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Plasma gonadotropin, prolactin levels and hypothalamic tyrosine hydroxylase activity in rats during estrous cycle, after overiectomy and after blockade of catecholamine biosynthesis

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Abstract. Plasma gonadotropin, prolactin levels and hypothalamic tyrosine hydroxylase activity were evaluated at 0900, 1200 and 1700 h during diestrus, proestrus and estrus, ovariectomized and after systemic administration of reserpine or α-methyl *p*-tyrosine, which interfere with catecholamine biosynthesis, in rats. Gonadotropin and prolactin levels showed peak values during the afternoon of proestrus, while hypothalamic tyrosine hydroxylase activity was markedly lowered at 1200 on proestrus. Gonadotropin levels were slightly lowered whereas prolactin concentrations and hypothalamic tyrosine hydroxylase activity were significantly increased by reserpine. Depletion of hypothalamic dopamine by reserpine apparently resulted in significant elevation of prolactin levels which inturn induce tyrosine hydroxylase. Gonadotropin levels and hypothalamic tyrosine hydroxylase activity were significantly suppressed after the administration of α-methyl *p*-tyrosine. Prolactin levels, however, were elevated significantly. These results indicate that catecholamines are involved in the control of gonadotropin and prolactin release during estrous cycle and inhibition of catecholamines biosynthesis by α-methyl *p*-tyrosine could result in suppression of gonadotropin levels, whereas removal of tonic inhibition of hypothalamic dopamine by α-methyl-*p*-tyrosine elevate prolactin levels.

Keywords. Estrous cycle; ovariectomy; reserpine, α-methyl *p*-tyrosine; gonadotropins; prolactin; tyrosine hydroxylase.

Introduction

Catecholamine (CA) containing neuronal systems terminating in the hypothalamus and other areas of central nervous system regulate or modulate the release of gonadotropins and PRL (Schneider and McCann, 1970; Vijayan and McCann, 1978). Noradrenergic pathways in particular, appear to stimulate the release of luteinizing hormone (LH) (Martinovic and McCann, 1977; Hancke and Wuttke, 1979). α-Methyl-*p*-tyrosine (α-MPT) specifically inhibits tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis, inhibition of that enzyme being competitive with the natural substrate tyrosine. As a result, dopamine as well as noradrenaline levels are lowered in both peripheral adrenergic tissues and in the CNS.

Abbreviations used: PE, Proestrus; E, estrus; DE, diestrus; LH, luteinizing hormone; FSH, follicle stimulating hormone; PRL, prolactin; DA, dopamine; α-MPT, α-methyl-*p*-tyrosine; L-dopa, L-dihydroxyphenylalanine; dopamine, 3, 4 dihydroxy phenylethylamine.

The activity of hypothalamic tyrosine hydroxylase (EC 1.14.16.2), the rate limiting enzyme in catecholamine biosynthesis, whose activity changes correlate well with alterations in dopamine levels as well as plasma gonadotropin and prolactin (PRL) levels, were measured during different stages of the estrous cycle and after ovariectomy. The effects of reserpine, an alkaloid which depletes brain catecholamines from storage granules, and α-MPT were evaluated by measuring plasma gonadotropin and PRL levels in order to determine if a correlation exists between the changes in hormone levels and hypothalamic tyrosine hydroxylase activity.

Materials and methods

Animals

Sexually mature, virgin, Wistar female rats weighing 180-200 g were housed under controlled conditions of light (12 h light: 12 h dark) and temperature (22 ± 2°C). They were fed on food pellets obtained from Hindustan-Lever, Bombay, and had access to water *ad libitum.*

Vaginal smear was checked in these rats daily in the morning. Animals showing at least three consecutive 4 day cycles, were killed by decapitation at 0900, 1200 and 1700 h on the days of diestrus (DE), proestrus (PE) and estrus (E). Trunk blood was collected and plasma was separated at 4°C and stored frozen for the assay of gonadotropins and PRL. Brains were quickly removed and hypothalami were dissected out to measure tyrosine hydroxylase activity.

A group of rats were ovariectomized (OVX) under light ether anesthesia and used for experimentation 2-3 weeks later. Reserpine (Sigma Chemical Co., St. Louis, Missouri, USA. Lot 97C-0033) was dissolved in 20% ascorbic acid (10mg/ 0.5ml) and was administered, sc, at a dose of 10mg/kg body wt/day, for 3 days. Controls were given equal volume of vehicle. The animals were killed by decapitation, 24 h after the last injection.

α-MPT (Sigma Chemical Co., St. Louis, Missouri, USA. Lot. 58C-0371) dissolved in 0.1N HCl (200 mg/ml) (pH adjusted to 5.6-6.0 with 5N NaOH) was administered, ip, at a dose of 400 mg/kg body wt. Controls received equal volume of vehicle. Animals were decapitated 2 h after the injection. Trunk blood was collected, plasma was separated at 4°C and stored frozen for the later assay of gondatropins and PRL by radioimmunoassay (RIA).

Radioimmunoassay

Plasma LH, FSH and PRL concentrations were determined by a double antibody radioimmunoassay, using kits supplied by NIAMDD-National Institutes of Health, Bethesda, Maryland, USA, pituitary hormone distribution programme and according to the guidelines provided with the kit. The results were expressed with reference to the reference preparations, NIH-LHRP-1, FSH-RP-1, and PRL-RP-2 respectively. All samples were run in one assay, each in duplicate, to avoid interassay variation.

Hypothalamic tyrosine hydroxylase assay

Hypothalami (which include pre-optic area, medial basal hypothalamus and median eminence) were dissected out as a single block according to the procedure described earlier (Vijayan, 1974). Tyrosine hydroxylase activity was assayed by the colorimetric method of Shiman *et al.* (1971) as described earlier (Babu and Vijayan, 1981). The activity of tyrosine hydroxylase was expressed as nmol of 3,4 dihydroxyphenylalanine (dopa) formed/mg of protein/h. Protein was estimated by the method of Lowry *et al.* (1951).

Statistics: Statistical evaluation of the data was done by student's *'t'* test.

Results

Luteinizing hormone

During diestrus, the concentrations of LH were low at each time studied (0900, 1200 and 1700 h). During proestrus, however, the morning and noon values were low but by 1700 h the hormone levels were elevated significantly ($P<0.001$). There were no significant differences between the morning, noon and afternoon LH levels on estrus (figure 1).

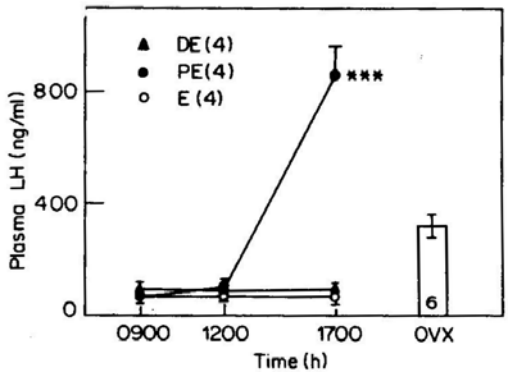

Figure 1. Plasma LH levels at different times during the estrous cycle and after ovariectomy.

In this and subsequent figures, numbers at the base of each column or in parentheses indicate the number of animals in each group and/or at each point. Vertical lines above and/or below the mean represent mean ± SEM. ★★★ $P< 0.001$ *vs* 1200 PE value.

Follicle stimulating hormone

Plasma FSH levels were low at all times during diestrus. The proestrus values were low during 0900 and 1200 but at 1700 h the levels were significantly elevated ($P<0.05$) which persisted through estrus (figure 2).

Prolactin

Plasma PRL levels were low during diestrus and during the morning (0900) and noon (1200) of proestrus. There was a significant ($P<0.001$) surge of PRL during the afternoon (1700) of PE. No significant alterations in PRL levels were observed at various times (0900,1200 and 1700 h) of estrus, though the values were slightly

higher than DE and PE morning and noon concentrations (figure 3).

Tyrosine hydroxylase

Hypothalamic tyrosine hydroxylase activity was significantly higher at 1200 and 1700 h during DE, when compared to PE and E values at the same time. However,

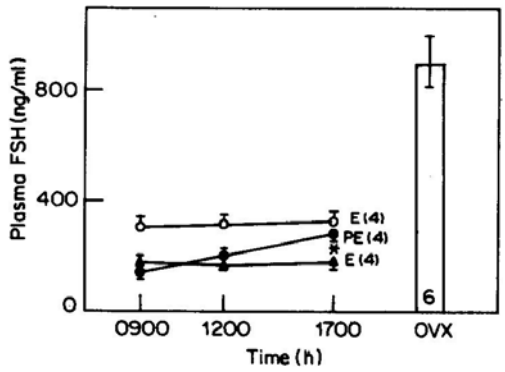

Figure 2. Plasma FSH levels at different times during the estrous cycle and after ovariectomy.

★ P<0.05 *vs* 0900 PE levels.

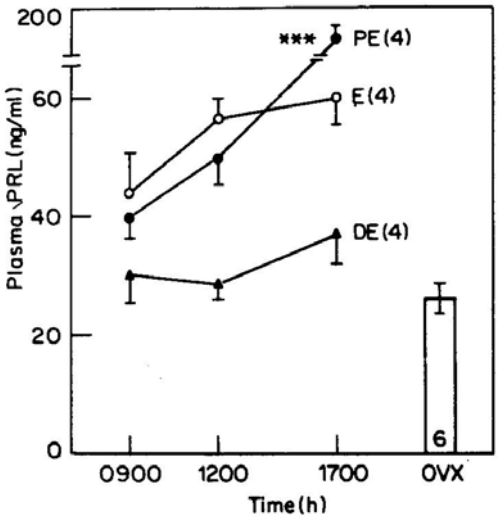

Figure 3. Plasma PRL concentrations at different times during the estrous cycle and after ovariectomy.

★★★ P<0.001 *vs* 0900 and 1200 PE levels.

there was no difference in the enzyme activity between 0900, 1200 and 1700 h during DE or E. On the contrary, the enzyme activity was significantly lowered at 1200 h during PE with respect to 0900 h or DE 1200 h value (figure 4).

Gonadotropin, PRL levels and tyrosine hydroxylase activity after ovariectomy

Plasma gonadotropin levels were significantly elevated (P<0.001) while PRL titers were relatively low in OVX rats (figures 1,2 and 3). Hypothalamic enzyme

activity was comparable to those of DE and E values, but significant increase in enzyme activity was evident only with respect to DE 1200 h value (figure 4).

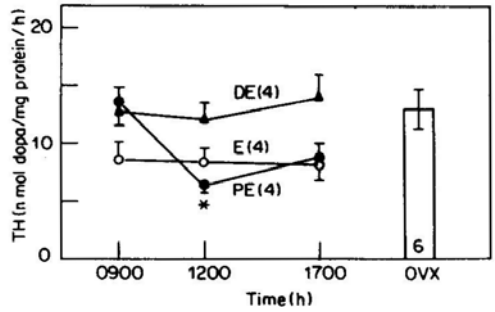

Figure 4. Hypothalamic tyrosine hydroxylase activity at different times during the estrous cycle and after ovariectomy.

★ P<0.05 *vs* 0900 PE value and 1200 DE value.

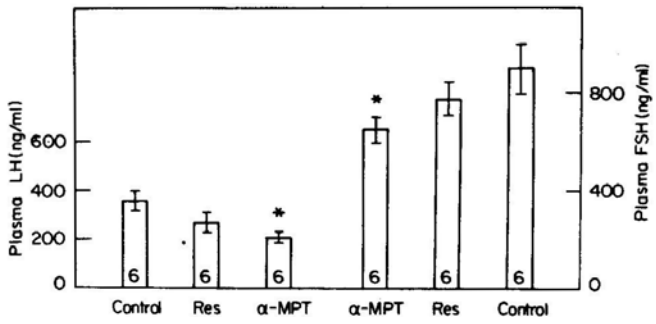

Figure 5. Plasma LH and FSH levels following the administration of reserpine or α-MPT, in OVX rats.

★ P < 0.05 *vs* control.

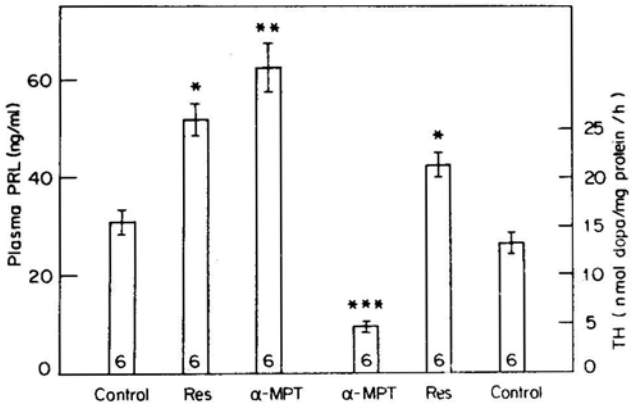

Figure 6. Plasma PRL levels and hypothalamic tyrosine hydroxylase activity following the administration of reserpine or α-MPT, in OVX rats.

★ P<0.05 ★★ P<0.01 *vs* control ★★★ P<0.001.

Gonadotropin, PRL and hypothalamic tyrosine hydroxylase activity after reserpine

Ascorbic acid diluent administration had no effect on plasma gonadotropin levels. Reserpine administration produced only a slight decrease in plasma LH and FSH levels (figure 5) while prolactin levels were significantly ($P<0.05$) elevated (figure 6).

Hypothalamic tyrosine hydroxylase activity was unaltered after the injection of the diluent. On the other hand, tyrosine hydroxylase activity was significantly elevated ($P < 0.05$) following reserpine injections (figure 6).

Gonadotropin, PRL and hypothalamic tyrosine hydroxylase activity after α-MPT

Administration of α-MPT, an inhibitor of tyrosine hydroxylase, significantly suppressed ($P<0.05$) plasma LH levels at 2 h after injection in OVX animals (figure 5). Plasma FSH levels were also significantly ($P<0.05$) suppressed following α-MPT injection with respect to the control values (figure 5). α-MPT, significantly elevated ($P<0.01$) plasma prolactin levels and inhibited tyrosine hydroxylase activity (figure 6).

Discussion

Plasma LH and PRL levels during the afternoon of proestrus agree well with the earlier findings. FSH levels showed peak values during afternoon of PE, and throughout estrus. There were no significant differences in LH and PRL at different times of diestrus and estrus. Hypothalamic tyrosine hydroxylase activity showed a significant decrease at 1200 h followed by an increase of LH during the afternoon of proestrus. The decrease in tyrosine hydroxylase activity at 1200 h on PE may reflect lowered dopaminergic activity, precipitating the series of events leading to the afternoon LH surge. However, dopamine has been shown to stimulate LH release via an action of LHRH (Vijayan and McCann, 1978).

It is well accepted that DA exerts an inhibitory influence on PRL secretion. In the present study, tyrosine hydroxylase activity was significantly lowered at 1200 h on P E , which was followed by an elevation in PRL concentrations at 1700 h.

As expected, plasma LH and FSH levels were significantly elevated and PRL levels were lowered following ovariectomy. The rise in gonadotropins was obviously due to the removal of negative feedback by ovarian steroids. Since estrogens are stimulatory to prolactin release, ovariectomy resulted in decreased PRL levels.

Reserpine, a drug which blocks the uptake of catecholamines into storge vesicles, slightly suppressed plasma gonadotropin levels, but elevated PRL levels and hypothalamic tyrosine hydroxylase activity. The depletion of hypothalamic dopamine reserves by reserpine apparently elevates PRL levels. Reserpine depletes the brain of dopamine, norepinephrine and also serotonin (Shore *et al.,* 1955).The enhanced hypothalamic tyrosine hydroxylase activity is attributed to autoregulatory feedback mechanism of PRL release, by which increased levels of prolactin stimulate the synthesis of tyrosine hydroxylase. Cycloheximide, an inhibitor of protein synthesis, is reported to disrupt the PRL mediated stimulation of dopamine synthesis in these neurons (Johnston *et al.,* 1980).

When pituitary glands are incubated in the presence of reserpine, there is a significant dose-related inhibition of PRL release, suggesting that pituitary action of this alkaloid is opposite to that of CNS action, and this direct effect of reserpine to inhibit PRL release is reported to be independent on any interaction with catecholamine systems and is mediated by other, presently undefined mechanisms (Login and MacLeod, 1981).

α-MPT, a specific inhibitor of the tyrosine hydroxylase, inhibited the elevated gonadotropin levels in ovariectomized rats. Plasma prolactin levels, on the other hand, were significantly elevated. α-MPT has earlier been reported to block prevolulatory discharge of LH and progesterone induced LH release in estradiol primed rats (Kalra and McCann, 1975).

The suppression of LH and FSH release following α-MPT may be due to the inhibition of noradrenaline synthesis in different areas of brain by this compound. Noradrenaline which strongly stimulates LH release, is implicated in the physiological control of LHRH-LH release (Clifton and Sawyer, 1979). Prolactin levels were significantly elevated, apparently due to the inhibition of hypothalamic dopamine synthesis as evidenced by suppression of tyrosine hydroxylase activity. The present results provide additional evidence on the regulatory role played by the enzyme tyrosine hydroxylase and the involvement of catecholamines in pituitary gonadotropin and PRL release.

Acknowledgements

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