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RAPID COMMUNICATION

ELEVATION OF PLASMA LH IN RESPONSE TO SYSTEMIC INJECTION OF β-ENDORPHIN ANTISERUM IN ADULT MALE RATS

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Abstract

A single injection of β-endorphin antiserum into mature male rats produced approximately a 3-fold rise in plasma LH levels by 10 to 50 min, and declined by 90 min, but remained significantly elevated above pre-injection values. Control rats injected with normal rabbit serum showed no elevation in plasma LH values. These results indicate that β-endorphin is one of the endogenous opiates that tonically depresses basal release of LH in mature male rats.

Footnotes

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Introduction

Our laboratory first reported that a single injection of naloxone (NAL), a specific antagonist of opiates, rapidly elevated serum levels of LH and FSH in intact male rats (1), an observation confirmed by others (2,3). We suggested that this constituted evidence that the endogenous opioid peptides (EOP) chronically depressed gonadotropin release from the pituitary of rats (1,2). It also was shown that NAL can counteract the inhibitory feedback of estradiol, progesterone, and testosterone on LH release (4,5), indicating that endogenous opiates are involved in feedback inhibition by gonadal steroids on gonadotropin release. Apparently NAL promotes LH and FSH release by stimulation of hypothalamic noradrenergic activity and GnRH release (6). Since β-endorphin is present both in the hypothalamus and pituitary, and inhibits LH release, it was of interest to determine whether systemic administration of a specific antiserum to β-endorphin could increase serum LH levels in intact male rats. If so, this would constitute evidence that β-endorphin is one of the endogenous opiates that depresses basal LH secretion.

Materials and Methods

Animals

Male rats (250-300 gm) were obtained from Harlan Industries (Indianapolis, IN). They were maintained on a 14/10 hr light/dark cycle (lights on at 0500 h), at a temperature of 23±2°C. Animals received food (Purina Lab Chow) and water, ad libitum.

Surgery

Each animal was implanted with a silastic catheter (0.1mm x 0.2mm) into

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the right atrium via the external jugular vein under ether anesthesia. The distal end of the catheter was channeled subcutaneously to a point of exit 1 cm posterior to the base of the skull. Immediately after surgery, the animals were injected with 0.2 ml of penicillin (150,000 units, Longicil Fortified, Ft. Dodge Laboratories, Fort Dodge, IA) to prevent infection, and returned to individual stainless steel cages (18x12x 24cm). Animals were weighed prior to surgery and again before the experiment. Animals that showed a reduction in body weight were not used in the experiments.

Antiserum to β-Endorphin

The antiserum to β-endorphin was the generous gift of Dr. H. Friesen, University of Manitoba, Winnipeg, Canada. The procedure followed for the generation of the antiserum, and the properties of the antiserum have been described in detail (7). The antiserum to β-endorphin and normal rabbit serum (NRS) were injected iv at a dose of 1.4ml/350gm BW. A 1:32 solution of this antiserum binds 70% of 125 I-β-endorphin (Dr. H. Friesen, personal communication). NRS (Pelfreeze, Rogers, AK), reconstituted in sterile 0.87% NaCl, was used as a control serum.

Experimental Procedures

Animals were brought into the experimental room for two successive days in order to adapt them to the room. On the third day, the rats were brought into the experimental room and a silastic leader (20cm in length) was attached to each catheter and exited through the wire mesh on top of the cage. During the experiment, the rats were allowed free access to food and water. After removal of the void volume (0.2 ml), blood samples (0.75ml) were drawn into heparinized syringes and centrifuged for 3 min at 2000xg. The plasma was removed and frozen at -20°C until LH was measured by radioimmunoassay (RIA). The red blood cells were resuspended in sterile 0.87% NaCl and reinjected into the animals from which they were removed.

Radioimmunoassay of LH

Plasma LH was measured with the NIADDK kit provided by the Hormone Distribution Agency, NIH, using the double antibody method (8). Values were expressed in terms of the standard NIADDK LH-RP-1.

Statistics

Analysis of variance and the Student-Newman-Keuls procedure were used to analyze the data. A difference of $p<0.05$ was considered to be significant.

Results

Systemic injection of antiserum to β-endorphin produced significant elevation ($p<0.01$) of plasma LH over pre-injection values by 10 min, remained at peak levels until 50 min, and declined by 90 min (Fig. 1). However, even at 90 min, LH remained significantly elevated above pre-injection values and above saline-injected control levels. Control animals injected with NRS showed no increase in plasma LH over pre-injection values during the entire 90 min experiment.

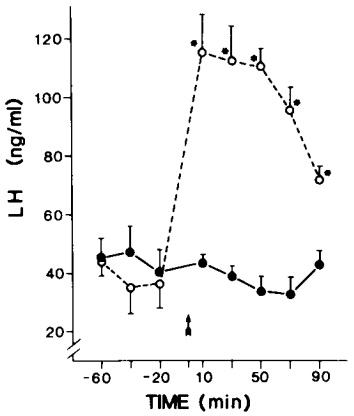

Fig. 1. Effects of β-endorphin antiserum (dashed line) and normal rabbit serum (solid line, controls) on plasma LH levels in adult male rats. Vertical bars indicate standard errors of mean, and stars indicate significant differences from control and pre-injection values. Each solid point is the average of assays from six rats. Each open circle is the average value from seven rats.

Discussion

In the present study, passive immunization with antiserum to β-endorphin in sexually mature male rats resulted in a significant elevation in plasma

levels of LH that lasted for at least 90 min. In a previous study, intraventricular injection of an antiserum to β-endorphin or dynorphin into 12 day old female rats also produced an elevation of plasma LH, whereas an antiserum to met-enkephalin was ineffective (9). β-endorphin antiserum was much more active in elevating plasma LH than dynorphin. The present results indicate that a specific antiserum to β-endorphin injected iv is effective in elevating plasma LH in adult male rats. This study therefore indicates that one of the endogenous opiates responsible for depressing basal LH secretion in adult male rats is β-endorphin. β-endorphin may be the most important endogenous opiate involved in depressing LH release, since it is present in relatively high concentrations both in the hypothalamus and pituitary, and appears to be the most potent of the endogenous opiates that inhibit LH release in rats (Van Vugt DA, Sylvester PW, Meites J, unpublished).

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