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The Effects of Sodium Pentobarbital or Ether Anesthesia on Spontaneous and Electrochemically-Induced Gonadotropin Release[1] (39469)

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It is well established in rats, that LH and FSH rise concomitantly on the afternoon of proestrus (1-3) and that injection of sodium pentobarbital (Nembutal) shortly before the onset of the spontaneous gonadotropin surge prevents this release from occurring (Nembutal-blockade) (4-7). Although electrochemical stimulation (ECS) (8) of the medial preoptic area (MPOA) or arcuate-median eminence region (ARC-ME) is effective in inducing the release of gonadotropins in Nembutal-blocked proestrous rats, the temporal patterns of LH and FSH secretion do not mimic those which occur spontaneously (6, 9). In most studies, following ECS of the MPOA, plasma LH levels have peaked and are approaching basal concentrations prior to the commencement of a plasma rise in FSH. Conversely, administration of hypothalamic extracts induced an immediate elevation of both gonadotropins *in vivo* (10) and *in vitro* (11). The following studies were undertaken to determine if the temporal dissociation of LH versus FSH release following central nervous ECS was the result of the depressant effects of Nembutal on preoptico-hypothalamic function or if it was due to other factors.

Material and methods. The adult female Sprague-Dawley rats (200-250 g) used in this study were purchased (A/R Madison, Wisc.) and maintained in a light and temperature (22-24°C) controlled room for 2-3 weeks prior to use. Lights were on from 0400-1800. Daily vaginal lavages were made and only those rats which exhibited at least two consecutive 4-day estrous cycles were selected for study. On the day of vaginal proestrus, animals were divided into four groups:

Group A. spontaneous proestrous LH and FSH release. At 1100 on proestrus, 10 cyclic rats were anesthetized with ether and the right external jugular vein was exposed. A polyethylene cannula (o.d. 0.038 in.) was inserted to the level of the right atrium. The cannula was coursed beneath the skin to the back of the neck, leaving 2-3 inches exposed for subsequent blood collections. Beginning at 1300, hourly blood collections (0.5 cc) were made for 6 h.

Group B. electrochemical stimulation of either the dorsal anterior hypothalamic area (DAHA) or the medial preoptic area (MPOA) at 1300 employing Nembutal anesthesia. Fifteen minutes before the anticipated spontaneous surge of LH/FSH, which occurs in our rat colony between 13-1500, an ovulation-blocking dose of Nembutal (30 mg/kg ip) was administered to a group of 31 animals. The right external jugular vein was cannulated as described in Group A. The animals were immediately placed in a stereotaxic apparatus and a blood sample (0.5 cc) was collected. A concentric bipolar stainless steel electrode [specifications previously described (4)] was stereotaxically oriented into either the right DAHA (n = 23) or the right MPOA (n = 8). A current intensity of 60 μA/60 sec was supplied by a constant current stimulator (Electronics for Life Sciences, Rockville, Md.) and monitored by a cathode ray oscilloscope. Following stimulation, blood samples (0.5 cc) were collected at 60, 120, 180, 240, 300 and 360 min. An equal volume of 0.9% saline was reinfused after each blood collection. The following morning, the animals were sacrificed and the Fallopian tubes were examined for the presence of ova. Brains were perfused via the common carotid artery, first with 20 ml of 0.9% saline, saturated with potassium ferro-ferricyanide, and then with 20 ml of 10% formalin. Brains were removed, stored in 10% forma-

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lin and eventually were frozen-sectioned with a sliding microtome at a thickness of 60 μ to determine the stimulus size and diameter. Lesion diameters were measured with an ocular micrometer from the lateral extent of the area of electrocoagulation produced by ECS.

Group C. electrochemical stimulation (ECS) of either the MPOA or the DAHA 120 min after Nembutal injection. As in Group B, Nembutal (30 mg/kg ip) was administered to a group of 14 proestrous rats at 1245. The right external jugular vein of these animals was cannulated and a blood sample (0.5 cc) was collected at 1300 (0 time). Thereafter, the rats remained undisturbed (except for a blood collection at 60 min) until 1500 (120 min) at which time the MPOA of seven animals was unilaterally electrochemically stimulated (60 μA/60 sec). The DAHA of the remaining seven rats was electrochemically stimulated (60 μA/60 sec) and all experimental procedures described in Group B were then performed in both subgroups.

Group D. ECS of either the MPOA or the DAHA in ether-anesthetized proestrous rats. At 1100, 2 hrs before the anticipated onset of the spontaneous surge of gonadotropins, 11 rats were anesthetized with ether and the right external jugular vein was cannulated. Animals were immediately placed in a stereotaxic apparatus and a blood collection (0.5 cc) was taken (0 time). In each preparation, either the right MPOA ($n = 6$) or the right DAHA ($n = 5$) was electrochemically stimulated. Following ECS, blood samples were collected at 30, 60, 90, and 120 min. The following morning (estrus), animals were sacrificed and their Fallopian tubes were examined for the presence of ova; the brains were perfused, removed, and then were stored for future determination of stimulus size and location.

Radioimmunoassay procedures. Plasma LH was determined by radioimmunoassay according to the ovine:ovine procedure of Niswender *et al.* (10). A crude rat pituitary extract prepared to correspond with a B160 standard supplied to us by Dr. V. L. Gay was used as standard reference material; this preparation has been shown to have a LH potency of 0.17 × NIH-LH-S1. Plasma FSH was assayed by the rat radioimmunoassay kit materials generously provided by the NIAMDD and values are expressed in terms of the NIAMDD Rat-RP-1 which is 2.1 × NIH-FSH-S1 standard. The kit materials were employed using modifications previously described (4) to permit the simultaneous assay of LH and FSH in small volumes of plasma. Statistical evaluations between and among groups was performed using Duncan's multiple range test (11).

Results. Group A. Spontaneous release of FSH and LH on proestrus. At 1400 both LH/FSH were significantly elevated above the basal plasma concentrations obtained at 1300 proestrus (Fig. 1A). One hour later, a further significant increase in both gonadotropins was observed and at 1600 LH/FSH had reached peak concentrations. Thereafter, LH decreased to basal concentrations by 1900 while plasma FSH remained elevated. At autopsy the following morning, all animals had ovulated and the Fallopian tubes contained 8–13 ova.

Following the injection of Nembutal at 1245, these spontaneous surges were blocked and LH/FSH concentrations remained at basal levels throughout the afternoon of proestrus. These plasma patterns and concentrations of LH/FSH have been reported previously (4).

Group B. ECS of the DAHA or the MPOA of Nembutal-anesthetized rats. The first significant ($P < 0.05$) increase in plasma FSH in DAHA–ECS rats did not occur until 120 min poststimulation and peak plasma FSH concentrations (274 ± 11 ng/ml) were observed by 180 min poststimulation. Once such peak concentrations were achieved, plasma FSH remained elevated in this and all other experimental groups throughout the remainder of the collection periods.

In DAHA–ECS rats, plasma LH concentrations remained at basal levels except for a slight but significant ($P < 0.05$) rise 120 min poststimulation. These animals had not ovulated by the following morning (estrus).

Following MPOA stimulation of a second group of rats, plasma LH began to rise at 30 min and peak plasma LH concentrations were obtained at 60–120 min poststimulation (Fig. 1B). These levels had de-

creased to baseline by 180–240 min. In contrast, the first significant ($P < 0.05$) elevation in plasma FSH in these animals was not achieved until 120 min and peak concentrations were not reached until 180 min poststimulation. The Fallopian tubes of MPOA-electrochemically stimulated rats contained 8–12 ova at autopsy the following morning. The locations of MPOA and DAHA stimulation sites are illustrated in Fig. 3.

Group C. ECS of the MPOA or DAHA 120 minutes after Nembutal injection. Rats unilaterally electrochemically stimulated in the DAHA 120 min after Nembutal administration exhibited significant ($P < 0.05$) elevations in plasma FSH concentrations by 60 min and peak plasma levels (288 ± 14 ng/ml) by 120 min poststimulation. Plasma LH concentrations in these animals remained unaltered throughout the entire blood collection periods. None of these animals had ovulated at autopsy on estrous morning.

Unilateral ECS of the MPOA 120 min following the injection of Nembutal induced release patterns of FSH similar to those observed after DAHA-ECS. Significant ($P < 0.05$) plasma elevations of this gonadotropin were observed by 60 min and peak concentrations occurred (321 ± 56 ng/ml) by 120 min poststimulation (Fig. 2A). Peak FSH plasma concentrations in this MPOA-electrochemically stimulated group were significantly greater ($P < 0.05$) than those observed in the DAHA group stimulated 120 min after Nembutal treatment. The maximal plasma LH concentrations observed 60 min poststimulation (124 ± 26 ng/ml) had declined to basal levels by 180–240 min poststimulation. Examination of the oviducts the following morning revealed that full ovulations had occurred (8–12 ova).

Group D. ECS of the MPOA or DAHA in ether-anesthetized proestrous rats. Unilateral ECS of the DAHA in ether-anesthetized rats at 1100 induced a significant ($P < 0.05$) release of pituitary FSH by 60 min and maximal plasma concentrations (302 ± 40 ng/ml) were achieved by 120 min poststimulation. Not only had plasma LH levels risen significantly from baseline by 60 min but they were further elevated by 120 min poststimulation. All animals in this group had ovulated completely by estrous morning.

Unilateral ECS of the MPOA in ether-anesthetized rats elicited a significant ($P < 0.05$) release of both gonadotropins by 60 min poststimulation (Fig. 2B). Plasma LH and FSH concentrations increased simul-

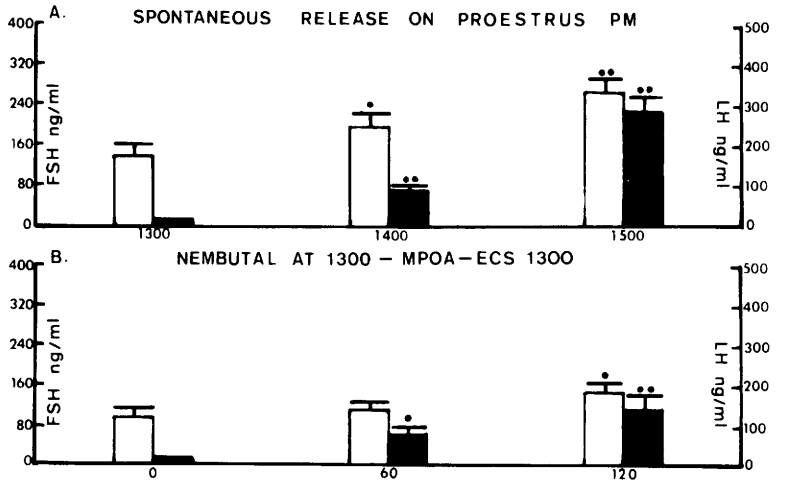

FIG. 1. Temporal and quantitative changes in plasma LH (shaded bars) and FSH (open bars) on the afternoon of proestrus at 13, 14, and 1500 (A), or 0, 60, and 120 min following electrochemical stimulation (ECS) of the medial preoptic area (MPOA) at 1300 in Nembutal-treated proestrous rats (B). Vertical bars represent the standard error of the mean. (. = $P < 0.05$; .. = $P < 0.01$)

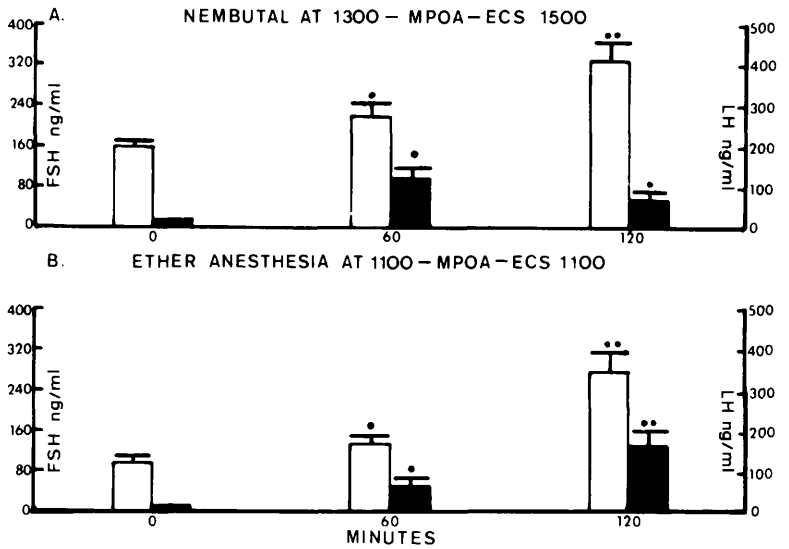

FIG. 2. Plasma concentrations of LH (shaded bars) and FSH (open bars) at 0, 60, and 120 min following electrochemical stimulation of the medial preoptic area (MPOA-ECS) in Nembutal-treated proestrous rats (A) or 0, 60, and 120 min after MPOA-ECS in etherized proestrous rats at 1100 (B). Vertical bars represent the standard error of the mean. (. = $P < 0.05$; .. = $P < 0.01$).

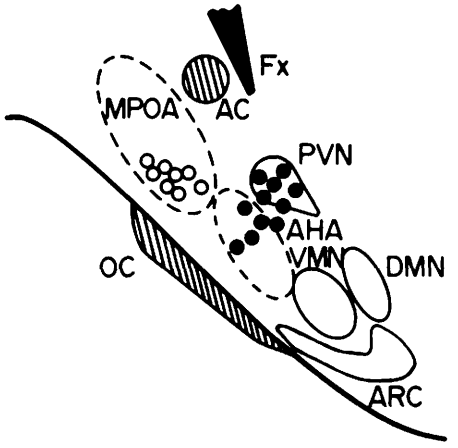

FIG. 3. Parasagittal section of the MPOA-hypothalamus of rats indicating centers of lesions produced following electrochemical stimulation (ECS) of the medial preoptic area (MPOA) (open circles) or the dorsal anterior hypothalamic area (DAHA) (black circles).

taneously thereafter. All animals had ovulated 8–13 ova by estrous morning.

Discussion. Electrochemical (9, 14) or electrical stimulation (5) of the MPOA elevates plasma LH concentrations within 30 min poststimulation and the present studies confirm these earlier observations. Since hypothalamic extracts injected directly into the pituitary portal vessels (10) provoke an increase in plasma LH within 10 min, the apparent delay in the LH plasma rise in MPOA-electrochemically stimulated animals must occur somewhere within the preoptico-hypothalamic system. Examination of the temporal patterns of plasma LH/FSH which occur spontaneously on the afternoon of proestrus has revealed that both gonadotropins rise concomitantly (1–3). In contrast, electrochemical stimulation of the MPOA, the arcuate-median eminence (ARC-ME), or the DAHA in Nembutal-anesthetized rats induces an increase plasma FSH but the lag time from stimulation to the first significantly detectable plasma elevation in this gonadotropin was 1.5–2 hr. (4, 6, 9). Since Nembutal does not alter the responsiveness of the pituitary gland to LH-RH injections (15), it must have a central site of action in affecting gonadotropin release. If the interval from injection of Nembutal to ECS of the MPOA or the DAHA is delayed for 120 min, the time to the first rise in plasma FSH is markedly reduced. At this time, the animals are recovering from the anesthetic properties of Nembutal for they will exhibit withdrawal reflexes to foot-pinch, exhibit corneal reflexes, and show other general indications of behavioral arousal. Electrochemical stimulation

of the CNS during this period effectively induces a more rapid release of pituitary FSH than that which occurs in the deeply anesthetized preparation.

Consequently, it seems apparent that those neural regions which regulate FSH are highly and selectively sensitive to the depressant actions of Nembutal. Since such drug actions occur regardless of whether the MPOA or the DAHA is electrochemically stimulated, and the stimulated release of pituitary LH is not appreciably altered by Nembutal, it is probable that this barbiturate affects MPOA or DAHA synaptic transmission at the level of the ARC-ME. Neuropharmacological studies have shown that central neural pathways which have abundant interneuronal connections such as the ascending reticular activating system are particularly vulnerable to the depressant actions of barbiturates (16).

It has been reported previously that the use of ether anethesia prior to the onset of the spontaneous surge of LH does not interfere with the release of this gonadotropin on the afternoon of proestrus (17). In the present study, ECS of DAHA at 1100 in etherized proestrous rats elicits a release of FSH within 60 min post-stimulation as compared to the 90–120 min delay observed in Nembutal-anesthetized preparation.

The results of these studies require a further evaluation of whether one or several releasing hormone(s) for LH/FSH exist. Since temporal plasma patterns of LH are unaltered by Nembutal in MPOA-electrochemically stimulated rats, and pituitary LH/FSH are released concomitantly following an i.v. injection of hypothalamic extracts, the present data support the concept that separate releasing hormones exist for these gonadotropins. Although the pituitary discharge of FSH eventually occurs in MPOA- or DAHA-electrochemically stimulated rats after induction of Nembutal anesthesia, it should be recalled that ECS produces an irritative lesion with iron deposits which continues to stimulate even in the absence of current flow (8). Thus, as recovery from anesthesia progresses, this stimulative lesion could activate those neural components responsible for release of FSH and this event takes from 90–120 min poststimulation to occur.

Summary. These studies have examined the effects of sodium pentobarbital or ether anesthesia on temporal plasma patterns and concentrations of FSH and LH following electrochemical stimulation (ECS) of the medial preoptic area (MPOA) or dorsal anterior hypothalamic area (DAHA) in proestrous rats. The injection of sodium pentobarbital at 1245 on proestrus and ECS of either the MPOA or DAHA at 1300 elicited a rise in plasma FSH 120 min poststimulation. Injection of this barbiturate at 1245 and ECS of either region at 1500, when the effects of anesthesia had begun to wane, reduced the time interval between stimulation and the first significant plasma FSH rise to 60 min. A third group of proestrous rats was anesthetized with ether at 1100 and the DAHA or the MPOA was electrochemically stimulated. In such preparations, FSH in plasma rose significantly 60 min poststimulation. In all MPOA-electrochemically stimulated groups, the first significant rise in plasma LH occurred 30 min poststimulation regardless of the time of treatment or anesthetic drug employed. In none of the groups did DAHA-ECS provoke a rise in plasma LH. These results suggest that the CNS systems controlling FSH but not LH secretion are sensitive to sodium pentobarbital and that the lag time between ECS and plasma rises in FSH apparently is related to the depressant effects of the barbiturate on neural FSH controlling systems.

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