EFFECTS OF SOME DOPAMINE ANTAGONISTS ON SPATIAL MEMORY PERFORMANCE IN RATS - EXPERIMENTAL RESEARCH

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EFFECTS OF SOME DOPAMINE ANTAGONISTS ON SPATIAL MEMORY PERFORMANCE IN RATS - EXPERIMENTAL RESEARCHES (Abstract)**:** Dopamine is a neurotransmitter with an important role in forming long-lasting memories for some time, especially in episodic memory. Literature data show that dopamine receptor stimulation may be detrimental to spatial working memory functions in lab animals. (R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride derivative - SCH-23390 is a synthetic compound that acts as a selective, high-affinity antagonist of D1 receptors. Experimental studies suggest that SCH 23390 may prevent the spatial working memory disturbances induced by the active substances of marijuana. Melperone is an atypic antipsychotic drug presenting also dopaminergic D2 and 5-HT2A receptor antagonistic activity. This neuroleptic agent is used in the treatment of some types of schizophrenia. **Aim:** Experimental research on the effects of two dopamine receptor antagonists on spatial memory performance in rats. **Material and methods:** The experiment was carried out in white Wistar rats (200-250g), divided into 3 groups of 7 animals each, treated intraperitoneally with the same volume of solution for 14 days, as follows: Group I (Control): saline solution 0.1ml/10g kbw; Group II (coded SCH): SCH-23390 0.3 mg/kbw; Group III (coded MLP): melperone 2 mg/kbw. The dopaminergic agent spatial memory performance was assessed by recording spontaneous alternation behavior in a single session in Y-maze. Each animal was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. Alternation was defined as a consecutive entry in three different arms. The alternation percentage was computed with the following formula: number of alternations divided by total number of arm visits minus 2. Data were presented as +/- standard deviation and significance was tested by SPSS Statistics for Windows version 13.0 and ANOVA method. P-values less than 0.05 were considered statistically significant compared to those in the control group. Experimental researches were carried out in compliance with the regulations of our University Committee for Research and Ethical Issues. **Results:** SCH-23390 (0.3 mg/kbw) and melperone (2 mg/kbw) intraperitoneal injection for 14 days determined a statistically significant ($p<0.05$ and $p<0.01$, respectively) increase in spontaneous alternation rate (compared to controls in Y-maze test). **Conclusions:** Our research revealed that the 14 consecutive days administration of these two dopamine receptor antagonists was associated with the improvement of short-term memory in rats, more intense for SCH-23390 compound. **Keywords:** SCH-23390, MELPERONE, Y-MAZE TEST, MEMORY

Substances mediating dopaminergic transmission have been clinically used in the treatment of different neurological and psychiatric disorders such as Parkinson and Huntington disease, schizophrenia, bipolar disorder, attention deficit hyperactivity disorder. Dopaminergic system, have been also known to play a major role in the emotional response to rewarding, as well to aversive stimuli (1).

SCH-23390 is a R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride derivative acting as a selective, high-affinity antagonist of D1 receptors. Experimental studies have shown that SCH 23390 abolished generalized seizures induced by the chemoconvulsants: pilocarpine and soman (2, 3).

Melperone is a dopaminergic D2 and 5-HT2A receptor antagonist. This butyrophenone derivative structurally related to haloperidol has the ability to produce an antipsychotic effect in man at doses that cause minimal extrapyramidal side effects (4). It is also known to possess anxiolytic properties (5).

Electrophysiological studies showed that this drug determined an increase of dopamine levels in the medial prefrontal cortex and nucleus accumbens and a release of acetycholine in the medial prefrontal cortex. Clinical trial revealed that melperone is used especially in the treatment of neuroleptic-resistant schizophrenia (6, 7).

The supposition that dopaminergic system is involved in modulation of recognition memory is based on different experimental reports indicating the enhancement of memory consolidation by activation of dopaminergic receptor (8).

To directly prove this hypothesis we investigated the effects of two dopamine receptor antagonists, SCH-23390 and melperone, on spatial memory performance in Y-maze, a behavioral test for measuring the eagerness of lab animals to explore new environments.

This standardized test is used to evaluate working memory in rodents by measuring spontaneous exploratory behavior. The substances were administered one hour before the beginning of a behavioral session.

MATERIAL AND METHODS

Male white Wistar rats (150–200g) were used in the study. Animals were housed in plastic cages in an animal room maintained at 23 ± 1°C on a 12-hour dark cycle (light period, 07:00-19:00).

Animals had water *ad libidum* and were maintained at 85% of their free feeding weight by controlling the amount of their single daily meal following the experimental session during 45 min. Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

The animals were distributed into 3 groups of 6 subjects each, and treated intraperitoneally as follows Group: I (Control): saline solution 0.1ml/10 kbw; II (coded SCH): SCH-23390 0.3 mg/kbw; III (coded MLP): melperone 2 mg/kbw.

The used drugs, SCH-23390 and melperone (Sigma-Aldrich Chemical Company), were dissolved with 0.9% saline, prepared immediately before use. All drugs were injected intraperitoneally in a constant volume of 0.1 ml/10 g body weight.

To assess the possible involvement of dopamine agents in the maintenance of spatial cognition, the present study investigated the effects of dopamine receptor antagonists SCH-23390 and melperone on rat memory performance on Y-maze (9). This device consists of 3 identical arms (40

x 9 x 16 cm) situated at 120° from each others, and a central triangular platform. Each arm has walls with specific motifs all over the inner area of the three walls allowing animals to distinguish it from the others.

This experimental model was used to tests if the rat remembered the arm it had just explored and would therefore enter one of the other arms of the maze. Each animal was placed at the end of one arm and allowed to move unreservedly through the maze during an 8 min session. The first 2 minutes were for habituation, and the last 6 minutes for the alternation between arms recorded with photo beam breaks positioned at midpoint of each arm. Repeated activation of the same detector was not considered as an arm-entry until the detector in another arm was activated.

Spontaneous alternation was defined as entry to an arm that was least occupied in recent times (10). Latency to leave the start arm, latency of first arm visit, number of arm visited, alternate arm returns, and the same arm returns were also counted.

A video camera set over the device and connected to a computer in another room was used to allow us to score behavior without distressing the animals. All trials were videotaped and analyzed by an observer unfamiliar with treatment condition. The tasks were performed between 8:00 and 13:00 a.m.

Data for each measure were evaluated separately by analysis of variance for repeated determinations. Results were expressed as arithmetic mean ± SD and represented graphically. All values were processed using SPSS for Windows version 17.0 and ANOVA method. P-value less than 0.05 were considered statistically significant compared to controls.

Experimental protocol was implemented following the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues guidelines for the handling and use of experimental animals, according to the ethical standards of the European Community.

Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical considerations all the animals were sacrificed at the end of the experiment (11).

RESULTS

Spontaneous alternation or free-running in rats refers to their natural tendency to spontaneously choose alternate arms in a Y-maze. Arm exploration was defined as entering at least the first third of an arm. The alternation percentage of saline-treated rats was 61.4 ± 2.28.

Intraperitoneal administration of SCH 22390 resulted in a decreased e number of arm entries (mean ± S.E.M. number of arm entries was 22.67±1.63), but statistically non-significant compared to control animals (mean ± S.E.M. number of arm entries was 28.17±2.14) (tab. I).

The 14-day treatment with SCH-23390 (0.3 mg/kbw) determined a 77.2±1.72 spontaneous alternation rate.

Statistical analysis of the results showed that the alternation rate of SCH-23390-treated rats was statistically ($p<0.05$) higher than that of control rats in Y-maze test, thus suggesting a facilitation of extinction learning (fig. 1).

The administration of melperone (2mg/kbw) resulted in a statistically significant ($p<0.01$) increase of spontaneous alternation rate (86.8±1.16) compared to controls, suggesting effects on spatial memory, especially on short-term memory (tab. II, III).

TABLE I

Descriptive statistics - Spontaneous alternation rate

	N	Mean	Std. Deviation	Std. Error	Min	Max
Controls	6	61.40	2.28	.206	58.40	64.50
SCH-23390	6	77.20	1.72	.118	75.70	80.50
Melperone	6	86.80	1.16	.154	85.70	88.90

* The mean difference is significant at the .05 level.

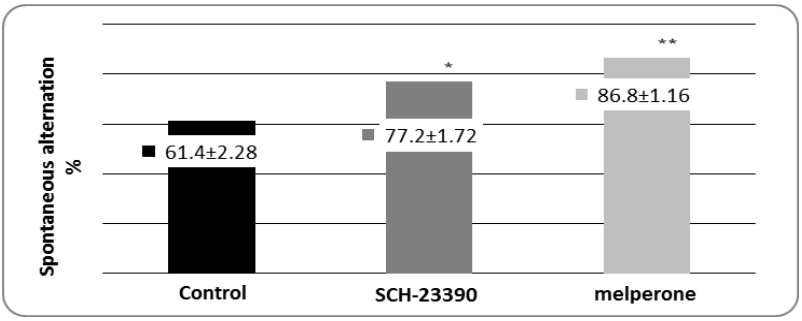

Fig. 1. Effects of SCH-23390 and melperone administration on spontaneous alternation percent/rate in Y-maze. Values were expressed as mean ± S.E.M of spontaneous alternation rate. *p<0.05, **p<0.01 vs control

TABLE II

ANOVA - Spontaneous alternation rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.048	6	1.112	11.103	.010
Within Groups	10.133	18	.464		
Total	25.181	24			

TABLE III

Multiple comparisons - Dependent variable.
Spontaneous alternation rate. Tukey HSD (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
SCH-23390	.40000	.118	**.046***	.3022	.7084
melperone	.30000	.154	**.007****	.1317	.8112

* The mean difference is significant at the .05 level.

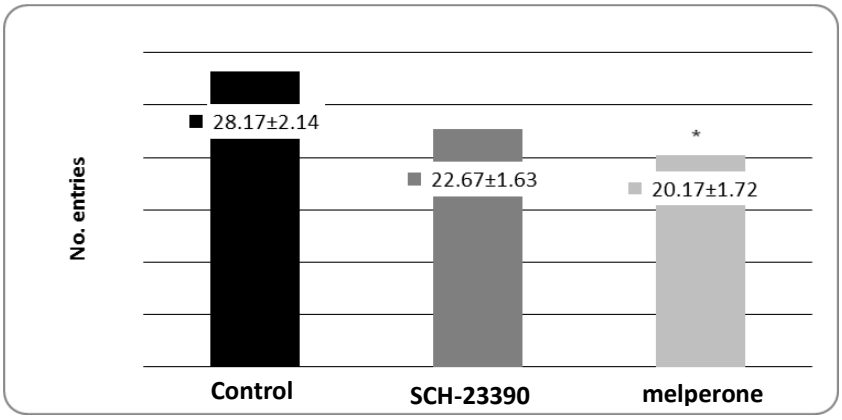

Fig. 2. Effects of SCH-23390 and melperone administration on the number of arm entries in Y-maze test. Values were expressed as mean ± S.E.M. of the number of arm entries. *p<0.05, **p<0.01 vs control

At the same time, only a tendency of reduced exploration capacity after the treatment with SCH 22390 in rats was noticed (tab. IV).

The treatment with melperone determined a statistically significant (p<0.05) diminution of the number of arm entries (mean ± S.E.M. number of arm entries was 20.17±1.72) compared to saline treated rats. These results suggested that melperone generated deficits of performance in Y-maze test (fig. 2) (tab. V, VI).

TABLE IV

Descriptive statistics - Number of arm entries

	N	Mean	Std. Deviation	Std. Error	Min	Max
Control	6	28.17	2.14	.168	26.00	31.00
SCH-23390	6	22.67	1.63	.102	21.00	25.00
melperone	6	20.17	1.72	.117	18.00	23.00

* The mean difference is significant at the .05 level.

TABLE V

ANOVA - Number of arm entries

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.886	6	1.045	10.563	.050
Within Groups	9.791	18	.312		
Total	24.677	24			

TABLE VI

Multiple comparisons - Dependent variable. Number of arm entries. Tukey HSD (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
SCH-23390	.30000	.102	.062	.2156	.6700
melperone	.50000	.117	**.048***	.1422	.7014

* The mean difference is significant at the .05 level.

This nonspecific depressant behavioral effect observed in Y-maze test could be due to the inhibition of D2 dopaminergic receptor.

The administration of dopaminergic receptor antagonists induced a reduction of the same arm return and alternate arm return number, statistically significant ($p<0.05$) more intense in melperone group compared to controls (tab. VII).

No changes in latency of the first arm visit were found between groups treated with SCH-23390, melperone and saline solution.

TABLE VII

The effect of SCH-23390 and melperone in Y-maze test (same arm return, alternate arm return, latency first arm visit). Values were expressed as mean ± S.E.M. *p<0.05, **p<0.01 vs control.

	Number of same arm return	Number of alternate arm return	Latency first arm visit (seconds)
Control	6.3±1.17	4.2±1.23	22.64±1.73
SCH-23390	4.5±0.73	3.6±0.64	21.17±0.90
melperone	2.4±0.33	1.2±0.17	21.37±1.25

DISCUSSION

The present research established the inhibition of exploratory and locomotors activity of rats induced by consecutive 14 days administration of melperone and only a tendency for reduced exploratory activity following SCH 22390 administrations. This nonspecific depressant behavioral effect could be due to the inhibition of D2 dopaminergic receptor.

Previous reports have described a facilitation of rat spatial working memory determined by D1 receptor antagonist SCH 22390 administrations in the radial arm maze. In this behavioral experimental model SCH-23390 influenced short-time memory, without affecting long-time memory of experimented animals (12).

Different studies using a variety of approaches suggest that dopamine pathway may be a key element in modulating activity in the central areas to facilitate behavioral flexibility (13, 14, 15).

Various researches indicated that dopamine D1 and D2 receptors have been involved in recognition memory processing but their implications in different phases of this cognitive process remains uncertain.

The central dopamine receptors, especially D1 receptors in the prefrontal cortex, may be very important for mediating dopamine effects on cognitive functions (8).

It has been visibly established the involvement of dopamine receptors subtypes in the mediation of cognitive functions and in spontaneous exploratory behavior in rodents (13, 16).

The contribution of different central nervous system areas, particularly hippocampus, in spatial memory and exploration capacity of laboratory animals is well recognized (17).

Experimental studies illustrate that SCH 23390 may prevent the spatial working memory disturbances induced by active substances of marijuana, block of apomorphine-induced stereotypy and methamphetamine-induced lethality in experimental models (18). Also it presented antistereotypic effects, cataleptogenic effect and inhibitory effect on amphetamine circling in different laboratory animal species (3).

Dopaminergic receptors (D1, D2, D3, D4, D5), described in 1979 are G-protein coupled receptors that mediate the activity of the neurotransmitter dopamine, being involved in spontaneous behavior, reward, in pleasure and motivation, blood pressure control and hormonal secretion regulation (19).

D_1 receptors are found at high levels in the typical dopamine rich regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercle, whereas the distribution of the D_5 receptors is more restricted (20, 21, 22). D_1 receptor mediated important actions of dopamine to control movement, cognitive function and cardiovascular system activity. The functions of D_5 receptors are not very well known until now (23). D_1-like receptors show high affinity for SCH 23390 and SKF 83566 which are selective antagonists for these subtypes and also moderate affinities for typical dopamine agonists such as SKF 82958, SKF 38393, SKF82526 and dihydrexidine (21, 24).

D_2-like receptors show high affinity for selective antagonists such as: butyropenones (e.g. haloperidol) and the substituted benzamides (e.g. sulpiride), phenothiazines and thioxanthines (23). Most antagonists show a stronger affinity for the D_2 receptor (e.g. pimozid) compared with the D_3 and D_4 receptors. The D_2-like subtypes manifest moderate affinities for typical dopamine agonists with the D_3 receptor generally showing higher affinities for agonists than the other subtypes (25).

The D_2 receptor is the principal subtype in the brain and is found at higher levels in typical dopamine rich zones. D_3 and D_4 receptors are found at much lower levels and in a more restricted distribution, especially in limbic areas in the central nervous system (24, 26). The D_2 receptor mediates the effects of dopamine to control movement, some aspects of behavior in the brain and prolactin secretion from the anterior pituitary gland (23).

The functions of the D_3 and D_4 receptors are not clear, although their localization suggests roles in cognitive, affection and behavior function (27).

CONCLUSIONS

The present research indicates that 14 consecutive days administration of D2 dopaminergic receptor antagonist melperone is associated with the enhancement of cognitive functions in rats. This was proved to be more efficient in facilitating extinction learning. Moreover, the experiment revealed the inhibition of exploratory locomotor

activity induced by consecutive 14 days administration of melperone in rats.

The Y-maze test used to assess aspects of cognitive function and learning in rats showed an improvement of spatial memory in the rats exposed to D1 dopamine receptor antagonist SCH 23390 indicated by an increase of spontaneous alternation rate compared to controls, suggesting significant effects on short-term memory.

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NEWS

CANDIDA ALBICANS BIOFILM

Candida albicans is a major human fungal pathogen causing mucosal and deep tissue infections of which the majority is associated with biofilm formation on medical implants. Biofilms have a huge impact on public health, as fungal biofilms are highly resistant against most antimycotics. Animal models of biofilm formation are indispensable for improving our understanding of biofilm development inside the host, their antifungal resistance and their interaction with the host immune defence system. The study's authors have demonstrated for the first time that non-invasive, dynamic imaging and quantification of *in vitro* and *in vivo C. albicans* biofilm formation including morphogenesis from the yeast to hyphae state is feasible by using growth-phase dependent bioluminescent *C. albicans* strains in a subcutaneous catheter model in rodents. They have shown the defect in biofilm formation of a bioluminescent *bcr1* mutant strain. This approach has immediate applications for the screening and validation of antimycotics under *in vivo* conditions, for studying host–biofilm interactions in different transgenic mouse models and for testing the virulence of luminescent *C. albicans* mutants, hereby contributing to a better understanding of the pathogenesis of biofilm-associated yeast infections (Greetje Vande Velde,Sonˇa Kucharíková, Sanne Schrevens,Uwe Himmelreich and Patrick Van Dijck Towards non-invasive monitoring of pathogen–host interactions during *Candida albicans* biofilm formation using *in vivo* bioluminescence. *Cellular Microbiology*, 2014, 16(1): 115–130)

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