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Effect of β-Adrenergic Drugs on LH, FSH, and Growth Hormone (GH) Secretion in Conscious, Ovariectomized Rats (41732)

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Abstract. The effects of third ventricular (3V) injection of the β-adrenergic antagonist, propranolol (PROPR), a selective β_1-antagonist, metoprolol (MET), a selective β_2-antagonist, IPS 339, and a β-adrenergic agonist (−) isoproterenol (ISOPR), on plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and growth hormone (GH) were studied in conscious, ovariectomized (OVX) rats. Samples were removed from unrestrained rats which had been previously implanted with atrial and 3V cannulae, and plasma hormone levels were determined by radioimmunoassay (RIA). Intraventricular injection of PROPR (30 μg), MET (40 μg), or IPS 339 (20 μg) induced a gradual elevation in plasma GH concentrations, whereas ISOPR (30 μg) reduced plasma GH. ISOPR (30 μg) brought about a decrease in plasma LH concentrations, but PROPR, MET and IPS 339 had no effect on LH levels. PROPR (30 μg) increased plasma FSH concentrations, but there was no significant effect of MET, IPS 339 or ISOPR on FSH secretion. The results indicate that the β-adrenergic system can inhibit the release of GH, LH, and FSH. This system appears to have a tonic inhibitory effect on GH and FSH but not LH release in the OVX rat.

The role of α-adrenergic receptors in regulation of luteinizing hormone (LH), follicle stimulating hormone (FSH), and growth hormone (GH) secretion has been extensively studied, while the possible action of β-adrenergic receptors on the release of these hormones has received little attention. There is evidence that blockade of α- (by phenoxybenzamine) but not β-adrenergic receptors (by propranolol, etc.) inhibits progesterone-induced LH surges in ovariectomized (OVX), estradiol-treated rats or dopamine-induced FSH secretion from the pituitary *in vivo* and *in vitro* (1–3). Weick (4) observed suppression of the pulsatile secretion of LH in OVX rats by α-adrenergic blockers (phenoxybenzamine, phentolamine) but not by the β-adrenergic blocker, propranolol. Malas *et al.* (5) also reported the negligible action of propranolol on LH secretion in patients. On the other hand, there is evidence that activation of β-adrenergic receptors can inhibit LH release (6–8). Leung *et al.* (9, 10) supported the hypothesis that the release of LH is under dual control by α-adrenergic (stimulatory) and β-adrenergic (inhibitory) mechanisms.

The action of β-adrenergic receptors in the regulation of GH secretion is contradictory. Some investigators (11, 12) reported a slow increase in human plasma GH after intravenous administration of propranolol. Others found no significant change in GH concentration after isoproterenol or propranolol administration (13–16). Imura *et al.* (17) demonstrated that either α-adrenergic agonists or a β-adrenergic antagonist caused a rise in plasma GH concentrations in man.

Recently in the γ-hydroxybutyric acid anesthetized rat, Bluet-Pajot *et al.* (18) have reported that the β-adrenergic agonist, isoprenaline, and the β-antagonist, propranolol, had no effect on the delayed secretion of GH consistently observed in rats anesthetized in this way. To our knowledge no experiments have been carried out in the unanesthetized rat.

So far there is no report concerning the *in vivo* action of β_1- or β_2-adrenergic antagonists on pituitary secretion. The object of the present study was to investigate the gonadotropin and GH responses to third ventricular (3rd V) administration of a β-antagonist, propranolol, a selective β_1-antagonist, metoprolol, a

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selective β_2-antagonist, IPS 339, and the β-adrenergic agonist, (−) isoproterenol (19, 20).

Materials and Methods. *Animals.* Virgin female Sprague–Dawley rats (Simonsen Lab., Gilroy, Calif.) weighing 200–240 g were housed under controlled conditions of light (lights on 0500–1900 hr) and temperature with free access to Purina rat chow and water. One week after arrival, they were OVX under light ether anesthesia. Three to five weeks after ovariectomy the animals were used for the experiments.

Experimental procedure. Six to eight days prior to experimentation, stainless-steel cannulae (23-gauge, 17 mm in length) were inserted into the 3rd V of the brain using a stereotaxic instrument (Kopf) (21). Twenty four hours before initiation of an experiment, a silastic cannula was inserted into the right external jugular vein to the level of the right atrium to facilitate blood withdrawal in conscious, unrestrained animals (22). On the day of experimentation, an extension of polyethylene tubing (PE 50; 12 in. in length) filled with heparin–0.9% NaCl was attached to the distal end of the jugular cannula and the animals were left undisturbed for 45–60 min. The preinjection blood sample (0.6 ml) was withdrawn just before the 3rd V injection of drug or 0.9% NaCl (saline).

Each substance for 3rd V administration was microinjected through a 30-gauge inner cannula made of stainless steel which was guided to its site in the 3rd V by the 23-gauge outer cannula placed earlier. All such injections were made in a volume of 5 μl saline injected slowly over a period of 1 min.

Heparinized blood samples were collected from the external jugular vein cannula at varying intervals (see Results). The volume of all samples was replaced immediately after each bleeding by an equal volume of saline. Plasma was separated by centrifugation at 4°C and stored frozen until the day of assay.

Chemicals. The chemicals used were: (±) propranolol HCl (PROPR) and (−) isoproterenol (ISOPR) (both from Sigma Chemical Co.), Metoprolol (MET), a gift from Ciba-Geigy Pharmaceutical Company, and IPS 339 (Hassle, Göteborg, Sweden).

Radioimmunoassay. LH concentrations in the plasma were measured by the radioimmunoassay (RIA) procedure of Niswender *et al.* (23) using RP-1 rat pituitary LH reference standard and LH titers were expressed in terms of the NIH-LH-S1 reference preparation to be comparable to previous results from this laboratory. FSH and GH levels in plasma were measured by the respective RIA kits supplied by NIADDK for each hormone, and the results were expressed in terms of the RP-1 reference standard provided with the kits.

Significance of the differences between pre- and postinjection plasma hormone levels in each experimental and control group was calculated by analysis of variance with repeated measure followed by the Neumann–Keul's multiple comparison test.

Results. *Effect of intraventricular saline injection on plasma hormone levels.* The intraventricular injection of 5 μl of 0.9% NaCl failed to modify significantly growth hormone, LH or FSH levels, although there was a tendency for GH levels to fall in the initial 5 min following the injection.

Effects of β-adrenergic drugs on GH release. Injection of either the β-adrenergic antagonist, PROPR (30 μg), or a selective β_2-antagonist, IPS 339 (20 μg), into the 3rd V induced a slow increase in plasma GH levels (Fig. 1). The increase was significant ($P < 0.005$) at 60 min after injection. On the other hand, 3rd V injection of MET (40 μg), the selective β_2-antagonist, induced an initial significant decrease ($P < 0.05$) in plasma GH levels at 5–15 min after injection, which was followed by an increase ($P < 0.0025$) at 60 min after injection (Fig. 1).

Injection of ISOPR (30 μg), the β-agonist, into the 3rd V brought about a significant decrease in plasma GH levels ($P < 0.005$) at 15–30 min, with a return to the preinjection levels by 60 min after injection (Fig. 2).

Effect of β-adrenergic drugs on LH release. Injection of ISOPR (30 μg) into the 3rd V significantly decreased plasma LH levels at 15–60 min after injection ($P < 0.05$ at 15 and 60 min, $P < 0.001$ at 30 min) (Fig. 3B), but 3rd V injection of PROPR (30 μg), MET (40 μg), or IPS 339 (20 μg) had no significant effect on plasma LH concentrations (Figs. 3A, B).

Effect of β-adrenergic drugs on FSH release. In contrast to its lack of effect on LH, 3rd V injection of PROPR (30 μg) induced a significant elevation in plasma FSH concentration at 5 min after injection ($P < 0.05$) (Fig.

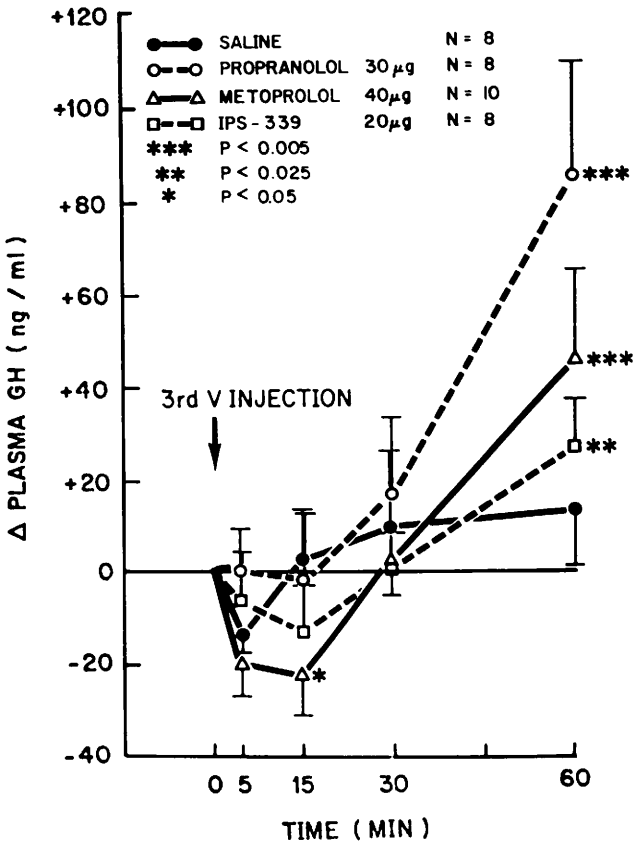

FIG. 1. Effect of third ventricle (3rd V) injection of 0.9% NaCl (3 μl), PROPR (30 μg), MET (40 μg), and IPS 339 (20 μg) on plasma GH levels in OVX rats. Vertical lines = mean ± SEM. In this and subsequent figures *P* values are versus initial (0 time) hormone concentrations.

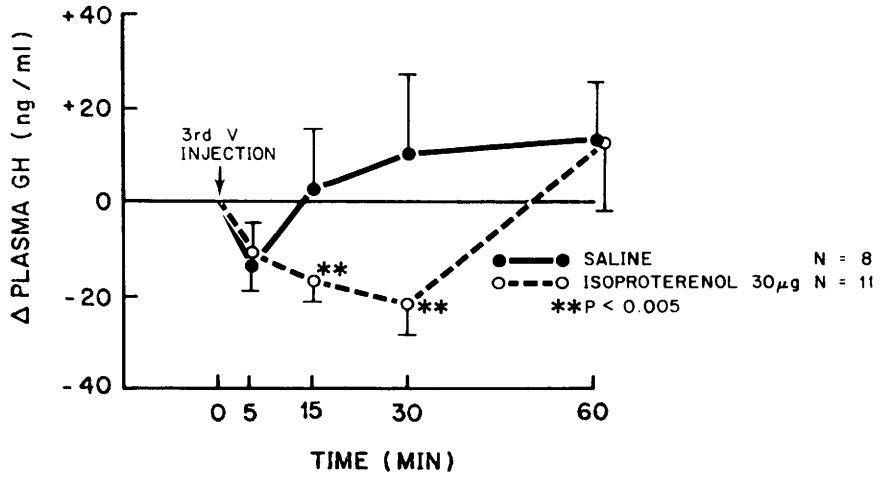

FIG. 2. Effect of 3rd V injection of 0.9% NaCl (5 μl), and ISOPR (30 μg) on plasma GH levels in OVX rats.

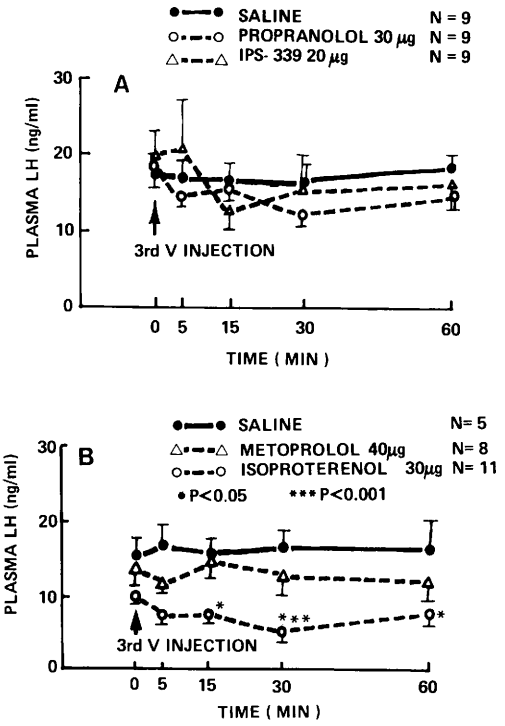

FIG. 3. (A) and (B). Effect of 3rd V injection of 0.9% NaCl (5 μl), PROPR (30 μg), IPS 339 (20 μg), MET (40 μg), and ISOPR (30 μg) on plasma LH levels in OVX rats.

4), but 3rd V injection of MET (40 μg), IPS 339 (20 μg), or ISOPR (30 μg) had no effect on plasma FSH levels (data not shown).

Discussion. Third V injection of the β-adrenergic antagonist, PROPR, the β_1-antagonist, MET, and the β_2-antagonist, IPS 339, significantly elevated the plasma GH levels at 60 min after injection, whereas the β-adrenergic agonist, ISOPR, decreased plasma GH concentration. These results, corresponding to the results of some others (11, 12, 17), support the view that the β-adrenergic system may play a tonic inhibitory role in the regulation of GH secretion.

The effect of the β_1-antagonist, MET, differed slightly from the action of PROPR and the β_2-antagonist, IPS 339, in that MET brought about an initial decrease which was followed by an increase in GH levels, whereas PROPR and IPS 339 only elevated plasma GH. The reason for this difference is not apparent. The mechanical effects of injection of 5 μl into the 3rd V may have been responsible for a small transient and nonsignificant decrease in GH seen in saline-injected animals.

Injection of ISOPR into the 3rd V significantly reduced plasma LH concentrations, which supports the results of Caceres and Taleisnik (6) and Leung *et al.* (9, 10) and indicates that β-adrenergic stimulation inhibits LH release. Nevertheless, 3rd V injection of β-antagonists had no significant effect on plasma LH levels. Thus, it appears that there is no significant inhibitory β-receptor tone on LH release in OVX rats. Since the release of LH is pulsatile in the castrate, our use of only a few samples could have obscured an effect of β-receptor tone. Subsequent results by Bedran de Castro in which more frequent samples were taken before and after administration of β-receptor blocking drugs have not revealed any effect on pulsatile release of LH (Bedran de Castro and McCann, unpublished data).

In striking contrast to the result with LH, 3rd V injection of the β blocker, PROPR, significantly increased plasma FSH which suggests that the β-adrenergic system tonically inhibits FSH secretion. Since LH release was unaffected, PROP administration may bring about release of a specific FSH-releasing factor (24). However, the failure of 3rd V injection of MET, IPS 339, and ISOPR to alter FSH secretion significantly is puzzling and cannot be explained at present.

It must be kept in mind that the β_1 and β_2 receptor blockers are not completely specific. For example in *in vitro* binding assays the ratio of K_ds for β_1/β_2 binding is 25 for the β_1-antagonist, IPS 339, and 1/20 for the β_2-antagonist, metoprolol (19). Nonetheless, the actions of these drugs on pituitary hormone

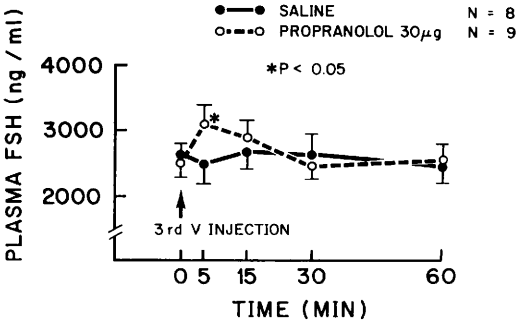

FIG. 4. Effect of 3rd V injection of 0.9% NaCl (5 μl), and PROPR (30 μg) on plasma FSH levels in OVX rats.

release are presumably mediated by the specific β-receptors recently demonstrated in the hypothalamus (25). Roughly equal numbers of recepors of the β_1 and β_2 types were found.

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