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Behavioural evidence of agonist-like effect of isoteoline at 5-HT_{1B} serotonergic receptors in mice

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Abstract

Isoteoline is a compound of aporphine structure derived from the alkaloid glaucine. Previous studies with isoteoline have shown antagonistic activity at 5-HT_{2C} serotonergic receptors. We have investigated whether isoteoline interacts with 5-HT_{1B} receptors. An isolation-induced social behavioural deficit test in mice was used as a model of stimulation of these receptors. The deficit in the behaviour of isolated mice in this experimental procedure was reported to be sensitive to 5-HT_{1B}-receptor stimulation, since agonists at these receptors are capable of reversing it. In our study, we used N-(3-trifluoromethylphenyl)piperazine (TFMPP) (2 mg kg^{-1}) as a reference agonist at these receptor sites. TFMPP completely restored the normal behaviour of the isolated mice. Its effect was prevented by propranolol (4 mg kg^{-1}), a β-adrenergic receptor antagonist with a high affinity for 5-HT_{1B} receptors, which was inactive by itself. When isoteoline was given before TFMPP, it did not prevent the effect of the latter. Given alone at doses of 0.25, 1, 4 or 8 mg kg^{-1}, isoteoline showed an effect of its own to normalize the behaviour of isolated mice. The effect of isoteoline (1 mg kg^{-1}, i.p.) was antagonized by pretreatment with propranolol, indicating that it was mediated through stimulation of 5-HT_{1B} receptors. Repeated treatment with isoteoline (1 mg kg^{-1}, 2×3 days, i.p.) produced tolerance to its effect and significantly attenuated the effect of TFMPP, when animals were tested 16 h after the last injection. In conclusion, the results provided functional evidence of agonist-like activity of isoteoline at the 5-HT_{1B} receptors.

Introduction

Isoteoline is an aporphine molecule derived from the alkaloid glaucine. Its pharmacological profile is characterized by anti-hypertensive (Markov et al 1984; Zhelyazkov et al 1984) and anxiolytic (Zhelyazkova-Savova 1998a; Zhelyazkova-Savova 1999) effects believed to be mediated through adrenergic/dopaminergic and anti-serotonergic mechanisms, respectively.

Isoteoline has been studied previously for interaction with serotonergic neurotransmission in experimental models, involving different receptor subtypes. The results have demonstrated that isoteoline acts as an antagonist at 5-HT_{2C} receptors (Zhelyazkova-Savova et al 1997, 1999; Zhelyazkova-Savova 1998b, 2000; Zhelyazkova-Savova & Negrev 2000). In tests of functional stimulation of 5-HT_{2A} (Zhelyazkova-Savova & Negrev 2000) and 5-HT_{1A} (Zhelyazkova-Savova 2000; Zhelyazkova-Savova & Negrev 2000) receptors isoteoline was devoid of any effects.

In an attempt to elucidate further the relationship of isoteoline to central serotonergic functions, mediated by different receptors, we investigated whether isoteoline interacted with 5-HT_{1B} receptors. We used a behavioural model of stimulation of these receptors, developed by Frances (1988a) and Frances et al (1990b), which is a test suitable for detecting 5-HT_{1B} ligands. Those authors found that through 5-HT_{1B} receptors serotonin was involved in a behavioural modification induced by isolation, and proposed this paradigm as a model useful for screening 5-HT_{1B} agonists. We tested isoteoline in this model for antagonistic and agonistic effects and compared it with N-(3-trifluoromethylphenyl)piperazine (TFMPP) as a reference agonist for 5-HT_{1B} receptors.

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Materials and Methods

Animals

Male white mice (30–40 g) were used in the experiments. The animals were kept under the standard conditions of the animal house with 12-h light–dark cycle (lights on at 07 00 h). Isolated mice were housed one per cage for seven days and the grouped mice ten per cage. 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 isoteoline was checked by means of HPLC analysis by comparing the compound with an authentic sample and its purity was 98% (Zhelyazkov et al 1984). N-(3-trifluoromethylphenyl)piperazine hydrochloride (TFMPP) was kindly provided by Research Biochemicals International (USA) as part of the Chemical Synthesis Program of the National Institute of Mental Health, Contract 278-90-0007. Propranolol hydrochloride was purchased from Imperial Chemical Industries PLC.

Experimental procedure

Mice were tested in pairs (one isolated and one grouped-housed) by introducing them under a glass beaker (height 14 cm, diam. 10 cm), inverted over a rough glass surface. The test duration was 2 min. During this period, the number of escape attempts by the mice were counted by a person unaware of the treatment. In the first experiment the number of escape attempts of both control mice (isolated and grouped) were counted, and in the subsequent experiments only isolated mice were treated, observed and processed. An escape attempt was defined as any of the following: the two forepaws being leant against the wall of the beaker; the mouse sniffing, with its nose in the spout of the beaker; or the mouse scratching the glass floor. No minimal duration for one attempt was limited; for attempts lasting longer than 3 s, a new attempt was counted (Frances et al 1990b). All mice were tested once only. Drugs were administered intraperitoneally (i.p.) and treatment was given to the isolated mice only. Agonists were injected 30 min before the test and the antagonists 30 min before the agonists. Isoteoline was administered at doses of 0.25, 1, 4 and 8 mg kg^{-1} body weight, which were behaviourally active in other experiments. TFMPP was used at a dose of 2 mg kg^{-1} and propranolol at a dose of 4 mg kg^{-1}. These doses were selected from literature sources (Frances 1988b; Frances et al 1990b). All drugs were dissolved in distilled water and injected in a volume of 10 mL kg^{-1} body weight. The control isolated mice received distilled water in the same volume. The experimental groups consisted of 5–15 mice.

Subchronic treatment

Isolated mice were treated repeatedly during three consecutive days (the last three of the seven-day period of isolation), twice daily, at 0830 and 1730 h each day, with isoteoline at a dose of 1 mg kg^{-1} (i.p.). Control mice received distilled water. The animals were tested 16 h after the last injection, by giving the test treatment 30 min before performing the test.

All procedures concerning animal treatment and experimentation were in accordance with the Guiding Principles in the Care and Use of Animals, approved by the Council of the American Physiological Society, with European Communities Council Directives 86/609/EEC and with the National regulations, adopted by the local Ethical Commission in the Varna Medical University.

Statistical analysis

The results were assessed by means of one-way analysis of variance, followed by Dunnett's multiple comparison post test and, where appropriate, by post test for linear trend. Two independent groups were compared by Student's *t*-test. GraphPad Prism statistical software was used. Results are presented as mean $\pm$ s.e.m.

Results

When control isolated mice were tested together with grouped mice, their mean number of escape attempts was roughly twice less than that of non-isolated mice (Student's *t*-test, $P = 0.0005$). TFMPP (2 mg kg^{-1}) increased the number of escape attempts to approximately the value of the non-isolated mice (Student's *t*-test, $P = 0.0003$ vs control) (Table 1).

When isoteoline was given 30 min before TFMPP, it was incapable of reversing its effect (one-way analysis of variance, $P = 0.7704$) (Table 2).

The effect of isoteoline alone was similar to, although less marked than, that of TFMPP. It resulted in the increase of the number of escape attempts as compared with the control group. The effect was dose-dependent and statistically significant (one-way analysis of variance, $P = 0.0201$; Dunnett's post test, $P < 0.05$ for isoteoline 1 mg kg^{-1} and $P < 0.01$ for isoteoline 8 mg kg^{-1}; post test for linear trend, $P = 0.0036$) (Table 3).

Propranolol (4 mg kg^{-1}) was inactive by itself. It antagonized the effect of TFMPP, decreasing significantly the number of attempts (Student's *t*-test, $P = 0.0016$ vs TFMPP alone). Propranolol prevented the effect of isoteoline 1 mg kg^{-1} as well (Student's *t*-test, $P < 0.0001$ vs isoteoline alone) (Table 4).

After three days of treatment, on the fourth day, mice receiving isoteoline (1 mg kg^{-1}) as a test treatment after vehicle pretreatment (water+isoteoline 1), had significantly more escape attempts than control animals (water+water) (Student's *t*-test, $P = 0.0015$). Mice receiving TFMPP (2 mg kg^{-1}) as a test treatment after vehicle pretreatment (water+TFMPP 2), gave a similar result (Student's *t*-test, $P = 0.0002$ vs control). Mice treated with isoteoline (1 mg kg^{-1}) for three consecutive days and re-

Table 1 Isolation-induced social behavioural deficit in mice. Effect of isolation on the behaviour of mice and restoration of normal behaviour of isolated mice by TFMPP (2 mg kg^{-1}, i.p.).

Groups	Control grouped		Control isolated		TFMPP-treated isolated	
Number of escape attempts	14.9±1.3	n = 10	8.0±1.3***	n = 13	15.4±1.4$^{\#\#\#}$	n = 10

Values are mean±s.e.m. ***$P < 0.001$ vs control grouped mice, $^{\#\#\#}P < 0.0001$ vs control isolated mice. n, number of mice per group.

Table 2 Effect of isoteoline on the effect of TFMPP in the isolation-induced social behavioural deficit in mice.

Pretreatment (mg kg^{-1}, i.p.)	Treatment (mg kg^{-1}, i.p.)	Number of escape attempts	n
Isoteoline 0	TFMPP 2	15.4±1.4	10
Isoteoline 0.25	TFMPP 2	15.1±2.0	7
Isoteoline 1	TFMPP 2	14.6±1.6	10
Isoteoline 4	TFMPP 2	12.9±0.8	13
Isoteoline 8	TFMPP 2	14.6±2.3	10

Values are mean±s.e.m. n, number of mice per group.

Table 3 Effect of isoteoline on the isolation-induced social behavioural deficit in mice.

Treatment (mg kg^{-1}, i.p.)	Number of escape attempts	n
Isoteoline 0	8.0±1.3	13
Isoteoline 0.25	11.2±1.5	6
Isoteoline 1	12.1±1.4*	14
Isoteoline 4	11.1±1.3	15
Isoteoline 8	13.4±0.9**	10

Values are mean±s.e.m. *$P < 0.05$ and **$P < 0.01$ vs control. n, number of mice per group.

Table 4 Effect of propranolol alone and on the effect of TFMPP and isoteoline on the isolation-induced social behavioural deficit in mice.

Pretreatment (mg kg^{-1}, i.p.)	Treatment (mg kg^{-1}, i.p.)	Number of escape attempts	n
Propranolol 0	Water	8.0±1.3	13
Propranolol 4	Water	7.9±1.3	10
Propranolol 0	TFMPP 2	15.4±1.4***	10
Propranolol 4	TFMPP 2	8.14±1.7$^{\#\#}$	7
Propranolol 0	isoteoline 1	12.14±1.4*	14
Propranolol 4	isoteoline 1	3.6±0.93^{+++}	5

Values are mean±s.e.m. *$P < 0.05$ and ***$P < 0.0001$ vs control. $^{\#\#}P < 0.01$ vs TFMPP alone. $^{+++}P < 0.0001$ vs isoteoline alone. n, number of mice per group.

Table 5 Effect of repeated treatment with isoteoline (2×3 days) on the effect of a test dose of isoteoline and TFMPP, given 16 h after the last injection.

Repeated treatment (mg kg^{-1}, i.p.)	Test treatment (mg kg^{-1}, i.p.)	Number of escape attempts	n
Water	Water	6.5±1.1	6
Water	Isoteoline 1	13.5±1.2**	6
Water	TFMPP 2	16.3±1.3***	6
Isoteoline 1	Isoteoline 1	7.2±1.7°	6
Isoteoline 1	TFMPP 2	12.2±1.1$^{\#}$	6

Values are mean±s.e.m. **$P < 0.01$ and ***$P < 0.001$ vs water+water. °$P < 0.05$ vs water+isoteoline 1. $^{\#}P < 0.05$ vs water+TFMPP. n, number of mice per group.

ceiving the same test treatment (isoteoline 1+isoteoline 1) produced escape attempts not significantly different from control animals (water+water), but significantly less than the mice treated with water+isoteoline 1 (Student's *t*-test, $P = 0.0115$). Mice treated with isoteoline (1 mg kg^{-1}) for three consecutive days and receiving TFMPP (2 mg kg^{-1}) as a test treatment (isoteoline 1+TFMPP 2) made significantly less escape attempts than those treated with water+TFMPP 2 (Student's *t*-test, $P = 0.0354$) (Table 5).

Discussion

Based on previous studies showing isoteoline to behave heterogeneously towards different 5-HT-receptor subtypes such as 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{2C}$, it was the aim of this study to determine the relationship of isoteoline to 5-HT$_{1B}$ receptors. The isolation-induced social behavioural deficit test in mice was a suitable and simple tool to use for this purpose. In this model, mice subjected to a brief (seven day) period of isolation display a behavioural modification, evident when pairs of animals (one isolated and one grouped-housed) are observed under an inverted beaker. Under these circumstances the number of "escape attempts" made by isolated mice is nearly half those of grouped animals. This phenomenon has been termed "isolation-induced social behavioural deficit". Analysis of this syndrome has shown that antidepressants (tricyclic,

selective serotonin reuptake inhibitors, atypical) (Frances et al 1989; Frances & Khidichian 1990) or tranquilizers (Frances & Lienard 1989) are ineffective in antagonizing the behavioural deficit. Agonists at 5-HT_{1B} serotonergic receptors turned out to be the only drugs capable of normalizing the behaviour of isolated mice (Frances et al 1990b). The authors thus suggested that the isolation-induced social behavioural deficit test could serve as a behavioural model sensitive to 5-HT_{1B} agonists. We used TFMPP as a reference 5-HT agonist, active as an agent counteracting the effect of isolation on the behaviour of mice. Frances et al (1990b) showed that this effect of TFMPP was prevented by 5-HT_{1B} receptor antagonists. In our experiments, as expected, TFMPP was active in normalizing the behaviour of isolated mice, since it restored the number of their escape attempts. This effect was completely antagonized by the β-adrenoceptor blocker propranolol with high affinity for 5-HT_{1B}-receptor subtype (Middlemiss & Hutson 1990; Zifa & Fillion 1992), while incapable of altering the behavioural deficit by itself. This finding is in accordance with the data reported by Frances (1988b), who found similar results with propranolol and the relationship between propranolol and TFMPP in the studied behavioural paradigm.

Isoteoline was first tested for antagonistic activity towards the effect of TFMPP. The results showed that it was ineffective in antagonizing this effect. This finding was interpreted as a lack of antagonist-like action of isoteoline at the 5-HT_{1B} receptors. TFMPP is known also to act as an agonist at 5-HT_{2C} (formerly 5-HT_{1C}) receptors (Glennon et al 1988; Kennett & Curzon 1987) and isoteoline, as already mentioned, has been shown to act as an antagonist at these receptors in a number of experimental models. Similarly to mianserin and cyproheptadine, used by Frances et al (1990b) as 5-HT_{2C} receptor antagonists, isoteoline was without effect. This fact agrees with the conclusion of the authors that 5-HT_{2C} receptors were not involved in the isolation-induced behavioural deficit in mice.

The administration of isoteoline alone dose-dependently and significantly increased the escape attempts made by the isolated mice, though the effect was less pronounced than with TFMPP. Frances (1988a), Frances et al (1990b) and Frances & Monier (1991a) found that of all psychoactive drugs tested, those able to restore the behaviour of isolated mice shared the common property of activating 5-HT_{1B} receptors. Therefore, we assumed that isoteoline was acting in an agonist-like manner at these receptors. To test this hypothesis, we used propranolol as a 5-HT_{1B}-receptor antagonist, behaviourally inactive in the present model. Propranolol antagonized significantly the effect of isoteoline at a dose of 1 mg kg^{-1} and this supported the proposed agonistic effect of isoteoline at the 5-HT_{1B} receptors.

Frances & Monier (1991b) observed tolerance to the effects of 5-HT_{1B} receptor agonists in the behavioural deficit induced by isolation in mice. There was also cross-tolerance between such agents. To test whether isoteoline would induce tolerance to its effect and also to the effect of TFMPP, we performed a subchronic experiment. We treated isolated mice with either water or isoteoline at a dose of 1 mg kg^{-1} for three consecutive days and on the fourth day we gave the animals a test treatment. The results showed that after three-days pretreatment with isoteoline, the same test dose of the drug given 16 h after the last injection failed to reverse the effect of isolation, unlike the single treatment, suggesting that tolerance had developed. The effect of a test dose of TFMPP (2 mg kg^{-1}) was also reduced significantly after pretreatment with isoteoline e.g. signs of cross-tolerance were found. However, the effect of TFMPP was not completely prevented by repeated administration of isoteoline. This may be due to the fact that isoteoline was used in a relatively low, albeit active, dose. Frances & Monier (1991b) used the 5-HT_{1B} agonist RU 24969 to induce tolerance at a dose larger than the test dose. They found also that a test dose twofold that of the one used in single experiments could overcome tolerance. It is clear that manipulations of the doses used may be a factor altering drug effects in the explored event. Further investigation is required into the role of dose in inducing tolerance. Nevertheless, the cross-tolerance to the effect of TFMPP, though incomplete, adds arguments in favour of the agonistic action of isoteoline at the 5-HT_{1B} receptors.

The clinical relevance of the isolation-induced social behavioural deficit and the pharmacological manipulations on it were not completely clear. The tolerance to the effect of 5-HT_{1B} receptor agonists in this paradigm, resulting from repeated treatment, was due possibly to desensitization of the receptors (Frances & Monier 1991b). The acute administration of benzodiazepines (Frances et al 1990a) and the chronic administration of antidepressants (Frances & Khidichian 1990) have led to reversal of the TFMPP effect. These findings fit the hypothesis of serotonin receptor hypersensitivity in panic disorders (Kahn et al 1988), where benzodiazepines and antidepressants are clinically useful, implying that the receptors of the 5-HT_{1B} subtype may be the ones that are sensitized. In this respect, chronic treatment with 5-HT_{1B} agonists may have therapeutic potential in this clinical condition (Frances & Monier 1991b). The tolerance that was found with repeated administration of isoteoline in the isolation-induced social behavioural deficit may also be viewed in this light. It may be of additional benefit to its anxiolytic effect due to 5-HT_{2C}-receptor antagonism (Zhelyazkova-Savova 1998a, 1999).

Conclusion

The results provided functional evidence of a possible agonist-like action of isoteoline at the 5-HT_{1B} receptors. This was because isoteoline demonstrated behaviour in which other 5-HT_{1B} agonists have been shown to be involved; its effects were antagonized by a 5-HT_{1B} receptor antagonist; and cross-tolerance was observed between isoteoline and another 5-HT_{1B} receptor agonist. Confirming these results by other approaches, such as receptor binding and/or using 5-HT_{1B} receptor knockout mice (Ramboz et al 1996), would be highly valuable.

Putting together our results with previous data of the interaction of isoteoline with 5-HT_{2C} serotonergic receptors, we have characterized isoteoline as a putative mixed agonist/antagonist at the 5-HT_{1B} and 5-HT_{2C} serotonergic

receptors, respectively. These properties may be potentially useful in clinical disorders such as anxiety and panic attack.

References

Frances, H. (1988a) New animal model of social behavioral deficit: reversal by drugs. *Pharmacol. Biochem. Behav.* **29**: 467–470

Frances, H. (1988b) Psychopharmacological profile of 1-(m-(trifluoromethyl)phenyl) piperazine (TFMPP). *Pharmacol. Biochem. Behav.* **31**: 37–41

Frances, H., Lienard, C. (1989) Isolation-induced social behavioral deficit test: effect of tranquillizing drugs. *Pharmacol. Biochem. Behav.* **34**: 293–296

Frances, H., Khidichian, F. (1990) Chronic but not acute antidepressants interfere with serotonin (5-HT1B) receptors. *Eur. J. Pharmacol.* **179**: 173–176

Frances, H., Monier, C. (1991a) Isolation increases a behavioral response to the selective 5-HT1B agonist CGS 120 66B. *Pharmacol. Biochem. Behav.* **40**: 279–281

Frances, H., Monier, C. (1991b) Tolerance to the behavioural effect of serotonergic (5-HT1B) agonists in the isolation-induced social behavioural deficit test. *Neuropharmacology* **30**: 623–627

Frances, H., Danti, S., Fermanian, J. (1989) Effect of tricyclic antidepressant drugs in the isolation-induced social behavioral deficit test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **13**: 229–235

Frances, H., Khidichian, F., Monier, C. (1990a) Benzodiazepines impair a behavioral effect induced by stimulation of 5-HT1B receptors. *Pharmacol. Biochem. Behav.* **35**: 841–845

Frances, H., Lienard, C., Fermanian, J. (1990b) Improvement of the isolation-induced social behavioral deficit involves activation of 5-HT1B receptors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **14**: 91–102

Glennon, R. A., Pierson, M. E., McKenney, J. D. (1988) Stimulus generalization of 1-(3-trifluoromethyl-phenylpiperazine (TFMPP) to propranolol, pindolol and mesulergine. *Pharmacol. Biochem. Behav.* **29**: 197–199

Kahn, R. S., Asnis, G. M., Wetzler, S., Van Praag, H. M. (1988) Neuroendocrine evidence for serotonin receptor hypersensitivity in panic disorder. *Psychopharmacology* **96**: 360–364

Kennett, G. A., Curzon, G. (1987) Evidence that the 5-HT agonists m-CPP and TFMPP cause hypolocomotion by stimulation of 5-HT1C receptors. *Br. J. Pharmacol.* **92** (Suppl.): 563P

Markov, M., Zhelyazkov, D., Georgiev, N. (1984) Antihypertensive effect of isoteoline in hypertension models. *Exp. Med. Morphol.* **23**: 134–138

Middlemiss, D. N., Hutson, P. H. (1990) The 5-HT1B receptors. *Ann. N. Y. Acad. Sci.* **600**: 132–147

Ramboz, S., Saudou, F., Amara, D. A., Belzung, C., Segu, L., Misslin, R., Buhot, M. C., Hen, R. (1996) 5-HT1B receptor knock out – behavioral consequences. *Behav. Brain Res.* **73**: 305–312

Zhelyazkov, D., Mollov, N., Markov, M., Philipov, S., Georgiev, N., Zhelyazkova-Savova, M., Petrov, O., Tarkolev, N. (1984) An agent for treatment of hypertonic disease and a method for its synthesis. Invention Authorship Certificate No 43200

Zhelyazkova-Savova, M. (1998a) Effect of isoteoline in a test for detecting anxiolytic activity in mice. *Eksper. Klin. Farmakol.* **61**: 19–21

Zhelyazkova-Savova, M. (1998b) Isoteoline in two models of serotonin 5-HT2C receptor stimulation. *IMAB Ann. Proc.* **4**: 297–299

Zhelyazkova-Savova, M. (1999) Effect of IST in two animal models of anxiety in the rat. *IMAB Ann. Proc.* **5**: 240–242

Zhelyazkova-Savova, M. (2000) Differential behavior of isoteoline in models of 5-HT1A and 5-HT2C receptor stimulation. *IMAB Ann. Proc.* **6**: 187–188

Zhelyazkova-Savova, M., Negrev, N. (2000) Isoteoline, a putative serotonin antagonist, inhibits mCPP-, but not 8-OH-DPAT- and DOI-induced increase in serum prolactin levels. *Pharmacol. Res.* **42**: 93–99

Zhelyazkova-Savova, M., Giovannini, M. G., Pepeu, G. (1997) Increase of cortical acetylcholine release after systemic administration of mCPP in the rat: an in vivo microdialysis study. *Neurosci. Lett.* **236**: 151–154

Zhelyazkova-Savova, M., Giovannini, M. G., Pepeu, G. (1999) Systemic mCPP increases acetylcholine release from rat hippocampus—implication of 5-HT2C receptors. *Pharmacol. Res.* **40**: 165–170

Zifa, E., Fillion, G. (1992) 5-Hydroxytryptamine receptors. *Pharmacol. Rev.* **44**: 401–458