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Scripta Scientifica Medica, Vol. 35 (2003), pp 33-36 Copyright © Medical University, Varna

EFFECT OF ISOTEOLINE ON SEIZURE SUSCEPTIBILITY IN PENTYLENETETRAZOLE-TREATED MICE

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ABSTRACT

The present work was designed to examine the effect of isoteoline on neuronal excitability in mice. Isoteoline has previously been shown to behave as a 5-HT2 antagonist in a number of experimental settings with results indicating possible selectivity of the compound for 5-HT2C receptors. These serotonergic receptors are implicated, amongst many other functions, in modulating seizure susceptibility. Mutant mice lacking 5-HT2C receptors have been shown to suffer spontaneous seizures and higher mortality. This has lead to the notion that serotonin may be involved in the pathogenesis of epilepsy. However, no 5-HT2 antagonist has so far been shown to possess pro-convulsive activity. We used the model of pentylnetetrazole (PTZ)-induced seizures to assess the anticonvulsive effect of the 5-HT2C agonist chlorophenylpiperazine (mCPP). It proved to prevent significantly the clonic and tonic seizures induced by a sub-maximal dose of PTZ. IST which was expected if anything to act pro-convulsively was tested against PTZ at a dose equal to its ED_{50}. No such effect was observed; on the contrary, a slight insignificant anticonvulsive tendency was noted. On the other hand, IST tended to antagonize the anticonvulsive effect of mCPP, but the effect failed to reach statistical significance. The results are discussed in the light of similar data from the literature concerning 5-HT2C antagonists. In addition, the failure of IST to significantly antagonize the protective effect of mCPP is sought to be explained, possibly by its dopaminomimetic activity.

Key words: isoteoline, seizure susceptibility, pentylenetetrazole, chlorophenylpiperazine, mice

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic neurotransmitter that is known to modulate numerous sensory, motor and behavioural processes in the central nervous system. The activation of a large family of receptor subtypes mediates the diverse responses of this neurotransmitter. Serotonergic mechanisms are implicated in the patho-biochemistry of a number of neuropsychiatric disorders such as depression, anxiety, eating disorders, etc. as well as in the mode of action of many clinically used drugs. Amongst the functions modulated by serotonin is the neuronal excitability. Early works point to evidence that the 5-HT protects from seizures induced by different chemo- and electroconvulsive treatments. Literature data show that depletion of endogenous serotonin lowered the threshold for induction of seizures and increased severity and incidence of seizures (15). On the other hand, pharmacological manipulations enhancing the serotonin function in the brain decreased the intensity of experimental seizures of different mechanism and genesis (20). The nature of the receptors involved in this inhibiting effect of serotonin on the excitability of the brain is gradually getting revealed during the last decade. Experiments with relatively selective ligands indicated the role of 5-HT2 receptors. More recent studies with mutant mice specify that animals lacking 5-HT2C receptor subtype are extremely susceptible to seizures of different nature. This lead has opened discussions about the role of similar spontaneous mutations in epileptogenesis (13).

Isoteoline (IST) is a compound derived from glaucine. Pharmacological studies have shown it to possess antihypertensive effects, on the one side (3), and anxiolytic activity, on the other (2,23). The latter has been demonstrated to depend on blockade of 5-HT2C receptors. In addition, IST has behaved as a 5-HT2C receptor antagonist in a number of other experimental settings known to involve this subtype of receptors (22,24,25).

In line with the above data, it was pertinent for us to ask the question if IST could lower the threshold of neuronal excitability. This is why the aim of the present work was to examine IST for possible pro-convulsive action. We used the model of pentylenetetrazole-induced seizures in mice and investigated the modulating potential of the 5-HT2C receptor agonist chlorophenlpiperazine (mCPP) and that of IST

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as an antagonist. In addition to being used alone, IST was also tested as a pre-treatment against mCPP.

MATERIAL AND METHODS

Animals

Male albino mice of 30-40g weight were used. Animals were kept under standard conditions in the animal facility with a 12:12h light-dark period provided and had their usual access to food and water.

Pentylenetetrazole (PTZ)*-induced seizures in mice*

PTZ is a commonly used chemoconvulsant to generate a model of clonic-tonic seizures. To define the doses of PTZ used in the present study, we constructed a dose-response curve in the range of 10-90mg/kg injected subcutaneously (s.c.). ED_{50} was determined graphically to equal approximately 65mg/kg. In experiment 1, where mCPP was applied as a pre-treatment PTZ was injected at a sub-maximal dose of 85mg/kg, since anticonvulsive effect of mCPP was expected. mCPP was injected intraperitoneally (i.p.) at two intervals - 20 and 30min prior to PTZ. In experiment 2, where pro-convulsive effect with IST pre-treatment was eventually proposed we used the ED_{50} of PTZ. IST was also given i.p., 30 min prior to PTZ. In experiment 3, mCPP and PTZ were given i.p. and s.c., 30 and 60min, respectively, after the i.p. administration of IST. Control mice in all experiments received distilled water i.p.

Two types of measures were followed: the latency times till the onset of the clonic and the tonic phase, as well as the frequency of the seizures. The clonic phase was defined by a myoclonus of the forepaws and the rear paws with loss of the writhing reflex for more than 15sec. The tonic phase was defined as the tetanic contraction of the fore- and rear paws followed as a rule by a lethal exit.

Drugs

Isoteoline hydrobromide has been synthesized in the Department of Pharmacology of the Higher Medical Institute of Varna (1). It was used at a dose of 4mg/kg, which has been effective as anxiolytic one both in mice and in rats (2,23). Pentylenetetrazole was used in the form of the commercial preparation under the trade name of Corazol (Pharmachim).

Statistics

The results were analyzed by chi square test with a level of significance p=0,05. GraphPad Prism software was used.

RESULTS AND DISCUSSION

Throughout the experiments, there have been observed no statistical differences in latencies to the clonic and tonic phases of the seizures between treated and control groups (not shown). For this reason, only the results from the frequency of clonic and tonic seizures are considered below.

Experiment 1. The effect of mCPP was tested at the background of the sub-maximal dose of PTZ, 85mg/kg. With pre-treatment time of 20min mCPP at doses 5 and 7,5mg/kg did not alter significantly the frequency of the seizures, either clonic or tonic (not shown). With the 30min pre-treatment interval at doses of 2,5 and 5mg/kg mCPP produced a dose-dependent reduction in the number of mice displaying clonic and tonic seizures. The effect was statistically significant at the dose 5 mg/kg ($p<0,05$ and $p<0,001$, respectively) (Table 1).

Table 1. Effect of mCPP (30 minutes pre-treatment time) on the frequency of clonic and tonic seizures induced by sub-maximal dose of PTZ $p<0,05$ and $p<0,001$ vs. vehicle pre-treated mice

Pre-treatment [mg/kg]	Treatment [mg/kg]	Clonic seizures			Tonic seizures		
		Frequency	%	P	Frequency	%	P
Vehicle [2 ml/kg]	PTZ 85	10/10	100		10/10	100	
mCPP 2.5	PTZ 85	9/10	90	NS	9/10	90	NS

Experiment 2. The effect of IST was tested against PTZ at the ED_{50} of 65mg/kg. In this setting IST at the doses used of 1 and 4mg/kg caused a slight and dose-independent increase in the number of the non-convulsive mice (Table 2).

Table 2. Effect of IST on the frequency of clonic and tonic seizures induced by PTZ at the dose of ED_{50} NS - non-significant vs. vehicle pretreated mice

Pre-treatment [mg/kg]	Treatment [mg/kg]	Clonic seizure frequency	%	P
Vehicle [2 ml/kg]	PTZ 65	6/10	60	
IST 1	PTZ 65	3/10	30	NS
Vehicle [2 ml/kg]	PTZ 65	5/10	50	
IST 4	PTZ 65	3/10	30	NS

Experiment 3. At the dose of 4mg/kg IST reversed the anticonvulsive effect of mCPP against sub-maximal PTZ dose increasing the number of the convulsive mice. The effect, though insignificant, was observed both with clonic and tonic seizures (Table 3).

Considerable evidence exists in the literature demonstrating the role of serotonin in reducing neuronal excitability. Consistent with this, our results with the serotonergic agonist mCPP acting to antagonize the convulsive action of PTZ in mice was not unexpected. It was shown to reduce the incidence both of clonic and tonic seizures induced by

sub-maximal doses of PTZ. Although mCPP binds to several serotonrgic receptors, it displays a relative preference to the 5-HT2C subtype and is often used for functional studies of these receptors (9). Another work with the same agonist has demonstrated similar anticonvulsive results, though the effect has been shown to depend on the nature of the model (electroshock vs. PTZ-induced seizures) as well as on the animal species (mouse vs. rat) (18). We chose mCPP because of the evidence that amongst the numerous serotonergic receptors in the brain 5-HT2C is the one most probably involved in the protective effect of serotonin agents against increased seizure susceptibility. Recent investigations with 5-HT2C knock-out mice show that deletion of this gene results in a syndrome characterized by spontaneous seizures, lowered seizure threshold, enhanced seizure propagation, sound-induced seizure susceptibility and increase mortality (4,5,8,16,17). These findings have lead to the idea that mutation in 5-HT2C receptors might be implicated in epileptogenesis (19).

Table 3. Effect of IST on the anticonvulsive action of mCPP towards PTZ at its sub-maximal dose. NS - non-significant vs. mCPP-treated mice

Pretreatment [mg/kg]	Treatment [mg/kg]	Clonic seizures			Tonic seizures		
		Frequency	%	P	Frequency	%	P
Vehicle [2ml/kg]	MCPP 0 + PTZ 85	10/10	100		10/10	100	
Vehicle [2ml/kg]	MCPP 5 + PTZ 85	5/10	50	<0.05	2/10	20	<0.001
IST 4	MCPP 5 + PTZ 85	7/10	70	NS	5/5	50	NS

Having in mind the functional pharmacological data that has been accumulated about IST as an anti-anxiety agent acting through inhibition of 5-HT2C receptors (2,23), it was worth testing this compound for ability to provoke increased seizure susceptibility. We treated mice with IST at a dose of 4mg/kg known from our previous works to be anxyolitic in this animal species (2). For PTZ we chose a dose inducing convulsions in 50% of the treated mice, in order to be able to observe eventually a pro-convulsive effect with IST pre-treatment. The results proved to be negative, i.e., we found no increase in the number of convulsing mice, neither with clonic, nor with tonic seizures. Our results were similar to those of other analogous studies. The apprehension that selective 5-HT2C antagonist may behave pro-convulsively has made it necessary for such products to be checked accordingly. Recently developed selective antagonists have been tested in screening seizure models. In spite of the expectations based on data with mutant animals, no one of these studies found pro-convulsive effects for 5-HT2C receptor antagonists (10,18,19). It was concluded that, in normal adult animals, this receptor subtype may usually be subjected to only a low level of 5-hydroxytryptamine tone and its inhibition can not account for lowered seizure susceptibility (18). Obviously, other mechanisms operate in knock-out animals, when this protective function is completely absent.

Our results showed further the inability of IST to completely reverse the anticonvulsive effect of mCPP. This effect was slight and insignificant. In many other experiments the antagonism of IST vs. mCPP-mediated effects was readily demonstrated (2,22,24,25). The present results, however, are intriguing in one another aspect and this is the tendency of IST to act in an anticonvulsive manner by itself. Though insignificant, this effect seems worth noting for two reasons. First, it means that no fear of increased neuronal excitement should exist in terms of the putative clinical use of IST as an anti-anxiety agent. Second, this tendency to display synergistic effect with mCPP may explain why IST failed to antagonize its effect. Indeed, it is difficult to speculate about the mechanism of this effect. In there was this respect it is interesting that a protective effect of apomorphine against PTZ-induced seizures and found it to be mediated through activation of both D1 and D2 receptors (14). There is functional evidence of IST stimulating dopamine receptors (21). In this way, the anti-PTZ-convulsive tendency of IST might be due to its dopaminomimetic activity, and the same mechanism could oppose functionally the antagonism of IST vs. mCPP. In other words, if we assume that IST displays at the same time serotonin antagonist and dopamine agonist-like actions, these may interact and mutually antagonize each other leading to reduced anticonvulsive effect (as a dopamine agonist) and ability to inhibit the effects of mCPP (as serotonin antagonist). This presumption is, however, uncertain, since Lazarova and Roussinov have found, though considerably earlier, that the dopaminergic and serotoniergic systems have an antagonist effect on the convulsive reactivity in the case of PTZ convulsion model (11).

In conclusion, the present study has provided evidence that the 5-HT2C receptor antagonist IST lacks pro-convulsive activity in the PTZ model. This is important in view of its anxiolytic activity. The week anticonvulsive effect of IST observed and its inability to fully antagonize the effect of the 5-HT2C receptor agonist mCPP may reflect interference of central dopamine receptors stimulation.

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