THE EFFECTS OF CERTAIN STEROID HORMONES ON THE ACTIVITY OF OVINE HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) – DEGRADING ENZYMES

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1. Introduction

Peptidases capable of degrading luteinizing hormone-releasing hormone (LH-RH) have been shown to exist in the hypothalamus of the rat [1], rabbit [2] and sheep [3,4]. The activity of these peptidases has also been reported to be stimulated in the rat by repeated injection of oestradiol and progesterone [5]. We wish to describe here the effects on the activity of ovine hypothalamic LH-RH-degrading enzymes of low doses of 17β-oestradiol, progesterone and corticosterone applied directly to enzyme-containing extracts in vitro in the presence of synthetic LH-RH.

2. Materials and methods

2.1. *Extraction of hypothalamic peptidases*

Hypothalamic tissue was removed from seasonally anoestrous ewes slaughtered at a local abattoir. The tissue was placed in chilled medium 199 (Difco Laboratories) for transport to the laboratory. The method of enzyme extraction was basically that in [6] with minor modifications [4]. The protein content of the dialysed extract was determined by a microbiuret method using bovine serum albumin (Sigma Chemicals, fraction V; 15% N_2) as standard and was corrected to the experimental concentration by the addition of a 3:1 mixture of 0.01 M phosphate buffer (containing 0.9% NaCl, 0.01% sodium merthiolate and 0.25% egg albumin (pH 7.2)) and 0.05 M phosphate buffer (pH 7.25). The dialysed extract was adjusted to either 100 μg or 300 μg non-diffusable protein/ml.

2.2. *Incubation of synthetic LH-RH with hypothalamic peptidases*

Incubations (either 15 or 30 min) were performed at 37°C in the 3:1 mixture of phosphate buffers in 1 ml total vol. Controls were arranged in three ways: extract and buffer; buffer and 500 ng synthetic LH-RH; preboiled (15 min) extract and 500 ng synthetic LH-RH. The extract and 500 ng synthetic LH-RH were incubated in the experimental tubes either alone or in the presence of steroid hormones. The latter were added to the extract by resuspending the dried residues from aliquots of the steroids in ethanol, in phosphate buffer mixture to give correct concentrations in the incubation media (1 pg, 10 pg and 100 pg 17β-oestradiol/ml; 0.1 ng, 1 ng and 10 ng progesterone/ml; 0.1 ng, 1 ng and 10 ng corticosterone/ml). Incubations were terminated by boiling (10 min) in a water bath. The tubes were sealed and stored at −20°C until required for assay of residual LH-RH. Each incubation was performed as 4 replicates and on 2 occasions with different hypothalamic preparations.

2.3. *Assay of LH-RH*

The incubation media were diluted 1:50 before

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radioimmunoassay for LH-RH. Either an antiserum donated by Dr S. L. Jeffcoate, St Thomas's Hospital, London (final dilution 1:80 000) or an antiserum (Lot R42) donated by Dr T. M. Nett, Colorado State University (final dilution 1:100 000) was used. The details of the assay method are in [4].

3. Results

The incubation of 500 ng synthetic LH-RH with hypothalamic extract at a 300 μg non-diffusable protein/ml for 30 min resulted in a 95% degradation of LH-RH, consistent with [3]. Preboiled hypothalamic extract did not degrade LH-RH significantly. There were no statistically significant differences between the LH-RH contents of the media when they were assayed using the Jeffcoate or Nett antisera. The presence of 17β-oestradiol in the medium greatly enhanced the enzymic activity of the hypothalamic extract and when 10 pg/ml oestradiol were added no residual LH-RH was detected when either antiserum was used. The reduction both of the protein content of the medium (to 100 μg non-diffusable protein/ml) and of the duration of the incubation (to 15 min) resulted in less degradation of LH-RH (40% degradation). Under these conditions, the addition of 10 pg/ml and 100 pg/ml (but not 1 pg/ml) 17β-oestradiol significantly increased the degradation of LH-RH ($P < 0.01$ and $P < 0.001$, respectively). The addition of 10 ng/ml progesterone and 10 ng/ml corticosterone also increased LH-RH degradation significantly ($P < 0.01$ in each case) while lower concentrations of both were ineffective. The results of one 15 min incubation at 100 μg non-diffusable protein/ml when assayed with the Jeffcoate antiserum are shown in fig.1.

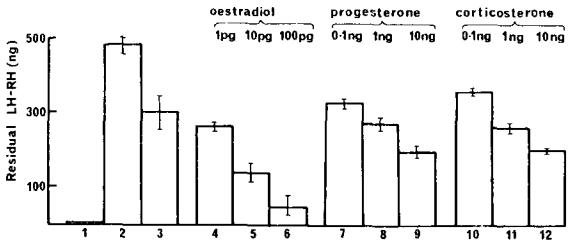

Fig.1. Effects of 17β-oestradiol, progesterone and corticosterone on the degradation of synthetic LH-RH (500 ng) when incubated with hypothalamic tissue extract at 100 μg non-diffusable protein/ml for 15 min. Each column is the mean of 4 incubations. Vertical bars represent ± SEM. Column (1) endogenous LH-RH present in the extract; (2) residual LH-RH when synthetic LH-RH was incubated with pre-boiled extract; (3) residual LH-RH when synthetic LH-RH was incubated with extract; (4–6) residual LH-RH when synthetic LH-RH was incubated with extract containing 1, 10 or 100 pg/ml of 17β-oestradiol, respectively; (7–9) residual LH-RH when synthetic LH-RH was incubated with extract containing 0.1, 1 or 10 ng/ml of progesterone, respectively; (10–12) residual LH-RH when synthetic LH-RH was incubated with extract containing 0.1, 1 or 10 ng/ml of corticosterone, respectively. The statistical significance of differences (P) between columns: 1 and 2, < 0.001; 1 and 3, < 0.001; 2 and 3, < 0.05; 3 and 4, NS; 3 and 5, < 0.01; 3 and 6, < 0.001; 3 and 7, NS; 3 and 8, NS; 3 and 9, < 0.01; 3 and 10, NS; 3 and 11, NS; 3 and 12, < 0.01; NS, not significant.

4. Discussion

The stimulation of hypothalamic peptidase activity obtained by treating animals with steroid hormones has been imitated in this study by adding steroids directly to enzyme-containing extracts. That steroids can act directly on these enzymes has not, to our knowledge, been reported previously. New enzyme molecules could not have been formed under the conditions of these incubations since there was no nuclear material present. The rapidity with which stimulation of activity occurred suggests that the steroids may either remove an inhibitor from the enzyme molecules or stimulate the enzyme activity allosterically. Work is in progress using a greater range of steroid molecules in order to examine further the specificity of this effect which may play a role in the feedback of steroid hormones on gonadotrophin secretion.

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