Effect of starvation on gonadotrophin secretion and on in vitro release of LRH from the isolated median eminence of the male rat

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Abstract. A perfusion system was developed in which isolated median eminences (ME) were stimulated in vitro by depolarizing agents such as potassium and veratridine. Potassium concentrations between 30 and 80 mM released increasing amounts of luteinizing hormone-releasing hormone (LRH) from the MEs of starved and control rats. Veratridine at a concentration of 50 μM caused a more prolonged LRH release in both starved and control animals. LRH secretion in vitro was slightly, though not under all conditions, significantly greater in rats starved for 5 days.

The testosterone (T)-LH feedback was studied by castrating the animals and substituting various doses of T through implantation of T-releasing capsules of different sizes. The T concentration in plasma, which can prevent the castration-induced increase of LH, is much smaller in starved than in control rats. The in vitro release of LRH evoked by 80 mM potassium was not different for starved and fed rats under various feedback conditions. Both groups revealed decreased in vitro release of LRH when castrated animals were not substituted with T.

The effect of castration was studied from 1 to 28 days. The plasma LH values rapidly increased in starved and control animals, indicating that the hypothalamic response to castration is not delayed by starvation. The release in vitro of LRH decreased from the first to the fifth day and remained constant thereafter. No significant difference between starved and fed rats was observed. The experiments indicate that the 'releasable pool' of LRH in vitro is greater under conditions of reduced LH release in vivo. The basic mechanism of depolarization-induced exocytosis of LRH from the ME is intact in starved animals.

In patients with anorexia nervosa the function of the hypothalamic-pituitary-gonadal system is greatly impaired (Mortimer et al. 1973; Beumont et al. 1976; Pirke et al. 1979a). Since voluntarily starving healthy subjects demonstrate the same alterations in gonadotrophin secretion as patients with anorexia nervosa (Pirke et al. 1982), it can be assumed that the decrease of gonadotrophin secretion in this disease is a consequence of weight loss. In order to evaluate the underlying mechanisms, we studied the male rat as an animal model, because also in this species starvation causes a decrease in gonadotrophin secretion (Srebnik & Fletcher 1972; Root & Russ 1972; Campbell et al. 1977; Howland & Skinner 1973). We demonstrated earlier (Pirke & Spyrka 1981) that the LRH content of the ME is increased in the starved rat and that the in vivo stimulation with synthetic LRH shows unaltered responsiveness of the pituitary during starvation. This means that the release of LRH from the ME is impaired.

We address here the question whether the basic mechanism of LRH exocytosis induced by depolarization of the LRH neuron is disturbed during starvation. For this purpose we developed a perfusion system in which we exposed isolated MEs to depolarizing agents such as potassium and veratridine. These substances were earlier shown to release LRH from the basal hypothalamus in vitro (Drouva et al. 1981; Harter & Ramirez 1980; Gallardo & Ramirez 1977). Since the sensitivity of
the testosterone-luteinizing hormone (T-LH) feedback is greatly increased in the starved rat (Pirke & Spyra 1981), we studied the in vitro release of LRH from the ME of animals in which various conditions of the T-LH feedback were artificially achieved by castration and implantation of T-releasing capsules of different sizes.

Materials and Methods

Animals and tissue preparation

Male Wistar rats weighing 200–220 g were obtained from 'Mus rattus' (Brunnthal, Germany). The rats were kept on a 10 h dark and 14 h light schedule. Light was on from 5:00–19:00 h. Rat food and water were available ad libitum for the control rats, while starved rats only got water. The animals starved for 5 days.

The animals were sacrificed by decapitation between 9:00 and 11:00 a.m., immediately after removal from their cages. Trunk blood was collected, serum was separated by centrifugation and stored at −20°C until analyzed. The MEs were dissected at once, as described by Negro-Vilar et al. (1979). Under the stereomicroscope, the stump of the stalk was held with a fine pair of forceps. With a microscissor, two longitudinal cuts were made along the lateral limits of the infundibular recess forward towards the optic chiasm. Finally, a frontal cut was made behind the optic chiasm. To control the dissection technique some of the MEs were immunohistochemically stained using an antibody against LRH (Weindl & Sofroniev 1981). The MEs were transported to the perfusion chamber in plastic vials containing ice-cold perfusion medium.

Perfusion system

The perfusion of the MEs was performed in plastic chambers (vol 130 µl). Cellulose nitrate membrane filters (Sartorius SM 11347 pore size 0.3 µm) were inserted on both entrance and exit of the perfusion chambers. To prevent adsorption of released LRH at these filters, the filters were first perfused with a bovine-serum-albumin solution (2% bovine serum albumin in perfusion medium).

Five MEs were kept in one chamber. The chamber was then perfused at 37°C with Krebs-Ringer-bicarbonate, containing glucose (11 mM), ascorbic acid (0.6 mM) and bacitracin (2 × 10⁻⁵ M), the latter to prevent degradation of LRH (Rotsztejn et al. 1976). Shortly before perfusion, this medium was saturated with 95% O₂:5% CO₂, the pH value was adjusted to 7.2 in a glass container. From there the medium was pumped by a Minipuls II pump (Gilson) at a flow rate of 100 µl/min through the chambers. Fractions of 500 or 1000 µl were collected and stored at −20°C until analyzed.

After 30 min of perfusion, the MEs were stimulated for 5 min by either elevating the potassium concentration in exchange for sodium in the perfusion medium (15–80 mM) or by adding veratridine (50 µM). Additional stimulations of 5 min duration were applied at intervals of 30–45 min.

In preliminary perfusion experiments the time course of the LRH release from the MEs was tested, stimulating them for 5–30 min at time intervals of 5–45 min. When the stimulation with potassium lasted longer than 5 min, a continuous release of LRH was observed following the initial peak-reaching about 2/3 of the peak concentration. When stimulating repeatedly, a time interval of at least 10 min was needed in order to gain separate peaks of LRH release. For stimulation, the potassium concentration of the perfusion medium was elevated to 80 mM. In some experiments the potassium concentration in each fraction was measured. The 5 MEs from each chamber were collected at the end of the perfusion. They were stored in perfusion medium at −20°C until analyzed for LRH content.

Radioimmunoassays

The LRH content in the fractions of the perfusion medium and in the MEs was measured as described earlier (Pirke et al. 1979b). The precision was 11.7% at an average concentration of 21.3 pg/200 µl. LRH in serum was measured using reagents kindly provided by the Pituitary Agency of the National Institute of Health. The method was described earlier (Pirke et al. 1979c). The results were expressed in ng of LH-RP-1 standard per ml. The precision was 9% at an average concentration of 113 ng/ml. The LRH release was given as ng/5 min/5 MEs.

Testosterone in serum was measured by radioimmunoassay without chromatography (Pirke 1973). The precision was 9% at an average concentration of 1.64 ng/ml.

Experiments

Experiment 1. Effect of starvation on LRH release induced by constant doses of potassium (a) and veratridine (b).

a. The MEs of 20 starved and 20 fed rats were perfused. Four chambers contained the MEs of the starved, 4 chambers those of the fed animals. The MEs were stimulated 7 times at intervals of 40 min. For stimulation, the potassium concentration in the perfusion medium was elevated to 80 mM.

b. Forty starved and 40 control rats were used in this experiment. The MEs were treated as described above. Three stimulations were applied by adding veratridine in a final concentration of 50 µM to the perfusion medium. Intervals between stimulation were 45 min.

Experiment 2. Effect of starvation on LRH release induced by various doses of potassium.

The MEs of 40 starved and 40 fed rats were perfused.
Eight chambers contained the MEs of the starved rats, 8 chambers those of the fed ones. The MEs were stimulated 4 times at intervals of 30 min. The stimuli were applied in ascending concentrations of potassium (15 mM, 30 mM, 50 mM, 80 mM).

**Experiment 3.** Effect of starvation on LRH release under different conditions of the T-LH feedback.

Six groups of 20 rats each were castrated under ether anaesthesia. One starved and 1 control group were not substituted with T. The other groups received implants of T-filled silastic capsules (internal diameter 2 mm, external diameter 4 mm), releasing T at a constant rate (Damassa et al. 1976; Pirke et al. 1978), immediately after castration. In order to achieve T values between 1.0 and 1.8 ng/ml, the starved group received capsules 0.3 cm in length, the control group capsules 0.65 cm in length. In order to achieve T values above 1.8 ng/ml, a starved group was implanted with capsules 1.6 cm long and a control group with capsules 2.6 cm long. The different relationship between capsule length and T serum concentrations in starved and fed rats was discussed in an earlier study (Pirke & Spyr 1981). Starvation began immediately after castration. All animals were killed 5 days later. The MEs of these groups were perfused as described above. They were stimulated 3 times at intervals of 30 min by elevating the potassium concentration in the perfusion medium to 80 mM. Serum of the trunk blood was assayed for T and LH.

**Experiment 4.** Effect of starvation on LRH release at different times after castration.

Rats were castrated under ether anaesthesia at different intervals before decapitation. Food was withdrawn from the starved rats 5 days prior to decapitation. The interval from castration to decapitation was:

- 1 day: 20 starved and 20 fed rats
- 2 days: 20 starved and 20 fed rats
- 5 days: 20 starved and 20 fed rats
- 14 days: 20 starved and 20 fed rats
- 28 days: 20 starved and 20 fed rats

The MEs of these groups were perfused and stimulated 3 times at intervals of 30 min by potassium (80 mM). Serum of trunk blood was assayed for LH. Statistical comparison between groups was done using the Mann-Whitney-U test.

**Results**

After 5 days of starvation, the weight loss was 62 ± 11 g. During this time weight in the control animals increased by 20 ± 11 g. The protein content of the dissected MEs did not differ between starved and control rats and was, on the average 23 ± 4.5 µg.

Fig. 1 shows a typical time course of LRH release under increased potassium content of the perfusion medium. During stimulation a sharp onset and termination of the LRH release was observed. The LRH release closely followed the course of potassium concentrations in the perfusion medium. About 1% of the LRH content of the MEs (7.6 ± 0.8 ng/5 ME) was released during a single stimulation.

Fig. 2a shows the time course of LRH release from the MEs of starved and control rats repeatedly stimulated with potassium over a period of 5 h. The size of the peaks remained constant for about 2½ h and then gradually decreased. During the whole experiment the LRH release was significantly (P < 0.01) greater in the starved animals. Stimulation with veratridine (Fig. 2b) resulted in a different release pattern. The release peaks were broader and showed a shoulder after the initial
Stimulation of LRH release from MEs of starved (····) and fed (——) rats. Potassium concentration during stimulation was 80 mM. Each point represents the mean value ± SEM of 4 chambers.

Stimulation of LRH release from MEs of starved (····) and fed (——) rats by veratridine (50 µM). Each point represents the mean value ± SEM of 8 chambers.
maximum. Already during the first three stimula-
tions a decline of the peak size was seen. The MEs
from the starved rats again showed a greater
secretion of LRH, the difference, however was not
significant.

Stimulation of the MEs by increasing doses of
potassium caused increasing responses of the LRH
release (Fig. 3). A potassium concentration of 15
mM was not effective in either group. The LRH
release peaks of the starved animals were higher
than those of the fed animals, but this difference
was not significant.

Fig. 4 shows the effect of starvation in animals
under different T-LH feedback conditions.

At T levels above 1.8 ng/ml, equally low LH
values were seen in starved and fed rats. The LRH
release was not significantly different between
groups. In the range between 1.0 and 1.8 ng T per
ml the LH levels were increased in the control
animals but low in the starved rats. The corre-

csponding LRH release in vitro was smaller in the fed
groups (Fig. 4), although the difference did not
reach significance.

Castration of the animals without T substitution
caused high LH serum levels in starved and fed
rats. The LH values of the starved animals were
significantly lower ($P < 0.01$) than those in the fed
rats.

LRH release was nor significantly different in
both groups, but it was significantly lower
($P < 0.01$) than the release from MEs of the sub-
stituted animals.

Fig. 5 shows the effect of starvation on LH serum
levels and on LRH release in vitro at different
times after castration. The LH increase after
starvation was not delayed in starved animals. On
days 1 and 5 after castration, the LH values were
significantly higher in the control rats than in the
starved animals. There was no significant diffe-
rence in LRH release between the two treatment
groups at any time after castration. After 14 and 28
days, a further increase of LH occurred in starved
and fed rats. The LRH release in vitro decreased
significantly ($P < 0.01$) from day 1 to day 5 and
remained unchanged thereafter in both starved
and control groups.

![Fig. 3](image_url)

*Fig. 3.*

Stimulation of LRH release from MEs of starved (– – –) and fed (——) rats with increasing doses of potassium.

Each point represents the mean value ± SEM of 8 chambers.
I

CAPSULE LENGTH (cm)

TESTOSTERONE (ng/ml)

Fig. 4.

LH serum levels and LRH release from MEs. The average values of the T concentration in serum, which were achieved by implanting T-filled silastic capsules into the castrated rats, are indicated by the location of the columns of LH serum levels and LRH release on the x-axis (black columns: fed rats, white columns: starved rats). The animals of the two groups characterized with the 'capsule length 0.00' were castrated and a sham operation performed, instead of implanting any capsule. The MEs were stimulated 3 times at 30 min intervals, elevating the potassium concentration to 80 mM. The LRH values of each column represent the mean value ± sem of the 3 successive release peaks in 4 chambers. * = P < 0.01.

Discussion

The effect of starvation on the hypothalamic-pituitary-gonadal axis is not fully understood. Since the gonadal hormone production can be restored by giving gonadotrophic hormones (Pirke & Spyra 1981) and since the pituitary gland can be stimulated by synthetic LRH in the starved rat (Root & Duckett 1973; Campbell et al. 1977; Pirke & Spyra 1981), it is assumed that food deprivation must mainly influence the hypothalamic control. In agreement with earlier findings (Root & Russ 1972; Pirke & Spyra 1981), we observed here that the hypothalamic stimulation of the LH release can be restored in starved rats when the animals are castrated. Although the initial increase of LH is significantly smaller in the starved rats (Fig. 5), the response to castration does not seem to be delayed.

The T-LH feedback functions in the starved rat, but a greater reduction in T serum concentration is necessary to raise LH values in serum, as was demonstrated in experiment 3 (Fig. 4). In the testosterone range from 1.0–1.8 ng/ml, the LH values are increased in the control but not in the starved rats.

The aim of this study was to look for possible
alterations in the depolarization-induced exocytosis of LRH in the starved rat under various functional conditions of the LRH neurons.

The MEs which were perfused contain only the axons and the terminals of the LRH neuron. The pericaria, which are located in the preoptic-septal region and in the diagonal band of Broca (Kawano & Daikoku 1981), are separated. Nevertheless, this part of the LRH neuron is able to release LRH in response to a depolarizing agent (Fig. 1). The advantages of using a perfusion system are: the possibility to observe the time course of LRH release and to avoid accumulation of metabolic and secretory products which may influence the LRH release (Gallardo & Ramirez 1977).

Fig. 2a demonstrates that constant amounts of LRH are released by stimulation with potassium over a period of 2½ h. After this time, the LRH release declines, maybe due to an impairment of the metabolism of the nerve terminals.

Stimulation with veratridine (Fig. 2b) shows a different LRH release pattern. The prolonged

![Graph](image)

**Fig. 5.**
LH serum levels and LRH release from MEs in castrated starved and fed rats at different times after castration. LH values were 55 ± 11.0 ng/ml (x ± SEM) in intact control and 2.1 ± 1.5 ng/ml in intact starved rats. The MEs were stimulated 3 times at intervals of 30 min in the perfusion experiment with potassium (80 mM). The LRH release values of each column represent the mean value ± SEM of the 3 successive LRH release peaks in 4 chambers. * = P < 0.01.
release may be caused by prolonged binding of veratridine to the sodium channels.

We reported earlier that the LRH content in the MEs of the starved rats is greater than in control animals (Pirke & Spyra 1981). Here we observed that the LRH release in vitro is at least as great – under all conditions – as in control animals, and in some cases (experiment 1) even significantly higher. This observation may be explained on the basis of data from experiments 3 and 4. In experiment 3, a reduced LRH release in vitro was observed in castrated animals, as compared to castrated and T-substituted animals. We may assume that the increased LH secretion is caused by an increased release of LRH in vivo. In experiment 4 we observed declining LRH release in vitro from the first to the fifth day post-castration.

This indicates that an enhanced LRH release in vivo may lead to a reduction of the pool of releasable LRH. An alternative explanation could be that the LRH neuron is less sensitive to depolarizing stimuli. This assumption is, however, not favoured by the results of experiment 2 (Fig. 3), in which it was shown that the dose-response curve (K ➔ LRH release) was similar for MEs of starved and control rats.

From day 5 to day 14 and further on to day 28 there is a second increase in the LH serum levels. This observation is in agreement with the data of Badger et al. (1978), who also demonstrated that the LH rise after castration in male rats takes place in two phases, of which the first is caused by an increased LRH release. The authors suggested a further increase of LRH release into the portal vessels or alternatively, an increased sensitivity of the pituitary gland to LRH as an explanation for the second LH rise. The LRH release in vitro is reduced from day 1 to day 5 after castration and remains unchanged thereafter. Assuming that a reduction in the releasable pool of LRH is a consequence of an enhanced LRH release in vivo, our results support the assumption of an increased sensitivity of the pituitary gland to LRH during the second phase of the castration-induced LH rise.

The starvation-induced suppression of LH release, which is also detectable in the castrated animals on days 1–5 after castration, is abolished on days 14 and 28 after castration. On days 14 and 28, LH concentrations are even significantly higher in the starved rats. This may be a consequence of an altered LH metabolism during starvation.

In conclusion, the depolarization-induced release of LRH in vitro is not affected by starvation of the animals. The greater in vitro release from the ME of the starved rat may be explained by the reverse relationship between LRH secretion in vivo and the releasable amount of LRH in vitro reported here.

References


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