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EFFECT OF ISOTEOLINE IN ANIMAL MODELS PREDICTIVE OF ANTIDEPRESSANT ACTIVITY

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ABSTRACT

Isoteoline, a compound of aporphine structure, was studied for antidepressant activity. Two animal models were used: the behavioral despair test and the clonidine-induced hypothermia. Isoteoline failed to alter the immobility time in rats subjected to forced swimming, but antagonized the immobility-reducing effect of desipramine, both after a single and multiple administration. In rats made hypothermic by i.p. injection of clonidine, Isoteoline did not antagonize the effect on rectal temperature, but rather accentuated it. The conclusion was made that Isoteoline was devoid of antidepressant activity. The results were analyzed in terms of previously demonstrated interactions of Isoteoline with subtypes of serotonergic receptors and were found to provide further evidence in support of these interactions.

Key words: isoteoline. behavioral despair test, clonidine-induced hypothermia, antidepressant activity, rats

INTRODUCTION

Isoteoline (IST) is a compound of aporphine structure derived from the alkaloid glaucine. It has been shown to possess cardiovascular and central nervous system effects, the most prominent of which being the blood pressure lowering (3) and the anxiolytic (2,19) ones. The vascular effects are believed to be of adrenergic and/or dopaminergic nature. The anxiolytic effect of IST demonstrated on rats and mice is probably mediated by 5-HT2 receptor antagonism, possibly of the 5-HT2C subtype. IST has been shown to behave as a 5-HT2C antagonist in a number of experimental models (2,18,20). It has been also found to act in a 5-HT1B agonistic manner in a specific test (unpublished data).

The 5-HT neurotransmission is well recognized to play a role in the mechanism of action of different groups of antidepressants. With many of them, along with the leading mechanism of reuptake inhibition of 5-HT and/or noradrenalin, a direct interaction with 5-HT receptors has been found (9,14). It is not known if this is a major mechanism, or it merely contributes to the additional characteristics of the drugs.

Taking into consideration the interaction of IST with the serotonergic neurotransmission, the present study was undertaken to test IST for antidepressant activity. For this purpose we utilized two experimental models, used routinely for screening of antidepressant drugs: the behavioral despair test of Porsolt (13) and the clonidine-induced hypothermia test (6).

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MATERIAL AND METHODS

Animals. Male Wistar rats of 160-185g mean body weight were used. The animals were kept under the standard conditions of the animal house, with 12:12 h light-dark cycle and lights on at 7:00 a.m. The animals had free access to food and water.

Drugs. Isoteoline hydrobromide was synthesized at the Department of Pharmacology of the Higher Medical Institute in Varna (the identity of IST was checked by means of HPLC analysis by comparing the compound with an auhentic sample and its purity was 98% (1). Desipramine (DMI) was used in the form of the commercial preparation Petylyl (Germed). Clonidine hydrochloride was purchased from Sigma.

Behavioral despair test (forced swimming test).
The animals were placed into a glass cylinder filled with water (of temperature 25°C) up to 18cm in height. After the first burst of active movements in seeking a way out, the animals stop fighting and acquire a passive posture, keeping their nose above the water only by slight supportive movements. This immobility is regarded as an index of behavioral despair, that is, as a reaction of the animals to an inescapable stress situation. The time in which the rats remain in this position is the quantifiable criterion of this 'desperate' condition.

The immobility time was measured during 5min sessions. IST was used intraperitoneally (i.p.) at doses of 0.25, 1 and 4mg/kg. The treatment of IST was given either acutely 60

min prior to test, or repeatedly. In the latter case, IST was injected twice daily over three consecutive days and the test was performed 16h after the last injection. In addition to being given on its own, IST was also administered in combination with DMI, which was used as a reference drug. DMI (10mg/kg) was injected i.p. 60min before the test or 60min after the administration of IST and was also given as a single or multiple (of the same duration) treatment.

Clonidine-induced hypothermia.

The rectal temperature of the rats was measured by means of a transistor thermoresistant thermometer. made in the laboratory for medicinal instruments in the Higher Medical Institute of Plovdiv, Bulgaria. The lubricated thermoelectrode was inserted into the rectum at a depth of 3cm. The measurements were taken at an ambient temperature of an average of 20°C.

The initial rectal temperature was read twice before the application of the drugs at an interval of 60min. The temperatures were measured at the 60th, 90th, 120th, 150th, 210th, 240th min after the injection of the pharmacological agent. IST (0.25, 1 and 4mg/kg) was given i.p. on its own, or 30min before clonidine (0.2mg/kg, i.p.). For a better comparability, the results are represented as absolute differences between the mean initial temperature and the temperature taken after each of the treatments.

Statistics. The results are represented as a mean ±SEM. The statistical assessment of the data was performed by means of one way ANOVA, followed by Dunnett's multiple comparison test. Two separate groups were compared by means of Student's *t*-test. GraphPad Prism software packet was used.

RESULTS AND DISCUSSION

Effect of IST on the behavioral despair test

With both regimens of application - acute or multiple, IST alone had no effect on the immobility time of rats subjected to forced swimming (Fig. 1).

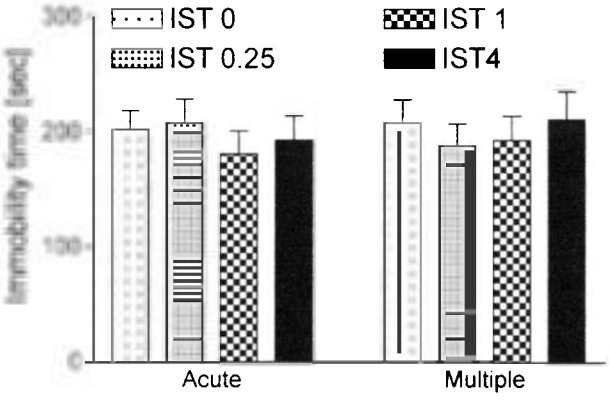

Fig. 1. Effect of IST on immobility time of rats in the test of behavioral despair

DMI given i.p at 10mg/kg 60min before the start of the test, lead to a statistically significant decrease of the immobility time: 156 ±9.8sec (n=10) vs. control values of 202 ±16.8 sec (n =12) (p =0.0364).

Multiple (2x3 days) administration of DMI resulted in 138 ±15 sec (n =6) vs. 208 ±20 sec (n =6) for the control animals (p =0.0188) (Fig. 2).

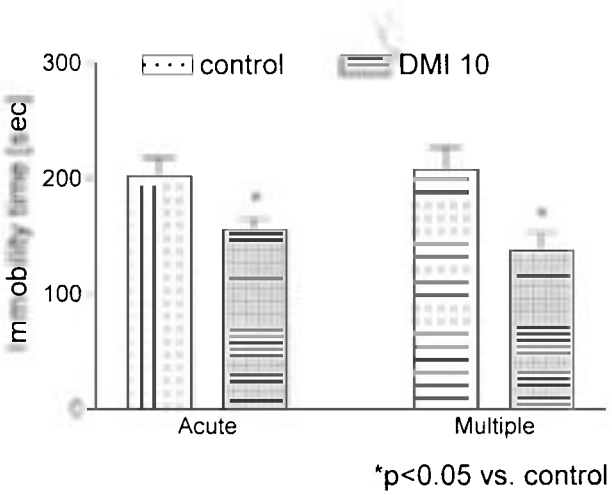

Fig. 2. Effect of DMI on immobility time of rats in the test of behavioral despair

The pretreatment with IST reduced the effect of DMI both with acute and multiple treatment. The single administration of IST resulted in immobility time of 214 ±10.3 sec (n=11) at 0.25mg/kg, 200 ±16.5 sec (n=11) at 1mg/kg, and 190 ±18 sec (n=11) at 4mg/kg (one way ANOVA, p=0.0486; Dunnett's multiple comparison test, p<0.05 for IST 0.25mg/kg). With multiple administration of IST the results were: 128 ±17 sec (n=6) at 0.25mg/kg, 160 ±18sec (n=4) at 1mg/kg, and 224 ±22 sec (n=4) at 4mg/kg (one way ANOVA, p=0.0096; Dunnett's multiple comparison test, p<0.05 for IST 4mg/kg) (Fig. 3).

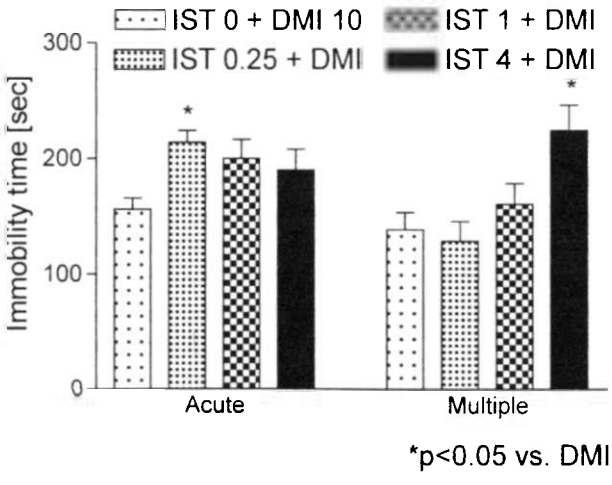

Fig. 3. Effect of IST on the anti-immobility effect of DMI

Effect of IST on clonidine-induced hypothermia

Clonidine (0.2mg/kg) caused a marked decrease in the rats' rectal temperature with a trough of about -2°C at the 120thmin. The 30min pre-treatment with IST accentuated the effect of clonidine in an inverted dose-dependent manner, the smallest dose (0.25mg/kg) being the most effective. (Fig. 4A).

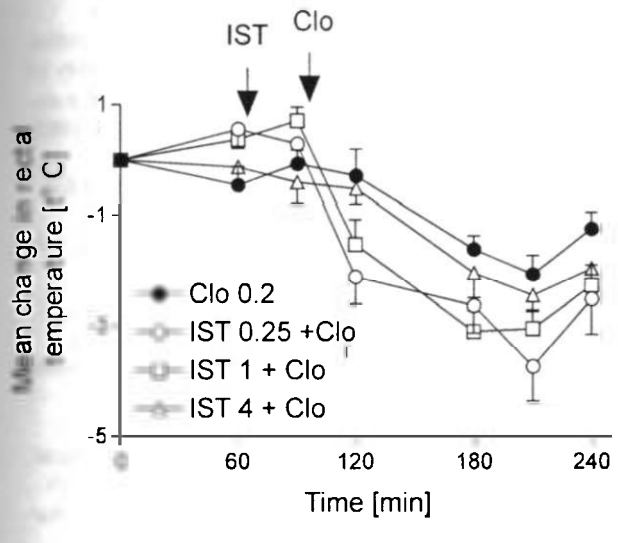

Fig. 4A. Effect of IST on clonidine-induced hypothermia. Time-effect of IST throughout the

At the 210th min clonidine induced a temperature drop of -2.07 ±0.34°C (n=7), and the pretreatmet with IST deepened this effect to -3.73 ±0.41°C with 0.25mg/kg (n=6), to -3.05 ±0.31°C with 1mg/kg (n=6), and to -2.45 ±0.26°C with 4mg/kg (one way ANOVA, p=0.0112; Dunnett's multiple comparison test, p<0.01 for IST 0.25mg/kg) (Fig. 4B).

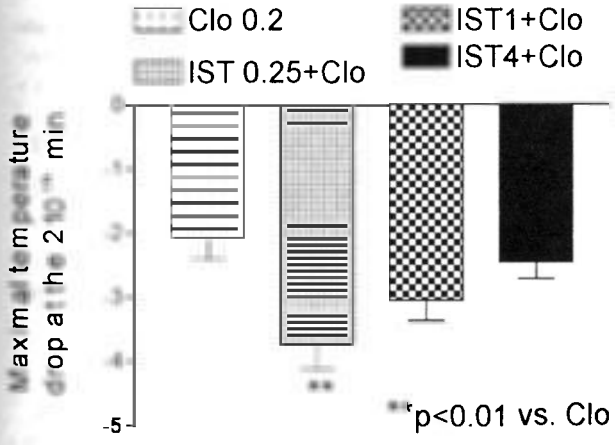

Fig. 4B. Effect of IST on clonidine-induced hypothermia at the 120th min (the time of maximal effect of clonidine)

Interaction with serotonergic and other receptors has been described with different antidepressants. One mechanism of this interaction is 5-HT2 receptor antagonism found with representatives of the tricyclic, selective serotonin reuptake inhibitors and atypical group (14). Some azapirones are agonists at 5-HT1A receptors and display antidepressant activity, at least in experimental conditions, in addition to their clinically useful anxiolytic activity (9).

Our interest to study IST for potential antidepressant activity was dictated by the findings of antagonistic behavior of IST at 5-HT2C receptors and the data of 5-HT1B some agonist-like effects.

The model described by Porsolt is one of the commonly used tests to study antidepressant drugs (13). Many of the clinically used antidepressants give positive results in this test. They typically reduce the immobility time, during which the animals, forced to swim, cease to actively search for escape and acquire an immobile position. IST was tested on rats treated acutely and sub-acutely (for three days). With both treatments IST was devoid of any effect on immobility time. As opposed to IST, DMI used as a positive control decreased the immobility time after a single and multiple administration, through increasing the time spent in fighting. When IST pre-treatment was given to DMI-treated animals, the anti-immobility effect of DMI was reduced. The effect was of similar magnitude with the three doses used, but only with the smallest one (0.25mg/kg) were the results statistically significant. After multiple treatment the effect of IST was dose-dependent with significant antagonism reached at 4mg/kg. The different dose-effect relationship of IST according to the duration of treatment may reflect some tolerance developing during the course of longer administration.

Different explanations of the present effect of IST are possible:

According to Espejo and Minano (7), the depression-like symptoms in the forced swimming of rats are due to the enhanced dopaminergic brain activity. Suhara *et al.* (15) have found that DMI inhibits the effects of dopamine probably by reducing its receptor binding. This would mean that activating dopaminergic activity would antagonize the effect of DMI. IST could activate dopaminergic activity in two possible ways:

1. There is evidence suggesting a tonic inhibition of dopaminergic neurotransmission by 5-HT2C stimulation (10,11). Correspondingly, the 5-HT2C antagonist SB-242084 has been found to increase the dopamine level in a dialysate from frontal cortex (10). Being a putative 5-HT2C receptor antagonist, IST could have similar effects and this might account for its effects found in the present study.
2. A direct dopaminomimetic action of IST itself cannot be ruled out (17).

Cervo *et al.* (4) reported inhibition of the DMI anti-immobility effect by serotonin agonists and found this effect to be dependent on 5-HT1B receptor stimulation. By analogy, we could assume the same mechanism for IST, based on the ability of IST to behave as a 5-HT1B agonist in a specific experimental set (personal unpublished data).

Theoretically, yet another option exists to explain the effect of IST. Kitada *et al.* (8) have demonstrated that the anti-immobility effect of DMI can be prevented by al-

pha-adrenergic blockers. Data exist that boldine (an isomer of IST) and other closely related aporphine derivatives display alpha-adrenolytic activity (16). IST could behave similarly, as evidenced by its blood pressure reducing properties. However, IST was found to be ineffective in inducing ptosis in rats as an index of alpha-adrenolytic function, which makes the possibility of IST acting as an alpha-blocker to prevent the effect of DMI unlikely.

Altogether, various factors should be taken into account when looking for interpretation of the anti-DMI effect of IST in the rat behavioral despair test.

Most probably a complex mechanism involving interaction with different receptors for serotonin and/or dopamine underlies the modifying effect of IST in respect to the DMI action.

Clonidine hypothermia represents another model for antidepressant screening (6). Antagonism to this effect of clonidine correlates with antidepressant activity. IST was ineffective in reversing the hypothermia induced by clonidine in agreement with the negative results from the forced swimming test. Moreover, an enhancement of clonidine hypothermia was found. This finding was intriguing and stimulated our search for an explanation. Data from the literature show an existence of serotonergic component in the hypothermia caused by clonidine (12).

The authors have found that this hypothermic effect was increased at the background of serotonin depletion by chlorophenylalanine or with destruction of the median raphe nucleus; it was reduced by indirect (fenfluramine) or direct (chlorophenylpiperazine) 5-HT agonists. The present effect of IST is similar to that of the factors reducing the serotonergic activity. Having in mind the 5-HT2C antagonist action of IST and the fact that most effects of chlorophenylpiperazine are mediated through the same receptors (5), the augmentation of clonidine hypothermia by IST may serve another indirect piece of evidence favoring antagonism at the 5-HT2C receptors. A puzzling moment in the present results is the inverse dose-effect relationship of IST. The smallest dose was the most active. It is possible that the selectivity of the effect is lost with higher doses due to interference of other mechanisms.

CONCLUSION

The results from the present study demonstrate that IST is devoid of antidepressant activity in the tests used. At the same time they provide further, albeit indirect, evidence pointing at interaction of IST with central serotonergic receptors of 5-HT2C and/or, probably, 5-HT1B subtype.

REFERENCES

1. Желязков, Д., Н. Моллов, М. Марков, Х. Филипов и др. Средство за лечение на хипертонична болест и метод за неговата синтеза. Авт. свид. №43200/13.IV.1984г. МПК А61 31/485. Държ. рег. №55261/1982.
2. Желязкова-Савова, М. Влияние изотеолина на поведение мышей в условиях теста для выявляния анксиолитической активности. - *Эксперим. клин. фармакол.*, **61**, 1998. № 3, 19-21
3. Марков, М., Д. Желязков, Н. Георгиев. Антихипертензивен ефект на изотеолина при моделни хипертонии. - *Експерим. мед. морфол.*, 1984, № 3, 134-138.
4. Cervo, L., G. Grignaschi, R. Samanin. 8-Hydroxy-2-(di-n-propylamino) tetralin, a selective 5-HT1A receptor agonist, reduces the immiobility of rats in the forced swimming test by acting on the nucleus raphe dorsalis.- *Eur. J. Pharmacol.*, **158**, 1988, 53-59.
5. Curzon, G., G. A. Kennet. m-CPP: a tool for studying behavioural responses associated with 5-HT1C receptors.- *Trends Pharmacol. Sci.*, **11**, 1990, 181-182.
6. Danysz, W., A. Plaznik, W. Kostowski, E. Malatynska *et al.* Comparison of desipramine, amitriptyline, zimelidine and alaproctate in six animal models used to investigate antidepressant drugs.- *Pharmacol. Toxicol.*, **62**, 1988, 42-50.
7. Espejo, E. F., F. J. Minano. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test.- *Neuroscience*, **88**, 1999, 609-615.
8. Kitada, Y., T. Miyauchi, Y. Kanazawa, H. Nakamichi *et al.* Involvement of alpha- and beta 1-adrenergic mechanisms in the immobility-reducing action of desipramine in the forced swimming test.- *Neuropharmacology*, **22**, 1983, 1055-1060.
9. Leonard, B. E. Mechanisms of action of antidepressants.- *CNS Drugs*, **4**, 1995, Suppl. 1, 1-12.
10. Millan, M. J., A. Dekeyne, A. Gobert. Serotonin (5-HT)2C receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not serotonin, release in the frontal cortex in vivo.- *Neuropharmacology*, **37**, 1998, 953-955.
11. Pehek, E. A. Local infusion of of the serotonin antagonists ritanserin or ICS 205,930 increases in vivo dopamine release in the rat medial prefrontal cortex.- *Synapse*, **24**, 1996, 12-18.
12. Plaznik, A., W. Kostowski, W. Danysz. The involvement of brain serotonin in the clonidine- induced hypothermia in rats.- *Pol. J. Pharmacol. Pharm.*, **35**, 1983, 301-308.
13. Porsolt, R. D., G. Anton, N. Blavet, M. Jalfre. Behavioral despair in rats: a new model sensitive to antidepressant treatments.- *Eur. J. Pharmacol.*, **47**, 1978, 379-391.
14. Sanchez, C., J. Hyttel. Comparison of the effects of antidepressants and their metabolites on the reuptake of biogenic amines and on receptor binding.- *Cell. Mol. Neurobiol.*, **19**, 1999, 467-489.
15. Suhara, T., O. Inoue, K. Kobayasi. Effect of desipramine on dopamine receptor binding in vivo.- *Life Sci.*, **47**, 1990, 2119-2126.
16. Valiente, M., S. Martinez, M. D. Ivorra, B. K. Cassels *et al.* Functional study on rat aorta and tail artery of a series of aporphine derivatives showing

selective affinity for alpha-1A adrenoceptors.- *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **351**, 1998, Suppl. 2, R605.

17. Zhelyazkov, D., M. Markov, A. Maleeva, N. Visheva *et al.* Neuro-endocrine effects of isoteoline.- *Acta Physiol. Pharmacol. Bulg.*, **10**, 1984, No 4, 53-58.
18. Zhelyazkova-Savova, M. Isoteoline in two models of serotonin 5-HT2C receptor stimulation.- *Annu. Proc. IMAB*, **4**, 1998, No 2, 297-299.
19. Zhelyazkova-Savova, M. Effects of isoteoline in two animal models of anxiety in the rat.- *Annu. Proc. IMAB*, 5, 1999, No 2, 240-242.
20. Zhelyazkova-Savova, M., N. Negrev. Isoteoline, a putative serotonin antagonist, inhibits m-CPP, but not 8-OH-DPAT and DOI-induced increase in serum prolactin levels.- *Pharmacol. Res.*, **42**, 2000, 93-96.