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β-Adrenergic Suppression of Progesterone-Induced Luteinizing Hormone Surge in Ovariectomized, Estrogen-Primed Rats (41326)

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Abstract. This study reveals that in OVX, estrogen-primed animals isoproterenol can interrupt a progesterone-induced LH surge already in progress and lower blood LH to control levels. The results support the notion that the noradrenergic influence may contain both α-adrenoceptor (stimulatory) and β-adrenoceptor (inhibitory) components and that an LH surge is triggered only when the balance is tipped in favor of the α-adrenoceptor component, presumably by the modulatory action of ovarian steroids.

A stimulatory role for norepinephrine (NE) in gonadotropin release was first demonstrated in the rabbit (1) when it was observed that intraventricular infusion of NE results in release of luteinizing hormone (LH) and ovulation. It is believed that the excitatory influence of NE on the release of LH is mediated by α-adrenergic receptors, since the effect can be prevented by α-adrenoceptor blocking agents such as dibenamine, which also block copulation-induced ovulation in the rabbit (2, 3). Likewise, intraventricular administration of NE was effective in inducing ovulation and/or LH release in the constant-estrous rat (4), and the ovariectomized steroid-primed rat (5–7) as well as in sheep (8). Administration of α-adrenergic blocking agents not only prevents the initiation of the LH surge induced by estrogen and progesterone in OVX rats (9), but also inhibits spontaneous ovulation in the proestrous rat when given before the "critical period" (10). All of these data support an α-receptor-mediated stimulatory action of NE on LH release, while β-adrenoceptors in the CNS are likely to have little or no effect in this regard (9, 11, 12). However, Cáceres and Taleisnik (13) have recently presented evidence that activation of β-receptors can, in fact, inhibit LH release.

Quite recently we reported that while both α-adrenergic agonists and isoproterenol, a β-adrenergic agonist, have the ability to suppress the pulsatile rhythm of LH when given intraventricularly to OVX rats, only the α-agonists were effective in inducing an LH surge in OVX estrogen–progesterone-primed animals; isoproterenol gave negative results relative to stimulation (14). We postulated that with regard to adrenergic modulation of LH release, β-adrenoceptors are, if anything, inhibitory, while the stimulation of LH secretion is primarily the function of an α-adrenoceptor. We present here evidence that isoproterenol, when administered into the third ventricle of the OVX estrogen-primed rat, not only fails to stimulate but actually suppresses the induction of an LH surge by progesterone. These results are consistent with the hypothesis that the release of LH is under dual control by α-adrenergic (stimulatory) and β-adrenergic (inhibitory) mechanisms, and that an LH surge occurs only when the balance of α-adrenergic:β-adrenergic influence is tipped in favor of the former, presumably as a result of the modulatory action of ovarian steroids.

Materials and Methods. Female Sprague–Dawley rats (Simonsen Laboratories, Gilroy, Calif.) were housed under controlled temperature (22°) and lighting (14 L:10 D, lights on 0500–1900 hr) conditions. Food and water were supplied *ad libitum*. Vaginal smears were taken daily and only those rats showing two or more consecutive 4-day estrous cycles were used for further study. The animals were ovariectomized 3 weeks prior to stereotaxic surgery, at which time the rats weighed 250–

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300 g. Cannulation of the third ventricle was performed according to a procedure adapted from Krieg and Sawyer (5). To prevent leakage of cerebrospinal fluid, a 28-gauge stainless steel stylette was inserted into the 22-gauge guide tube and its handle cemented in place until the day of the experiment.

Infusion experiments were begun no sooner than one week after completion of the stereotaxic surgery, at which time all animals appeared healthy. Beginning 3 days prior to the infusion sessions, each rat received a daily sc injection of estradiol benzoate (EB, 5 μg/100 g body wt) in sesame oil for 2 days. On the third day an sc injection of progesterone (P, 2 mg) in sesame oil was given at 0800. At 2-hr intervals from 1300 to 1900 hr serial blood samples (0.5 ml) were withdrawn via cardiac puncture under light ether anesthesia. The blood samples were collected in heparin-coated tubes and centrifuged. The plasma was separated and stored at −50° until assayed.

Isotonic saline as a control solution and 0.3 μmole DL-isoproterenol hydrochloride (Sigma) dissolved in isotonic saline were adjusted to pH 5.5 for infusion into the third ventricle of the rat. Five minutes prior to the onset of infusion, the inner stylette was replaced by a 28-gauge cannula connected to polyethylene (PE20) tubing containing the appropriate infusion solution. All infusions were delivered between 1530 and 1600 hr in a 2-μl volume over 2 min using a Hamilton microliter syringe and infusion pump.

Plasma samples were analyzed for LH with radioimmunoassay reagents provided by the NIAMDD Rat Pituitary Hormone Distribution Program. The reference preparation was NIAMDD Rat LH-RP-1, with a biological potency of 0.03 × NIH-LH-S1 (Ovarian Ascorbic Acid Depletion Assay).

At the conclusion of the bleeding period, the animals were given a deep anesthetic dose of pentobarbital, and their brains were perfused with 10% formalin in saline. Brains were subsequently removed from the skulls and sectioned at 100 μm to determine the location of the third ventricle cannula.

Mean plasma LH levels were determined for the pre- and postinfusion periods. Differences within the same or different groups of rats were determined by analysis of variance followed by paired or unpaired *t* tests. Values for LH given in the text represent the mean ± SE.

Results. The patterns of LH secretion following progesterone treatment and intraventricular infusion of either saline or isoproterenol in ovariectomized, estrogen-primed rats can be seen in Fig. 1. In all animals, progesterone treatment at 0800 hr caused a highly significant rise in plasma LH levels by 1500 when compared with those seen at 1300 hr ($P < 0.01$). The LH values reached at 1500 hr were not significantly different between the two groups of rats ($P > 0.05$) in which either saline or isoproterenol was to be administered into the third ventricle following blood sampling at this time (1530–1600 hr). In the saline-infused group the LH levels at 1700 and 1900 were elevated when compared with the LH values at 1300 ($P < 0.01$), but were not different from those seen at 1500 in the same animals ($P > 0.05$). In contrast, intraventricular infusion of isoproterenol caused a dramatic suppression of LH levels at 1700 when compared with LH values at 1500 in the same animals ($P < 0.01$). Also, at 1900 hr while the plasma LH values of the saline-infused group were still elevated, those of the isoproterenol-infused group at 1900 were not significantly different from

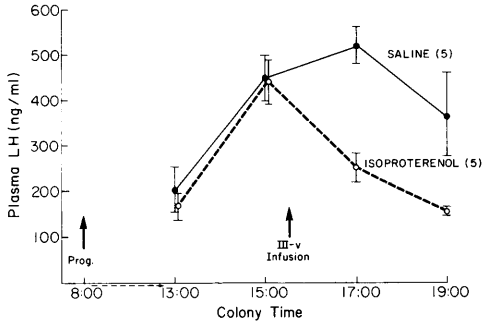

FIG. 1. Effects of intraventricular infusion of saline and the β-adrenoceptor agonist isoproterenol on the progesterone-induced LH surge in ovariectomized, estrogen-primed rats. Five rats were employed in each series. Further details in text.

the baseline values seen at 1300 in the same animals ($P > 0.05$). Thus, when compared with the surge in the saline-infused animals, the LH rise in the isoproterenol-infused rats was interrupted and markedly suppressed, with LH values dropping to approximately 50% of those for the saline-infused group at 1700 (254 ± 30 vs 579 ± 44 ng/ml) and at 1900 (158 ± 7 vs 365 ± 94 ng/ml).

Discussion. We have recently reported that the β-adrenergic agonist isoproterenol, when administered intraventricularly, has the ability to suppress the pulsatile pattern of LH release in long-term ovariectomized rats but fails to initiate an increase in LH secretion in ovariectomized, estrogen–progesterone-primed animals (14). The lack of effect of isoproterenol in OVX, steroid-primed rats is at variance with the action of NE, which has the ability to stimulate an LH surge in ovariectomized rats primed previously with estrogen and progesterone (5, 7) while inhibiting pulsatile LH release in ovariectomized, unprimed animals (7). Thus, we postulated that the stimulatory action of NE on LH release in the presence of ovarian steroids is probably mediated by an α-adrenergic mechanism, whereas β-adrenergic receptors in the CNS may be primarily inhibitory with regard to LH secretion. Results of the present study show for the first time than when administered during the rising phase of a progesterone-induced LH surge in the ovariectomized, estrogen-primed rat, isoproterenol clearly suppresses LH release, thereby further supporting the hypothesis that central adrenergic neurons may exert an inhibitory influence on LH release through β-adrenoceptors, an action distinct from their known stimulatory role on ovulation and LH secretion via α-adrenoceptors.

Our finding is of special interest in light of the earlier report, mentioned above, by Cáceres and Taleisnik (13) that in the ovariectomized, estrogen-primed rat the surge level of LH attained following intraventricular NE was markedly enhanced if the animals were pretreated with propranolol (a β-adrenergic blocking agent) 1 hr prior to NE administration. Propranolol had no effect on the level of LH in the absence of NE. Therefore, it is possible that the excitatory action of NE on LH release may conceal an inhibitory influence and that this inhibitory effect can be revealed by suppressing it with a β blocker, thereby releasing an unopposed stimulatory action. Indeed, results of the present study are quite complementary to the findings of Cáceres and Taleisnik (13) and further support the presence of a hidden inhibitory component in the modulatory role of NE in the induction of an LH surge by ovarian steroids.

The locus of action for the proposed inhibitory β-adrenergic mechanism remains to be elucidated, although NE delivered into the third ventricle has been shown to distribute mainly to areas close to the ventricle (15) and β-adrenergic receptors have been identified within the hypothalamus (16). Assuming that some of the hypothalamic β-adrenergic receptors are located on LHRH neurons, it can be postulated that the effect of intraventricular isoproterenol is exerted directly on the LHRH neurons, perhaps by inducing a hyperpolarization of cell membranes, resulting in a suppression of neuronal activity in a manner similar to that observed for cerebellar Purkinje cells or hippocampal pyramidal cells responding to iontophoretic application of NE (17). Alternatively, β-adrenergic activation may involve an indirect mode of action such as recruitment of putative interneurons which are inhibitory to LH release (18).

In conclusion, our results clearly demonstrate the ability of a central β-adrenoceptor agonist to suppress LH secretion not only in the ovariectomized, unprimed rat (14), but also in the presence of ovarian steroids. Thus, although it is currently believed that NE plays a critical role in the steroid-induced rise in LH levels (19, 20) as well as in the preovulatory LH surge on proestrus (21), our results suggest a possible influence of inhibitory NE receptors (which are, presumably, β-adrenoceptors) in addition to the well-documented stimulatory action of NE on LH release.

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