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 pM or 1 mM 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 pM 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 μM 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 μM 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.,
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 pM DA did not inhibit PRL accumulation at any stage of the estrous cycle.

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