The luteinizing hormone response to luteinizing hormone-releasing hormone, prostaglandin E2 and naloxone is modulated by divergent sensitivity to testosterone feedback

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Abstract. Testosterone (T) levels necessary to suppress LH secretion are reduced in starvation, and increased feedback sensitivity to T is therefore postulated. The luteinizing hormone (LH) response to naloxone (Nal) is more easily suppressed by starvation than is its response to prostaglandin E2 (PGE2) and to luteinizing hormone-releasing hormone (LRH). If the divergent suppressibility is due to altered feedback sensitivity in starvation, it should be feasible to reproduce this phenomenon in normally nourished rats by increasing T levels. Adult male Wistar rats were castrated and implanted with silicone capsules (0–2.6 cm) filled with T. Indwelling jugular cannulae were implanted. On days 4 to 8 post operation rats were injected iv with LRH (25–400 ng/kg body weight), PGE2 (0.05–1.0 mg/kg body weight) or Nal (0.5–50 mg/kg body weight). Blood samples were drawn before and 10, 20 and 30 min after injection. Results show that the response to Nal was already suppressed at medium T levels. The LH response to PGE2 was diminished to a greater extent than the response to LRH but was never completely suppressed by increasing steroid levels. These data are compatible with the hypothesis that steroid feedback sensitivity augments with increasing levels of regulation of the hypothalamic-pituitary-gonadal axis.

Administration of luteinizing hormone-releasing hormone (LRH), prostaglandin E2 (PGE2) or naloxone (Nal) results in release of luteinizing hormone (LH) under standard conditions (for review see McCann & Ojeda 1979; Van Vugt et al. 1981). LRH acts directly on the pituitary gland. PGE2 is thought to act at the hypothalamic level via activation of LRH neurons (for review see Warberg 1982). This mechanism is not influenced by blockade of adrenergic receptors or other neurotransmitters (Harms et al. 1976). There was no demonstrable direct influence of PGE2 on the pituitary. Nal activates hypothalamic structures by removing endorphinergic inhibition. Its action on LH release is critically dependent on intact catecholamine (Kalra & Simpkins 1981) and prostaglandin (Pirke, unpublished observation) release and synthesis. Prostaglandins are essential to the formation of cyclic AMP by alpha-adrenergic receptors (Partington et al. 1980). Intact function of the LRH neurons is another prerequisite of Nal action.

A hierarchial structure for the modulation of LRH neuron function has been postulated on the basis of these experiments, beginning at the top with inhibition by opioid peptides, followed by a stimulatory noradrenergic influence, itself again dependent on a prostaglandin mechanism. A quantitative relationship between testosterone (T) levels and the level of LH has been reported by Damassa et al. (1976). Aromatization of T does not seem to be an essential factor in this feedback action (Pirke et al. 1982). Feedback modulation of the LRH effect on LH release is well known (Debeljuk et al. 1972); an essential factor of this modulation being the LRH receptor number in vivo (Conne et al. 1982) and in vitro (Drouin & Labrie 1976) in the pituitary. Feedback modulation of the PGE2 effect on LH release by progesterone during the oestrous cycle in female rats in vitro has been described by DePaolo et al. (1982). Cicero et al. (1980) found
parallels in the action of morphine and T, describing a competitive antagonism between Nal and the short-term influences of T substitution.

In rats these levels of regulation of LH secretion are affected in different ways by starvation (Küderling et al. 1984). LH release in response to a LRH stimulus is mildly diminished in the course of starvation. The response to PGE₂ is diminished to a greater extent but is never completely suppressed. The response to Nal is rapidly decreased, and is completely abolished after 5 days of total starvation. In humans, low body weight in anorexia nervosa leads to a blunted LH response to LRH (Beumont et al. 1976). The response to Nal is abolished or largely suppressed in weight-loss related amenorrhea (Grossman et al. 1982).

Starvation is accompanied by altered feedback sensitivity to T. In the course of starvation in the rat the T level necessary to suppress the post-castration rise of LH is substantially diminished after 5 days of starvation (Pirke & Spyra 1981). In the unsubstituted rat the post-castration rise of LH is unimpaired by starvation.

The experiments reported here were designed

![Time curve of the LH response to stimulation with different doses of LRH in rats with capsule lengths 0.0 (▲), 0.7 (□), 1.1 (■), 1.7 (Δ), 2.6 cm (●).](image_url)
to provide a firmer basis for evaluation of the following hypotheses:

1) There is a steroid feedback modulation of the modulators of LRH neuron function.

2) Sensitivity to steroid hormone feedback augments with increasing level of regulation in the hypothalamic-pituitary-gonadal axis.

3) The sequence of events in starvation can be explained by increasing sensitivity to steroid hormone feedback.

**Materials and Methods**

**Animals**

Male Wistar rats (200–220 g) were obtained from Mus rattus GmbH (Brunnthal, FRG) and from Süddeutsche Versuchstierfarm GmbH & Co. KG (Tuttlingen, FRG). Rats were kept in cages of 5 and had free access to Altrumin® standard rat chow and water. Lighting scheme was 12 h light, 12 h dark with lights on at 6 a.m. Experiments were performed between 8 and 12 a.m.

**Materials**

LRH was purchased from Hochst AG, Frankfurt FRG, prostaglandin E₂ and naloxone from Sigma Chemie GmbH Taufkirchen, FRG, testosterone from Merck AG, Darmstadt, FRG, and silastic tubes from Deutsch & Neumann, Berlin.

**Methods**

T in serum was measured by radioimmunoassay without chromatography as described by Pirke (1973). The precision was 6.9% at an average concentration of 484.4 ng/100 ml. LH was measured using reagents kindly provided by the Pituitary Agency of the National Institute of Health as described earlier (Pirke et al. 1979). The results are expressed as ng of the LH-RP-1 standard per ml. Intra-assay variability was 9.1%. Inter-assay variability was 16.4% at an average concentration of 21.6 ng/ml.

**Statistical analysis**

Unpaired data were analyzed by the Kruskal and Wallis H-test followed by multiple comparisons with the Mann-Whitney U-test where appropriate. Paired data were
analyzed using the Wilcoxon test. The cumulated response (CR) as a parameter for the area under the curve was calculated using the formula:

$$CR = (10' - 0') + (20' - 0') + (30' - 0')$$

in which 0' signifies the level shortly before stimulation, 10' the level 10 min after stimulation and so on.

**Fig. 2A.**
Cumulated response (index of area under the LH response curve) after stimulation with different doses of LHRH in rats with different capsule lengths (mean ± SEM).
Cumulated response after stimulation with different doses of PGE₂ in rats with different capsule lengths (mean ± SEM).

**Experiments**

After a minimum of 3 days of adaptation rats were anaesthetized using 0.01 mg fentanyl, 1 mg xylazine and 30 mg hexobarbital-Na. Indwelling jugular cannulae were implanted according to the procedure described by Küderling et al. (1984). Rats were castrated and silicon capsules (internal diameter 2 mm, external 3 mm) filled with crystalline T were implanted, as described by Damassa et al. (1976). Capsule lengths (CL) were 0.0, 0.7, 1.1, 1.7 and 2.6 cm. This resulted in a linear correlation between capsule length and T level, as described by Pirke & Spyra (1981). The formula of the regression equation was $y = 100x + 61$.

Animals were used for a maximum of 5 stimulations between days 4 and 8 after operation. LRH (25–400 ng/kg body weight), PGE₂ (0.05–1.0 mg/kg body weight) and Nal (0.5–50 mg/kg body weight) were dissolved in 200 µl of 0.9% saline and were rapidly injected iv. Blood samples (0.45 ml) were drawn immediately before and 10, 20 and 30 min after injection. Blood samples were allowed to clot at room temperature, centrifuged and stored at −30°C until assay. Each point of the dose-response curves represents the values of $n = 8$ to $n = 22$ animals.

**Results**

Fig. 1A shows the time curve of the LH response to LRH stimulation. LH levels after 10 and 20 min were all significantly higher ($P < 0.05$) than baseline with the exception of the 25 ng LRH stimulus at 0.7, 1.1 and 1.7 cm CL.

Fig. 1B shows the time course of the LH response to stimulation with PGE₂. The rise of LH levels after 10 min was significant ($P < 0.05$) except in 0.0 with 0.05 mg PGE₂ stimulation and after 20 min with the following exceptions: CL 0.0 with
0.05 and 0.25 mg PGE₂ stimulation and CL 1.1 with 0.05 PGE₂ stimulation.

Fig. 1C shows the time course of the LH response after stimulation with Nal. LH levels were significantly \( (P < 0.01) \) increased after 10 min in CL 0.0 with all doses of Nal. Increases in CL 0.7 were only marginally significant. In longer capsules no significant increase could be attained at any dose of Nal.

Fig. 2A shows the cumulated response (CR) as an index of the area under the response curve after LRH stimulation. CR was significantly higher after 400 ng LRH than after 25 ng LRH in all substitution groups \( (P < 0.05) \). The difference between CL 0.0 and 2.6 was significant with all doses of LRH. In all substitution groups a plateau of LH response was reached at higher doses of LRH. Between stimulations with 200 and 400 ng LRH the difference was insignificant and from the shape of the curve, no further substantial rise in LH level with increasing stimulation could be presumed.

Fig. 2B shows the CR to PGE₂. The difference in CR following stimulation with 0.05 and 1.0 mg PGE₂ in CL 0.0, 1.1 and 2.6 was significantly different \( (P < 0.05) \). Between CL 0.0 and 2.6 the difference was significant \( (P < 0.05) \) with a stimulation of 1.0 mg PGE₂. With the exception of CL 0.0, the shapes of the curves were such that no further substantial increase in LH levels could be expected with rising doses of PGE₂.

Fig. 2C shows the CR after Nal stimulation. Differences between doses were not significant; a plateau of drug action had already been reached at 0.5 mg Nal. The difference between CL 0.0 and the other substitution groups is significant at all doses of Nal \( (P < 0.01) \). Differences between the higher substitution groups were not significant.

\[ \text{Fig. 2C.} \]

Cumulated response after stimulation with different doses of Nal in rats with different capsule lengths (mean ± SEM).
Discussion

Nal and PGE₂ have no direct effect on the pituitary, and their effect depends therefore on transmission of the stimulus by the LRH neuron and does not outweigh feedback suppression at the pituitary level. The dose response curves of stimulation with LRH are the basis for discussion of the curves obtained for PGE₂ and Nal.

The decreasing ratio of PGE₂ to LRH action and Nal to PGE₂ and LRH action that could be demonstrated with increasing T levels favours the concept of a T modulation of the modulators of LH release and supports the hypothesis that the divergent suppressibility of LRH, PGE₂ and Nal action is an effect of increased steroid feedback sensitivity in starvation. This feedback action is non-competitive in that neither PGE₂, Nal nor T competes directly in modulating the release of LRH.

This is in contrast to the findings of Cicero et al. (1980) and Van Vugt et al. (1982) who reported full reversibility of T feedback inhibition of LH secretion by high doses of Nal. They concluded that T feedback was mediated by an increased secretion of opioid peptides which could be antagonized competitively by Nal. The present results do not support the hypothesis, and the differences between them and those cited above may perhaps be ascribed to differing experimental conditions. Cicero et al. (1980) and Van Vugt et al. (1982) studied short-term (membrane) effects after single dose injections of T, whereas the present study was concerned with long-term (genomic) effects of constant release substitution.

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