Effects of prolactin on *in vivo* striatal monoaminergic activity are modulated by a previous reproductive experience

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Abstract

Central prolactin (PRL) modulates neuronal activity, which is physiologically relevant and behaviorally meaningful. The stimulatory or inhibitory behavioral effects of exogenous PRL are strongly associated with dose and time of treatment. Central PRL injections produce a dual modulation of striatal dopaminergic responses in males. The activity of the striatal monoaminergic system can be modulated by a previous reproductive experience in females. The objective of the present study was to test *in vivo* the acute and 5 day-treatment effects of central PRL injections on the striatal dopaminergic and serotoninergic terminals activity in age-matched nulliparous and primiparous females. Seven primiparous and 6 nulliparous rats were stereotaxically implanted with guide cannulas into the lateral ventricle and into the contralateral striatum. Five daily intracerebroventricular injections of ovine prolactin (oPRL;10 mg/5 ml) were performed. On days 1 and 5, females were submitted to striatal microdialysis sessions. The concentrations of dopamine and serotonin metabolites in the dialysate were measured by HPLC-ED. Acute oPRL injection induced a decrease in extracellular levels only for HVA concentrations which was more intense in primiparous than in nulliparous dams. DOPAC concentrations were increased by PRL injection in primiparous compared to nulliparous dams on day 5. On this day DOPAC, HVA and 5HIAA primiparous baseline dialysate concentrations were significantly higher than in nulliparous animals. These data suggest that reproductive experience can modulate *in vivo* striatal dopaminergic responses to PRL and reveal a relation between striatal dopaminergic and serotoninergic responses that is suggestive of a similar PRL modulation of both neurotransmitter terminals.

Key words:
Pregnancy.
Microdialysis.
Dopamine.
Serotonin.
Striatum

Introduction

Prolactin (PRL) is a peptide that acts centrally and influences grooming [1,2,3], sexual [4, 5, 6, 7, 8], maternal [9, 10, 11] and stereotyped behavior [12, 13]. PRL's effects on behavior are strongly associated with dose and time of treatment [6, 7, 14]. The low-dose concept was introduced in the late 1970s to describe the effects of some neuropeptides such as PRL, vasopressin, oxytocin and analogues that can cross the blood - brain barrier at low doses and induce strong and long-lasting behavioral changes in male and female animals [15]. Intracerebroventricular (icv) or subcutaneous injections of PRL produce an inverted U-shape dose-response curve [14, 16]. Thus, acute or long-term central PRL injections respectively have stimulatory and inhibitory effects on male sexual behavior possibly by opposite effects on dopaminergic neurotransmission [6, 7, 14]. The effect of PRL on sexual behavior has been widely investigated and has attracted great interest because of its relationship with sexual impotence. Hyperprolactinaemia is associated with sexual disturbances in men [17, 18] and women [19]. In contrast, PRL is

increased before mating in males [20] and during sexual intercourse in men [21]. Dual modulation of PRL central effects after icv injections in male rats was reported earlier by our group [7]. Acute central PRL injection increases, while a 5 day treatment with central PRL injections decreases, extracellular striatal levels of various dopamine metabolites as measured by *in vivo* microdialysis [7]. These changes were correlated with respectively facilitatory and inhibitory effects on sexual behavior in male rats [7]. Paradoxical data were obtained in females, which showed that PRL release after mating and genitosensory stimulation [22]. Lordosis inhibition after central PRL injection or chronic hyperprolactinaemia has been demonstrated [4, 23]. The physiological loss of sexual activity during lactation is related to PRL levels [24]. Also, hyperprolactinaemic women have low sexual activity [25]. On the other hand, lordosis can be stimulated after PRL infusion into the midbrain [26]. Dual modulation of PRL is also observed in grooming behavior. Within this context, low physiological concentrations of PRL are behaviorally meaningful, while high doses are not effective [14]. In addition, PRL is effective in inducing maternal behavior after central injections [10]. In females, serum PRL levels are decreased as well as dopaminergic activity can be increased by a previous reproductive experience [12, 13, 27, 28, 29, 30]. The reproductive experience itself is also behavioraly meaningful [12, 31, 32, 33]. Circulating PRL levels in women are reduced as a function of reproductive experience for over a decade [34,35], reducing the incidence of breast cancer in parous women [35]. Further, PRL release elicited by dopaminergic antagonists such as haloperidol and metoclopramide are less intense in women [35] and rats [36] after a reproductive experience. The haloperidol-induced increase in striatal dopaminergic activity is less intense in experienced females and in multigravid animals as compared to virgin and primigravid animals, respectively [28, 29, 30]. Suckling-induced PRL secretion is reduced during lactation in multiparous females [37]. Also, diurnal and nocturnal PRL surges are less intense in multigravid than in primigravid female rats [27]. In addition, the influence of parity on monoaminergic activity, including dopamine and serotonin, was demonstrated for the olfactory bulb [38, 39], hypothalamus [30], and striatum [28, 29].

Taken together, these data suggest that PRL induces neural adjustments especially in monoaminergic neurotransmission as well as reproductive experience in females. In order to assess the influence of reproductive experience on PRL-induced changes in striatal monoaminergic activity as already tested in males [7], age-matched virgin and experienced dams were compared *in vivo* using freely moving animals. The present set of experiments was designed to test the acute and 5-treatment day effects of central PRL on the striatal dopaminergic and serotoninergic activity in parous females.

Material and Method

Subjects

Age-matched female Wistar rats, 90 days old at the beginning of the experiments, were randomly housed in polypropylene cages under a 12/12 h light-dark cycle (lights on at 0600 h). Food and water were available *ad libitum* throughout the experiment. Experimental procedures were in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brasil. Half of the original set of females was mated, allowed to give birth (litters were culled to 6 pups), to raise their litters to weaning (21 days) and to rest for 15 days. This was called the primiparous group while the nulliparous group was kept waiting so that we obtained 2 age-matched groups. After the resting period, both groups were anaesthetized (xylazine, 5 mg/kg and ketamine, 25 mg/kg) and stereotaxically implanted with two guide cannulas: one for intracerebroventricular injections (Plastics One, USA) directed at the lateral ventricle according to the stereotaxic co-ordinates of the Paxinos and Watson Atlas [40]:

(AP) = -0.8; (DV) = +1.2 and (ML) = -1.2; and the other used for microdialysis into the striatum of the contralateral brain hemisphere (Bioanalytical Systems Inc. USA, CMA/11) according to the caudatus putamen co-ordinates [40]: (AP) = +0.2; (DV) = -3.4 and (ML) = +3.0. Females were not ovariectomized and estrus cycles were registered and randomized within groups.

Drugs

Prolactin from ovine pituitary glands (oPRL; SIGMA; 32 UI/mg) was prepared as an aqueous solution for icv injection. Used dose of oPRL was 10 mg icv, which was earlier showed to be significant in terms of behavior [10].

Microdialysis procedures

Seven primiparous and six nulliparous females were submitted to two microdialysis sessions separated by a 5 day interval. Microdialysis probes were inserted through the guide cannulas immediately before each session. Baseline was obtained after 3 consecutive perfusates with less than 5% variation between them on dopaminergic and serotoninergic metabolites concentrations. On day 1, during the first microdialysis session, after baseline, the females first received an icv injection of 5 ml of saline, followed by ovine prolactin (oPRL; 10 mg/5 ml) 2 h later. Injections were performed slowly (Harvard Pump, USA) over a period of 10 minutes. Saline injection was used to as an injection control. Microdialysis samples were obtained by perfusing Ringer (1.5 ml/min; Harvard Pump, USA) and collected every 20 min into *eppendorf* tubes containing perchloric acid. Perfusates were than frozen to -80°C for later HPLC-ED quantification of dopamine, serotonin and their metabolites. On days 2, 3 and 4, only icv oPRL injections (10 mg/5 ml/day) were performed. On day 5, the last icv oPRL injection (10 mg/5 ml) was performed during the second microdialysis session after baseline and saline injection (5 ml) as performed on day 1. Saline injection was performed to observe possible influences of injections itself on neurotransmitters extracellular concentrations so that each female group was its own control. During the microdialysis sessions the animals were conscious and freely moving. Cannula placements were checked after all experimental procedures.

HPLC-ED neurotransmitters quantifications

The chromatography system (Shimadzu 10A) was of reversed phase with ionic pairment with a line filter. A C18 column (Supelco), a sample injector of 20 ml and an electrochemical detector (Decade, Antec Leyden) were attached to the system. The mobile phase was a citrate-phosphate buffer adjusted to pH 2.8 with ortophosphoric acid with sodium EDTA (40 mg/ml) and heptanosulfonic acid (556 mg/ml) and methanol (80 ml/L). The mobile phase was degassed with helium and the flow rate was 1.4 ml/min. The column temperature was kept at 55°C. Dopamine (DA) metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and serotonin (5HT) metabolite 5-hydroxyindoleacetic acid (5HIAA) were recognized by their retention time compared to standards. The time for each sample was 8 min. The limit of detection was 0.52 ng/ml (DOPAC), 0.59 ng/ml (DA), 0.59 ng/ml (5HIAA), 2.4 ng/ml (5HT) and 0.56 ng/ml (HVA). DA and 5HT were below limit of detection and therefore data were not presented.

Statistical Analysis

Statistical analysis software package was used (GraphPad Instat Version 3.01, 1998). The extracellular concentrations (ng/ml) of DOPAC, HVA and 5HIAA obtained after striatal microdialysis during dialysis (400 min; dialysates collected every 20 min), were compared by Repeated Measures ANOVA followed by Tukey-Kramer Multiple Comparisons Test. Baseline period and after icv oPRL injections average neurotransmitters metabolites concentrations (ng/ml) obtained in nulliparous and primiparous on day 1

compared to day 5 were compared by One-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test. Also, Student *t* Test was used to compare nulliparous and primiparous average metabolites concentrations. No interactions between estrus cycle and reproductive experience were found by a Two-Way ANOVA, so that estrus cycle was not considered. Results were considered significant if $p < 0.05$. Data from all animals were included in the analysis, although when dialysates concentrations were not detected in a specific sample, data were excluded, thus number of animals and degrees of freedom can be different depending on specific metabolite or day.

Result

During the first microdialysis session, acute icv oPRL injection induced no significant changes in extracellular concentrations of nulliparous and primiparous rats for 5HIAA. Nevertheless, a significant decrease in the extracellular concentrations of HVA after oPRL injection was observed in primiparous ($p < 0.0001$, $F_{19,95} = 5.71$; Repeated Measures ANOVA). After the second microdialysis session, the effects of 5 days of oPRL treatment revealed significant differences in extracellular concentrations of DOPAC in experienced females ($F_{19,114} = 8.71$, $p < 0.0001$; Repeated Measures ANOVA). Thus, prolactin-induced significant differences between nulliparous and primiparous females were showed on day 1, a significant decrease in average HVA concentrations in primiparous (78.70 ± 4.19 ng/ml) compared to nulliparous (118.99 ± 15.56 ng/ml; $p = 0.0082$; $t_{97} = 2.70$; Student ***t*** Test; Figure 1). On day 5, DOPAC average extracellular concentration (52.18 ± 4.28 ng/ml) in primiparous group was higher than in nulliparous females (34.61 ± 2.97 ng/ml; $p = 0.0001$; $t_{88} = 3.36$; Student ***t*** Test; Figure 1). Also, average concentrations of metabolites after 5 day oPRL injection are reduced in both groups, it is less intense in primiparous group (DOPAC: $p < 0.001$; $F_{3,176} = 21.306$; HVA: $p < 1.001$, $F_{3,185} = 48.68$; 5HIAA: $p = 0.0005$, $F_{3,185} = 6.153$; One-way ANOVA; Figure 1)

When we look at the baseline and

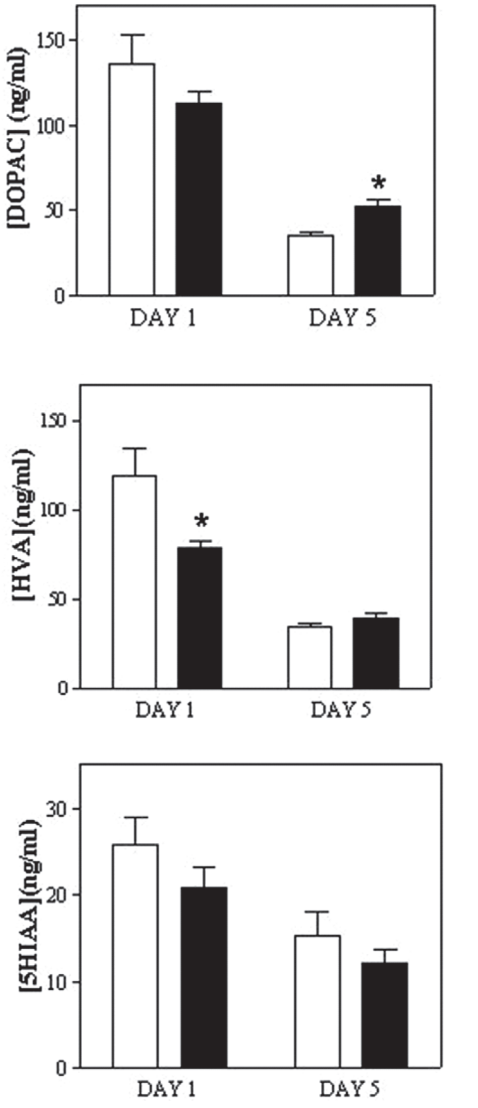

Figure 1 - Average extracellular striatal concentrations (ng/ml) of DOPAC, HVA and 5HIAA after PRL injection quantified by HPLC-ED in nulliparous (open bars; n = 6) and primiparous (solid bars; n = 7) female rats. On DAY 1, animals were submitted to the first microdialysis session simultaneously with the first icv oPRL injection. On days 2, 3 and 4 only icv oPRL daily injections were performed. On DAY 5, animals were submitted to the second microdialysis simultaneously with the fifth daily icv oPRL injection. Data are reported as means ± SEM of dialysates obtained during the microdialysis sessions. (*) indicates significant differences compared to nulliparous rats on day 1 or day 5

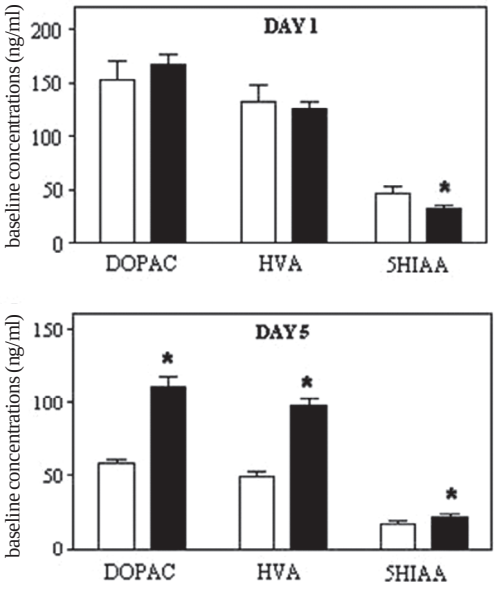

Figure 2 - Average striatal extracellular concentrations (ng/ml) of DOPAC, HVA and 5HIAA quantified by HPLC-ED during the microdialysis baseline period in nulliparous (open bars; n = 6) and primiparous (solid bars; n = 7) female rats. On DAY 1, animals were submitted to the first microdialysis session simultaneously with the first icv oPRL injection. On days 2, 3 and 4 only icv oPRL daily injections were performed. On DAY 5, animals were submitted to the second microdialysis simultaneously with the fifth daily icv oPRL injection. Data are reported as means ± SEM of dialysates obtained during the microdialysis sessions. (*) indicates significant differences compared to nulliparous rats on day 1 or day 5

after saline injections metabolites concentrations (Figure 2), on the first day of the test, average baseline concentrations of 5HIAA were significantly lower in perfusates from primiparous (30.66 ± 2.61 ng/ml) than from nulliparous (45.31 ± 6.06 ng/ml) animals ($p = 0.021$; $t_{75} = 2.35$, Student *t* Test) only for baseline period. On the other hand, baseline concentrations during the second microdialysis session after 5 days of daily central oPRL injections were increased in primiparous compared to nulliparous females for DOPAC (nulliparous: 58.44 ±3.07 ng/ml; primiparous: 110.58 ± 7.18 ng/ml; $p = 0.0009$; $t_{74} = 3.45$; Student ***t*** Test), HVA (nulliparous: 17.27 ± 1.94 ng/ml; primiparous: 21.42 ± 2.20 ng/ml; $p = 0.0001$; $t_{75} = 4.24$; Student ***t*** Test) and 5HIAA (nulliparous: 49.55 ± 3.62 ng/ml; primiparous: 98.65 ± 4.01 ng/ml); $p = 0.0125$; $t_{75} = 2.56$; Student ***t*** Test; Figure 2). Thus, at the beginning of the second microdialysis session, primiparous rats differed from nulliparous rats so that, both nulliparous and primiparous rats showed decreased DOPAC and HVA concentrations compared to the first dialysis session (DOPAC: $p < 0.001$, $F_{3,76} = 22.95$; HVA: $p < 0.0001$, $F_{3,84} = 12.38$; One-Way ANOVA followed by Tukey-Kramer; Figure 2). For 5HIAA only in nulliparous group differences between day 1 and day 5 were found ($p = 0.015$, $F_{3,80} = 3.71$; One-Way ANOVA followed by Tukey-Kramer; Figure 2).

Discussion

PRL actions in the brain may be influenced by sex, period of treatment and also by reproductive experience, as it can be suggested by these results. Central PRL actions are relevant to striatal monoaminargic neurons [28, 29, 30]. The present results obtained from awaked freely moving females showed that striatal terminals activity can be modulated by reproductive experience both after acute and long term oPRL icv treatment. Soon after oPRL injection there was a decreased striatal concentration of the final dopamine metabolite HVA in primiparous animals which suggests that this group is more sensitive to acute PRL-induced modulation of striatal dopaminergic activity compared to virgin animals. On the other hand, after 5 day oPRL icv injections elicited increased striatal concentrations of another dopamine metabolite DOPAC in primiparous which in turn suggests that this group of animals is less sensitive to long-term PRL-induced modulation of striatal dopaminergic activity compared to nulliparous females. It was previously reported that icv PRL could modify striatal monoaminergic activity so that neurochemical parameters were reduced [41]. The same effect was observed after acute PRL icv injection. Further, parity could increase this effect.

In males, it was showed that after

acute or 5 day icv oPRL injection there was an increase and a decrease in striatal monoaminergic activity, respectively [7], which was not observed in females. This is probably due to differences in dopaminergic activity and circulating PRL levels between male and female rats. Lower PRL circulating levels [42, 23] and decreased TIDA activity [43, 44] are found in males compared to females. Neuroleptics also have a less intense effect in males compared to females [23, 45]. Thus, TIDA activity is more intense in females, possibly as a compensatory mechanism consequent to the high circulating levels of PRL. Thus, more dopamine is necessary to control more circulating PRL. If dopaminergic activity is increased in the hypothalamus, it may be modified in other brain areas as well. In fact, endogenous hyperprolactinaemia enhances amphetamine and apomorphine induced stereotypy [46], which is related to increased striatal dopaminergic activity [12, 47, 48]. The effects of PRL on striatal dopaminergic activity differ between females and males. Taken together, these facts can explain the different responses of females and males, so that, as also reported in previous studies, the acute responses of females to PRL were similar to the responses observed in males after 5 days of icv PRL injections [7, 49].

The decrease in striatal dopaminergic activity (HVA) after acute oPRL injection was more intense in primiparous than in nulliparous females. This difference may have been related to increased striatal dopaminergic activity [12, 13, 29] and lower circulating PRL levels [34, 35, 36] in this group. Thus, the effect of acute icv injection was more intense in experienced compared to virgin females. On the other hand, dopaminergic activity after long term oPRL treatment suggested a hyposensitivity of this neural mechanism in experienced females which is in accordance with previous data on hyperprolactinaemia [5].

We could also detect that on the day of acute PRL injection, no significant differences were found comparing average concentrations of dopamine metabolites between primiparous and nulliparous rats during baseline or after saline injection. A significant decrease in 5HIAA average concentration is observed in baseline concentration in primiparous females that can be a consequence of reproductive experience neural adaptations. Serotonin and 5HIAA tissue concentrations were studied before in striatal homogenates (unpublished data). Although no significant differences were detected then, taken together the present results could reveal that serotonin synthesis is not modified by reproductive experience, but its release can be modulated. This hypothesis also remains to be tested.

At the beginning of the second microdialysis session, baseline concentrations of neurotransmitter metabolites were higher in experienced females compared to virgin females. Saline did not elicit any striatal activity responses. This result could be a consequence of 5 days of central oPRL injections. The number of central PRL receptors is directly related to the plasma concentrations of this hormone [50]. Thus, increased PRL levels determine increased PRL binding sites [50] and this mechanism is responsible for hypothalamic modulation of TIDA activity [36]. Also, PRL is related to morphological and physiological functional changes in other brain areas, which can also affect behavior [4, 14, 24, 25, 46, 51, 52, 53]. Five days of central PRL injections reduced basal neurotransmitter concentrations in both experienced and virgin animals. This may have been a consequence of the five days of increased central oPRL levels. This reduction was less intense in primiparous females, suggesting that long-term oPRL treatment was less effective in this group or primiparous striatal dopaminergic terminals were less responsive then terminals of virgin rats to 5 days of oPRL injections. Also, on day 5 after oPRL injection during microdialysis, this peptide seemed to elicit only responses in primiparous females, so that differences observed during baseline and saline period disappeared after oPRL injection, especially because of its effect on primiparous. Thus, still primiparous are more sensitive to acute

injection, so that terminals respond immediately to injections itself, but they tend to be more resistant to long term adaptations. This could be an interesting hypothesis to be tested.

Also, if we look at PRL-induced changes after acute or long term treatments, we can observe that HVA average concentrations were decreased in primiparous after acute oPRL injection. This shows that observed changes in DOPAC concentrations were not so intense. In the second microdialysis, what we see is an increase in DOPAC average concentrations in primiparous compared to nulliparous females, which is an opposite result comparing to the first microdialysis session. This could reflect effects of 5 daily oPRL injections and differences in mechanisms of neural adaptation. These modifications in extracellular metabolite concentrations could also reflect changes in neurotransmitters synthesis.

These results also show a reproductive experience-induced modulation of extracellular concentrations of 5HIAA. These changes can result from compensatory mechanisms since serotonin is correlated to dopamine and changes in one neurotransmitter system could elicit alterations in the other one. Compensatory increase in 5HT turnover results from treatment with a dopamine uptake inhibitor [54]. Furthermore, the integrity of serotoninergic neurotransmission is important for dopaminergic neuronal activity, which can also increase PRL release as a consequence of decreased tyrosine hydroxylase a ctivity [55, 56]. On the other hand, since our results showed similar serotoninergic and dopaminergic responses after acute or 5 day oPRL injection, they suggest that PRL modulation of both neurotransmitter terminals is similar in striatal targets.

In conclusion, reproductive experience modulates PRL-induced changes *in vivo* extracellular striatal concentrations of dopamine and serotonin metabolites. This modulation is rather different than the one observed in males tested with the same experimental design [7]. Changes in peptide-induced striatal monoaminergic responses in experienced females may reflect adaptive mechanisms that may be relevant to physiological and pathological processes.

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Efeitos da prolactina na atividade monaminérgica estriatal in vivo são modulados por uma experiência reprodutiva prévia

Resumo

A prolactina (PRL) tem efeito modulatório sobre a atividade neuronal no sistema nervoso central, fato este relevante do ponto de vista fisiológico e comportamental. Os efeitos estimulatórios e inibitórios da PRL exógena estão fortemente relacionados com a dose e o tempo de tratamento. A injeção de PRL diretamente no sistema nervoso central pode levar a modulação da atividade dopaminérgica tanto aguda como prolongadamente, como já foi observado anteriormente em ratos. Por outro lado, esse sistema de neurotransmissão também pode ser modificado por uma experiência reprodutiva anterior em ratas. Portanto, o objetivo do presente estudo é verificar *in vivo* o efeito de uma única injeção de PRL ou injeções repetidas sobre a atividade

Palavras-chave:
Gestação.
Microdiálise.
Dopamina.
Serotonina.
Corpo estriado

dos terminais dopaminérgicos e serotoninérgicos em fêmeas nulíparas e primíparas da mesma idade. Para tanto, 7 ratas primíparas e 6 nulíparas foram submetidas a cirurgias estereotáxicas para a implantação de cânulas-guia para injeção intracerebroventricular (i.c.v.) no ventrículo lateral e para microdiálise no corpo estriado contralateral. Após o período de recuperação pós-cirúrgico, as ratas foram submetidas a injeções i.c.v. durante 5 dias consecutivos com PRL ovina (oPRL; 10 mg/ 5 ml). Nos dias 1 e 5, as fêmeas foram também submetidas a sessões de microdiálise que se iniciavam antes da injeção i.c.v.. As concentrações de dopamina, serotonina e seus respectivos metabólitos foram quantificadas nos dialisatos coletados a cada 20 min por meio de HPLC-ED. A injeção aguda de oPRL induziu uma diminuição nas concentrações extracelulares de HVA de maneira mais intensa em fêmeas primíparas comparadas as nulíparas. As concentrações de DOPAC estavam aumentadas no dia 5 após as injeções consecutivas de oPRL no grupo de primíparas comparadas às nulíparas. No dia 5, as concentrações basais de DOPAC, HVA e 5HIAA em primíparas foram significantemente maiores do que em nulíparas. Estes resultados são sugestivos que a experiência reprodutiva pode modular a atividade dopaminérgica estriatal *in vivo* em conseqüência a injeção de PRL e revelou uma relação entre a atividade dopaminérgica e serotoninérgica nesta região cerebral, o que pode ser sugestivo de que ambos os sistemas de neurotransmissão neste local podem ser modulados pela PRL.

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