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Turnover rate and stimulus-evoked release of dopamine by progesterone and *N*-methyl-D-aspartic acid in rat striatum during pregnancy

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Abstract

The proposed modulatory role of progesterone on dopaminergic nerve terminal activity in the striatum was examined in pregnant rats. Endogenous dopamine concentration and the in vitro effect of exogenous progesterone in association with *N*-methyl-D-aspartic acid (NMDA) upon [^{3}H]dopamine release from striatal slices were determined. Striatal dopamine and 3,4-dihidroxyphenylacetic acid (Dopac) contents on day 5 of pregnancy were significantly higher than those found at the other stages of pregnancy and proestrus. On days 5 and 15 of pregnancy, progesterone (400 nM) was able to enhance [^{3}H]dopamine release stimulated by NMDA (50 μM). A similar effect was found in striatal slices from proestrus rats. In contrast, progesterone was without an effect on days 1, 10 and 20 of pregnancy and postpartum. The results suggest that an increased synthesis and/or release of dopamine takes place on certain days of pregnancy and, simultaneously, that there is a significant increase in the responsiveness of striatal dopaminergic nerve terminals to excitatory inputs. They provide further support for a modulatory role of progesterone in relation with a glutamatergic action on dopaminergic activity in the corpus striatum.

Keywords: Striatum; Pregnancy; Dopamine; Progesterone; NMDA (*N*-methyl-D-aspartic acid); Glutamate

1. Introduction

There is growing evidence that the gonadal steroids influence dopaminergic transmission in the striatum (Becker, 1990; Morissette et al., 1990a,b; Pasqualini et al., 1995). In these studies, progesterone was suggested as a hormonal modulator of the nigrostriatal dopaminergic system (Dluzen and Ramírez, 1985). The action of progesterone seems to be partially of non-genomic nature and exerted on membrane binding sites (Tischkau and Ramírez, 1993). However, it is well known that excitatory amino acidergic pathways are closely related with the nerve endings of the nigrostriatal system in the striatum (Iversen, 1995).

In a recent paper from our laboratory we provided evidence of a possible interaction between glutamate and progesterone on dopamine release in the striatum. It was shown that progesterone enhanced the stimulatory effect of the glutamate agonist *N*-methyl-D-aspartate (NMDA) on dopamine release from striatal slices of proestrus rats superfused in vitro (Cabrera and Navarro, 1996) and, therefore, we proposed that progesterone may influence the afferent excitatory synaptic inputs.

In order to evaluate this hypothesis, the aim of this study was to test the effect of NMDA on dopamine release from rat striatal slices in vitro under a physiological condition whereby endogenous progesterone levels are elevated, such as the pregnancy. Superfusion experiments were done throughout pregnancy, from day 1st to day 20th. Dopamine concentration and its turnover in striatum were also determined.

2. Materials and methods

Adult Sprague–Dawley rats of 90–120 days old from our own breeding colony were used. They were housed in a temperature (22–23°C) and light (14 h light schedule; lights on at 06:00 a.m.) controlled environment with food and water available ad libitum. Females were mated in the evening of the proestrus day. On the following morning, pregnancy was confirmed by the presence of spermatozoa in the vaginal smears. This was designated as day 1 of pregnancy.

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All animal experiments were carried out in accordance with the guidelines of the National Institutes of Health (NIH), USA. Newborn pups were transferred to foster mothers.

2.1. Dopamine assays

The animals were decapitated in the morning (11:00 a.m.) and the brains were rapidly placed in a cold plate for dissection. The striata were removed and homogenized in a test tube containing 0.2 M perchloric acid, sonicated and centrifuged for 15 min. Dopamine and its primary metabolite 3,4-dihidroxyphenylacetic acid (Dopac) were determined in the clear supernatant by using a reverse-phase ion-paired HPLC column (C 3 μ) with electrochemical detection (LKB). The mobile phase consisted of 45 mM sodium phosphate, 0.43 mM sodium octyl sulphate, 0.34 mM EDTA and 20% acetonitrile, pH 3.5. The oxidation potential was set at 0.55 V. Tissue pellets were dissolved in 0.1 M NaOH for protein assay (Lowry et al., 1951). Results are expressed as nanograms of compound per mg protein.

2.2. Superfusion experiments

Animals were decapitated and the striatum was dissected out. Coronal slices 240 μm thick were obtained with a McIlwain tissue chopper. Six to 8 slices were preincubated in a Dubnoff shaker at 37°C for 30 min in Krebs–Ringer-bicarbonate-glucose buffer (KRB), pH 7.4, containing 25 nM [^{3}H]dopamine (specific activity 57 Ci/mmol) and saturated with 95% O_2 and 5% CO_2. The slices were then transferred to a superfusion chamber and superfused at 0.7 ml/min with KRB to washout the dopamine not incorporated into the tissue (30 min). After the washing period, 5 fractions of 2.5 min were collected and considered as basal release. Thereafter, tissue was superfused with KRB containing either progesterone (0.3 mg/ml; final concentration 400 nM) or its vehicle (propylene glycol) until the end of the experiment. Starting at time 12.5 min, the slices were exposed for 7.5 min to NMDA (50 μM). For superfusions, Mg^{2+}-free KRB buffer was used. At the end of the experiments, slices were homogenized in 3 ml 0.2 M perchloric acid, centrifuged, and aliquots of the supernatant of each fraction were collected and mixed with scintillation fluid to measure the radioactivity.

Total radioactivity was calculated as the sum of total tritium collected during the superfusion and the amount remaining in the tissue. The effects of NMDA on basal efflux were expressed either as a percentage of [^{3}H]dopamine evoked release or as a percentage of net release (for details, see Cabrera and Navarro, 1996).

2.3. Chemicals

Analytical grade reagents were purchased from Merck (Darmstadt, Germany). NMDA, dopamine and Dopac were purchased from RBI (Natick, MA, USA). Progesterone was from Sigma (St. Louis, MO, USA) and [^{3}H]DA from New England Nuclear (Boston, MA, USA).

2.4. Statistical analysis

Data were expressed as the means ± S.E.M. and analysed using the analysis of variance followed by Student–Newman–Keuls test for comparison between groups. Values of $P < 0.05$ were considered as significant.

3. Results

3.1. Endogenous dopamine in the striatum during pregnancy

During pregnancy, endogenous dopamine concentrations in striatum fluctuated ($F(6,39) = 6.65$, $P < 0.0001$) (Fig. 1A). They reached the highest level on day 5 ($P <$

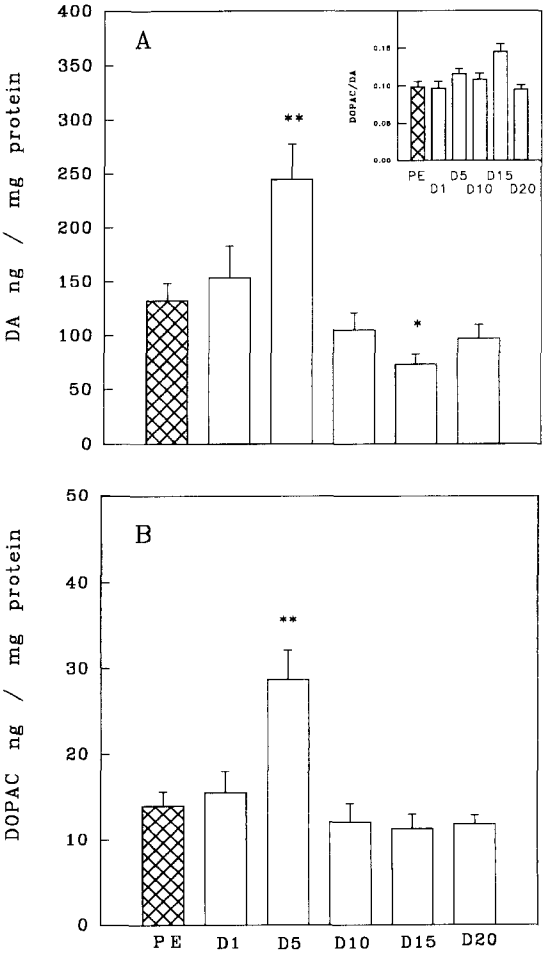

Fig. 1. Dopamine (A) and Dopac (B) concentrations in the rat striatum at proestrus (PE) and throughout the pregnancy. At bottom: day of pregnancy (D); PP: postpartum. Each column represents the mean ± S.E.M. for 8–9 rats. * $P < 0.05$ and ** $P < 0.01$ versus proestrus. The inset in panel A shows the Dopac/Dopamine ratio at PE and during the pregnancy.

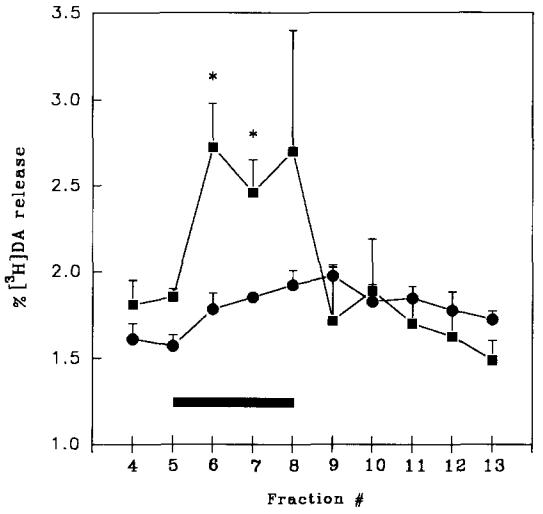

Fig. 2. Representative profiles of NMDA-induced [^{3}H] dopamine release from superfused rat striatal slices. (•) proestrus; (■) 15th day of pregnancy. Tritium efflux was evaluated in fractions collected every 2.5 min (bottom). As indicated by the horizontal bar, the NMDA (50 μM) stimulus was applied for 7.5 min. As depicted, NMDA markedly increased [^{3}H]dopamine release at day 15 of pregnancy. The points represent the mean value ± S.E.M. of 3 superfusions for each group. * $P < 0.05$ versus proestrus.

0.01 versus proestrus and other days of pregnancy), in coincidence with a peak of Dopac concentration ($P < 0.01$) (Fig. 1B). On day 15, dopamine levels decreased ($P < 0.05$) and turned back to proestrus levels on day 20.

3.2. NMDA and NMDA / progesterone-evoked [^{3}H]dopamine release

NMDA-evoked [^{3}H]dopamine release from rat striatal slices progressively increased from proestrus to the 10th day of pregnancy ($F(6,34) = 4.88$, $P < 0.001$). The magnitude of this effect was similar on days 10,15 and 20 of pregnancy ($P < 0.05$ versus proestrus). The profile of a superfusion experiment,in slices from proestrus and pregnancy day 15, depicted in Fig. 2, clearly shows the increase in tritium efflux as soon the slices were exposed to NMDA.

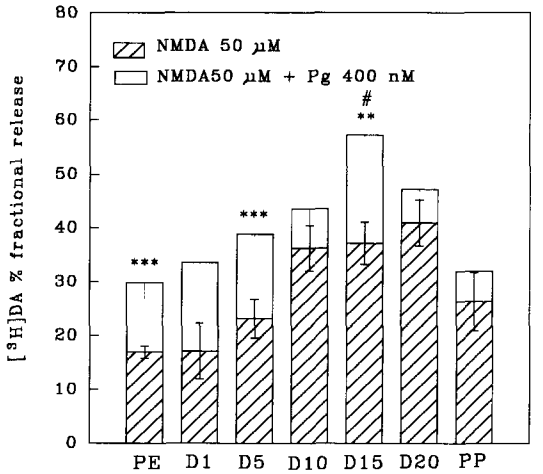

Fig. 3. Histograms illustrating the effects of NMDA (50 μM) and NMDA plus progesterone (400 nM) on [^{3}H]dopamine release from superfused striatal slices of proestrus (PE) and pregnant rats. At bottom, day of pregnancy (D); PP: postpartum. Each column represents the mean value ± S.E.M. for 5–7 experiments. * * $P < 0.01$ versus NMDA alone. * * * $P < 0.001$.

Progesterone (400 nM) enhanced the effect of NMDA on dopamine release ($F(7,38) = 5.32$, $P < 0.001$). The increase was over 50% on days 5 ($P < 0.001$) and 15 of pregnancy ($P < 0.01$) (Fig. 3). On days 1, 10 and 20 of pregnancy and postpartum, the same concentration of progesterone failed to modify the releasing action of NMDA (Fig. 3). In agreement with previous results (Cabrera et al., 1993), progesterone also increased the fractional [^{3}H]dopamine release at proestrus ($P < 0.001$),but the in absence of NMDA it was ineffective during pregnancy and proestrus (data not shown).

4. Discussion

In this paper, we report modifications in the responsiveness of nigrostriatal nerve terminals in rats during pregnancy. These results give further support to progesterone as a modulator of the glutamatergic–dopaminergic interactions in the striatum.

The study reveals a significant increase in dopamine and Dopac content in the striatum on day 5, in coincidence with the highly significant increase of [^{3}H]dopamine release evoked by NMDA and further, its enhancement by progesterone. The increased levels of dopamine and its metabolite on day 5 of pregnancy could be interpreted as an increased activity of the dopaminergic system as a consequence of gonadal hormonal action. Estrogen can modulate the dopaminergic activity by inhibiting dopamine uptake in cortical synaptosomes (Michel et al., 1987). Low doses of 17-beta-estradiol increase the number, but not the affinity, of reuptake sites in striatal dopaminergic terminals (Morissette et al., 1990b). Among the acute effects of estradiol, changes in dopamine turnover and affinity states of D_2 receptors have been described in the striatum and n. accumbens of rats (Levesque and Di Paolo, 1988). Furthermore, it has been found that exogenous progesterone injected at low doses is able to increase striatal dopamine and Dopac concentrations (Di Paolo et al., 1986) and extracellular dopamine concentrations (Petitclerc et al., 1995). Moreover, at higher doses, progesterone did augment the density of striatal dopamine D_2 receptors (Fernández-Ruiz et al., 1989).

[^{3}H]dopamine release from striatal slices was used as an index of responsiveness to the excitatory amino acid NMDA and to the combined exposure to NMDA and progesterone. Throughout pregnancy the action of progesterone was discriminative. In spite of the maximal stimulatory properties of NMDA on dopamine release from days

10 to 20, progesterone 'in vitro' was effective in enhancing the action of NMDA only on days 5 and 15 of pregnancy. The effect of NMDA after day 10 of pregnancy may be explained by an increase in synaptic activity as a result of the action of gonadal hormone on the neuronal network (Brinton, 1993; Gould et al., 1990; Woolley and McEwen, 1992).

A possible explanation for the variability in the 'in vitro' responses to progesterone could be an interactive action among the rapid and slow mechanisms of action of the gonadal hormone (Minami et al., 1990; Pfaff, 1989). These interactions could result in enhanced responses during selected days of pregnancy or during the female reproductive cycle (Smith et al., 1988). Another important point to be considered is a change in the priming action of estrogen which may modify the sensitivity to the action of progesterone 'in vitro' (Cabrera et al., 1993). It is probable that the failure of exogenous progesterone to facilitate NMDA-induced [^{3}H]dopamine release on days 1 and 10 of pregnancy and postpartum is associated with a decreased priming action of estrogen due to low ovarian secretion rate (Shaikh, 1971; Yoshinaga et al., 1969).

However,this point merits further investigation since, in an opposite situation on day 20 of pregnancy, when estrogen and progesterone secretion reach high levels (Tellería and Deis, 1994; Shaikh, 1971), no enhancement of NMDA action by progesterone was found. Therefore, other factors than estrogen should be taken into consideration.

The physiological significance of the dopamine-releasing effects of excitatory amino acids on the striatum is partially known. There is consistent evidence in favour of a glutamatergic stimulatory action, mediated mainly by NMDA receptors, upon dopaminergic nigrostriatal afferents (Krebs et al., 1991; Westerink et al., 1992; Iversen, 1995). In vivo microdialysis studies have indicated that the endogenous excitatory transmitter glutamate does not exert a direct tonic effect on striatal dopamine release (Moghadan and Gruen, 1991; Keefe et al., 1993), but it is possible that under conditions where endogenous progesterone secretion is increased, glutamate is an important trigger for enhanced dopamine release. In addition, as has been observed for $GABA_A$ receptors (Lan et al., 1991; Gee and Lan, 1991), the various subunits of the NMDA receptor could have different sensitivities to the non-genomic membrane effects of ovarian steroids throughout pregnancy. Therefore, certain subunits of these receptors could be expressed during day 5 and day 15 in coincidence with increases in endogenous progesterone between days 3–6 and days 15–17 of pregnancy (Forcelledo et al., 1982; Morishige et al., 1973). We speculate that on days 1, 10 and 20 the NMDA receptors lack the progesterone sensitive subunits.

We conclude that on certain days of pregnancy, there is a lowering of the threshold for glutamate stimulation of nigroestriatal dopaminergic nerve terminals and this may be a consequence of fluctuations in the responses of both the glutamatergic and dopaminergic systems to the activity of gonadal hormones.

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