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Proadrenomedullin N-Terminal 20 Peptide Inhibits Adrenocorticotropin Secretion from Cultured Pituitary Cells, Possibly via Activation of a Potassium Channel

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Preproadrenomedullin is processed into at least two biologically active peptides, adrenomedullin (AM) and proadrenomedullin N-terminal 20 peptide (PAMP). Both peptides are hypotensive; however, they exert this action via differing mechanisms. In pituitary cells in culture, both basal and releasing factor-stimulated adrenocorticotropin (ACTH) secretion is inhibited by AM. Here we report that basal, but not stimulated, ACTH secretion from cultured rat pituitary cells is also inhibited by PAMP. The effect is dose-related, occurs in a physiologically relevant dose range that is similar to that of AM, and is blocked by the potassium channel blocker, glybenclamide. The failure of glybenclamide to inhibit AM's effects on ACTH secretion indicates that in pituitary, as in other tissues, these two products of the same prohormone can exert similar biologic activity, although via differing mechanisms.

Key Words: Pituitary; vasoactive peptides; corticotropin.

Introduction

The adrenomedullin gene *(1,2)* encodes two biologically active peptides, adrenomedullin (AM) and proadrenomedullin N-terminal 20 peptide (PAMP). Both peptides are potent hypotensive agents. In the periphery, AM acts directly on the vasculature, stimulating nitric oxide (NO) formation *(3,4)*. The vasodilatory effect of PAMP is indirect via an inhibitory action on sympathetic fibers innervating the vasculature *(5)*. In many tissues, these two peptides do share, as in the vasculature, complementary actions. For instance, in the adrenal gland, both peptides inhibit angiotensin II-induced aldosterone secretion *(6,7)*. The effect of PAMP is more potent than that of AM, and unlike AM, the inhibitory action *(8)* on mineralocorticoid secretion is not blocked by the calcitonin gene-related peptide (CGRP) antagonist $CGRP_{8-37}$ *(7)*. However, not all of the actions of AM are shared by PAMP. The renal effects of AM to increase blood flow and to stimulate urinary salt and water excretion *(9)* are not shared by PAMP. Furthermore, central nervous system (CNS) actions to inhibit water drinking *(10)* and salt appetite *(11)* are unique to AM. In some cases, the opposite is also true. In cultured adrenal medullary cells, release of catecholamines in response to carbachol is accompanied by PAMP secretion *(12)*, and the PAMP released exerts autocrine feedback effects to decrease the effect of carbachol on sodium influx *(13)*, a key step in acetylcholine-induced catecholamine release.

The actions of the proadrenomedullin-derived peptides seem to contribute to the homeostatic mechanisms by which blood volume and pressure are regulated. This is true in anterior pituitary gland as well. There AM exerts potent inhibitory effects on basal and corticotropin-releasing hormone- (CRH) stimulated adrenocorticotropin (ACTH) release *(14)*. These effects do not appear to be mediated via an action on the characterized AM receptor *(15)*, and the mechanism of the ACTH inhibition is unknown. Expression of the adrenomedullin gene has been demonstrated in anterior pituitary gland *(16,17)*, and it is possible that proadrenomedullin-derived peptides exert local autocrine or paracrine actions in the tissue. In this article, we characterize the effect of PAMP on ACTH secretion from cultured rat anterior pituitary cells. It is clear from the work of Wang and Greer *(18)* that alteration of potassium channel activity can affect ACTH secretion. Since activation of an inward rectifying potassium channel appears to underlie the action of PAMP in PC-12 cells *(13)*, and in fact, activation of potassium channels may explain as well the ability of AM to dilate cerebral vessels *(19)*, we also examined the potential role of potassium channels in the ACTH-inhibiting effects of AM and PAMP.

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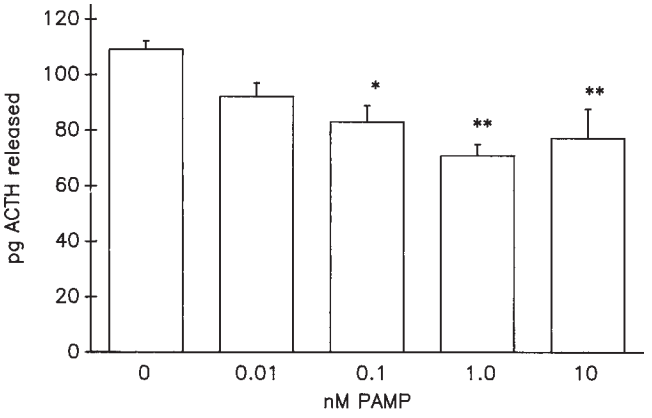

Fig. 1. PAMP inhibits basal ACTH release from cultured anterior pituitary cells. Results presented are the means of 6 replicates from 4 separate cell harvests. *$p < 0.05$, **$p < 0.01$ vs 0 n*M* PAMP.

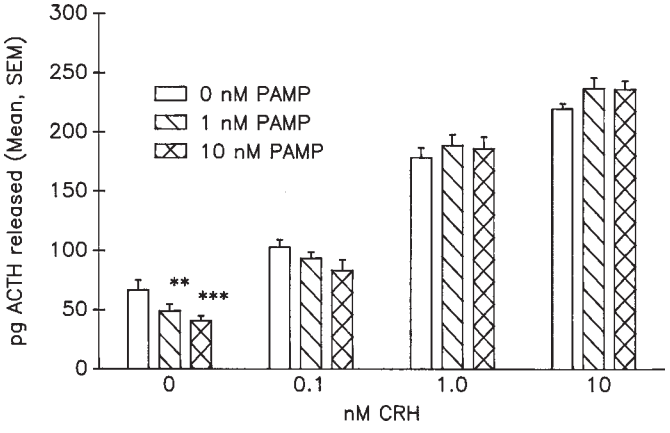

Fig. 2. Effect of PAMP on basal and CRH-stimulated ACTH release from dispersed anterior pituitary cells. *$p < 0.01$, **$p < 0.001$ vs 0 n*M* PAMP/0 n*M* CRH.

Results

In four separate cell harvests, PAMP in log-molar doses ranging from 0.01 to 1.0 n*M*, significantly inhibited basal ACTH release. Maximal inhibition was observed with the 1.0-n*M* dose of PAMP, and no further inhibition occurred in the presence of 10 n*M* peptide (Fig. 1). The effect was specific for ACTH secretion, since growth hormone release was not significantly affected by these doses of PAMP (data not shown). In additional cell harvests, PAMP again inhibited basal ACTH secretion (Fig. 2), but no significant effect of PAMP on CRH-stimulated ACTH secretion was observed.

Glybenclamide by itself at doses of 1.0, 10, and 100 n*M* did not significantly alter basal ACTH secretion. However, in a preliminary study, higher (micromolar) doses of the blocker resulted in stimulation of hormone secretion (data not shown). This agrees with prior reports that detail the threshold for activation of calcium channels by glybenclamide to be in the micro- to millimolar range *(20)*. Thus, we chose a dose of the potassium channel blocker

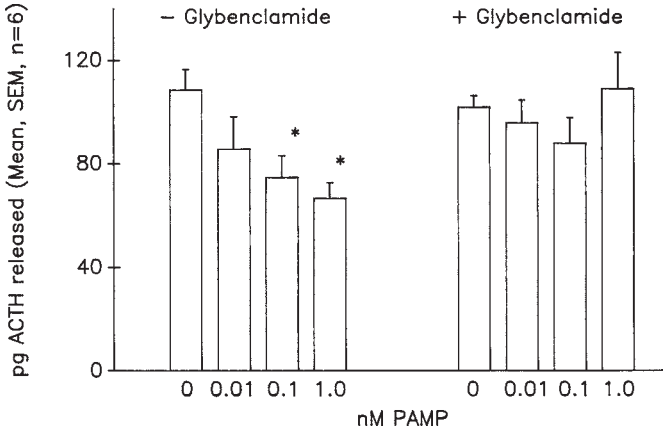

Fig. 3. Blockade of the ATP-sensitive potassium channel by glybenclamide prevents the inhibitory effect of PAMP on basal ACTH release from cultured anterior pituitary cells. *$p < 0.05$, **$p < 0.01$ vs 0 n*M* PAMP.

that did not activate calcium channels in our target cells. The 100-n*M* dose of potassium channel blocker completely abolished the ACTH-inhibiting action of PAMP (Fig. 3). In an additional cell harvest, 100 n*M* glybenclamide reversed the inhibitory action of 1.0 n*M* PAMP (control: 86.8 ± 3.2 pg ACTH released; 1.0 n*M* PAMP, 70.7 ± 4.6, $p < 0.05$ vs control; 100 n*M* glybenclamide, 86.8 ± 2.3; PAMP + glybenclamide, 82.5 ± 4.7; $n = 6$), but not that of 1.0 n*M* AM (control: 87.3 ± 2.1 pg ACTH released; 1.0 n*M* AM, 74.4 ± 3.9, $p < 0.05$ vs control; 100 n*M* glybenclamide, 81.7 ± 3.1; AM + glybenclamide, 74.2 ± 0.9, $p < 0.01$ vs control; $n = 6$).

Discussion

Both AM *(14)* and PAMP exert significant inhibitory effects on basal ACTH secretion in vitro. The doses of AM *(14)* and PAMP required for this action (0.01–0.1 n*M*, or approx 25–250 pg/well for PAMP and 57–570 pg/well for AM) are well within the reported levels of the peptides in anterior pituitary extracts *(16,17)*. Thus, these in vitro results may have physiologic relevance. In the in vivo setting peripherally, but not centrally, administered AM lowers circulating ACTH levels in conscious animals *(21)*, suggesting again the physiologic relevance of our in vitro findings. It is not yet know whether similar peripheral administration of PAMP will also lower plasma ACTH levels.

As in the vasculature, the apparently similar actions of AM and PAMP on basal ACTH secretion are expressed via differing mechanisms. Indeed, the effect of PAMP on basal ACTH secretion, presumably through hyperpolarization of the resting membrane potential, did not interfere with subsequent ligand-stimulated hormone release. This isolation of effects on basal vs ligand-stimulated hormone secretion has been demonstrated previously in the case of prolactin release *(22)*. In pituitary, an involvement of ATP-

sensitive potassium channels in the inhibitory effect of PAMP, but not AM, can now be hypothesized. In the case of AM, however, both effects on basal and ligand-stimulated hormone release were observed *(14)*. Although glybenclamide did not block the effect of AM on basal ACTH secretion, we cannot rule out the possible involvement of a potassium channel, which might be uncovered with other blocking agents. At least when glybenclamide was employed, the effects of PAMP and AM on basal hormone secretion could be distinguished.

The presence in anterior pituitary of G-protein-linked, inward-rectifying potassium channels has been reported *(23)*, and future studies will determine the possible involvement of G-proteins in this effect of PAMP. It will also be important to determine the potential ability of PAMP and AM to control proopiomelanocortin gene transcription in anterior pituitary gland. This will allow for a comparison of the inhibitory actions of the two peptides with those of the conventional ACTH suppressor, dexamethasone. Finally, the stimuli for local peptide release must be determined, and the effects of removal of endogenous peptide on ACTH secretion studied.

Clearly, there are differences between the actions of AM and PAMP. In addition to not being affected by glybenclamide, AM's ability to inhibit CRH-stimulated ACTH release is not shared by PAMP. Thus, these two posttranslational products of adrenomedullin gene transcription exert complementary actions in anterior pituitary through unique mechanisms. As in the vasculature, these products of the same gene appear to act in concert. Although the physiologic relevance of these pituitary actions of AM and PAMP has not yet been established, the data presented here suggest a role for the peptides in pituitary function in general and specifically in the hormonal response to stress, as well as the endocrine regulation of fluid and electrolyte homeostasis *(24)*. In a physiologic sense, it will be important to determine the role played by locally produced AM and PAMP in basal and ligand-stimulated ACTH secretion. In a larger sense, it may be therapeutically important to gain insight into the mechanism(s) of action of endogenous compounds that may hold hormone secretion in check.

Materials and Methods

Male rats (250–300 g, Harlan Sprague Dawley, Indianapolis, IN) were sacrificed by decapitation as approved by the university animal care and use committee. Anterior pituitary glands were collected and mechanically dispersed in the presence of trypsin *(25)*. Cells were aliquoted into 24-well plates (approx 300,000 cells/well) and incubated for 72 h in Medium 199 (pH 7.3) containing 20 m*M* HEPES, 10% horse serum, and 1% antibiotic/antimycotic (all Gibco-BRL, Grand Island, NY) in room air at 37°C. On the day of experimentation, cells were washed with fresh medium and exposed for 1 h to test medium (0.5 mL) containing test substances diluted in test medium (Medium 199 containing 400 mg/L KCl and 60 mg/L KH_2PO_4, 20 m*M* HEPES, 1% penicillin-streptomycin [all GIBCO], and 0.1% BSA and 0.02 n*M* bacitracin [both Sigma, St. Louis, MO], pH 7.3, 37°C). When glybenclamide was employed, it was added to the cells 10 min prior to the addition of AM or PAMP. Incubations were terminated by removal of medium.

Peptides were purchased from Phoenix Pharmaceuticals, Inc. (Mt. View, CA). The selective blocker of ATP-sensitive potassium channels, glybenclamide (glyburide), was obtained from Calbiochem (La Jolla, CA). Accumulation of ACTH in the culture medium was determined by radioimmunoassay employing the rat ACTH kit obtained from DiaSorin (Stillwater, MN). Growth hormone levels in medium were determined using the kit provided by the National Hormone and Pituitary Program, NIDDK. Significant differences ($p < 0.05$) within and between groups were determined by ANOVA and multiple-comparison testing (Neuman-Keuls). Data presented are means and SEM.

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