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Effects of "Stress" on Pulsatile Luteinizing Hormone Release in Ovariectomized Rats[1] (38638)

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Circulating concentrations of LH fluctuate at high levels in a pulsatile fashion in long-term ovariectomized (OVX) rats (1). In contrast to cycling rats in which surgical interruption of anterior afferents to the medial basal hypothalamus (MBH) blocks the ovulatory surge of LH on proestrus, the pulsatile rises in plasma LH in OVX rats appear to be the result of autonomous neural activity in the MBH (2). This difference may explain why some of the neuropharmacologic drugs which definitively block the LH rise on proestrus do not substantially inhibit LH release in OVX rats (3, 4). Although the ovulation-blocking actions of these drugs are not believed to be the result of "stressing" the animals (5, 6), "stress" does exert complex effects on LH release in cycling rats (5–10). The present study examines the effects of a variety of "stresses" on pulsatile LH release in OVX rats.

Methods and Materials. Female Sprague–Dawley (Simonsen) rats were kept for 2 wk in a room with the lights on from 0500 to 1900 hr. At this time, the rats were bilaterally ovariectomized and kept for 4–7 wk before experimentation. The animals weighed 272–334 g at the time of experimentation.

In all rats, a cannula was inserted through the right external jugular vein into the right atrium of the heart under ether anesthesia as previously described (11). In three rats, the distal end of the cannula was stored in a backpack sewn to the skin for 3 days prior to blood collection. The rest of the rats were cannulated without a backpack 4–5 hr prior to blood withdrawal. Blood (0.2 ml) was withdrawn through the cannulas over a period of approximately 20 sec at 10 min intervals for a total of 20 collections. After each bleeding, 0.2 ml of heparinized saline (10 units/ml) was injected through the cannula. All blood collections were made between 1130 and 1800 hr. Experimental groups included rats which were: (a) Sham ovariectomized at the time of cannulation, (b) subjected to a leg break procedure in which the right tibia and fibula were manually broken after the seventh blood collection (c) injected with 10 units of ACTH (Parke Davis) or 200 μg of corticosterone (ICN) through the cannula after the sixth blood collection, and (d) immobilized by being placed in a narrow clear plastic injection box for 30 or 60 min after the seventh blood collection.

All blood samples were centrifuged at 4°C to separate plasma from cells and the plasma was stored frozen at −25° until subsequent radioimmunoassay of LH. Purified LH (LER-1056-C2; Leo Reichert, Jr.) and an anti-ovine LH antibody (No. 15; Gordon D. Niswender) were used in a modified assay system (13) of Niswender *et al.* (14). Values are expressed in terms of NIAMD-Rat LH-RP-1 (Rat Pituitary Hormone Program, NIH) which has a biological potency equivalent to 0.03 × NIH-S1.

Results. Regular fluctuating patterns in plasma LH concentration were measured in blood withdrawn over the 190 min period in all 3 OVX rats cannulated 3 days prior to blood collection (Fig. 1A). Cannulation 4–5 hr prior to blood collection was ineffective in altering pulsatile LH rhythms in three additional control OVX rats (Fig. 1B). Sham ovariectomy, the leg break procedure, injection of ACTH or injection of corticosterone had no observable effect on pulsatile rhythms in five rats receiving each treatment. Plasma LH concentrations for the first three rats to receive each treatment are plotted in Figs. 2 and 3, and they are representative of each group as a whole. On the other hand, 30 or 60 min of immobilization in 12 additional rats interfered with LH release (Figs. 4, 5). In all cases, plasma LH rhythms were inter-

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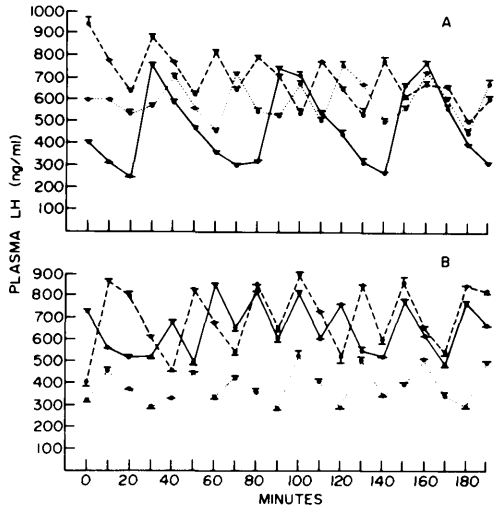

FIG. 1. Plasma LH concentration is plotted at 10 min intervals in individual long-term ovariectomized rats. The solid circles represent the mean LH values and the vertical lines the SEM for each blood sample in which triplicate determinations were made in a single assay. In A the rats were cannulated 3 days previously and the distal end of the cannula was stored in a backpack. In B the rats were cannulated without a backpack under ether anesthesia 4–5 hr prior to blood collection.

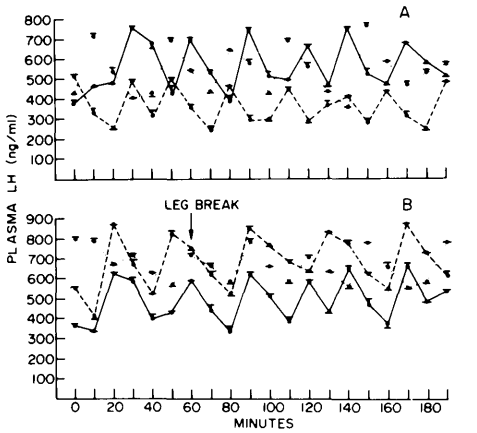

FIG. 2. As in Fig. 1B, rats were cannulated 4–5 hr previously and either sham ovariectomized at that time (A) or subjected to the leg break procedure after the seventh blood collection (B).

rupted with LH levels decreasing during the immobilization period. This decrease was followed by pulsatile fluctuations in plasma LH concentration after the end of the immobilization period.

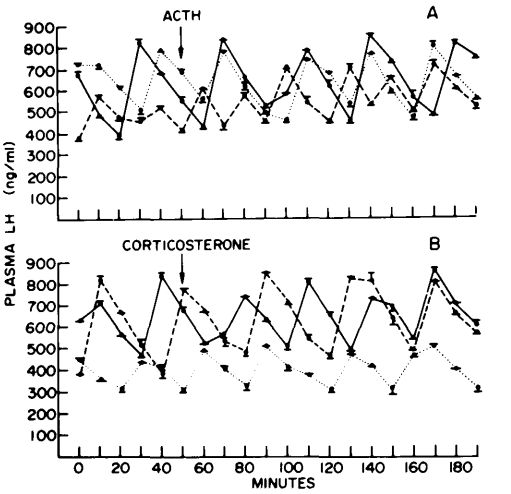

FIG. 3. As in Fig. 1B, rats were cannulated 4–5 hr previously and either injected with 10 units of ACTH (A) or 200 μg of corticosterone (B) through the cannula after the sixth blood collection.

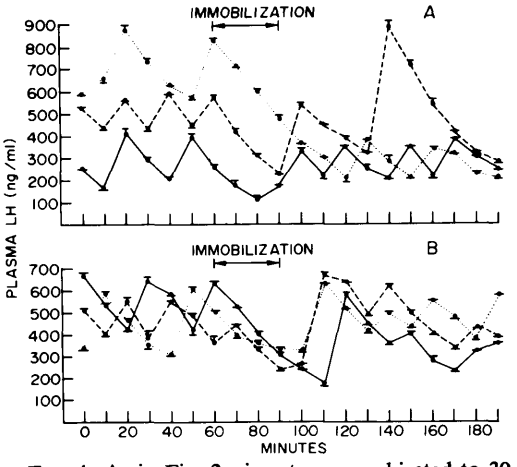

FIG. 4. As in Fig. 2, six rats were subjected to 30 min of immobilization after the seventh blood collection. Plasma LH concentrations are plotted for three individual rats in both A and B.

Discussion. Seyler and Reichlin (10) found blood volume depletion did not affect plasma LH concentration in long-term OVX rats. However, identical procedures were highly effective in elevating plasma LH in diestrous rats and estrogen–progesterone–thyroxine treated OVX animals and volume replacement reduced the effects of hemorrhage on LH release. It is therefore not surprising that withdrawal of 0.2 ml of blood at 10 min intervals followed by volume replacement

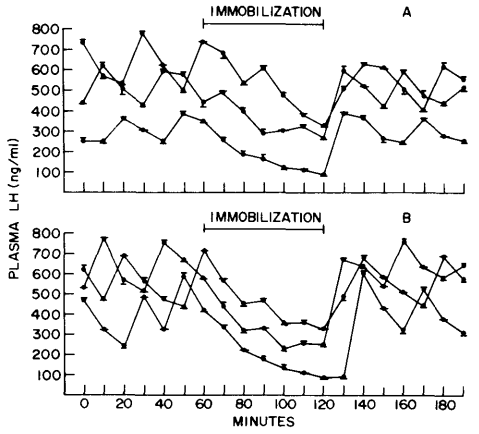

FIG. 5. As in Fig. 4, six rats were subjected to 60 min of immobilization.

had no effect on pulsatile LH rhythms as seen in this study after 20 collections and as reported in a previous study after 37 collections (12). ACTH, corticosterone and a variety of "stresses" were also ineffective in altering the pulsatile LH release mechanism in OVX rats.

On the other hand, immobilization did inhibit pulsatile LH release in OVX rats. A temporary interruption of the pulsatile rhythm has also been observed in a small percentage of animals after sc injection of oil (15), and Euker *et al.* (16) have reported restraint stress to block the proestrous LH surge. The mechanism by which immobilization inhibits LH release is unknown but it does not appear to involve ACTH or corticosterone release. It is possible that immobilization stress results in release of an unknown substance into the bloodstream that could inhibit pituitary LH release in response to endogenous luteinizing hormone releasing factor (LRF). Since pulsatile increases in plasma LH occur shortly after the immobilization period, it is more likely that hypothalamic LRF release is suppressed as a consequence of the immobilization procedure.

Summary. The effects of various "stresses" on pulsatile LH release in ovariectomized rats were investigated. Blood was withdrawn through atrial cannulas and replaced with saline at 10 min intervals for 190 min. Plasma LH concentration was suppressed in rats subjected to 30 or 60 min of immobilization during the collection period. On the contrary, sham ovariectomy 4 hr prior to blood collection, leg break or iv injection of ACTH or corticosterone during the collection period did not alter pulsatile patterns in LH release. The results indicate that LH release mechanisms are highly resistant to "stresses" but that immobilization can suppress LH release by an unknown mechanism.

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