585.

Жарковский А. М., Алликметс Л. Х. Влияние нейролептиков на рецепторы ЦНС при однократном и повторном применении. — В сб.: Всесоюз. симп. «Химия, фармакология и клиника нейролептиков». Тарту, 1986, 47—49.

Нурк А. М., Майметс М. О., Рягo Л. К., Васар Э. Э. Адаптационные изменения в ГАМК-ергической системе после отмены хронического применения галоперидола. — В сб.: Механизм действия и клиника производных гамма-аминомасляной кислоты. Уч. зап. ТГУ, вып. 687, Тарту, 1984, 17—27.

Bunney, B. S., Chiodo, L. A., Freeman, A. S. Further studies on the specificity of proglumide as a selective cholecystokinin antagonist in the central nervous system. — Ann. N. Y. Acad. Sci., 1985, **448**, 435—441.

Chiodo, L. A., Bunney, B. S. Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. — J. Neurosci. Res., 1983, **3**, 1607—1619.

Costall, B., Naylor, R. J. Stereotyped and circling behaviour induced by dopaminergic agonists after lesions of the midbrain raphe nuclei. — Eur. J. Pharmacol., 1974, **29**, 206—222.

Frey, P. Cholecystokinin octapeptide levels in rat brain are changed after subchronic neuroleptic treatment. — Eur. J. Pharmacol., 1983, **95**, 87—92.

Hommer, D. W., Stoner, G., Crawley, J. N., Paul, S. M., Skirboll, L. Cholecystokinin-dopamine coexistence: electrophysiological actions corresponding to cholecystokinin receptor subtype. — J. Neurosci. Res., 1986, **6**, 3039—3043.

Hökfelt, T., Skirboll, L., Rehfeld, J. F., Goldstein, M., Markey, K., Dann, O. A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence for immunochemistry combined with retrograde tracing. — J. Neurosci. Res., 1980, **5**, 2093—2124.

Kosaka, T., Kosaka, K., Tateishi, K., Hamaoka, Y., Yanaihara, N., Wu, J.-Y., Hama, K. GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivies in the rat hippocampus and dentate gyrus. — J. Comp. Neurol., 1985, **239**, 420—430.

Nishikawa, T., Tanaka, M., Tsuda, A., Kuwahara, H., Koga, I., Uchida, Y. Effect of ceruletide on tardive dyskinesia: a pilot study of quantitative computer analysis on electromyogram and microvibration. — Psychopharmacol., 1986, **90**, 5—8.

Owen, F., Bourne, R. C., Poulter, M., Crow, T. J., Paterson, S. J., Kosterlitz, H. Tritiated etorphine and naloxone binding to opioid receptors in caudate nucleus in schizophrenia. — Brit. J. Psychiat., 1985, **146**, 507—509.

Praissman, M., Martinez, P. A., Saladino, C. F., Berkowitz, J. M., Steggles, A. W., Finkelstein, J. A. Characterization of cholecystokinin binding sites in rat cerebral cortex using a ^{125}I-CCK-8 probe resistant to degradation. — J. Neurochem., 1983, **40**, 1406—1413.

Vasar, E., Maimets, M., Nurk, A., Soosaar, A., Allikmets, L. Comparison of motor depressant effects of caerulein and N-propylnoraporphine in mice. — Pharmacol. Biochem. Behav., 1986, **24**, 469—478.

Voigt, M., Wang, R. Y., Westfall, T. C. Cholecystokinin octapeptides alter the release of endogenous dopamine from the rat nucleus accumbens in vitro. — J. Pharmacol. Exp. Ther., 1986, **237**, 147—153.

Тартуский государственный университет

Поступила в редакцию
28/IV 1987

Eero VASAR, Andres SOOSAAR, Aavo LANG

KOLETSÜSTOKINIINI RETSEPTORITE OSALEMINE HALOPERIDOOLI PIKAAJALISE MANUSTAMISE KÄITUMUSLIKE JA BIOKEEMILISTE EFEKTIDE REALISEERUMISEL

Katsetes valgete isaste rottidega on leitud tihe seos dopamiini retseptorite afiinsuse ning koletsüstokiniini (CCK-8) ja bensodiasepiini retseptorite arvu vahel eesajus haloperidooli (0,5 mg/kg päevas) 15-päevase manustamise järel. CCK-8 ja bensodiasepiini retseptorite vähenemisel tõusis dopamiini retseptorite tundlikkus, kuid nende arvu suure-

nemine viis dopamiini retseptorite afiinsuse vähenemisele. CCK-8 retseptorite agonist tseruleiin kõrvaldas mõlemad haloperidooli kroonilise manustamise efektid: dopamiini retseptorite tundlikkuse tõusu ühtedel rottidel ja languse teistel. Haloperidooli (0,5 mg/kg päevas) ja tseruleiini (0,1 mg/kg päevas) 15-päevane manustamine põhjustasid analoogseid käitumuslikke ja biokeemilisi efekte valgetel isastel hiirtel. Haloperidooli ja tseruleiini kroonilise manustamise järel suurenes fenamiini (3 mg/kg) motoorikat stimuleeriv toime, kuid arenes tolerantsus mustsimooli ($GAVH_A$-retseptorite agonisti) ja bensodiasepiini antagonisti Ro 15-1788 efektide suhtes. Paralleelselt suurenes dopamiin$_2$- ja opioidretseptorite arv hiire aju subkortikaalsetes struktuurides, samal ajal kui CCK-8 retseptorite tihedus vähenes eesaju kortikaalsetes osades. Muutused bensodiasepiini retseptorite arvus olid sõltuvuses uuritud ajustruktuurist. Kui eesaju kortikaalsetes osades retseptorite arv vähenes, siis ajutüves suurenes see haloperidooli ja tseruleiini mõjul. Saadud tulemustest järeldub, et neuroleptikumi kroonilise manustamise biokeemiliste ja käitumuslike muutuste formeerumisel etendavad väga olulist osa CCK-8-ergilised mehhanismid ajus.

Eero VASAR, Andres SOOSAAR, Aavo LANG

THE INVOLVEMENT OF CHOLECYSTOKININ RECEPTORS IN THE REALIZATION OF BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF LONG-TERM HALOPERIDOL ADMINISTRATION

Experiments with male albino rats have shown the dependence of dopamine receptors affinity on the density of cholecystokinin (CCK-8) and benzodiazepine receptors after a 15-day-long haloperidol (0.5 mg/kg daily) treatment. In case the number of CCK-8 and benzodiazepine receptors decreased, the affinity of dopamine receptors increased, but an increase in CCK-8 and in the density of benzodiazepine receptors led to the reduction of dopamine receptors affinity. An acute administration of caerulein, an agonist of CCK-8 receptors, antagonized both effects of the long-term haloperidol medication: the increase of the dopamine receptors affinity in one group and the decrease in the other. The haloperidol (0.5 mg/kg daily) and caerulein (0.1 mg/kg daily) treatment during 15 days caused similar behavioural and biochemical effects on male albino mice. The motor stimulant effect of amphetamine (3 mg/kg) increased, but the tolerance developed to the effects of muscimol (1 mg/kg), the agonist of $GABA_A$-receptors, and Ro 15-1788 (10 mg/kg), the antagonist of benzodiazepine receptors, after a long-term administration of caerulein and haloperidol. Simultaneously the number of dopamine$_2$-receptors in striatum and opioid receptors in limbic structures increased, whereas the density of CCK-8 receptors significantly reduced in forebrain cortical structures. The changes in the number of benzodiazepine receptors depended on the brain structures studied. In forebrain cortical structures the number of benzodiazepine receptors decreased, but in brainstem their density was increased by caerulein and haloperidol. In conclusion, it seems very probable that CCK-8-ergic mechanisms in the brain play a significant role in the formation of behavioural and biochemical effects of a long-term neuroleptic medication.

Sov. Med. Rev. G. Neuropharm., Vol. 1, 1990, pp. 101–126
Photocopying permitted by license only

Printed in the United Kingdom

ADAPTATIONAL CHANGES IN GABA, BENZODIAZEPINE AND CHOLECYSTOKININ RECEPTORS ELICITED BY LONG-TERM HALOPERIDOL ADMINISTRATION

L.H. ALLIKMETS[1] and E.E. VASAR[2]

[1]*Department of Pharmacology, Tartu University* [2]*Laboratory of Psychopharmacology, Institute of General and Molecular Pathology, Tartu University*

Abstract

The authors' investigations into the effects of prolonged haloperidol treatment on the GABA- and CCK8-ergic systems of the brain and into the roles of these neurochemical systems in adaptational changes in response to long-term administration of antipsychotic drugs are reviewed. Chronic haloperidol treatment, while reducing the density of dopamine$_2$ receptors, is shown to reduce the density of $GABA_A$ receptors and of the associated benzodiazepine (BZ) receptors, without affecting $GABA_B$ receptor numbers. As a result, the 'stimulatory' $GABA_A$ and benzodiazepine receptors become predominant, and the behavioral effects of muscimol and Ro 15-1788 are reversed. Administration of Ro 15-1788, together with haloperidol, blocks the development of dopamine$_2$ receptor hypersensitivity and the modification of $GABA_A$ and BZ receptors. Cerulein, a CCK8 receptor agonist, destabilizes the interaction of dopaminergic ligands with dopamine$_2$ receptors. Long-term haloperidol treatment made the animals (mice) tolerant to the behavioral effects of cerulein. Adaptational changes in the CCK8-ergic systems under chronic haloperidol treatment have been found to be associated with alterations in dopamine$_2$-ergic and opioid receptors.

Contents

1. Introduction

Neuroleptics (drugs with antipsychotic activity), are known to be potent antagonists of dopamine receptors. A direct correlation has been demonstrated between a neuroleptic's affinity for dopamine$_2$-receptors in the striatum of experimental animals and the clinically effective daily dose of the neuroleptic (Seeman, 1980). Adaptational changes that occur in the dopaminergic system after prolonged treatment with various neuroleptics have been fairly well investigated. Dopamine$_2$ receptor density has been shown to increase during prolonged dopamine receptor blockade with the result that a hypersensitivity to behavioral effects of dopamine agonists develops (Seeman, 1980). However, long-term administration of a neuroleptic also causes substantial alterations in other neurotransmitter systems, including among others the serotonergic γ-aminobenzoic acid (GABA)-ergic and cholinergic systems (Allikmets *et al.*, 1984). Of particular interest are alterations in the GABA-ergic and cholecystokinin (CCK8)-ergic systems which are closely linked up morphologically and functionally with the dopamine systems of the brain. CCK8 has been shown to act as a dopamine cotransmitter in mesolimbic and mesocortical structures (Hökfelt *et al.*, 1980), and to be co-present with GABA in neurons of the hippocampus and cerebral cortex (Wise, 1985). GABA- and CCK8- ergic mechanisms play important roles in regulating dopaminergic processes (Haefely *et al.*, 1983; Wang *et al.*, 1984)

The present studies were designed to examine adaptational changes in the

GABA- and CCK8-ergic systems during prolonged neuroleptic treatment and to see how such changes relate to the overall adaptation of the organism to the neuroleptic. Haloperidol was chosen because it is a typical neuroleptic with potent antipsychotic activity and has gained wide use in clinical practice.

2. Involvement of GABA and Benzodiazepine Receptors in Haloperidol Actions

In the forebrain and diencephalon, the dopaminergic and GABA-ergic systems are closely interrelated morphologically and functionally, so that both GABA and benzodiazepine receptors are observed to be considerably altered after long-term administration of various neuroleptics that block dopamine receptors (Allikmets *et al.*, 1984). Altered activity of GABA-ergic mechanisms contributes significantly to tardive dyskinesia, a severe side-effect of neuroleptic treatment. Under such treatment, $GABA_A$- and benzodiazepine-receptor densities fall in many forebrain structures (Allikmets *et al.*, 1984), although the substantia nigra shows increased $GABA_A$-receptor numbers together with supersensitivity of its neurons to GABA. The adaptation of GABA-ergic mechanisms and benzodiazepine receptors on long-term neuroleptic treatment is not, therefore, a straightforward process. We have examined adaptational changes in GABA and benzodiazepine receptors in response to long-term haloperidol administration and the possibility of preventing these changes with diazepam and the benzodiazepine antagonist Ro 15-1788 (flumazepil; Hofmann-La Roche, Basel, Switzerland).

2.1 Comparative Effects of Haloperidol and 6-Hyaroxydopamine (6-OHDA) on GABA and Benzodiazepine Receptors

The effect of long-term intraperitoneal haloperidol administration on these receptors in mice and rats was compared with that of single-dose intraventricular injection of 6-OHDA (60 μg per mouse and 200 μg per rat). 6-OHDA was chosen because its actions after intraventricular administration are very similar to those of neuroleptics after a single intraperitoneal dose: both strongly inhibit various forms of behavior and considerably increase the sensitivity of postsynaptic dopamine receptors (Seeman, 1980). As can be seen in Figure 1, muscimol ($GABA_A$-receptor agonist) and baclofen ($GABA_B$-receptor agonist) caused significant decreases in the motor activity of mice. Muscimol enhanced rather than depressed the activity of mice treated with haloperidol for 15 days and restored it to the control level in 6-OHDA-treated animals. The sedative action of baclofen was not altered by

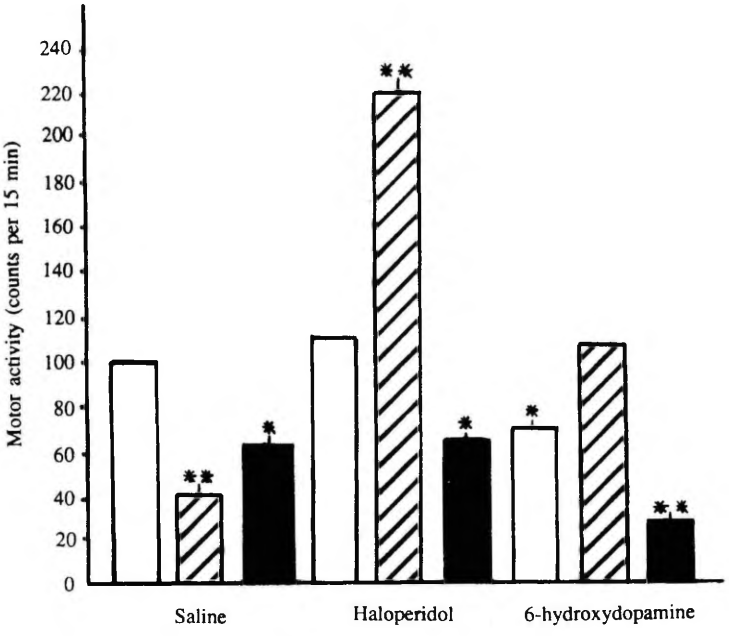


FIGURE 1 Effects of muscimol (0.75 mg kg^{-1}) and baclofen (3 mg kg^{-1}) on motor activity of mice after long-term haloperidol treatment (0.25 mg kg^{-1}, i.p., twice daily for 15 days) or a single intraventricular 6-hydroxydopamine (6-OHDA) injection (60 μg). Assays were carried out 48 h after the last haloperidol injection and 7 days after the 6-OHDA injection. Each mouse was placed in a photoelectric actometer 15 min after an intraperitoneal injection of the GABA mimetic, and the motor activity was measured over a 15-min period. *White bars*, physiologic saline; *hatched bars*, baclofen. * $P < 0.05$; ** $P < 0.01$ *vs* the control (saline + saline-treated) group by Mann-Whitney *U* test.

haloperidol but was significantly enhanced by 6-OHDA.

In rats, we examined the behavioral effects of Ro 15-1788 (5 mg kg^{-1}),a benzodiazepine antagonist (Table 1). In control animals, the drug had stimulatory effects, while in those treated with haloperidol it antagonized the increase in orienting exploratory activity following the discontinuation of prolonged haloperidol treatment. Preinjecting rats with 6-OHDA (200 μg) intraventricularly resulted in a significant diminution of this activity. Ro 15-1788, like muscimol in mice, reversed the effect of 6-OHDA, most likely by accelerating dopamine metabolism, as is indicated by the observations that Ro 15-1788 (5 mg kg^{-1}) raises the level of 3,4-dihydroxyacetic acid, a

Table 1 Effect of a single dose (5 mg kg^{-1}) of the benzodiazepine antagonist Ro 15-1788 on behavioral responses of rats after 15 days' treatment with haloperidol (0.25 mg kg^{-1} twice daily) or a single dose (200μg; intraventricular) of 6-OHDA.[a]

Group	Motor activity		Rearings		Head dips	
	Counts	%	No.	%	No.	%
Saline + saline	30 ± 4.2	100	5.2 ± 1.6	100	6.7 ± 0.7	100
Saline + Ro 15-1788	39 ± 4.4	131	8.5 ± 1.7*	163	11.5 ± 1.6**	172
Haloperidol + saline	42 ± 4.8*	142	5.1 ± 1.3	98	11.8 ± 1.5**	176
Haloperidol + Ro 15-1788	26 ± 3.8	88	4.8 ± 1.4	92	6.0 ± 0.7	90
6-OHDA + saline	14.2 ± 2.6**	46	1.6 ± 0.6**	31	2.6 ± 0.5**	39
6-OHDA + Ro 15-1788	32 ± 4.8	110	3.2 ± 1.2*	62	4.8 ± 0.8	72

[a]Behavior was assessed by the open field method. The test animal was placed in the center of an open field (measuring 100 × 100 × 40 cm) at 30 min after i.p. injection of Ro 15-1788. Motor activity was measured using five independent photoelectric channels. All behavioral assays were for 5 min periods.
In comparison with the saline + saline group $p <$: *0.05; **0.01 (Mann–Whitney U test).

dopamine metabolite, in the rat caudate nucleus (Allikmets and Rägo, 1983) and, when preinjected into rats, attenuates the sedation caused by a low dose of apomorphine (Vasar *et al.*, 1984*a*).

In parallel with the behavioral experiments outlined above we carried out radioligand-binding tests—^{3}H-muscimol, ^{3}H-GABA, and ^{3}H-flunitrazepam binding to $GABA_A$, $GABA_B$ and benzodiazepine receptors, respectively. It has been found (Table 2) that long-term haloperidol treatment decreases $GABA_A$- and benzodiazepine receptor numbers in the forebrain without affecting $GABA_B$ receptors but that, in contrast, intraventricularly administered 6-OHDA has no significant effect on $GABA_A$ and benzodiazepine receptor numbers while reducing those of $GABA_B$ receptors.

Such contrasting adaptational changes produced by haloperidol and 6-OHDA notwithstanding their rather similar behavioral and biochemical effects, may be attributed to differences in their mechanisms of action on the GABA and benzodiazepine receptors. Intraventricularly injected 6-OHDA appears to reduce $GABA_B$ receptor density by destroying presynaptic terminals, as is attested by our and other studies where these receptors have been shown to occur, in particular, on monoaminergic nerve endings in the forebrain and to participate, via calcium-dependent mechanisms, in the regulation of monoamine release (Bowery *et al.*, 1980; Allikmets and Rägo, 1983). The enhanced sedative effect of baclofen after 6-OHDA, observed despite a fall in $GABA_B$ receptor density, may be accounted for by a considerable decrease in the brain content of dopamine for which baclofen

Table 2 Binding parameters of ^{3}H-ligands with GABA and benxodiazepine receptors in mouse and rat forebrains after 15 days' haloperidol treatment (0.25 mg kg^{-1}, twice daily) or a single intraventricular 6-OHDA injection (60 µg per mouse, 200µg per rat).[a]

Group	$GABA_A$ Receptors (mice)		$GABA_B$ Receptors (mice)		Benzodiazepine Receptors (rats)	
	K_D	B_{max}	K_D	Bmax	K_D	Bmax
Saline	9.8 ± 0.8	805 ± 22	52 ± 4.8	203 ± 17	2.5 ± 0.3	710 ± 45
Halo-peridol	10.5 ± 0.9	508 ± 32**	49 ± 4.4	242 ± 22	2.6 ± 0.34	482 ± 38**
6-OHDA	9.3 ±0.6	708 ± 36	56 ± 5.2	122 ± 12*	3.0 ± 0.28	644 ± 42

[a]Values are the results of three separate experiments. Binding tests with ^{3}H-muscimol ($GABA_A$ receptors) ^{3}H-GABA ($GABA_B$ receptors) and ^{3}H-flunitrazepam (benzodiazepine receptors) were performed as described in Nurk *et al.* (1984), Bowery *et al.* (1980) and Möhler and Okada (1978), respectively. K_D = dissociation constant, nmol l^{-1}; B_{max} = binding site density, fmol (mg protein)$^{-1}$.
In comparison with the saline-treated group, $p <$: *0.05; **0.01 (Student's t test).

is a functional antagonist. As for haloperidol, it predominantly blocks dopamine$_2$ receptors and, as indicated by *in vitro* evidence, prolonged haloperidol administration significantly increases their density (Seeman, 1980), which is paralleled by decreases in $GABA_A$ and benzodiazepin receptor densities. A balance therefore appears to exist in the forebrain between $GABA_A$ and benzodiazepine receptors, on the one hand, and dopamine$_2$ receptors on the other, so that the densities of these receptors are negatively correlated. One result of these molecular transformations is that the behavioral effects of the $GABA_A$ receptor agonist muscimol and the benzodiazepine antagonist Ro 15-1788 are reversed. However, the reductions in $GABA_A$ and benzodiazepine receptor numbers cannot fully explain the altered behavioral effects of muscimol and Ro 15-1788.

Muscimol injected into the raphe nuclei or substantia nigra, but not into forebrain structures, is known to have a stimulatory effect on animal behavior, the effect being potentiated by benzodiazepine agonists. We have therefore compared ^{3}H-muscimol and ^{3}H-flunitrazepam binding in the forebrain and afterbrain of rats following their long-term haloperidol treatment. The density of both $GABA_A$ and benzodiazepine receptors was found to be increased in the forebrain and decreased in the afterbrain (Table 3). These changes in receptor density appear relevant to the reversal of behavioral

Table 3 Effect of long-term(15 days) haloperdiol treatment (0.25 mg kg^{-1}, twice daily) on ^{3}H-muscimol and ^{3}H-flunitrazepam binding in rat forebrain and brain stem.[a]

Group		^{3}H-muscimol		^{3}H-flunitrazepam	
		Forebrain	Brain stem	Forebrain	Brain stem
Saline:	K_D	9.6 ± 1.6	12.6 ± 1.3	2.1 ± 0.3	3.2 ± 0.3
	B_{max}	908 ± 83	380 ± 36	1060 ± 92	420 ± 43
Haloperidol:	K_D	10.2 ± 1.8	13.2 ± 1.3	1.8 ± 0.4	3.4 ± 0.4
	B_{max}	640 ± 50*	508 ± 36*	780 ± 76*	606 ± 42*

[a]See footnote to Table 2. For forebrain binding tests, a frontal incision was made along the optic chiasm line, and the brain structures anterior to the incision lines were used; for brain stem tests,a frontal incision was made along the posterior line of the diencephalon, and the stem structures were then teased off from the cerebellum and cortical formations.
In comparison with saline group *$p < 0.05$ (Student's t test).

effects of muscimol and Ro 15-1788. It would seem that $GABA_A$ and benzodiazepine receptors in the afterbrain differ functionally from those of the forebrain in that the 'stimulatory' receptors preponderate in the former and 'inhibitory' ones in the latter and that long-term haloperidol administration increases 'stimulatory' receptor numbers, with the result that the muscimol and Ro 15-1788 actions are reversed. Different rats, however, may

Table 4 Effects of diazepam, Ro 15-1788 and naloxone administered twice daily for 10 days together with apomorphine on apomorphine-induced aggresiveness in male rats.[a]

Drug	Day 3	Day 7	Day 10
Apomorphine + saline	0	1.9 ± 0.31	3.0 ± 0.22
Apomorphine + diazepam (2.5 mg kg^{-1})	0.5 ± 0.28	0.8 ± 0.42	1.3 ± 0.41*
Apomorphine + Ro 15–1788 (5 mg kg^{-1})	0	1.3 ± 0.39	2.3 ± 0.34
Apomorphine + naloxone (0.5 mg kg^{-1})	0.8 ± 0.42	2.9 ± 0.18*	3.2 ± 0.25
Apomorphine + diazepam + Ro 15–1788	0	2.3 ± 0.17	3.5 ± 0.18
Apomorphine + diazepam + naloxone	1.9 ± 0.38*	3.2 ± 0.24*	4.0 ± 0.0*

[a]Values are points scored for intensity of aggressiveness using the scoring system described in Allikmets *et al.* (1979). All drugs were given 15 min before apomorphine (1 mg kg^{-1}).
In comparison with the apomorphine + saline group *$p < 0.05$ (Mann–Whitney U test).

already differ in pretreatment sensitivity of $GABA_A$ and benzodiazepine receptors, as is indicated by the differential effects of diazepam on apomorphine-induced aggressive behavior. Thus, diazepam at 2.5 mg kg^{-1} had a striking accelerating effect on this behavior in some male rats while blocking it in others (Table 4). As shown in this table, Ro 15-1788 at 5 mg kg^{-1} had a very weak antagonistic effect on apomorphine-induced aggression, while the opiate antagonist naloxone blocked only the antiaggressive action of diazepam. These findings indicate that Ro 15-1788, like haloperidol on chronic administration, is capable of blocking the functionally predominant subtype of benzodiazepine receptors. This may explain the reversal of the behavioral effect of Ro 15-1788, as seen after long-term haloperidol treatment, and the elimination by this drug of both the anti- and proaggression effects of diazepam in male rats.

2.2 Effects of Diazepam and Ro 15-1788 on Adaptational Changes in the GABA-ergic System and Benzodiazepine Receptors During Long-term Haloperidol Treatment

The benzodiazepine antagonist diazepam has been shown to alleviate symptoms of tardive dyskinesia. In animal experiments, administration of diazepam in combination with haloperidol was effective in countering the development of behavioral supersensitivity in dopamine receptors and the haloperidol-induced increase in $dopamine_2$ receptor density in the caudate nucleus (Zharkovsky and Allikmets, 1986). In the studies reported here, we examined how diazepam or Ro 15-1788, given to mice in combination with haloperidol, influence the latter's ability to cause adaptational changes in the GABA-ergic system and benzodiazepine receptors. Ro 15-1788 plus haloperidol administration was found to block the development of hypersensitivity to amorphine-induced stereotypy and Quipazine-induced head twitchs, i.e. of $dopamine_2$ and $serotonin_2$ receptor hypersensitivity. Diazepam (2.5 mg kg^{-1}) plus haloperidol (0.25 mg kg^{-1}) administration failed to prevent adaptational changes in $GABA_A$ receptors, but was effective in countering the decrease in benzodiazepine receptors in the forebrain (Table 5). Diazepam like haloperidol, reversed the sedative effect of muscimol in mice. Long-term administration of the benzodiazepine antagonist Ro 15-1788 enhanced the sedative effect of muscimol and eliminated the haloperidol's reversal of this effect (see Table 5). In our radioligand binding experiments, Ro 15-1788 diminished ^{3}H-muscimol and ^{3}H-flunitrazepam binding in mouse forebrain while significantly diminishing the effect of haloperidol on $GABA_A$ and benzodiazepine receptors on long-term administration.

These findings indicate that the benzodiazepine antagonist Ro 15-1788,

Table 5 Effect of 15 day treatment of mice with diazepam or the benzodiazepine antagonist Ro 15-1788 in combination with haloperidol-induced (0.25 mg kg^{-1}) adaptational changes in $GABA_A$and benzodiazepine receptors.[a]

Treatment	Effect of muscimol (0.75 mg kg^{-1}) on motor activity (counts/30 min)	^{3}H-muscimol binding in mouse forebrain		^{3}H-flunitrazepam binding in rat forebrain	
		K_D	B_{max}	K_D	B_{max}
Saline (control)	$144 \pm 18^{**}$	9.6 ± 1.6	900 ± 120	2.5 ± 0.22	810 ± 48
Haloperidol	$308 \pm 32^{**}$	10.2 ± 1.8	$510 \pm 50^{**}$	2.6 ± 0.18	$570 \pm 42^{**}$
Haloperidol + diazepam (2.5 mg kg^{-1})	$320 \pm 28^{**}$	9.8 ± 1.4	$520 \pm 60^{**}$	2.7 ± 0.25	760 ± 60
Diazepam (2.5 mg kg^{-1})	$354 \pm 36^{**}$	9.7 ± 1.6	$500 \pm 54^{**}$	2.6 ± 0.20	$620 \pm 50^{*}$
Haloperidol + Ro 15-1788 (5 mg kg^{-1})	128 ± 15	9.4 ± 1.2	890 ± 100	2.8 ± 0.21	670 ± 65
Ro 15-1788 (5 mg kg^{-1})	83 ± 12	9.4 ± 1.6	$630 \pm 50^{*}$	2.5 ± 0.19	670 ± 50

[a]See footnotes to Tables 1 and 2. The tests were carried out 48 h after withdrawal from haloperidol treatment.
In comparison with control $p \leq$: $^{*}0.05$; $^{**}0.01$.

unlike the benzodiazepine agonist diazepam, strongly counteracts adaptational changes in $GABA_A$ and benzodiazepine receptors during prolonged haloperidol treatment.

3. CCK8-ergic Mechanisms in Haloperidol Actions

As already noted, dopaminergic and cholecystokinin (CCK8)-ergic mechanisms in the forebrain are closely interrelated morphologically and functionally. Convincing evidence exists that CCK8 is a dopamine cotransmitter in neurons supplying its mesolimbic and mesocortical structures (Hökfelt *et al.*, 1980). On the other hand, blockade of these dopaminergic mechanisms explains the antipsychotic action of neuroleptics (Carlsson, 1983), which are the most potent antipsychotic drugs known today. CCK8 has been reported to exert a marked influence on dopaminergic processes by altering dopamine metabolism and release and to affect the affinity of dopamine receptors (Zetler, 1985).

Dopamine, in turn, has been shown to regulate CCK8 release in the caudate nucleus. Prolonged blockade of dopamine receptors by a neuroleptic (haloperidol, chlorpromazine or clozapine) results in elevated CCK8 levels in forebrain subcortical structures and appreciably increases CCK8 receptor density in the forebrain.

We have investigated the effects of cerulein (a CCK8 receptor agonist) on dopaminergic mechanisms and of long-term haloperidol administration on CCK8-ergic mechanisms.

3.1 Effect of Cerulein on Dopaminergic Mechanisms

The decapeptide amide cerulein, which is related to CCK8 in chemical structure, is a potent agonist of central CCK8 receptors (Zetler, 1985). We have found ^{3}H-CCK8 (1 nmol l^{-1}) binding to be 50% inhibited by cerulein in a concentration of 4 nmol l^{-1} by sulfated CCK8 at 15 nmol l^{-1} and by proglumide, a CCK8 receptor antagonist, at 650 μmol l^{-1}. These findings, and also behavioral experiments (Zetler, 1985), indicate that cerulein has a markedly higher affinity for CCK8 receptors than other ligands.

It has been documented by many authors that systemically administered CCK8 and cerulein eliminate the stereotypic behavior elicited by dopaminomimetics and inhibit the motor excitation caused by phenamine, an indirectly acting dopaminomimetic (Zetler, 1985).

We thought it worthwhile to examine the mechanism by which the antidopaminergic action of cerulein is mediated. To this end, cerulein was

Table 6 Effects of cerulein (50–125 μg kg^{-1}) and proglumide (25–100 mg kg^{-1}) on phenamine-induced (3 mg kg^{-1}) excitation in mice.[a]

Drugs		Motor activity	
		Counts/15 min	Counts/30 min
Saline		184 ± 22	315 ± 36
Phenamine		280 ± 42	565 ± 76
Phenamine + cerulein:	50 μg kg^{-1}	244 ± 46	528 ± 62
	75 μg kg^{-1}	112 ± 20*	237 ± 34*
	100 μg kg^{-1}	74 ± 16**	152 ± 26**
	125 μg kg^{-1}	70 ± 14**	144 ± 22**
Phenamine + proglumide:	25 mg kg^{-1}	328 ± 46	604 ± 66
	50 mg kg^{-1}	313 ± 50	575 ± 78
	100 mg kg^{-1}	288 ± 44	555 ± 69
Phenamine + proglumide (50 mg kg^{-1}) + cerulein (75 μg kg^{-1})		181 ± 28***	332 ± 40***
Phenamine + proglumide (100 mg kg^{-1}) + cerulein (100 μg kg^{-1})		162 ± 36***	268 ± 65*

[a]Phenamine was administered 15 min before the test, while cerulein and proglumide were given immediately before it.
In comparison with the phenamine only group $p <$: *0.01; **0.001; ***0.0001 (Mann–Whitney *U* test).

examined for its impact on phenamine (amphetamine)-induced excitation in male mice and rats and on the behavioral effects of apomorphine in male rats. In mice, cerulein injected at 15 min after phenamine (3 mg kg^{-1}) caused a dose-dependent inhibition of the latter's excitatory effects on motor behavior (Table 6), while the CCK8 receptor antagonist proglumide, did not modify these effects in doses of 25–100 mg kg^{-1}. Proglumide only weakened the inhibitory action of cerulein in doses of 50 mg kg^{-1} and 100 mg kg^{-1}. In rats, cerulein did not alter the major behavioral effects of phenamine (2.5 mg kg^{-1}) such as stereotypy and motor activity enhancement. Prolonged treatment with cerulein (100 μg kg^{-1}) plus apomorphine (1 mg kg^{-1}) suppressed aggressive behavior in rats given apomorphine and had an attenuating effect on apomorphine-induced stereotypy. The animals thus treated were sluggish and drowsy. However, cerulein (100 or 200 μg kg^{-1}) failed to suppress

Table 7 Effect of single-dose treatment with cerulein or haloperidol on phenamine (3 mg kg^{-1}) sensitivity in mice.[a]

Drugs	Motor activity	
	Counts/15 min	Counts/30 min
Saline + saline	199 ± 24	314 ± 36
Saline + phenamine	280 ± 38	549 ± 62
Haloperidol (0.25 mg kg^{-1}) +phenamine	390 ± 42*	702 ± 64*
Cerulein (50 μg kg^{-1}) + phenamine	325 ± 39	540 ± 61
Cerulein (100 μg kg^{-1}) + phenamine	488 ± 68**	914 ± 82**
Cerulein (50 μg kg^{-1}) + haloperidol (0.25 mg kg^{-1}) + phenamine	439 ± 54*	844 ± 76**
Cerulein (100 μg kg^{-1}) + haloperidol (0.25 mg kg^{-1}) + phenamine	438 ± 48*	845 ± 82**

[a]Cerulein and haloperidol were given 48 h before phenamine; motor activity was measured 15 min after phenamine injection.
In comparison with saline + phenamine group $p <$: *0.05; **0.01 (Mann–Whitney U test).

aggressive behavior in rats that had been sensitized to apomorphine aggressiveness by 10-day administration of apomorphine (1 mg kg^{-1} twice daily). Cerulein, therefore, influenced only the development of apomorphine-mediated aggressiveness.

In further experiments, cerulein was found to reinforce phenamine's behavioral effects in mice (although only in the dose of 100 μg kg^{-1}) (Table 7) rather than weakening them as in the previous experiments where it was given after phenamine (see Table 6); this sensitizing action of cerulein was stronger than that of haloperidol (0.25 mg kg^{-1}). When cerulein (50 μg kg^{-1}) was combined with haloperidol (0.25 mg kg^{-1}), the sensitizing action of the latter with respect to phenamine-mediated excitation was markedly enhanced, as is evident from Table 7. In rats, on the contrary, the excitatory effect of phenamine (2.5 mg kg^{-1}) was attenuated both by cerulein alone (40 μg kg^{-1}) and, even more, by cerulein plus haloperidol (0.25 mg kg^{-1}) (Figure 2). Proglumide (50 mg kg^{-1}) did not alter the effect of cerulein plus haloperidol administration on the excitatory activity of phenamine.

These studies indicate that the effects of cerulein on phenamine-induced excitation in mice are very different from those in rats: in mice cerulein, like haloperidol, exerts a strong antiphenamine action if administered after phenamine and causes hypersensitivity to the latter if given before it; in rats cerulein either has no effect on phenamine activity or, when given before

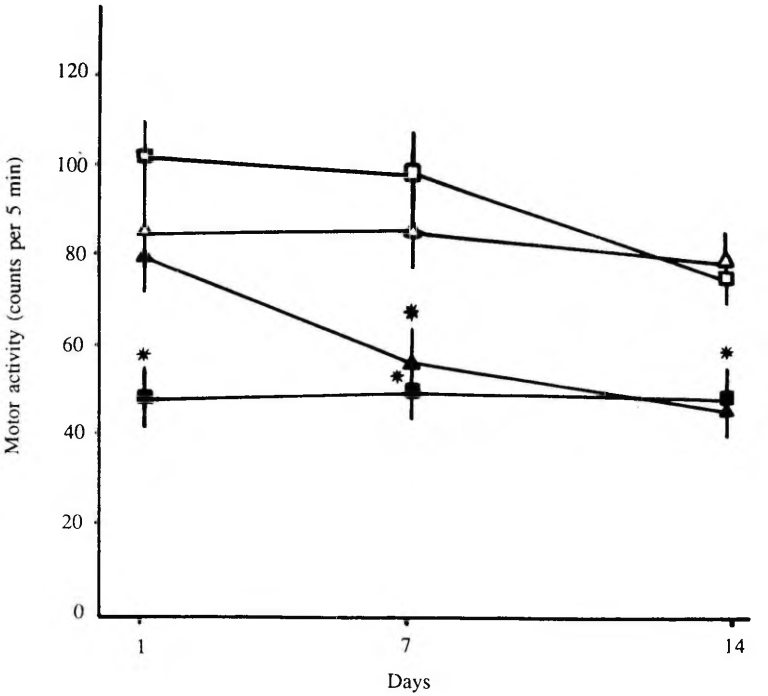


FIGURE 2 Effects of single-dose treatment with cerulein (40 μg kg^{-1}), haloperidol (0.25 mg kg^{-1}), and cerulein plus haloperidol on phenamine-induced (2.5 mg kg^{-1}) motor excitation in rats. Phenamine was given at 1, 7 or 14 days after haloperidol or cerulein. Motor activity was measured in an open field (see footnote *a* to Table 1). Δ ,Saline + phenamine; ❑, haloperidol + phenamine;▲, cerulein + phenamine; ■ , cerulein + haloperidol + phenamine. * $P < 0.05$ *vs* the saline + phenamine group by Mann-Whitney *U* test.

phenamine, weakens the phenamine-mediated excitation. The mechanisms of antiphenamine action by cerulein may therefore be different in these two species.

In an attempt to identify the mechanisms of cerulein action in mice and rats, we studied cerulein for its effects on the binding of ^{3}H-spiroperidol and ^{3}H-lysergic acid diethylamide (^{3}H-LSD). *In vivo*, cerulein inhibited in a dose- dependent manner the binding of these monoaminergic ligands in the forebrain of mice (the data for ^{3}H-spiroperidol are shown in Figure 3), but it

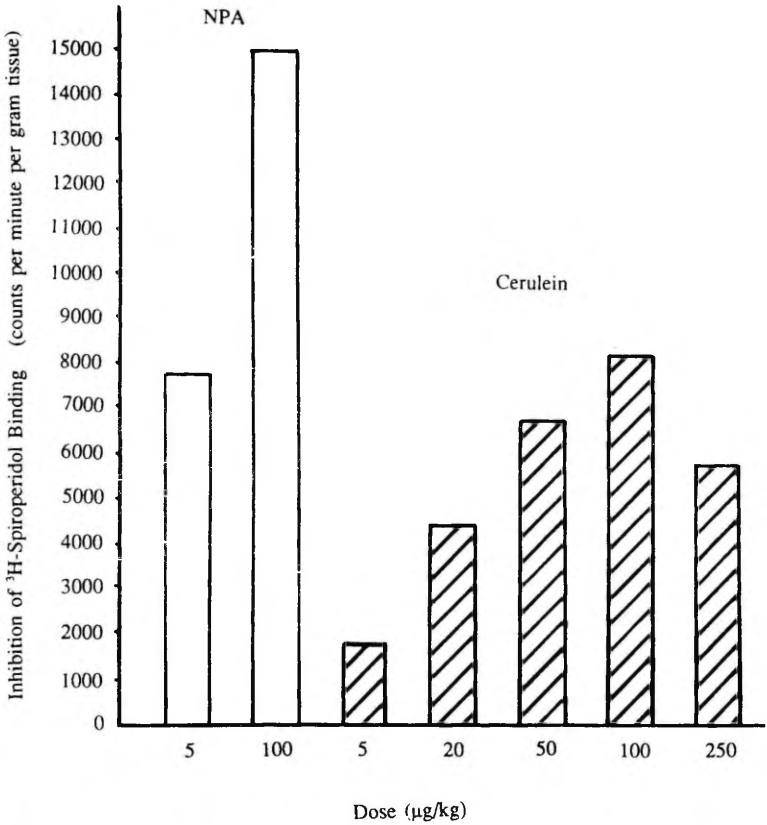


FIGURE 3 Effects of cerulein and *N*-propylnorapomorphine (NPA) in various doses on ^{3}H-spiroperidol (5 μg kg^{-1}) binding in mouse forebrain subcortex *in vivo*. Mean values from three separate experiments performed as described previously (Vasar *et al.*, 1984b). Cerulein and NPA were given 15 min before ^{3}H-spiroperidol, and the animals were decapitated 20 min after being injected with the radioligand.

should be noted that it only interacted with a portion of dopamine receptors. Thus, cerulein mainly acted on those ^{3}H-spiroperidol binding sites with which *N*-propylnorapomorphine (NPA) interacted when given in the low dose of 5 μg kg^{-1}. These results suggest that, in mice, cerulein passes across the blood-brain barrier with relative ease and, moreover, acts primarily on high-affinity dopamine$_2$ receptors.

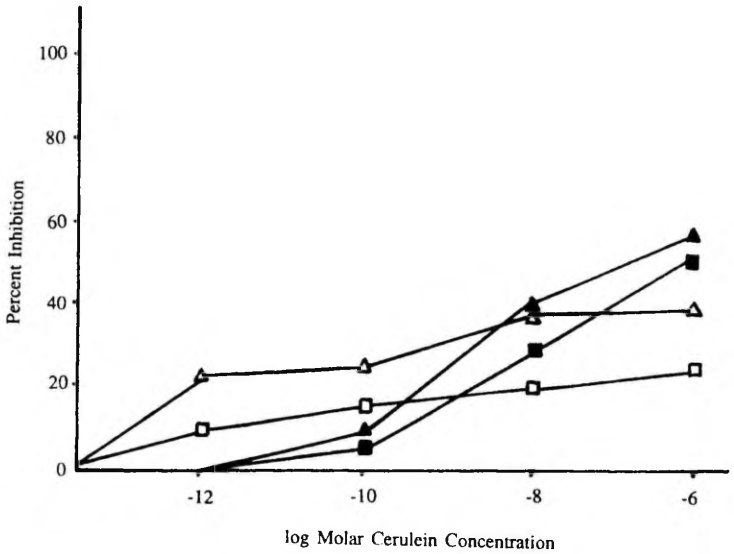


FIGURE 4 Effects of cerulein in various concentrations on ^{3}H-spiroperidol and ^{3}H-lysergic acid diethylamide (^{3}H-LSD) *in vitro* binding in the association state in rat forebrain. Mean values from four separate experiments performed as described by Creese *et al.*, (1977). Brain membranes were preincubated with the indicated cerulein concentrations for 20 min at 25°C, after which a radioligand was added, followed by further incubation for 8 min at the same temperaure. Δ, ^{3}H-LSD, 0.25 nmol l^{-1} ;□, ^{3}H-LSD, 5 nmol l^{-1};▲, ^{3}H-spiroperidol, 0.125 nmol l^{-1} ; ■, ^{3}H-spiroperidol, 1 nmol l^{-1}.

In vitro, cerulein had virtually no effect on the binding of either ^{3}H-spiroperidol of ^{3}H-LSD to dopamine receptors in forebrain samples from rats if it was in a state of equilibrium, but did inhibit the binding when in a state of association (Figure 4). These findings suggest that cerulein in the association state is capable of destabilizing the interaction of dopaminergic ligands with dopamine$_2$ receptors. This mechanism appears to underlie the inhibitory effect of cerulein on ^{3}H-spiroperidol binding *in vivo*. Similarly, cerulein may be thought to exert potent antidopamine activity by destabilizing the interaction of endogenous dopamine with dopamine$_2$ receptors. It is the antidopaminergic action of cerulein that is responsible for the behavioral hypersensitivity developing to phenamine after a single cerulein injection (see Table 7). The molecular basis behind this phenamine hypersensitivity

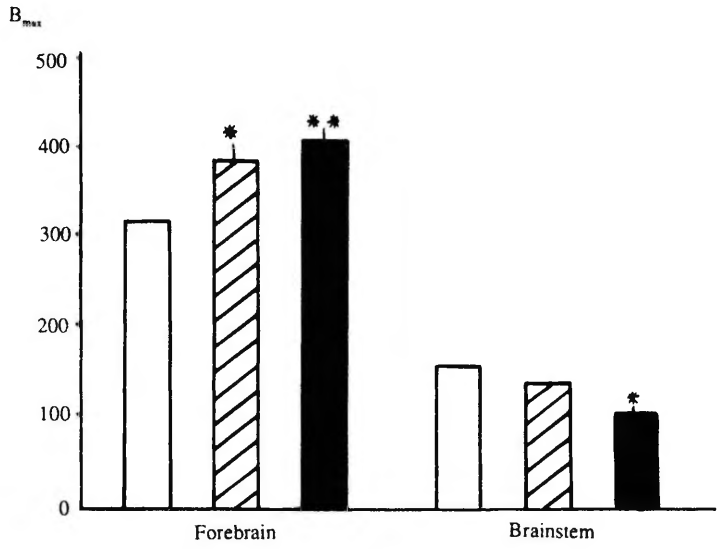


FIGURE 5 Effects of single-dose and multiple dose (10 injections once daily) treatments with cerulein (100 μg kg $^{-1}$) on *in vitro* ^{3}H-LSD binding in mouse forebrain and brainstem. Mean values from three separate experiments. Assays were done as described in Creese *et al.* (1977) 24 h after the single or last cerulein injection. B_{max} = ^{3}H-LSD binding site density in fmol (mg protein)$^{-1}$. ❒,10-day treatment with saline; ▨ , single dose of cerulein; ■, cerulein for 10 days. * $P < 0.05$; ** $P < 0.02$ *vs* the saline-treated group by Student's *t* test.

was an increase in dopamine$_2$ receptor numbers in the forebrain and a reduction in dopamine autoreceptor density in the brainstem (Figure 5). It should be noted that the increase in dopamine$_2$ receptor numbers in the forebrain and the decrease in dopamine autoreceptor density in the brainstem were more marked after multiple cerulein injections.

The inhibition of apomorphine aggressiveness in rats by cerulein may likewise be accounted for by its destabilizing effect on apomorphine interaction with dopamine$_2$ receptors. That cerulein is able to exert antidopaminergic effects in rats (and not only in mice) has been indicated by Dumbrille-Ross and Seeman (1984) who found that single-dose cerulein treatment elicited a sustained elevation of dopamine$_2$ receptor density in the nucleus accumbens and striatum. However, when injected into rats that had been presensitized to apomorphine-mediated aggressiveness, cerulein was without effect even in a dose as high as 200 μg kg^{-1}. Cerulein, therefore, is capable of blocking the development of hypersensitivity in dopamine$_2$ recep-

Table 8 Effects of cerulein and haloperidol, 48 h after a single injection, on ^{3}H-etorphine binding in the accumbens and caudate nuclei of rats.[a]

Treatment	*Nucleus accumbens*		*Nucleus caudatus*	
	K_D	B_{max}	K_D	B_{max}
Saline	0.53 ± 0.04	606 ± 32	0.61 ± 0.04	493 ± 30
Haloperidol (0.25 mg kg^{-1})	0.54 ± 0.03	632 ± 36	0.7 ± 0.03	581 ± 31*
Cerulein (40 μg kg^{-1})	0.54 ± 0.04	502 ± 28*	0.60 ± 0.03	489 ± 32
Haloperidol + cerulein	0.51 ± 0.03	450 ± 32**	0.60 ± 0.04	562 ± 36

[a]Results of three separate experiments; the binding tests were performed as described in Owen *et al.* (1985). See footnote to Table 2 for definition of K_D and B_{max}.
In comparison with saline-treated group $p <$: *0.05; **0.001 (Student's t test).

tors in response to repeated apomorphine injections but fails to counteract the effect of apomorphine if these receptors are already hypersensitive.

As shown by Matsubara and Matsushita (1986), cerulein interacts with phenamine (amphetamine) in rats in a different way than in mice. When given to rats in a single dose together with haloperidol, cerulein was found to exert a long-lasting antagonistic effect on the excitatory action of phenamine. These authors have clearly demonstrated that this antagonistic effect is mediated through release of β-endorphin and its interaction with opioid receptors in the nucleus accumbens. β-Endorphin stimulation of opioid receptors in this nucleus strongly inhibited presynaptic dopaminergic activity in the mesolimbic system (Matsubara and Matsushita, 1986). These results have been confirmed by our radioligand binding experiments in which preinjecting rats with a single cerulein dose (40 μg kg^{-1}) led to a significant decrease in the amount of bound ^{3}H-etorphin, an opioid receptor ligand, in the nucleus accumbens; still less ^{3}H-etorphin, was bound when cerulein was given together with haloperidol (Table 8). Cerulein failed to exert a similar effect on opioid receptors in the nucleus caudatus.

The findings presented above indicate that cerulein can mediate its anti-dopaminergic effects not only by enhancing the sensitivity of dopamine autoreceptors (Zetler,1985) but also by causing CCK8 receptors to interact with post-synaptic dopamine$_2$ receptors in forebrain structures such as the striatum and mesolimbic system, as well as by inducing more β-endorphin to be released in the nucleus accumbens. The augmented β-endorphin release strongly inhibits the activity of presynaptic dopaminergic mechanisms in the mesolimbic system. In mice, cerulein does not elicit enhanced β-endorphin release and thus fails to exert a sustained antiphenamine effect.

3.2 Adaptational Changes in the CCK8-ergic System on Long-Term Haloperidol Treatment

The results discussed above indicate that the CCK8 receptor agonist cerulein acts much in the same way as do neuroleptics. Although these do not interact with CCK8 receptors, chronic neuroleptic treatment is known to alter substantially the activity of CCK8-ergic mechanisms in the brain. Prolonged (two weeks) administration of haloperidol or reserpine increases the density of binding sites for ^{125}I-CCK-33 in mouse forebrain while 2-week treatment with haloperidol, clozapine or chlorpromazine raises CCK8 levels in the forebrain subcortex.

Our previous studies have shown that mice treated long-term with haloperidol become tolerant to the sedative action of cerulein, and that this effect correlated well with the reversal of the effect of cerulein on ^{3}H-spiroperidol binding (Vasar *et al.*, 1986).

We have now examined in detail the changes that occur in the CCK8-ergic

Table 9 Modification of behavioral effects of cerulein in mice by long-term haloperidol treatment (0.25 mg kg^{-1} twice daily for 15 days).[a]

Treatment	Long-term treatment	
	Saline	Haloperidol
Orienting/exploratory activity (counts/30 min)		
Saline	359 ± 39	276 ± 17
Cerulein (20 μg kg^{-1})	198 ± 23*	252 ± 27
Electric pain sensitivity (no. of aggressive contacts/2 min)		
Saline	13.5 ± 0.95	8.6 ± 0.93
Cerulein (50 μg kg^{-1})	6.8 ± 0.82**	21.8 ± 2.99**
Picrotoxin-induced convulsions (survival time; min)		
Saline	17.4 ± 1.7	18.1 ± 1.5
Cerulein (125 μg kg^{-1})	26.1 ± 1.6*	21.5 ± 1.5

[a]All tests were started 48 h after the last haloperidol injection. Orienting/exploratory activity was measured with a photoelectric actometer; cerulein was given immediately before the measurements. Aggressiveness was assayed in foot-shock boxes each containing a pair of mice which received 48 electric shocks (40 V) over a 2 min period, with cerulein being given 20 min before the assay. In the group with picrotoxin-induced (8 mg kg^{-1}) convulsions, it was given 10 min before picrotoxin.

In comparison with saline-treated group $p <$: *0.05; **0.01 (Mann–Whitney *U* test).

Table 10 Effects of intraventricular cerulein injection on orienting/exploratory activity in rats pretreated with haloperidol for 15 days (0.25 mg kg^{-1} twice daily).[a]

Treatment	No. of counts		No. of rearings		No. of head dips	
	Saline	Haloperidol	Saline	Haloperidol	Saline	Haloperidol
Saline	40.4 ± 3.1	58.4 ± 7.5	2.2 ± 0.38	4.2 ± 1.07	5.9 ± 0.92	4.8 ± 0.77
Cerulein (5 ng)	28.8 ± 4.4*	45.5 ± 10.9	2.7 ± 0.70	4.5 ± 0.87	4.8 ± 0.69	8.2 ± 1.45*
Cerulein (50 ng)	27.4 ± 3.6*	93.0 ± 9.2*	0.8 ± 0.26*	12.0 ± 2.86*	4.2 ± 1.03	11.3 ± 1.83**
Cerulein (500 ng)	52.0 ± 6.6	53.0 ± 5.6	3.0 ± 1.39	3.7 ± 0.55	6.2 ± 1.10	5.0 ± 0.61

[a]The assays were started 48 h after the last haloperidol administration using the open field method: 1 min after saline or cerulein injection, the rats were placed in the center of an open field, measuring 100 × 100 × 40 cm.
In comparison with control (intraventricular injection of physiological saline) $p <$: *0.05; **0.01 (Mann–Whitney U test).

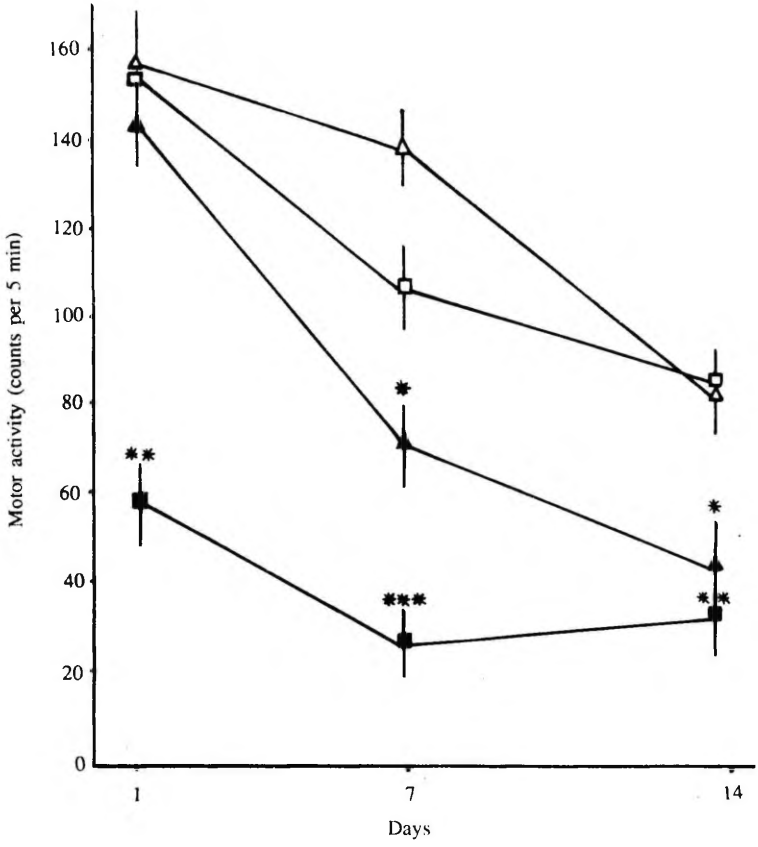


FIGURE 6 Enhanced antiphenamine action of cerulein after 15-day haloperidol treatment (0.25 mg kg^{-1} twice daily). Cerulein was given in a dose of 40 μg kg^{-1} 48 h after withdrawal from haloperidol treatment, followed by phenamine (2.5 mg kg^{-1}) 1,7 or 14 days later. Δ, saline + saline + phenamine; □, haloperidol + saline + phenamine; ▲, saline + cerulein + phenamine; ■, haloperidol + cerulein + phenamine. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ *vs* the saline + saline + phenamine-treated group by Mann-Whitney *U* test.

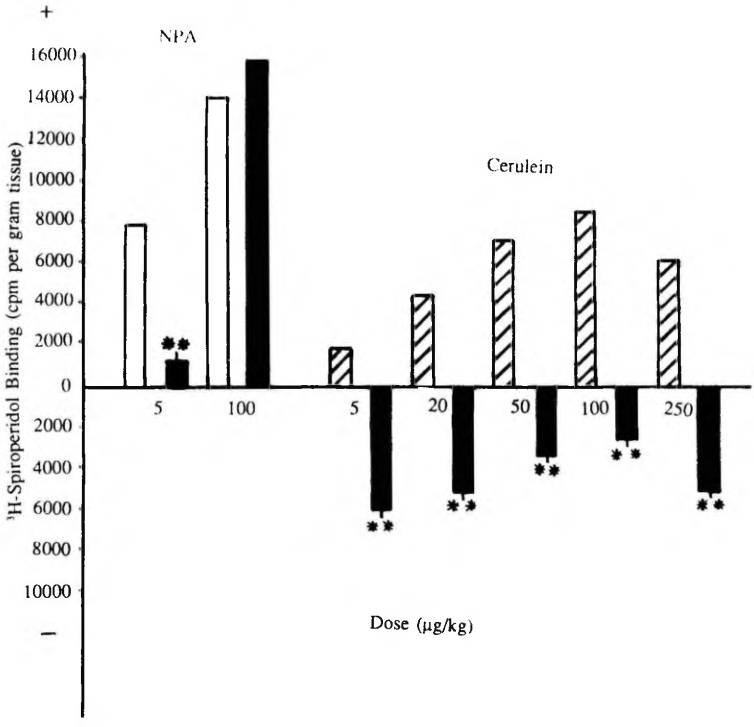


FIGURE 7 Effect of cerulein and *N*-propylnorapomorphine (NPA) in various doses on *in vivo* ^{3}H-spiroperidol (5 μg kg^{-1}) binding in mouse forebrain subcortex after 15-day haloperidol treatment (0.25 mg kg^{-1} twice daily). Assays were carried out 48 h after withdrawal from haloperidol treatment using the previously described procedure (Vasar *et al.*, 1984b). *Ordinate*: inhibition (−) or stimulation (+) of ^{3}H-spiroperidol binding. □, NPA after 15 days of saline; ▩, cerulein after 15 days of saline; ■, NPA or cerulein after 15 days of haloperidol. (See also legend to Figure 3.) * $p < 0.01$ *vs* the saline-treated group by Student's *t* test.

system during prolonged blockade of dopamine and serotonin receptors by haloperidol, a typical neuroleptic. Fifteen-day haloperidol treatment (0.25 mg kg^{-1} twice daily) altered all the major behavioral effects of cerulein in mice (Table 9). Thus, the sedative and anticonvulsant actions of cerulein were considerably attenuated, and cerulein (50 μg kg^{-1}) enhanced aggressive behavior rather than exerting the moderate antiaggression effect observed for animals not treated with haloperidol. As for rats (Table 10), an intraventricular cerulein injection in a dose of 5 or 50 mg weakened orienting/exploratory activity in untreated animals and (after the 50 mg dose) did not affect or

markedly stimulated this activity in those treated with haloperidol for 15 days.

In contrast, the long-lasting antagonistic effect of cerulein on phenamine-mediated excitation was significantly increased by the prolonged haloperidol treatment, the rats thus treated being stimulated by phenamine much less than control animals (Figure 6).

To identify the mechanisms by which the observed changes in cerulein's action are brought about by long-term haloperidol treatment, we studied ^{3}H-CCK8 binding in rat forebrain *in vitro*. At 48 h after discontinuation of haloperidol treatment, the affinity of ^{3}H-CCK8 binding sites had changed

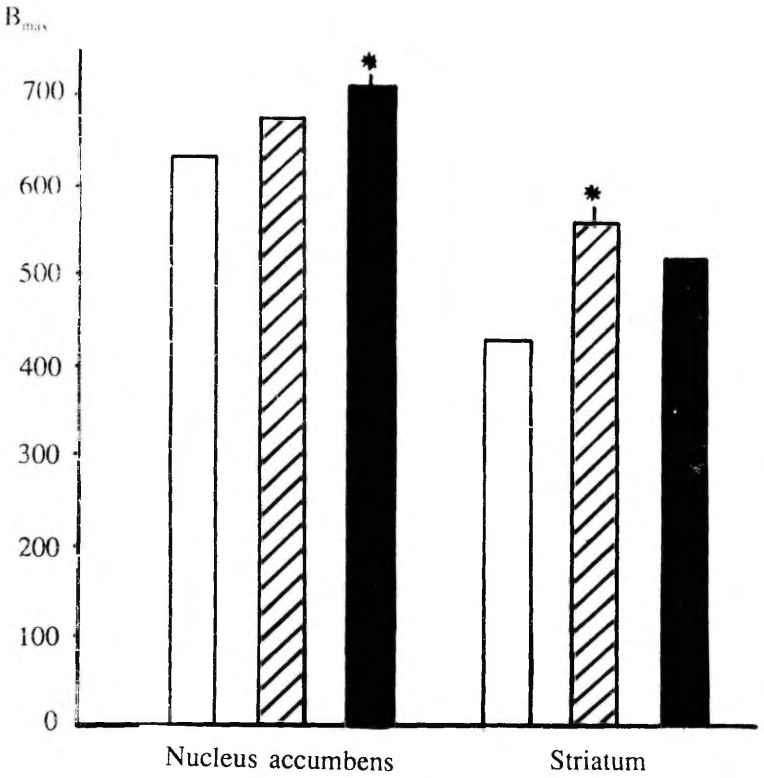


FIGURE 8 Effect of single *vs* multiple dose (0.25 mg kg^{-1}) haloperidol treatment on ^{3}H-etorphin bending in rat nucleus accumbens and striatum. Assays were carried out 48 h after haloperidol treatment. *Ordinate* ^{3}H-etorphin binding site density in fmol (mg protein)$^{-1}$. □, saline; ▩, single-dose haloperidol treatment; ■, 15-day haloperidol treatment. $^{*}P < 0.05$ *vs* 15-day treatment with saline by Student's *t* test.

but slightly (the dissociation constant being 0.62 ± 0.05 *vs* 0.78 ±0.05 nmol l^{-1} in the control), whereas their density had decreased significantly (21.7 ± 1.8 *vs* 29.1 ± 1.3 fmol (mg protein)$^{-1}$ in the control). In mice, long-term haloperidol treatment reversed the inhibitory effect of cerulein on ^{3}H-spiroperidol binding *in vivo*: the forebrains of mice thus treated bound more ^{3}H-spiroperidol after cerulein injection (Figure 7). The reversal of the cerulein effect was associated with a decrease in the density of high-affinity dopamine$_2$ receptors since the long-term haloperidol treatment also attenuated the effect of NPA (5 μg kg^{-1}).

Prolonged treatment with neuroleptics is known to cause behavioral supersensitivity to opioid peptides in limbic structures (Stinus *et al.*, 1986). As shown above, the sustained antagonism of phenamine-induced excitation by cerulein is associated with a decrease in opioid receptor numbers in the nucleus accumbens. Our study of how long-term haloperidol treatment affects ^{3}H-etorphin binding indicates that such treatment results in a significant elevation of opioid receptors in this nucleus (Figure 8), suggesting their heightened sensitivity in mesolimbic structures, which in turn may account for the enhanced antiphenamine action of cerulein observed under such haloperidol treatment.

To sum up, long-term haloperidol treatment attenuates or reverses the inhibitory behavioral effects of cerulein with one exception: the sustained antagonist effect of cerulein on phenamine- induced excitation is enhanced rather than weakened. Critical to the attenuation of many cerulein effects is a reduction in the densities of CCK8 receptors and high-affinity dopamine$_2$ receptors. Long-term haloperidol administration probably changes the latter receptors to a low- affinity state in the test forebrain structures. The high-affinity dopamine$_2$ receptors occur, in the main, on interneurons of the caudate nucleus and limbic structures. This indicates that long-term haloperidol administration most likely reduces the functional activity of interneurons in subcortical structures of the forebrain.

An antagonistic effect of cerulein on phenamine excitation occurs only when the endopioid system is intact, but not when it has been destroyed or blocked, which implies that β-endorphins act as intermediaries between CCK8 and dopaminergic mechanisms in the mesolimbic system. Single- or multiple-dose cerulein administration elicits cerulein hypersensitivity in opioid receptors of the nucleus accumbens, and this in turn leads to enhanced antiphenamine action of cerulein.

4. Conclusions

The foregoing discussion clearly demonstrates that the effects resulting from long-term administration of the typical neuroleptic haloperidol are due to changes it causes not only in dopaminergic system but also in the GABA- and CCK8-ergic systems which are closely related morphologically and functionally with the latter. The studies described above warrant the following conclusions:

1. Alterations in the GABA-ergic system under the action of haloperidol are specific in that they occur on $GABA_A$ receptors and the closely related benzodiazepine receptors.

2. Haloperidol-induced changes in $GABA_A$ and benzodiazepine receptors may be of two kinds because of the existence of two functional types of these receptors. One type, the so-called inhibitory receptors, occur in forebrain structures, are closely linked with post synaptic dopamine receptors and inhibit their activity and thus the activity of the dopaminergic system. The other type, 'stimulatory' receptors, are found in the brainstem, are linked with monosynaptic autoreceptors, and inhibit the latter's activity to augment the function of monoaminergic systems. Long-term haloperidol administration causes stimulatory GABAA and benzodiazepine receptors to preponderate with the result that the behavioral effects of muscimol (a $GABA_A$ receptor agonist) or of Ro 15-1788 (a benzodiazepine receptor antagonist) may be reversed.

3. The benzodiazepine antagonist Ro 15-1788, unlike the agonist diazepam, is capable of rectifying the changes induced by long-term haloperidol treatment in $GABA_A$ and benzodiazepine receptors.

4. Cerulein, a CCK8 receptor agonist, resembles haloperidol in its behavioral (antidopaminergic action) and biochemical (changes in the affinity of $dopamine_2$) effects, but unlike haloperidol, it acts on $dopamine_2$ receptors indirectly, via CCK8 receptors, thereby apparently destabilizing the interaction of dopamine and dopamine agonists with $dopamine_2$ receptors. Some of the effects seen on long-term haloperidol treatment may therefore be mediated via CCK8-ergic mechanisms which are closely linked morphofunctionally with dopaminergic systems.

5. Adaptational changes in the CCK8 systems under the action of prolonged haloperidol treatment are directly dependent on alterations in other

neurotransmitter (dopaminergic and endopioid) systems with which they have close morphofunctional links. Such haloperidol treatment decreases CCK8 receptor density in rat forebrain (in the cerebral cortex, mesolimbic structures, and nucleus accumbens). Behavioral analysis, however, indicates that haloperidol lowers sensitivity in only a fraction of CCK8 receptors. The hypersensitivity of these CCK8 receptors is probably associated with the haloperidol-induced transition of a proportion of high-affinity dopamine$_2$ receptors to a low-affinity state. The other CCK8 receptors, which are closely associated with endopioids, develop hypersensitivity, as is indicated by the augmented antiphenamine activity of cerulein after repeated haloperidol injections. It should be noted that such interactions between CCK8, endopioids, and dopaminergic mechanisms occur in rats, but not in mice.

6. The adaptational alterations in dopaminergic, GABA-ergic, and CCK8-ergic systems described above underlie those side-effects and antipsychotic actions of haloperidol seen on its long-term administration.

References

Allikmets, L.H. and Rägo, L.K. (1983) The effect of benzodiazepine antagonist Ro 15-1788 on the effects of GABA-ergic drugs. *Naunyn-Schmiedeberg's Arch. Pharmacol*, 324, 235-237

Allikmets, L.H., Stanley, M. and Gershon, S. (1979) The effect of lithium on chronic haloperidol-enhanced apomorphine aggression in rats. *Life Sci.*, 25, 165-170

Allikmets, L.H , Zharkovsky, A.M., Nurk, A.M. *et al.* (1984) Effect of long-term treatment with neuroleptics on the plasticity of CNS receptors. *Vestn. Med. Nauk. SSSR*, No. 11, 37-42 (In Russian)

Bowery, N.G., Hill, D.R., Hudson, A.L. *et al.* (1980) (-) Baclofen decreases neurotransmitter release in mammalian CNS by an action at a novel GABA receptor. *Nature* 283, 92-94

Carlsson, A. (1983) Antipsychotic agents: elucidation of their mode of action. In: Parknam, M.J. and Bruinvels, J. (Eds) *Discoveries in Pharmacology* Vol. 1: *Psycho- and Neuropharmacology*, Amsterdam: Elsevier, pp. 197-206 Creese, I., Schneider, R. and Snyder, S.H. (1977) ^{3}H-spiroperidol labels dopamine receptors in pituitary and brain *Eur. J. Pharmacol.*, 46: 377-381

Dumbrille-Ross, A. and Seeman, P. (1984) Dopamine receptor elevation by cholecystokinin. *Peptides*, 5, 1207-1212

Haefely, W., Polc, P., Pieri, L. *et al.*, (1983) Neuropharmacology of benzodiazepines: synaptic mechanisms and neural basis of action. In: Costa, E. (Ed.), *The Benzodiazepines: From Molecular Biology to Clinical Practice* New York: Raven Press, pp. 21-66

Hökfelt, T.L., Skirboll, L., Rehfeld, J.F. *et al.*, (1980). A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing *Neuroscience* 5, 2093-2124

Matsubara, K. and Matsushita, A. (1986) β-endorphin involvement in the antidopaminergic effect of caerulein. *Jpn. J. Pharmacol.*, 40, 417-422

Möhler, H. and Okada, T. (1978) Biochemical identification of the site of action of benzodiazepines in human brain by ^{3}H-diazepam binding *Life Sci.*, 22 985-996

Nurk, A.M., Maimets, M.O., Rägo, L.K. and Vasar, E.E. (1984) Adaptational changes in the GABA-ergic system after discontinuation of chronic haloperidol treatment. In: *Mekhanizm deistviya i klinika proizvodnykh gamma-aminomaslyanoi kisolty* [Mechanism of Action and Clinical Effects of GABA Derivatives] (*Uchenye Zapiski Tartuskogo Gosuniversiteta [Transactions of Tartu State University]*, 687, 71-78 (In Russian)

Owen, F., Bourne, R.C., Poulter, M. *et al.* (1985) Tritiated etorphine and naloxone binding to opioid receptors in caudate nucleus in schizophrenia. *Br. J. Psychiat*, 146, 507-509

Seeman, P., (1980) Brain dopamine receptors. *Pharmacol. Rev.*, 32, 229-313 Stinus, L., Nadand, D., Jauregui, J. and Kelley, A.E. (1986) Chronic treatment with five different neuroleptics elicits behavioral supersensitivity to opiate infusion into the nucleus accumbens. *Biol. Psychiatr.*, 21, 34-48

Vasar, E.E., Maimets, M.O., Rägo, L.K. *et al.* (1984*a*) Effect of imidazobenzodiazepine (Ro 15-1788) on aggressive behavior in rats *Byul. Eksp. Biol. Med.*, 99, 441-443 (In Russian)

Vasar, E., Maimets, M., Nurk, A. and Allikmets, L. (1985) Stimulation of ^{3}H- spiroperidol binding by cerulein (analogue of the octapeptide cholecystokinin) after long-term Neuroleptic treatment *Byul. Eksp. Biol. Med.*, 100, 72-74 (In Russian)

Vasar, E., Maimets, M., Nurk, A. and Allikmets, L. (1984*b*) Caerulein stimulates ^{3}H-spiperone binding *in vivo* after long-term haloperidol administration. *Psychopharmacol. Bull.*, 20, 691-692

Vasar, E.E., Maimets, M., Nurk, A. *et al.*, (1986) Comparison of motor depressant effects of caerulein and *N*-propylnorapomorphine in mice. *Pharmacol. Biochem. Behav.*, 24, 469-478

Wang, R.Y., White, F.J., Voight, M.M. (1984) Cholecystokinin, dopamine and schizophrenia. *Trends Pharmacol. Sci*, 9, 436-438

Wise, S.P., (1985) Co-localization of GABA and neuropeptides in the cerebral cortex. *Trends Neurosci.*,3, 92-95

Zetler, G. (1985) Neuropharmacological profile of cholecystokinin-like peptides *Ann. N.Y. Acad. Sci.*, 448, 448-469

Zharkovsky, A.M. and Allikmets, L.H. (1986) Analysis of dopamine receptor supersensitivity after chronic neuroleptic treatment. In Valdman, A.V. (Ed.) *Drug Dependence and Emotional Behaviour* New York: Plenum, pp.286-302

Pharmacology Biochemistry & Behavior, Vol. 35, pp. 855–859. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Similar Behavioral and Biochemical Effects of Long-Term Haloperidol and Caerulein Treatment in Albino Mice

EERO VASAR, LEMBIT ALLIKMETS, ANDRES SOOSAAR AND AVO LANG

Laboratory of Psychopharmacology, Institute of General & Molecular Pathology
Tartu University, 34 Veski Street, 202400 Tartu, Estonia, USSR

Received 6 June 1988

VASAR, E., L. ALLIKMETS, A. SOOSAAR AND A. LANG. *Similar behavioral and biochemical effects of long-term haloperidol and caerulein treatment in albino mice.* PHARMACOL BIOCHEM BEHAV 35(4) 855–859, 1990.—Behavioral and biochemical experiments on male albino mice have revealed similar effects after the cessation of repeated (15 days) haloperidol (0.5 mg/kg daily IP) and caerulein (0.1 mg/kg daily SC) treatment. Tolerance developed to the action of muscimol (a GABA-A agonist, 1 mg/kg IP), caerulein (a CCK-8 agonist, 15 μg/kg SC) and flumazenil (a benzodiazepine antagonist, 10 mg/kg IP). Muscimol and caerulein were not able to suppress the motor activity of mice after 15 days treatment with haloperidol and caerulein. Flumazenil, which increased motor activity in saline-treated animals, also failed to affect activity after extended haloperidol or caerulein treatment. In contrast, the motor excitation induced by amphetamine (an indirect dopamine agonist, 3 mg/kg IP) was increased after haloperidol or caerulein administration. In radioligand binding studies the density of dopamine-2-receptors in striatum, opioid receptors in mesolimbic structures, and benzodiazepine and GABA-A receptors in brainstem was significantly elevated after long-term haloperidol or caerulein treatment. Simultaneously, the number of CCK-8, benzodiazepine and GABA-A receptors in cerebral cortex was decreased. It is probable that CCK-8-ergic mechanisms are involved closely in the action of repeated haloperidol treatment. CCK-8 seems to modulate the action of haloperidol through altering the sensitivity of dopamine, opioid, GABA-A and benzodiazepine receptors.

Long-term treatment Haloperidol Caerulein Exploratory activity Radioligand binding

IT is generally accepted that the antipsychotic potency of neuroleptic drugs is correlated with their affinity to dopamine-2-receptors in striatum (21,22). However, long-term treatment with neuroleptic drugs causes significant changes not only in dopamine receptors, but also in receptors for the other neurotransmitters. For example, the administration of haloperidol for 15 days increases the number of glutamate receptors in striatum (26), but decreases the density of GABA-A and benzodiazepine receptors in forebrain structures of rat (1,2). Recently, the involvement of cholecystokinin octapeptide (CCK-8) in the action of neuroleptic drugs has been established. Repeated administration, but not acute treatment, of different neuroleptic drugs (clozapine, chlorpromazine and haloperidol) evidently increases the amount of CCK-8 in striatum and mesolimbic structures (11). Chang *et al.* (6) have shown that long-term treatment with haloperidol increases the density of CCK-8 receptors in cortical and limbic structures of mice. In addition, Bunney *et al.* (3, 4, 7) have demonstrated that repeated, but not acute, administration of different neuroleptic drugs (haloperidol, chlorpromazine, clozapine, etc.) induces depolarization and subsequent inactivation of dopamine neurons in midbrain. Acute treatment with CCK-8 causes the same effect and proglumide, an antagonist of CCK-8, reverses completely the effect of neuroleptic drugs (4). The above studies support the idea that CCK-8 is playing an obvious role in the mediation of biochemical and behavioral effects of neuroleptic treatment. The aim of the present study was a further clarification of the involvement of CCK-8 in the action of neuroleptic drugs. To study this problem the behavioral and biochemical effects of long-term haloperidol and caerulein, an agonist of CCK-8 receptors, treatment were compared. The changes in dopamine-2-, opioid, GABA-A, benzodiazepine and CCK-8 receptors were studied in both behavioral and radioligand experiments. Carlsson (5) has suggested that neuroleptic drugs cause their antipsychotic effect by blocking mesolimbic dopamine receptors. Other investigators have reported that GABA- and CCK-8-ergic systems have dense morphofunctional connections with dopaminergic system in mesolimbic structures (12,14). Because the normal functioning of the mesolimbic dopamine system appears to be critical for the regulation of locomotor activity in rodents (8) we used the exploratory locomotor activity of mice to determine the long-term behavioural effects of haloperidol and caerulein. Haloperidol was chosen as the neuroleptic for investigation in this study, because it is a potent and widely used antipsychotic drug. It is also noteworthy that in drug discrimination experiments there is substantial generalization between haloperidol and CCK-8 (9). Caerulein was selected for investigation because it is most effective among the available CCK-8 analogs.

METHOD

Animals

Male albino mice unknown strain, weighing 25 ± 3 g, were used. Mice were maintained at 20 ± 2°C and on 12-hr light, between 8 a.m. and 8 p.m. with food and water ad lib.

Determination of Exploratory Activity

Exploratory activity was measured in individual cages. The cage for registration of exploratory activity was a cylinder with an inner diameter 40 cm and 2 photocells (located in walls) for detection of motor activity. Exploratory activity was counted between 15 and 45 min after intraperitoneal administration of amphetamine (an indirect dopamine agonist, 3 mg/kg), muscimol (a GABA-A agonist, 1 mg/kg) and flumazenil (a benzodiazepine antagonist Ro 15-1788, 10 mg/kg), or between 0 and 30 min in the case of subcutaneous treatment of caerulein (a CCK 8 agonist, 15 μg/kg). The doses of amphetamine, caerulein, muscimol and flumazenil were chosen according to results of our previous studies. These doses cause only moderate, but statistically evident, changes in exploratory activity. Thus, an increase or decrease in activity due to the action of these drugs can be detected after repeated treatment with haloperidol or caerulein.

Preparation of Brain Membranes for Radioligand Studies

Following decapitation (between 10 and 12 a.m.) the whole brain was rapidly removed from skull. The different brain regions (cerebral cortex, striata, mesolimbic structures/nucleus accumbens and tuberculum olfactorium/and brainstem) were dissected on ice. Freehand method was used for dissection of brainstem, whereas the other structures were dissected according to the method of Glowinski and Iversen (13). Brain regions from ten mice were pooled and homogenized in 10 volumes of ice-cold 50 mM Tris HCl, pH 7.4 at 4°C, using motor-driven Teflon-glass homogenizer for 12 strokes. The homogenate was centrifuged at 40000 × g for 15 min, resuspended in the same volume of buffer and again centrifuged for 15 min. The membrane preparation for all radioligand studies was the same, except for [^{3}H]-etorphine binding. In this case the homogenate of the mesolimbic structures was incubated for 45 min at 37°C between two centrifugations (for elimination of endogenous opioid peptides). In the case of [^{3}H]-muscimol binding the membranes were washed (centrifuged) 7 times at 40000 × g for 15 min.

Radioligand Binding Studies

Different incubation mixtures were used for the radioligand binding experiments. The binding of [^{3}H]-etorphine (36 Ci/mmole, Amersham International, U.K.), [^{3}H]-flunitrazepam (81 Ci/mmole, Amersham International, U.K.) and [^{3}H]-muscimol (19 Ci/mmole, Amersham International, U.K.) were performed in 50 mM Tris HCl (pH 7.4 at 4°C). [^{3}H]-Spiroperidol (77 Ci/mmole, Amersham International, U.K.) binding was determined in an incubation buffer consisting of the following: 50 mM Tris HCl (pH 7.4 at 4°C), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $EDTANa_2$, 50 μM pargyline and 0.1% ascorbic acid. [^{3}H]-Pentagastrin (81 Ci/mmole, NEN-Dupont, USA) binding was studied in the following incubation medium: 10 mM HEPES-KOH (pH 6.8 at 4°C), 5 mM $MgCl_2$, 1 mM $EDTANa_2$, 0.2% bovine serum albumin.

For the binding experiments each polypropylene tube (1.5 ml) received 50 μl of [^{3}H]-ligand, 50 μl of incubation medium or displacing compound and 400 μl of brain membrane homogenate (1–4 mg of original tissue wet weight). [^{3}H]-Flunitrazepam was added in concentrations from 0.6 to 16 nM. The nonspecific binding was determined by using 1 μM flunitrazepam. The membranes of cerebral cortex and brainstem were incubated at 0°C for 60 min. [^{3}H]-Muscimol was used in concentrations from 1 to 80 nM. The nonspecific binding was measured by 100 μM muscimol. The membranes of cerebral cortex and brainstem were incubated for 40 min at 0°C. [^{3}H]-Etorphine was added in concentrations from 0.05 to 3 nM, the nonspecific binding was detected by adding naloxone (10 μM). The incubation of membrane membranes was performed at 25°C for 45 min. [^{3}H]-Spiroperidol was used in concentrations from 0.1 to 2 nM and the nonspecific binding was measured by adding 1 μM spiroperidol. The membranes of murine striata were incubated for 30 min at 37°C. [^{3}H]-Pentagastrin was added to the incubation medium in concentrations from 0.1 to 20 nM, nonspecific binding was detected with 1 μM caerulein. Incubation of [^{3}H]-pentagastrin was performed for 75 min at 25°C.

In all cases the binding experiment was stopped by rapid centrifugation (Beckman microfuge model 12) for 3 min at 11000 × g. The supernatant was carefully discarded and remaining pellet was washed with ice-cold incubation buffer and the tips of polypropylene tubes were cut into counting vials. Radioactivity of samples was counted after stabilization in scintillation cocktail within 24 hours using a Beckman LS 6800 (counting efficacy 50–54%). The binding experiments were repeated at least three times and the data analyzed using the Scatchard method (19).

Drugs and Their Administration

The drugs used in the present investigation are caerulein (Ceruletide, Farmitalia Carlo Erba, Italy), haloperidol (Gedeon Richter, Hungary), spiroperidol (Janssen Pharmaceutica, Belgium), naloxone (Dupont, USA), flunitrazepam and flumazenil (Ro 15-1788) (Hoffmann-La Roche, Switzerland), muscimol (Serva, FRG), pargyline (Sigma, USA), amphetamine (USSR). Caerulein, muscimol, amphetamine and commercial solution of haloperidol were prepared in saline. The injection solution of flumazenil was made soluble in saline by adding some drops of Tween-80. Each injection was done in a volume of 0.1 ml/10 g body weight. Haloperidol (0.5 mg/kg IP) and caerulein (0.1 mg/kg SC) were injected once daily for 15 days. The doses of haloperidol and caerulein were chosen according to our previous studies. Acute administration of haloperidol (0.5 mg/kg) and caerulein (0.1 mg/kg) caused significant neuroleptic effects in mice (catalepsy and the reversal of the behavioral effects of dopamine agonists). The behavioral and radioligand experiments were performed 72 hours after the cessation of haloperidol and caerulein treatment.

Statistics

The results of the binding studies were evaluated by the Student's *t*-test. The results of the behavioral experiments were analyzed by the Mann-Whitney *U*-test.

RESULTS

According to our preliminary experiments the cessation of long-term administration of haloperidol and caerulein did not cause significant signs of withdrawal. The basal motor activity of mice was unaltered 72 hours after the last injection of repeated treatment with saline and haloperidol as well as caerulein (Table 1). In addition, we found that quinolinic acid- and picrotoxin-induced seizures were identical after the withdrawal of long term saline, haloperidol or caerulein administration. The behavioral effects of ketamine (motor excitation, stereotyped behavior) remained unchanged after the withdrawal of haloperidol and caerulein (data not presented). In addition, there were no significant differences in the binding values of [^{3}H]-spiroperidol, [^{3}H]-flunitrazepam and [^{3}H]-pentagastrin if the tissues were obtained 2 or 72 hours after the last injection of haloperidol and caerulein. Consequently, the changes in mice behavior and radioligand binding described below were not caused by the withdrawal of haloperidol and caerulein, but rather were induced by the repeated administration of both drugs.

TABLE 1

THE EFFECT OF CAERULEIN, AMPHETAMINE, MUSCIMOL AND FLUMAZENIL ON EXPLORATORY ACTIVITY AFTER 15-DAY HALOPERIDOL OR CAERULEIN TREATMENT IN MICE

	Long-Term Treatment					
	Saline		Haloperidol		Caerulein	
	Motor Activity Counts During 30 Min					
Drug/dose		%		%		%
Saline	171 ± 15	100	188 ± 14	110	184 ± 18	108
Caerulein 15 μg/kg	104 ± 10	100	172 ± 15*	165	190 ± 15*	183
Amphetamine 3 mg/kg	409 ± 30	100	598 ± 45*	146	704 ± 62*	172
Muscimol 1 mg/kg	89 ± 10	100	203 ± 36*	228	170 ± 28*	191
Flumazenil 10 mg/kg	261 ± 17	100	162 ± 15*	62	193 ± 16*	74

The study was performed 72 hours after the cessation of haloperidol, caerulein or saline treatment. The mean values ± S.E.M. are shown. *$p<0.05$ (U-test Mann-Whitney, compared to mice, receiving saline injections for 15 days).

Seventy-two hours after the cessation of 15 days of haloperidol (0.5 mg/kg daily) and caerulein (0.1 mg/kg daily) treatment the effects of different drugs on mice motor activity were changed. The motor excitation induced by amphetamine (3 mg/kg) was evidently increased after haloperidol or caerulein treatment (Table 1). However, tolerance developed to the action of muscimol (1 mg/kg), caerulein (15 μg/kg) and flumazenil (10 mg/kg). Muscimol and caerulein were not able to suppress the motor activity of mice after haloperidol or caerulein administration (Table 1). Flumazenil, which increased the motor activity in saline-treated animals, failed to affect activity after 15 days of haloperidol or caerulein treatment.

The prolonged haloperidol and caerulein treatment also affected the binding of different radioligands to washed brain membranes in a similar way. They changed mainly the number of binding sites of different radioligands, but failed to affect the affinity of the radioligands for their sites. The density of [^{3}H]-spiroperidol binding sites in striatum (mainly dopamine-2-receptors) was significantly increased after the administration of both drugs (Table 2). Similar increase of [^{3}H]-etorphine (labelling mu-, delta- and kappa-opioid receptors) binding sites was detected in mesolimbic structures. Differently from [^{3}H]-spiroperidol and [^{3}H]-etorphine binding the number of [^{3}H]-pentagastrin (a ligand interacting with central CCK-8 receptors) binding sites was evidently decreased in cerebral cortex. The changes in [^{3}H]-flunitrazepam and [^{3}H]-muscimol binding were dependent on the brain region studied. In cerebral cortex their number was reduced, whereas in brainstem the density of [^{3}H]-flunitrazepam and [^{3}H]-muscimol binding sites was increased after 15-day treatment of haloperidol and caerulein (Table 2).

DISCUSSION

Zetler (27) has shown in his experiments on mice that caerulein causes haloperidol-like behavioral effects, but the further pharmacological analysis revealed marked differences in the action of haloperidol and caerulein. Haloperidol, the potent antipsychotic drug, preferentially blocks dopamine-2-receptors (18), whereas

TABLE 2

THE EFFECT OF 1st DAYS OF HALOPERIDOL OR CAERULEIN ADMINISTRATION ON PARAMETERS OF DOPAMINE-2-, BENZODIAZEPINE, GABA$_A$, OPIOID AND CCK-8 RECEPTORS IN MOUSE BRAIN

	K_d (nM)			B_{max} (pmoles/g tissue)		
Radioligand, Brain Structure	Saline	Haloperidol	Caerulein	Saline	Haloperidol	Caerulein
[^{3}H]-spiroperidol, striatum	0.47 ± 0.05	0.62 ± 0.05	0.63 ± 0.05	34.8 ± 3.0	45.0 ± 2.5*	49.2 ± 3.2*
[^{3}H]-flunitrazepam, cerebral cortex	1.70 ± 0.25	1.60 ± 0.25	1.50 ± 0.18	198 ± 12	144 ± 15*	138 ± 14*
[^{3}H]-flunitrazepam, brainstem	2.42 ± 0.20	1.92 ± 0.18	2.62 ± 0.17	103 ± 8	125 ± 12	142 ± 16*
[^{3}H]-muscimol, cerebral cortex	9.6 ± 1.6	10.2 ± 1.8	11.0 ± 1.2	91 ± 8	64 ± 5*	63 ± 6*
[^{3}H]-muscimol, brainstem	12.6 ± 1.3	13.2 ± 1.3	14.3 ± 1.3	38 ± 4	51 ± 4	50 ± 5
[^{3}H]-etorphine, mesolimbic area	0.62 ± 0.05	0.61 ± 0.05	0.77 ± 0.05	33 ± 2.4	42 ± 2.5*	46 ± 3.2*
[^{3}H]-pentagastrin, cerebral cortex	3.50 ± 0.40	3.20 ± 0.30	3.20 ± 0.32	5 ± 0.4	3.5 ± 0.3*	3.2 ± 0.3*

The study was performed 72 hours after the cessation of haloperidol, caerulein or saline treatment. The mean values of three independent experiments are shown. Statistically evident differences from saline-treated mice: *$p<0.05$ (Student's t-test). K_d, constant of dissociation (nM); B_{max}, apparent number of binding sites (pmoles/g wet weight tissue).

caerulein stimulates CCK-8 receptors (27). Despite the significant differences in the molecular action of the two drugs, long-term treatment with haloperidol and caerulein has a similar effect on behavior and causes similar changes in radioligand binding to washed brain membranes. Our data suggest that both compounds increase the number of dopamine-2-receptors in striatum and opioid receptors in mesolimbic structures. The increased sensitivity of mice to motor stimulating effect of amphetamine, a compound that increases the release of dopamine, probably reflects the enhancement of dopamine-2-receptors density after haloperidol or caerulein treatment. Some authors have demonstrated (17,23) that opioid receptors in limbic structures play an important role in the regulation of dopamine receptors' sensitivity. The prolonged administration of different neuroleptic drugs (haloperidol, sulpiride, flupenthixol, etc.) leads to the hypersensitivity not only of dopamine receptors, but also of opioid receptors in mesolimbic structures (20,23). It seems probable that the increased sensitivity of opioid receptors is obligatory for the development of hypersensitivity in dopamine receptors in mesolimbic area.

After 15 days of haloperidol and caerulein administration a marked decrease in CCK-8 receptors density in cerebral cortex is found. The significant reduction of motor depressant effect of caerulein after haloperidol or caerulein treatment is probably related to the decrease of CCK-8 receptor number in brain. Consequently, haloperidol and caerulein treatment cause a subsensitivity of CCK-8 receptors. A similar subsensitivity (decrease of [^{3}H]-CCK-8 binding sites in mouse and rat brain, tolerance or inversion of caerulein's behavioral effects) of CCK-8 receptors was found after long-term haloperidol treatment in our previous experiments (25). Many behavioral studies now support the idea that CCK-8 acts as a functional antagonist of dopamine and endogenous opioid peptides in brain (10, 16, 27). Accordingly, the subsensitivity of CCK-8 receptors seems to be necessary for the development of hypersensitivity of dopamine and opioid receptors. However, Chang *et al.* (6) have shown the opposite effect, the increase of the number of CCK-8 receptors, after repeated haloperidol treatment in mice. They have used [^{125}I]-CCK-33 for labelling of CCK-8 receptors and they have administered significantly higher dose of haloperidol (2–3 mg/kg) to mice. These factors may explain the differences between our study and that of Chang *et al.* (6). Despite the discrepancy the above-mentioned results would support the idea that CCK-8-ergic mechanisms play a crucial role in the mediation of the effects of prolonged neuroleptic treatment.

The changes in benzodiazepine and GABA-A receptors differ from those of the other neurotransmitter receptors after long-term haloperidol or caerulein administration. In frontal cortex the density of benzodiazepine and GABA-A receptors is reduced and it is parallel to the reduction of CCK-8 receptors. The number of benzodiazepine and GABA-A receptors in brainstem, on the contrary, is increased after haloperidol or caerulein treatment. The similar alteration of CCK-8 and benzodiazepine-GABA-A receptors in cerebral cortex may be linked to the finding that CCK-8 and GABA are co-mediators in the same neurons of cerebral cortex and hippocampus (15). The molecular changes in benzodiazepine and GABA-A receptors are probably associated with tolerance of behavioral effects of GABA-A agonist muscimol and benzodiazepine antagonist flumazenil. Muscimol did not suppress and flumazenil did not increase the motor activity of mice after long-term treatment of haloperidol and caerulein. The possible explanation for these changes may consist of the existence of functionally different benzodiazepine and GABA-A receptors in forebrain and brainstem structures (24). The results of present study show that CCK-8 may have, through CCK-8 receptors indeed, a modulating action on the sensitivity of GABA-A-benzodiazepine, opioid and dopamine receptors. This opinion is supported not only by present study, but also by other investigators. CCK-8 and caerulein inhibit not only the action of amphetamine and methylphenidate, interacting with presynaptic dopaminergic mechanisms, but also the effects of apomorphine, a direct agonist of dopamine receptors (27).

In conclusion, the similar actions of haloperidol and caerulein after long-term treatment seem to be related to the fact that the effects of haloperidol are effected not only through dopaminergic, but also via CCK-8-ergic mechanisms. The effect of CCK-8 seems to be related to the modulation (through CCK-8 receptors) of the sensitivity of different neurotransmitter receptors (dopamine, endogenous opioid peptides and GABA).

ACKNOWLEDGEMENTS

We would like to thank Farmitalia Carlo Erba, Janssen Pharmaceutica, Hoffmann-La Roche and Dupont for the generous gifts of drugs.

REFERENCES

1. Allikmets, L. H.; Zarkovsky, A. M.; Nurk, A. M.; Vasar, E. E.; Maimets, M. O.; Rago, L. K. Effect of prolonged administration of neuroleptics on CNS receptors plasticity. Proc. Med. Acad. Sci. USSR 11:37–42, 1984.
2. Allikmets, L. H.; Vasar, E. E.; Nurk, A. M.; Maimets, M. O. Adaptive changes on GABA and benzodiazepine receptors after prolonged treatment with haloperidol. In: Babkov, J. G., ed. Pharmacological regulation of desadaptation. Moscow: Institute of Pharmacology Press; 1986:36–43 (in Russian).
3. Bunney, B. S.; Grace, A. A. Acute and chronic haloperidol treatment: comparison of effects on nigral dopaminergic cell activity. Life Sci. 23:1715–1728; 1978.
4. Bunney, B. S.; Chodo, L. A.; Freeman, A. S. Further studies on the specificity of proglumide as a selective cholecystokinin antagonist in the central nervous system. Ann. NY Acad. Sci. 448:345–351; 1985.
5. Carlsson, A. Antipsychotic drugs, neurotransmitters and schizophrenia. Am. J. Psychiatry 135:164–173; 1978.
6. Chang, R. S. L.; Lotti, V. S.; Martin, G. E.; Chen, T. B. Increase in brain [^{125}I]-cholecystokinin (CCK) receptor binding following chronic haloperidol treatment, intracisternal 6-hydroxydopamine and ventral tegmental lesions. Life Sci. 32:871–878; 1983.
7. Chiodo, L. A.; Bunney, B. S. Typical and atypical neuroleptics: differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminegic neurons. J. Neurosci. 3:1607–1609; 1983.
8. Costall, B.; Domeney, A. M.; Naylor, R. J. The continuity of dopamine receptors antagonism can dictate the long-term behavioral consequences of a mesolimbic infusion of dopamine. Neuropharmacology 24:193–197; 1985.
9. De Witte, P.; Swanet, E.; Gewiss, M.; Goldman, S.; Roques, E.; Vanderhaeghen, J.-J. Psychopharmacological profile of cholecystokinin using the self-stimulation and the drug-discrimination paradigms. Ann. NY Acad. Sci. 448:470–487; 1985.
10. Faris, P. L.; Komisaruk, B. R.; Watkins, L. R.; Mayer, D. J. Evidence for the neuropeptide cholecystokinin as an antagonist of opiate analgesia. Science 219:310–312; 1983.
11. Frey, P. Cholecystokinin octapeptide levels in rat brain are changed after subchronic neuroleptic treatment. Eur. J. Pharmacol. 95:87–92; 1983.
12. Garbutt, J. C.; VanKammen, D. P. The interaction between GABA and dopamine: implications for schizophrenia. Schizophr. Bull. 9: 336–353; 1983.
13. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in rat brain. J. Neurochem. 13:655–664; 1966.
14. Hokfelt, T.; Rehfeld, J. F.; Skirboll, L.; Ivemark, B.; Goldstein, M.; Markey, K. Evidence for coexistence of dopamine and CCK in mesolimbic neurons. Nature 285:476–478; 1980.

15. Kosaka, T.; Kosaka, K.; Tateishi, K.; **Hamaoka, Y.**; Yanaihara, N.; Wu, J.-Y., Hama, K. GABAergic neurons containing CCK-8-like or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J. Comp. Neurol. 239:420–430; 1985.
16. Matsubara, K.; Matsushita, A. Long-lasting reduction of amphetamine induced hyperactivity in rats after combined coadministration of caerulein with haloperidol. Eur. J. Pharmacol. 101:157–158; 1984.
17. Matsubara, K.; Matsushita, A. β-endorphine involvement in the antidopaminergic effect of caerulein. Jpn. J. Pharmacol. 40:417–422; 1986.
18. Ortmann, R.; Bischoff, S.; Radeke, E.; Buesh, O.; **Delini-Stula, A.** Correlations between different measures of antiserotonin activity of drugs: study with neuroleptics and serotonin blockers. Naunyn Schmiedebergs Arch. Pharmacol. 321:265–270; 1982.
19. Scatchard, G. The attraction of protein for small molecules and ions. Ann. NY Acad. Sci. 51:660–672; 1949.
20. Seeger, T. F.; Nazzaro, J. M.; Gardner, E. L. Selective inhibition of mesolimbic behavioral supersensitivity by naloxone. Eur. J. Pharmacol. 65:435–438; 1980.
21. Seeman, P.; Lee, T.; Chau-Wong, M.; Wong, K. Antipsychotic drug doses and dopamine/neuroleptic receptors. Nature 261:717–719; 1976.
22. Seeman, P. Brain dopamine receptors. Pharmacol. Rev. 32:229–313; 1980.
23. Stinus, L.; Nadaud, D.; Jauregui, J.; Kelley, A. E. Chronic treatment with five different neuroleptics elicits behavioral supersensitivity to opiate infusion into nucleus accumbens. Biol. Psychiatry 21:34–48; 1986.
24. Vasar, E. E.; Soosaar, A. H.; Nurk, A. M.; Maimets, M. O.; Allikmets, L. H. Distinct effect of long-term haloperidol treatment on GABA-A and benzodiazepine receptors in different brain areas. Bull. Exp. Biol. Med. 101:433–435; 1986.
25. Vasar, E. E.; Soosaar, A. H.; Maimets, M. O.; Allikmets, L. H. Reduced sensitivity of the brain cholecystokinin receptor after the prolonged treatment with haloperidol. Bull. Exp. Biol. Med. 101: 583–585; 1986.
26. Zarkovsky, A. M.; Allikmets, L. H. The action of acute and repeated administration of neuroleptics on CNS receptors. In: Abstracts of All-Union Symposium "Chemistry, Pharmacology and Clinical Use of Neuroleptics." Tartu: Tartu University Press; 1986:47–49 (in Russian).
27. **Zetler, G.** Neuropharmacological profile of cholecystokinin-like peptides. Ann. NY Acad. Sci. 448:448–469; 1985.

In: Psychosomatic Disorders. (V. Vasar ed.). Acta et Commentationes Universitatis Tartuensis, 1991, 891: 215-223

THE CHANGES AT CHOLECYSTOKININ RECEPTORS AFTER LONG-TERM TREATMENT WITH DIAZEPAM

E.Vasar, J.Harro, A.Soosaar, A.Lang

Laboratory of Psychopharmacology, Tartu University

202400 TARTU, Estonia

SUMMARY

The effect of benzodiazepine withdrawal was studied on CCK-8 receptors in experiments on male rodents. Benzodiazepine anxiolytic diazepam (5 mg/kg i.p.) was injected for two-weeks. The behavioural and radioligand binding studies were performed 24-72 hours after the last injection of diazepam. The significantly suppressed exploratory activity of mice in elevated plus-maze was reflecting the obvious anxiety after benzodiazepine withdrawal. CCK-8 agonist caerulein (500 ng/kg), which induced anxiogenic-like effect in control animals, was not able to change the exploratory activity of mice pretreated with diazepam for two-weeks. The sedative effect of caerulein (15 μg/kg) was reduced after withdrawal of benzodiazepine treatment. The mild antiaggressive effect of caerulein (40 μg/kg) was replaced by proaggressive action in male mice pretreated with diazepam. The anticonvulsant effect of caerulein (125 μg/kg) against picrotoxin-induced seizures was completely reversed after withdrawal of diazepam treatment. These results suggest that the subsensitivity is developing at CCK-8 receptors after long-term diazepam treatment. However, according to radioligand studies the number of ^{3}HpCCK-8 binding sites was increased in frontal cortex, piriform cortex and hippocampus of rat after benzodiazepine withdrawal. It seems possible that repeated treatment with diazepam is causing the opposite changes at different subtypes of CCK-8 receptors. The sensitivity of one subtype is reduced, whereas the affinity of others is increased to CCK-8. Probably the described changes at CCK-8 receptors are related to increased anxiety after withdrawal of long-term diazepam treatment.

KEY WORDS: DIAZEPAM; BENZODIAZEPINE WITHDRAWAL; CCK-8 RECEPTORS; CAERULEIN; ANXIOGENIC-LIKE EFFECT; SEDATIVE EFFECT; AGGRESSIVENESS; ANTICONVULSIVE EFFECT; MOUSE; RAT

INTRODUCTION

Cholecystokinin octapeptide (CCK-8), neuropeptide widely distributed in CNS, is shown to colocalize with major inhibitory transmitter GABA in cerebral cortex and hippocampus (Kosaka et al., 1985). Interaction between CCK-8 and GABA seems to be antagonistic, because several behavioural effects of CCK-8 and caerulein, its structural analogue, can be antagonized by the administration of benzodiazepine anxiolytics (Kubota et al., 1985; 1986). It is widely accepted that benzodiazepine anxiolytics exert their action through the facilitation of GABAergic neurotransmission in the brain (Haefely et al., 1985). Two benzodiazepines, diazepam and lorazepam, depress at very low doses selectively the CCK-8-induced excitation of rat hippocampal pyramidal cells (Bradwejn, De Montigny, 1984). It has been suggested that this effect of benzodiazepine tranquillizers might be related to their anxiolytic properties (Bradwejn, De Montigny, 1985). The blockade of CCK-8 receptors by CCK antagonist lorglumide is shown to potentiate several behavioural effects of diazepam (Panerai et al., 1987). In our previous studies the potent anxiogenic-like effect of CCK-8 agonists (caerulein, pentagastrin) is established (Harro et al., 1989b). Subchronic, but not acute, treatment with diazepam is able to antagonize the anxiogenic-like action of caerulein (Harro et al., 1989a). Recent evidence suggests that repeated treatment with benzodiazepine anxiolytics reduces electrophysiologically measured neuronal responsiveness to CCK-8 in hippocampus (Bouthillier, De Montigny, 1988). The above described studies probably reflect very significant role of CCK-8 in the action of benzodiazepine tranquillizers. The aim of present study was to reveal the role of CCK-8 receptors in benzodiazepine withdrawal. The different behavioural effects of caerulein (anxiogenic, sedative, antiaggressive and anticonvulsant) and ^{3}HpCCK-8 binding were studied after withdrawal of two-weeks diazepam administration in rodents.

MATERIALS AND METHODS

Male unstrained albino laboratory mice (20-25 g) and male Wistar rats (220-250 g) were used in this study. Every experimental group consisted of 8-10 animals. Diazepam (5.0 mg/kg i.p. daily, Seduxen, commercial solution, *Gedeon Richter*, Hungary) or diazepam vehicle (40% propylene glycol, 10% ethyl alcohol, 5% sodium benzoate, 1,5% benzyl alcohol) were injected for 14 days. The behavioural and radioligand binding experiments were performed 24-72 hours after the last injection of drug or vehicle. The anxiogenic-like effect of CCK-8 agonist caerulein (*Farmitalia-Carlo Erba*, Italy) was studied according to the original method of Pellow et al. (1985) (so-called 'elevated plus-maze') in our slight modification (Harro et al., 1989a). Exploratory activity of mice in elevated plus-maze was detected 15 min after the injection of caerulein (500 ng/kg i.p.). The locomotor activity of animals was measured in individual cages. The cage for registration of motor activity was a cylinder with an inner diameter 40 cm and 2 photocells (located in walls) for detection of motor activity.

Locomotor activity was counted between 0 and 30 min after subcutaneous treatment with caerulein (15 μg/kg). The action of caerulein on aggressive behaviour was determine according to the method of foot-shock-elicited aggressiveness. Each pair of mice received during 2 min 48 foot-shocks (2 mA) and number of aggressive contacts (bitings, boxings etc.) was counted. Caerulein 40 μg/kg was injected 15 min before the experiment. In the case of study of anticonvulsive effect of caerulein the mice were placed in individual observation boxes 15 min before the start of the experiment. After this habituation period each animal was injected with caerulein (125 μg/kg) or saline 10 min prior to picrotoxin (10 mg/kg, *Sigma*, USA). Mice were observed for 30 min and the latencies to onset of clonic seizures, tonic extension and death were registered. ^{3}HpCCK-8 (60 Ci/mmole, *Amersham International plc*, UK) binding was measured by the method of Praissman et al. (1983) with slight modifications. The rats were killed by cervical dislocation respectively 30 min and 24 h after the last injection of diazepam and the brains immediately removed from the skull. The different brain regions - frontal cortex, piriform cortex and hippocampus - were dissected on ice. Brain regions from 5 rats were pooled. Saturation curves of ^{3}HpCCK-8 binding (0.05-2 nM) were analyzed using the ENZFITTER program on IBM microcomputers (Leatherbarrow, 1987).

RESULTS

The exploratory activity of mice in elevated plus-maze was significantly suppressed after withdrawal of repeated diazepam administration in comparison with animals receiving vehicle (table 1). The administration of caerulein (500 ng/kg) caused the anxiogenic-like effect (reduction of exploratory activity) in vehicle pretreated mice, whereas after repeated treatments with diazepam it did not change the behaviour of mice. After withdrawal of diazepam injections the motor activity of mice was somewhat reduced in comparison with vehicle treated group (table 2). 15 μg/kg caerulein suppressed significantly the motor activity in control group, whereas in benzodiazepine pretreated mice the sedative effect of caerulein was reduced. In control mice 40 μg/kg caerulein slightly reduced the number of aggressive contacts between animals. However, after diazepam withdrawal caerulein increased markedly the aggressiveness (table 3). 125 μg/kg caerulein evidently antagonized seizures induced by picrotoxin in control mice, but not in animals after diazepam withdrawal (table 4). Long-term treatment with diazepam or withdrawal of diazepam administration did not alter the affinity (K_d) of ^{3}HpCCK-8 binding sites in different forebrain structures (table 5). Two-weeks treatment with diazepam elevated the number of CCK-8 binding sites in piriform cortex. After the withdrawal of diazepam treatment the density of CCK-8 binding sites was increased in all three brain structures studied.

Table 1

Effect of caerulein (500 ng/kg) on exploratory activity of mice after repeated (14 days) treatment with diazepam in elevated plus-maze.

Pretreatment/ drug	Latency of first open part entry (s) in plus-maze	No of sectors crossed in open part of plus-maze	Total time spent in open part of plus-maze(s)
Vehicle+saline	10±2	31±2.8	96±7
Vehicle+caerulein	53±29	16±4.1**	61±12*
Diazepam+saline	16±3	16±3.7**	64±12*
Diazepam+caerulein	41±15	17±4.3	49±11

The last treatment was given 72h prior to the experiment. Saline and caerulein were administered 15 min prior to experiment. * - $p < 0.05$ significantly different from vehicle+saline; ** - $p < 0.01$ significantly different from vehicle+saline, Duncan's test following significant ANOVA.

Table 2

Effect of long-term treatment with diazepam on motor depressant effect of caerulein (15 μg/kg) in mice

Long-term treatment+ drug	Number of motor activity counts during 15 min	%	during 30min	%
Vehicle + saline	184±17	100	325±39	100
Vehicle + caerulein	115±19*	63	198±35*	61
Diazepam + saline	177±19	100	263±22	100
Diazepam + caerulein	116±16**	66	214±32	81

The experiment was performed 48 hours after the last injection of diazepam. Caerulein (15 μg/kg) was injected immediately before the experiment. The number of mice in each group was 10-12. * - $p < 0.05$ (Student's t-test, in comparison with vehicle+saline treated group); ** - $p < 0.05$ (Student's t-test, as compared with diazepam+saline).

Table 3

Effect of caerulein (40 μg/kg) on foot-shock induced aggressiveness after long-term treatment with diazepam.

Long-term treatment + drug	Number of aggressive contacts during 2 min
Vehicle + saline	14±1.0
Vehicle + caerulein	9±1.6
Diazepam + saline	13±2.5
Diazepam + caerulein	28±3.2*,**

The experiment was performed 48 hours after the last injection of diazepam. Caerulein (40 μg/kg) was injected 20 min before the experiment. The number of mice in each group was 10-12. * - $p < 0.002$ (Newman-Keuls test following significant ANOVA as compared with diazepam + saline).** - $p < 0.0001$ (Newman-Keuls test following significant ANOVA, in comparison with vehicle + caerulein).

Table 4

Anticonvulsant effect of caerulein (125 μg/kg) against picrotoxin (10 mg/kg) induced seizures after repeated treatment with diazepam in mice.

	Latency			
Long-term treatment+ drug	Clonic seizures (sec.)	Tonic seizures (min.)	Death (min.)	Number of mice survived seizures
Vehicle + picrotoxin	442±25	16.1±1.5	17.4±1.7	0/9
Vehicle + caerulein + picrotoxin	775±141*	23.0±2.0*	24.3±2.5*	5/9
Diazepam + picrotoxin	615±151	21.0±2.1	21.2±2.0	1/10
Diazepam + caerulein + picrotoxin	564±61	19.5±2.6	19.8±2.5	1/10

The experiment was performed 48 hours after the last injection of diazepam. Caerulein (125 μg/kg) was injected 10 min prior to picrotoxin (10 mg/kg). * - $p < 0.05$ (Newman-Keuls test following significant ANOVA, as compared with vehicle + picrotoxin).

Table 5

3H-CCK-8 binding with rat brain homogenates after repeated treatment and after withdrawal of diazepam (5 mg/kg per day i.p. for two weeks) or vehicle.

	Repeated treatment		Withdrawal	
Brain region/group	K/d	B/max	K/d	B/max
Frontal cortex				
VEHICLE	0.27±0.01	23.4±0.7	0.19±0.02	20.6±1.3
DIAZEPAM	0.22±0.03	24.2±1.3	0.22±0.04	27.2±2.1*
Piriform cortex				
VEHICLE	0.29±0.04	30.4±2.4	0.27±0.03	31.5±1.7
DIAZEPAM	0.33±0.04	47.0±4.2*	0.39±0.07	38.7±3.4
Hippocampus				
VEHICLE	0.46±0.09	9.5±1.5	0.66±0.16	10.5±1.7
DIAZEPAM	0.31±0.14	9.8±2.6	0.70±0.12	15.6±1.9*

Rats were decapitated 30 min or 24 h after last pretreatment injection respectively. Results are from pooled tissue of 5 animals. B/max expressed as fmol/mg protein; K/d expressed as nM. * - $p < 0.05$, Student's t-test, as compared to corresponding vehicle group.

DISCUSSION

The present results show the significance of CCK-8 receptors in the development of withdrawal signs after long-term diazepam treatment. All the studied behavioural effects of CCK-8 agonist caerulein are changed after withdrawal of long-term diazepam treatment. The significantly suppressed exploratory activity of mice in elevated plus-maze is reflecting obvious anxiety in these animals after benzodiazepine withdrawal. It seems to be the main reason why diazepam pretreated mice did not react to anxiogenic-like effect of caerulein in elevated plus-maze. The reduction of sedative effect of caerulein is probably related to decreased basal motor activity of mice after diazepam withdrawal. The mild antiaggressive effect of caerulein was replaced by proaggressive action after 14 days diazepam medication. The anticonvulsant action of caerulein against picrotoxin-induced seizures was reversed by repeated diazepam pretreatments for two weeks. These results suggest that subsensitivity is developing at CCK-8 receptors after withdrawal of long-term diazepam treatment. This opinion is supported by the study of Bouthillier and De Montigny (1988). They have shown that 14-day treatment with either diazepam or flurazepam reduced the responsiveness of rat dorsal hippocampus pyramidal neurons to CCK-8. However, according to ^{3}HpCCK-8 binding studies the number of CCK-8 receptors is elevated in different structures of rat forebrain after 14-day treatment with benzodiazepine anxiolytic. Probably two opposite processes are taking place at CCK-8 receptors during repeated benzodiazepine treatment. The sensitivity of one subtype of CCK-8 receptors is reduced, whereas the other subtype is becoming more sensitive to CCK-8 after diazepam withdrawal. The existence of different subtypes of CCK-8 receptors is described in radioligand (Moran et al., 1986; Wennogle et al., 1988) and electrophysiological experiments (Mac Vicar et al., 1987). Mac Vicar et al. (1987) have shown that low concentrations of CCK-8 are inhibiting the activity of hippocampal pyramidal neurons, whereas only very high concentrations of CCK-8 are causing the excitation of these cells (Dodd, Kelly, 1981). From radioligand binding studies the existence of CCK-A ("visceral") and CCK-B ("brain") receptors is described in different brain regions (Moran et al., 1986; Hill et al., 1987; Barrett et al., 1989). But the relation of CCK-A and CCK-B receptors to subtypes of CCK-8 receptors, in which long-term diazepam treatment is evoking respectively sub- and hypersensitivity, is still unclear.

In conclusion, the present study is supporting the idea of Bouthillier and De Montigny (1988) that long-term diazepam treatment is affecting the sensitivity of CCK-8 receptors. But differently from this study it is possible that repeated treatment with diazepam is causing the opposite changes at different subtypes of CCK-8 receptors. Two-weeks administration of diazepam is reducing the sensitivity of one subtype, but increasing the affinity of others to CCK-8. It is probable that described changes at CCK-8 receptors are related to increased anxiety after benzodiazepine withdrawal.

REFERENCES

Barrett R.W., W.E.Steffey and C.A.W.Wolfram (1989) Type-A CCK binding sites in cow brain: characterization using (-)-^{3}H-L-364,718 membrane binding assays. Mol.Pharmacol. 36: 285-290.

Bouthillier A. and C.DeMontigny (1988) Long-term benzodiazepine treatment reduces neuronal responsiveness to cholecystokinin: an electrophysiological study in the rat. Eur.J.Pharmacol. 151: 135-138.

Bradwejn J. and C.DeMontigny (1984) Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurones. Nature 312: 363-364.

Bradwejn J. and C.DeMontigny (1985) Effects of PK 8165, a partial benzodiazepine receptor agonist, on cholecystokinin-induced activation of hippocampal pyramidal neurons: a microiontophoretic study in the rat. Eur.J.Pharmacol. 112; 415-418.

Dodd J. and J.S.Kelly (1981) The actions of cholecystokinin and related peptides on pyramidal neurons in the mammalian hippocampus. Brain Res. 205: 337-350.

Haefely W., E.Kyburz, M.Gerecke and H.Möhler (1985) Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists. Adv.Drug Res. 14: 165-322.

Harro J., M.Põld and E.Vasar (1989a) Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: influence of acute and subchronic diazepam treatment. Naunyn-Schmiedeberg's Arch. Pharmacol. (in press).

Harro J., M.Põld, E.Vasar and L.Allikmets (1989b) The role of brain cholecystokinin receptors in generation of anxiety states in mice and rats. In: Sudakov K.V. (ed.) Systems Research in Physiology, vol. 5, Gordon & Breach, London (in press).

Hill D.R., N.J.Campbell, T.M.Shaw and C.N.Woodruff (1987) Autoradiographic localization and characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J.Neurosci. 7: 2967-2976.

Kosaka T., K.Kosaka, K.Tateishi, Y.Hamaoka, N.Yanaihara, J.-Y.Wu and K.Hama (1985) GABAergic neurons containing CCK-8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J.Comp.Neurol, 239: 420-430.

Kubota K., K.Sugaya, I.Matsuda, Y.Matsuka and M.Itonaga (1985) Caerulein antagonism by

benzodiazepines in the food intake in mice. Jpn.J.Pharmacol. 39: 120-122.

Kubota K., F.-Y.Sun, K.Sugaya, and N.Sunagane (1986) Reversal of antinociceptive effect of caerulein by benzodiazepine. J.Pharmacobio-Dyn 39: 428-431.

Leatherbarrow R.J. (1987) Enzfitter, a non-linear regression data analysis program for the IBM PC. Elsevier Science Publisher, Amsterdam, The Netherlands.

MacVicar R.A., J.P.Kerrin and J.S.Davison (1987) Inhibition of synaptic transmission in the hippocampus by cholecystokinin (CCK) and its antagonism by a CCK analog (CCK27-33). Brain Res. 406: 130-135.

Moran T.H., P.H.Robinson, M.Goldrich and P.R.McHugh (1986) Two brain CCK receptors: implications for behavioral actions. Brain Res. 362: 175-179.

Panerai A.E., L.C.Rovati, M.Bianchi, S.R.Bareggi and P.Mantegazza (1987) Effect of a cholecystokinin antagonist on some effects of diazepam. Neuropharmacology 26: 1285-1287.

Pellow S., P.Chopin, S.E.File and M.Briley (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J.Neurosci.Meth. 14: 149-167.

Praissman M., P.A.Martinez, C.F.Saladino, J.M.Berkowitz, A.W.Steggles and J.A.Finkelstein (1983) Characterization of cholecystokinin binding sites in rat cerebral cortex using 125I-CCK-8 probe resistant to degradation. J.Neurochem. 40: 1406-1414.

Wennogle L., H.Wysowskyi, D.J.Steel and B.Petrack (1988) Regulation of central cholecystokinin recognition sites by guanyl nucleotides. J.Neurochem. 50: 954-959

In: Psychopharmacology, 1991, 105: 393-399

DIFFERENTIAL INVOLVEMENT OF CCK-A AND CCK-B RECEPTORS IN THE REGULATION OF LOCOMOTOR ACTIVITY IN THE MOUSE

Eero Vasar*, Jaanus Harro, Aavo Lang, Anu Pôld,
and Andres Soosaar

Psychopharmacology Lab, Institute of General
and Molecular Pathology, Tartu University,
34 Veski Street, 202400 TARTU, Estonia.
Telefax: (USSR code) 01434 30365
* - To whom all correspondence should be addressed

ABSTRACT

The influence of the CCK-A antagonist devazepide and the CCK-B/gastrin antagonist L-365,260 on the locomotor activity of mice was studied. Devazepide and L-365,260 had opposite effects on spontaneous locomotor activity, and on caerulein- and apomorphine-induced hypomotility in the mouse. Devazepide in high doses (0.1-1 mg/kg IP) reduced spontaneous motor activity, whereas L-365,260 at a high dose (1 mg/kg IP) increased the activity of mice. Devazepide (0.1-10 µg/kg) moderately antagonized the sedative effect of apomorphine (0.1 mg/kg SC) and caerulein (25 µg/kg SC), whereas L-365,260 (1-10 µg/kg) significantly potentiated the actions of dopamine and CCK agonists. Concomitant administration of caerulein (15 µg/kg SC) and apomorphine (0.1 mg/kg SC) caused an almost complete loss of locomotor activity in the mouse. Devazepide and L-365,260 (0.1-10 µg/kg) were completely ineffective against caerulein-induced potentiation of apomorphine's hypomotility. Devazepide in high doses (0.1-1 mg/kg), reducing the spontaneous motor activity of mice, counteracted the motor excitation induced by d-amphetamine (5 mg/kg IP). The CCK agonist caerulein (100 µg/kg SC) had a similar antiamphetamine effect. Devazepide (1-100 µg/kg) and L-365,260 (1 µg/kg) reversed completely the antiamphetamine effect of caerulein.

The results of present study reflect apparently distinct role of CCK-A and CCK-B receptors in the regulation of motor activity. The opposite effect of devazepide and L-365,260 on caerulein- and apomorphine-induced hypolocomotion is probably related to the antagonistic role of CCK-A and CCK-B receptor subtypes in the regulation of mesencephalic dopaminergic neurons. The antiamphetamine effect of caerulein is possibly linked to the stimulation of CCK-A receptors in the mouse brain, whereas the blockade of both subtypes of the CCK-8 receptor is involved in the antiamphetamine effect of devazepide.

Key words: Caerulein - CCK-A receptors - CCK-B receptors - Devazepide - L-365,260 -

INTRODUCTION

Dopamine coexists with cholecystokinin octapeptide (CCK-8) in some mesencephalic neurons, innervating mesolimbic and cortical regions (Hökfelt et al., 1980). Mesolimbic dopamine is known to have a significant role in the regulation of motor activity in rodents (Bradbury et al., 1983; Costall et al., 1985). Systemic treatment with CCK agonists (CCK-8 and caerulein) in low doses significantly suppresses locomotor activity in rodents (Zetler, 1985) and in higher doses the compounds are able to block stereotyped behaviour and hyperlocomotion induced by dopamine agonists (Zetler, 1985; Matsubara and Matsushita, 1986; Vasar et al., 1988). It has been suggested that several behavioural effects of CCK-8 and caerulein are generated through peripheral mechanisms (Morley, 1987). It is thought that the motor depressant effect of CCK-8 and the suppression of dopaminergic activity by large doses of CCK agonists are peripheral origin since they could be abolished by abdominal vagotomy in rats (Crawley and Kiss, 1985; Hamamura et al., 1989). The highly selective antagonist at peripheral CCK (CCK-A subtype) receptors devazepide completely reversed the motor depression induced by CCK-8 in mice (Khosla and Crawley, 1988) and in rats (Soar et al., 1989). Nevertheless, not all authors have been able to reproduce the finding that vagotomy can reverse the behavioural effects of CCK agonists in rodents. Moroji and Hagino (1987) have demonstrated that bilateral subdiaphragmatic vagotomy does not prevent the behavioural effects of systemically administered caerulein in mice. The suppression of electrical self-stimulation by caerulein is completely insensitive to vagotomy in rats (De Witte et al., 1986). Altar and Boyar (1989) have shown that peripherally injected CCK-8 interacts through CCK-B receptors (brain or central subtype) with central dopaminergic mechanisms. Recently two different subtypes of the CCK receptor (CCK-A and CCK-B) have been shown to occur in the brain of rodents (Moran et al., 1986; Dourish and Hill, 1987). The CCK-B subtype is ubiquitous in the brain, whereas CCK-A receptors were shown to be localized in certain discrete regions of brain, including the area postrema, nucleus of the solitary tract and the interpeduncular nucleus (Moran et al., 1986; Hill et al., 1987). However, recent behavioural, electrophysiological and homogenate radioligand binding studies (Crawley et al., 1985; Rovati, 1988; Barrett et al., 1989; Gerhardt et al., 1989; Vickroy and Bianchi, 1989) show CCK-A receptors to have a more widespread distribution in the mammalian brain than suggested by above CCK autoradiographic studies.

The aim of present study was to analyze further the role of CCK-A and CCK-B receptors in the regulation of motor activity of mice. Therefore, two highly selective CCK antagonists devazepide (CCK-A antagonist) (Chang and Lotti, 1986) and L-365,260 (CCK-B/gastrin antagonist) (Lotti and Chang, 1989) were used to examine the role of CCK receptor subtypes in the regulation of motor activity and in the action of peripherally injected caerulein, an agonist at CCK receptors. The action of devazepide and L-365,260 was studied on spontaneous motor

activity, apomorphine-induced hypolocomotion and amphetamine-induced hyperlocomotion, and on the behavioural effects of caerulein (caerulein-induced hypolocomotion, potentiation of apomorphine-induced hypomotility by caerulein, antiamphetamine effect of caerulein) in mice.

METHODS

Animals. Male albino mice, weighing 20-25 g, were used throughout the study. Mice were maintained at 20±3°C and on 12- hr light, between 9 a.m. and 9 p.m., with food and water ad lib. All the experiments were performed between 3 and 9 p.m.

Procedure. Spontaneous locomotor activity and hypolocomotion induced by apomorphine and caerulein were studied in an open-field. Animals were placed singly into the centre of the open-field area (30x30x18 cm, divided by lines into 16 equal squares) and observed during 3 min. The number of line crossings, rearings and head-drippings into holes was counted. Apomorphine (a dopamine agonist, 0.1 mg/kg) and caerulein (a potent CCK-8 agonist, 15 and 25 µg/kg) were given subcutaneously 15 min before the experiment. CCK antagonists (devazepide and L-365,260) were administered intraperitoneally 30 min prior to open-field test. Amphetamine-induced hyperlocomotion and antiamphetamine effect of caerulein were measured in individual photocell cages. The cage for registration of motor activity was a cylinder with an inner diameter 40 cm and 2 photocells (located in walls) for detection of motor activity. Motor activity was counted between 15 and 45 min after intraperitoneal administration of d-amphetamine (an indirect dopamine agonist, 5 mg/kg). CCK antagonists were given intraperitoneally 15 min before the injection of d-amphetamine. Caerulein (100 µg/kg) was given subcutaneously 5 min after the administration of amphetamine.

Drugs. The following drugs were used in the present study: caerulein (*Bachem*), d-amphetamine (*Sigma*), apomorphine (*Sigma*), devazepide and L-365,260 (*Merck Sharp & Dohme*). Caerulein, d-amphetamine and apomorphine were prepared in saline. Some drops of 0.001 N HCl was added for stabilizing the injection solution of apomorphine. Devazepide (1-methyl- 3-(2- indoloyl)amino-5-phenyl-3H-1,4-benzodiazepin-2-one) and L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H- 1,4-benzodiazepin-3-yl)-N'-(3methyl-phenyl)urea) were made soluble in saline by adding 1-2 drops of Tween-85. The same vehicle, 1-2 drops of Tween-85 in saline, was the control injection for CCK antagonists. Each treatment was given in a volume of 0.1 ml/10 g body weight.

Statistical analysis. Results are expressed in the tables and figures as means ± S.E.M. The behavioural data were analyzed using one-way analysis of variance (ANOVA). Post hoc comparisons between individual groups were made by using Newman-Keuls test.

RESULTS

In the behavioural studies CCK antagonists (devazepide and L-365,260) in low doses failed to affect the locomotor activity of mice in an open-field (figure 1), only in high doses were they able to change the behaviour of animals. Devazepide (0.1-1 mg/kg) decreased the number of line crossings in an open-field [$F(5,54)=2.88$, $p<0.05$] (figure 1), whereas L-365,260 (1 mg/kg) had the opposite effect [$F(5,54)=2.52$, $p<0.05$]. The systemic administration of caerulein in a moderate dose (25 µg/kg) reduced the number of line crossings and head-dips in the open-field test (table 1). The pretreatment of animals with devazepide (0.1-10 µg/kg) only partially antagonized the effect of the CCK agonist, particularly on head dips. However, a high dose of devazepide (100 µg/kg) reduced the spontaneous locomotor activity of animals and enhanced the effect of caerulein [$F(5,54)= 2.62$, $p<0.05$ for crossings; $F(5,54)=4.08$, $p<0.005$ for head-dips]. L-365,260 (0.1-1000 µg/kg) enhanced the sedative effect of caerulein [$F(6,63)= 3.86$, $p< 0.01$ for crossings; $F(6,63)= 3.72$, $p<0.01$ for rears; $F(6,63)= 6.86$, $p<0.0001$ for head-dips] (table 1). The dopamine agonist apomorphine in low dose (0.1 mg/kg) reduced the motor activity of mice (table 2). L-365,260 (1-10 µg/kg) significantly enhanced the sedative effect of apomorphine in the mouse [$F(6,63)=4.06$, $p<0.005$ for crossed lines; $F(6,63)=2.36$, $p<0.05$ for rears; $F(6,63)=8.15$, $p<0.00001$ for head-dips]. Small doses of devazepide (1-10 µg/kg) only partially attenuated the effect of apomorphine, whereas high doses (100 and 1000 µg/kg) enhanced the effect of the dopamine agonist [$F(5,54)=4.68$, $p<0.001$ for line crossings; $F(5,54)=2.83$, $p<0.05$ for rears] (table 2). Pretreatment with caerulein (15 µg/kg) significantly potentiated apomorphine-induced hypolocomotion in the mouse [$F(3,36)=38.4$, $p<0.000001$ for line crossings, $F(3,36)=20.7$, $p<0.00001$ for rears, $F(3,367)=5.01$, $p<0.01$ for head-dips] (figure 2). The coadministration of apomorphine and caerulein caused nearly complete loss of motor activity in mice. Several animals lay motionless in the centre of open-field area. Neither devazepide, nor L-365,260 could antagonize the effect of concomitant treatment with apomorphine and caerulein (data not shown).

An indirect dopamine agonist d-amphetamine (5 mg/kg) caused a three fold increase in the number of motor activity counts (figure 3). L-365,260 had no effect on d-amphetamine induced hyperactivity, whereas devazepide in high dose (1 mg/kg) suppressed spontaneous motor activity and completely antagonized the motor stimulation induced by d-amphetamine [$F(9,86)=3.1$, $p<0.005$ for 30 min period] Caerulein (100 µg/kg) also potently reversed the motor excitation induced by d- amphetamine (figure 4). The pretreatment of mice with devazepide over a wide dose range (1-100 µg/kg) completely blocked the antiamphetamine effect of caerulein [$F(7,104)=9.56$, $p<0.000001$ for 30 min period]. The administration of L-365,260 at low dose (1 µg/kg) also counteracted the antiamphetamine effect of CCK agonist [$F(7,104)=4.48$, $p<0.0001$ for 30 min period].

LEGENDS TO THE FIGURES

Figure 1. THE EFFECT OF CCK-8 ANTAGONISTS ON THE SPONTANEOUS MOTOR ACTIVITY OF MICE IN AN OPEN-FIELD. L-365,260 (0.0001-1 mg/kg, i.p.) and devazepide (0.0001-1 mg/kg, i.p.) were administered 30 min before the experiment. The number of crossed lines during 3 min is presented in the figure. Each bar represents the mean ± S.E.M. for 10 animals. Data subjected to one-way analysis of variance and Newman-Keuls test. * - $p<0.05$ (significantly different from vehicle treated animals). - Vehicle; - Devazepide; - L-365,260

Figure 2. THE EFFECT OF CAERULEIN ON APOMORPHINE-INDUCED HYPOLOCOMOTION. Apomorphine (0.1 mg/kg, s.c.) was given 15 min and caerulein (15 µg/kg, i.p.) 10 min prior to the open-field test. The number of crossings, rears and head-dips during 3 min is presented here. Each bar represents the mean ± S.E.M. for 10 animals. Data were subjected to one-way analysis of variance and followed by Newman-Keuls test. * - $p< 0.05$; ** - $p<0.01$ (statistically evident difference from vehicle treated mice). - Vehicle; - Apomorphine (0.1 mg/kg); - Caerulein (15 µg/kg); Apomorphine + caerulein

Figure 3. THE INTERACTION OF CCK ANTAGONISTS WITH AMPHETAMINE-INDUCED HYPERLOCOMOTION. CCK antagonists (0.001-1 mg/kg, i.p.) were given 15 min before d-amphetamine (5 mg/kg, i.p.), whereas d-amphetamine was injected 15 min prior to the experiment. The locomotor activity of mice was measured in the individual cages. The number of counts was registered during 30 min. Each bar represents the mean ± S.E.M. for 10 animals. Data were subjected to one-way analysis of variance and Newman-Keuls test. * - $p<0.05$ (significantly different from vehicle treated group); ** - $p<0.01$ (if compared to vehicle+d-amphetamine). - Vehicle; - Vehicle + d-amphetamine; - Devazepide + d-amphetamine; - L-365,260 + d-amphetamine

Figure 4. THE INTERACTION OF CCK ANTAGONISTS WITH ANTIAMPHETAMINE EFFECT OF CAERULEIN. CCK antagonists (0.0001-1 mg/kg, i.p.) were injected 30 min, d-amphetamine (5 mg/kg, s.c.) 15 min and caerulein (0.1 mg/kg, s.c.) 10 min before the experiment. The number of motor activity counts was registered in the individual cages during 30 min. Each bar represents the mean ± S.E.M. for 10 animals. Data were subjected to one-way analysis of variance and followed by Newman-Keuls test. * - $p<0.05$ (significantly different from vehicle+saline); ** - $p<0.05$ (if compared to vehicle + d-amphetamine); *** - $p<0.05$; **** - $p<0.01$ (if compared to d-amphetamine+caerulein). - Vehicle; - Vehicle + d-amphetamine; - Devazepide + caerulein + d-amphetamine; - L-365,260 + caerulein + d-amphetamine

FIGURE 1

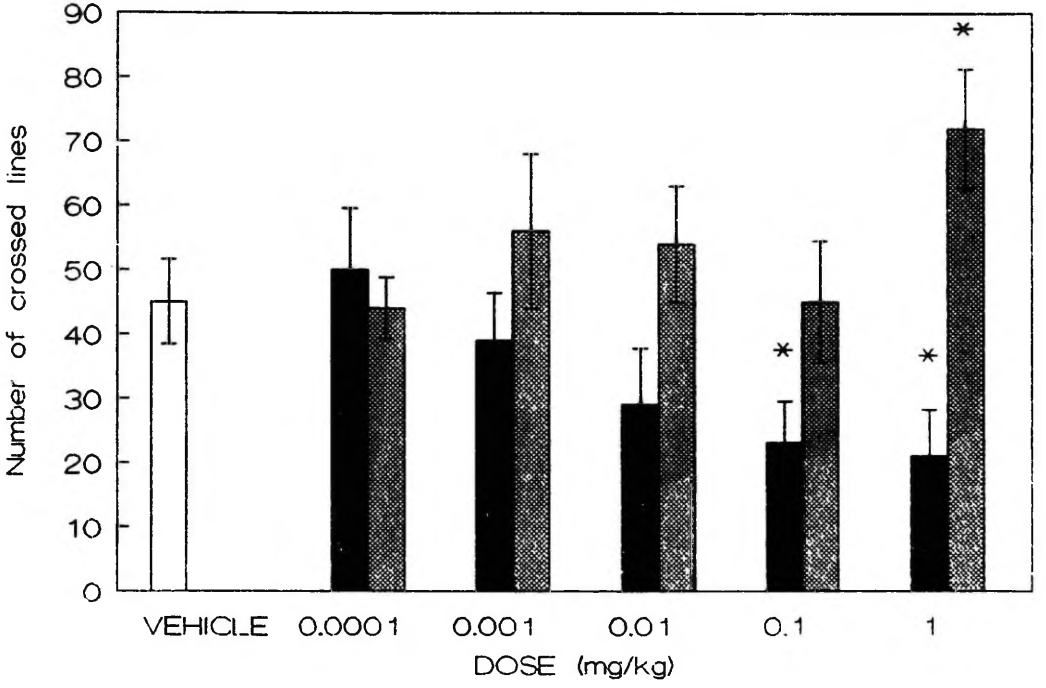

90
80
70
60
50
40
30
20
10
0
Number of crossed lines
*
*
*
VEHICLE
0.0001
0.001
0.01
0.1
1
DOSE (mg/kg)

FIGURE 2

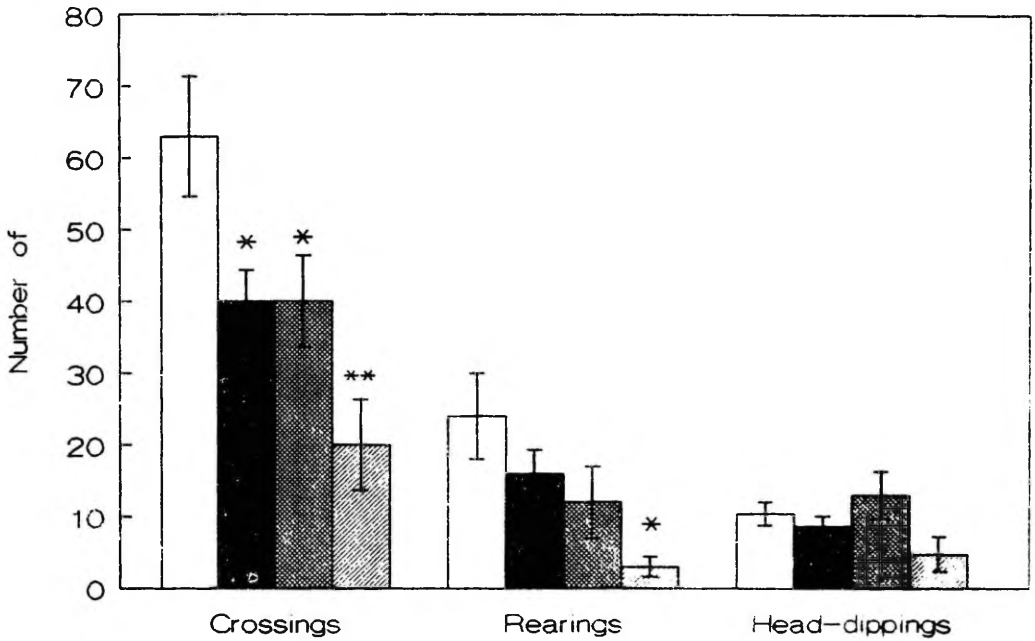

80
70
60
50
40
30
20
10
0
Number of
*
*
**
*
Crossings
Rearings
Head-dippings

FIGURE 3

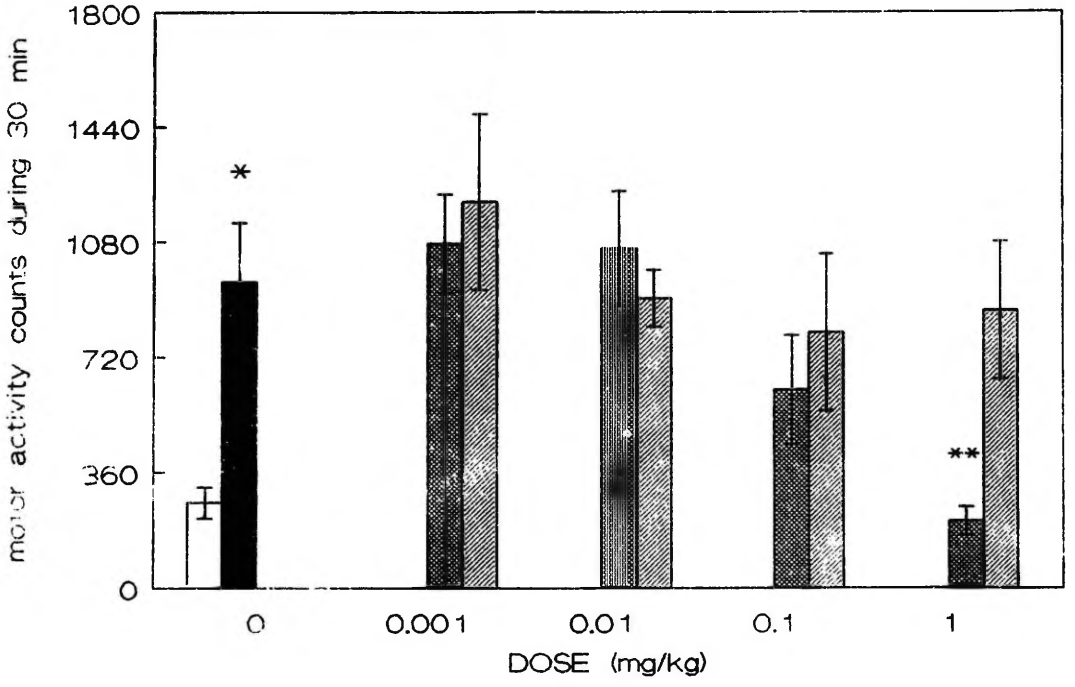

1800
1440
1080
720
360
0
motor activity counts during 30 min
*
**
0
0.001
0.01
0.1
1
DOSE (mg/kg)

FIGURE 4

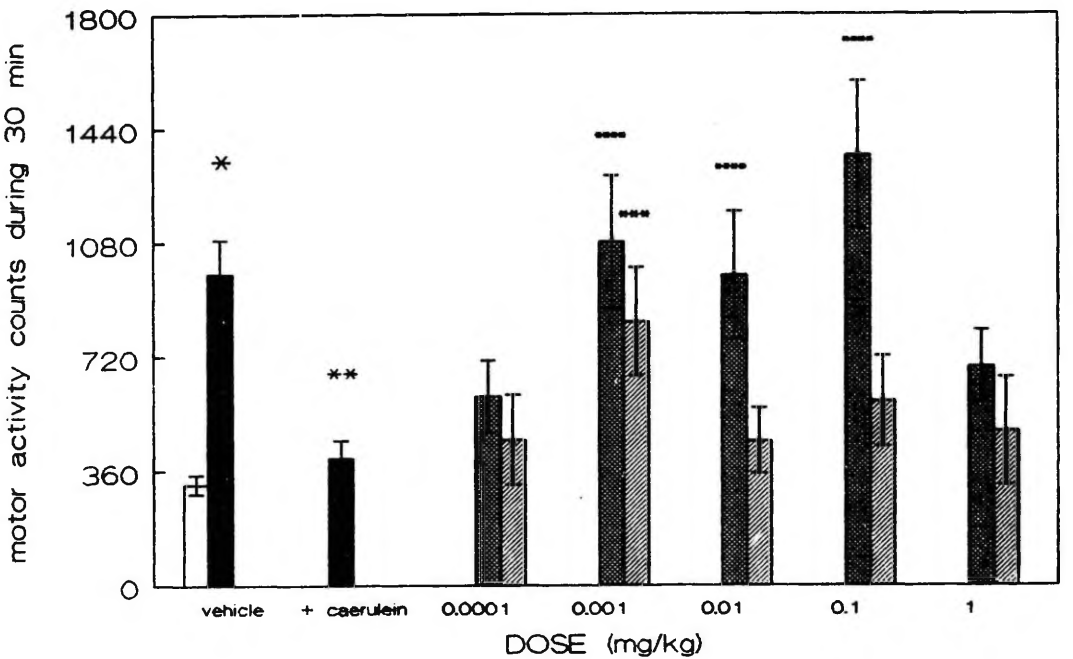

1800
1440
1080
720
360
0
motor activity counts during 30 min
*
**
****
***
****
****
vehicle
+ caerulein
0.0001
0.001
0.01
0.1
1
DOSE (mg/kg)

Table 1

The interaction of L-365,260 and devazepide with caerulein-induced hypolocomotion in the mouse.

Drug / dose	Line crossings (during 3 min) Mean values ± S.E.M.	Rears	Head-dips
Vehicle	77±6.2	29±6.3	22±2.9
Vehicle + caerulein 25 µg/kg	56±5.6*	16±4.3	9±1.4*
Devazepide 0.1 µg/kg + caerulein 25 µg/kg	54±5.4*	13±4.8	16±3.3#
Devazepide 1 µg/kg + caerulein 25 µg/kg	58±12.4	16±5.7	18±3.4#
Devazepide 10 µg/kg + caerulein 25 µg/kg	66±7.5	19±3.2	14±1.5#
Devazepide 100 µg/kg + caerulein 25 µg/kg	38±8.0*	13±5.7	10±1.8*
L-365,260 0.1 µg/kg + caerulein 25 µg/kg	52±6.0*	15±3.6	11±2.2*
L-365,260 1 µg/kg + caerulein 25 µg/kg	35±6.8*,#	5±1.8*,#	10±1.8*
L-365,260 10 µg/kg + caerulein 25 µg/kg	41±8.5*	8±4.6*	13±2.5*
L-365,260 100 µg/kg + caerulein 25 µg/kg	40±10.5*	10±5.2*	8±1.6*
L-365,260 1000 µg/kg + caerulein 25 µg/kg	56±9.4	14±4.8	14±1.4#

CCK antagonists were administered 30 min and caerulein 15 min before the experiment. * - $p<0.05$ (Newman-Keuls test after significant one-way ANOVA, if compared to vehicle treated mice). # - $p<0.05$ (Newman-Keuls test, in comparison to vehicle + caerulein treatment).

Table 2

The effect of devazepide and L-365,260 on apomorphine-induced hypolocomotion in mice.

Drug / dose	Line crossings (during 3 min) Mean values ± S.E.M.	Rears	Head-dips
Vehicle	60±8.8	14.2±3.0	7.4±0.9
Vehicle + apomorphine 0.1 mg/kg	40±3.3*	10.3±3.0	7.5±1.2
L-365,260 0.1 µg/kg + apomorphine 0.1 mg/kg	38±3.6*	9.2±2.6	5.2±2.0
L-365,260 1 µg/kg + apomorphine 0.1 mg/kg	29±8.5*	5.4±1.6*	2.6±0.7*,#
L-365,260 10 µg/kg + apomorphine 0.1 mg/kg	23±5.8*,#	5.7±1.5*	2.3±0.7*,#
L-365,260 100 µg/kg + apomorphine 0.1 mg/kg	32±5.7*	7.5±2.4	2.8±0.8*,#
L-365,260 1000 µg/kg + apomorphine 0.1 mg/kg	36±5.6*	9.6±2.5	4.0±0.6*
Vehicle	79±10.2	22±4.8	7.9±2.6
Vehicle + apomorphine 0.1 mg/kg	45±5.6*	11±2.9	5.5±1.8
Devazepide 1 µg/kg + apomorphine 0.1 mg/kg	55±6.6	13±2.6	5.1±1.6
Devazepide 10 µg/kg + apomorphine 0.1 mg/kg	54±5.8	12±2.6	6.1±2.0
Devazepide 100 µg/kg + apomorphine 0.1 mg/kg	32±10.0*	6±2.7*	2.8±0.9
Devazepide 1000 µg/kg+ apomorphine 0.1 mg/kg	34±7.5*	8±2.3*	3.0±1.0

CCK antagonists were given 15 min prior to apomorphine, whereas apomorphine was injected 15 min before the experiment.

* - p<0.05 (Newman-Keuls test, following significant one-way ANOVA, in comparison to vehicle-treated mice); # - p<0.05 (if

REFERENCES

Altar CA, Boyar WC (1989) Brain CCK-B receptors mediate the suppression of dopamine release by cholecystokinin. Brain Res. 483:321-326

Barrett RW, Steffey ME, Wolfram CAW (1989) Type-A CCK sites in cow brain: characterization using (-)-[^{3}H]-L-364,718 membrane binding assays. Mol.Pharmacol. 36:285-290

Bradbury AJ, Costall B, Naylor RJ, Neumeyer JL (1983) Motor inhibition induced by apomorphine derivatives in the mouse. J.Pharm.Pharmacol. 35: 494-499

Chang RSL, Lotti VJ (1986) Behavioral and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc.Natn.Acad.Sci. USA 83: 4923-4926

Costall B, Domeney AM, Naylor RJ (1985) The continuity of dopamine receptors antagonism can dictate the long-term behavioural consequences of a mesolimbic infusion of dopamine. Neuropharmacol. 24:193-197

Crawley JN (1989) Microinjection of cholecystokinin into the rat ventral tegmental area potentiates dopamine-induced hypolocomotion. Synapse 3: 346-355.

Crawley JN, Schwaber JS (1984) Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of parvocellular subdivision of the nucleus tractus solitarius. Brain Res. 295:289-299

Crawley JN, Kiss JZ (1985) Tracing the sensory pathway from the gut to brain regions mediating the actions of cholecystokinin on feeding and exploration. Ann.N.Y.Acad.Sci. 448:586-588

Crawley JN, Stivers JA, Blumstein LK, Paul SM (1985) Cholecystokinin potentiates dopamine-mediated behaviors: evidence for modulation specific to a site of coexistence. J.Neurosci. 5:1972-1983

De Witte P, Goldman S, Gewiss M, Poels JF, Van Boxez P, Van Der Veken E, Vanderhaeghen JJ (1986) Similar effect of caerulein on intracranial self-stimulation in vagotomized and non-vagotomized rats. Neurochem.Int. 8:339-343

Dourish CT, Hill DR (1987) Classification and function of CCK receptors. Trends Pharmacol.Sci. 8:207-208

Dourish CT, Rycroft N, Iversen SD (1989) Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. Science 245: 1509-1511

Dourish CT, O'Neill MF, Coughlan J, Kitchener SJ, Hawley D, Iversen SD (1990) The selective CCK-B antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat: evidence for mediation of CCK/opioid interactions by CCK-B receptor. Eur.J.Pharmacol. 176:35-44

Gerhardt GA, Friedmann M, Brodie MS, Vickroy TW, Gratton AP, Hoffer BJ, Rose GM (1989) The effect of cholecystokinin (CCK-8) on dopamine-containing nerve terminals in the

the mouse and the rat (Vasar et al., 1988). Namely, systemic treatment with caerulein reversed the behavioural effects (stereotyped behaviour and motor excitation) of amphetamine and ketamine in the mouse, but not in the rat. On the other hand, devazepide at a moderate dose (10 μg/kg) completely reversed amphetamine-induced stereotypy and motor excitation in the rat (Vasar et al., unpublished data), whereas in the mouse it potently antagonized the antiamphetamine effect of caerulein (the present study).

In conclusion, the results of present study reflect apparently distinct role of CCK-A and CCK-B receptors in the regulation of motor activity. The opposite effect of devazepide and L-365,260 on caerulein- and apomorphine-induced hypolocomotion is probably related to the antagonistic role of CCK-A and CCK-B receptor subtypes in the regulation of mesencephalic dopaminergic cells. The CCK-A receptors, at which caerulein acts to reduce locomotor activity, are possibly in the periphery and associated in some way through the vagal afferent pathways with dopaminergic neurons in the mesencephalon (Crawley and Schwaber, 1984; Crawley and Kiss, 1985). The antiamphetamine effect of caerulein seems to be linked to the stimulation of CCK-A receptors in the mouse brain, whereas probably the blockade of both subtypes of the CCK-8 receptor is involved in the antiamphetamine effect of devazepide.

Acknowledgement. The authors wish to thank Dr. S.D. Iversen from *Merck Sharp & Dohme* for the generous gift of devazepide and L-365,260.

caudate nucleus and nucleus accumbens of the anaesthetized rat: an in vivo electrochemical studies. Brain Res. 499:157-163

Hagino Y, Moroji T, Iizuka R (1989) A behavioural pharmacological study on intracerebroventricularly administered CCK-8 related peptides in mice. Neuropeptides 13: 107-113

Hamamura T, Kazahaya Y, Otsuki S (1989) Ceruletide suppresses endogenous dopamine release via vagal afferent system studied by in vivo intracerebral dialysis. Brain Res. 483:78-83

Hamilton MH, Rose IC, Herberg LJ, De Belleroche JS (1990) Effect of intracerebroventricular and systemic injections of caerulein, a CCK analogue, on electrical self-stimulation and its interaction with the CCK-A receptor antagonist, L-364,718 (MK-329). Psychopharmacology 101:384-389

Hill DR, Campbell NJ, Shaw TM, Woodruff GN (1987) Autoradiographic localization and characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. J.Neurosci. 7:2967-2976

Hökfelt T, Rehfeld JF, Skirboll L, Ivemark B, Goldstein M, Markey K (1980) Evidence for coexistence of dopamine and CCK in mesolimbic neurones. Nature (London) 285:476-478

Hommer DW, Palkovits M, Crawley JN, Paul SM, Skirboll LR (1985) Cholecystokinin-induced excitation in the substantia nigra: evidence for peripheral and central components. J.Neurosci. 5:1387-1397

Hommer DW, Stoner G, Crawley JN, Paul SM, Skirboll LR (1986) Cholecystokinin-dopamine coexistence: electrophysiological actions corresponding to cholecystokinin receptor subtype. J.Neurosci. 6:3039-3043

Khosla S, Crawley JN (1988) Potency of L-364,718 as an antagonist of the behavioural effects of peripherally administered cholecystokinin. Life Sci 42:153-159

Lotti VJ, Chang RSL (1989) A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. Eur.J.Pharmacol. 162:273-280

Matsubara K, Matsushita A (1986) ß-endorphin involvement in the antidopaminergic effect of caerulein. Jap.J.Pharmacol. 40:417-422

Moran TH, Robinson PH, Goldrich M, Mc Hugh PR (1986) Two brain CCK receptors: implications for behavioral actions. Brain Res. 362:175-179

Morley JE (1987) Behavioral effects of administered cholecystokinin. ISI Atlas Pharmacol. 1:49-51

Moroji T, Hagino Y (1987) Bilateral subdiaphragmatic vagotomy does not prevent the behavioural effects of systematically administered ceruletide in mice. Neuropeptides 9:217-224

Rovati L (1988) New pentanoic acid derivatives with potent CCK antagonistic properties: different activity on periphery vs. central nervous system. In: Wang RY, Schoenfeld R (eds) Cholecystokinin antagonists. Alan R. Liss, Inc., New York, pp. 1-11

Soar J, Hewson G, Leighton GE, Hill RG, Hughes J (1989) L-364,718 antagonizes the

cholecystokinin-induced suppression of locomotor activity. Pharmacol.Biochem.Behav. 33:637-640

Strömbom U (1977) Antagonism by haloperidol of locomotor depression induced by small doses of apomorphine. J.Neural Transm. 40:191-194

Vasar E, Allikmets L, Ryzhov I, Prakhye I, Soosaar A, Mirzayev S (1988) Intraspecies differences in the behavioural effects of caerulein, an agonist of CCK-8 receptors, in mice and rats. Bull.Exp.Biol.Med. 105:168-171

Vickroy T.W., Bianchi BR (1989) Pharmacological and mechanistic studies of cholecystokinin-facilitated [^{3}H]-dopamine efflux from rat nucleus accumbens. Neuropeptides 13: 43-50

Zetler G (1985) Neuropharmacological profile of cholecystokinin-like peptides. Ann.N.Y.Acad.Sci. 448:448-469

DISCUSSION

In these behavioural studies the CCK-B/gastrin antagonist L-365,260 and the CCK-A antagonist devazepide had opposite effects on spontaneous locomotor activity, and on apomorphine- and caerulein-induced hypolocomotion in mice. The spontaneous motor activity was affected only by high doses of CCK antagonists, but the hypolocomotion induced by caerulein and apomorphine was changed by low doses of devazepide and L-365,260. Devazepide (0.1-1 mg/kg) reduced the spontaneous motor activity of mice, whereas L-365,260 (1 mg/kg) increased this behaviour. It is very puzzling that CCK antagonists affect in a similar way apomorphine- and caerulein-induced hypolocomotion. Devazepide partially antagonized the sedative effect of low doses of apomorphine and caerulein, whereas L-365,260 significantly potentiates the action of caerulein and apomorphine. According to the existing data the motor suppressant effect of apomorphine and caerulein are thought to be related to the decreased activity of dopaminergic cells in the mesencephalon (Strömbom, 1977; Zetler, 1985). The behavioural effects of CCK antagonists probably reflect the distinct role of CCK-A and CCK-B receptors in the regulation of presynaptic dopaminergic activity in the mouse brain. The blockade of CCK-B receptors by L-365,260 decreases the dopaminergic activity, whereas the interaction of devazepide with CCK-A receptors increases it in the mouse brain. It seems probable that the CCK-A receptors at which caerulein and CCK-8 act to reduce locomotor activity are in the periphery and associated in some way through the vagal afferent pathway with dopaminergic neurons (Crawley and Schwaber, 1984; Crawley and Kiss, 1985; Hommer et al., 1985). However, it is important to stress that in the present study devazepide, in contrast to the investigation of Khosla and Crawley (1988), only moderately antagonized the motor depressant effect of caerulein. Hamilton et al. (1990) have shown that devazepide only partially antagonizes the suppression of self-stimulation induced by caerulein in the rat. All these experiments support the idea that not only the CCK-A receptor subtype is involved in mediating the effect of caerulein. The concomitant treatment with a low dose of apomorphine and caerulein causes nearly complete loss of motor activity in the mice. Several animals lay motionless in the middle of the open field. Devazepide and L-365,260 in low doses, which do not affect locomotor activity of animals per se, were completely ineffective against the motor depression induced by the simultaneous administration of caerulein and apomorphine. According to the studies of Hommer

et al. (1986) and Crawley (1989) the CCK-8 receptors that mediate the potentiation of dopamine-induced hypolocomotion and suppression of the electrical activity of dopamine neurons in the rat mesencephalon by CCK-8 belong to the CCK-B subtype. Aftar and Bova (1989) have found that the antagonistic effect of centrally or peripherally administered CCK-8 agonists (CCK-8, desulfated CCK-8 and CCK-4) on amphetamine evoked dopamine release in the mouse striatum is also related to the CCK-B receptor subtype. Nevertheless, it is not clear whether CCK-B receptors are involved in the potentiation of apomorphine-induced

hypolocomotion by caerulein in the mouse and it remains to be elucidated.

The interaction of CCK antagonists with amphetamine-induced hyperlocomotion and the antiamphetamine effect of caerulein is somewhat different from their action on caerulein and apomorphine elicited hypolocomotion. It is suggested that the different pharmacology of CCK-8 against dopamine-induced hypolocomotion and hyperlocomotion is related to the involvement of distinct brain regions in the development of two opposite behavioural effects of dopamine in the rat (Crawley, 1989). The potentiation of dopamine-induced hypolocomotion is linked to the interaction of CCK-8 with dopamine "autoreceptors" in the ventral tegmental area, whereas the potentiation of dopamine-induced hyperlocomotion is related to an interaction with post-synaptic dopaminergic receptors in the posteromedial part of the nucleus accumbens (Crawley et al., 1985; Crawley, 1989). The CCK-B/gastrin antagonist L-365,260 did not significantly change amphetamine-induced hyperlocomotion, but paradoxically it reversed (at a low dose) the antiamphetamine effect of caerulein. Devazepide antagonized the antiamphetamine effect of caerulein at low doses, where it probably interacts selectively with CCK-A receptors. However, at a high dose (1 mg/kg), which also interacts with CCK-B receptors (see Dourish et al., 1989; 1990), devazepide per se reverses the motor excitation induced by d-amphetamine. The antiamphetamine effect of devazepide is in good agreement with our previous studies in which the unselective CCK antagonist proglumide (5-15 mg/kg) also blocked the effect of d-amphetamine (Vasar et al., unpublished data). According to the studies of Moroji and Hagino (1987) the antiamphetamine effect of caerulein in mice is completely resistant to the vagotomy. It is worthy to note that nearly 10 times higher doses of caerulein are required for blocking amphetamine-induced hyperlocomotion in comparison to the sedative effect of caerulein. Accordingly, it seems very probable that the CCK-A receptors involved in the antiamphetamine effect of caerulein are distinct from the CCK-A receptors related to caerulein and apomorphine-induced hypolocomotion. The above mentioned study (Moroji, Hagino, 1987) suggest the possibility that these CCK-A receptors are located in the mouse brain. This idea is supported also by the study of Hagino et al. (1989) in which the intracerebroventricular administration of CCK-8 and caerulein, but not desulfated CCK-8 and CCK-4, antagonizes amphetamine induced motor excitation in the mouse. The recent behavioural, electrophysiological and radioligand binding studies (Rovati, 1988, Crawley, 1989; Vickroy and Bianchi, 1989, Gerhardt et al., 1989) have established that there is a wider distribution of CCK-A receptors in the rat brain than was previously thought (Moran et al., 1986; Hill et al., 1987). The possible mediation of the antiamphetamine effect of caerulein through the CCK-A receptors in the mouse brain may reflect the substantial difference between CCK-A receptors in the mouse and rat brain. Crawley and colleagues (1985; Crawley, 1989) have shown that CCK-8 by interacting with CCK-A receptors facilitates dopamine-induced hyperlocomotion in the posteromedial part of the nucleus accumbens of the rat. The different pharmacology of CCK-A receptors in the mouse and the rat brain seems to account for the interspecies differences in the behavioural effects of caerulein in

European Journal of Pharmacology, 202 (1991) 385–390

ADONIS 001429999100629S

EJP 52043

Changes in motor activity and forebrain [propionyl-^{3}H]propionylated-CCK-8 binding in mice after repeated administration of drugs affecting cholecystokinin receptors

Eero Vasar [1], John D. Stephenson and Brian S. Meldrum

[1] *Psychopharmacology Laboratory, Tartu University, Tartu, Estonia and Departments of Neuroscience and Neurology, Institute of Psychiatry, London SE5 8AF, U.K.*

Received 25 June 1991, accepted 9 July 1991

The effects of acute or repeated treatment of male albino BKW mice with caerulein, a cholecystokinin octapeptide (CCK-8) agonist, and with devazepide (MK-329) and L-365,260, antagonists at CCK_A ('peripheral') and CCK_B ('central') receptors respectively, on motor activity and [propionyl-^{3}H]propionylated-CCK-8 ([^{3}H]pCCK-8) binding were studied. Acute treatment with a large dose of caerulein (100 μg/kg s.c.) suppressed motor activity (line crossings and rearings) whereas devazepide (2 mg/kg i.p.) had the opposite action. L-365,260 (2 mg/kg i.p.) increased only the number of rearings. Tolerance developed to the locomotor effects of caerulein and devazepide when these same doses were administered once daily (caerulein) or twice daily (devazepide) for 10 days. Twice daily administration of L-365,260 (2 mg/kg) for 10 days did not significantly alter the locomotor activity of mice. The sedative effect of caerulein (20 μg/kg s.c.) was markedly reduced in mice receiving repeated injections of either a larger amount of caerulein (100 μg/kg) or devazepide but not after L-365,260. The stimulant effect of (+)-amphetamine (2 mg/kg s.c.) on motor activity was increased by subchronic administration of either devazepide or caerulein, but not by L-365,260. All three compounds (caerulein, devazepide and L-365,260) increased the number of ([^{3}H]pCCK-8 binding sites in mouse forebrain but the increase was only significant after L-365,260. The effects of long-term treatment with caerulein are probably related to the stimulation of CCK_A receptors, whereas the paradoxically similar action of devazepide may be linked to the blockade of both subtypes of the CCK-8 receptor. The results of the present study can therefore be explained by postulating different roles for CCK_A and CCK_B receptors in the regulation of motor activity, the CCK_A receptors being the more important.

Caerulein; Devazepide; L-365,260; CCK receptors; Amphetamine; Motor activity

1. Introduction

Dopamine coexists with cholecystokinin octapeptide (CCK-8) in the neurones of the ventral tegmental area, innervating mesolimbic structures of rats (Hökfelt et al., 1980). Mesolimbic dopamine is known to have a crucial role in the regulation of motor activity in rodents (Bradbury et al., 1983; Costall et al., 1985). Systemic treatment with low doses of either CCK-8 or caerulein significantly suppressed locomotor activity of rodents (Zetler, 1985) and at higher doses hyperlocomotion induced by dopamine agonists was also blocked (Zetler, 1985; Matsubara and Matsushita, 1986; Vasar et al., 1988a). According to some reports, peripheral CCK-8 receptors linked with the vagus nerve have significance for motor depressant and antidopaminergic activity of CCK-8 (Crawley and Schwaber, 1983; Kawasaki et al., 1983; Hamamura et al., 1989). However, Altar and Boyar (1989) showed that peripherally injected CCK-8 affected central dopaminergic mechanisms directly. Two different subtypes of the CCK-8 receptor have now been described in the brains of rodents (Moran et al., 1986; Dourish and Hill, 1987). CCK_A ('peripheral' type) receptors were shown to be localized in certain discrete regions of brain, including the area postrema, the nucleus of the solitary tract and the interpeduncular nucleus (Moran et al., 1986; Hill et al., 1987). However, electrophysiological and homogenate radioligand binding studies (Barrett et al., 1989; Gerhardt et al., 1989; Vickroy and Bianchi, 1989) have shown the CCK_A receptors to be more widely distributed in the brain than suggested by the above autoradiographic studies. The aim of the present study was to examine the role of CCK_A and CCK_B receptors in the regulation of motor activity of mice. Motor

Correspondence to: B.S. Meldrum, Department of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, U.K. Tel. (1) 71 703 5411, ext. 3398.

activity was suppressed by caerulein, a CCK-8 agonist, and stimulated by amphetamine, an indirectly acting dopamine agonist. Sensitivity of the CCK receptors was changed by 10 days daily treatment with the highly selective CCK_A and CCK_B antagonists, devazepide and L-365,260 respectively, and with caerulein. The binding of [propionyl-^{3}H]propionylated-CCK-8 ([^{3}H]pCCK-8) to forebrain nuclei was determined concurrently with the behavioural studies.

2. Materials and methods

Male BKW mice (25-30 g) obtained from Bantin and Kingman (Hull) were used throughout the experiments. Caerulein (100 μg/kg once daily), devazepide (2 mg/kg twice daily) and L-365,260 (2 mg/kg twice daily) were administered for 10 days. The effects of caerulein and amphetamine on motor activity were studied 24 h after the last injection of CCK-8 antagonists and caerulein. [^{3}H]pCCK-8 binding experiments were carried out 2 h after the last injection of CCK-8 antagonists and 24 h after the last injection of caerulein. The shorter time interval for the CCK-8 antagonists was selected because [^{3}H]pCCK-8 binding had returned to normal by 24 h after the last of the twice daily injections of devazepide.

2.1. Measurement of motor activity in mice

Motor activity of mice was quantified in an 'open-field' (35 × 25 × 20 cm) divided by lines into six equal rectangles. The total number of line-crossings and rearings were counted for either 3 or 5 min. In the case of repeated treatments, the motor activity of mice was registered 20 min after the first and last injection of caerulein, devazepide, L-365,260 or vehicle. After 10-day treatment with CCK-8-ergic drugs the effect of caerulein (20 μg/kg) and (+)amphetamine (2 mg/kg) on motor activity was studied 15 min after injection of caerulein and 30 min after administration of (+)-amphetamine. Vehicle-injected control animals were always paired with drug-treated animals to control for diurnal fluctuations in motor activity. The results were analyzed using a one-way ANOVA followed by Newman-Keuls test for significance.

2.2. [^{3}H]pCCK-8 binding experiments

[^{3}H]pCCK-8 binding was measured by the method of Praissman et al. (1983). The mice were killed by cervical dislocation prior to decapitation and the brains immediately removed and bisected across the optic chiasma. The structures anterior to this cut (cortical regions, striata, mesolimbic structures, septum, etc.) were used for the binding studies. The brain material from three mice was pooled and homogenates prepared by homogenization in 75 volumes of 50 mM Tris-HCl (Sigma, pH 7.4 at 20°C) using a polytron homogenizer (Silverson) for 15 s. The homogenates were centrifuged at 37000 × g for 20 min, resuspended in the same volume of buffer and recentrifuged as described above. The final pellet was homogenized in a standard incubation buffer: HEPES (Sigma, 10 mM), NaCl (Sigma, 130 mM), KCl (Sigma, 5 mM), $MgCl_2$ (Sigma 5 mM), sodium EDTA (Sigma, 1 mM), bovine serum albumin (Sigma, 0.4 mg per ml, pH 6.7 at 20°C). Ten millilitres of incubation buffer was routinely used for each 100 mg of original tissue wet weight. The binding experiments were performed in polypropylene tubes (1.5 ml), each containing 50 μl of [^{3}H]pCCK-8 (73.2 Ci/mmol, Amersham International plc, 0.03-2.0 nM), 50 μl of incubation buffer or 200 nM CCK-8 (Bachem) and 400 μl freshly prepared brain membranes. The brain membranes were preincubated for 25 min at 23°C with or without 200 nM CCK-8, after which the radiolabelled ligand was added and the samples were carefully mixed. The membranes were incubated in the presence of radioligand for 2 h at 23°C and incubation was terminated by rapid centrifugation in a Beckman microfuge (11000 × g) for 3 min at room temperature. The supernatants were carefully aspirated and the pellets washed three times with 250 μl of ice-cold incubation buffer before transfer to scintillation vials. A tissue solubilizer (Soluene-350, Canberra-Packard) was added (300 μl) to the vials which were then incubated at 60°C for 1 h. Scintillation cocktail (Optiphase, Hisafe II, LKB-Wallac) was then added and radioactivity was counted by liquid scintillation spectrometry after a 12 h stabilization period. The specific binding of [^{3}H]pCCK-8 was defined as the difference between the degree of binding in the absence and presence of 200 nM of CCK-8. The specific binding of [^{3}H]pCCK-8 was 70-80% of total binding in the case of lower ligand concentrations (0.03-0.25 nM) and approximately 50% with higher concentrations (above 1 nM). Saturation curves were analyzed using a non-linear regression program (ENZFITTER, Leatherbarrow, 1987).

2.3. Drugs

Caerulein (Bachem) and (+)-amphetamine (Sigma) were dissolved in saline (0.9% NaCl solution w/v). Devazepide (MK-3291, 1-methyl-3-(2-indoloyl)amino-5-phenyl-3H-1,4-benzodiazepin-2-one) and L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N′-(3-methyl-phenyl)urea were suspended in saline with 1-2 drops of Tween-80. The same vehicle was used as the injectate for the control experiments. Devazepide and L-365,260 (Merck

Sharp & Dohme) were given i.p. whereas (+)-amphetamine and caerulein were injected s.c.

Spontaneous motor activity and motor activity induced by caerulein, apomorphine and (+)-amphetamine in the albino mouse were affected differently by doses of devazepide and L-365,260 in the range 0.0001-1 mg/kg (Vasar et al., 1991). For the present, chronic study, larger doses of the two antagonists were used (2 mg/kg) to ensure complete blockade of CCK_A and CCK_B receptors throughout the interval between injections.

3. Results

A single injection of a large dose of caerulein (100 μg/kg) significantly suppressed the motor activity of mice ($F(1,24) = 5.10$, $P < 0.05$, Newman-Keuls $P < 0.05$ for rearings; $F(1,24) = 8.80$, $P < 0.01$, Newman-Keuls $P < 0.01$ for line-crossings, as compared to vehicle treatment; table 1). The CCK_A antagonist, devazepide (2 mg/kg) increased the frequencies of rearings and line-crossings ($F(1,24) = 7.43$, $P < 0.05$, Newman-Keuls $P < 0.05$ for rearings; $F(1,24) = 6.18$, $P < 0.05$, Newman-Keuls $P < 0.05$ for line-crossings), whereas the CCK_B antagonist, L-365,260 (2 mg/kg) only increased the number of rearings ($F(1,24) = 5.36$, $P < 0.05$, Newman-Keuls $P < 0.05$). Tolerance developed to the locomotor effects of caerulein and the antagonists after their repeated administration (table 1). However tolerance to devazepide was not seen in all mice, about 20% becoming aggressive with repeated treatment. These mice attempted to bite the backs of other mice in the cage.

Administration of a moderate dose of caerulein (20 μg/kg) 24 h after the last injection of CCK-8-ergic drugs reduced motor activity only in control animals pretreated with either vehicle ($F(1,20) = 9.08$, $P < 0.01$, Newman-Keuls ns. for rearings, $F(1,20) = 13.02$, $P < 0.01$, Newman-Keuls $P < 0.01$ for line-crossings, as compared to vehicle + saline treatment) or with L-365,260 ($F(1,20) = 4.59$, $P < 0.05$, Newman-Keuls ns. for rearings; $F(1,20) = 12.86$, $P < 0.01$, Newman-Keuls $P < 0.01$ for line-crossings; table 2). The sedative effect of caerulein (20 μg/kg) was significantly reduced in mice pretreated with devazepide ($F(1,20) = 4.50$, $P < 0.05$; Newman-Keuls ns. for line-crossings compared with vehicle + saline treatment; table 2) and was reversed in mice pretreated with a larger dose of caerulein, 100 μg/kg. Thus, caerulein, 20 μg/kg, increased motor activity both when compared with a group receiving caerulein after chronic administration of vehicle ($F(1,18) = 19.88$, $P < 0.001$, Newman-Keuls $P < 0.01$ for rearings; $F(1,18) = 8.57$, $P < 0.01$, Newman-Keuls $P < 0.05$ for line-crossings, as compared to vehicle + caerulein treatment) and when compared with a group receiving saline after chronic administration of vehicle ($F(1,20) = 4.59$, $P < 0.05$, Newman-Keuls ns. for rearings, as compared to vehicle + saline group; table 2).

Administration of (+)-amphetamine produced a 54% increase in the number of line-crossings ($F(1,20) = 6.04$, $P < 0.05$, Newman-Keuls $P < 0.05$ for line-crossings, as compared to vehicle + saline treatment,

TABLE 1

The effect of repeated treatment (for 10 days) with caerulein (100 μg/kg, once daily), devazepide (2 mg/kg, twice daily) and L-365,260 (2 mg/kg, twice daily) on motor activity of mice. The number of rearings and line-crossings was counted over a 3-min period 20 min after the first and last injection of drug or vehicle.

Treatment	n	Rearings		Line-crossings	
		First	Last	First	Last
Vehicle	14	41 + 3.3	37 + 4.2	132 + 7.8	129 + 13.2
Caerulein	12	30 + 3.4 [a]	40 + 4.2	80 + 6.8 [b]	99 + 11.7
Devazepide	12	54 + 3.3 [a]	46 + 5.7	159 + 7.4 [a]	110 + 10.6
L-365,260	12	53 + 3.0 [a]	49 + 4.4	131 + 7.9	117 + 10.0

[a] $P < 0.05$, [b] $P < 0.01$ compared with vehicle-treated animals, by Newman-Keuls analysis following significant ANOVA). n = number of animals in the group

TABLE 2

The effect of caerulein (20 μg/kg) on mouse motor activity given 24 h after the last of a series of injections of devazepide (2 mg/kg, twice daily), L-365,260 (2 mg/kg, twice daily) and caerulein (100 μg/kg, once daily). Rearings and line-crossings were recorded over a 3-min period 15 min after caerulein injection.

Treatment	n	Rearings	Line-crossings
Vehicle + saline	12	41 ± 3.3	132 ± 7.8
Vehicle + caerulein	10	24 ± 5.0	85 ± 11.2 [c]
Devazepide + caerulein	10	35 ± 5.5	104 ± 11.1 [a]
L-365,260 + caerulein	10	28 ± 6.4	84 ± 11.7 [c]
Caerulein + caerulein	10	53 ± 3.8 [b]	125 ± 6.0 [a]

[a] $P < 0.05$; [b] $P < 0.01$ (in comparison with vehicle + caerulein group); [c] $P < 0.01$ (in comparison with vehicle + saline group) (Newman-Keuls analysis following significant ANOVA). n = number of mice in the group.

TABLE 3

The effect of (+)-amphetamine (2 mg/kg) on mouse motor activity given 24 h after the last of a series of injections of caerulein, devazepide and L-365,260. Rearings and line-crossings were measured over a 5-min period 30 min after amphetamine.

Treatment	n	Rearings	Line-crossings
Vehicle + saline	12	58 + 5	189 + 12
Vehicle + amphetamine	10	77 + 14	292 + 26 [a]
Devazepide + amphetamine	10	111 + 14 [b]	372 + 36 [b]
Caerulein + amphetamine	10	110 + 8 [b]	365 + 31 [b]
L-365,260 + amphetamine	10	89 + 17 [a]	298 + 29 [a]

[a] $P < 0.05$; [b] $P < 0.01$ (compared with vehicle + saline group, Newman-Keuls analysis following significant ANOVA) n = number of animals in the group.

TABLE 4

The effect of repeated treatment with caerulein, devazepide and L-365,260 on [^{3}H]CCK-8 binding in mouse forebrain. The studies were done 24 h after the last injection (10 days treatment) of CCK-8 antagonists and 24 h after the last administration of caerulein. The values are the means ± S.E.M. of six independent studies.

Treatment	B_{max} (pmol/g tissue)	K_d (nM)
Vehicle	8.67 ± 0.60	0.68 ± 0.16
Caerulein	7.31 ± 0.42	0.53 ± 0.02 [a]
Devazepide	7.41 ± 0.43	0.47 ± 0.02 [b]
L-365,260	10.38 ± 0.95	0.95 ± 0.09

[a] $P < 0.05$; [b] $P < 0.01$ (statistically significant ANOVA) [c] $P < 0.001$ (in comparison with vehicle-treated animals, Student's t-test).

table 3) and this effect was not altered by 10 days pretreatment with L-365,260. Injection of (+)-amphetamine in animals pretreated with devazepide increased motor activity more than in animals pretreated with vehicle ($F(1,18) = 2.83$, $P = 0.10$ for rearings; $F(1,18) = 3.60$, $P = 0.075$ for line-crossings). This increase was more marked when compared with the saline + vehicle group ($F(1,20) = 13.58$, $P < 0.01$, Newman-Keuls $P < 0.01$ for rearings; $F(1,20) = 18.63$, $P < 0.001$, Newman-Keuls $P < 0.01$ for line-crossings). The effects of repeated treatment with caerulein were similar to those of repeated treatment with devazepide inasmuch as (+)-amphetamine produced a motor stimulation which was more pronounced when compared with the vehicle + saline-treated animals ($F(1,20) = 17.06$, $P < 0.001$, Newman-Keuls $P < 0.01$ for rearings; $F(1,20) = 19.10$, $P < 0.001$, Newman-Keuls $P < 0.01$ for line-crossings) than with the vehicle + amphetamine treated animals ($F(1,18) = 3.60$, $P = 0.075$ for rearings; $F(1,18) = 2.95$, $P = 0.10$ for line-crossings, table 3).

Repeated treatment with each of the three CCK-8-ergic compounds altered [^{3}H]pCCK-8 binding in mice forebrain (table 4). Treatment with devazepide and caerulein slightly increased the affinity of [^{3}H]pCCK-8 binding sites ($F(1,10) = 7.66$, $P < 0.05$, for caerulein; $F(1,10) = 12.05$, $P < 0.01$, for devazepide) whereas after L-365,260 there was no significant change. All the chronic drug treatments tended to increase the number of [^{3}H]pCCK-8 binding sites, but only the 83% increase after L-365,260 was significant (table 4).

4. Discussion

The present study demonstrates significant differences between the effects of selective CCK_A and CCK_B antagonists on the regulation of CCK receptor density suggesting that the two receptor types differently affect the regulation of locomotor activity in mice. The CCK_A receptor antagonist, devazepide administered daily for 10 days, increased locomotor responses to (+)-amphetamine but reduced sensitivity to caerulein without significantly affecting the number of forebrain CCK receptors. In contrast, repeated injections of the CCK_B antagonist, L-365,260 markedly increased receptor density but did not affect motor responses to caerulein and (+)-amphetamine. The effects of devazepide were similar, surprisingly, to those produced by chronic administration of the non-selective CCK agonist, caerulein. However the present study used large doses of devazepide (2 mg/kg twice daily) and it cannot be assumed that its effects were solely due to prolonged CCK_A receptor blockade. In rats for example, large doses of devazepide (1 mg/kg) enhanced morphine analgesia in the paw pressure and tail flick tests (Dourish et al., 1988; O'Neill et al., 1989) effects which are mediated by CCK_B receptors (Dourish et al., 1990).

Administration of a single high dose of caerulein (100 μg/kg) suppressed the motor activity of mice, whereas devazepide (2 mg/kg) had the opposite effect. Tolerance to the motor effects of both compounds developed after their repeated administration, although approximately 20% of animals receiving devazepide did not develop tolerance, but became aggressive with repeated injections. These mice frequently attacked the other animals in the cage usually biting the victim's back.

The sedative effect of a moderate dose of the CCK-8 agonist, caerulein (20 μg/kg) was prevented by chronic caerulein pretreatment suggesting that the CCK-8 receptors became desensitized to caerulein although this was not reflected in changes to the binding of [^{3}H]pCCK-8. The sedative effect of caerulein was also reduced after repeated injections of the CCK_A antagonist, devazepide. This was unexpected, because chronic administration of an antagonist usually leads to receptor up-regulation and an enhanced sensitivity to agonists. There are no obvious reasons why chronic devazepide treatment did not render the animals more sensitive to caerulein but the sedative effect of caerulein was also reduced after administration of proglumide (10 mg/kg twice daily), a non-selective antagonist of CCK-8 (Vasar et al., unpublished data). Devazepide has previously been reported to antagonize the locomotor effects produced by CCK-8 (Khosla and Crawley, 1988). One explanation for these findings is antagonism of both receptor sub-types by the dose-schedule of devazepide used in this study (see Dourish et al., 1990). It has been shown that the motor depressant actions of caerulein and apomorphine are reduced by low doses of devazepide (0.1-10 μg/kg) whereas low doses of L-365,260 (1-10 μg/kg) have the opposite effect (Vasar et al., in press). Since CCK_A and CCK_B receptors appear to affect the regulation of motor activity differently, an interaction of devazepide with both CCK-8 receptor subtypes might explain the otherwise paradoxical reduction of the motor depressant

effect of caerulein after chronic treatment with devazepide. An alternative explanation for this finding is that CCK_A receptors located on either side of the blood-brain barrier may have opposite effects on mouse motor activity. The receptors responsible for the sedative effects of CCK are located in the nucleus of the solitary tract and vagus nerve (outside the blood-brain barrier). Stimulation of these receptors suppresses dopamine release in basal ganglia induced by peripherally injected CCK-8 agonists (Crawley and Schwaber, 1983; Hamamura et al., 1989). In contrast, CCK_A receptors in the striatum and mesolimbic structures promote dopamine release and potentiate dopamine-induced hyperlocomotion (Crawley et al., 1985; Vickroy and Bianchi, 1989). Chronic treatment with proglumide increased the activity of dopaminergic cells in the mesolimbic system (Chiodo et al., 1987). A similar effect occurring after repeated treatment with devazepide might explain the reduced sedative effect of caerulein. The motor stimulant effect of (+)-amphetamine was increased after repeated administration of caerulein and devazepide, but not after L-365,260. This was most likely due to increased sensitivity of striatal and mesolimbic dopamine D_2 receptors because long-term administration of CCK antagonists (proglumide, devazepide) and agonists (CCK-8 and caerulein), increased the number of dopamine D_2 receptors in the basal ganglia of rodents (Dumbrille-Ross and Seeman, 1984; Csernansky et al., 1987; Vasar et al., 1988b; Vasar et al., unpublished data) but probably by different mechanisms. Increased dopamine D_2 receptor sensitivity would also account for the increased aggressiveness seen in some mice during repeated treatment with devazepide.

It has been shown that the two subtypes of the CCK receptor have opposite effects on mesolimbic dopaminergic activity. Stimulation of CCK_A receptors potentiates dopamine-induced hyperlocomotion from the medial nucleus accumbens (Crawley et al., 1985) and increases the release of dopamine from the same region (Vickroy and Bianchi, 1989). Injection of CCK-8 into the dorsomedial accumbens also increased the frequency of apomorphine-induced jaw movements, an effect prevented by the selective CCK_A antagonist, lorglumide (Koshikawa et al., 1990). In contrast, CCK_B receptors potentiated apomorphine-induced inhibition of dopaminergic ventral tegmental cells and the reduction in motor activity produced by dopamine applied to the same area (Hommer et al., 1986; Crawley, 1989). Injections of CCK-8 into the ventrolateral accumbens did not affect the frequency of apomorphine-induced jaw movements, in contrast to the facilitation seen after its injection into the medial part of the nucleus accumbens (Koshikawa et al., 1990). These opposite effects of the two receptor sub-types on the activity of dopaminergic systems could explain why repeated administration of the agonist, caerulein and the antagonist, devazepide affected behaviour similarly if it is assumed that high doses of caerulein also affected CCK_B receptors. In recent acute studies, low doses of devazepide (1-100 $\mu g/kg$) have been shown not to affect amphetamine-induced hyperlocomotion but to completely prevent (Vasar et al., in press) the antagonistic effect of caerulein (Zetler, 1985; Allikmets and Vasar, 1990). However, large doses of devazepide alone antagonized the effects of amphetamine (Vasar et al., in press), effects similar to those of caerulein and which have been shown to be due to selective CCK_A receptor stimulation. This suggests that caerulein and large doses of devazepide would also be expected to have similar effects on sensitivity to (+)-amphetamine after chronic administration. The mechanism of the anti-amphetamine effect of large doses of devazepide is not clear because in contrast to selective CCK_B receptor agonists, devazepide (10 mg/kg) did not decrease amphetamine-induced dopamine release, measured as 3-methoxytyramine (Altar and Boyar, 1989).

In conclusion, repeated treatment with caerulein and devazepide significantly affected the locomotor activity of mice and their behavioural responses to caerulein and (+)-amphetamine. The results are interpreted in the light of recent suggestions that CCK_A and CCK_B receptors have opposite effects on dopamine-mediated behaviours (Crawley, 1989; Koshikawa et al., 1990; Vasar et al., in press). It is difficult to explain the discrepancy between the increase in the density of CCK-8 receptors and the lack of any changes in behaviour after 10 day treatment with L-365,260. It may be that the behaviours studied are more dependent on CCK_A receptors and that L-365,260 does not affect these receptors even at high doses.

Acknowledgement

E.V. was supported by a British Council Fellowship.

References

Allikmets, L. and E. Vasar, 1990, Adaptational changes at GABA, benzodiazepine and cholecystokinin receptors elicited by long-term haloperidol administration, Sov. Med. Rev. G. Neuro-pharm. 1, 101.

Altar, C.A. and W.C. Boyar, 1989, Brain CCK-B receptors mediate the suppression of dopamine release by cholecystokinin, Brain Res. 483, 321.

Barrett, R.W., M.F. Steffey and C.A.W. Wolfram, 1989, Type-A CCK binding sites in cow brain: characterization using ( )-[^{3}H]-L-364,718 membrane binding assays, Mol. Pharmacol. 36, 285.

Bradbury, A.J., B. Costall, R.J. Naylor and J.L. Neumeyer, 1983, Motor inhibition induced by apomorphine derivatives in the mouse, J. Pharm. Pharmacol. 35, 494.

Chiodo, L.A. and B.S. Bunney, 1987, Population response of mid brain dopaminergic neurons to neuroleptics: further studies on time course and nondopaminergic neuronal influences, J. Neurosci. 7, 629.

Costall, B., A.M. Domeney and R.J. Naylor, 1985, The continuity of dopamine receptors antagonism can dictate the long term behavioural consequences of a mesolimbic infusion of dopamine, Neuropharmacology 24, 193.

Crawley, J.N., 1989, Microinjection of cholecystokinin into the rat ventral tegmental area potentiates dopamine-induced hypolocomotion, Synapse 3, 346.

Crawley, J.N. and J.S. Schwaber, 1983, Nucleus tractus solitarius lesions block the behavioural actions of cholecystokinin, Peptides 4, 743.

Crawley, J.N., J.A. Stivers, L.K. Blumstein and S.M. Paul, 1985, Cholecystokinin potentiates dopamine-mediated behaviors: evidence for modulation specific to a site of coexistence, J. Neurosci. 5, 1972.

Csernansky, J.G., S. Glick and S. Mellentin, 1987, Differential effects of proglumide on mesolimbic and nigrostriatal dopamine function, Psychopharmacology 91, 440.

Dourish, C.T. and D. R. Hill, 1987, Classification and function of CCK receptors, Trends Pharmacol. Sci. 8, 207.

Dourish, C.T., D. Hawley and S. D. Iversen, 1988, Enhancement of morphine analgesia and prevention of morphine tolerance in the rat by the cholecystokinin antagonist L-364,718, Eur. J. Pharmacol. 147, 469.

Dourish, C.T., M.F. O'Neill, J. Coughlan, S.J. Kitchener, D. Hawley and S.D. Iversen, 1990, The selective CCK-B antagonist L-365,260 enhances morphine analgesia and prevents morphine tolerance in the rat: evidence for mediation of CCK/opioid interactions by CCK-B receptor, Eur. J. Pharmacol. 176, 35.

Dumbrille-Ross, A. and P. Seeman, 1984, Dopamine receptor elevation by cholecystokinin, Peptides 5, 1207.

Gerhardt, G.A., M. Friedmann, M.S. Brodie, T.W. Vickroy, A.P. Gratton, B.J. Hoffer and G.M. Rose, 1989, The effects of cholecystokinin (CCK-8) on dopamine-containing nerve terminals in the caudate nucleus and nucleus accumbens of the anesthetized rat: an in vivo electrochemical studies, Brain Res. 499, 157.

Hamamura, T., Y. Kazahaya and S. Otsuki, 1989, Ceruletide suppresses endogenous dopamine release via vagal afferent system, studied by in vivo intracerebral dialysis, Brain Res. 483, 78.

Hill, D.R., N.J. Campbell, T.M. Shaw and G.N. Woodruff, 1987, Autoradiographic localization and characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists, J. Neurosci. 7, 2967.

Hökfelt, T., L. Skirboll, J.F. Rehfeld, M. Goldstein, K. Markey and O. Dann, 1980, A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing, Neuroscience 5, 2093.

Hommer, D.W., G. Stoner, J.N. Crawley, S.M. Paul and J.R. Skirboll, 1986, Cholecystokinin dopamine coexistence: electrophysiological actions corresponding to cholecystokinin receptor subtype, J. Neurosci. 6, 3039.

Kawasaki, K., M. Kodama and A. Matsushita, 1983, Caerulein, a cholecystokinin-related peptide, depresses somatic function via the vagal afferent system, Life Sci. 33, 1045.

Khosla, S. and J.N. Crawley, 1988, Potency of L-364,718 as an antagonist of the behavioral effects of peripherally administered cholecystokinin, Life Sci. 42, 153.

Koshikawa, N., K. Kikuchi de Beltrán, M. Kobayashi, M. and J.D. Stephenson, 1990, Facilitatory effects of CCK-8 in the dorsomedial nucleus accumbens on apomorphine-induced jaw movements in rats, Eur. J. Pharmacol. 183, 1118.

Leatherbarrow, R.J., 1987, Enzfitter, Non-linear Regression Data Analysis Program for the IBM PC (Elsevier Science Publisher, Amsterdam, The Netherlands).

Matsubara, K. and A. Matsushita, 1986, β-Endorphin involvement in the antidopaminergic effect of caerulein, Jap. J. Pharmacol. 40, 417.

Moran, T.H., P.H. Robinson, M. Goldrich and P.R. McHugh, 1986, Two brain CCK receptors: implications for behavioral actions, Brain Res. 362, 175.

O'Neill, M.F., C.T. Dourish and S.D. Iversen, 1989, Morphine-induced analgesia in the rat paw pressure test is blocked by CCK and enhanced by the CCK antagonist MK-329, Neuropharmacology 28, 243.

Praissman, M., P.A. Martinez, C.F. Saladino, J.M. Berkowitz, A.W. Steggles and J.A. Finkelstein, 1983, Characterization of cholecystokinin binding sites in rat cerebral cortex using [^{125}I]-CCK-8 probe resistant to degradation, J. Neurochem. 40, 1406.

Vasar, E., L. Allikmets, I. Ryzhov, I. Prakhye, A. Soosaar and S. Mirzaev, 1988a, Intraspecies differences in the behavioural effects of caerulein, an agonist of CCK-8 receptors, in mice and rats, Bull. Exp. Biol. Med. 105, 168.

Vasar, E., A. Soosaar and A. Lang, 1988b, The involvement of cholecystokinin receptors in the realization of behavioural and biochemical effects of long-term haloperidol administration, Proc. Acad. Sci. Estonian S.S.R. Biol. 37, 131.

Vasar, E., J. Harro, A. Lang, A. Põld and A. Soosaar, Differential involvement of CCK-A and CCK-B receptors in the regulation of locomotor activity in the mouse, Psychopharmacology (in press).

Vickroy, T.W. and B.R. Bianchi, 1989, Pharmacological and mechanistic studies of cholecystokinin-facilitated [^{3}H]dopamine efflux from rat nucleus accumbens, Neuropeptides 13, 43.

Zetler, G., 1985, Neuropharmacological profile of cholecystokinin-like peptides, Ann. N.Y. Acad. Sci. 448, 448.

IN: Molecular Pharmacology of Receptors. IV. (L. Allikmets, ed.). Acta et Commentationes Universitatis Tartuensis, 1991, 929: 34-41.

PILOCARPINE-INDUCED LIMBIC SEIZURES - AN INVOLVEMENT OF CCK RECEPTORS.

E. Vasar, J. Harro, A. Lang, A. Soosaar

Psychopharmacology Lab, Tartu University

SUMMARY

A muscarinic agonist pilocarpine (380 mg/kg) induced in all injected male mice the fatal seizures. The pretreatment of mice with CCK-8 (25-200 μg/kg) antagonized significantly the effect of pilocarpine, whereas the CCK-B/gastrin agonist pentagastrin (CCK-5, 2500 μg/kg) only moderately inhibited the action of muscarinic agonist. Devazepide (10-1000 μg/kg), a selective antagonist at CCK-A receptors, and L-365,260 (10- 1000 μg/kg), a selective antagonist at CCK-B receptors, antagonized the anticonvulsant effect of CCK-8. However, only a high dose (1 mg/kg) of devazepide and L-365,260 reversed significantly the action of CCK-8.

In rats the administration of pilocarpine (380 mg/kg) decreased significantly the number of [3H]-pCCK-8 binding sites in the several forebrain structures (the frontoparietal cortex, striatum and hippocampus). The comparison of [3H]-pCCK-8 binding in the brain structures of rats with and without seizures revealed evidently higher decrease of CCK-8 receptors' density in animals experiencing seizures. In the hippocampus the difference between the values of responders and non-responders was statistically evident. The significant reduction of [3H]-pCCK-8 binding density in the rat brain during pilocarpine-induced seizures probably reflects the involvement of CCK-B receptors. However, the weak reversal of pilocarpine-induced seizures by CCK-5, and nearly similar action of L-365,260 and devazepide against the anticonvulsant effect of CCK-8 in the mouse seems to support the involvement of both subtypes of the CCK-8 (CCK-A and CCK-B) receptor in the modulation of pilocarpine-induced limbic seizures in rodents.

KEY WORDS: LIMBIC SEIZURES; CCK-8 RECEPTORS; PILOCARPINE; DEVAZEPIDE; L-365,260; CCK-8 ; MOUSE; RAT.

An involvement of cholecystokinin octapeptide (CCK-8) in the regulation of seizure activity has been suggested by numerous pharmacological studies. Thus, systemic or intracerebral administration of CCK-8 and its analogue caerulein inhibits seizures with different genesis [6, 20, 21]. On the other hand, the unspecific CCK-8 antagonist proglumide reverses the anticonvulsant effect of caerulein against picrotoxin and quinolinate-induced seizures, and potentiates seizures induced by quinolinate, an agonist at N-methyl-D-aspartate receptors [19, 20]. The highest levels of CCK-8 immunoreactivity and receptors are found in the different limbic and cortical structures (piriform cortex, amygdala, hippocampus etc.) [5, 12, 17], which are known to be involved in the regulation of seizure activity [4, 14]. Limbic seizures with varied genesis have been demonstrated to cause nearly complete loss of CCK-8 immunoreactivity from hippocampal mossy fiber system [3]. The potent convulsant picrotoxin is shown to reduce CCK-8 immunoreactivity in the several limbic regions [7]. The systemic treatment with muscarinic agonist pilocarpine is shown to cause very typical limbic seizures in rodents [18]. Magnani et al. [10, 11] have shown that the systemic treatment with CCK-8 and caerulein significantly affects the release of acetylcholine from the cerebral cortex of the rat "in vivo". Therefore, the aim of present work was to establish the role of CCK-8 receptors in the regulation of limbic seizures induced by pilocarpine in mice and rats. CCK-8, CCK-B/gastrin agonist pentagastrin (CCK- 5) and two selective antagonists at CCK-8 receptors L-365,260 (antagonist of "brain" or CCK-B receptors) and devazepide (antagonist of "visceral" or CCK-A receptors) [1, 9] were used for clarifying of this problem. Simultaneously with the behavioural experiments, the effect of pilocarpine-induced seizures was studied on the parameters of CCK-8 receptors in the different brain structures of the rat.

MATERIALS AND METHODS

Male albino mice (25-30 g) and male Wistar rats (250-300 g) were used throughout the experiment. The mice were placed into individual observation boxes 15 min before the start of experiment. After this habituation period CCK antagonists - L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N'-(3-methyl-phenyl)urea, CCK-B antagonist, 0.01-1 mg/kg) and devazepide (formerly MK-329, CCK-A antagonist, 0.01-1 mg/kg) - were injected 15 min. and CCK-8 (25-200 μg/kg) and pentagastrin (CCK-5, 2.5 mg/kg) 10 min prior to muscarinic agonist pilocarpine (380 mg/kg). Mice were observed for 60 min and the latencies of onset of tremor, and tonic seizures and death were registered. In the radioligand binding experiments with [propionyl-3H]propionylated CCK-8 (3[H]-pCCK-8) scopolamine methylnitrate (an antagonist at peripheral muscarinic receptors) was injected 30 min prior to saline or pilocarpine (380 mg/kg). Two subgroups of rats - responders and non-responders to pilocarpine-induced limbic seizures -

were selected for radioligand binding studies. Animals, respectively with and without seizures, were killed by decapitation 60 min after the administration of pilocarpine. The brains were removed rapidly from the skulls and the frontoparietal cortex, mesolimbic structures (nucleus accumbens, tuberculum olfactorium), piriform cortex, striata and hippocampus were dissected [15]. The method of Praissman et al. [16] in our slight modification was used for 3[H]-pCCK-8 binding studies. Saturation curves were analyzed using ENZFITTER program for IBM microcomputers [8].

RESULTS.

Systemic treatment with muscarinic agonist pilocarpine (380 mg/kg) evoked in all injected male mice (n=39) the fatal seizures. The pretreatment of mice with CCK-8 (25-200 μg/kg) significantly antagonized the effect of 380 mg/kg pilocarpine (figure 1). 50 μg/kg CCK-8 obviously reversed the effect of muscarinic agonist, the further increase of CCK-8 dose did not enhance the effect of neuropeptide. 13 mice from 39 tested survived pilocarpine- induced seizures after administration of 200 μg/kg CCK-8. CCK- 8 antagonist devazepide in the high dose (1 mg/kg) evidently antagonized the anticonvulsant effect of CCK-8 (figure 2). CCK-B antagonist L-365,260 also after the administration of high dose (1 mg/kg) reversed the anticonvulsant action of CCK- 8 (figure 3). However, L-365,260 (10-1000 μg/kg), differently from devazepide, completely blocked the antagonism of CCK-8 against the pilocarpine-induced lethality.

Pilocarpine up to 1 mM did not interact with 3[H]-pCCK-8 binding in the radioligand studies "in vitro". The administration of high dose of pilocarpine (380 mg/kg) changed the parameters of 3H-pCCK-8 binding sites in the several forebrain structures (table). Pilocarpine reduced significantly the number of 3[H]-pCCK-8 binding sites in the striatum, frontoparietal cortex and hippocampus (table). In the hippocampus affinity of 3H-pCCK-8 binding sites was also increased after administration of pilocarpine. The comparison of [3H]-pCCK-8 binding parameters in the animals, responding and non-responding to pilocarpine induced seizures, revealed more significant changes in the brain structures of rats, experiencing seizures (table). In the hippocampus the difference between the values of [3H]-pCCK-8 binding in responders and non-responders was statistically evident (table).

DISCUSSION

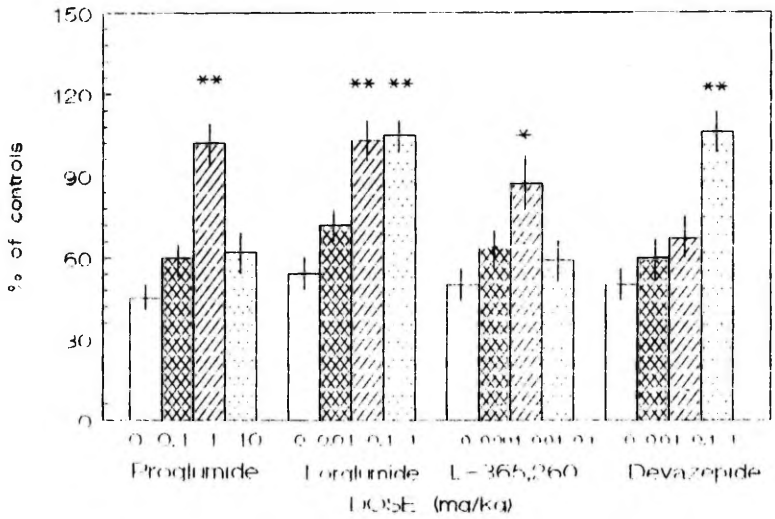
The results of present study are reflecting a significant role of CCK-8 receptors in the modulation of epileptogenic effect of a muscarinic agonist pilocarpine. CCK-8 potently antagonizes the seizures induced by the lethal dose of pilocarpine. One third of mice survive pilocarpine induced seizures after pretreatment with 200 μg/kg CCK-8. CCK- b/gastrin agonist pentagastrin only

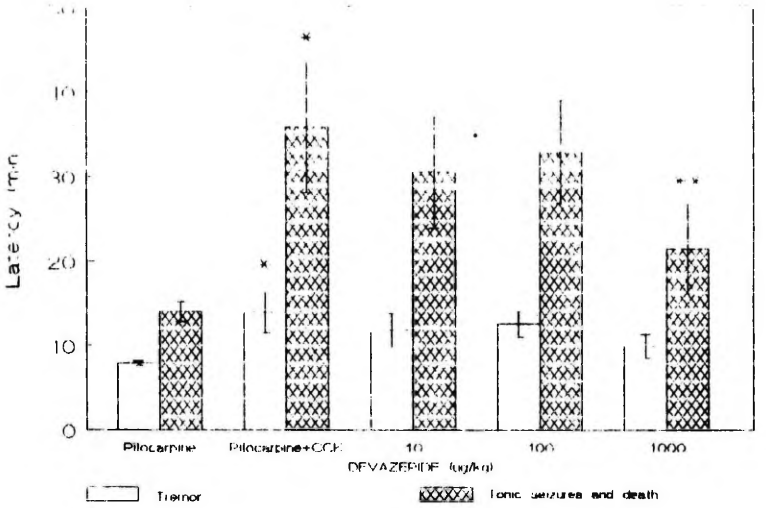
LEGEND TO THE FIGURES

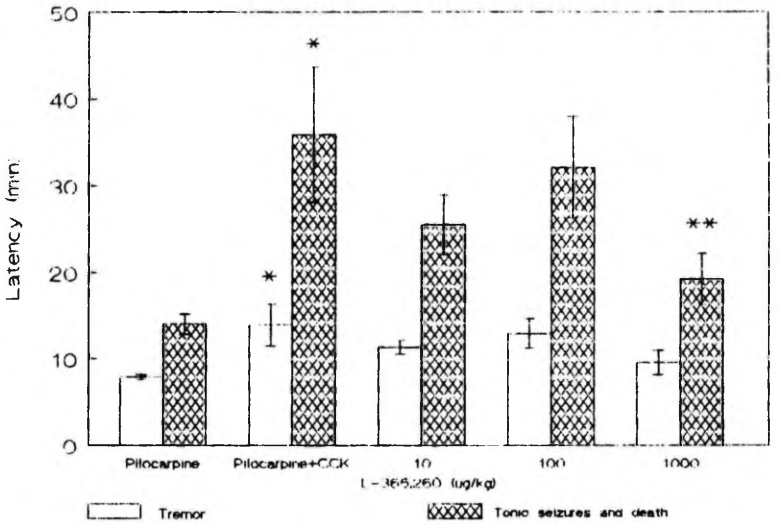
Figure 1. The effect of CCK-8 (25-200 μg/kg) and pentagastrin (CCK-5, 2500 μg/kg) on pilocarpine-induced seizures in mice. CCK-8 and CCK-5 were given 10 min prior to pilocarpine (380 mg/kg). The animals were observed for 60 min after the administration of pilocarpine. Significant differences between vehicle/pilocarpine and CCK-8 or CCK- 5/pilocarpine treated groups were determined by Newman-Keuls test after significant ANOVA. $F_{5,116}= 8.71$, $p<0.0001$ (for tremor); $F_{5,116}= 10.46$, $p<0.000001$ (for tonic seizures and death). * - $p< 0.05$; ** - $p< 0.01$; *** - $p<0.005$ (if compared to pilocarpine treated mice).

Figure 2. The effect of devazepide (10-1000 μg/kg) on the anticonvulsant action of CCK-8 (200 μg/kg) against pilocarpine-induced seizures in mice. Devazepide was injected 15 min and CCK-8 10 min prior to pilocarpine. Significant differences between pilocarpine, CCK-8/pilocarpine and devazepide/CCK-8/pilocarpine treated groups were determined by Newman-Keuls test after significant ANOVA. $F_{4,77}=2.4$, $p<0.05$ (for tremor), $F_{4,77}=2.5$ (for tonic seizures and death). * - $p<0.05$ (if compared to pilocarpine treated animals); ** - $p<0.05$ (if compared to CCK-8/pilocarpine treated mice).

Figure 3. The effect of L-365,260 (10-1000 μg/kg) on the anticonvulsant effect of CCK-8 (200 μg/kg) against pilocarpine-induced seizures in mice. L-365,260 was injected 15 min and CCK-8 10 min prior to pilocarpine. Significant differences between pilocarpine, CCK-8/pilocarpine and L-365,260/CCK-8/pilocarpine treated groups were determined by Newman-Keuls test after significant ANOVA. $F_{4,77}= 2.86$, $p<0.05$ (for tremor); $F_{4,77}= 3,69$, $p<0.01$ (for tonic seizures and death). * - $p<0.05$ (if compared to pilocarpine treated moce); ** - $p<0.05$ (if compared to CCK-8/pilocarpine treated animals).


150
120
90
60
30
0
% of controls
**
** **
*
**
0 0.1 1 10
Progliumide
Loxiglumide
L-365,260
Devazepide
Dose (mg/kg)


Latency (min)
0
10
20
30
Pilocarpine
Pilocarpine+CCK
10
100
1000
DEVAZEPIDE (µg/kg)
Tremor
Tonic seizures and death


Latency (min)
50
40
30
20
10
0
*
*
**
Pilocarpine
Pilocarpine+CCK
10
100
1000
L-365,260 (ug/kg)
Tremor
Tonic seizures and death

Table

The binding parameters of [3H]-pCCK-8 in the brain structures of responding and non-responding rats to pilocarpine (380 mg/kg) seizures.

Brain structures		Saline	Non-responders	Responders
Mesolimbic area	Kd	0.42±0.02	0.40±0.03	0.50±0.15
	Bmax	4.51±0.32	4.13±0.22	3.55±0.53
Piriform cortex	Kd	0.43±0.02	0.63±0.06	0.68±0.08
	Bmax	6.30±0.20	5.22±0.29	5.58±0.75
Frontoparietal	Kd	0.33±0.02	0.41±0.03	0.26±0.02
cortex	Bmax	5.15±0.30	3.76±0.76	2.71±0.23a
Striatum	Kd	0.27±0.01	0.45±0.07	0.36±0.04
	Bmax	5.23±0.28	4.25±0.19a	3.74±0.24b
Dorsal hippo-	Kd	0.63±0.04	0.37±0.05a	0.15±0.02b,c
campus	Bmax	1.87±0.17	1.18±0.28a	0.56±0.05b,c

The brain structures of 4-5 rats have been pooled. The mean values ± S.E.M. of 4 independent experiments are presented in table. Kd - dissociation constant in nM; Bmax - apparent number of binding sites in pmoles per gram original tissue wet weight a - $p < 0.05$; b - $p < 0.01$ (compared to saline treated rats, Student's t-test); c - $p < 0.05$ (compared to non-responders, Student's t-test).

moderately reduces the convulsant action of pilocarpine. Accordingly, it seems probable that the peripherally injected CCK-8 affects the cholinergic neurotransmission in the brain. It is suggested that several behavioural effects of CCK-8 and caerulein are generated through primarily peripheral mechanisms [13]. It is thought that the sedative effect of large doses of CCK-8 is of peripheral origin and could be abolished by abdominal vagotomy [2]. Magnani et al. [10, 11] have shown that CCK-8, in the doses 10 μg/kg and higher, potently inhibits the release of acetylcholine from the rat cerebral cortex. This effect of CCK-8 is not affected by bilateral vagotomy or by the lesion of dopaminergic cells in the substantia nigra. The selective CCK antagonists devazepide and L-365,260 reverse the anticonvulsant effect of CCK-8. However, it happens only after the administration of very high dose (1 mg/kg) of CCK antagonists. It is noteworthy that the effect of L-365,260 is somewhat stronger.

L-365,260, in wide dose range (10-1000 μg/kg), antagonize also the effect of CCK-8 on pilocarpine-induced lethality. Nevertheless, the both subtypes of CCK-8 (CCK-A and CCK-B) seems to be involved in the anticonvulsant effect of CCK-8. According to the radioligand binding studies "in vitro", pilocarpine (up to 1 mM) does not interact directly with CCK-8 receptors in the brain. However, the systemic administration of very high dose of pilocarpine (380 mg/kg) is reducing the density of CCK-B receptors in the frontoparietal cortex, striatum and hippocampus of the rat brain. The comparison of 3[H]-pCCK-8 binding parameters in rats, responding and non- responding to the administration of pilocarpine with seizures, reveals markedly higher reduction of CCK-B receptors in animals with seizures. It supports the idea that CCK-B receptors are involved in the modulation of seizures induced by muscarinic agonist.

In conclusion, it is very likely that the both subtypes of CCK-B receptor are involved in the modulation of limbic seizures induced by the muscarinic agonist pilocarpine. This idea is supported by the findings that CCK-B/gastrin agonist pentagastrin only moderately antagonized the effect of pilocarpine, the selective CCK-8 antagonists devazepide and L- 365,260 have nearly similar effect on the anticonvulsant effect of CCK-8, and during pilocarpine-induced seizures the density of CCK-8 receptors is significantly reduced in the several brain regions.

Acknowledgment. The authors are grateful to Merck, Sharp and Dohme Ltd. for the generous gift of devazepide and L-365,260.

REFERENCES

1. Chang R.S.L. and Lotti V.J. (1986) Biochemical and pharmacological characterization of an extremely potent and selective nonpeptide cholecystokinin antagonist. Proc.Natn.Acad.Sci.U.S.A. 83: 4923-4926.

2. Crawley J.N. and Kiss J.Z. (1985) Tracing the sensory pathway from gut to brain regions mediating the actions of cholecystokinin on feeding and exploration. Ann.N.Y.Acad.Sci. 448: 586-588.

3. Gall C. (1988) Seizure induce dramatic and distinctly different change in enkephalin, dynorphin and CCK immunoreactivities in mouse hippocampal mossy fibers. J.Neurosci. 8: 1852-1862.

4. Handforth A. and Ackermann R.F. (1988) Electrically induced limbic status and kindled seizures. In: Anatomy of epileptogenesis. (eds. Meldrum B.S., Ferrendelli J.A. and Wieser H.G.). John Libbey and Co Ltd., London, pp. 71-88.

5. Harris Q.L.G., Lewis S.J., Shulkes A. and Vajda F.J.E. (1988) Regional concentrations of cholecystokinin in the rat: the effects of kindled and non-kindled seizures. Neuropharmacol. 27: 547-549.

6. Kadar T., Pesti A., Penke B. and Telegdy G. (1984) Inhibition of seizures induced by picrotoxin and electroshock by cholecystokinin octapeptides and their fragments in rats after intracerebroventricular administration. Neuropharmacol. 23: 955-961.

7. Kato T., Emi Y., Takita M. and Kushima Y. (1988) Time dependent changes in the concentrations of immunoreactive cholecystokinin octapeptide sulfate in rat brain regions after systemic injection of picrotoxin. Neurochem.Int. 12: 493-497.

8. Leatherbarrow R.J. (1987) Enzfitter, a non-linear regression data analysis program for the IBM PC. Elsevier Science Publisher, Amsterdam.

9. Lotti V.J. and Chang R.S.L. (1989) A new potent and selective non-peptide gastrin antagonist and brain cholecystokinin receptor (CCK-B) ligand: L-365,260. Eur.J.Pharmacol. 162: 273-280.

10. Magnani M., Mantovani P. and Pepeu G. (1984) Effect of cholecystokini octapeptide and ceruletide on release of acetylcholine from cerebral cortex of the rat in vivo. Neuropharmacology 23: 1305-1309.

11. Magnani M., Florian A., Casamenti F. and Pepeu G. (1987) An analysis of cholecystokinin-induced increase in acetylcholine output from cerbral cortex of the rat. Neuropharmacology 26: 1207-1210.

12. Marley P.D., Rehfeld J.F. and Emson P.C. (1984) Distribution and chromatographic characterisation of gastrin and cholecystokinin in the rat central nervous system. J.Neurochem. 42: 1523-1530.

13. Morley J.E. (1987) Behavioural effects of administered cholecystokinin. ISI Atlas Pharmacol. 1: 49-51.

14. Patel S. (1988) Chemically induced limbic seizures in rodents. In: Anatomy of epileptogenesis. (eds. Meldrum B.S., Ferrendelli J.A. and Wieser G.H.), John Libbey and Co. Ltd., London, pp. 89-106.

15. Paxinos G. and Watson C. (1982) The rat brain in stereotaxic coordinates. Academic Press Australia, North Ryde.

16. Praissman M., Martinez P.A., Saldino C.F., Berkowitz J.M., Steggles A.W. and Finkelstein J.A. (1983) Characterization of cholecystokinin binding sites in rat cerebral cortex using a [125I]-CCK-8 probe resistant to degradation. J.Neurochem. 40:1406-1413.

17. Saito A., Sankaran K., Goldfine D. and Williams J.A. (1980) Cholecystokinin receptors in the rat brain: characterization and distribution. 208: 1155-1156.

18. Turski W.A., Cavalheiro E.A., Schwarz M., Czuczwar S.J., Kleinrok Z. and Turski L. (1983) Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalic and neuropathological study. Behav.Brain Res. 9: 315-322.

19. Vasar E., Rägo L., Soosaar A., Nurk A. and Maimets M. (1985) Modulatory effect of caerulein on benzodiazepine receptors. Bull.Exp.Biol.Med. 102: 711-713.

20. Vasar E., Allikmets L., Ryzhov I., Prakhye I., Soosaar A. and Mirzayev S. (1988) Intraspecies differences in the behavioral effects of caerulein, an agonist of CCK-8 receptors, in mice and rats. Bull.Exp.Biol.Med. 105: 168-170.

21. Zetler G. (1985) Caerulein and its analogues: neuropharmacological properties. Peptides 6: 33-46.

In: Multiple Cholecystokinin Receptors in the CNS (S.T. Cooper and C.D. Dourish, eds.), Oxford University Press, 1991 (in press)

CCK-8 RECEPTORS AND ANXIETY IN RATS

Eero Vasar, Jaanus Harro, Anu Põld and Aavo Lang

Psychopharmacology Lab, Tartu University,

34 Veski Str., 202400 TARTU, Estonia

The agonists at CCK-8 receptors are shown to induce or potentiate fear-related behaviours in rats. Caerulein and pentagastrin (CCK-5) have at very low doses an anxiogenic-like effect on rats in an elevated plus-maze (Harro, Põld, Vasar and Allikmets, 1989). CCK-8 antagonist proglumide completely reversed the anxiogenic-like action of caerulein and CCK-5 in rodents (Harro et al., 1989; Harro, Põld and Vasar, 1990). Intraventricular administration of CCK-4 significantly increases the intensity of foot-shock elicited aggressiveness in male rats (Vasar, Maimets and Allikmets, 1984). Very recently, the intravenous administration of CCK-4 is shown to cause very severe anxiety and panic-like attacks in healthy volunteers (De Montigny, 1989). However, it is not clear where the primary target of anxiogenic-like action of CCK-8 related peptides lies: in the brain or periphery. Therefore, the present study was dedicated to reveal the primary target of anxiogenic-like effect of CCK-8 agonists on the rat.

The male and female rats (weighing 180-220 grams) were used throughout the study. The animals were used only once. The anxiogenic-like effect of CCK-8 agonists (caerulein, CCK-5 and CCK-4) was studied according to the method of Pellow, Chopin, File and Briley (1985) in an elevated plus-maze. The lowest dose of caerulein to cause the anxiogenic-like effect on the rat was 100 ng/kg. CCK-5 had the similar effect after administration of 500 ng/kg. The subcutaneous treatment with 10 µg/kg CCK-4 in some experiments also significantly decreased the exploratory activity of rats. The maximal reduction of animals behaviour was seen after injection of 25 and 50 µg/kg of CCK-4 (table 1). In higher doses (100 µg/kg and 1 mg/kg) CCK-4 failed to affect the rats'

behaviour. The anxiogenic-like effect of CCK agonists was in good accordance with their potency to inhibit [^{3}H]-propionylated-CCK-8 ([^{3}H]-pCCK-8, 0.3 nM) binding in the cerebral cortex, but not in pancreas (table 2). According to these results it is very probable that CCK-B (central subtype) receptors have a significance in the anxiogenic-like action of CCK-8 agonists on the rat.

Further, the interaction of different CCK antagonists (lorglumide, proglumide, devazepide and L-365,260) with anxiogenic-like effect of CCK-4 (50 µg/kg) was studied. The pretreatment with 0.01 mg/kg L-365,260, the selective antagonist at CCK-B/gastrin receptors, caused statistically evident antagonism with the effect of CCK-4 (figure 1). L-365,260 antagonized the action of CCK-4 in 10 times smaller dose than lorglumide (the effective dose 0.1 mg/kg). CCK-B/gastrin antagonist was 100 times more effective than the selective CCK-A (peripheral subtype) antagonist devazepide (1 mg/kg) and proglumide (1 mg/kg). It is worthy noting that the antagonism of glutaramic acid derivatives (proglumide, lorglumide) against CCK-4 was more pronounced if compared to the effect of 1,4- benzodiazepines (devazepide, L-365,260).

CCK-8 is shown to localize in some brain regions (cerebral cortex, hippocampus) mostly in GABA-ergic neurons (Kosaka, Kosaka, Tateishi, Hamaoka, Yanaihara, Wu and Hama, 1985). There is existing the clear antagonistic interaction between benzodiazepine tranquillizers and CCK-8 in the electrophysiological experiments (Bradwejn and De Montigny, 1984). Picrotoxin, the potent anatgonist at chloride channel, at anxiogenic dose (0.5 mg/kg) increased the density of CCK-8 receptors in frontal cortex and hippocampus (figure 2). In higher doses (1 and 2.5 mg/kg) picrotoxin induced seizures and apparently decreased the density of CCK-8 receptors (figure 2). Picrotoxin failed in the acute experiments to affect the parameters of benzodiazepine receptors. On the other hand, the anxiogenic doses of CCK-8 agonists caerulein (100 ng/kg) and CCK-5 (500 ng/kg) did not change the affinity and density of CCK-8 receptors, but decreased (respectively 34 % and 38 %) the apparent number of benzodiazepine receptors in frontal cortex. It is quite probable that at least in the frontal cortex the negative interaction is existing between CCK-8- and GABA-ergic systems in the regulation of anxiety. This opinion is supported also by the experiments, where the rats were selected according to their behaviour in the elevated plus-maze. There was possible to find the animals from the population of rats, which exploratory activity differed very significantly. The radioligand binding experiments with [^{3}H]-pCCK-8 and [^{3}H]-flunitrazepam revealed very evident differences between the high ("non- anxious") and low activity ("anxious") rats in the density of CCK-8 and benzodiazepine receptors in the frontal cortex (figure 3). "Non-anxious" rats had obviously lower density of CCK-8 and higher density of benzodiazepine receptors in the frontal cortex if compared with "anxious" animals.

In conclusion, the anxiogenic-like effect of peripherally administered CCK-8 agonists is probably related to the central subtype of CCK receptor in the rat. At least in the frontal cortex CCK-B receptors seem to have a strong negative interaction with GABA-benzodiazepine receptor complex.

Thus, the balance between CCK-8- and GABA-ergic systems has the significance in the genesis of anxiety.

Devazepide and L-365,260 are the generous gifts from *Merck, Sharp and Dohme*. Proglumide and lorglumide were donated by *Rotta Pharmaceutici*.

TEXT TO THE FIGURES

Figure 1 THE INTERACTION OF CCK ANTAGONISTS WITH ANXIOGENIC-LIKE EFFECT OF CCK-4 ON RATS IN AN ELEVATED PLUS- MAZE.

Data presented in the figure are % of control values of each separate experiment. The scores are mean (±S.E.M.) percentage of crossed lines in the open part. In the control group the mean value of crossed lines during 4 min was between 10 and 14 in the different experiments. Proglumide (0.1-10 mg/kg, i.p.) and lorglumide (0.01-1 mg/kg, i.p.) were administered 10 min before the CCK-4 (50 µg/kg, s.c.) treatment, whereas devazepide (0.01-1 mg/kg, i.p.) and L-365,260 (0.001-0.1 mg/kg, i.p.) were given 15 prior to the CCK-4 dose. Lorglumide, devazepide and L-365,260 were suspended with the help of some drops of Tween-85 in distelled water. The same vehicle (some drops of Tween-85 in distilled water) was given to control rats. * - $p < 0.05$; ** - $p<0.01$, as compared to respective CCK-4 group values.

Figure 2. THE EFFECT OF PICROTOXIN ON [^{3}H]-pCCK-8 BINDING IN THE RAT BRAIN.

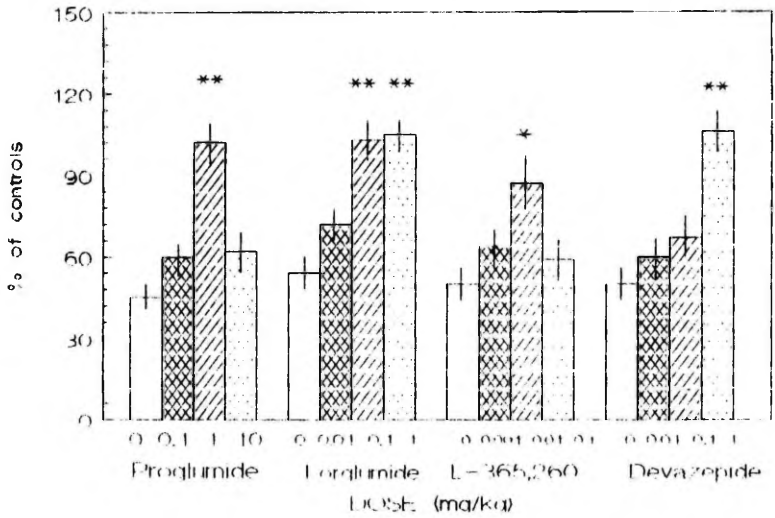
Data are expressed as the apparent number of binding sites (B/max) in pmol/g original tissue wet weight of the frontal cortex and hippocampus of the rat. The mean values of three independent studies (±S.E.M.) are presented in the table. Picrotoxin (0.1-2.5 mg/kg, s.c.) was injected 30 min before the decapitation. * - $p< 0.05$; ** - $p<0.01$, as compared to control group (Student's t-test).

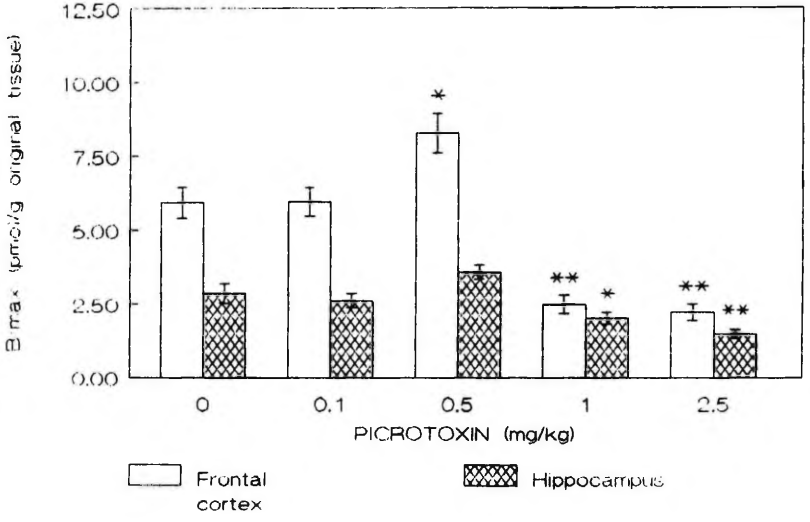
Figure 3. [^{3}H]-pCCK-8 AND [^{3}H]-FLUNITRAZEPAM BINDING IN THE FRONTAL CORTEX OF THE RATS, SELECTED ACCORDING TO THEIR RESPONSE IN THE ELEVATED PLUS-MAZE.

In the low activity group the number of crossed lines in the open part was 0.9±0.6. In the intermediate group it was 8.5±0.5 and in the high activity group 18.2±1.5. The differences between three selected groups are statistically evident ($p< 0.01$, Mann- Whitney U-test). The mean values of three independent selection experiments are presented in the figure. Data are % of home-cage control values in the frontal cortex. The apparent number of [^{3}H]-pCCK-8 binding sites in the frontal cortex of home-cage controls was 3.66±0.34 pmol/g original tissue, and for [^{3}H]-flunitrazepam it was 137±12 pmol/g original tissue.

* - $p< 0.05$, significant difference from home-cage controls;

** - $p< 0.05$, significant difference from high activity group (Student's t-test).


150
120
90
60
30
0
% of controls
**
** **
*
**
0 0.1 1 10
0 0.01 0.1 1
0 0.001 0.01 0.1
0 0.01 0.1 1
Proglumide
Loxiglumide
L-365,260
Devazepide
Dose (mg/kg)


12.50
10.00
7.50
5.00
2.50
0.00
0
0.1
0.5
1
2.5
PICROTOXIN (mg/kg)
Frontal cortex
Hippocampus

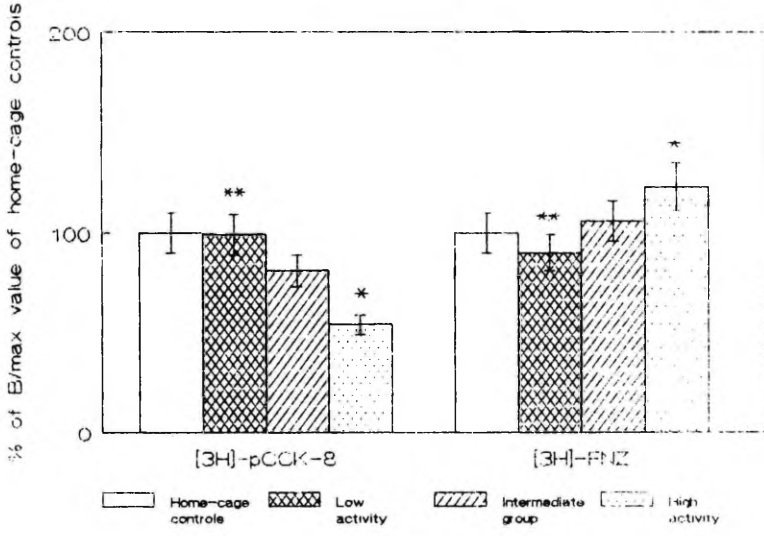

% of B/max value of home-cage controls
200
100
0
**
*
**
*
[3H]-pCCK-8
[3H]-PNZ
Home-cage controls
Low activity
Intermediate group
High activity

Table 1

Effect of CCK-4 on the exploratory activity of the rat in an elevated plus-maze

Test dose	Latency of first open part entry (s)	No of lines crossed in open part	Total time spent in open part (s)
Vehicle	42±8	10.3±1.1	57±6
CCK-4 1 µg/kg	36±24	11.3±2.6	69±12
10 µg/kg	57±10	7.4±2.6	45±6
25 µg/kg	81±8*	6.2±1.2 *	39±5
50 µg/kg	86±12*	5.5±1.0 *	33±5
100 µg/kg	69±14	9.0±2.2	63±10
1000 µg/kg	39±24	10.9±2.1	58±10

All values are means ± S.E.M. The test time was 4 min. CCK-4 was administered s.c. 15 min prior to the experiment. *- $p<0.05$ (significantly different from vehicle treated group, Newman-Keuls test after ANOVA, one-way ANOVA for entries $F(1,153)=2.18$, $p<0.05$; for crossings $F(1,153)=2.36$, $p<0.05$; for total time $F(1,153)=1.92$, $p=0.08$).

Table 2

Correlation between anxiogenic-like effect of CCK-8 agonists and their affinity at CCK-8 receptors in cerebral cortex and pancreas of the rat.

CCK-8 agonists	Suppression of exploratory activity in elevated plus-maze (pmol/kg)	IC_{50} values against [^{3}H]-pCCK-8 cerebral cortex (nM)	pancreas
Caerulein	0.074	1.1	0.6
CCK-5	0.670	10	6200
CCK-4	43.3	411	>10000
Pearson's γ		0.99998 p= 0.004	0.797 p= 0.41

The doses of CCK agonists presented in the table are inducing the statistically evident anxiogenic-like effect in the elevated plus-maze. The radioligand binding studies were performed according to the method of Praissman, Martinez, Saldino, Berkowitz, Steggles and Finkelstein, 1983.

REFERENCES

1. Bradwejn J. and De Montigny C. (1984) Benzodiazepines antagonize cholecystokinin-induced activation of rat hippocampal neurones. Nature 312: 363-364.

2. De Montigny C. (1989) Cholecystokinin tetrapeptide induces panic-like attacks in healthy volunteers: preliminary findings. Arch. Gen. Psychiatry 46: 511-517.

3. Harro J., Pôld M., Vasar E. and Allikmets L. (1989) The role of CCK-8-ergic mechanisms in the regulation of emotional behaviour in rodents. Zh.Vyss.Nerv.Deyat. 39: 877-883.

4. Harro J., Pôld M. and Vasar E. (1990) Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: influence of acute and subchronic diazepam treatment. Naunyn-Schmiedebrg's Arch.Pharmacol. 341: 62-67.

5. Kosaka T., Kosaka K., Tateishi K., Hamaoka Y., Yanaihara N., Wu J.-Y. and Hama K. (1985) GABAergic neurons containing CCK-8- like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J.Comp.Neurol. 239:420-430.

6. Pellow S., Chopin P., File S. and Briley M. (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods. 14: 149-167.

7. Praissman M., Martinez P.A., Saldino C.F., Berkowitz J.M., Steggles A.M. and Finkelstein J.A. (1983) Characterization of cholecystokinin binding sites in rat cerebral cortex using a [^{125}I]-CCK-8 probe resistant to degradation. J.Neurochem. 40:1406-1413.

8. Vasar E., Maimets M. and Allikmets L. (1984) Role of the serotonin$_2$-receptors in regulation of aggressive behaviour. Zh.Vyss.Nerv.Deyat. 34: 283-289.