Atrial natriuretic peptide enhances cortisol secretion from guinea-pig adrenal gland: Evidence for an indirect paracrine mechanism probably involving the local release of medullary catecholamines

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Received November 7, 2005; Accepted December 18, 2005

Abstract. Atrial natriuretic peptide (ANP) is a regulatory hormone widely expressed, along with its receptors, in organs and body tissues. ANP is well known to inhibit aldosterone secretion from mammalian adrenals, but its effect on glucocorticoid-hormone production is controversial. *In vivo* experiments showed that prolonged ANP administration raised the plasma concentration of cortisol in both normal and dexamethasone/captopril-treated guinea pigs (i.e. in animals with pharmacologically interrupted hypothalamic-pituitary-adrenal axis and renin-angiotensin system). ANP did not affect cortisol secretion from dispersed guinea pig zona fasciculata-reticularis cells, but enhanced catecholamine release from adrenomedullary cells. ANP stimulated cortisol output from guinea pig adrenal slices containing medullary chromaffin tissue, and the ß-adrenoceptor antagonist *l*-alprenolol blocked this effect. The conclusion is drawn that ANP, when the structural integrity of the adrenal gland is preserved, is able to enhance glucocorticoid secretion in guinea pigs, through an indirect mechanism involving the rise in the catecholamine release, which in turn, acting in a paracrine manner, stimulate secretion of inner adrenocortical cells.

Introduction

Atrial natriuretic (ANP) is the first member of a family of peptides originally discovered in the late 1970s in the secretory granules of atrial myocytes, and including brain natriuretic peptide and C-type natriuretic peptide (reviewed in ref. 1). ANP acts via two G protein-coupled receptors, named A and B, that are coupled to guanylate cyclase (reviewed in refs. 2,3). Subsequent studies showed that ANP and its receptors are present in several extra-atrial tissues, among which are heart ventricles, blood vessels, brain, lungs, kidneys and endocrine glands.

Mammalian adrenal zona glomerulosa possesses A and B subtypes of ANP receptors, and many lines of evidence indicate that ANP, via the cyclic-GMP pathway, inhibits either basal or agonist-stimulated aldosterone secretion (reviewed in refs. 4,5). The possible effects of ANP on the zona fasciculata and glucocorticoid secretion have been far less investigated. Although ANF receptors have been demonstrated in the rat zona fasciculata (6), most studies did not report any effect of ANP on glucocorticoid secretion in this species (1,4). However, findings suggest that ANP is able to lower basal and especially ACTH-stimulated glucocorticoid production from cultured human and cow zona fasciculata cells (7,11). The A subtype of ANP receptors is expressed also in the adrenal medulla (12,13), and the bulk of evidence indicates that ANP inhibits catecholamine release (14-17). However, ANP has been more recently reported to induce tyrosine hydroxylase mRNA expression and to raise catecholamine content in rat pheochromocytoma PC12 cells, via the guanylate cyclase-dependent cascade (18).

Therefore, it seemed worthwhile to investigate the *in vivo* and *in vitro* effects of ANP on glucocorticoid and catecholamine secretion from the guinea-pig adrenal gland. Guinea pig was chosen because its main glucocorticoid hormone is cortisol, as in humans and cows.

Materials and methods

Animals and reagents. Adult male guinea pigs, either bred in our laboratory facilities (*in vivo* experiments) or purchased from Charles-River (Como, Italy) (*in vitro* experiments), were

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Key words: atrial natriuretic peptide, adrenal gland, corticosteroid secretion, catecholamine secretion, guinea pig

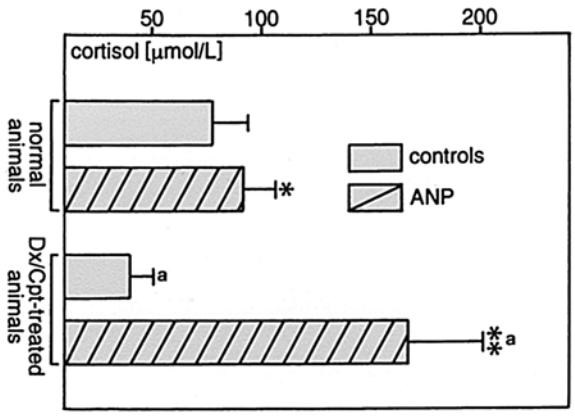

Figure 1. Effect of the prolonged administration of ANP on the blood concentration of cortisol in normal and dexamethasone (Dx)/captopril (Cpt)-treated guinea pigs. Bars are means ± SEM (n=20). *P<0.05 and **P<0.01 from the respective control group; aP<0.01 from the respective normal animal group.

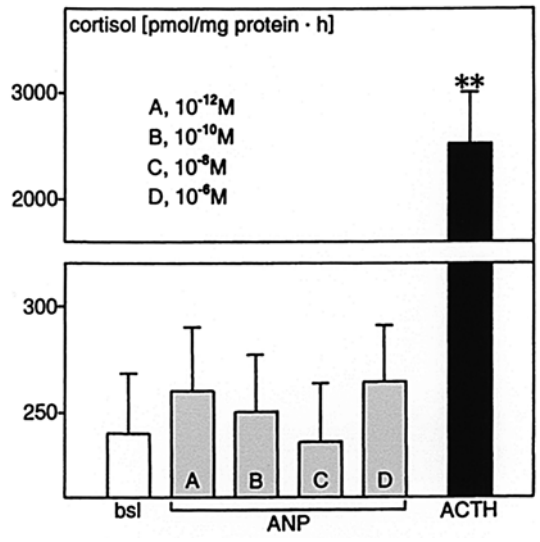

Figure 2. Effect on ANP and ACTH on cortisol secretion from dispersed guinea pig zona fasciculata-reticularis cells. Bars are means ± SEM (n=6). **P<0.01 from baseline (bsl).

kept under a 12:12 h light/dark cycle (illumination onset at 8:00 a.m.) at 23˚C, and maintained on a standard diet and tap water *ad libitum*. The study protocol was approved by the local Ethics Committees for Animal Studies. The angiotensin-converting enzyme inhibitor captopril (Capoten) was obtained from Squibb (Milan, Italy). ANP, ACTH, bovine serum albumin (BSA), and all other chemicals and reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

In vivo experiments. Animals were divided into two groups (n=40). One group was subcutaneously injected for 14 days with dexamethasone (2.5 mg/kg) and captopril (2.5 mg/kg). The other group was given daily injections of 0.9% NaCl. On the 6th day, half the animals in each group received ANP (0.1 mg/kg). At the end of the treatment, blood samples were collected from retro-orbital vein, and stored at -30˚C until hormonal assay.

In vitro experiments. Guinea pigs were sacrificed by cervical dislocation, and their adrenal glands were gently decapsulated to separate zona glomerulosa from the inner zones and then hemisected; adrenal halves were enucleated for removal of adrenal medulla. Dispersed zona fasciculata-reticularis and adrenomedullary cells were obtained by collagenase digestion and mechanical disaggregation, and the purity of cell preparations was checked under the phase microscope (19,20). Freshly dispersed cells obtained from 6 guinea pigs were pooled to obtain a single cell suspension, and 6 cell suspensions for each incubation experiment were employed. Aliquots of each cell suspension (10^4 cells/ml in Krebs-Ringer bicarbonate buffer with 0.3% glucose and 0.2% BSA) were incubated with ANP (from 10^{-12} to 10^{-6} M), or in the absence of ANP (baseline value). Adrenocortical cells were also incubated in the presence of 10^{-8} M ACTH. Adrenal glands from other guinea pigs were sliced, and slices (containing both cortical and medullary tissues; 6-8 mg tissue/ml) were incubated, in replicates of 6 each, as follow: i) ANP (from 10^{-12} to 10^{-6} M); and ii) *l*-alprenolol (10^{-6} M) alone or in the presence of 10^{-8} M ANP. The incubation was carried out in a shaking bath at 37˚C for 60 min (cortisol secretion) or 20 min (catecholamine secretion), in an atmosphere of 95% air-5% CO_2. Supernatants were stored at -80˚C until hormonal assays, and protein concentration of dispersed cells and tissue slices was measured by the BCA protein assay kit (Sigma-Aldrich Corp.).

Hormone assays. Cortisol blood concentration was measured by enzyme immunoassay (EIA), as previously detailed (21). Cortisol was extracted from supernatants and purified by HPLC (22-24), and its concentration was estimated by radio-immunoassay (RIA), using a commercial kit purchased from IRE-Sorin (Vercelli, Italy) (20). The catecholamine (epinephrine, E; and nor-epinephrine, NE) concentrations in the supernatants were measured by HPLC, using a reverse phase column and a glassy carbon electrochemical detector (22,25,26).

Statistics. Data were expressed as means ± SEM, and their statistical comparison was done by the paired sample t-test (cortisol blood concentration) or by ANOVA, followed by Duncan's multiple range test.

Results

The prolonged dexamethasone/captopril administration lowered by about 45% the blood concentration of cortisol in guinea pigs. ANP treatment for 6 days increased the plasma level of cortisol by about 20% in normal guinea pigs and by about 3.5-fold in dexamethasone/captopril administered animals (Fig. 1).

ANP did not affect cortisol secretion from dispersed guinea pig inner adrenocortical cells displaying a normal response to ACTH (Fig. 2). In contrast, ANP (10^{-8} and 10^{-6} M) enhanced E and NE release from medullary cells by about 3-fold, lower concentrations of ANP being ineffective (Fig. 3).

ANP (10^{-8} and 10^{-6} M) raised cortisol production from guinea pig adrenocortical slices containing adrenomedullary tissue by about 45% (Fig. 4), as well as evoked a significant increase in E and NE release (data not shown). The cortisol

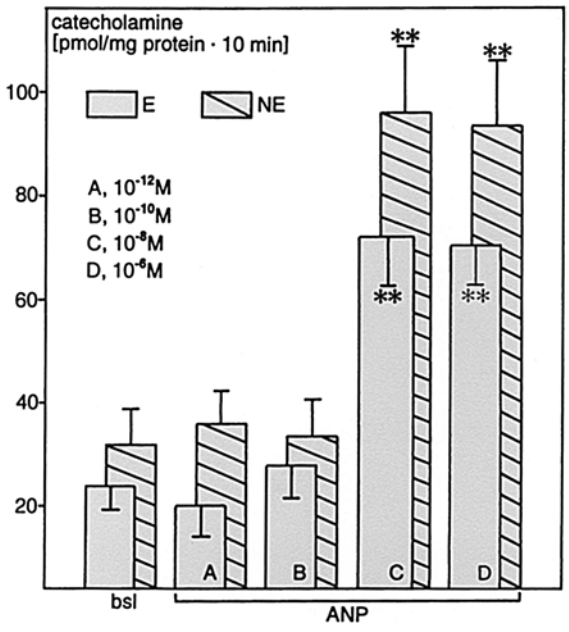

Figure 3. Effect of ANP on catecholamine secretion from dispersed guinea pig adrenomedullary cells. Bars are means ± SEM (n=6). **P<0.01 from baseline (bsl).

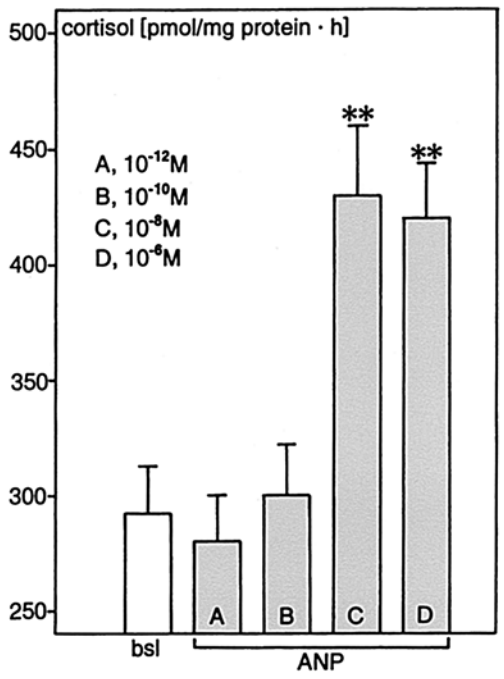

Figure 4. Effect of ANP on cortisol secretion from guinea pig adrenal slices containing medullary chromaffin tissue. Bars are means ± SEM (n=6). **P<0.01 from baseline (bsl).

secretagogue effect of 10^{-8} M ANP was annulled by 10^{-6} M *l*-alprenolol, which per se was ineffective (Fig. 5).

Discussion

Our present *in vivo* experiments show that ANP is able to raise the blood level of cortisol not only in intact guinea pigs, but also in animals where the hypothalamic-pituitary-adrenal axis and renin-angiotensin system had been pharmacologically blocked by dexamethasone and captopril. This finding rules out the possibility that ANP effect was due to a stimulating action on ACTH and/or angiotensin II release, and strongly suggests that this peptide acts directly on the adrenal gland. However, a direct action of ANP on zona fasciculata-reticularis cells can be excluded, inasmuch as this peptide does not exert any cortisol secretagogue effect on dispersed cell preparations. In this connection, we wish to stress that this last observation is at variance with the reported direct inhibitory effect of ANP on the secretory activity of inner adrenocortical cells of other cortisol-secreting species, like cows and humans (7-11). Despite ineffective on inner adrenocortical cells, ANP is able to stimulate cortisol secretion from guinea pig adrenal slices containing medullary tissue. Thus, it seems conceivable to postulate that an indirect effect, mediated by medullary chromaffin cells, underlies this cortisol secretagogue action of ANP. Compelling evidence indicates that catecholamines are able to stimulate steroidogenesis, adrenocortical cells being provided with ß-adrenoceptors (5,27). Several regulatory peptides have been reported to enhance steroid secretion by eliciting the release of catecholamines from chromaffin cells, that in turn stimulate adrenocortical cells acting in a paracrine manner: VIP and PACAP (reviewed in ref. 28), neuropeptide-Y (reviewed in ref. 29), tachykinins (reviewed in ref. 30), endothelins (reviewed in ref. 31), and adrenomedullin and related peptides (reviewed in ref. 32).

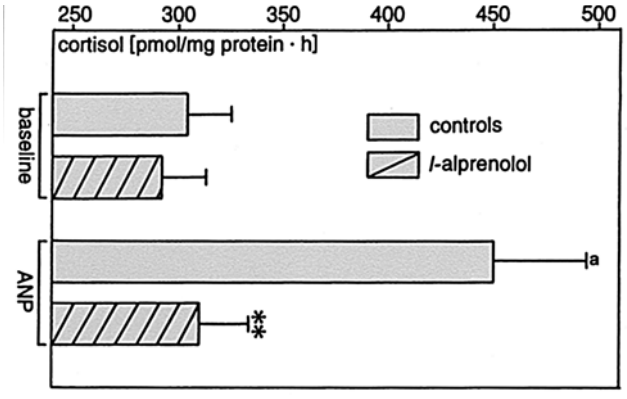

Figure 5. Effect of *l*-alprenolol (10^{-6} M) on baseline and ANP(10^{-8} M)-stimulated cortisol secretion from guinea pig adrenal slices containing medullary chromaffin tissue. Bars are means ± SEM (n=6). **P<0.01 from the respective control group; aP<0.01 from baseline.

The hypothesis that in guinea pigs the mechanism mediating the glucocorticoid secretagogue action of ANP involves an increased release of catecholamines is supported by the following evidence: i) ANP enhances E and NE release from guinea pig adrenomedullary cells, a finding stressing again the species-related differences between guinea pigs and cows, dogs and rats, where ANP appears to inhibit catecholamine release (14-17); and ii) *l*-alprenolol, a specific ß1-receptor antagonist, completely suppresses cortisol response of guinea pig adrenal slices to ANP, without per se affecting baseline cortisol secretion. This last observation makes unlikely the possibility that the *l*-alprenolol effect was due to a nonspecific inhibitory effect on the steroidogenic machinery of inner adrenocortical cells.

Collectively, our present findings allow us to draw the following conclusions: i) when the structural integrity of adrenal gland is preserved, ANP is able to stimulate glucocorticoid secretion in guinea pigs through an indirect paracrine mechanism involving adrenomedullary catecholamine release; and ii) relevant species-specific differences occur in the adrenal cytophysiology of guinea pig and other mammalian species, especially as far as catecholamine response of adrenomedullary cells to ANP is concerned.

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