ADRENOMEDULLIN AND PROADRENOMEDULLIN N-TERMINAL 20 PEPTIDE INHIBIT AGONIST-STIMULATED ALDOSTERONE SECRETION OF HUMAN ADRENOCORTICAL CELLS, THROUGH A MECHANISM PROBABLY INVOLVING THE IMPAIRMENT OF CALCIUM INFLUX

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ABSTRACT

Adrenomedullin (ADM) and proadrenomedullin N-terminal 20 peptide (PAMP) are two hypotensive peptides, which are able to inhibit agonist-stimulated aldosterone secretion from adrenal zona glomerulosa. In this study we examined the effects of these two peptides on the aldosterone response of dispersed human adrenocortical cells to maximal effective concentrations of their main agonists. ADM (10^{-7} M) and PAMP (10^{-8} M) did not affect either basal or ACTH-stimulated aldosterone secretion, but they counteracted the secretory response to both 10^{-9} M angiotensin-II (ANG-II) (partial inhibition) and 10mM K^{+} (complete inhibition). The Ca^{2+} ionophore A23187 (10^{-5} M) partially reversed the inhibitory effect of both peptides on aldosterone response to ANG-II, and abolished that on aldosterone response to K^{+} . Collectively these findings provide indirect evidence that the mechanism underlying the aldosterone anti-secretagogue action of ADM and PAMP may involve the impairment of the agonist-induced rise in the cytosolic Ca^{2+} concentration, and especially of Ca^{2+} influx.

Adrenomedullin (ADM) and proadrenomedullin N-terminal 20 peptide (PAMP) are two hypotensive peptides, which are produced by the post-translational processing of a 185-amino acid pro hormone, named preproadrenomedullin (for review, see 10). Both peptides are expressed in the mammalian adrenal medulla, and the presence of specific receptors for ADM and PAMP has been demonstrated in the adrenal cortex, and especially in the zona glomerulosa (for review, see 9).

Like other regulatory peptides contained in adrenal medulla (for review, see 8). ADM and PAMP were found to modulate the secretory activity of the adrenal cortex. They specifically inhibit

angiotensin-II (ANG-II)-stimulated aldosterone production by dispersed rat (1,5,6,11) and human zona glomerulosa cells (2,3), without affecting either basal secretion or ACTH-stimulated one. There is also evidence that ADM hampers aldosterone response of rat zona glomerulosa cells to K^{+} (11), and that the mechanism underlying the aldosterone antisecretagogue action of ADM may involve the impairment of Ca^{2+} influx (2,5,11).

PAMP was found to be significantly more effective than ADM in inhibiting ANG-II-elicited aldosterone secretion of rat and human adrenocortical cells (1,3), but no data are at present available dealing with its possible mechanism of action. Therefore, it seemed worthwhile to study the effects of ADM and PAMP on the aldosterone response of human adrenocortical cells to their main agonists (ACTH, ANG-II and K^{+}), and to ascertain whether the inhibitory effect of both peptides is

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Fig. 1 Effects of ADM (10^{-7} M) and PAMP (10^{-8} M) on basal and agonist-stimulated aldosterone secretion of dispersed human adrenocortical cells. Data are means $\pm$ SEM ($n=3$). + $P<0.05$ and * $P<0.01$ from the respective baseline values (B); ^ $P<0.01$ from the respective control groups.

counteracted by a Ca^{2+} ionophore.

MATERIALS AND METHODS

Human ADM(1-52), human PAMP, ACTH and ANG-II were purchased from Peninsula Labs (St. Helene, U.K.); the Ca^{2+} ionophore A23187, human serum albumin (HSA) and other laboratory reagents from Sigma (St. Louis, MO, U.S.A.); and Medium 199 from DIFCO (Detroit, MI, U.S.A.). RIA kit for aldosterone was purchased from IRE-Sorin (Vercelli, Italy). Fragments of adrenal glands were obtained from six consenting adult patients (40–50-year-old) undergoing unilateral nephrectomy for kidney cancer. The study protocol followed the local Ethical Committee guidelines for human studies.

Adrenal tail fragments were employed to obtain dispersed cell preparations by collagenase digestion and mechanical disaggregation, as detailed previously (2). Dispersed cells obtained from each adrenal were placed in Medium 199 and Krebs-Ringer bicarbonate buffer with 0.2% glucose, containing 5 mg/mL HSA, and incubated (3×10^4 cells/mL, in replicates of three each) as follows: (i) 10^{-9} M ANG-II, 10 mM K^+ or 10^{-9} M ACTH in the presence or absence of 10^{-7} M ADM (1-52) or 10^{-8} M human PAMP; and (ii) 10^{-9} M ANG-II or 10 mM K^+ plus 10^{-7} M ADM or 10^{-8} M PAMP in the presence or absence of 10^{-5} M

A23187. The concentrations of ADM, PAMP and A23187 were those previously found to be the maximal effective ones *in vitro* (2,3), and the same is true for the concentrations of ANG-II, K^+ and ACTH (4). The incubation was carried out for 90 min in a shaking bath at 37°C in an atmosphere of 95% air–5% CO_2 . Aldosterone was extracted from the incubation media and purified by high performance liquid chromatography (7), and its concentration measured by the ALDOCTK2 kit (sensitivity, 5 pg/mL; intra- and inter-assay variation coefficients, 7.5% and 8.8%, respectively).

Data obtained from each adrenal gland were averaged and expressed as mean $\pm$ SEM of three separate experiments. The statistical comparison of results was performed using ANOVA, followed by the Duncan's Multiple Range Test.

RESULTS

ADM and PAMP did not affect either basal or ACTH-stimulated aldosterone secretion of dispersed human adrenocortical cells, but they counteracted the stimulatory effect of both 10^{-9} M ANG-II and 10 mM K^+ . Aldosterone response to ANG-II was only partially reversed (by about 60%), while the response to K^+ was abolished. A23187 (10^{-5} M) partially blocked (by about 40–50%) the inhibitory effect of both ADM and PAMP on aldosterone response to ANG-II, and annulled that

Fig. 2 Effect of A23187 (10^{-5} M) on the ADM- or PAMP-induced inhibition of aldosterone response of dispersed human adrenocortical cells to 10^{-9} M ANG-II (upper panels) or 10 mM K^{+} (lower panels). Data are means $\pm$ SEM ($n=3$). $^{*}P<0.05$ and $^{*}P<0.01$ from the respective control groups (C, secretory response to agonists in the absence of ADM or PAMP); $^{a}P<0.05$ and $^{a}P<0.01$ from ADM or PAMP only exposed groups; $^{b}P<0.05$ and $^{b}P<0.01$ from the respective baseline values.

on aldosterone response to K^{+} (Fig. 2).

DISCUSSION

The present results confirm that ADM and PAMP specifically inhibit the aldosterone secretagogue effect of the agonists, whose signaling mechanism involves the rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), which in turn activates protein kinase-C (PKC) cascade (for review, see 9).

It is current knowledge that the mechanisms whereby ANG-II and K^{+} elicit the increase in

$[Ca^{2+}]_i$ are quite different (for review, see 4). ANG-II, by interacting with specific AT1 receptors, activates phospholipase C and consequently raises the production of IP_3 , which enhances Ca^{2+} release from intracellular stores, and of diacylglycerol, which directly activates PKC. Moreover, ANG-II increases Ca^{2+} influx. Conversely, the mechanism of action of K^{+} exclusively involves the opening of voltage-gated Ca^{2+} channels. Hence, the demonstration that ADM and PAMP abolish the aldosterone response to K^{+} , but only partially reversed that to ANG-II, strongly suggests that both proadrenomedullin-derived peptides exert their antimineralocorticoid action mainly by blocking Ca^{2+} influx.

This contention is supported by the finding that a potent ionophore, at a concentration which has been previously found to induced a sizable increase in aldosterone secretion of human adrenocortical cells (2), annuls the inhibitory effect of ADM and PAMP on aldosterone response to K^{+} , but only partially counteracts that on the response to ANG-II, in which PKC activation is not exclusively mediated by the rise in $[Ca^{2+}]_i$.

In conclusion, our study provides indirect evidence that ADM and PAMP are able to impair Ca^{2+} influx in human adrenocortical cells, and investigations are under way to ascertain which type of Ca^{2+} channels (T- or L-type) is blocked by these regulatory peptides. However, the possibility cannot be ruled out that both proadrenomedullin-derived peptide may also somehow interfere with the IP_3 -mediated Ca^{2+} release from intracellular stores.

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