Effect of atrial natriuretic peptide on gonadotropin release in superfused rat pituitary cells
(luteinizing hormone/follicle-stimulating hormone/adenohypophysis)

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ABSTRACT Cardiac atrial muscle cells produce a polypeptide hormone that plays a role in the control of water and electrolyte balance and blood pressure. The circulating form of this hormone is the atrial natriuretic peptide (ANP), which contains 28 amino acids. Various immunohistochemical studies have shown that ANP is present in many areas of the central nervous system, including the median eminence. In our studies, we investigated the effect of ANP in a superfused rat pituitary cell system. When ANP was administered at increasing concentrations (0.01 μM to 1 μM), it caused a significant dose-related stimulation of the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The lowest effective dose of ANP in our system was 0.03 μM. When ANP and LH-releasing hormone were administered together, the response was prolonged and had the characteristics of ANP-stimulated LH and FSH release. In contrast with some previous reports, ANP in high concentration (1 μM) consistently induced a small but significant stimulation of the release of corticotropin. ANP did not influence the basal release of prolactin, growth hormone, and thyrotropin.

DeBold et al. (1–3) were the first to report that the heart atrial muscle cells produce a polypeptide that they named the atrial natriuretic factor (ANF). These findings were confirmed by others (4, 5). Recently, other groups isolated rat and human atrial peptides and determined their amino acid sequences. (For review, see ref. 6.) The cloning and sequence analysis of the complementary DNA encoding the ANF precursor revealed that ANF is synthesized in the rat in a prepro form containing 152 amino acids (7, 8). Different peptides consisting of the C-terminal 25–30 amino acids of prepro-ANP have full biological activity and have been also called atrial natriuretic factors (9). However, the circulating form of ANF appears to be cardionatri I or α-rat atrial natriuretic peptide (ANP), a 28-amino acid peptide that comprises residues 123–150 of prepro-ANP (9). ANP is the most potent endogenous natriuretic/diuretic substance described to date; it decreases blood pressure and has an inhibitory effect on renin and aldosterone secretion (1, 3, 10). Recent immunohistochemical studies and tests based on RIAs indicate that ANF is present in the central nervous system. ANF-like immunoreactivity has also been detected in whole hypothalamic extracts (11). Jacobowitz et al. reported that ANF-positive nerve fibers and cell bodies can be observed in the preoptic area, hypothalamus, mesencephalon, and pons of rats (12). In colchicine-treated animals, they found a large number of ANF-immunoreactive cell bodies also in the organon vasculosum of the lamina terminals and hypothalamic nuclei. In addition, they detected ANF-positive fibers in the external zone of the median eminence. Immunohistochemical results of Saper et al. (13) and Nakao et al. (14) confirmed these findings. Since ANF is present immunocytochemically in the median eminence, there is a possibility that it may act also on the pituitary through the portal circulation. Anand-Srivastava et al. (14) demonstrated that ANF has an inhibitory effect on pituitary adenylate cyclase and suggested that ANF inhibits the release of corticotropin (ACTH) at high concentration. In view of these findings and the growing evidence that some peptides discovered in the peripheral organs may play a physiological role in the central nervous system and the anterior pituitary gland, we decided to investigate the effect of ANP in the superfused rat pituitary cell system.

MATERIALS AND METHODS

Pituitary cells originating from 6 or 12 female Sprague-Dawley rats (160–180 g) at random stages of the estrus cycle were used in each chamber of the superfusion apparatus. The preparation of the cells and the superfusion apparatus were very similar to that described by Vigh and Schally (15). The rats were decapitated, and the anterior pituitaries were removed, cut into small pieces, incubated with collagenase (Worthington, type I), dispersed, mixed with 0.9 ml of swollen Sephadex G-10 that had been preequilibrated with oxygenated tissue culture medium (medium 199, Sigma), and transferred into the superfusion chamber. The cells were perfused overnight with the medium at a flow rate of 20 ml/hr. Collection of 1-ml fractions started the next morning. The test materials were administered for 6 or 15 min. On the basis of preliminary experiments, the period between administering the test materials ranged from 30 to 60 min. The fractions were acidified with 30 μl of 1 M HCl and stored at 4°C until analyzed by RIAs for luteinizing hormone (LH), follicle-stimulating hormone (FSH), ACTH, growth hormone (GH), thyrotropin (TSH), and prolactin. Our results are based on five independent superfusions.

RIAs. RIAs were performed on aliquots of the undiluted superfusion medium. The sample sizes were as follows: LH, 50 μl; FSH, 200 μl; ACTH and TSH, 100 μl; prolactin and GH, 10 μl. All of the antisera (a-rLH S-8, a-rFSH S-11, a-hACTH batch no. 2, a-rTSH S-5, a-rPRL S-9, a-rGH S-4) and the hormones (rLH RP-2, rFSH RP-2, hACTH 1-39, rTSH RP-2, rPRL RP-3, rGH RP-2) were provided by the National Hormone and Pituitary Program (National Institute

Abbreviations: ANF, atrial natriuretic factor; ANP, atrial natriuretic peptide; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; ACTH, corticotropin (adrenocorticotropic hormone); TSH, thyroid-stimulating hormone (thyrotropin); RH, releasing hormone.

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of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health).

**Test Materials.** LH-releasing hormone (LH-RH), ovine corticotropin-releasing factor (CRF), and hGH-RH<sub>44</sub> were synthesized by solid-phase methods and purified in our laboratory. Thyrotropin-releasing hormone (TRH) was a gift from Takeda (Osaka, Japan). ANP was obtained from Bachem (Torrance, CA) (ANP, rat, 28 amino acids). For testing the adsorption of ANP on the Sephadex G-10 column, 2 μg of ANP was iodinated by the chloramine-T method (16).

**RESULTS**

In the superfused rat pituitary cell system, ANP elevated LH and FSH secretion in a dose-related manner (Fig. 1). LH release and FSH release were stimulated to a greater extent with increasing doses of ANP. The lowest effective dose was 0.03 μM ANP. When the LH and FSH peaks elicited by ANP (Fig. 1) and LH-RH (Fig. 2) were compared, the effect of ANP on LH and FSH release was found to be more prolonged. Although the effect of LH-RH was concluded in 9–12 min, it required 24–36 min to recover a stable baseline after administering ANP at different dose levels. When LH-RH and ANP were given together, the response was prolonged and had the characteristics of ANP-stimulated LH and FSH release. However, the secretion in the first 9 min was additive only when larger doses of LH-RH and ANP (1 nM and 0.3 μM, respectively) were infused onto the column (Fig. 2).

![Fig. 1.](image1.png)

**Fig. 1.** Release of LH (Upper) and FSH (Lower) from superfused rat pituitary cells. (In this experiment we used 12 pituitaries per channel.) Double arrows indicate 6-min pulses of ANP: 1, 0.01 μM; 2, 0.03 μM; 3, 0.1 μM; 4, 0.3 μM; 5, 1 μM.

![Fig. 2.](image2.png)

**Fig. 2.** Effect of LH-RH and ANP alone or in combination on the release of LH (Upper) and FSH (Lower) in the superfused rat pituitary cell system. (Cells from 12 pituitaries per channel.) Double arrows indicate 6-min pulses of the following samples: 1, LH-RH, 1 nM; 2, ANP, 0.3 μM; 3, LH-RH, 1 nM, and ANP, 0.3 μM.

In contrast to the results of Anand-Srivastava et al. (14), we did not detect any inhibition of the release of ACTH from the anterior pituitary cells by ANP, even at very high (1 μM) concentration. On the contrary, in our system at this dose, ANP slightly, but consistently, elevated the ACTH level. Simultaneous infusion of 1 μM ANP did not influence the ACTH release elicited by 0.2 nM CRF (Fig. 3). Administr-
of ANP at different concentrations had no effect on prolactin (Fig. 4), GH, and TSH release (data not shown).

To exclude the possibility that the prolonged effect of ANP was due to its adsorption to Sephadex G-10, we tested the clearance of iodinated ANP from the column. No adsorption was found when [125I]-labeled ANP and unlabeled ANP were administered in a 1:4 ratio, at a final concentration of 0.1 μM or 0.3 μM for 6 min. Three minutes after stopping the infusion, 95% of the iodinated ANP was cleared from the column.

**DISCUSSION**

ANF is reported to inhibit adenylate cyclase in different tissues (17), including the anterior and posterior pituitary (14). The vasorelaxant action of ANF is associated with an increase in cyclic GMP in plasma and vascular smooth muscle (18). Waldman et al. (19) showed that ANF selectively activates particulate guanylate cyclase and elevates cyclic GMP in homogenates of different rat tissues but not in brain cells. However, Friedl et al. found that ANF elevates the level of cyclic GMP in astroglia-rich brain cell cultures (20). No data have been reported so far on the effect of ANF on pituitary guanylate cyclase.

The activation of gonadotropin secretion by LH-RH involves calcium mobilization (21). The role of cyclic nucleotides in mediating the action of LH-RH is not clear. Since LH-RH increases the cyclic AMP level in the pituitary and concomitantly stimulates LH and FSH release, it was proposed that cyclic AMP could be the mediator of LH-RH action on LH and FSH release (22). However, the results of Naor et al. suggest that the stimulation of pituitary cyclic AMP formation by LH-RH is not an obligatory step in the mechanism of LH release, since the inhibition of cyclic AMP with flufenamic acid abolishes LH-RH stimulation of cyclic AMP but does not affect its action on LH release (23). LH-RH also stimulates pituitary cyclic GMP production (24).

Nakano et al. reported that dibutyryl guanosine 3',5'-cyclic monophosphate stimulates the release of gonadotropins in cultured rat anterior pituitary cells (25), but Naor and Catt observed no effect of cyclic GMP and its active analogs upon LH release (26).

Our findings show that ANP stimulates the release of LH and FSH from rat anterior pituitary cells. However, the mechanism through which ANP releases LH and FSH remains to be elucidated. The involvement of cyclic nucleotides in this process is not known. At present, Ca2+ seems to be the only certain mediator of gonadotropin release, but there are no data indicating whether ANP is involved in the modulation transmembrane mobilization of Ca2+ in the anterior pituitary. Relatively high concentrations of ANF that were needed to release LH and FSH in vitro suggest that ANF synthesized in the hypothalamus, which could possibly reach the pituitary through the portal circulation, would not affect the pituitary gonadotrophs under physiological conditions. New questions as to the possible role of ANF in regulating the pituitary gonadotrophs are raised by recent results of McKenzie et al., who presented immunocytochemical evidence that ANF is present in the pituitary (27). Analyzing serial sections of rat adenohypophysis they demonstrated ANF-like immunoreactivity in the LH- and FSH-positive cells. They suggest that this endogenously synthesized stored ANF may play a role in the secretion of ACTH by corticotroph cells. Our results do not support the role of ANF in regulating ACTH secretion, although this would have been most plausible. However, the immunocytochemical presence of ANF in gonadotrophs and the fact that in the superfused pituaray cell system ANF stimulates the release of LH and FSH will require additional research to clarify the possible role of ANF in these mechanisms.

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**Fig. 4.** Prolactin (PRL) release from superfused rat pituitary cells (six pituitaries per channel). Double arrows indicate 15-min pulses of ANP: 1, 0.01 μM; 2, 0.1 μM; 3, 1 μM.