Infusion of Dopamine at Low Concentrations Stimulates the Release of Prolactin from α-Methyl-*p*-Tyrosine-Treated Rats (43573)

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Abstract. **Prolactin (PRL) secretion from the anterior pituitary gland is inhibited by dopamine (DA) released into the hypophysial portal vasculature from neurons in the hypothalamus. We have shown previously that DA also stimulates PRL secretion *in vitro*. Here we report that DA has a dual effect on PRL release *in vivo*. Injection of rats with α-methyl-*p*-tyrosine (200 mg/kg, ip) induced an immediate 35-fold enhancement of PRL secretion which reached a plateau by 90 min after injection on diestrus 1. When DA was infused intravenously at varying doses beginning at 90 min after α-methyl-*p*-tyrosine, differing effects on PRL secretion were observed. These effects were dose dependent: higher doses of DA (1000 ng/kg/min) inhibited and lower doses (10 ng/kg/min) stimulated PRL secretion. These data suggest that DA may be an important stimulator of PRL secretion *in vivo*.** [P.S.E.B.M. 1993, Vol 203]

Prolactin (PRL) is found in many vertebrates and is important to the regulation of such diverse physiologic processes as reproduction, immune responses, osmoregulation, and promotion of growth as well as behaviors such as migration and nurturing of young. Secretion of PRL from the anterior pituitary gland is under dominant inhibitory control by the hypothalamus, presumably due to dopamine (DA) of hypothalamic origin (1). DA is able to potently inhibit PRL secretion both *in vitro* and *in vivo* (2–4). DA is present in hypophysial portal plasma (5) in concentrations great enough to account for the low basal secretion of PRL (3, 6). The concentration of DA in portal plasma is inversely related to PRL concentrations in peripheral plasma (5, 7–9). DA secretion into the portal vasculature decreases in response to simulated PRL-releasing stimuli such as mammary nerve stimulation or copulomimetic stimulation, but this decrease is not great enough to account for the magnitude of PRL released (7–10). Thus, it has been proposed that physiologic PRL secretion occurs as a result of a delicate balance between a diminution of DA release into portal blood and an enhanced secretion of a hypothalamic PRL-releasing factor(s) (7–10).

The inhibitory effect of DA on PRL secretion is mediated by specific DA receptors located on the plasma membrane of PRL-secreting cells, lactotrophs, in the anterior pituitary (11, 12). These DA receptors have been further characterized by ligand-binding studies to be exclusively of the D_2 subtype (13, 14). Our laboratory (15) and others (16–19) have shown that DA is also capable of *stimulating* PRL release from lactotrophs in culture. The stimulatory effects of DA are dose dependent and mediated by a D_2 receptor unique from that which mediates the inhibitory effects (15, 17). However, the physiologic significance of these observations is questionable in the absence of evidence of this dual regulatory effect *in vivo*. Neill's laboratory has demonstrated that α-methyl-*p*-tyrosine (αMPT) treatment lowers the concentration of DA in portal plasma to undetectable levels as measured by high-performance liquid chromatography and eliminates 89% of the catecholamine oxidation current peak in the anterior pituitary gland using *in vivo* electrochemistry (3, 20).

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Peripheral infusion of DA at a dose of 1000 ng/kg/min in αMPT-treated rats not only resulted in portal plasma levels of DA equivalent to those of diestrous rats, but also returned PRL levels to those of diestrous rats (3). Using this same experimental paradigm, we demonstrate here that relatively low concentrations of DA *stimulate* PRL secretion *in vivo*.

Materials and Methods

Effect of DA Infusion in Varying Doses on PRL Secretion in αMPT-Treated Rats. Only female rats exhibiting at least two consecutive 4-day estrous cycles were used in this study. Right atrial and left carotid arterial cannulae were surgically implanted in female rats on estrus as described previously (21). The following day, Diestrus 1, rats were treated with the catecholamine synthesis inhibitor αMPT (200 mg/kg, ip, Sigma, St. Louis, MO) at 0930 hr. Ninety minutes after αMPT injection, the rats were infused intravenously for 45 min with saline containing varying concentrations of DA (1, 10, 100, and 1000 ng/kg/min) using a peristaltic pump (Gilson, Middleton, WI) at a flow rate of 4 μl/min. DA solutions were made immediately before infusion. Infusion of saline solution alone served as the control. Serial blood samples were obtained from the arterial cannula immediately before αMPT treatment, immediately before infusion (Time 0) and at 5, 15, 30, 45, 60, and 75 min after initiating the infusion. Serum was collected from blood samples by centrifugation and assayed for PRL by radioimmunoassay, as described previously (22).

Statistical Analyses. Secretory profiles of PRL were evaluated by analysis of variance with repeated measures. Differences between time points between treatment groups were evaluated by analysis of variance and Duncan's multiple range test.

Results

Effect of DA infusion in Varying Doses on PRL Secretion in αMPT-Treated rats. α-Methyl-*p*-tyrosine, when injected into female rats on Diestrus 1, caused an immediate 35-fold elevation of PRL secretion which achieved a plateau by 90 min after injection (Fig. 1). There was no further change of PRL secretion in Diestrus 1 rats infused with saline (Fig. 1). All experiments were started 90 min after αMPT injection, when PRL levels were stable. Diestrous female rats were infused with DA in various concentrations 90 min after intraperitoneal injection of αMPT (Fig. 1). DA infused at a dose of 1 ng/kg/min had no effect on PRL secretion. However, in rats infused with 10 ng/kg/min DA, PRL secretion increased within 5 min of the start of the infusion. This increase in PRL secretion peaked by 15 min and then quickly declined thereafter, although the infusion continued for an additional 30 min. The peak PRL response was approximately two times preinfusion

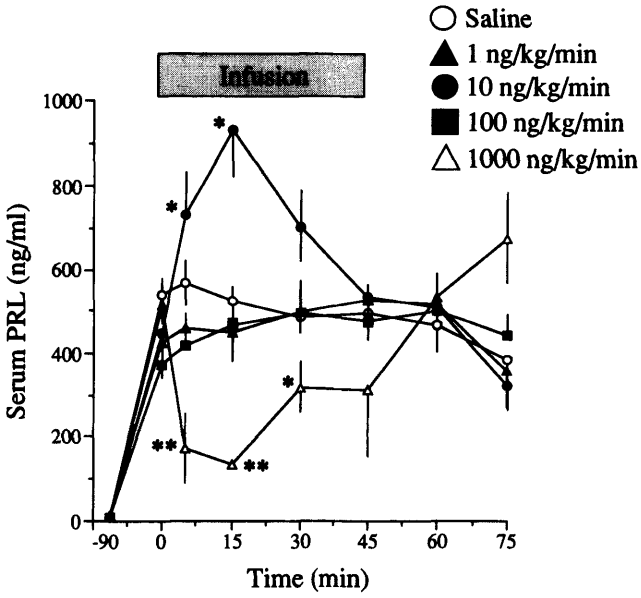

Figure 1. Effect of infusing varying concentrations of DA on PRL secretion in αMPT-treated rats. DA was infused (shaded box) into the atrial cannula at either 1, 10, 100, or 1000 ng/kg/min for 45 min using a peristaltic pump at a flow rate of 4 μl/min. Saline infusion served as the control. Serial blood samples were collected from the arterial cannula. Infusion of either saline (open circles), 1 ng/kg/min DA (closed triangles), or 100 ng/kg/min DA (closed squares) had no effect on PRL secretion. Infusion of 10 ng/kg/min DA (closed circles) significantly stimulated PRL release, whereas 1000 ng/kg/min DA (open triangles) significantly inhibited PRL release (n = 7–8 rats/group). *$P < 0.05$ versus saline-treated group at the same sampling time by analysis of variance. **$P < 0.01$ versus saline-treated group at the same sampling time by analysis of variance.

values. DA infused at a concentration of 100 ng/kg/min had no effect on PRL release. As expected from previous studies (3), infusion of 1000 ng/kg/min DA significantly inhibited PRL release. This dose of DA (1000 ng/kg/min) was capable of inhibiting PRL secretion by approximately 80%.

Discussion

Using the experimental paradigm first described by Gibbs and Neill (3) to demonstrate that DA inhibits PRL secretion *in vivo*, we have shown that relatively low concentrations of DA stimulate PRL secretion. Endogenous DA levels in the anterior pituitary were pharmacologically lowered using the catecholamine synthesis inhibitor αMPT. Depletion of DA caused a precipitous increase in peripheral plasma PRL concentrations which reached a maximum by 90 min after injection. After reaching a maximum 90 min after αMPT injection, PRL levels remained constant for at least another 75 min. DA was infused at various concentrations after PRL levels had reached this plateau. As has been reported previously, DA delivered at a concentration of 1000 ng/kg/min inhibited PRL secretion to approximately 20% of the initial value (3). DA delivered at a concentration of one tenth of this potently inhibitory dose had no effect on PRL secretion. How-

ever, DA at a concentration 100 times lower than that required for inhibition potently *stimulated* PRL secretion (two times preinfusion controls). Concentrations of DA 10 times lower than the stimulatory concentration had no effect. The inability of 100 ng/kg/min DA to affect PRL secretion was probably due to the stimulation of both excitatory and inhibitory cascades by this dose of DA, thus canceling either effect. The stimulatory concentration of DA (10 ng/kg/min) had a short-lived effect on PRL secretion consisting of an initial rapid increase in PRL secretion which peaked within 15 min of initiating the infusion, followed by a return to levels not significantly different from controls by 30 min, even in the presence of continued infusion. This short-lived response to stimulatory concentrations of DA is consistent with our previous *in vitro* studies, in which dispersed pituitary cells were perifused with various concentrations of DA (15). In that study (15), stimulatory concentrations of DA also induced a short-lived stimulatory PRL secretory response.

The physiologic relevance of a stimulation of PRL secretion caused by DA 100 times lower than the concentration required for maximal inhibition can be questioned. Gibbs and Neill (3) reported that DA concentration in the long portal vessels of the hypophysial stalk is 6 ng/ml (30 n*M*) in diestrous rats. αMPT injection reduced DA levels in the stalk plasma to undetectable levels as determined by high-performance liquid chromatography (3) and reduced the catecholamine oxidation peak current by 89% in the anterior pituitary as determined by *in vivo* electrochemistry (30). Infusion of approximately 1000 ng/kg/min DA returned DA concentrations in stalk plasma to near normal levels while inhibiting PRL secretion by 70% (3). Several laboratories have measured DA concentrations in stalk plasma responding to PRL releasing stimuli. Generally, stalk plasma DA concentrations were only slightly decreased in response to PRL-releasing stimuli. Cervical stimulation reduced stalk plasma DA by 36% (7), whereas simulation of the suckling stimulus by electrical stimulation of the mammary nerve decreased stalk plasma DA by 70% (9). When collecting stalk plasma, the contribution of the tuberohypophysial dopaminergic neurons to the total DA concentration within the anterior pituitary gland via the short portal vessels is neglected and, as demonstrated by Mulchaney and Neill (20), these neurons contribute at least 50% of the total anterior pituitary DA content. Therefore, stalk plasma concentrations of DA, coming entirely from long portal vessels, do not necessarily reflect the DA levels perceived by the lactotrophs in the anterior pituitary gland. Since 50% of the DA acting on the anterior pituitary gland arrives through the short portal vessels, the important information is not stalk plasma concentration, but total anterior pituitary content of DA. At the moment, we do not know what changes in DA content occur in the anterior pituitary gland in response to PRL-releasing stimuli.

It has been demonstrated that the relief of inhibition expected due to a decrease in DA in the portal vasculature during PRL-releasing stimuli is not great enough to account for the magnitude of PRL released (7–10). It has been proposed that there is an increased release of PRL-releasing factors or a decrease in the release of PRL-inhibiting factors superimposed upon the decrease in DA secretion into portal blood (7–10). Our data suggest that DA may play a dual role in the physiologic release of PRL secretion. When DA secretion is decreased in response to a PRL-releasing stimulus, this allows for a relief of inhibition while the minute levels of DA still present further stimulate PRL secretion.

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