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THE GLYCOPEPTIDE MOIETY OF VASOPRESSIN-NEUROPHYSIN PRECURSOR IS NEUROHYPOPHYSIAL PROLACTIN RELEASING FACTOR

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SUMMARY: All of the classically-described hypothalamic, hypophysiotropic factors that regulate anterior pituitary hormone secretion have now been isolated and identified except for prolactin releasing factor. We report here that the 39-amino acid glycopeptide comprising the carboxyterminus of the neurohypophysial vasopressin-neurophysin precursor stimulates prolactin release from cultured pituitary cells as potently as does thyrotropin releasing hormone but has no effect on the secretion of other pituitary hormones. Furthermore, antisera to the glycopeptide administered to lactating rats attenuated suckling-induced prolactin secretion. Thus, this glycopeptide appears to be the neurohypophysial prolactin releasing factor.

All of the originally postulated hypothalamic, hypophysiotropic hormones that regulate anterior pituitary hormone secretion have now been isolated and identified (1-4) except for prolactin releasing factor (5). Considerable evidence supports the existence of a peptidic prolactin releasing factor which is thought to be essential for the large release of prolactin that follows suckling (5). Recent experiments showing that surgical removal of the posterior pituitary gland specifically blocks suckling-induced prolactin release suggest that the unidentified prolactin releasing factor resides in the neurohypophysis (6-7), in contrast to all other hypophysiotropic factors which arise from the median eminence of the hypothalamus (1-4).

In the present study, we have investigated a prolactin-releasing role for the 39 amino acid glycopeptide moiety (8) of the vasopressin-neurophysin precursor (9), an abundant neurohypophysial peptide with previously unassigned function.

MATERIALS AND METHODS

The effect of the glycopeptide on pituitary hormone secretion by cultured pituitary cells was measured with reverse hemolytic plaque assays

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as described previously (10, 11). This assay permits microscopic visualization of hormone release as areas of hemolysis (plaques) surrounding pituitary cells, and is based on complement-mediated hemolysis of protein A-coupled ovine erythrocytes in the presence of hormone antibody. Our previous studies have demonstrated that plaque area is positively and linearly related to the amount of hormone secreted and that the assays are specific (10, 11).

Immunoneutralization studies with glycopeptide antiserum were performed on lactating rats of the Holtzman strain at days 8-11 of lactation. Mothers weighing approximately 300 g were anesthetized with pentobarbital sodium and surgically implanted with a cannula in the right jugular vein 24-48 hr before the experiment. On the day of the experiment, the litter (which had been reduced to 8 pups on day 2 of lactation) was separated from the mother for 4 hr before initiation of the suckling experiments. A control blood sample (0.2 ml) for prolactin analysis by radioimmunoassay was obtained immediately before the administration of antiserum (0.45 ml) or normal rabbit serum (0.5 ml) into the jugular vein; 15 min later the litters were returned to the mothers for suckling. Additional blood samples (0.2 ml) were obtained at the indicated intervals during a 1-hr suckling period.

Prolactin concentrations in serum were measured by radioimmunoassay using reagents supplied by Dr. A. F. Parlow through the National Pituitary Agency, NIAMDK, NIH. Values are expressed as ng equivalents of the RP-3 standard (30 IU/mg protein) and were run as duplicates in two separate assays. To accomodate the presence of rabbit serum in test sera from rats, we increased sheep anti-rabbit immunoglobulin to the amount required to maximally precipitate antibody-bound iodinated prolactin in the radioimmunoassay.

The specificity of the antiserum for glycopeptide was investigated by determining its cross-reaction with various neurohypophysial peptides using an enzyme-linked immunosorbent assay as described (12). Alkaline phosphatase conjugated goat anti-rabbit immunoglobulin (Sigma) and p-nitrophenyl phosphate disodium were used for color development which was scanned for absorbance at 405nm in an enzyme-linked immunosorbent assay spectrophotometer.

Synthetic thyrotropin releasing hormone, corticotropin releasing factor, growth hormone releasing factor, and luteinizing hormone releasing hormone were obtained from Sigma Chemical Co. (St. Louis, Mo.). The 39-amino acid glycopeptide was isolated from ovine pituitary glands as previously described (8). Its amino acid sequence has been reported (8). The glycopeptide was stored lyophilized until use in these experiments. The antiserum to vasopressin related glycopeptide was prepared in the laboratory of D.G.S. by S. Zakarian; the ovine glycopeptide was administered in 50% complete Freund's adjuvant into the foot pads and hind legs of New Zealand strain white rabbits. One month later booster injections in 50% incomplete Freund's adjuvant were given intracutaneously into the abdomen, and the procedure was repeated at two-week intervals. Blood was collected from the ear vein and the sera obtained was stored at -70°C. The antibody reacted with the 39 residue ovine, bovine and porcine glycopeptides with high affinity but did not react to a significant extent with their NH_2- or COOH- terminal fragments.

RESULTS

Addition of the ovine glycopeptide to rat anterior pituitary cells in culture (Fig. 1A) stimulated prolactin release to a similar extent as thyrotropin releasing hormone, the most intensively studied and potent of the peptides that release prolactin. The glycopeptide released prolactin even more potently in the presence of prolactin-inhibitory concentrations of dopamine (Fig. 1B), which is physiologically appropriate since

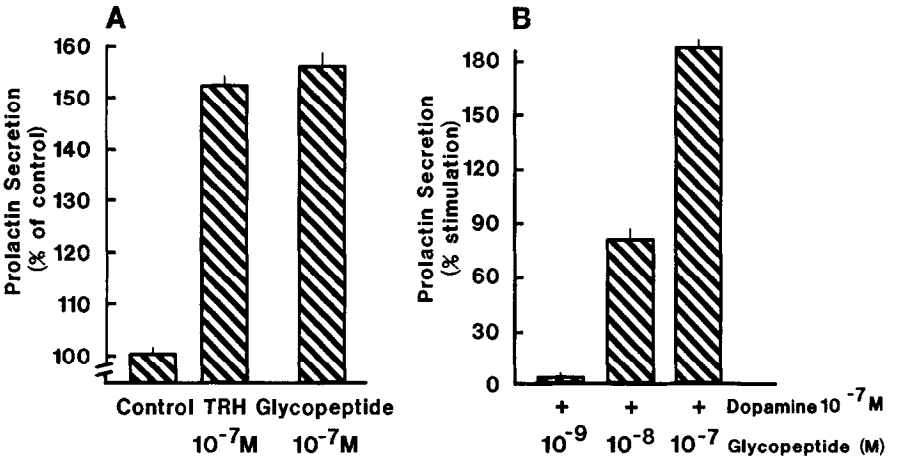

Figure 1. Stimulation of prolactin release from cultured rat anterior pituitary cells by vasopressin-neurophysin associated glycopeptide either alone (A) or in the presence of dopamine (B). Prolactin secretion was measured by reverse hemolytic plaque assay, and the results shown are mean ± S.E.M. of a single experiment. The mean stimulation expressed as % of control for 10^{-7}M glycopeptide in all experiments was 169% (n=6), for 10^{-8}M glycopeptide was 137% (n=5), and for 10^{-7}M thyrotropin releasing hormone was 189% (n=4).

suckling-induced prolactin release occurs in the absence of prolonged, major changes in dopamine secretion (5). This stimulation is specific to prolactin since similar concentrations of the glycopeptide did not alter luteinizing hormone, adrenocorticotropic hormone, thyroid stimulating hormone, or growth hormone release from cohort rat anterior pituitary cells in culture that were demonstrably responsive to their hypothalamic, hypophysiotropic hormones (Table 1).

Table 1. Effect of vasopressin-neurophysin associated glycopeptide on luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH) and growth hormone (GH) secretion [a]

Pituitary Hormone (Hypothalamic Hormone)[b]	Control[c]	Glycopeptide (10^{-7}M)[c]	Hypothalamic Hormone[c]
LH (LHRH 10^{-9}M)	1,945±193	1,956±149	21,874±2,674
ACTH (CRF 10^{-7}M)	791±133	1,003±355	6,231±602
TSH (TRH 10^{-7}M)	1,159±251	1,693±349	5,478±1,041
GH (GHRF 10^{-9}M)	2,099±193	2,644±315	13,894±3,871

[a] Separate reverse hemolytic plaque assays for each of the pituitary hormones listed were used to measure their secretion in the presence of no secretagogue (control), in the presence of glycopeptide, or in the presence of their specific hypothalamic regulatory hormones to serve as positive controls.

[b] Abbreviations: LHRH, luteinizing hormone releasing hormone; CRF, corticotropin releasing factor; TRH, thyrotropin releasing hormone, and; GHRF, growth hormone releasing factor.

[c] Mean (± S.E.M.) total hemolytic plaque area (μm^2 of 200 plaques) which is positively and linearly related to the amount of pituitary hormone secreted.

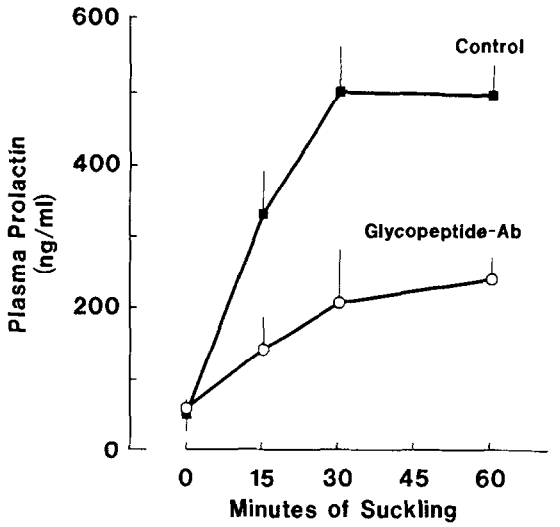

Figure 2. Inhibition of suckling-induced prolactin secretion by antiserum to the vasopressin-neurophysin associated glycopeptide. Plasma prolactin levels were measured by radioimmunoassay; the antiserum (0.45 ml) or normal rabbit serum (0.5 ml) was administered intravenously to the mothers 15 min before sucking by the pups began. The data are means ± S.E.M. for n=3 in the antiserum-treated group and n=11 in the normal rabbit serum (control) group.

To investigate a role for the glycopeptide in prolactin release in vivo, we administered glycopeptide antiserum to lactating rats intravenously immediately before permitting their previously isolated pups to suckle (Fig. 2). Radioimmunoassayable plasma prolactin levels were significantly lower than in control rats receiving a similar amount of normal rabbit serum (Fig. 2). A prolonged and profound suppression of milk secretion apparently occurred during subsequent sucklings since the litters from antiserum-treated mothers weighed only 78% of those from control mothers after a week (litter weights in the 3 antiserum-treated mothers were 192 ± 13.4 gm, mean ± S.E.M. vs. 244.7 ± 12.3 gm for 4 control litters).

The antiserum was tested for immunoreactivity in an enzyme-linked immunosorbent assay with several neurohypophysial peptides: ovine glycopeptide, vasopressin, oxytocin, neurophysins I and II, vasoactive intestinal peptide, angiotensin II, dynorphin A, atrial natriuretic factor, and thyrotropin releasing hormone. The limit of detection of the antiserum for glycopeptide was 0.5 ng and the amount of glycopeptide yielding an optical density reading of 1.0 was 10 ng. None of the other peptides was detected in amounts up to 100 ng/well, demonstrating the specificity of the glycopeptide antiserum.

DISCUSSION

Prolactin is unusual among hormones of the anterior pituitary gland in that its secretion occurs at a high spontaneous rate in the absence of

hypothalamic influence (5). Thus, an important mode of regulation of its secretion is the tonic inhibition exercised by dopamine (13), secreted by the hypothalamus into hypophysial stalk blood (5). Nevertheless, considerable evidence supports the existence of a prolactin releasing factor (5). Recent experiments suggest that the prolactin releasing factor arises from the neurohypophysis: lesions of the hypothalamic paraventricular nucleus (14) and surgical removal of the posterior pituitary lobe (6) block sucking-induced prolactin release. Crude extracts of the neural lobe of the pituitary gland also have been reported to stimulate prolactin release (7, 15). Although several peptides that stimulate prolactin release such as vasoactive intestinal peptide, angiotensin II, thyrotropin releasing hormone, and oxytocin are known to reside in the neurohypophysis, evidence was presented that the releasing factor was unique and not identical with these or 17 other neurohypophysial peptides (7).

An abundant peptide in the neurohypophysis not previously investigated for prolactin release is the 39 amino acid glycopeptide that arises from the vasopressin-neurophysin precursor. This peptide, discovered by Smyth and Massey (8) before its assignment to the vasopressin precursor by cDNA sequencing (9), has no known function. As expected, immunocytochemical studies of the glycopeptide show a localization in the posterior pituitary gland and hypothalamus that is identical to vasopressin and neurophysin II (16). Thus, co-secretion of vasopressin and its neurophysin are expected to occur with the glycopeptide since they are derived from the same precursor (9) and packaged together in the same secretory granules (16).

Our results suggest that the vasopressin-neurophysin associated glycopeptide is the neurohypophyseal prolactin releasing factor. Although increased secretion of oxytocin classically is considered to be associated with the suckling stimulus, there is nevertheless evidence of increased secretory activity of the vasopressinergic neuronal system during lactation: a) the biosynthesis of both vasopressin and oxytocin is enhanced in lactating rats (17); b) antidiuresis, the hallmark of vasopressin action, occurs during suckling in rabbits, goats, and cows (cf. 18), and; c) suckling induces release of vasopressin associated neurophysin in some sheep, cows and women (cf. 18).

Several peptides previously have been nominated to serve as prolactin releasing factors based on their activity to stimulate prolactin release and on the ability of their antisera to attenuate suckling-induced prolactin release. These peptides include vasoactive intestinal peptide (19), oxytocin (20), and thyrotropin releasing hormone (21). These findings together with our evidence of a physiological prolactin-releasing role for the vasopressin-related glycopeptide suggest that prolactin release may be

mediated by a complex of interacting hypothalamic-neurohypophysial peptides.

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