The Influence of Spiperone on Oxidative Stress and Memory

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Memory is a cognitive domain showing various deficits in schizophrenia. Spiperone, a potent D2-like receptor antagonist, is also a typical antipsychotic belonging to the butyrophenone chemical class, which is licensed for clinical use in some countries for the treatment of schizophrenia. However, very few studies in the literature used spiperone for the blockade of the dopaminergic system. In this way, considering these very few information about the effects of spiperone on memory processes and also on oxidative stress status, we decided to study the effects of spiperone (0.4 mg/kg body weight) pre-testing intraperitoneally administration in normal rats on spontaneous alternation behavior and number of arm entries in Y-maze task and the latency time in the passive avoidance performance. Also, we were interested in seeing the effects of spiperone administration on the oxidative stress status from the hippocampus, by assessing two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX, as well as a lipid peroxidation marker: malondialdehyde-MDA. The administration of spiperone impaired memory processes in normal rats (especially a significant decrease of the latency time on both 24 and 72 h in pre-test spiperone treated rats, as compared to the control group), while also generating increased levels of lipid peroxidation markers such as MDA and decreased enzymatic antioxidants in the hippocampus. Moreover, we found significant correlations between the behavioral parameters we determined in the passive avoidance tasks and the levels of all oxidative stress markers which we determined, as a result o spiperone administration.

Keywords: spiperone, superoxide dismutase, gluthatione peroxidase, oxidative stress, memory.

Spiperone, a potent D2-like receptor antagonist, is also a typical antipsychotic belonging to the butyrophenone chemical class, which is licensed for clinical use in some countries for the treatment of schizophrenia [1]. In addition, spiperone is also most of the times cited as a derived of the most common used haloperidol [2].

As it is already known, memory is a cognitive domain showing various deficits in schizophrenia [3, 4] and several studies actually investigated the effects of the antipsychotic drugs on memory function, with several controversial results, stating both positive and negative effects [5, 6]. However, very few studies used the specific D2 antagonist spiperone for the blockade of the dopaminergic system. In this way, most of the studies regarding spiperone were connected to the so-called spiperone binding assay on the dopaminergic receptors [7] or in even fewer cases in studying the effects of some neuroleptic ameliorative administration on the memory process, in different combinations after a phencyclidine–induced model of schizophrenia [8].

However, it seems that there still some controversies regarding the effects of spiperone on memory processes, since some authors reported an ameliorative effect of his administration [9], while in other situations it seems to block the mechanisms implicated in memory and especially learning processes [10].

In addition, oxidative stress is also cited for its implications in the pathophysiology of schizophrenia [11-13]. Moreover, the effects of spiperone administration on the oxidative stress status are not completely understood to this date. In this way, in some studies spiperone abolished the neuroprotective effect of cabergolinein cortical neurons [14]. Moreover, the addition of spiperone alone had no toxic influence in the presence or absence of H_2O_2, suggesting that a receptor-mediated mechanism is involved in the survival-promoting effect of cabergoline [14]. In contrast, Zheng et al. showed that spiperone significantly decreased the production of the nitric oxide in lipopolysaccharide and adenosine 5'-triphosphate (ATP)-stimulated microglia cells, primary microglia and primary astrocyte cultures [2].

In this way, considering these very few information about the effects of spiperone on memory processes and oxidative stress status, we decided to study the consequences of pre-testing intraperitoneally spiperone administration in normal rats on spontaneous alternation behavior and number of arm entries in Y-maze task and the latency time in the passive avoidance performance, as well as the effects of spiperone administration on the oxidative stress status from the hippocampus. Moreover, we were interested in studying if there is a correlation between the behavioral parameters we determined in Y maze or passive avoidance tasks and the levels of the oxidative stress markers which we determined (two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX, as well as a lipid peroxidation marker: malondialdehyde-MDA), as a result o spiperone administration.

Experimental part

Material and methods

Animals. Adult male Wistar (n = 20) rats, weighing 200-250 g at the start of the experiment, were housed in groups of five animals per cage and kept in a room with controlled temperature (22°C) and a 12:12-h light/dark cycle (starting at 08:00 h), with food and water ad libitum.

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The animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). This study was approved by the local Ethics Committee and also efforts were made to minimize animal suffering and to reduce the number of animals used.

Treatment. The administration of spiperone was performed in a dosage of 0.4 mg/kg body weight, intraperitoneally (i.p.), 30 min before Y maze and also 30 minutes before acquisition phase in passive avoidance.

Ymaze task. Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviours was calculated as total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) × 100. Spontaneous alternation behaviour is considered to reflect spatial working memory, which is a form of short-term memory [15].

Step-through passive avoidance task. In brief, a step-through type passive avoidance apparatus (Coulbourn Instruments) consisting of two compartments (25×15×15 cm high), one illuminated and one dark, both equipped with a grid floor was used. The two compartments were separated by a guillotine door. In the acquisition trial, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable foot shock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the foot shock and was placed back into the light compartment. The door was again opened 30 s later to start the next trial. The training continued until the rat stayed in the light compartment for a 120-s period on a single trial. The rats were given 3–5 trials and trained to avoid punishment (remain on shock-free zone). After 24 h, each rat was placed in the light compartment and the step-through latency was recorded until 300 s had elapsed (retention trial). The step-through latency in the retention trial was used as the index of retention of the training experience. Longer retention latencies were interpreted as indicating better retention of the training experience [16].

Tissue collection. After the behavioral test, all rats were anesthetized, rapidly decapitated and the whole brain was removed. The hippocampi were then collected. Each of the samples was weighed and homogenized with a Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in bidistilled water (1g tissue/10mL bidistilled water). Samples were centrifuged 15 min at 3000 rpm. Following centrifugation, the supernatant was separated and pipetted into tubes.

Biochemical estimations

Determination of malondialdehyde

Malondialdehyde (MDA) concentrations were determined by thiobarbituric acid reactive substances (TBARs) assay. 200µL of supernatant was added and briefly mixed with 1 mL of trichloroacetic acid at 50%, 0.9 mL of TRIS-HCl (*p*H 7.4) and 1 mL of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100^{O}C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve and the results were expressed as nmol/mg protein.

Determination of superoxide dismutase

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, product number: 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

Determination of glutathione peroxidase

Glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity [17-21].

Statistical analysis

The animal's behavior in Y maze (as expressed through spontaneous alternation and number of arm entries) and passive avoidance (as expressed through the step-through latency time) and the levels of oxidative stress markers (SOD, GPX and MDA) were statistically analyzed by using one-way analysis of variance (ANOVA). All results are expressed as mean ± SEM. F values for which $p < 0.05$ were regarded as statistically significant. Also, Pearson's correlation coefficient and regression analysis were used to evaluate the connection between the behavioral parameters in Y maze or passive avoidance tasks and the aforementioned oxidative stress markers, as a result of spiperone administration.

Results and discussions

In this way, table 1 shows the effects of pre-test intraperitoneally spiperone administration in rats in the passive avoidance task and Y maze task. In the passive avoidance task, we noticed a significant decrease of the latency time (long-term emotional memory), on both 24 ($F(1.18) = 23$, $p < 0.0001$) and 72 h ($F(1.18) = 14$, $p = 0.001$), as a result of spiperone administration, when compared to the control group. However, in the spatial task of the Y-maze, no significant differences between groups was noticed on the spontaneous alternation (immediate working memory) ($F(1.18) = 2$, $p = 0.1$), as well as the number of arm entries (locomotor activity) ($F(1.18) = 0.5$, $p = 0.4$).

In addition, table 2 shows the parameters of the oxidative stress status from the hippocampus. Thus, spiperone administration resulted in a significant increase of oxidative stress, as revealed by a very significant increase in the MDA concentration ($F(1.18) = 144$, $p < 0.0001$), which is an important marker of the lipid peroxidation processes. Moreover, the D2 receptor antagonist spiperone significantly decreased the specific antioxidant activity of both SOD ($F(1.18) = 10$, $p = 0.004$) and GPX ($F(1.18) = 16$, $p = 0.0007$) from the hippocampus.

Table 1

MEAN ± SEM VALUES FOR BEHAVIORAL PARAMETERS IN CONTROL AND PRE-TEST SPIPERONE TREATED RATS

Parameter	Groups		P value
	Control	Spiperone	
Step-through passive avoidance			
First-day (24 h) latency test (s)	300 ± 0	164.77 ± 44.79	< 0.0001
72 h latency test (s)	260.69 ± 30.91	131.50 ± 53.39	0.001
Y maze task			
Spontaneous alternation (%)	80.74 ± 6.53	73.27 ± 6.39	0.1
Number of arm entries	13.59 ± 0.78	14.09 ± 0.94	0.4

Table 2

MEAN ± SEM VALUES FOR OXIDATIVE STRESS PARAMETERS IN TEMPORAL LOBE FROM CONTROL AND PRE-TEST SPIPERONE TREATED RATS

Parameter	Groups		P value
	Control	Spiperone	
MDA (nmol/mg protein)	57.14 ± 4.46	259.83 ± 17.82	< 0.0001
SOD (U/mg protein)	0.9 ± 0.08	0.52 ± 0.038	0.004
GPX (U/mg protein)	0.31 ± 0.029	0.20 ± 0.02	0.0007

Also, Pearson's correlation coefficient and regression analysis, significant correlations were found in the case of first day (24 h) latency in the passive avoidance vs. the main markers of the oxidative from the hippocampus, which we determined in this study, as in the case of MDA (n = 20, r = -0.637, p = 0.003), or in the case of the first day latency vs. SOD (n = 20, r = 0.703, p = 0.001) or first day latency vs. GPX (n = 20, r = 0.398, p = 0.043) (fig. 1).

The same applies for the latency time in the passive avoidance task after 72 hours vs. MDA (n = 20, r = -0.590, p = 0.0036), SOD (n = 20, r = 0.405, p = 0.041) and also GPX (n = 20, r = 0.422, p = 0.0043) (fig. 1).

However, no significant correlations were obtained in the case of the behavioral parameters in the Y maze task (spontaneous alternation percentage and number of arms entries) vs. the oxidative stress makers, except for the spontaneous alternation vs. GPX (n = 20, r = 0.466, p = 0.0039).

These data could suggest that the increase we showed in the oxidative stress status could be correlated the memory deficits observed as a result of spiperone administration.

As mentioned before, the studies investigating the role of dopamine D2 receptors in the cognitive functions such as long-term memory, working memory and locomotor activity are quite limited. In this way, it seems that the systemic administration of drugs that stimulate dopamine D2 receptors has been reported to improve cognitive functions in rats [22, 23] and humans [24], while the blockade of dopamine D2 receptors impaired those functions in rats [25] and humans [26].

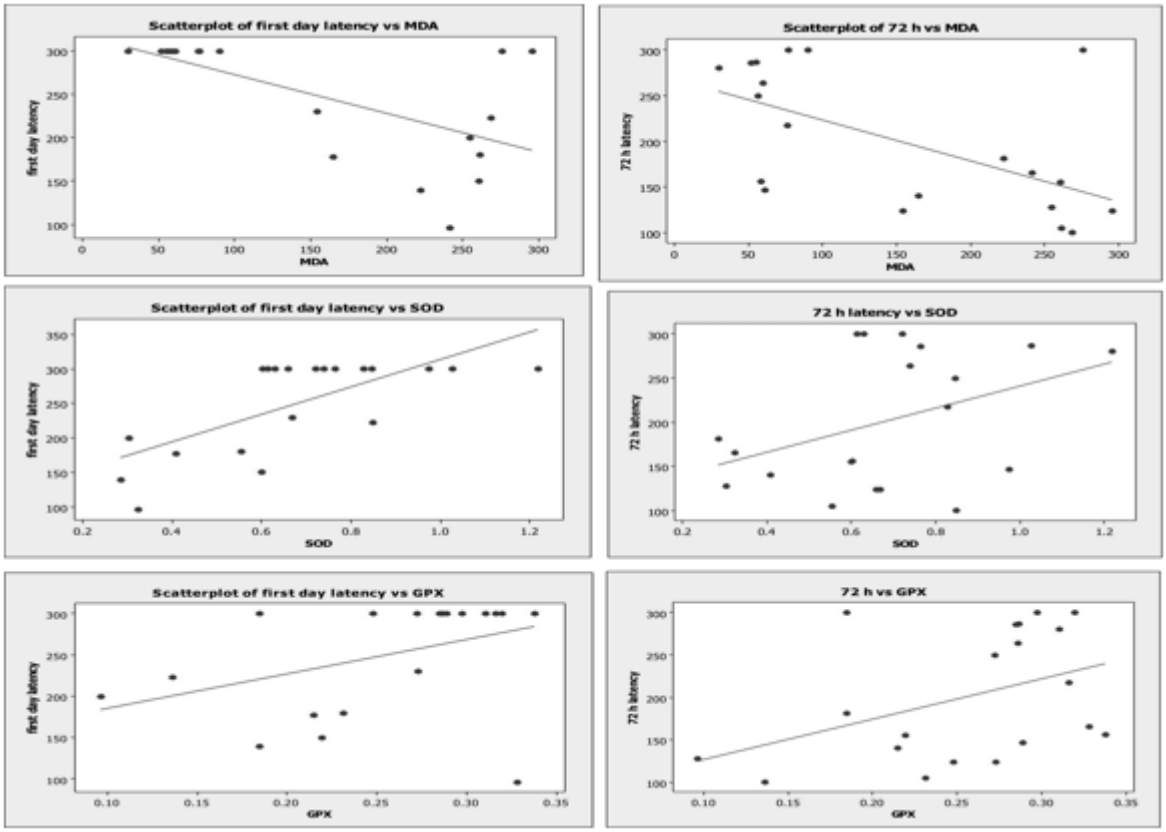

Fig. 1. The correlations between the behavioral parameters of the passive avoidance (24 h and 72 latency time) vs. the main markers of the oxidative from the hippocampus (superoxide dismutase-SOD, glutathione peroxidase-GPX and malondialdehyde-MDA).

In our study, we found that spiperone administration, a dopamine D2 antagonist, was associated only with long-term memory dysfunction in rats. Thus, we report here that pre-test intraperitoneally administration of spiperone resulted in significant decrease of the latency time (long-term emotional memory) in the passive avoidance task. However, no significant modifications were observed when spiperone was administrated before Y maze task. In this way, our results are suggesting reduced effect of spiperone on D2 receptors involved in spatial efficiency and motor activity in the Y maze task, and a predominant influence on D2 receptors implicated in consolidation of passive avoidance memory.

In contrast, other studies have demonstrated that another D2 receptor antagonist, sulpiride, intraperitoneally administrated in rats before the active place avoidance task, resulted in decreased locomotor activity and affected spatial behavior [23], while systemic post-test sulpiride administration enhanced retention in both the hidden and visible platforms of the Morris water maze [27].

In addition, the passive-avoidance test has been used previously to evaluate the effects of the antipsychotic drug haloperidol, which is also a D2 receptor antagonist, on learning and memory function in rodents, with the Japanese group of Ichihara et al. founding that pre-test haloperidol administration did not impair the passive avoidance response at doses lower than those inducing motor disturbances (e.g. sedation) during the training session [28]. Moreover, it seems that all these contrasting findings could be related to different binding characteristics of sulpiride and spiperone on D2 receptors [29].

On the other hand, our previous studies also showed that the administration of pergolide, an agonist for both D1 and D2 receptors, resulted in a decreased oxidative stress status in the temporal lobe of a 6-OHDA rat model of Parkinson's disease, as demonstrated especially by a significant reduction in the MDA levels, which was also correlated with an improvement of the spatial memory tested in specific behavioral tasks such as radial-8-arm maze and Y-maze [30].

In our present study, the intraperitoneal administration of spiperone in normal rats resulted in a significant increase for the oxidative stress status of the hippocampus, as demonstrated by the increase in the concentration of MDA, a lipid peroxidation marker, and the significant decrease for the specific activities of the main antioxidant enzymes SOD and GPX.

As already discussed, previous studies on the underlying mechanisms mediated by D2 receptors and related to the oxidative stress manifestations have revealed that the stimulation of D2 receptors by cabergoline is suppressing the ERK and p38 signaling pathways in cortical neurons. Also, spiperone abolished the neuroprotective effect of cabergoline, while the addition of spiperone alone had no toxic influence in the presence or absence of H_2O_2 [14]. As also mentioned before, it was demonstrated that spiperone could exert protective effects against inflammation-mediated neurodegeneration, mainly through a decreased the production of nitric oxide in microglia cells. In this way, spiperone attenuated the expression of inducible nitric oxide synthase and proinflammatory cytokines such as interleukin-1beta and tumor necrosis factor-alpha in microglia cells. The different dependency on receptor-mediated mechanisms may be attributed to differences in the cell-types used, cortical neurons, mesencephalic neurons/cell line or microglia cells [2].

Also, since we suggested in this paper a possible connection between oxidative stress modifications and memory consolidation, especially in the less known context of the spiperone administration in rats, we should also mention the increased relevance of the oxidative stress status in the main neuropsychiatric disorders, starting with dementia, as our group previously demonstrated in several different instances [25, 31].

Even more then that, it was previously shown that oxidative stress could be quite important also in the schizophrenic pathology [11-13] and there is also a significant correlation between the main markers of the oxidative stress status and some specific scales for schizophrenia such as PANSS and AIMS [32]. In this context, it is also worth mentioning the increased awareness from the current literature in understanding the cognitive and memory deficits in schizophrenia, ranging from alterations in memory, attention and motor skills deficiencies to a significantly affected intelligence for example [4].

In fact, similar aspects regarding a possible connection between the oxidative stress status and superior cognitive functions in most of the neuropsychiatric disorders were also demonstrated (including by the results of our group) in PD [24], depression [33] or even autism [34].

In addition, the biological importance of this connection is demonstrated by its presence even on the lower scale of evolution models, such as in species like Drosophila, as in the work of Haddadi et al. group which demonstrated quite recently the importance for the accumulation of oxidative damage and reduction of antioxidants in aging and functional senescence, by showing important correlations between consolidated forms of olfactory memory and a decrease in the antioxidant enzymes such as catalase and SOD or reduced glutathione level, as well as by an increase in the lipid peroxidation and reactive oxygen species (ROS) concentrations in the brain of the aforementioned Drosophilla age-related memory impairment model [35].

These consistent results regarding the connections that might exist between memory consolidation and oxidative stress status could be explained by the fact that the brain has low levels of antioxidants, as compared to the other organs, while on the other hand it is very rich in polyunsaturated fatty acids and catecholamines (important oxidizable substrates) and also it one of the most important oxygen consumer in the body [25, 35].

Of course, all these data is suggesting the potential benefits of the antioxidant therapy. However, most of studies are generally controversial in regards to the usefulness of these drugs in most of the neuropsychiatric disorders. Also, the usage of some antioxidants for these superior functions-related disorders prevention or treatment is extremely controversial, given also some additional speculated side effects of these compounds [25]. Still, there are data such as the one of Zhangh et al. from example, which showed in two different studies that adding a Ginkgo biloba extract to classical haloperidol treatment could result in better scores in the Scales for the Assessment of Positive and Negative Symptoms [36], while also enhancing the effectiveness of the antipsychotic and reducing some extrapyramidal side effects [37].

Conclusions

In conclusion, we found that the administration of spiperone, a D2 receptor antagonist, could impair memory processes in normal rats, while also generating increased levels of lipid peroxidation markers such as MDA and decreased enzymatic antioxidants in hippocampus. Moreover, we found significant correlations between the behavioral parameters we determined in the passive

avoidance tasks and the levels of all oxidative stress markers which we determined, as a result o spiperone administration, suggesting that oxidative stress could be related to some memory deficits induced by spiperone in normal rats.

There are also several important limitations to our study. Firstly, we did not designed this study to see the effects of spiperone in a rat model of schizophrenia (which was anyway previously studied on phencyclidine-induced rat model of schizophrenia – [3]), but only on normal rats (more like a model for the side effects of this antipsychotic administration), considering the general lack of literature in this area of research. However, present studies are being performed in our lab, in order to see the effects of spiperone administration in a ketamine-induced rat of schizophrenia (on some specific memory-related behavioral tasks and on oxidative stress levels). Also, the relevance of some synthetic SOD and GPX mimetics is right now tested in our lab in order to see if it can reverse the spiperone-induced memory impairments. In addition, we are planning to look to more specific areas of the brain in the future for the oxidative stress status modifications, such as hippocampus, amygdala or the striatum.

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