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PHOTOAFFINITY LABELING OF PITUITARY GONADOTROPIN RELEASING HORMONE RECEPTORS DURING THE RAT ESTROUS CYCLE

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A bioactive photoaffinity derivative of gonadotropin releasing hormone was used to identify pituitary gonadotropin releasing hormone receptors at various stages of the rat estrous cycle. Sodium dodecyl sulfate polyacrylamide gel electrophoresis resulted in the identification of a single specific component with an apparent molecular weight of 60,000 daltons throughout the estrous cycle. The amount of radioactivity incorporated into the 60K dalton band in diestrus and proestrus female rats increased 2.5-fold to that of metestrus and estrus female rats. These findings provide additional evidence for the identification of pituitary gonadotropin releasing hormone receptors.

INTRODUCTION

Gonadotropin releasing hormone (GnRH) stimulates gonadotropin release from the pituitary by interacting with specific receptors on gonadotrope cells. However, pituitary responsiveness to GnRH varies during various endocrine states (1). Studies on the binding of GnRH to pituitary receptors throughout the estrous cycle have indicated that while receptor affinity remains unchanged, marked alterations are observed in receptor content (2-6).

Recently, we have identified a specific GnRH receptor protein from pituitary membrane preparations, with an apparent molecular weight of 60,000 daltons, using an ^{125}I-labeled photoreactive GnRH derivative (7). In the present study, this radiolabeled derivative has been used to label pituitary GnRH receptors during the rat estrous cycle.

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MATERIALS AND METHODS

Synthesis and iodination of photoreactive analog

[Azidobenzoyl-D-Lys^6]GnRH was prepared by reaction of [D-Lys^6]GnRH with (4-azidobenzoyl)-N-hydroxysuccinimide (8). The analog was iodinated by the lactoperoxidase method, applied to a Sephadex G-25 column (4x35 cm) previously equilibrated and eluted with 0.01 M acetic acid (7). The specific activity of the labeled peptide was approximately 1.0 mCi/μg.

Pituitary membrane preparations

Wistar-derived female rats (150-200 g; 60 days old) which exhibited at least two consecutive 4-day estrous cycles, as assayed by daily vaginal smears, were used. The rats were housed in air-conditioned quarters, illuminated between 05:00 and 19:00 h. Pituitary membranes from each stage of the cycle (4 rats) were prepared as described previously (7,8). Briefly, the glands were homogenized gently with a Dounce homogenizer at 4°C in assay buffer (10 mM Tris.HCl, pH-7.4, 0.1% BSA) containing 1 mM dithiothreitol and centrifuged for 10 min at 1000 x g. The supernatant was then centrifuged for 20 min at 20,000 x g. The pellet was resuspended in assay buffer, centrifuged at 20,000 x g for 20 min and finally suspended in assay buffer.

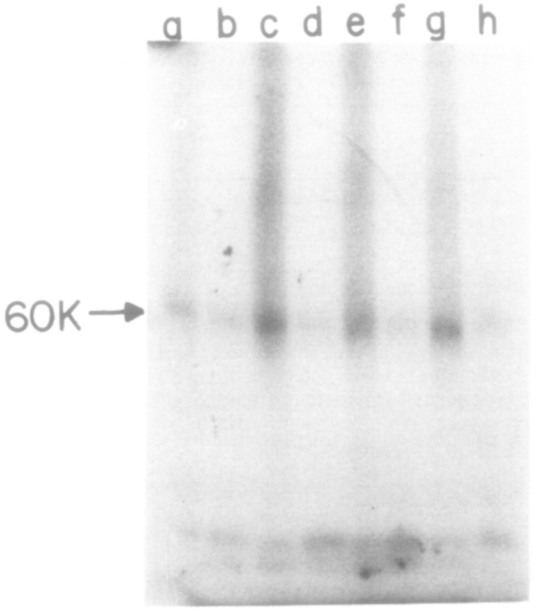
Photolysis and SDS-polyacrylamide gel electrophoresis

Pituitary membranes derived from 4 female rats at various stages of the estrous cycle (1.6-2.0 mg protein/ml) were incubated with ^{125}I-labeled [azidobenzoyl-D-Lys^6]-GnRH (10^5 cpm) in the presence or absence of 10^{-6}M Buserelin ([D-Ser(tBu)^6,des-Gly^{10},ethylamide]GnRH) in 1.5 ml of assay buffer at 4°C in the dark. After 90 min the membranes were washed by centrifugation and photolysed (7 min at 4°C, optimal conditions) with a mercury lamp at a distance of 10 cm. The membranes were then washed (x2) with assay buffer by centrifugation and the pellet boiled in 1% SDS containing 10 mM dithiothreitol. Aliquots were prepared and analyzed in 7.5% slab gel. After staining with Coomassie blue and destaining, the gels were dried for autoradiography.

RESULTS AND DISCUSSION

[Azidobenzoyl-D-Lys^6]GnRH binds with high affinity (apparent Kd value of 0.4 nM) to a single class of receptors (8). These binding sites represent physiological receptors, since photoactivation of the analog with cultured rat pituitary cells results in a long term release of luteinizing hormone (unpublished results).

Photoactivation of the ^{125}I-labeled [azidobenzoyl-D-Lys^6]GnRH after preincubation (90 min at 4°C) with pituitary membrane preparations derived from various stages of the rat estrous cycle resulted (Figure) in the identification of a single specific band with an apparent molecular weight of 60,000 daltons. We have previously shown that the 60K component represents specific binding sites for GnRH in immature rats (7). Thus, photoaffinity



Autoradiogram of sodium dodecyl sulfate polyacrylamide gel electrophoresis of pituitary membrane proteins, labeled with ^{125}I[azidobenzoyl-D-Lys^{6}]GnRH in the absence (lanes a, c, e and g) and presence (lanes b, d, f and h) of 10^{-6}M Buserelin. Lanes a and b, metestrus; lanes c and d, diestrus; lanes e and f, proestrus and lanes g and h, estrus. For experimental details see Materials and Methods.

labeling of GnRH receptors in immature and cycling rats revealed identical molecular weight of 60K daltons.

The amount of radioactivity incorporated into the 60K dalton band throughout the rat estrous cycle is shown in the Table. In diestrus and proestrus female rats there was a 2.5-fold increase in the amount of radioactivity incorporated into the 60K dalton band, as compared to that of metestrus and estrus female rats. The differences in radioactivity incorporated into the 60K dalton band is due to changes in receptors content, since all previous binding studies during the estrous cycle (2-6) have shown a single population of binding sites without any alteration in binding affinity. Similar patterns of receptor changes throughout the rat estrous cycle have been reported by other investigators using binding assays (2-5).

We have recently identified specific GnRH receptor proteins of membrane preparations from rat pituitary glands (7) as well as rat ovarian granulosa cells (9), using the ^{125}I-labeled photoreactive GnRH derivative,

Table

Radioactivity incorporated into the 60K dalton band during the rat estrous cycle

Animal status[a]	Specific cpm incorporated[b]
Metestrus	1220 ± 130[c]
Diestrus	2930 ± 240
Proestrus	2780 ± 170
Estrus	1350 ± 150

[a] The rats were sacrificed between 10.00 to 11.00 a.m.

[b] Pituitary membranes were photolabeled as described in Materials and Methods. The membranes were then subjected to SDS-polyacrylamide gel electrophoresis and the amount of specific radioactivity (cpm in the absence of Buserelin minus cpm in the presence of 10^{-6}M Buserelin) incorporated into the 60K dalton band determined (n=3).

[c] The values were corrected with respect to protein concentrations.

[azidobenzoyl-D-Lys^6]GnRH. The specificity of photolabeling was established by the following lines of evidence: (a) Various GnRH analogs exhibited similar potency in inhibiting the binding of ^{125}I-labeled GnRH analogs and in decreasing the intensity of radioactive bands. (b) Other hormones (e.g. thyrotropin releasing hormone) did not affect the photolabeling of the specific bands. The present studies provide additional evidence for the specificity of labeling of the 60K dalton band since physiological alterations in pituitary GnRH receptor content during the rat estrous cycle are accompanied by similar changes in the radioactivity incorporated into this band.

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