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EFFECTS OF ISOTEOLINE IN EXPERIMENTAL TESTS FOR MEMORY AND SEDATION IN MICE

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ABSTRACT

Isoteoline (IST) has been previously shown to possess anxiolytic activity in models of mice and rats. The aim of the present study was to examine this compound for potential memory-impairing and sedative effects. IST was compared with scopolamine and diazepam known for their anterograde amnesia, in respect to information acquisition. Diazepam served also as a sedative standard. Passive avoidance test was used to explore the effect of the drugs on the cognitive processes. Memory components such as acquisition, consolidation and retrieval were assessed depending on the time of drugs' administration relative to the training session and the retention session 24h later. We measured open field locomotion as an index of possible sedation. The results showed that IST was devoid of adverse effects on cognition. Unlike diazepam and scopolamine, IST tended to improve acquisition. It had no effect on memory consolidation and retrieval. IST did not reduce locomotion as well as thus differing from diazepam also in this respect. The lack of amnesic and sedative effects of IST adds favourably to its anxiolytic activity.

Key words: isoteoline, passive avoidance, memory, sedation, mice

INTRODUCTION

Isoteoline (IST) is a glaucine derivative. Early studies with this compound have revealed antihypertensive and hypotensive activity (2,3,5). Besides IST has been shown to affect some neuroendocrine and behavioural indices through acting on the serotonergic neurotransmission (21,24,25). Among these, IST has been demonstrated to possess anxiolytic-like activity in mice subjected to the light-dark choice procedure (4) and in rats examined in the social interaction test (22). As with most of the other effects of IST, the anxiolytic effect is supposed to be due to 5-HT2C receptors blockade. In addition, microdialysis experiments have shown IST to increase the release of acetylcholine from hippocampus of freely moving rats, and at the same time to inhibit the release evoked by mCPP through stimulation of 5-HT2C receptors (23).

Taking into account the anxiolytic activity of IST and the last findings mentioned, it was attempted to answer the question whether IST would adversely affect memory and learning. In addition, having in mind another side effect of benzodiazepines - the sedative one, it was of interest to check if IST possessed the capacity of inducing sedation.

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Thus the aim of the present study was to assess the behaviour of IST in experimental tests detecting memory impairment and sedation. Diazepam and scopolamine were used as amnestic comparator drugs and diazepam served also as a sedation-causing agent.

MATERIAL AND METHODS

Animals

Male white mice weighing 25-40g were used, divided in groups of 5-10. The animals were kept under standard conditions in the animal house with a 12:12 light:dark period and had their usual access to food and water.

Methods

1. A single trial passive avoidance (PA) test was used to study the substances' effects on the cognitive functions in mice. We made use of a PA device constructed according to a modified procedure, in which the painful punishment (foot-shock) was replaced by a non-painful one (11). This represented a wooden box made of two chambers - a light one and a dark one. The two chambers communicated through a small hole allowing the free passage of the mouse. The dark chamber had a floor consisting of two wings, whose quick opening downwards caused the mouse to fall into cold water immediately after stepping into the chamber.

The mouse was gently placed in the center of the light chamber. After a few ambulations, the animal made himself to the dark compartment. On stepping there, the mouse was subjected to the stress of a sudden fall in cold water.

The latency (in seconds) was recorded from the moment of entering the light chamber till the moment of leaving it by stepping into the dark compartment with all the four paws.
After accomplishing the first, training session (T1), the test was repeated 24h later (retention session, T2), during which the same latencies were recorded without applying punishment.
Control mice had a significant prolongation of the T2 relative to T1 latencies, as a result of memory retention. Shortening of T2 in respect to control values marked impairment of cognitive functions and vice versa. Which cognitive process was particularly affected - acquisition, memory consolidation or retrieval, depended on the time when the drug treatment was given (8). Treating the animal before punishment was indicative of changes in learning or acquisition. Giving the drug immediately after the training session reflected the consolidation process. Finally, drug administration immediately prior to the retention session was used to get information about the retrieval process.
2. Open field test was used to study the locomotion of mice. Animals were gently placed in the center of an arena with dimensions of 60x60cm, the white floor of which was divided by black lines in 9 quadrates of 20x20cm. The locomotor activity was measured by counting the number of the lines crossed by the animal during 5-min sessions. The hypolocomotion was considered a measure of sedation.

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 (4, 22). Diazepam (Sopharma) was used at dose of 2mg/kg and scopolamine hydrobromide (Sopharma) - at a dose of 1mg/kg. The doses of scopolamine and diazepam were selected from the literature as effective doses in the mouse PA paradigm (7,18).

Statistics

The results were represented as a mean ±SEM and the means were compared by Student's t-test. GraphPad Prism software was used.

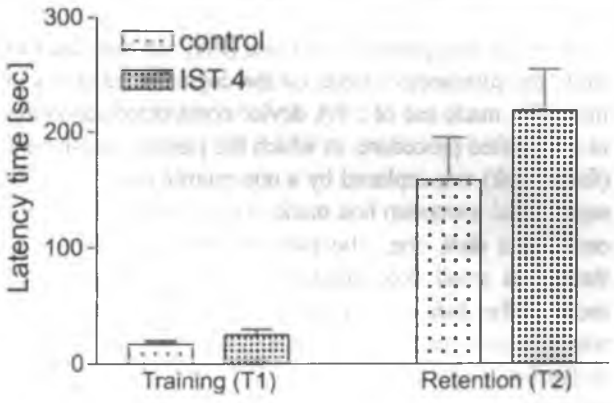

*Fig. 1. A. Effect of drugs on information acquisition. The drugs were injected i.p. 30 min before T1. Effect of IST (4 mg/kg, i.p.); *p<0,05, **p<0,01 vs. control*

RESULTS AND DISCUSSION

The effects on acquisition of the drugs studied were as follows: IST (4mg/kg), given by intraperitoneal (i.p.) route 30min before the training session, slightly prolonged the T2-latency (p=0,2537 vs. control) (Fig. 1A). Under the same conditions diazepam (2mg/kg, i.p.) and scopolamine (1mg/kg, i.p.) caused significantly shorter T2-latencies (p=0,0112 and p=0,0086, respectively) vs. control value (Fig. 1B).

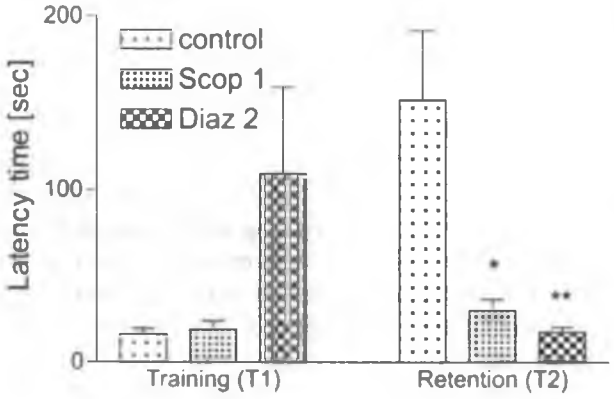

*Fig. 1. B. Effect of drugs on information acquisition. The drugs were injected i.p. 30 min before T1. Effects of diazepam (2 mg/kg, i.p.) and scopolamine (1 mg/kg, i.p.) *p<0,05, **p<0,01 vs. control*

In the test for memory consolidation IST treatment was given immediately after the training session. It resulted in a retention latency that was insignificantly shorter in respect to that of control animals (p=0,3632) (Fig. 2).

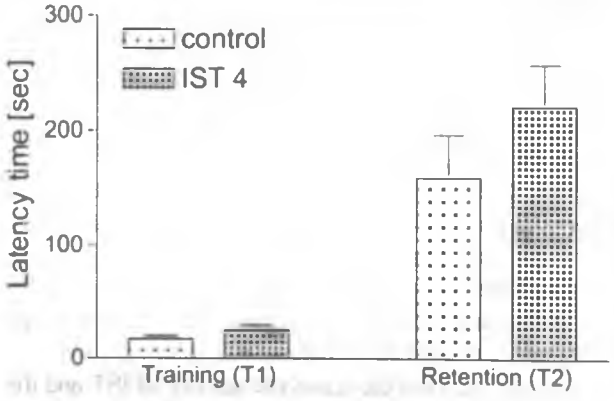

Fig. 2. Effect of IST (4 mg/kg, i.p.) on memory consolidation. The drug was injected i.p. immediately after T1

In the experiment for memory retrieval IST was administered 30min before the retention session. There was no difference between the T2-latency values of IST-treated and control mice (p=0,8376) (Fig. 3).
In the open field test IST caused no change in the horizontal locomotion (p=0,678 vs. controls), while diazepam significantly decreased the number of lines crossed in 5min (p=0,0261) (Fig. 4).

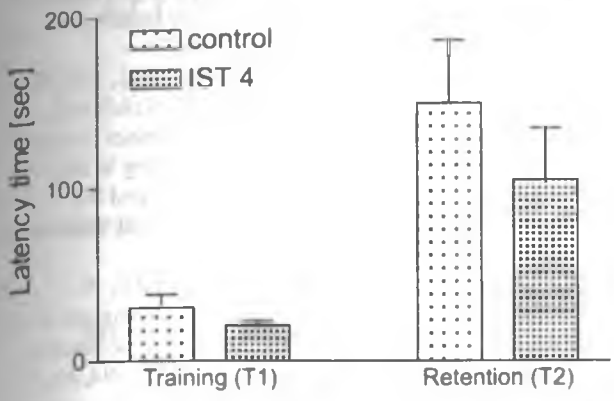

Fig. 3. Effect of IST (4 mg/kg, i.p.) on memory retrieval. The drug was injected i.p. 30 min before T2

The results of the present experiments showed that at a dose endowed with anxiolytic activity IST did not produce cognitive deficits. The lack of memory impairment was particularly prominent when comparing IST with diazepam and scopolamine in the sub-test of learning or acquisition. These drugs are long known for the anterograde amnesia that they induce both clinically and experimentally (6,7,10,12,17,18,19). This amnestic effect was confirmed in our experiments by the significant reduction of the retention latencies when the drugs were given prior to the training session. On the contrary, IST in the same schedule tended to increase, though insignificantly, the latency in the retention test.

*Fig. 4. Effect of IST (4 mg/kg, i.p.) and diazepam (2 mg/kg, i.p.) on open field locomotion *p<0.05 vs. control*

It is well known that the central cholinergic system is implicated in memory and learning (13 and others). The amnestic effect of scopolamine is mediated by blockade of central muscarinic receptors. The memory-impairing effect of diazepam has been shown to depend on specific benzodiazepine receptors on the GABA-ionophore complex, as well as on other neurotransmitter systems (9,20). At least in part, it is mediated by inhibition of the central cholinergic neurotransmission, being prevented by anticholinesterase drugs and compounds increasing the release of acetylcholine (6,7,16). We could thus speculate that the observed tendency of IST to improve learning in the PA paradigm might be due to its effect of enhancing central cholinergic neurotransmission (23). However, IST has also been found to antagonize the 5-HT2C receptor-mediated increase in acetylcholine efflux in hippocampus (23), which might account for the failure of the compound to reach statistically significant enhancement of acquisition. As far as the serotonergic system is concerned, it is also known to affect cognitive processes (14). Multiple 5-HT receptors are involved in PA with roles that probably differ at various stages of information processing (15). It has been traditionally accepted that 5-HT2A/2C receptor blockade improves learning (14), but due to the relative lack of selective ligands discriminating between the two receptors, the exact contribution of each of them is still poorly defined. Still, an important regulatory role was suggested for the 5-HT2C receptor based on the ability of a selective 5-HT2C receptor antagonist, Ro 60-0491, to reverse the memory-impairing effect of mCPP when injected before training (15). Since IST has the potential of antagonizing 5-HT2C receptors (4,21,24,25), this action could also be involved in the effect on learning. Thus IST may influence PA acquisition in a complex manner, by different and perhaps opposing mechanisms, resulting finally in a small positive answer.

On the other hand, IST did not significantly affect the processes of memory consolidation. There was a slight insignificant tendency to impair the retention performance. According to some literature data, there are 5-HT2A/2C antagonists that improve memory storage and others that do not [14]. IST had no effect on retrieval as well, as assessed by the PA task.

IST induced no sedation according to the results in the open field test. In this respect it differed from diazepam, which typically induced locomotor depression, indicative of sedative effect.

In conclusion, the lack of negative results on memory and the absence of sedation at effective doses favorably add to the anxiolytic activity of IST in mice and rats. Thus the present study provides evidence of IST being an experimental anxiolytic agent with promising and favorable adverse effects profile as compared with the clinically used benzodiazepines.

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