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Effects of the Estrous Cycle Stage on the Prolactin Secretory Response to Dopamine In Vitro

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Dopamine (DA) will both stimulate and inhibit prolactin (PRL) secretion from the anterior pituitary gland in vitro and in vivo. The present study was designed to determine if there are selected times during the estrous cycle of the rat when one function is favored over the other. Anterior pituitary glands collected on diestrus-1 (D1), diestrus-2 (D2), the morning of proestrus (Pro-AM), the afternoon of proestrus (Pro-PM), and estrus (E) were enzymatically dissociated and placed in monolayer culture. On the fourth day in culture, cells were challenged for 10, 20, 30, 60, 120, 180, or 240 min with media alone or media containing either 100 p*M* or 1 μ*M* DA. The concentration of PRL in the media was determined by radioimmunoassay. Regression analysis revealed that in the absence of DA, PRL secretion from cultured cells differed significantly depending on the stage of the estrous cycle during which they were obtained. Cells obtained during the morning of diestrus-2 secreted PRL at the greatest rate compared to other stages of the cycle. When all stages were compared, the rates of PRL secretion were: D2>E>D1>Pro-AM>Pro-PM (each significantly different from the others, $P < 0.01$). By 20–30 min of exposure to 100 p*M* DA, the rate of PRL secretion from cells obtained during each stage of the cycle was significantly enhanced. This enhanced secretion persisted in cells obtained during D2 and Pro-PM but was short-lived in cells obtained during other stages. No inhibition of PRL secretion was induced by this dose of DA. PRL secretion was inhibited when treated with 1 μ*M* DA in cells obtained at all stages of the estrous cycle. Inhibition was more prolonged in cells obtained on D1, D2, and Pro-AM. DA was least effective as an inhibitor of PRL secretion in cells obtained during Pro-PM and E. Prior to inhibiting PRL secretion in cells obtained during Pro-PM, 1 μ*M* DA rapidly stimulated PRL secretion. This effect persisted for 60 min. These data suggest that in the absence of DA, the dynamics of PRL secretion from anterior pituitary cells in vitro differ depending on the stage of the estrous cycle during which the cells were obtained. Moreover, the in vivo environment of the cell determines the direction and magnitude of the PRL-secretory response to DA.

Key Words: Prolactin; estrous cycle; dopamine.

Introduction

Prolactin (PRL) secretion from anterior pituitary cells is controlled by hypothalamic releasing and inhibiting hormones as well as ovarian hormones. Dopamine (DA), released from hypothalamic tuberoinfundibular dopaminergic neurons (TIDA), acts directly on the lactotroph at high doses to inhibit (Ben-Jonathan, 1985; Ben-Jonathan et al., 1989) and at low doses to stimulate PRL secretion (Shin, 1978; Denef et al., 1980, 1984; Burris et al., 1991, 1992; Hill et al., 1991; Arey et al., 1993; Burris and Freeman, 1993; Freeman and Burris, 1993; Kineman et al., 1994; Porter et al., 1994).

The amount of DA arriving at the anterior pituitary, the PRL-secretory response to DA, and the number of DA receptors differs during the estrous cycle of the rat. Indeed, DA levels in hypophysial portal blood are at their lowest when PRL secretion increases during the afternoon of proestrus (Ben-Jonathan et al., 1977). Moreover, the sensitivity of the lactotroph to the inhibitory effects of DA in vivo (Brandi et al., 1990) is least at this same time. The number of DA receptors have been reported to either increase prior to (Heiman and Ben-Jonathan, 1982) or decrease coincident with (Pasqualini et al., 1984) the initiation of the preovulatory surge of PRL on proestrus. These data suggest that the changes in the steroid backgrounds during the estrous cycle determine the secretory response of the lactotroph to DA.

Given that, under appropriate conditions, PRL secretion can be either stimulated or inhibited by DA (Shin, 1978; Denef et al., 1980, 1984; Burris et al., 1991, 1992; Hill et al., 1991; Arey et al., 1993; Burris and Freeman, 1993; Freeman and Burris, 1993; Kineman et al., 1994; Porter et al.,

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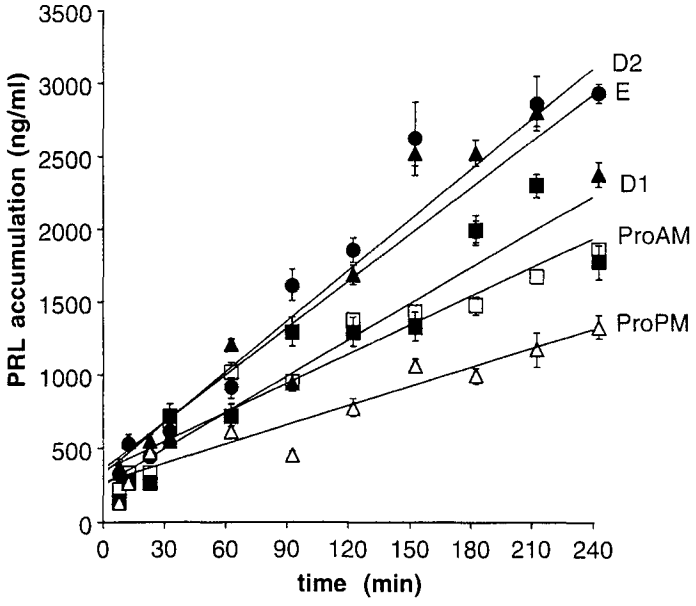

Fig. 1. The mean (± SE) accumulation of PRL secreted over 4 h in vitro into media of cells obtained at diestrus-1 (D1), diestrus-2 (D2), the morning of proestrus (ProAM), the afternoon of proestrus (ProPM), and the morning of estrus (E). Data were evaluated by multiple linear regression using the simplest equation, $y = b + mx$. The correlation coefficient (R^2) for each fitted line was highly significant. ($P < 0.001$). The slopes (m) of the lines differed significantly from each other ($P < 0.01$). □, D1, $y = 322.09 + 6.6610x$, $R^2 = 0.933$, $P < 0.001$; ●, D2, $y = 308.51 + 11.582x$, $R^2 = 0.936$, $P < 0.001$; ■, ProAM, $y = 225.47 + 8.2716x$, $R^2 = 0.892$, $P < 0.001$; △, ProPM, $y = 235.04 + 4.3664x$, $R^2 = 0.901$, $P < 0.001$; ▲, E, $y = 340.94 + 10.708x$, $R^2 = 0.898$, $P < 0.001$.

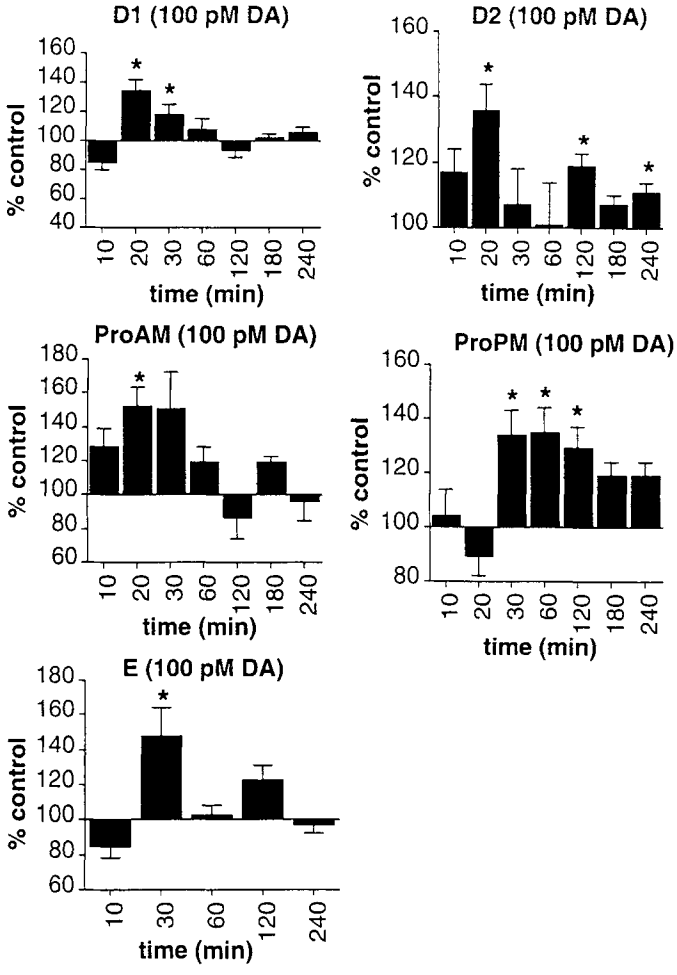

Fig. 2. Effect of treatment with 100 p*M* DA on PRL secretion from anterior pituitary cells collected at various stages of the estrous cycle. Data are expressed as percent of the untreated controls at the corresponding times. Asterisk represents significant differences from controls ($P < 0.01$).

1994), the present study was designed to assess the effect of the stage of the estrous cycle from which the pituitary cells were obtained on the stimulatory or inhibitory PRL-secretory response to DA in vitro.

Results

Figure 1 illustrates PRL accumulation in wells containing anterior pituitary cells obtained from rats during discrete phases of the 4-d estrous cycle. Unweighted linear regression revealed that PRL accumulated in the wells in a linear manner (all correlation coefficients $P < 0.001$) but at varying rates (as indicated by the differing slopes of the fitted lines, $P < 0.01$) depending on the stage of the estrous cycle. In the absence of DA, the rate of PRL accumulation was greatest on D2 and D2>E>D1>Pro-AM>Pro-PM.

Accumulation of PRL secreted from cells obtained during different phases of the estrous cycle varied in response to the concentration ($P < 0.01$) and duration ($P < 0.01$) of DA exposure (Figs. 2 and 3). One hundred picomolar DA enhanced PRL accumulation by 20–30 min after initiation of the challenge at all cycle stages (Fig. 2). This enhanced release persisted with some variation; disappearing by 30 min on D1 and Pro-AM, 180 min on Pro-PM, and persisting through 4 h on D2. In addition, 100 p*M* DA did not inhibit PRL accumulation at any stage of the estrous cycle.

In contrast, 1 μ*M* DA produced a dramatically different PRL secretory profile (Fig. 3). Enhanced PRL accumulation was elicited on Pro-PM within 10 min exposure to 1 μ*M* DA ($P < 0.01$). The enhancement produced by this concentration persisted through 60 min. PRL accumulation was not enhanced by 1 μ*M* DA at any other stage of the estrous cycle. By 60 min exposure to 1 μ*M* DA, PRL accumulation on D1, D2, and Pro-AM was effectively inhibited (Fig. 3). On the other hand, 1 μ*M* DA inhibited PRL accumulation by 2 h on E but not until 4 h of exposure on Pro-PM.

Discussion

Several novel aspects of PRL secretory control are revealed by this study: (1) In the absence of DA, the rate of secretion of PRL in vitro differs depending on the stage of the estrous cycle during which the cells are obtained. (2) The direction (stimulatory or inhibitory) and the timing of its onset, the sensitivity, and the magnitude of the PRL-secretory response to DA also differs in cells obtained during differing stages of the estrous cycle.

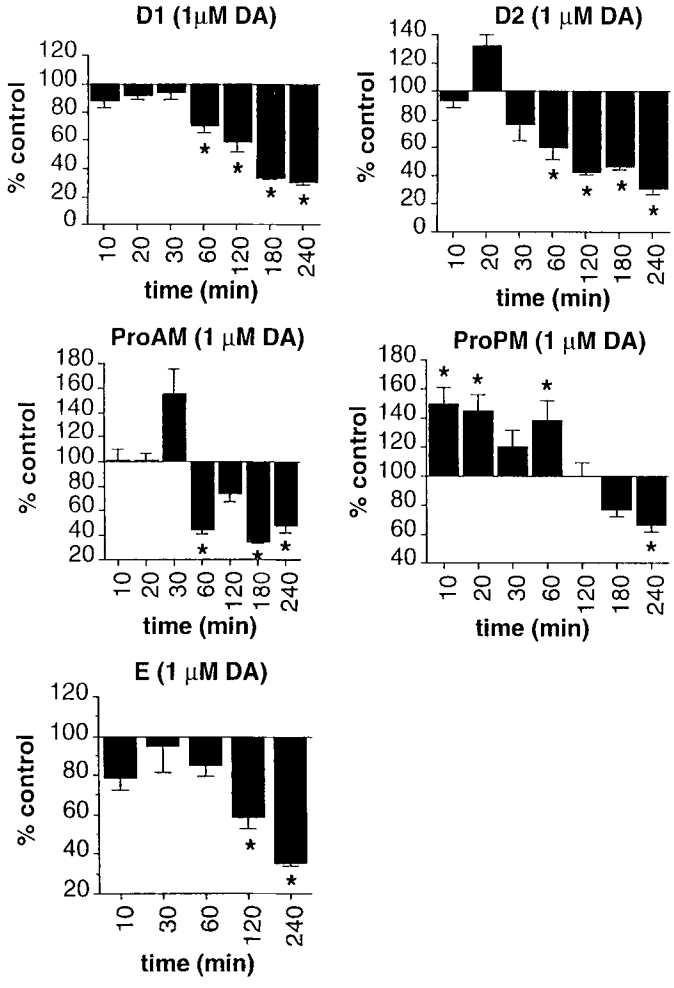

Fig. 3. Effect of treatment with 1 μ*M* DA on PRL secretion from anterior pituitary cells collected at various stages of the estrous cycle. Data are expressed as percent of the untreated controls at the corresponding times. Asterisk represents significant difference from controls ($P < 0.01$).

The PRL-secretory response in the absence of DA of cultured cells obtained on each day of the cycle differed significantly. Specifically, cells obtained on D2 secreted the highest levels of PRL as compared to other cycle days (Fig. 1), and cells obtained on Pro-PM secreted the least amount of PRL over the 240-min challenge period (Fig. 1). By removing the lactotrophs from their in vivo environment and placing them in culture, they are being deprived of the influence of hypothalamic DA. This should result in enhanced secretion of PRL, which indeed it does (Neill and Nagy, 1994). It is well established that ovariectomy of rats results in low levels of PRL in the circulation, which are equivalent regardless of the stage of the cycle during which the surgery was performed (Amenomori et al., 1970). Under these circumstances, one would anticipate that the secretion of PRL in vitro would be exceedingly low and not differ by stage of the cycle of the donor rats since they have essentially been deprived of ovarian steroid support. Indeed, Brandi et al. (1990) reported that, within the first 30 min of perifusion, there was no difference in PRL secretion from cells obtained during differing stages of the estrous cycle. Surprisingly, this is not the case in our experiments (Fig. 1). Though the levels of PRL secreted are all exceedingly high as a result of DA withdrawal, the levels clearly differ by stage of the donor rat. This suggests that, even after 4 d in culture, the lactotroph retains the ability to secrete PRL in a pattern dependent upon its in vivo milieu. Though the present experiments are not sufficient to offer an explanation for the difference, they do suggest estrous cycle stage-dependent differences in secretory controls of the lactotroph. In addition, these observations suggest that anterior pituitary cells cultured for up to 4 d, retain characteristics imprinted by their environmental milieu on the day during which they were obtained.

In order to compare the effects of DA on PRL secretion from cells obtained during different stages of the cycle, we exposed the cells to a relatively low (100 p*M*, Fig. 2) or high (1 μ*M*, Fig. 3) concentration of DA for intervals as brief as 10 min to as long as 4 h (Fig. 2). Such an approach confirmed our previous observations that low concentrations of DA sustain stimulation of PRL secretion in vitro (Burris et al., 1991, 1992; Freeman and Burris, 1993; Arey et al., 1993; Burris and Freeman, 1993). On the other hand, as expected, low concentrations of DA are incapable of inhibiting PRL secretion at any stage of the estrous cycle. This enhanced PRL-secretory response to low dose DA seems to be dependent on the stage of the cycle during which the cells are obtained since cells harvested on D2 or Pro-PM seem to be the most responsive (Fig. 2). The response is rapid-in-onset and relatively short-lived: appearing as early as 20 min on D1 or D2 and persisting for up to 2 h during Pro-PM or 4 h on D2 (Fig. 2). The rapid relative enhancement of PRL accumulation by DA and its rapid disappearance (Fig. 2; D1, Pro-AM, E) is a reflection of a late lag of PRL accumulation in DA-treated wells relative to the ongoing accumulation in control wells. It is interesting to note that Pro-PM is one of the most responsive stages of the cycle (Fig. 2) yet, at the same time, is the period during which the secretion of PRL in vitro in the absence of DA is the lowest (Fig. 1). Thus DA may be awakening a process (stimulation of PRL secretion) that is, by virtue of the cells' environment during Pro-PM, normally quiescent. Similarly, it may be enhancing a secretory process that is most active on D2. The basis for the response may be tied to the ovarian steroid environment that existed at the time the pituitary glands were harvested. We are currently studying the nature of the environment during the various stages and the process(es) that is (are) awakened at this time.

Another finding of this study is that a 1-μ*M* concentration of DA, which is a well established inhibitor of PRL secretion, initially stimulates secretion prior to inhibiting it. Parker and Lawson (1993) have shown that pituitary fragments obtained from estradiol-treated Fischer 344 rats responded to 0.1 μ*M* DA with enhanced PRL secretion. Our data suggest that this effect is also dependent on the stage

of the estrous cycle during which the cells were obtained. Just as cells obtained during Pro-PM were the most responsive to the stimulatory effects of 100 p*M* DA (Fig. 2), cells obtained during the same time responded to μ*M* DA with an initial early stimulation of PRL secretion followed by the later inhibition of PRL secretion (Fig. 3). In agreement with the results of others (Brandi et al., 1990), these cells obtained during Pro-PM were also least responsive to the inhibitory effects of μ*M* DA on PRL secretion when compared to other stages of the estrous cycle. (Fig. 3). Thus something about the environment of the lactotroph on Pro-PM in vivo determines that it responds to DA in a dose-independent stimulatory manner in vitro. Again, the best candidates for the determinants are the ovarian steroids. Alternatively, since Pro was the only PM time of the cycle studied, we cannot be sure that this may not be a circadian response capable of expressing itself during the afternoon of each day of the estrous cycle.

The observations of the present study were made in monolayers of primary cultured cells, whereas our previous observations were made in a perifusion system (Burris et al., 1991). Any disagreement between our previous observations and those of the present study can be attributed to the fact that all of our previous studies were performed in cells obtained from random cycling female rats. Thus cells from the most responsive stages of the estrous cycle would be diluted by cells that are less responsive and thus may potentially give varying results.

These studies, taken together, provide evidence that the environment of the lactotroph determines the direction and magnitude of its PRL-secretory response to DA. Previous studies have suggested that this may be through activation of different DA receptors (Burris et al., 1991; Burris and Freeman, 1994; Porter et al., 1994) or perhaps differing G-proteins (Burris et al., 1992; Kineman et al., 1994). The role of the ovarian steroids in these responses is currently under investigation in our laboratory.

Materials and Methods

Animals

Female Sprague-Dawley rats (200–250 g; Charles River, Wilmington, MA) were maintained in a controlled temperature and humidity environment, with a light period of 0600–1800 h. Food and water were supplied ad libitum. Estrous cycles were monitored by vaginal smear. Animals showing at least three consecutive 4-d estrous cycles were used. Experimental groups consisted of 10 animals per dispersion in the same phase of the estrous cycle, from which pituitary glands were obtained during each of five times: diestrus-1 (1000 h; D1), diestrus-2 (1000 h; D2), the morning of proestrus (1000 h; PRO-AM), the afternoon of proestrus (1700 h; Pro-PM), and estrus (1000 h; E). All procedures performed on animals were approved by the Florida State University Animal Care and Use Committee.

Enzymatic Dissociation of Anterior Pituitary Glands

Unanesthetized rats, each in the same stage of the estrous cycle, were rapidly decapitated and the anterior pituitary glands were collected and pooled in 10% fetal bovine serum (FBS)-supplemented DMEM (pH 7.4) (Gibco/BRL; Grand Island, NY). Under a sterile hood, glands were washed several times with filtered HEPES-buffered saline (HBS; pH 7.4), placed in a 100-mm sterile dish, and cut into 1.0-mm cubes. Tissue pieces were washed in HBS and transferred to a sterile centrifuge tube. A mixture of collagenase (700 U/mL; Worthington, Freehold, NJ), hyaluronidase (765 U/mL; Sigma, St. Louis, MO), and bovine serum albumin (2%; Sigma) was solubilized in HBS (1.0 mL solution/gland), filtered into the tube containing the tissue pieces, and the entire contents decanted into a sterile Spinner flask and incubated at 37°C for 70–75 min. Immediately after the incubation, cells were gently triturated resulting in a suspension of monodispersed cells. The cell suspension was washed in 20 mL HBS and centrifuged at 400*g* for 15 min. The supernatant was decanted and the pellet resuspended in 10% FBS-supplemented DMEM (pH 7.4) to a final concentration of 6×10^4 cells/200 μL media.

Bioassay of PRL-Secretory Response to Challenge with DA

Monodispersed anterior pituitary cells obtained at defined stages of the estrous cycle were plated in 96-well flat bottom plates (Corning; Fisher Scientific, Atlanta, GA) at a density of 6×10^4 cells/200 μl 10% FBS-supplemented DMEM containing 17.72 m*M* H^+ HEPES, 7.28 m*M* Na^+ HEPES, 100 U/mL Na^+ penicillin G, 100 U/mL Streptomycin sulfate (Sigma), 44 m*M* $NaHCO_3$ (Fisher), and 2.5 mg/L Fungizone (Gibco), and pH adjusted to 7.4. Cell cultures were maintained in a humidified Steri-Cult incubation chamber (Forma Scientific, Marietta, OH) for 4 d. Dopamine (Calbiochem, La Jolla, CA) solutions were prepared immediately prior to the bioassay and stored in darkness during the bioassay to decrease the rate of oxidation and inactivation.

On the fourth day in culture, the conditioned media was removed by gentle aspiration, and the cells were washed three times with unsupplemented DMEM (pH 7.4). Immediately after the last washing step, 100-p*M* or 1-μ*M* DA solutions were added to the cells for intervals of 10, 20, 30, 60, 120, 180, and 240 min. Cells treated with media alone served as controls. Media were collected after each challenge interval and stored at –20°C until radioimmunoassay. Each time point was represented by cells from at least three independent dispersions. Within each time point, 12 wells were challenged with media alone and 24 wells were challenged with either of the 2 concentrations of DA.

PRL RIA

The double antibody radioimmunoassay method was used for detection of PRL in conditioned media collected

after bioassay of cultured AP cells. Materials for RIA were provided by Albert F. Parlow and the National Hormone and Pituitary Program of the NIDDK. The RIA was performed using a previously described protocol (Freeman and Sterman, 1978). Hormone concentrations were expressed in terms of NIH rat PRL RP-3 with a sensitivity of 0.01 ng. The inter- and intraassay coefficients of variation were <8%.

Statistical Analysis and Graphic Presentation

Differences in secretion of PRL from cells obtained at various stages of the estrous cycle were evaluated by linear regression plotting accumulation of PRL against time in culture (n = 3 experiments for each stage). Curves were fitted by Cricketgraph. The slopes of the regression lines reflecting rates of PRL accumulation were evaluated for linearity and significant differences by analysis of variance (ANOVA) using StatView II.

Effects of DA on PRL accumulation in incubation media from cells obtained during various stages of the cycle were also evaluated. These experiments (n = 3) were planned as a nested design, with concentration of DA crossed with length of incubation nested within stage of the estrous cycle. Effect of DA on PRL accumulation over time was statistically evaluated by ANOVA and Fisher's LSD post hoc test (StatView II). The data revealed differences in the rate of PRL accumulation over time within stage of the cycle (controls) as well as significant effects of DA (treaments) on accumulation. Consequently, for clarity of presentation, the data on effects of DA are graphically presented as percent of their respective media-treated control.

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