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Striatal dopamine release stimulated by amphetamine or potassium: influence of ovarian hormones and the light–dark cycle

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The release of endogenous dopamine (DA) from rat striatal tissue was studied in an *in vitro* superfusion system following hormonal manipulations *in vivo*. Progesterone treatment in estrogen-primed ovariectomized female rats potentiated DA release stimulated either by amphetamine or potassium (K^+). In addition, the amount of striatal DA released in response to K^+ -stimulation was influenced by the light–dark cycle. We conclude that striatal DA release is modulated by ovarian hormones, and suggest that ovarian hormone modulation of presynaptic striatal DA activity may contribute to well-known estrous cycle dependent variations in some non-reproductive behaviors.

Endogenous ovarian steroid hormones may influence the functional activity of mesostriatal dopamine (DA) neurons by modulating presynaptic DA activity^{1,4}. For example, in the female rat, treatment with a synthetic estrogen–progesterone combination increases DA synthesis and turnover¹. In addition, the amphetamine (AMPH)-stimulated release of endogenous DA from striatal tissue *in vitro* varies with the estrous cycle, and is attenuated by ovariectomy (OVX)⁴. Progesterone treatment in estrogen-primed OVX rats reinstates normal levels of AMPH-stimulated striatal DA release⁴. However, it is not known whether this effect of ovarian hormones is specific to AMPH-stimulated DA release. To understand how ovarian hormones influence striatal DA release, it is important to determine if they also modulate the release of DA stimulated by other mechanisms. In the experiment reported here we compared the influence of progesterone treatment in estrogen-primed OVX rats on AMPH-stimulated non-vesicular DA release¹⁵ with the exocytotic DA release induced by depolarization with high extracellular concentrations of potassium (K^+). In preliminary studies, we noted that the light–dark cycle had an influence on DA release. Therefore, the effects of ovarian hormones on

striatal DA release were studied under 2 different lighting conditions.

Adult female Holtzman rats (Madison, WI) were individually housed on a 14 h–10 h, light–dark cycle in 1 of 2 colony rooms: (1) normal light–dark cycle, lights on at 06.00 h; (2) reversed light–dark cycle, lights off 08.30 h. Two weeks after OVX the animals received either 3 daily injections (s.c.) of peanut oil (0.1 ml) or 5.0 μ g estradiol benzoate (EB) in peanut oil. On the 4th day they received an injection of either oil or 1.2 mg of progesterone (P) in oil between 06.00 and 09.00 h, and were then decapitated 4 h later. Therefore, half of the animals were killed when lights were on in the colony room (OVX[oil]:light, $n = 6$; and OVX[EB+P]:light, $n = 5$) and half when the lights were off (OVX[oil]:dark, $n = 5$; and OVX[EB+P]:dark, $n = 5$).

Following decapitation, striatal tissue was rapidly removed from the brain, chopped into 1 mm³ pieces and placed in a superfusion chamber that was positioned in a constant temperature bath (37 °C). A modified Krebs-Ringer phosphate medium (pH 7.4) flowed through the chamber at a rate of 100 μ l/min. After a 75-min stabilization period, samples of the effluent were collected over 5-min intervals. These ef-

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fluent samples were assayed for DA concentrations by high performance liquid chromatography with electrochemical detection. (For a detailed description of techniques see ref. 7). Normal medium was infused for the first 5 min (interval 1). At the beginning of interval 2, medium containing 10 μ M D-amphetamine sulfate (AMPH) was infused for 2.5 min, followed by normal medium through interval 6. At the beginning of the 7th interval, medium containing 60 mM K⁺ (Na⁺ in the medium was reduced) was infused for 2.5 min, followed by normal medium through interval 9. These doses and infusion times were based on extensive dose-response studies conducted previously⁷. Release rate during each 5-min interval was expressed as pg DA/mg tissue/min. The data were analyzed by 2 separate three-way analyses of variance, the 3 factors being: (1) hormone treatment (oil vs EB+P); (2) light cycle (light vs dark); and (3) time (intervals 1–5 were analyzed to evaluate the effect of AMPH on DA release, and intervals 6–9 were analyzed to evaluate the effects of K⁺ stimulation).

There were no differences between the 4 groups in the basal rate (interval 1) of endogenous DA efflux (OVX[oil]:light 12.8 \pm 2.3, pg/mg/min \pm S.E.M.; OVX[EB+P]:light 13.5 \pm 3.3; OVX[oil]:dark 10.1 \pm 1.6; OVX[EB+P]:dark 10.4 \pm 2.3; $F(3,17) = 0.56$). As expected, 10 μ M AMPH infusion stimulated the release of DA in all groups ($F(3,51) = 45.3$, $P < 0.001$; Fig. 1). However, animals pretreated with estrogen plus progesterone released significantly more DA in response to AMPH stimulation than did oil-treated controls (hormone treatment \times time interaction, $F(4,68) = 3.22$, $P = 0.017$; see Fig. 1). Although this effect of hormone treatment was independent of the light cycle (hormone treatment \times light cycle \times time interaction, $F(4,68) = 0.68$), there was a non-significant trend for AMPH-stimulated DA release to be higher in animals killed in the light than in those killed in the dark ($F(4,68) = 2.03$, $P = 0.098$).

Depolarization of striatal tissue with K⁺ also stimulated the release of DA in all groups ($F(3,51) = 45.3$, $P < 0.001$; Fig. 1). However, K⁺-stimulated DA release was influenced by both hormone treatment and the light–dark cycle. Animals pretreated with estrogen plus progesterone released significantly more DA in response to K⁺-induced depolariza-

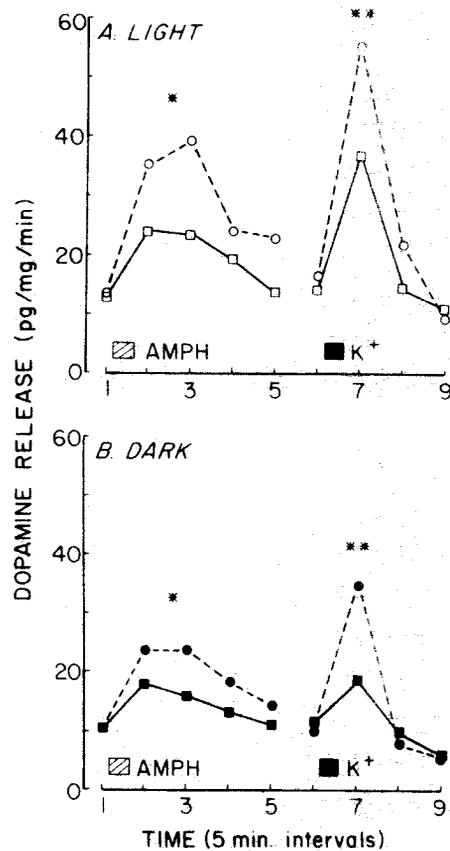


Fig. 1. The influence of light–dark cycle and estrogen plus progesterone (EB+P) treatment on striatal dopamine (DA) release stimulated by the infusion of 10 μ M D-amphetamine (▨ AMPH) or 60 mM KCl (■ K⁺). A: during the light period of the light–dark cycle (□–□ : EB+P; ○—○ : OIL); B: during the dark period of the light/dark cycle (■–■ : EB+P; ●—● : OIL). Animals pretreated with EB+P released significantly more DA in response to AMPH stimulation than did oil treated controls ($P = 0.017$). K⁺-stimulated DA release was significantly influenced by both hormone treatment and light cycle. There was greater K⁺-stimulated DA release in EB+P treated animals than in oil treated controls ($P = 0.004$) and there was a greater DA response to K⁺ stimulation during the light than during the dark ($P = 0.019$). See text for details.

tion than did oil-treated control animals, regardless of the light–dark cycle (hormone treatment \times time interaction, $F(3,51) = 4.88$, $P = 0.004$; Fig. 1). In addition, K⁺ induced more DA release in animals killed during the light than in those killed during the dark, regardless of hormone treatment (light cycle \times time interaction, $F(3,51) = 3.59$, $P = 0.019$; Fig. 1).

AMPH and K⁺ stimulate DA release by dissociable mechanisms. K⁺-stimulated DA release in vitro is calcium- and temperature dependent, and therefore

is thought to involve exocytosis^{3,7,17,19}. In contrast, AMPH-stimulated DA release is temperature- and sodium dependent, but calcium-independent^{7,10,17}. These observations have been interpreted as indicating that AMPH releases non-vesicular DA by an energy-dependent mechanism relying on the integrity of the neuronal membrane. Since both AMPH- and K⁺-stimulated DA release were potentiated by hormone treatment, the effect of estrogen plus progesterone is probably not on the vesicular/nonvesicular distribution of DA. Instead, it would seem that ovarian hormones produce a more general increase in the responsiveness of striatal DA neurons to stimulation. Whether gonadal hormones act directly on nigrostriatal DA neurons, or influence striatal activity only by indirect means remains to be determined.

The influence of the light-dark cycle on K⁺-stimulated DA release was not entirely unexpected because other indices of striatal DA activity are known to show ultradian rhythms. For example, striatal DA concentrations, synthesis and turnover are increasing when the lights go on and begin to fall when the lights go off^{9,14,18}. This is consistent with our observation that K⁺-stimulated DA release was greater when the lights were on, a time when metabolic activity is on

the increase, than when the lights were off and metabolic activity is decreasing. The finding that basal DA release was not influenced by the light cycle or hormonal treatment is not surprising, since basal DA efflux is both calcium- and temperature-independent⁷. Therefore, basal DA efflux would not necessarily be expected to reflect changes in striatal DA metabolism.

In closing, we would like to emphasize that the doses of estrogen and progesterone used in this experiment produce blood levels of these hormones within the normal physiological range^{8,12}. This is in contrast to the pharmacological doses required to produce measurable changes in striatal DA receptor binding^{11,13}. Therefore, it is very possible that ovarian hormone modulation of presynaptic striatal DA activity may underlie estrous cycle-dependent variations in behaviors mediated by the mesostriatal system^{5,6,16}, and in some other non-reproductive behaviors known to be influenced by ovarian hormones².

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