PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **186**, 344–347 (1987)

Effects of Hypothalamic Neurohormones on Prolactin Release from Pituitary Allografts in the Hamster (42623)

GARY T. CAMPBELL, MARK J. HORACEK, AND CHARLES A. BLAKE

Department of Anatomy, University of South Carolina School of Medicine, Columbia, South Carolina 29208

Abstract. Recent reports indicate that luteinizing hormone-releasing hormone (LHRH) releases prolactin (PRL) under some circumstances. We examined the chronic effects of LHRH, growth hormone-releasing hormone (GHRH), and corticotrophin-releasing hormone (CRH) on the release of PRL, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by pituitary allografts in hypophysectomized, orchidectomized hamsters. Entire pituitary glands removed from 7-week-old-male Golden Syrian hamsters were placed under the renal capsule of hypophysectomized, orchidectomized 12-week-old hamsters. Beginning 6 days postgrafting, hamsters were injected subcutaneously twice daily with 1 μg LHRH, 4 μg GHRH, or 4 μg CRH in 100 μl of vehicle for 16 days. Six hosts from each of the four groups were decapitated on Day 17, 16 hr after the last injection. Prolactin, LH, and FSH were measured in serum collected from the trunk blood. Treatment with LHRH significantly elevated serum PRL levels above those measured in the other three groups, which were all similar to one another. Serum LH levels in hosts treated with vehicle were elevated above those measured in the other three groups. Serum FSH levels in hosts treated with LHRH were greater than FSH levels in any of the other three groups. These results indicate that chronic treatment with LHRH can stimulate PRL and FSH release by ectopic pituitary cells in the hamster.

The release of prolactin (PRL) is thought to be regulated in a dual manner by a hypothalamic inhibitory dopaminergic system and a presumed PRL-releasing hormone. Results of some studies suggest that luteinizing hormone-releasing hormone (LHRH) or analogs can release PRL. For example, an analog stimulated the release of PRL from clonal pituitary cells (1). In humans, LHRH and an LHRH agonist increased serum PRL (2, 3). Denef and Andries (4) showed that LHRH treatment of cocultures of gonadotrophs and lactotrophs increased PRL and luteinizing hormone (LH) release. Recently, Hoeffler and Frawley (5) reported that LHRH, growth hormone-releasing hormone (GHRH), and corticotrophin-releasing hormone (CRH) increased the proportion of adenohypophyseal cells isolated from 5-day-old rats which released PRL in cultures. To investigate further the roles of neurohormones on PRL release, we determined the chronic effects of LHRH, GHRH, and CRH on serum PRL levels in hypophysectomized, orchidectomized hamsters with pituitary allografts beneath the renal capsule.

Material and Methods. Intact male and 9-week-old hypophysectomized and orchidectomized Golden Syrian hamsters were purchased from Charles River Laboratories, Inc. (Wilmington, MA). The animals were housed individually in a room with controlled lighting (14 hr light:10 hr darkness) and temperature (22–24°C). Food, and water supplemented with 5% sucrose, were constantly available.

Three weeks following hypophysectomy and orchidectomy, each hamster was anesthetized with 1.0 mg chloral hydrate/100 g body weight and exposure to ether. The fur over the right flank was shaved and the skin was cleansed with 70% alcohol and betadine. A 1-cm incision was made in the skin and muscle dorsal to the kidney. After gently exteriorizing the kidney, a pocket was created under the renal capsule by freeing the capsule from the underlying parenchymal tissue. Pituitary glands (the donor tissue) were removed from 7-week-old male hamsters which had been decapitated. One donor pituitary gland was placed in the subcapsular pocket and the kidney was returned to its

0037-9727/87 $1.50

normal position. The muscle was closed with silk suture, the skin was closed with metal wound clips, and the animal was allowed to recuperate without any special postoperative care.

Six days following the pituitary grafting, groups of six hamsters with allografts received subcutaneous injections of LHRH (1 μg per injection), GHRH (4 μg per injection), CRH (4 μg per injection), or vehicle (0.1% gelatin in 0.9% saline). Injections were given at 0700 and 1900 hr for 16 days. All releasing hormones were purchased from Peninsula Laboratories (Belmont, CA). The hamsters were decapitated on Day 17, 16 hr after the last injection. Six hypophysectomized, orchidectomized hamsters without allografts also were decapitated. The base of the skull was examined macroscopically for remnants of adenohypophyseal tissue. Trunk blood was collected and allowed to clot at room temperature. Serum was collected and assayed for LH, follicle-stimulating hormone (FSH) (6), and PRL (7). Data were analyzed by one-way analysis of variance and the post hoc Newman–Keuls test.

Results. Serum PRL immunoreactivity was undetectable (<1 ng/ml) in the hypophysectomized, orchidectomized hamsters without allografts (Fig. 1). PRL levels, 16 hr after the last injection, were similar in the hamsters with allografts and treated with vehicle, GHRH, or CRH. In contrast, serum PRL levels were elevated ($P < 0.01$) at 16 hr after the last injection of LHRH.

Serum LH levels in hosts treated with vehicle were above the levels measured in the hypophysectomized, orchidectomized hamsters without allografts ($P < 0.05$). Serum LH levels in the hosts treated wth LHRH, GHRH, or CRH were similar to the LH immunoreactivity measured in hypophysectomized, orchidectomized hamsters without allografts. Serum FSH levels in hosts treated with LHRH were greater than FSH levels in the hamsters without allografts or in any of the other hosts ($P < 0.05$). Standard curves prepared from pooled serum of the different groups with allografts were all parallel in the PRL assay.

Discussion. Present evidence suggests that either LHRH or a LHRH-induced product

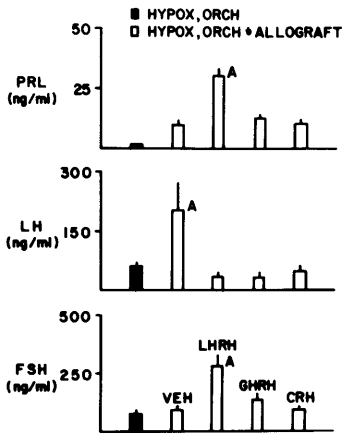

FIG. 1. Serum PRL, LH, and FSH levels in hypophysectomized, orchidectomized hamsters (solid bar) and in similar hamsters with a pituitary allograft and treated with vehicle, LHRH, GHRH, or CRH (open bars). Data are expressed as the means ± standard errors, except the PRL levels in hypophysectomized, orchidectomized hamsters without allografts which are plotted as 1 ng/ml. "A" indicates that the mean differs from the others at $P < 0.05$.

of the gonadotroph may influence PRL synthesis and release significantly. Our results substantiate other suggestions that LHRH releases PRL from malregulated (1, 2, 4, 5) or apparently normal adenohypophyseal cells (3). However, an agonist of LHRH inhibited PRL release from lactotrophs *in situ* or ectopic lactotrophs (8) and from a prolactin-secreting tumor (9). The physiological significance of the stimulatory action of LHRH on PRL release is unclear as the gonadotrophins and PRL usually are released in reciprocal fashion in adults. Begeot *et al.* (10, 11) have reported that LHRH or the α subunit of LH may participate in lactotroph differentiation. Thus, the stimulatory effect of LHRH on PRL release may be observed best with incompletely differentiated cells (5, 10, 11) or malregulated cells (1, 2, 4, 5). The differences between our results and others (8, 9) may reside in the fact that we used LHRH instead of an analog.

We interpret the serum LH data to indicate that all three neurohormones suppressed serum LH. We think that each injection of LHRH was followed by an immediate release of LH (6) and that the continued in-

jections of LHRH caused a decline in the basal release of LH (12). Thus, the supression of serum LH by injections of LHRH depended upon the time at which serum LH was measured. We do not have an explanation presently as to the mechanism by which GHRH and CRH suppressed serum LH.

A previous study showed that LHRH increased serum FSH in hypophysectomized, orchidectomized hosts (6). Preliminary unpublished data indicate that although cells containing FSH were present in the allografts in the hosts treated with vehicle in the present study, exposure of these allografts to the exogenous LHRH was needed for the maintenance of the FSH cell population. Such a condition has been suggested previously (13, 14). When pituitary tissue from perinatal hamsters is used as donor tissue in similar experiments, the gonadotrophs in the allografts contained almost exclusively LH. Treatment of the host with LHRH induced the appearance of FSH in the allograft cells (6). Thus, this model system may provide a means of determining the effects of different LHRH treatment regimes on the gonadotroph population.

We did not measure circulating growth hormone or adrenocorticotrophin in these hosts. However, we have evidence indicating that GHRH and CRH significantly altered the GH cell and ACTH cell populations, respectively (15). Thus, these neurohormones did affect the ectopic pituitary tissue. Hoeffler and Frawley reported that LHRH, GHRH, and CRH increased the proportion of cells releasing PRL when adenohypophyseal cells obtained from neonatal rats were cultured with these neurohormones (5). The discordance between our results and theirs (5) may reside in the facts that we studied the effects of the neurohormones on differentiated cells, whereas they studied the effects of the neurohormones on developing cells, and that we measured serum PRL at one time only. Moreover, because of the irregular shapes of the lactotrophs in the allografts, we may not be able to determine if any of the neurohormones induced changes in the percentages of allograft cells which are lactotrophs. In any case, effects of LHRH, GHRH, CRH, and thyrotropin-releasing hormone on the cell populations of a variety of preparations of pituitary cells, including the pituitary gland *in situ,* have now been reported (5, 6, 15, 16). Thus, evidence indicates that the neurohormones significantly affect the cell populations of the adenohypophysis.

This investigation was supported by grants from the National Institutes of Health (AM-19170 and HD-22687).

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Received April 14, 1987. P.S.E.B.M. 1987, Vol. 186.
Accepted September 2, 1987.