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Dynamics of Luteinizing Hormone Release after Intravenous Administration of Crude Stalk-Median Eminence Extract or Synthetic Gonadotropin-Releasing Hormone[1] (39473)

T. M. NETT AND G. D. NISWENDER

Reproductive Endocrinology Laboratory, Department of Physiology and Biophysics, Colorado State University, Fort Collins, Colorado 80523

The ability of acidic extracts of basal medial hypothalamic nuclei to induce the release of gonadotropins has been well documented (1-3). More recently a compound of hypothalamic origin capable of releasing gonadotropins has been isolated (4), identified (5), and chemically synthesized (6). This compound, gonadotropin-releasing hormone (Gn-RH) has been the subject of much intensive investigation in recent years. However, the dynamics of luteinizing hormone (LH) release following injection of Gn-RH (7-9) appear to be quite different than the release observed after injection of crude stalk-median eminence (SME) extracts (10, 11). Similar discrepancies have been observed following the *in vitro* perifusion of anterior pituitaries with partially purified hypothalamic extracts (12) or with crude hypothalamic extracts (13). Thus, the possibility existed that crude hypothalamic extracts contained substances, other than Gn-RH, which were capable of inducing the release of LH.

The following investigations were designed to: (i) compare the dynamics of LH release after injections of crude SME extracts or synthetic Gn-RH, and (ii) determine if hypothalamic factors other than Gn-RH contributed significantly to the release of LH.

Materials and methods. Mature Western range ewes in seasonal anestrus or that had been ovariectomized from 3 to 60 days were used. An indwelling polyvinyl cannula with a Teflon obturator (Safedwell, Becton-Dickinson, Co., Rutherford, New Jersey) were inserted into the jugular vein of each ewe for injections of test materials and for collection of blood samples. Crude extracts of porcine SME prepared as described by Schally *et al.* (14) and synthetic Gn-RH was generously supplied by Abbott Laboratories, North Chicago, Illinois.

Radioimmunoassay. All serum samples were assayed for LH and Gn-RH as described previously (15, 16). Acidic extracts of SME were neutralized with 1 *N* ammonium hydroxide, lyophilized, and reconstituted in phosphate-buffered saline containing 0.1% gelatin and 0.1% sodium azide (gel-PBS). The LH and Gn-RH content of these extracts were determined by assaying from 5×10^{-1} to 5×10^{-7} SME equivalents.

Experiment I. Four ewes which had been ovariectomized for 3 days were given an intravenous (iv) injection of 4 SMEs or 10 μg synthetic Gn-RH according to the schedule in Table I. Jugular blood samples were collected at −45, −30, −15, 0, 3, 6, 9, 12, 15, 18, 24, 30, 36, 42, 48, 54, 60, 72, 84, 96, 108, 120, 150, 180, 210, and 240 min relative to the administration of the material to be tested. The samples were placed in an ice-water bath immediately after collection and were allowed to clot overnight. Serum was separated by centrifugation and stored frozen until assayed for LH and Gn-RH.

Experiment II. Four ewes which had been ovariectomized for 60 days were given an iv injection of 4 SME equivalents or 300 ng synthetic Gn-RH in a design similar to that for Experiment I. Jugular blood samples were collected and processed as described for Experiment I.

The results of Experiments I and II indicated that the dynamics of LH release were different after administration of crude SME extract than after administration of synthetic Gn-RH. Radioimmunological analysis of the crude SME extract indicated a contamination of 15.8 μg LH per SME equivalent. Therefore, ammonium sulfate precipitation and gel-filtration were used to

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reduce the quantity of LH in the crude SME extract.

Experiment III. Four anestrous ewes were given an iv injection of 4 SME equivalents or 4 SME equivalents which had been partially purified by precipitating the large proteins with 50% saturated ammonium sulfate. The injection schedule and collection and processing of blood samples were similar to that described for Experiment I.

Experiment IV. Twenty SME equivalents were purified by gel-filtration on a 1 × 30 cm column of Sephadex G-25 eluted with 0.01 *N* acetic acid. Ten microliters of each 3 ml fraction were diluted to 1 ml with gel-PBS. Duplicate 200 μl aliquots of the diluted fractions were assayed for LH and Gn-RH. Fractions containing immunoreactive LH were discarded. The remaining fractions were neutralized with 1 *N* ammonium hydroxide, lyophilized, and reconstituted in physiological saline. Four anestrous ewes were given iv injections of 4 crude SME equivalents or 4 SME equivalents purified by gel-filtration. The injection schedule and collection and processing of blood samples were as described in Experiment I.

Results. The results from Experiment I are depicted in Figs. 1 and 2. Following the iv injection of 4 SME equivalents there was an immediate rise in levels of Gn-RH and LH in serum. Both hormones reached a peak (22 ± 3 ng/ml LH and 27 ± 13 pg/ml Gn-RH) within 3 min and then began to decline. Levels of Gn-RH had returned to baseline within 15 min whereas levels of LH did not return to baseline for 60 min after the injection of SME extract (Fig. 1). The increased levels of Gn-RH in samples collected at 30 and 120 min after injection were due to high levels in a single animal. The increase of Gn-RH noted at 120 min was associated with an increase in serum levels of LH in the same ewe. After an iv injection of 10 μg synthetic Gn-RH into the same ewes, levels of Gn-RH and LH in serum followed a different pattern. Levels of Gn-RH reached a peak within 3 min and declined to basal levels within 90 min (Fig. 2). The magnitude of the Gn-RH increase obtained after injection of 10 μg synthetic Gn-RH was 964 ± 216 pg/ml compared to an increase of 10.6 ± 2.2 pg/ml following the injection of 4 crude SME equivalents. The magnitude of the LH response follow-

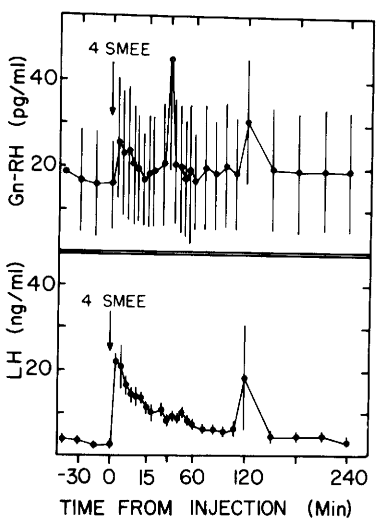

FIG. 1. Levels of Gn-RH and LH in serum of ewes following iv administration of 4 crude stalk-median eminence equivalents (SMEE). Each point represents the mean ± standard error ($n = 4$).

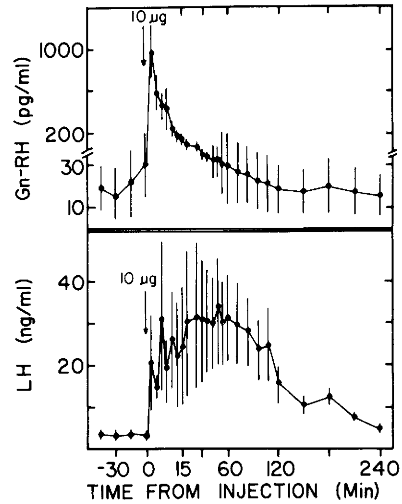

FIG. 2. Levels of Gn-RH and LH in serum of ewes following iv administration of 10 μg of synthetic Gn-RH. Each point represents the mean ± standard error ($n = 4$).

TABLE I. EXPERIMENTAL DESIGN FOR INJECTION OF SME EXTRACT OR SYNTHETIC Gn-RH INTO EWES

Treatment	Day 1	Day 2
SME	Ewe 1	Ewe 2
	Ewe 3	Ewe 4
Gn-RH	Ewe 2	Ewe 1
	Ewe 4	Ewe 3

ing the different treatments was similar (22.4 ± 0.5 ng/ml after administration of 4 crude SME equivalents compared to 33.6 ± 12.1 ng/ml after 10 μg synthetic Gn-RH). Although levels of LH began to increase immediately after injection of either Gn-RH or crude SME extract the time required to obtain maximal concentrations of LH was quite different (3.8 ± 0.6 min after crude SME vs 57.4 ± 13.3 min after synthetic Gn-RH). Futhermore, levels of LH remained elevated much longer after injection of synthetic Gn-RH.

The content of Gn-RH in crude SME extract was 68 ng/SME equivalent in Experiment II. The dosage of synthetic Gn-RH injected was similar to the amount of Gn-RH contained in 4 SME equivalents. After injection of 4 SME equivalents, levels of Gn-RH in serum increased 38 ± 8 pg/ml within 3 min and returned to baseline within 15 min. Levels of LH in serum increased 25.0 ± 8.2 ng/ml within 9 min and had returned to baseline within 150 min (Fig. 3). Following the injection of 280 ng synthetic Gn-RH the increase in levels of Gn-RH in serum was similar to that observed after injection of 4 crude SME equivalents (Fig. 4). However, the increase in levels of LH in serum (6 ± 3 ng/ml) was much smaller. The interval from injection until maximum levels of LH were observed averaged 4.5 ± 1.5 min after injection of crude SME compared to 9.7 ± 3.6 min after injection of synthetic Gn-RH.

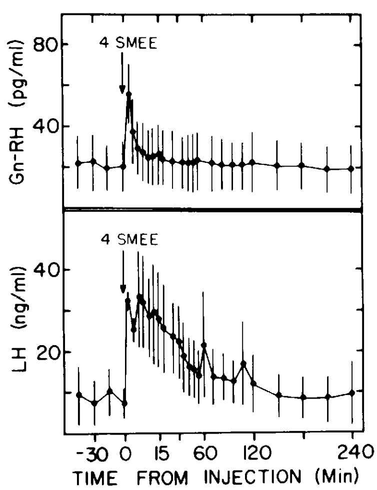

FIG. 3. Levels of Gn-RH and LH in serum of ewes following iv administration of 4 crude SMEE. Each point represents the mean ± standard error ($n = 4$).

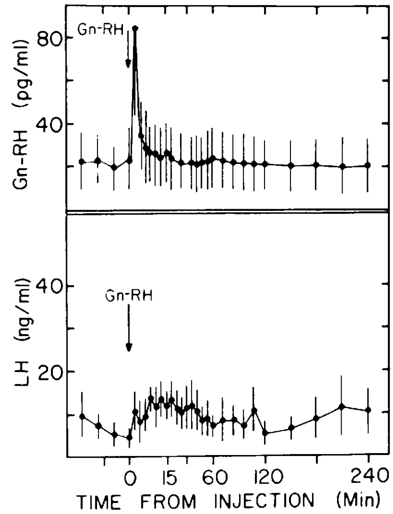

FIG. 4. Levels of Gn-RH and LH in serum of ewes following iv administration of 280 ng synthetic Gn-RH. Each point represents the mean ± standard error ($n = 4$).

In experiment III iv injection of 4 crude SME extracts into anestrous ewes resulted in increases in levels of LH and Gn-RH similar to those observed after injections of crude SME in Experiments I and II. Injection of crude SME extracts which had been treated with 50% saturated ammonium sulfate resulted in increases in levels of Gn-RH and LH similar to those observed after injection of 280 ng synthetic Gn-RH (Fig. 5).

In Experiment IV levels of Gn-RH and LH following injections of crude SME extract were similar to those observed in the previous experiments. Levels of Gn-RH in serum after injection of crude SME extracts which had been purified partially by gel-filtration were similar to levels observed after administration of crude SME extracts or after administration of 280 ng synthetic Gn-RH. The increase in the concentration of LH noted in these ewes was similar to that observed after injection of crude SME extract which had been treated with 50% saturated ammonium sulfate or after the injection of 280 ng synthetic Gn-RH (Fig. 6).

Discussion. Since the identification and subsequent synthesis of Gn-RH, several reports have suggested that other compounds of hypothalamic origin are capable of releas-

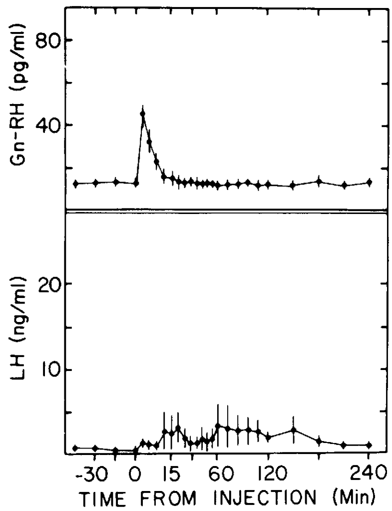

FIG. 5. Levels of Gn-RH and LH in serum of ewes following iv administration of 4 SMEE which were partially purified by ammonium sulfate fractionation. Each point represents the mean ± standard error (n = 4).

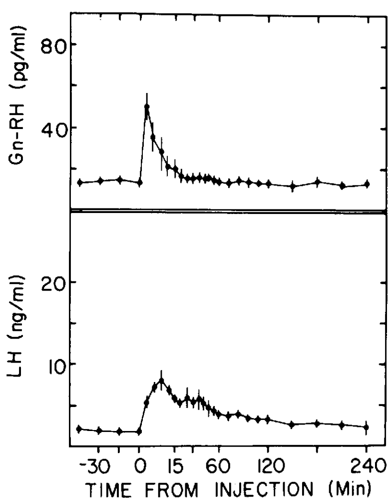

FIG. 6. Levels of Gn-RH and LH in serum of ewes following iv administration of 4 SMEE partially purified by gel-filtration. Each point represents the mean ± standard error (n = 4).

ing gonadotropins (17–19). Differences in the dynamics of LH release following injections of crude SME extract or Gn-RH seemed to support this concept. Furthermore, data obtained by Spies *et al.* (11) suggested that crude SME extracts induced release of LH much more effectively than Gn-RH in rhesus monkeys (9, 20). Data obtained from Experiments I and II of this study suggested that crude extracts of porcine SME were more effective in causing the release of LH in ewes than was synthetic Gn-RH. However, when the crude SME was partially purified by treatment with 50% saturated ammonium sulfate or by gel-filtration the releases of LH elicited by the SME extracts, or by a similar amount of synthetic Gn-RH, were similar. Subsequent analysis of the crude SME indicated that the factor resulting in increased levels of LH in serum that could be separated from Gn-RH by gel-filtration or by ammonium sulfate fractionation was, in fact, LH. It is possible that another factor capable of releasing LH was separated from Gn-RH by ammonium sulfate precipitation and by gel-filtration. However, this does not seem likely since the quantity of LH contamination in the crude SME extracts accounted for more than 90% of the increase in levels of LH in serum following injection of these substances, based on a volume of distribution for LH of 3.5 liters in the sheep (21).

That the release of LH after an injection of partially purified SME extracts or an equal amount of synthetic Gn-RH was similar (Experiments III and IV) suggests that Gn-RH is the only substance present in crude SME extracts in quantities sufficient to induce the release of LH following intravenous injections into sheep. This is further substantiated by reports indicating that both active and passive immunization against Gn-RH results in gonadal atrophy in rabbits (22), rats (23, 24) and sheep (Nett *et al.*, unpublished observation).

It seems likely that the rapid appearance of LH in the blood stream following injection of crude SME extracts reported by other investigators (10, 11) could have been the result of LH contamination in their extracts. Although Gay *et al.* (10) made no attempt to remove LH from their crude SME they reported that contamination accounted for less than 1% of the increase in serum levels of LH. Spies *et al.* (11) observed identical responses to crude SME and "LH-absorbed" SME. Similarly, when crude rat hypothalamic extract was used to superfuse rat hemipituitaries the release of LH was rapid and dramatic (13) even after correction for measurable LH contamination in the extract. In contrast, the release of LH from superfused bovine pituitary tissue was slight (12) when substances having a molecular weight of greater than 10,000 were removed from acidic extracts of bovine hypothalami by ultrafiltration.

The data obtained in this investigation and the available literature suggest that hypothalamic extracts contain substances of a molecular weight greater than 10,000 which increase immunoreactive levels of LH *in vivo* and in the medium used to perifuse pituitaries. In the present study this material appeared to be immunologically active LH.

From the levels of Gn-RH obtained after systemic injection of 10 μg Gn-RH or 280 ng Gn-RH and the resulting release of LH after these injections it is suggested that levels of Gn-RH in the hypothalamo-hypophyseal portal circulation of the ewe must increase to between 60 and 940 pg/ml, or remain elevated above baseline from 15 to 60 min, to induce a substantial release of LH. Direct measurement of the Gn-RH in portal blood collected from monkeys (25, 26) on the day of the expected LH surge indicated an increase in levels of Gn-RH to approximately 500 pg/ml. Similar data have been obtained in rats (27).

Summary. The comparative ability of crude acidic extracts of stalk-median eminence (SME) and of synthetic Gn-RH to induce the release of LH in ewes was examined. Intravenous injections of 4 SME equivalents resulted in a rapid increase in serum levels of LH ranging from 27 to 54 ng/ml and with maximal levels occurring from 3 to 6 min postinjection. Injection (iv) of 10 μg synthetic Gn-RH resulted in a prolonged release of LH into the circulation with serum levels of LH ranging from 21 to 49 ng/ml and with maximum levels occurring from 36 to 84 min postinjection. Administration of 280 ng synthetic Gn-RH (the quantity of Gn-RH contained in 4 SME equivalents) resulted in only a slight increase in serum LH (6 ± 3 ng/ml) with the maximum level at 9.7 ± 3.6 min post-injection. Injection of crude SME extracts after partial purification by ammonium sulfate precipitation or by gel-filtration resulted in only slight increases in serum levels of LH. Radioimmunoassay of the crude SME extracts indicated that over 90% of the increase in serum levels of LH observed after injection of crude SME could be accounted for by contamination of LH in the original extract.

Levels of Gn-RH in serum increased 10 to 60 pg/ml within 3 min after iv injection of crude SME extract or 280 ng synthetic Gn-RH and returned to baseline within 15 min. After iv administration of 10 μg synthetic Gn-RH serum levels of Gn-RH increased to 964 ± 216 pg/ml and returned to baseline within 60 min. Since a substantial release of endogenous LH occurred only after administration of 10 μg synthetic Gn-RH it is suggested that levels of Gn-RH in the hypophyseal portal circulation must approach 900 pg/ml to induce an increase in serum levels of LH comparable to the preovulatory surge.

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