Neuromodulatory effect of progesterone on the dopaminergic, glutamatergic, and GABAergic activities in a male rat model of Parkinson's disease

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Objectives: Progesterone has been reported to have a neuroprotective role in depression-like rats in a hemiparkinsonian model of the disease. In this work, we investigate if this hormone affects the three principal neurochemicals striatal systems (dopaminergic, glutamatergic, and GABAergic) that are involved in the physiopathology of the disease in a hemiparkinsonim male rat model at 8 weeks post-chemical injury.
Methods: For this purpose, we design three experimental groups: (1) sham group; (2) hemiparkinsonian group; and (3) hemiparkinsonian group subcutaneously injected with progesterone at 7 days post-chemical injury. Animals were tested in an automated rotational device at 8 weeks post-chemical injury. After behavioral test, K^+-evoked [^{3}H]-dopamine, [^{3}H]-glutamate, and [^{3}H]-gamma aminobutyric acid release from striatum slices were analyzed by superfusion experiments.
Results: The hemiparkinsonian group showed distinctive alterations that are produced by neurodegeneration of left nigrostriatal dopaminergic pathway by 6-hydroxydopamine hydrobromide (6-OHDA). On the other hand, the administration of progesterone 7 days after the injection of the neurotoxin was able to (1) improve the K^+-evoked [^{3}H]-dopamine release from the damaged striata (left); (2) avoid significant increase in the K^+-evoked [^{3}H]-glutamate release from the left striata; and (3) progesterone does not modify the K^+-evoked [^{3}H]-gamma aminobutyric acid release from the left striata.
Discussion: These results suggest that progesterone does have neuroprotective and neuromodulatory effects on striatal neurotransmission systems in the hemiparkinsonian male rats. The possible mechanisms would involve genomic and non-genomic actions of this neuroactive steroid which would modulate the activity of dopaminergic, glutamatergic, and GABAergic pathways.

Keywords: Hemiparkinsonism, Male rats, Progesterone, Dopamine, Glutamate, Gamma aminobutyric acid

Introduction

Parkinson's disease was first described as 'shaking palsy' by James Parkinson in 1817.[1] It is one of the most common late-life neurodegenerative diseases in developed countries all around the world, affecting about 1–2% of the population over 55 years old.[2] In Parkinson's disease, progressive loss of dopaminergirc neurons of the substantia nigra triggers complex functional modifications within the basal ganglia circuitry, which underlie the typical motor symptoms of the disease (tremor, rigidity, bradykinesia). Although a single causative factor has not been identified, a restricted number of converging pathogenic mechanisms have been suggested, including oxidative stress, mitochondrial dysfunction, protein mishandling, and inflammation.[3]

The degeneration of dopaminergic neurons has a central role in the physiopathology of the disease.[4] Thus, degeneration of the nigrostriatal dopaminergic pathway produces a significant reduction in the availability of dopamine in the striatum. This alteration on the functionality of the basal ganglia is compensated by an up-regulation of D1 and D2 striatal receptors and by increasing the metabolic activity of the corticostriatal glutamatergic pathway. The latter effect results in the death of striatal neurons by excitotoxicity.[5]

Despite the scientific and technological advances, there is no effective treatment to achieve the reversion

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or stop the progressive evolution of the disease. However, in recent years, the evidence of neuroprotective effects of progesterone has increased. This hormone is a neuroactive steroid that exhibits numerous important modulatory effects on normal or physiopathological brain functions.[6] Under physiological conditions, progesterone can affect a broad spectrum of behavioral functions, such as sexual and feeding behavior, responses to stress, emotion, memory, and cognition.[7,8] Under physiopathological conditions, it also plays important roles in the pathology and treatment of psychiatric disorders such as epilepsy, premenstrual syndrome, schizophrenia, depression, anxiety, multiple sclerosis, and other neurodegenerative diseases.[9]

At the molecular level, progesterone can bind to intracellular receptors that act as transcription factors and regulate gene expression,[10,11] as well as T-type Ca^{2+} channel, high-voltage activated Ca^{2+} channel, Na^+ channel, Ca^{2+}-activated K^+ channel, and anion channels[12]. On the other hand, at the cellular level, studies show that progesterone can modulate neuronal excitability.[13] In addition, this neuroactive steroid can modulate almost all kinds of classical synaptic transmission, including glutamatergic, GABAergic, cholinergic, and dopaminergic, by altering the responsiveness of postsynaptic receptors or the presynaptic release of neurotransmitters.[14]

Previous works of our group demonstrated that the neuroactive steroid progesterone and its metabolite allopregnanolone modulates striatal dopaminergic activity of female rats under different gonadal hormonal conditions[14,15] and that the systemic administration of progesterone to hemiparkinsonian male rats prevents depression-like behavior.[16]

Taking in consideration the evidence presented above, we hypothesize that progesterone could have a neuromodulatory role on neuroglial systems involved in the physiopathology of Parkinson's disease. The aim of this study was to investigate the neuromodulatory effects of progesterone on the main striatal neurotransmitters systems related to Parkinson's disease. We investigated whether subcutaneous treatment of progesterone affects the K^+-evoked [^{3}H]-dopamine, [^{3}H]-glutamate, and [^{3}H]-gamma aminobutyric acid release from striata slices in hemiparkinsonian male rats.

Materials and Methods

Animals

We used male Sprague–Dawley rats from our breeding colony. They were 60 days old at the beginning of the study, and their weights were from 280 to 340 g. Experimental subjects were housed under controlled temperature ($22 \pm 2^{\circ}C$) and lighting (12-hour light cycle beginning at 07.00 a.m.) conditions, with food and water made available *ad libitum*. Animals for these experiments were kept and handled according to the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academies, USA, 8th edition, 2011). All experiments were carried out under the approval of Universidad de Mendoza authorities.

Reagents

Progesterone and 6-hydroxydopamine hydrobromide (6-OHDA) were purchased from Sigma-Aldrich (St Louis, MO, USA). Apomorphine hydrochloride was obtained from Research Biochemicals International (Natik, MA, USA). Chloral hydrate was purchased from Anedra (Buenos Aires, Argentina). Desipramine HCl was obtained from Farmacia Sevilla (Mendoza, Argentina). [^{3}H]-dopamine, [^{3}H]-glutamate, and [^{3}H]-gamma aminobutyric acid were purchased from New England Nuclear (Boston, MA, USA).

Surgical procedures

In order to achieve unilateral lesions of the nigrostriatal system (chemical injury), rats received 6-OHDA injections into the left striatum. Animals were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal) and placed into a stereotaxic frame (David Kopf, USA). The neurotoxic 6-OHDA was dissolved at a concentration of 2 μg/μl saline in 0.1% ascorbic acid.[17] Two microliters of 6-OHDA solution were injected using a Hamilton syringe at the following coordinates: anterior-posterior, +1.2 mm; medio-lateral, +2.5 mm; dorso-ventral, −6.5 mm relative to bregma (Fig. 1A). The injection was conducted at a rate of 0.5 μl/min and the needle was left in place for another 5 minutes before it was slowly drawn back. To prevent uptake by noradrenergic neurons, animals were pre-treated with desipramine (25 mg/kg, intraperitoneal) 30–40 minutes before injection of 6-OHDA.[17]

Experimental design and drug-induced behavioral tests

Behavioural records were all performed by an observer blinded to the experimental condition of the group as well as to any previous performance of the subjects in other behavioral tests. Tests were performed at different time points after surgery according to the following outline (Fig. 1B): at time 0, adult rats were randomly selected in order to be surgically injected in left striatum with either the neurotoxic 6-OHDA or saline injection; 1 week later, the animals were assigned to one of the three experimental groups: (1) sham group: animals previously injected with saline instead of 6-OHDA; (2) hemiparkinsonian group: the same as in Group 1, but rats received 6-OHDA during surgery; and (3) hemiparkinsonian progesterone-treated group: the same as in Group 2, but rats were administered with progesterone 4 mg/kg subcutaneously (s.c.) at noon for 3 consecutive days starting

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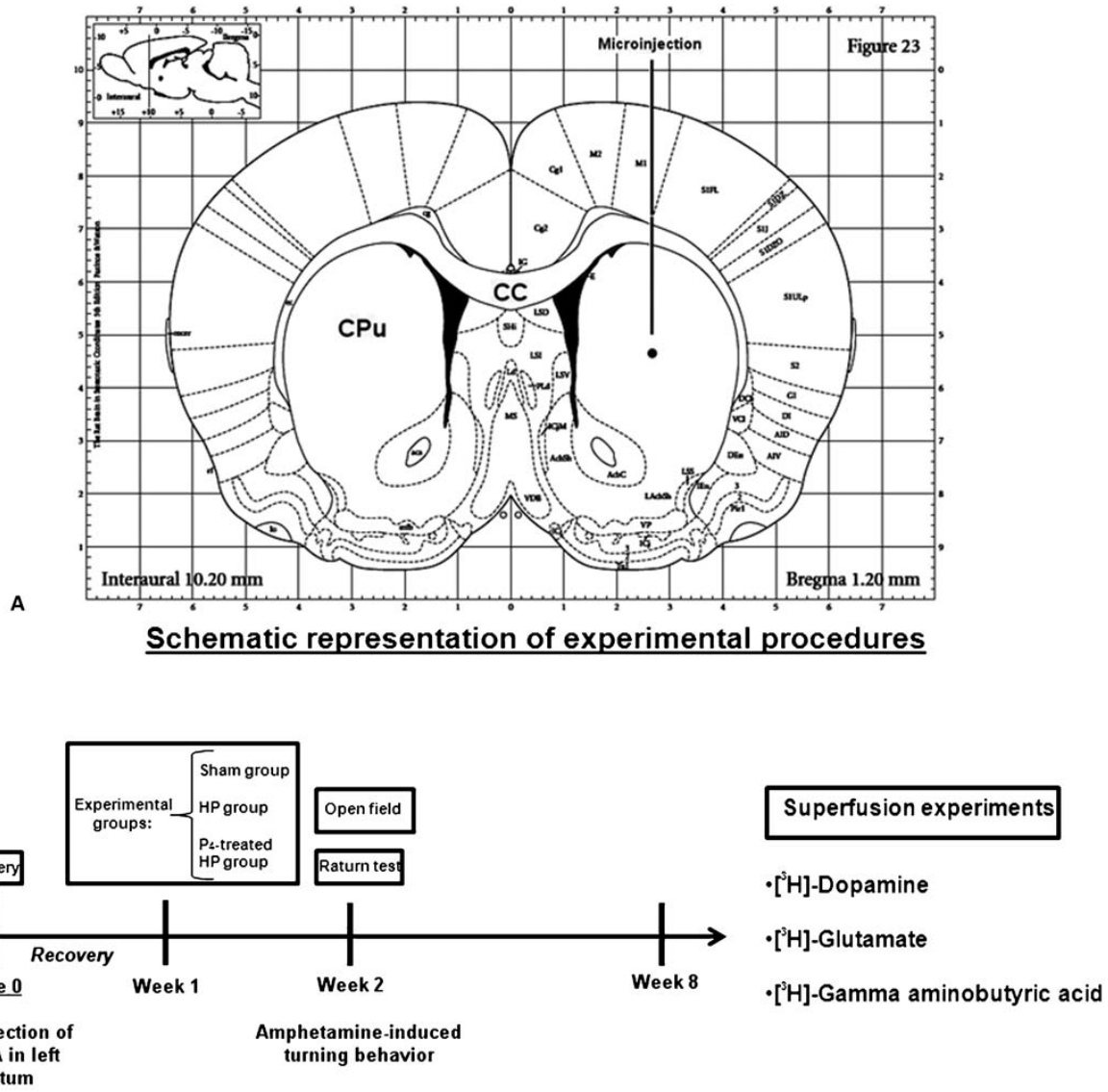

Figure 1 (A) Schematic representation of a brain coronary section showing the corpus striatum and the place where microinjections were performed. Coordinates: anterior-posterior, +1.2 mm; medio-lateral, + 2.5 mm; dorso-ventral, −6.5 mm relative to bregma. CC, corpus callosum; CPu, caudate putamen. Modified from Paxinos G and Watson C, 1997. The rat brain in stereotaxic coordinates. Compact third edition. (B) Schematic experimental procedures. HP group, hemiparkinsonian group; P_4-treated HP group, progesterone-treated hemiparkinsonian group. Time 0: surgery day.

1 week after 6-OHDA lesion, according to Gonzalez *et al.*[18] and Casas *et al.*[16] Two weeks after 6-OHDA injury, all groups were tested for amphetamine-induced turning behavior according to the protocol described below. Finally, 6 weeks after testing turning behavior, rats were killed by decapitation and their striata were dissected and evaluated by superfusion experiments to determine either the [^{3}H]-dopamine, [^{3}H]-glutamate, or [^{3}H]-gamma aminobutyric acid release.

Amphetamine-induced turning behavior

Two weeks after the 6-OHDA lesion, the rats received 1 mg/kg amphetamine intraperitoneally,[17] and were placed in individual plastic bowls with a diameter of 20 cm and attached via a specially adapted harness to an automated rotameter (Rotamex; Columbus Instruments, Columbus, OH, USA). They were allowed to habituate to their dimly lit environment for 10 minutes before contralateral and ipsilateral turns — regarding to the side of the lesion — were recorded over 60 minutes. The results of the turning behavior test were calculated as the difference between the number of contralateral and ipsilateral turns and were expressed as turns/h.[17] Rats previously treated with 6-OHDA that failed to show the ipsilateral turning behavior were considered as non-lesioned and therefore, dismissed from the experiment.

Open field test

In order to assess the locomotor and exploratory activity of the animals — avoiding potentially confounding variables affecting the turning behavior results — rats were tested by open field test to measure ambulatory and non-ambulatory activities, time spent, and vertical activity, according to Kaur

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et al.[19] The ethogram was registered with the free software Etholog v2.0.[20]

Superfusion experiments

The left and right striata were dissected out according to the procedures described by Cabrera and Bregonzio[15] and Giuliani *et al.*[21] In brief, each dissected striatum was longitudinally sliced at 240 μm with a McIIwain tissue chopper. Depending if the experiment was for dopamine, glutamate, or gamma aminobutyric acid release, each set of slices obtained per left or right striatum explant was exposed to 2 μl [^{3}H]-dopamine (specific activity 57 Ci/mmol) or 2 μl [^{3}H]-glutamate (specific activity 49.6 Ci/mmol), or 2 μl [^{3}H]-gamma aminobutyric acid (specific activity 76.2 Ci/mmol) in 2 ml of gassed (95% O_2 and 5% CO_2) KRBG Mg^{2+} free buffer for 15 minutes at 37°C in a Dubnoff metabolic shaker. The slices were transferred to superfussion chambers and superfused at 0.7 ml/min with KRBG Mg^{2+} free buffer for 30 minutes (washing period), to washout the [^{3}H]-neurotransmitter not incorporated into the tissue. Then, the experiments were started by superfusing KRBG Mg^{2+} free buffer. During this period, five fractions of 1.75 ml each one (2.5 min each fraction) were collected and considered as basal release. After that, the slices were superfused with KRBG Mg^{2+} free supplemented with KCl 28 mM and three 1.75 ml fractions were collected (K^+-evoked release). Then, the same solution used during the pre-stimulus period was superfused and five 1.75 ml fractions were collected. At the end of the experiments, the slices were homogenized in 2 ml of perchloric acid 0.2 M by sonication, and 0.5 ml aliquots of each fraction and homogenates were taken and mixed with scintillation fluid to measure the radioactivity. The percentage (%) of the [^{3}H]-dopamine, [^{3}H]-glutamate, or [^{3}H]-gamma aminobutyric acid released with respect to the amount of the [^{3}H]-dopamine, [^{3}H]-glutamate or [^{3}H]-gamma aminobutyric acid remaining in the tissue was calculated for each fraction. The results obtained in the different groups were expressed as % K^+-evoked [^{3}H]-neurotransmitter release that was calculated as the difference between the percents of neurotransmitter released by the tissue during K^+-evoked release (mean fractions: 6, 7, and 8) and the percentages of basal neurotransmitter release (mean fractions: 3, 4, and 5). Five to six animals were used per experimental group.[21]

Statistical analysis

For the statistical analysis, we utilized the software GraphPad Prism Version 5.01. We performed the test of Shapiro–Wilk in order to prove whether or not our data came from a normally distributed population, which was precisely the case. Data of percentage K^+-evoked dopamine, glutamate, and gamma aminobutyric acid release were analyzed using the one-way ANOVA. Each analysis was followed by multiple comparisons using a Bonferroni *post hoc* test. The significance level was set at $P<0.05$ for all statistical tests and is expressed as the mean ± SEM.

Results

Behavioral tests

To exclude the eventual effect of confounding variables related to locomotor and exploratory activity of the animals, we performed open field test. Statistical analysis showed there were no differences between the three experimental groups (Table 1).

As expected, the turning behavior in hemiparkinsonian and progesterone-treated hemiparkinsonian groups displayed strong ipsilaterality in response to amphetamine compared to sham group ($P<0.001$) (Table 1).

Effect of progesterone treatment on K^+-evoked [^{3}H]-dopamine release

K^+-evoked [^{3}H]-dopamine release from left striata was reduced in hemiparkinsonian group compared to

Table 1 Open field (A) and raturn (B) tests at 2 weeks post-6-OHDA lesion. Results are expressed as mean ± SEM of several types of movements recorded in the open field and raturn tests, from the sham, hemiparkinsonian (HP), and progesterone-treated hemiparkinsonian groups (P_4-treated HP)

A

Type of movements	Sham group	HP group	P4-treated HP group	ANOVA-1
Horizontal	4784 ± 65.33	4624 ± 245.3	4329 ± 355.5	$F=0.1088$; $P>0.05$
Vertical	30.4 ± 4.22	32.6 ± 7.89	32.8 ± 7.85	$F=0.0324$; $P>0.05$
Ambulatory	4230 ± 48.8	4307 ± 200	4199 ± 241.5	$F=0.0765$; $P>0.05$
Non-ambulatory	837 ± 34.53	845 ± 70.94	899.4 ± 70.03	$F=0.2857$; $P>0.05$
No. of movements	145.4 ± 28.14	122.2 ± 26.18	135.4 ± 40.4	$F=0.1216$; $P>0.05$

B

Turing behavior	Sham group	HP group	P4-treated HP group	ANOVA-1
Turns/h	5.83 ± 8.33	−120.83 ± 35.54***	−138.16 ± 53.54***	$F=15.32$; ***$P<0.001$

Note: Data were analyzed using a one-way analysis of variance (ANOVA).
***$P<0.001$.

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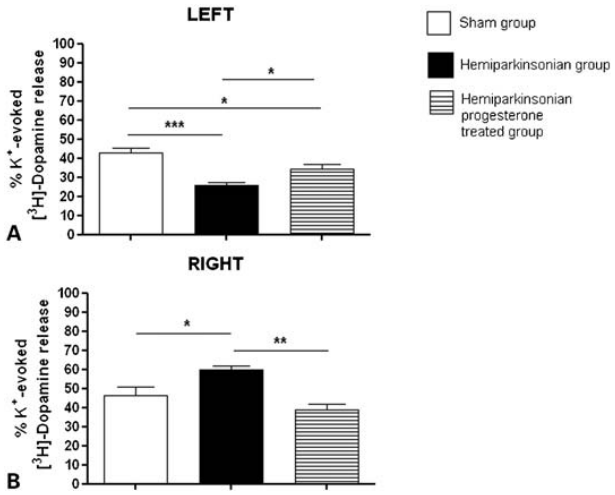

Figure 2 Percentage of K^+-evoked release of [^{3}H]-dopamine *in vitro* in the left (A) and right (B) striata. Tissues were superfused with KRBG Mg^{2+} free containing K^+ 28 mM (n=5–6 for each group). Results are expressed as mean ± SEM. F=17.67 (left striata); F=8.839 (right striata); ***P< 0.001; **P<0.01, *P<0.05 (one-way ANOVA).

sham group (P<0.001). Interestingly, in hemiparkinsonian progesterone-treated rats, the [^{3}H]-dopamine release from left striata was increased regarding to the hemiparkinsonian group (P<0.05). Even though this latter effect, the percentages of dopamine release were yet lower than the sham group (P<0.05) (Fig. 2A). On the other hand, in right striata, [^{3}H]-dopamine release was increased in the hemiparkinsonian group compared to the sham group (P<0.05) and the hemiparkinsonian progesterone-treated group (P<0.01) (Fig. 2B).

Effect of progesterone treatment on K^+-evoked [^{3}H]-glutamate release

In the hemiparkinsonian group, the K^+-evoked [^{3}H]-glutamate release by the left striata was increased compared to the sham and hemiparkinsonian progesterone-treated groups (P<0.01) (Fig. 3A). With respect to the right striata, in the hemiparkinsonian progesterone-treated group, the K^+-evoked [^{3}H]-glutamate release was decreased compared to the hemiparkinsonian group (P<0.05) (Fig. 3B). There were no statistical differences between the sham group and the other two groups (P>0.05) (Fig. 3B).

Effect of progesterone treatment on K^+-evoked [^{3}H]-gamma aminobutyric acid release

The K^+-evoked [^{3}H]-gamma aminobutyric acid release in the left and right striata was reduced in the hemiparkinsonian and hemiparkinsonian progesterone-treated groups compared to the sham group (P<0.001) (Fig. 4). Additionally, in the right striata, the [^{3}H]-gamma aminobutyric acid release was reduced in the hemiparkinsonian progesterone-treated group with respect to the hemiparkinsonian group (P<0.001) (Fig. 4B).

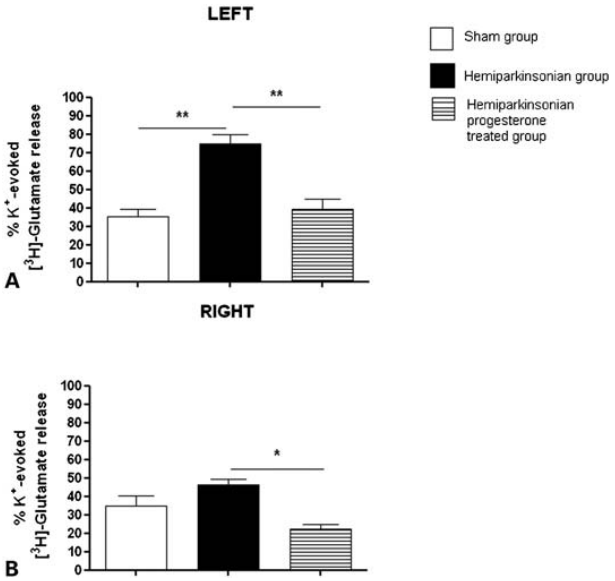

Figure 3 Percentage of K^+-evoked [^{3}H]-glutamate *in vitro* in the left (A) and right (B) striata. Tissues were superfused with KRBG Mg^{2+} free containing K^+ 28 mM (n=5–6 for each group). Results are expressed as mean ± SEM. F=20.32 (left striata); F=8.866 (right striata); **P<0.01, *P<0.05 (one-way ANOVA).

Discussion

The decrease in neuroactive steroid and neurosteroid levels during aging has been suggested to reduce brain neuroprotection.[22] This could be the basis for increased susceptibility to endogenous and environmental neurotoxic factors which may result in increased apoptosis and neuronal cell loss contributing to neurodegenerative processes.[22] Our results show that the neuroactive steroid progesterone modulates

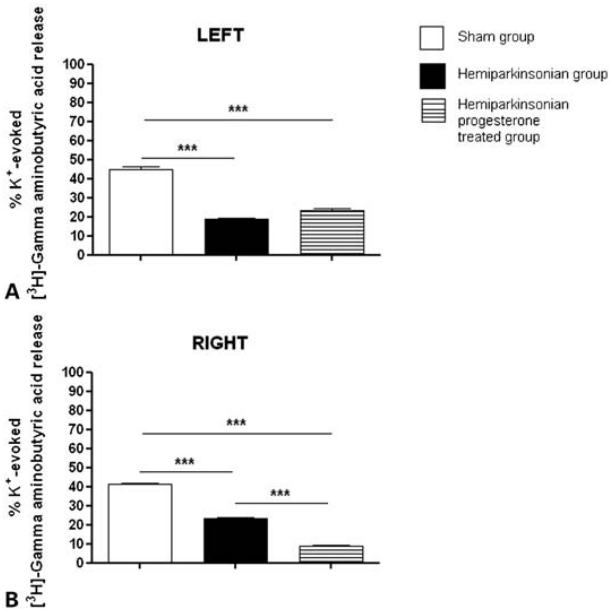

Figure 4 Percentage of K^+-evoked [^{3}H]-gamma aminobutyric acid *in vitro* in the left (A) and right (B) striata. Tissues were superfused with KRBG Mg^{2+} free containing K^+ 28 mM (n=5–6 for each group). Results are expressed as mean ± SEM. F=133 (left striata); F=408.4 (right striata); ***P<0.001 for one-way ANOVA.

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the main striatal neurotransmitter systems involved in the physiopathology of the Parkinson's disease.

Concerning the nigrostriatal dopaminergic pathway, progesterone appears to have a biological significance as a neuroprotective molecule. The decrease observed in [^{3}H]-dopamine release from the left striata due to 6-OHDA lesion, was improved after treatment with the hormone. On the other hand, the right striata of the hemiparkinsonian animals showed an increased neurotransmitter release that could be due to compensatory neuronal plastic changes.[23] According to our results, this counterbalance effect was also reverted when the hemiparkinsonian rats were treated with progesterone. The fact that these effects are maintained in the long term supports our hypothesis of an important biological function of progesterone as a neuroprotective steroid.

Progesterone might exert different actions and use different signaling mechanisms in normal and injured neural tissue.[24] The molecular mechanisms through the hormone acts include the increasing expression of anti-apoptotic proteins, down-regulation of proapoptotic gene expression, restoration of the mitochondrial function, and regulation of inflammation.[24] On the other hand, the increase in [^{3}H]-dopamine release due to progesterone treatment prompts us to consider the possibility of an increased synthesis of dopamine in dopaminergic neurons. It has been described that the gene expression of tyrosine hydroxylase is upregulated by this neuroactive steroid.[25]

The effects of progesterone appear also to affect the corticostriatal system. Progesterone treatment has a negative neuromodulatory effect on the left glutamatergic pathway. The diminished availability of dopamine in the hemiparkinsonian group could deregulate the activity of the glutamatergic neurons. It is well known that dopamine inhibits the glutamate release through action on the presynaptic dopamine receptors. The physiopathological glutamatergic alteration is also improved when the rats were treated with progesterone, reducing the [^{3}H]-glutamate release. This effect may be explained in part since progesterone protects dopaminergic neurons against the lesion with the neurotoxin 6-OHDA; therefore, the normal dopamine-dependent glutamate release inhibition could be preserved. On the other hand, in the right striata, the hemiparkinsonism appears not to influence the [^{3}H]-glutamate release but progesterone treatment decreases the neurotransmitter release. It has been reported that progesterone negatively modulates the glutamate release in prefrontal cortex through mechanisms that involve allosteric interaction with sigma 1 receptors in the glutamatergic terminals.[12] Our results in the right striata suggest to us also the possibility of a direct effect of progesterone on [^{3}H]-glutamate release through this latter mechanism.

Under the conditions of our experiments, [^{3}H]-gamma aminobutyric acid release was reduced in the hemiparkinsonian and hemiparkinsonian progesterone-treated rats for both the left and right striata. The release of this neurotransmitter in striatum appears to be regulated by dopamine in a dual way not yet fully understood. It has been suggested that dopamine might increase [^{3}H]-gamma aminobutyric acid release through its action on the D1 receptors, but reduce it through its action on the D2 receptors.[26,27] Although progesterone treatment appears not to have an effect on [^{3}H]-gamma aminobutyric acid release in the left striata, it was a significant decrease in the right ones. This disparity may be a consequence of differential sensibilities to the hormone in normal and damaged neural tissues. Thus, the significant reduction of [^{3}H]-gamma aminobutyric acid release in both the left and right striata due to the lesion with the neurotoxic and the differential response to progesterone appears to be the result of interplays of complexes mechanisms that could include deregulation and compensatory events associated with the reactivity to dopamine.

Progesterone and its metabolites seems to function as conspicuous neuroprotective molecules into the brain, particularly related to ischemic insults.[28] These steroids seem to reduce the oxidative stress,[25] decrease the cerebral edema,[24] and protect against glutamate toxicity.[25] These actions are thought to avoid the inflammatory process induced by 6-OHDA.[29] Since with our current experiments, we cannot ensure that the effects we observe are due to direct actions of progesterone or indirect action through its metabolites, we consider as an important issue to extend further studies to elucidate these possible differences.

Particularly pertinent in the context of the present study are the effects of progesterone on the activity of the main systems of neurotransmission involved in Parkinson's disease. These effects might implicate neuroprotective actions against physiopathological plastic changes associated with the neurodegenerative disease. These changes would include reduction of the density of dendritic spines on striatal neurons and alterations on the D1 and D2 receptors expression that alter corticostriatal transmission.[30] From our results, we can conclude that progesterone would protect the dopaminergic system to develop the changes described before and thus would prevent the consequent glutamatergic pathway deregulation. Taking into account that there is yet no effective treatment to stop the progression of Parkinson's disease, and that our present and previous[16] results indicate a positive modulatory effect of progesterone on the activity of the dopaminergic system, we are prompted to consider this hormone as a good candidate to further preclinical investigations tending to elucidate possible therapeutic actions.

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