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Research Report

Pargyline effect on luteinizing hormone secretion throughout the rat estrous cycle: Correlation with serotonin, catecholamines and nitric oxide in the medial preoptic area

Fernanda Barbosa Lima[a], *Raphael Escorsim Szawka*[a], *Janete Aparecida Anselmo-Franci*[b], *Celso Rodrigues Franci*[a,*]

[a]*Departamento de Fisiologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto-SP, Brazil*
[b]*Departamento de Morfologia, Estomatologia e Fisiologia, Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto-SP, Brazil*

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ABSTRACT

The neurons that produce gonadotrophin-releasing hormone (GnRH) are mainly found in the medial preoptic area (MPOA) and constitute a common final pathway to the control of luteinizing hormone (LH) surge on proestrus. The control of GnRH secretion depends on several neurotransmitters, such as serotonin (5-HT), noradrenaline (NA), dopamine (DA) and nitric oxide (NO). The aim of this work was to study the profile of 5-HT, catecholamines and their main metabolites in the MPOA throughout the estrous cycle and their interactions with NO system in this area to control LH surge. For this purpose, the following were evaluated: (I) the effect of pargyline (a monoamine oxidase inhibitor) acute treatment on plasma LH secretion throughout the estrous cycle, correlated with changes of 5-HT, DA and NA content as well as activity and expression of neuronal NO synthase (nNOS) within MPOA; (II) the effect of 5,7-dihydroxitriptamine (a drug that depletes 5-HT) microinjection into MPOA on plasma LH in ovariectomized rats treated with oil, estradiol (E_2) or E_2 plus progesterone (P_4). Pargyline prevented LH surge on proestrus without altering its basal secretion. Throughout the estrous cycle, pargyline augmented both 5-HT and DA contents in approximately 300% and NA content in 50% in the MPOA. During proestrus, pargyline stimulated nNOS activity at 9 h and inhibited it at 11 h. nNOS expression was inhibited by pargyline at 15 h. Depletion of 5-HT content in the MPOA increased LH secretion in ovariectomized rats treated with E_2 plus P_4, but it did not modify in rats treated with either oil or E_2. Therefore, the present data show that pargyline treatment can inhibit proestrus LH surge through a mechanism that may involve 5-HT and NO systems in the MPOA. Moreover, the effect of 5-HT in the MPOA for limiting LH surge seems to depend on plasma levels of E_2 and P_4.

* *Corresponding author*. Fax: +55 16 36330017.
E-mail address: crfranci@fmrp.usp.br (C.R. Franci).

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1. Introduction

Ovulation is a central event in the reproductive cycle of females, and it depends on the coordinated release of reproductive hormones. In female rats, the midcycle surge of gonadotrophins is triggered by a surge of gonadotrophin-releasing hormone (GnRH) into the hypothalamic hypophysial portal vasculature (Freeman, 1993). Moreover, the interaction of several inhibitory and excitatory neurotransmitters seems to occur on proestrus, modulating the preovulatory GnRH discharge. Accordingly, several neurotransmitters and neuropeptides, such as serotonin (5-HT) (Gouveia and Franci, 2004; Vitale and Chiocchio, 1993), noradrenaline (NA) (Negro-Vilar et al., 1979), dopamine (DA) (Herbison, 1998), nitric oxide (NO) (Moretto et al., 1993), angiotensin II (Dornelles and Franci, 1998) and gamma-aminobutyric acid (Masotto and Negro-Vilar, 1987) have been studied in order to understand the hypothalamic control of gonadotropins secretion.

Some investigators have explored the role of the serotoninergic system in the neuroendocrine control of the luteinizing hormone (LH) preovulatory surge (Kalra and Kalra, 1983). Serotoninergic efferents from raphe nuclei project extensively to forebrain structures, including the medial preoptic area (MPOA), which contains the cell bodies of GnRH neurons (Azmitia and Segal, 1978). It has been previously reported that serotoninergic innervation for MPOA stimulates while 5-HT in the medial basal hypothalamus (MBH) inhibits LH secretion (Johnson et al., 1996). Moreover, the effect of 5-HT upon LH release seems to depend on the experimental model used, including factors such as age and gender (Pinilla et al., 2003). Since GnRH neurons do not express 5-HT receptors (Wright and Jennes, 1993), the actions of 5-HT are likely to occur in a transynaptic way.

A lot of evidence indicates that noradrenergic neurotransmission in the hypothalamus is crucial to generate the LH preovulatory surge. This surge coincides with an increase in NA release in both MPOA (Mohankumar et al., 1994) and MBH (Thyagarajan et al., 1995), and it may be prevented by the inhibition of dopamine-beta-hydroxylase, the last enzyme in the NA biosynthesis pathway (Voogt and Carr, 1981). Estradiol (E_2) treatment in ovariectomized (OVX) rats increases NA release in the MPOA (Demling et al., 1985) and NA turnover in several hypothalamic areas (Wise et al., 1981), coinciding with the LH secretory surge during the afternoon.

Anatomical data have indicated that dopaminergic fibers maintain synaptic contact with GnRH neurons in MPOA and the anterior hypothalamic area (Silverman et al., 1994). DA seems to present a dual effect upon LH secretion, since its systemic infusion at low doses increases plasma LH in OVX rats treated with E_2 and progesterone (P_4) whereas the opposite occurs at high doses (Vijayan and McCann, 1978). Moreover, DA activity increase in MPOA on diestrus suggests an involvement of this neurotransmitter in the neuroendocrine events to induce the LH preovulatory surge and ovulation (Cruz et al., 2001).

Pargyline is a non-selective monoaminoxidase (MAO) inhibitor (inhibits either MAO-A and MAO B) and it is used as a tool to study aminergic systems. Since 5-HT and NA are preferentially deaminated by MAO-A and DA is a substrate for both, MAO A and B, pargyline is an effective tool used to study the effects of these neurotransmitters accumulation in the brain (Nakano and Mizuno, 1996). As pargyline provokes 5-HT accumulation, it is used in clinical context, in studies with models of depression (Zazpe et al., in press). Moreover it has been demonstrated that the propargylaminas, which include pargyline, present an effective neuroprotective role in Parkinson's Disease (Olanow, 2006).

In the brain, NO is produced from the oxidation of L-arginine to L-citrulline, catalyzed by neuronal NO synthase (nNOS), and it is involved in a variety of physiological and pathological processes (Southam and Garthwaite, 1993). NO neurons have been shown to be in close apposition to GnRH neurons within the anterior hypothalamus (Bhat et al., 1995; Herbison et al., 1996). This association appears to be relevant since it has been reported that NO is involved in both the LH surge of proestrus and that induced by ovarian steroids in OVX rats (Bonavera et al., 1993; Bonavera et al., 1996). Furthermore, NOS inhibitors and NOS antisense oligonucleotides have been demonstrated to attenuate the LH preovulatory surge (Bonavera et al., 1994). Recently, it has been shown that the blockage of 5-HT_1 or 5-HT_2 receptors in the MPOA of ovarian steroid-treated OVX rats decreases both local NOS activity and plasma LH, suggesting that interaction between 5-HT and NO within MPOA is involved in the control of LH secretion (Gouveia and Franci, 2004).

Considering these data, the present study aimed at the investigation of the profile of 5-HT, catecholamines and their main metabolites in the MPOA throughout the estrous cycle and their interaction with NO system in this area to control LH secretion in female rats. Accordingly, the effect of acute treatment with pargyline (a monoamine oxidase inhibitor) on the following was evaluated: (1) LH secretion throughout the rat estrous cycle, correlated with 5-HT, DA and NA content in the MPOA; (2) nNOS activity and expression in MPOA during the proestrus phase; (3) the effect of 5-HT depletion in the MPOA on LH secretion in ovarian steroid-treated OVX rats was also evaluated.

2. Results

2.1. *Experiment 1: effect of pargyline on plasma LH secretion and 5-HT, DA, NA and their main metabolites (5-HIAA, Dopac and mHPG, respectively) contents in the MPOA during the estrous cycle*

Fig. 1 shows the effect of pargyline treatment on plasma LH levels during the estrous cycle. There was a significant interaction between pargyline treatment and estrous cycle day ($F_{4,64}=25.23$; $P<0.001$). Both pargyline treatment ($F_{4,64}=37.61$; $P<0.001$) and estrous cycle days ($F_{4,64}=39.20$; $P<0.001$) presented statistically significant effects on plasma LH. The results showed that plasma LH was significantly higher in control rats at 17 h on proestrus, confirming the occurrence of a preovulatory surge. Pargyline prevented the LH preovulatory surge on proestrus, but it did not modify basal LH secretion on the other times evaluated.

There was a significant interaction between pargyline treatment and estrous cycle days ($F_{4,58}=24.20$; $P<0.001$) on 5-