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Effects of Adrenomedullin on the Human Adrenal Glands: An *in Vitro* Study

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ABSTRACT

Numerous lines of evidence indicate that adrenal medulla exerts a paracrine control on the secretory activity of the cortex by releasing catecholamines and several regulatory peptides. Adrenomedullin (ADM) is contained in adrenal medulla of several mammalian species, including humans. Thus, we investigated whether human ADM1–52 exerts a modulatory action on steroid secretion of human adrenal cortex *in vitro*. Dispersed adrenocortical cells (obtained from the gland tail deprived of chromaffin cells) and adrenal slices (including both capsule and medulla) were employed. ADM specifically inhibited angiotensin II-stimulated aldosterone secretion of dispersed cells and enhanced basal aldosterone production by adrenal slices, minimal effective concentrations being 10^{-7} and 10^{-9} mol/L, respectively. These effects of ADM were suppressed by the CGRP1 receptor antagonist CGRP8–37 (10^{-5} mol/L). Neither basal and ACTH-stimulated aldosterone secretion of dispersed cells nor agonist-enhanced aldosterone production by adrenal slices were affected by ADM, which also did not alter cortisol secretion of both types of adrenal preparations. ADM (10^{-6} mol/L) blunted the aldosterone secretagogue action of the Ca^{2+} ionophore A23187 (10^{-5} mol/L) on dispersed cells and adrenal slices. The β-adrenoceptor antagonist *l*-alprenolol (10^{-6} mol/L) suppressed aldosterone response of adrenal slices to 10^{-7} mol/L isoprenaline and ADM. ADM concentration dependently raised epinephrine and norepinephrine release by adrenal slices, minimal effective concentration being 10^{-9} mol/L. Collectively, these findings suggest that ADM, acting via the CGRP1 receptor subtype, exerts a direct inhibitory effect on angiotensin II-stimulated aldosterone secretion, which, when the integrity of adrenal tissue is preserved, is overcome and reversed by an indirect stimulatory action, conceivably involving the release of catecholamines by adrenal chromaffin cells. (*J Clin Endocrinol Metab* **82:** 1167–1170, 1997)

ADRENOMEDULLIN (ADM) is a recently discovered 52-amino acid hypotensive peptide, originally isolated from human pheochromocytomas (1). Subsequently, ADM transcription and translation products have been demonstrated in adrenal medulla of several mammalian species, including humans (for review, see Refs. 2 and 3). ADM is processed from a 185-amino acid precursor, named preproadrenomedullin, which is also cleaved to give rise to proadrenomedullin N-terminal 20 peptide, exhibiting a moderate hypotensive action (3).

Like other regulatory peptides contained in adrenal medulla (for review, see Ref. 2), ADM affects the secretory activity of the adrenal cortex in the rat. It was found to specifically inhibit angiotensin-II (ANG-II)-stimulated aldosterone secretion of dispersed zona glomerulosa cells (4, 5), and *in vivo*, to lower aldosterone plasma concentration in sodium-depleted or bilaterally nephrectomized animals (6). However, using *in situ* perfused rat adrenals, Mazzocchi *et al.* (7) showed that ADM enhances aldosterone release through a mechanism that cannot completely be accounted for by the increase in the flow rate of the perfusion medium.

Investigations of the effects of ADM on steroid secretion in humans are not yet available. Therefore, it seemed worthwhile to examine whether *in vitro* ADM affects the secretory activity of human adrenal glands.

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Materials and Methods

Fragments of adrenal glands were obtained from consenting adult patients (30–50 yr old) undergoing unilateral nephrectomy for kidney cancer. Starting from 2 weeks before surgery, patients were kept on a normal diet. Only patients not requiring medications able to alter adrenal function and with histologically normal adrenal glands were selected for these experiments. Portions of the head and tail of each adrenal, which, respectively, contain and do not contain medullary tissue (8), were removed, placed in Krebs-Ringer bicarbonate buffer with 0.2% glucose at 4 C, and immediately carried to our laboratory. Head fragments were cut into slices, always including the gland capsule and medulla; tail fragments were employed to obtain dispersed adrenocortical cell preparations by collagenase digestion and mechanical disaggregation (9).

Adrenal slices and dispersed cells obtained from each gland were placed in medium 199 (Difco, Detroit, MI) and Krebs-Ringer bicarbonate buffer with 0.2% glucose, containing 5 mg/mL human serum albumin, and incubated (8–10 mg/mL or 3×10^5 cells/mL, in replicates of five each) as follows: 1) human ADM1–52 (from 10^{-10}–10^{-5} mol/L) alone or in the presence of 10^{-9} mol/L ACTH or ANG-II; 2) 10^{-5} mol/L CGRP8–37 alone (adrenal slices) or with 10^{-9} mol/L ANG-II (dispersed cells) in the presence or absence of 10^{-6} mol/L ADM1–52; 3) 10^{-6} mol/L ADM1–52 in the presence or absence of 10^{-5} mol/L A23187; and 4) 10^{-6} mol/L *l*-alprenolol in the presence or absence of 10^{-7} mol/L isoprenaline or ADM1–52 (adrenal slices). ADM1–52, the CGRP1 receptor antagonist CGRP8–37 (5), ACTH, and ANG-II were purchased from Peninsula Labs (Merseyside, UK); the Ca^{2+} ionophore A23187 and the β-adrenoceptor agonist isoprenaline and antagonist *l*-alprenolol were obtained from Sigma Chemical Co. (St. Louis, MO). The incubation was carried out for 90 min in a shaking bath at 37 C in an atmosphere of 95% O_2–5% CO_2. The medium was collected and kept frozen at −80 C until hormonal assays.

Aldosterone and cortisol were extracted from the incubation media and purified by high-pressure liquid chromatography, as described previously (8). Their concentrations were measured by RIA, using commercial kits purchased from IRE-Sorin [Vercelli, Italy; ALDO-CTK2 kit:

sensitivity, 5 pg/mL; cross-reactivity: aldosterone, 100%; 17-iso-aldosterone and other steroids (including 18OH-corticosterone), <0.1%; intra- and interassay variations, 7.1% and 8.5%. Cortisol-RIA kit: sensitivity, 30 pg/mL; cross-reactivity: cortisol, 100%; 11-deoxycortisol, 4.8%; corticosterone, 3%; progesterone, 0.5%; 11-deoxycorticosterone, 0.02%; other steroids, <0.01%; intra- and interassay variations, 6.2% and 7.4%]. The concentration of epinephrine and norepinephrine in the incubation medium was measured, without previous allumina purification and concentration, by high-pressure liquid chromatography using a reverse phase column (150 × 4 mm; BioSil ODS 5S, Bio-Rad Laboratories, Hercules, CA) and a glassy carbon electrochemical detector (TL-5, Bioanalytical Systems, Lafayette, IN), as detailed earlier (8). Epinephrine and norepinephrine were about 50% each of the total yield, and the sensitivity of the assay was approximately 3 pmol/L. The intraassay variation coefficient was 7%.

Data obtained from each adrenal gland were averaged and expressed as the mean ± SEM of three separate experiments (three adrenals from three patients). The statistical comparison of results was performed using ANOVA, followed by the multiple range test of Duncan.

Results

ADM concentration dependently inhibited ANG-II-stimulated aldosterone production by dispersed adrenocortical cells. Minimal and maximal effective concentrations (10^{-7} and 10^{-6} mol/L) induced about 25% and 50% inhibition, respectively. Neither basal nor ACTH-stimulated aldosterone secretion was affected (Fig. 1). In contrast, ADM concentration dependently raised basal aldosterone production by adrenal slices, without eliciting any significant change in agonist-stimulated secretion. Minimal and maximal effective concentrations (10^{-9} and 10^{-7} mol/L) evoked 2.1- and 3.7-fold increases, respectively (Fig. 2). ADM did not affect basal or agonist-stimulated cortisol production by both types of adrenal preparations (Figs. 1 and 2). All the effects elicited by 10^{-6} mol/L ADM were abolished by the simultaneous exposure to 10^{-5} mol/L CGRP8–37 (Fig. 3).

The Ca^{2+} ionophore A23187 (10^{-5} mol/L) significantly raised aldosterone secretion by both dispersed adrenocortical cells and adrenal slices (about 3-fold). ADM (10^{-6} mol/L) abolished the secretagogue effect of A23187 on dispersed

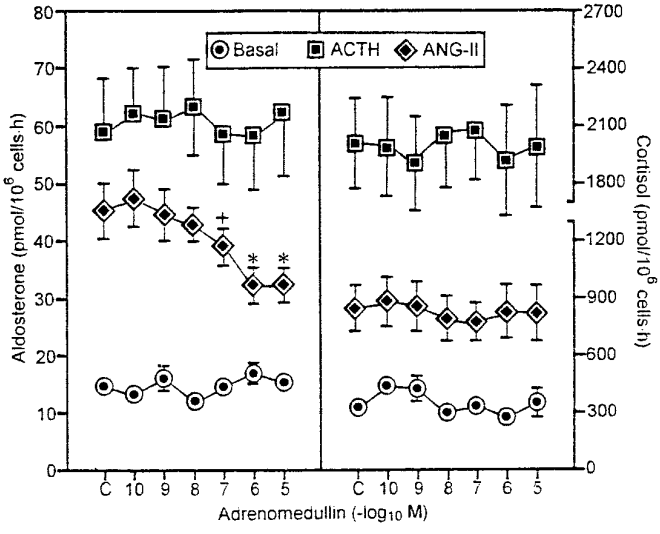

FIG. 1. Effects of ADM on basal and 10^{-9} mol/L agonist-stimulated aldosterone and cortisol production by dispersed human adrenocortical cells. Data are means ± SEM of three separate experiments. +, $P < 0.05$ and *, $P < 0.01$ *vs.* the respective control value (C).

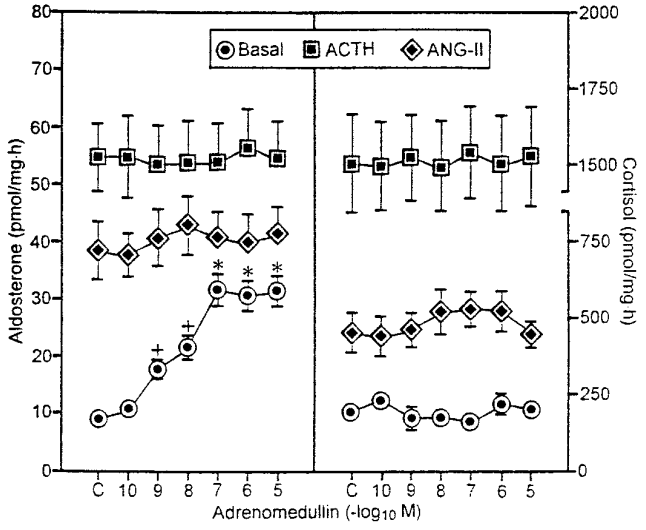

FIG. 2. Effects of ADM on basal and 10^{-9} mol/L agonist-stimulated aldosterone and cortisol production by human adrenal slices. Data are means ± SEM of three separate experiments. +, $P < 0.05$ and *, $P < 0.01$ *vs.* the respective control value (C).

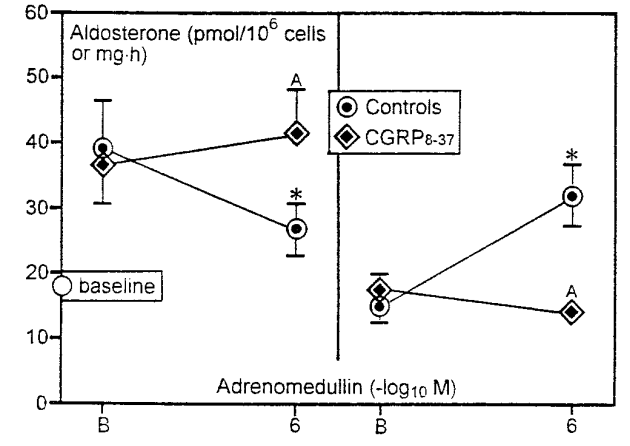

FIG. 3. Inhibitory effect of CGRP8–37 (10^{-5} mol/L) on ADM action on ANG-II-stimulated aldosterone production by dispersed human adrenocortical cells (*left panel*) and aldosterone secretion of human adrenal slices in the absence of ANG-II (*right panel*). Data are means ± SEM of three separate experiments. *, $P < 0.01$ *vs.* the respective basal value; A, $P < 0.01$ *vs.* the respective control value.

cells, but it only partially reversed (about 30%) that on adrenal slices, because it *per se* evoked a 3-fold rise in basal aldosterone production in these adrenal preparations (Fig. 4).

Isoprenaline (10^{-7} mol/L), like ADM, evoked a 3.3-fold increase in basal aldosterone production by adrenal slices. *l*-Alprenolol (10^{-6} mol/L) annulled aldosterone response of adrenal slices to both isoprenaline and ADM, without *per se* altering basal secretions (Fig. 5).

ADM raised epinephrine and norepinephrine release by human adrenal slices in a concentration-dependent manner. Minimal and maximal effective concentrations (10^{-9} and 10^{-7} mol/L) evoked 4- and 8-fold rises, respectively (Fig. 6).

Discussion

In keeping with the results of earlier studies carried out in the rat (4, 5, 7), we show that ADM exerts opposite secretory

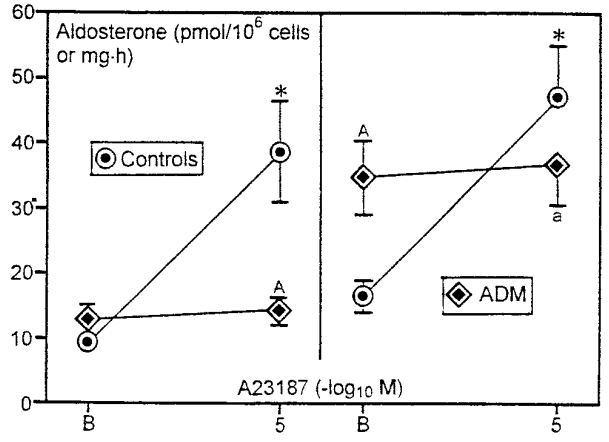

FIG. 4. Inhibitory action of ADM (10^{-6} mol/L) on A23187-induced stimulation of aldosterone production by dispersed cortical cells (*left panel*) and slices of human adrenal glands (*right panel*). Data are means ± SEM of three separate experiments. *, $P < 0.01$ *vs.* the respective basal value; a, $P < 0.05$ and A, $P < 0.01$ *vs.* the respective control value.

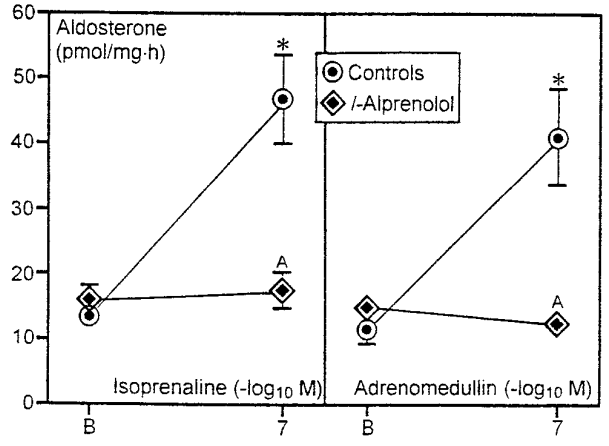

FIG. 5. Inhibitory effect of *l*-alprenolol (10^{-6} mol/L) on isoprenaline (10^{-7} mol/L)- and ADM (10^{-7} mol/L)-stimulated basal secretion of aldosterone by human adrenal slices. Data are means ± SEM of three separate experiments. *, $P < 0.01$ *vs.* the respective basal value; A, $P < 0.01$ *vs.* the respective control value.

effects *in vitro* according to the type of human adrenal preparation employed. In dispersed adrenocortical cells, ADM exerts a minor direct inhibitory action on ANG-II-stimulated aldosterone production. In adrenal slices, this effect is overcome and reversed by a major indirect stimulatory action requiring the integrity of adrenal tissue.

The complementary DNA for an ADM receptor has been cloned from rat lung tissue (10), and an orphan receptor gene has been identified, encoding a common CGRP1 receptor for both CGRP and ADM (11). Accordingly, evidence indicates that ADM competitively binds to CGRP receptors (12) and that the hypotensive effect of ADM in rats is, at least in part, mediated by CGRP1 receptors (13). The present study shows that both the direct and indirect effects of ADM on adrenal aldosterone secretion are abrogated by CGRP8–37, thereby suggesting that, not only in rats (5, 7), but also in humans, they are mediated by the type 1 of CGRP receptors.

The direct inhibitory effect of ADM on ANG-II-stimulated

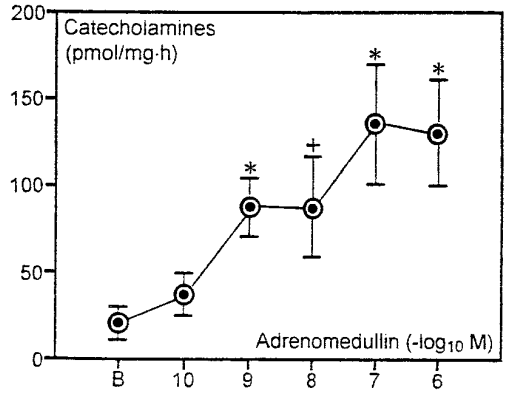

FIG. 6. Effect of ADM on catecholamine (epinephrine plus norepinephrine) release by human adrenal slices including medullary chromaffin tissue. Data are means ± SEM of three separate experiments. +, $P < 0.05$ and *, $P < 0.01$ *vs.* baseline (B).

aldosterone secretion conceivably involves the blockade of Ca^{2+} influx into human adrenocortical cells. In fact, ADM completely suppresses the aldosterone secretagogue action of the potent Ca^{2+} ionophore A23187 on dispersed cells. However, it only partially counteracts the stimulatory effect of ANG-II on adrenocortical cells, which involves the receptor-mediated activation of phospholipase C and thereby raises cytosolic Ca^{2+} concentration by increasing both Ca^{2+} influx and Ca^{2+} release from intracellular stores (for review, see Ref. 14). In agreement with this contention, preliminary data (not shown) indicate that ADM is able to suppress the aldosterone response of dispersed human adrenocortical cells to K^+, whose mechanism of action exclusively involves the opening of voltage-gated Ca^{2+} channels (for review, see Ref. 14). Obviously, these considerations easily may explain why ADM does not directly affect either basal secretion of dispersed cells or their aldosterone response to ACTH, which is relatively Ca^{2+}-independent (for review, see Ref. 14). It must be mentioned that our adrenal preparations respond to ANG-II also by raising their cortisol secretion, a finding in keeping with the presence of ANG-II receptors in human zona fasciculata-reticularis cells (15). The presence of functional specific receptors (of the CGRP1 subtype) for ADM in human zona glomerulosa, but not zona fasciculata-reticularis cells, could explain why ADM affects aldosterone, but not cortisol, response to ANG-II.

Our results strongly suggest that the indirect stimulatory effect of ADM on human adrenal aldosterone secretion is likely to be mediated by the release of epinephrine and norepinephrine by chromaffin cells contained in adrenal slices. Compelling evidence indicates that β-adrenoceptor agonists are able to enhance adrenal steroidogenesis in mammals, zona glomerulosa and mineralocorticoid secretion being their main targets in rodents, bovines, and humans (for review, see Ref. 2). Moreover, proofs are available that other intramedullary regulatory peptides, like pituitary adenylate cyclase-activating peptide (in humans and rats) and vasoactive intestinal peptide and neuropeptide Y (in rats), stimulate zona glomerulosa secretion through this indirect paracrine mechanism (for review, see Ref. 2). The contention that ADM may be included in this group of regulatory peptides is supported by the following lines of evidence: 1) *l*-alpre-

nolol, a specific β1-receptor antagonist, abolishes the aldosterone response of human adrenal slices, not only to the most potent β-receptor agonist isoprenaline (16) but also to ADM; and 2) ADM elicits a sizable catecholamine release by slices of the adrenal head including medullary tissue.

Our present demonstration that ADM is able to enhance aldosterone secretion in humans when the structural integrity of adrenal glands is preserved seems to be in contrast with earlier findings obtained *in vivo* in rats by Yamaguchi *et al.* (6); these investigators observed that sc-administered ADM decreases plasma aldosterone concentration in sodium-depleted or bilaterally nephrectomized rats. Apart from the obvious interspecific differences and the fact that aldosterone plasma level is the result of the balance between the rates of its production and metabolic clearance, it must be noted that it is always difficult to unequivocally interpret *in vivo* findings. In fact, ADM might have systemically affected other extraadrenal mechanisms involved in the regulation of adrenocortical secretion. For instance, ADM evokes a small reduction in plasma renin concentration (and conceivably ANG-II production) in sodium-depleted rats (6) and inhibits pituitary ACTH release in sheep and rats (17, 18). Accordingly, when ADM is directly and exclusively delivered to rat adrenal gland in *in situ* perfusion models, it seems not to depress, but to enhance aldosterone release (7).

In conclusion, our study shows that ADM exerts various effects on aldosterone secretion in humans. At micromolar concentrations, ADM, via specific receptors, directly inhibits aldosterone production elicited by those agonists that increase intracellular Ca^{2+} concentration. Concurrently, at nanomolar concentration, it indirectly stimulates aldosterone secretion by a mechanism involving epinephrine and norepinephrine release by chromaffin cells. The level of circulating ADM in humans is about 3×10^{-12} mol/L under basal conditions; however, even under pathological conditions, it does not exceed 1×10^{-11} mol/L (19, 20), thereby making unlikely the possibility that the peptide may act on adrenals as a true circulating hormone. Conversely, ADM content in human adrenal medulla averages 50 fmol/g fresh tissue (21): hence, upon maximal stimulation of its release, it could reach an intraadrenal concentration of about 10^{-8}/10^{-7} mol/L and therefore act as a paracrine regulatory peptide (2). In a recent review, Schell *et al.* (22) described ADM as a hormone mainly controlling the kidney excretion of water and electrolyte. Furthermore, increased plasma ADM levels have been reported in patients with arterial hypertension, where they were inversely correlated with GFR (23), and in congestive heart failure, where they were directly correlated with NYHA functional class (24). In this latter condition, a 4-fold increase of ADM plasma levels was likely to be caused not only by enhanced adrenal, but also by extraadrenal synthesis of the peptide in the heart. Hence, the dose-dependent biphasic paracrine effect of ADM on aldosterone secretion identified in this study might be of major relevance under pathophysiological conditions where a resetting of fluid and electrolyte homeostasis occurs.

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