Involvement of central cholinergic system in antinociception induced by sumatriptan in mouse.

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INVOLVEMENT OF CENTRAL CHOLINERGIC SYSTEM IN ANTINOCICEPTION INDUCED BY SUMATRIPTAN IN MOUSE

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Summary: *The antinociceptive effect of the antimigraine drug sumatriptan was assessed in mice (hot-plate and abdominal constriction tests). Antinociception induced by sumatriptan (10–30 mg kg^{-1} i.p.) was prevented by the muscarinic antagonist atropine (5 mg kg^{-1} i.p.), the ACh-depletor hemicolinium-3 (1 µg per mouse i.c.v.) and the 5-HT$_{1A}$ antagonist NAN-190 (0.5 mg kg^{-1} i.p.). Naloxone, CGP-35348 and reserpine administered in doses suitable for blocking analgesia respectively induced by morphine, baclofen and clomipramine did not modify sumatriptan antinociception. On the basis of the above findings, we can deduce that sumatriptan was able to induce antinociception by increasing cholinergic neurotransmission through the stimulation 0f 5-HT$_{1A}$ receptors.*

Introduction

The 5-HT$_{1A}$ agonists: 8-OH-DPAT, buspirone and gepirone, are able to induce an antinociception in rodents mediated by the amplification of cholinergic neurotransmission (1, 2). These observations are in agreement with the data of Bianchi *et al.* (3) showing an increase in ACh efflux from the cerebral cortex of freely moving guinea-pigs after administration of 8-OH-DPAT. Since the antimigraine drug sumatriptan (GR43175) is a 5-HT$_{1like}$ receptor agonist (4, 5) our aim consisted in ascertaining whether sumatriptan, like 8-OH-DPAT, buspirone and gepirone, is able to induce antinociception by increasing ACH release.

Materials and methods

Animals. Male Swiss-Webster mice (22–28 g), from Morini (San Polo d'Enza-Italy) were used. The animals were kept at 23 ± 1°C, with a 12-h light/dark cycle, light at 7 a.m., with food and water *ad libitum*. All experiments were carried out according to the guidelines of the European Community Council on animal care.

Hot-plate test. The method described by O'Callaghan & Holzman (6) was adopted, using a stainless-steel container (36 × 28 × 30 cm), thermostatically set at 52.5 ± 0.1°C, in a precision water-bath. Mice with a licking latency below 12 and over 18 s in the test before drug administration (30%) were rejected. an arbitrary cut-off time of 45 s was adopted.

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Abdominal-constriction test. The test was performed in mice according to Koster *et al.* (7). The number of stretching movements was counted for 10 min, starting 5 min after 0.6% acetic acid injection.

Rota-rod. The integrity of motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod according to Vaught *et al.* (8). The performance time was measured both before and 15, 30 and 45 min after treatment.

Drugs. The following drugs were used: atropine sulfate (Sigma); hemicholinium-3 hydrobromide, naloxone hydrochloride, NAN-190 hydrobromide, (RBI); CGP-35348 and reserpine (Ciba-Geigy), sumatriptan succinate (Glaxo). The doses given in the text are expressed as salts. All drugs were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use, except reserpine which was dissolved in a 20% solution of ascorbic acid. Intracerebroventricular (i.c.v.) administration was performed under short ether anaesthesia according to the method described Haley & McCormick (9).

Statistical analysis. Results are given as the mean ± SE. Analysis of variance, followed by Fisher's PLSD procedure for post-hoc comparison, was used to verify significance of differences between two means. *P* values of less than 0.05 were considered significant. Data were analysed with the StatView for the Macintosh computer program (1992).

Results

Effect on pain threshold

The antinociceptive effect of sumatriptan was investigated by means of the hot-plate test and the abdominal constriction test in mice. In the hot-plate test, sumatriptan injected i.p. at doses between 10 and 30 mg kg^{-1} induced a significant increase in the pain threshold (Table I). Sumatriptan antinociception was completely prevented by atropine (5 mg kg^{-1} i.p.), by the choline uptake blocker HC-3 (1 µg/mouse i.c.v.) and by the 5-HT_{1A} antagonist NAN-190 (0.5 mg kg^{-1} i.p.) (Table I). Conversely, no modification in sumatriptan antinociception was obtained by pretreating the mice with the opioid antagonist naloxone (1 mg kg^{-1} i.p.) and the $GABA_B$ antagonist CGP-35348 (100 mg kg^{-1} i.p.).

The dose-response curve of sumatriptan administered i.p. and i.c.v. on the abdominal constriction test is shown in Fig. 1. Sumatriptan, injected i.p. at the doses of 10, 20 and 30 mg kg^{-1}, statistically decreased the number of abdominal constrictions, with a maximum effect 15 and 30 min after treatment. Likewise, sumatriptan injected i.c.v. at 50–100 µg per mouse induced antinociception with the maximum effect 15 min after administration (Fig. 1). The antinociceptive effect of sumatriptan (20 mg kg^{-1} i.p.) was prevented, as in the hot-plate test, by pretreatment with HC-3 but not by reserpine (2 mg kg^{-1} i.p.), a monoamine store depletor, administered twice, 48 and 24 h before the test (Fig. 1). Sumatriptan, in the antinociceptive dose range, did not elicit any change of motor coordination as revealed by the rota-rod test (data not shown).

Discussion

Sumatriptan, like 8-OH-DPAt, is able to induce antinociception in mice in the presence of thermal (hot-plate test) or chemical (abdominal constriction test) stimuli. Sumatriptan exerts its antinociceptive effect by acting centrally for the following reasons: *i*) It is possible to reach the same intensity of analgesia by injecting doses (50–100 µg per mouse) of sumatriptan directly into the cerebral ventricles which are considerably lower than those needed parenterally; *ii*) i.c.v. administration of HC-3 is able to antagonize the increase in the sumatriptan pain threshold. Sumatriptan antinociception is dependent on central cholinergic activation mediated via 5-HT_{1A} receptor stimulation since it is prevented not only by the 5-HT_{1A} antagonist NAN-190, but also by the muscarinic antagonist atropine and the ACh depletor

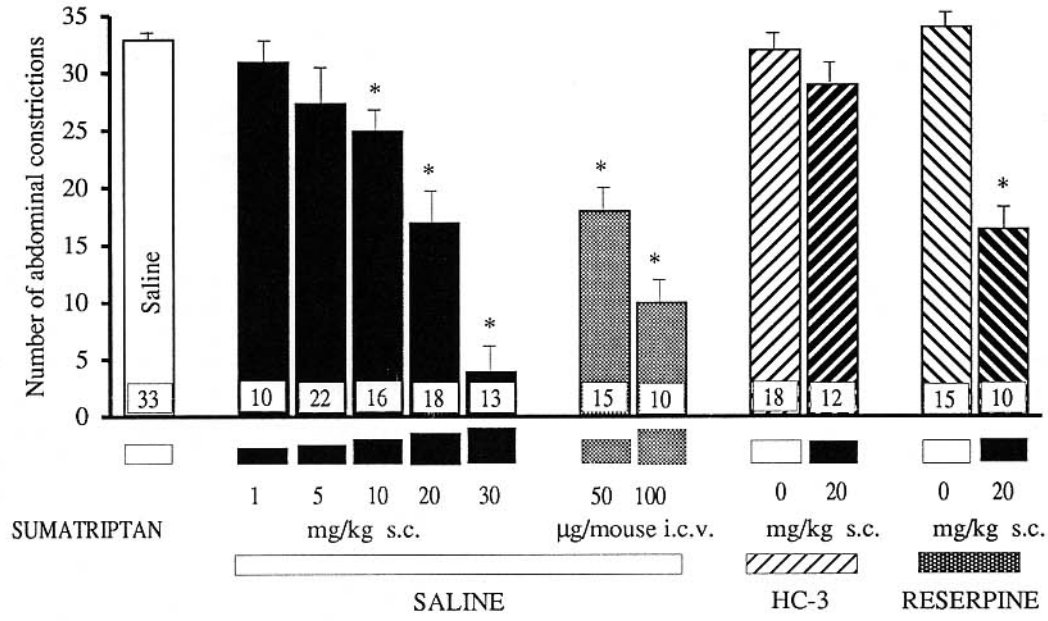

Fig. 1 Dose-response curves of sumatriptan i.p. and i.c.v. injected and effect of both hemicholinium-3 (HC-3) (1 µg per mouse i.c.v.) and reserpine (2 mg kg^{-1} i.p.) pretreatments on antinociception induced by sumatriptan in the mouse abdominal constriction test. HC-3 and reserpine were injected respectively 5 h and twice 48 and 24 h before test. The nociceptive responses were recorded 25–35 min and 15–25 min respectively after s.c. and i.c.v. sumatriptan administration. Vertical lines show S.E. of mean; * $P < 0.01$ in comparison with saline controls. Numbers inside the columns indicate the number of mice.

HC-3. The effectiveness of the central cholinergic system is, therefore, fundamental for sumatriptan antinociception. Other neurotransmitter systems are not involved in sumatriptan antinociception since the opioid antagonist naloxone, the $GABA_B$ antagonist CGP-35348 and the polyamine depletor reserpine are all unable to prevent the effect of sumatriptan. The doses and administration schedules of the above-mentioned drugs are suitable for preventing antinociception induced respectively by morphine (10), $GABA_B$ agonists (11) and by the antidepressant drug clomipramine (12). Skingle *et al.* (13) have reported that sumatriptan has little or no antinociceptive activity against a range of noxious stimuli in rodents. Since the doses of sumatriptan injected by these authors were in the same range as ours, it is probable that the striking discrepancy between our results and their depends on the excessive delay with which these authors detected the pain threshold. Since Skingle *et al.* (13) compared sumatriptan with morphine, we suppose that they have evaluated both drugs 30 min after administration, at wich time morphine reaches its maximum analgesic activity while the antinociceptive effect of sumatriptan almost disappears.

In summary, our results show that sumatriptan is able to potentiate endogenous cholinergic activity. It remains to be clarified whether the sumatriptan antinociception observed in rodents contributes to the antimigraine activity elicited by the drug in humans.

Table I *Dose-response curve of sumatriptan and effects of atropine, HC-3, naloxone, CGP-35348 and NAN-190 on sumatriptan antinociception in the mouse hot-plate test*

Pretreatment	Treatment	mg·kg^{-1} i.p.	Licking latency (s) Before pretreatment	After treatment 15min	30min	45min
Saline	Saline		13.7± 0.6	14.2± 0.8	14.5± 0.9	13.9± 0.9
	Sumatriptan	5	14.5± 1.1	15.7± 1.5	16.3± 1.4	14.3± 1.2
	Sumatriptan	10	13.6± 0.9	19.7± 1.7^	17.2± 1.3	14.8± 1.5
	Sumatriptan	20	14.1± 0.6	25.7± 2.1*	22.0± 1.5*	17.6± 1.4
	Sumatriptan	30	13.1± 1.0	25.1± 1.5*	24.4± 1.2*	19.5± 1.3
Atropine	Saline		14.8± 0.6	13.9± 0.9	14.1± 0.5	14.8± 0.8
5mg·kg^{-1}	Sumatriptan	20	13.9± 1.2	15.7± 1.9°	15.8± 2.2°	16.1± 1.6
HC-3	Saline		13.7± 1.8	13.5± 0.8	16.2± 0.6	15.3± 0.7
1µg/mouse i.c.v.	Sumatriptan	20	13.8± 1.1	14.0± 1.9°	13.6± 1.9°	13.0± 1.3
Naloxone	Saline		13.8± 0.4	14.4± 0.7	13.4± 0.9	15.0± 0.7
1mg·kg^{-1} i.p.	Sumatriptan	20	13.6± 0.5		25.1± 2.9*	
24.0± 2.2*	17.3± 1.7					
CGP 35348	Saline		13.6± 0.7	11.6± 0.9^	12.7± 1.1	12.3± 1.0
100mg·kg^{-1} i.p.	Sumatriptan	20	13.7± 0.5	24.5± 1.5*	18.7± 1.5	16.6± 1.3
NAN-190	Saline		15.6± 1.4	15.1± 2.1	16.3± 2.1	13.7± 1.8
0.5mg·kg^{-1} i.p.	Sumatriptan	20	14.4± 0.9	16.7± 1.6°	14.9± 1.7°	13.6± 1.8

Each value represent the mean of at least 8 mice.
Atropine, naloxone and NAN-190 were injected 15min before sumatriptan; HC-3 and CGP-35348 respectively 5h and 5min before sumatriptan.
* $p < 0.01$; ^ $p < 0.05$ in comparison with saline-saline; ° $p < 0.01$ versus saline-sumatriptan.

References

(1) Ghelardini C. et al. *The 5-HT$_{1A}$ agonist 8-OH-DPAT induces antinociception through a cholinergic mechanism.* Can. J. Physiol. Pharmacol., **72** (Suppl. 1), P13.9.43, 1994.

(2) Ghelardini C., Galeotti N., Fantetti L., Bartolini A. *Buspirone and gepirone: two anxiolytic drugs endowed with antinociception mediated by the cholinergic system.* Behav. Pharmacol., **6** (Suppl. 1), 42–43, 1995.

(3) Bianchi C., Siniscalchi A., Beani L. *5-HT$_{1A}$ agonists increase and 5-HT$_3$ agonists decrease acetylcholine efflux from the cerbral cortex of freely-moving guinea-pigs.* Br. J. Pharmacol., **101**, 448–452, 1990.

(4) McCarthy B.G., Peroutka S.D. *Comparative neuropharmacology of dihydroergotamine and sumatriptan (GR 43175).* Headache, 420–422, 1989.

(5) Dechant K.L., Clissold S.P. *Sumatriptan – a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the acute treatment of migraine and cluster headache.* Drugs, **43**, 776–798, 1992.

(6) O'Callaghan J.P., Holtzman S.G. *Prenatal administration of morphine to the rat: tolerance to the analgesic effect of morphine in the offspring.* J. Pharmacol. Exp. Ther., **197**, 533–544, 1976.

(7) Koster R., Anderson M., de Beer E.J. *Acetic acid for analgesic screening.* Fed. Proc., **18**, 412, 1959.

(8) Vaught J.L., Pelley K., Costa L.G., Setler P., Enna S.J. *A comparison of the antinociceptive responses to GABA-receptor agonists THIP and baclofen.* Neuropharmacology, **24**, 211–216, 1985.

(9) Haley T.J., McCormich W.G. *Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse.* Br. J. Pharmacol. Chemother., **12**, 12–15, 1957.

(10) Bartolini A. et al. *Antinociception induced by systemic administration of local anaesthetics depends on a central cholinergic mechanism.* Br. J. Pharmacol., **92**, 711–721, 1987.

(11) Malcangio M., Ghelardini C., Giotti A., Malmberg-Aiello P.,

Bartolini A. *CGP-35348, a new $GABA_B$ antagonist prevents antinociception and muscle-relaxant effect induced by baclofen.* Br. J. Pharmacol., **103**, 1303–1308, 1991.

(12) Galeotti N., Ghelardini C., Bartolini A. *Involvement of the serotoninergic system in the analgesic effect of tricyclic antidepressants.* Behav. Pharmacol., **6** (Suppl. 1), 20, 1995.

(13) Skingle M., Birch P.J., Leighton G.E., Humphrey P.P.A. *Lack of antinociceptive activity of sumatriptan in rodents.* Cephalalgia, **10**, 207–212, 1990.