EFFECT OF SYNTHETIC LUTEINIZING HORMONE RELEASING HORMONE ON PROLACTIN SECRETION FROM CLONAL PITUITARY CELLS

Damon C. Herbert and Edward G. Rennels

Department of Anatomy
The University of Texas Health Science Center at San Antonio
7703 Floyd Curl Drive
San Antonio, Texas 78284

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SUMMARY

The influence of LHRH, an analog of LHRH (hydroxy-PRO[1]) and inulin on prolactin (PRL) secretion was studied using a clonal strain of pituitary cells. At low concentrations, 0.08 ng to 8 ng/ml, LHRH stimulated PRL release while at higher concentrations the opposite effect was obtained. The analog of LHRH inhibited PRL secretion at all concentrations studied. No effect was measured with inulin.

The hypothalamus is known to regulate the anterior pituitary gland by hormones which either stimulate or inhibit pituitary cell secretion (1). Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are controlled by a single hormone, LHRH (luteinizing hormone releasing hormone) which is a decapeptide capable of stimulating the release of LH and FSH both in vivo as well as in vitro (1). In contrast, prolactin (PRL) secretion is believed to be modulated by a hypothalamic inhibiting factor. Of the large number of compounds known to influence PRL secretion, two of these, thyrotrophin releasing hormone (TRH) and dopamine, are present in the hypothalamus (2, 3). LHRH, which is present in high concentrations in the hypothalamus (4), has been reported to cause no change in serum PRL levels in rats (5) or humans (6). In the present study, synthetic LHRH and a biologically inactive analog of LHRH (7) have been employed to determine their effect on PRL secretion from a clone of normal rat pituitary cells previously shown to secrete only PRL (8).

MATERIALS AND METHODS

The 2B8 clonal strain of pituitary cells was derived from Rathke's pouch epithelium obtained from 11-13 day old fetal rats (8). Approximately 4 x 10^5 cells were placed into culture dishes (60x15 mm, Falcon #3002) each containing

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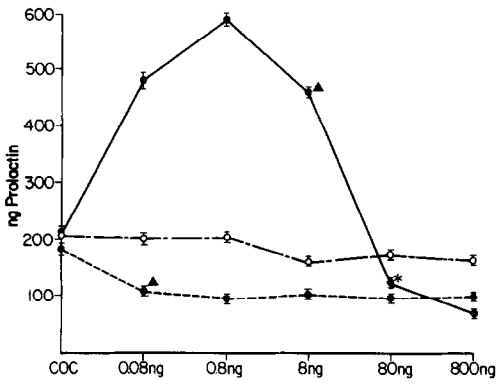

FIGURE 1. PRL concentration (mean ± SE) per ml of culture media obtained from clonal pituitary cells incubated with LHRH (————), an analog of LHRH (--------) and inulin (- — - — -).
*p < 0.005, ▲p < 0.0005 when compared to the respective "cells only" control (COC).

4 ml of 85% Ham's F10 synthetic medium and 15% "virus screened" fetal calf serum (Gibco Biological Supplies, Lot No. A952616). The cells were then maintained at room temperature for 2 hours before being transferred to a 37°C humidified CO_2 incubator for 10 days. Following this, the media were discarded and replaced with fresh media containing synthetic LHRH (Beckman Instruments, Inc.), hydroxy-PRO^1-LHRH [an LHRH analog with 0.001% activity (7)] or inulin (Sigma Chemical Corp.) in concentrations ranging from 0.08 to 800 ng/ml. After an additional 3 days in culture, the media were again discarded and replaced with fresh media containing LHRH, the analog or inulin. These were then maintained for 6 hours after which the media were collected and frozen at -20° C for analysis of PRL content. Two control cultures were also established; one designated "medium only" which consisted of dishes incubated with culture media only, and a second designated "cells only" consisting of dishes containing 2B8 cells and media but no LHRH, analog or inulin. The PRL concentrations in all studies were determined by radioimmunoassay using reagents supplied in kits by the NIAMDD. Values are expressed in terms of the rat PRL-RP-1 standard. All data were statistically analyzed by analysis of variance and the t test for comparison of differences between means.

RESULTS AND DISCUSSION

The effects of synthetic LHRH, an analog of LHRH, and inulin on PRL secretion are shown in figure 1. Concentrations of LHRH ranging from 0.08 ng to 8.0 ng/ml produced a marked stimulation of PRL secretion. The maximal effect was observed with 0.8 ng/ml which resulted in a 180% increase in the amount of PRL released by the 2B8 cells. When the cells were incubated with higher concentrations, 80 and 800 ng/ml, a significant reduction in the PRL

content of the medium was found. In contrast, the analog of LHRH produced a decrease in PRL secretion which was approximately 45% less than that observed in the "cells only" control, and was similar at each of the five concentrations employed. Inulin, on the other hand, failed to significantly alter PRL secretion at any of the concentrations examined. There was no detectable PRL present in the "medium only" control.

The changes observed in PRL release from the 2B8 cells incubated with LHRH or with the analog appear to be unrelated to the osmolarity of the culture medium. Inulin, a compound routinely employed to evaluate the influence of osmolarity on the physiology of tissues in culture, was without effect. We suspect that the inhibition of PRL release observed at the higher concentrations of LHRH may have no physiologic significance since such concentrations would not likely be encountered *in vivo* (9).

The nature of the inhibition of PRL by the analog, hydroxy-PRO^{1}-LHRH is not known. The observations suggest that pGlu, which is at the N-terminus of native and synthetic LHRH (1), may be necessary for the interaction between LHRH and the receptors that are involved in the stimulation of PRL release from the 2B8 cells. Nikolis *et al.* (7) have also indicated that pGlu is a key amino acid which is involved in the regulation of the biological activity of the hormone molecule.

There exists conflicting evidence in the literature regarding the effect of LHRH on PRL secretion. Sandow and Robyn (10) reported a significant decrease in peripheral PRL levels in male rats given subcutaneous or intravenous injections of 250 µg LHRH. The same results were observed after administering an intrapituitary injection of either 1 ng or 5 µg LHRH (10). LHRH, at a dose of 5 µg/kg, was also found to decrease serum PRL in adult female rats, androgenized females and in thyroidectomized animals (11). However, in thyroidectomized-androgenized rats, LHRH caused an elevation in PRL levels. In contrast, Suzuki *et al.* (12) failed to observe any changes in serum PRL in ovariectomized rats that received 0.02-200 ng of the decapeptide either

intravenously or directly into the pituitary gland. Likewise, Debeljuk et al. (5) found no effect in female rats given 0.1 μg LHRH after first being pretreated with reserpine. In contrast, when pregnant female rats were given 100 μg LHRH twice daily for the first seven days of pregnancy, serum PRL values were, with one exception, significantly lower than the values recorded in the controls during the first four days (13). On days five through seven, PRL was, in general, unaffected by the decapeptide. When 1 μg LHRH was administered to fetal rhesus monkeys, no change occurred in plasma PRL levels (14). However, a decrease in maternal plasma PRL was measured in three of seven animals, with a transient rise being observed in one of the females and no change in the other three (14). In humans, PRL levels have been reported to remain stable after an intravenous injection of 50 μg LHRH (6).

While several of these studies indicate that LHRH may affect PRL secretion, it is not known whether this presumptive action is directed at the PRL cell itself or is carried out indirectly through the modulation of steroid hormone secretion. Attempts by several laboratories to establish whether the decapeptide has a direct effect on the pituitary gland have been made using organ culture systems or cultures of dispersed pituitary cells (15-18). Significant changes in PRL secretion were not found; however, slight alterations in the concentration of PRL in the culture media were noted by Vaughan et al. (17) following the incubation of hemipituitaries with 20 ng/ml synthetic LHRH and by Blackwell et al. (18) who employed dispersed pituitary cells cultured in the presence of 10^{-10} to 10^{-7} M LHRH.

The fact that LHRH does effect PRL secretion in our clonal cells and in general has no effect in other in vitro systems or in vivo leads one to consider that the response of the 2B8 cells may be nonspecific. Müller et al. (19) found that growth hormone release could be stimulated from anterior pituitary transplants located under the kidney capsule of hypophysectomized female rats by TRH, LHRH or vasopressin. These same compounds failed to alter growth hormone in intact controls. In addition, they recorded a rise in

peripheral growth hormone levels following TRH administration to female rats bearing median eminence lesions; no change occurred in sham-operated controls. From these findings, they speculated that separation of the pituitary gland from its normal connections with the central nervous system "unmasks" receptors that are ordinarily unavailable for interaction with certain hypothalamic humors (19). Whether this hypothesis is a valid explanation for our experimental findings or whether LHRH is yet another regulator of PRL secretion in the intact animal remains to be elucidated.

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