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LACK OF ATRIAL NATRIURETIC PEPTIDE RECEPTORS IN HUMAN ALDOSTERONOMA

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The effect of synthetic alpha-human atrial natriuretic peptide (ANP) on aldosterone secretion was studied in human aldosterone producing adrenocortical adenoma obtained surgically from a patient with primary aldosteronism and in human apparently normal adjacent adrenal cortical tissues obtained from a patient with pheochromocytoma, in vitro. Apparently normal adrenal cortical tissue responded to ANP with the known inhibition of aldosterone secretion. In contrast, the aldosterone producing adenoma did not respond to ANP. When stimulated by either ACTH or angiotensin II, there is no inhibition by ANP in the adenoma tissue, whereas normal tissue was inhibited. Immunohistochemical examination utilizing an ANP-receptor antiserum demonstrated that there was no evidence of binding site in the cortical adenoma, in contrast, zona glomerulosa cells in the cortical tissues adjacent to either aldosterone producing adenoma or pheochromocytoma were densely stained. This apparent lack of ANP-receptors is an associated finding with the hypersecretion of aldosterone in the aldosterone prducing adenoma.

Atrial natriuretic peptides(ANP) in mammalian atria have been reported to have potent diuretic, natriuretic(1), vasorelaxant(2,3), and aldosterone-inhibitory activities (for review, see Ref. 4-7). Thus, ANP might play an important role in the regulation of extracellular fluid volume, electrolytes homeostasis, vascular tone and aldosterone production. Recently, binding sites or receptors for ANP have been demonstrated in these target tissues including the kidney(8,9,10), vascular smooth muscle(8),

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and adrenal gland(11), and pheochromocytoma(12). There are some reports related to the effect of ANP on human aldosterone producing adenoma(13,14,15). Two of them described inhibition of aldosterone secretion by ANP in vitro, and the other one described similar effects in normal adrenal cells but not in adenoma. On the other hand, it is known that several factors including adrenocorticotropic hormone(ACTH), angiotensin II and potassium stimulate aldosterone production in vivo and in vitro(16). We therefore investigated whether ANP receptors exist on cell membrane in human normal adrenocortical cells and aldosteronoma cells. Furthermore we investigated whether synthetic alpha-human ANP(h-ANP)(17) has an effect on aldosterone production in either human adrenocortical tissue or human aldosteronoma tissue and examined the effects of h-ANP on the dose-response curves for aldosterone in the presence of ACTH and angiotensin II.

Materials and Methods

Adrenocortical adenoma(aldosteronoma) was surgically removed from a patient with primary aldosteronism who had hypertension, hypokalemia, suppressed plasma renin activity, and increased plasma aldosterone concentration. The cortical adenoma was separated from the surrounding adrenal cortical tissue. As a control study, apparently normal adrenal cortical tissue was obtained from a patient with pheochromocytoma(12). Both aldosteronoma tisue and adrenal cortical tissue were sliced. After washing with medium 199 containing 5mM ethylenediaminetetraacetic acid (EDTA), the sliced tissues were incubated in triplicate in 1 ml of medium 199 containing 5mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride and 0.2 % bovine serum albumin in the absence or presence of 10^{-9}M and 10^{-8}M of concentration of ACTH, angiotensin II, and synthetic alpha human atrial natriuretic peptide (h-ANP) (Peptide Institute, Japan) for 40 minutes at 37 C in an atmosphere of 95% oxygen and 5% carbon dioxide. Aldosterone concentration in the bathing medium was measured directly by radioimmunoassay using Aldok-kit II(Dainabot, Japan)(18). The assay sensitivity of aldosterone was 1.0 pg/ml, and the intra-assay and inter-assay coefficients of variations were 4.8% and 7.2%. To rule out the possibility of interferences in the radioimmunoassay of aldosterone, we showed that there was no alteration of the standard curve for aldosterone when h-ANP, ACTH or angiotensin II were added. Binding assays for ANP receptor were carried out essentially as described previously using ^{125}I-ANP and membrane preparations(19). For the localization of ANP receptor, paraffin sections of aldosteronoma and adrenal cortices

surrounding aldosteronoma and pheochromocytoma were stained, after dewaxing and rehydration, by the avidin-biotin-peroxidase complex method(20) using anti-ANP receptor antiserum which was raised in rabbit by immunizing the ANP receptor purified from bovine lung by affinity chromatography(10).

Results

The basal amount of aldosterone released from the apparently normal cortical tissue obtained from a patient with pheochromocytoma was inhibited by h-ANP in a dose-dependent manner, whereas h-ANP had no effect on aldosterone release from the aldosteronoma tissue(Figure 1). h-ANP with concentration of 10^{-7}M lowered basal aldosterone release by 42 %(Figure 1; basal value=20.5±2.0 pg per ml of medium per 50mg wet tissue, 10^{-7}M value=8.6±1.7; $p<0.01$). Table 1 further shows that ACTH- and AII-stimulated releases of aldosterone from the adenoma were also unaffected by ANP while non-tumor adrenocortical tissues responded normally not only to the stimulators (ACTH and AII) but also to the inhibitor ANP.

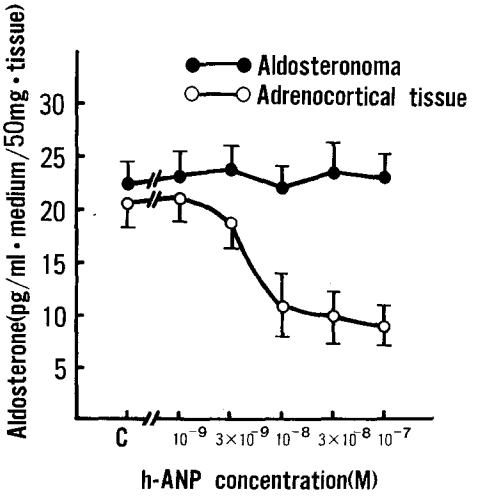

Figure 1. Effects of synthetic alpha human-atrial natriuretic peptide(h-ANP) on release of aldosterone from unstimulated normal adrenocortical tissue-slice(o) or aldosteronoma tissue-slice(●). Basal release of aldosterone from the normal tissue was inhibited by h-ANP in a dose-dependent manner. In aldosteronoma, however, h-ANP exerted no inhibitory effect on the release of aldosterone. Each point represents the mean+standard error of three experiments. In each experiment h-ANP concentrations are expressed as moles per liter of incubation medium.

Table 1. No effect of synthetic alpha human-atrial natriuretic peptide(h-ANP) on aldosterone secretion from unstimulated, ACTH-stimulated, and angiotensin II(AII)-stimulated aldosteronoma. As a positive control, normal human adrenal cortex capable of responding to h-ANP was included in samples

Addition		Aldosterone secretion (pg/ml/50 mg tissue)			
		Normal cortex		Aldosteronoma	
		-h-ANP	$+10^{-7}$M h-ANP	-hANP	$+10^{-7}$M h-ANP
None		18.5±3.5	10.0±3.0	20.2±4.3	18.8±3.6
ACTH	10^{-9}M	42.5±4.5	23.5±3.0	58.0±7.2	60.0±4.1
	10^{-8}M	65.1±4.7	41.5±4.6	84.4±5.8	83.3±4.9
AII	10^{-9}M	32.7±5.0	18.0±4.1	42.1±4.0	39.8±4.4
	10^{-8}M	48.0±6.2	37.4±3.2	53.5±4.5	56.1±4.1

Each value represents the mean±standard error of three determinations.

Binding assays in an effort to determine the receptor content in the adenoma failed to detect the binding sites for ^{125}I-ANP.

Aldosteronoma tissue was not stained with the anti-ANP-receptor antiserum, while intense staining was demonstrated in the normal zona glomerulosa cells surrounding the adenoma (Figure 2) and pheochromocytoma(12).

Discussion

In this study we found that h-ANP inhibited basal aldosterone production from normal adrenocortical tissue obtained from a patient with pheochromocytoma in a dose-dependent manner and reduced the sensitivity of the normal adrenocortical tissue to ACTH and angiotensin II, while h-ANP reduced neither basal nor ACTH- nor angiotensin II-stimulated aldosterone production from the aldosteronoma. These results suggest that the aldosteronoma has a defect in ANP receptors or the signal-transducing-processes following the receptor activation, which is an associated finding with uncontrolled oversecretion of aldosterone. The fact that the binding assay failed to detect the binding sites for ^{125}I-ANP

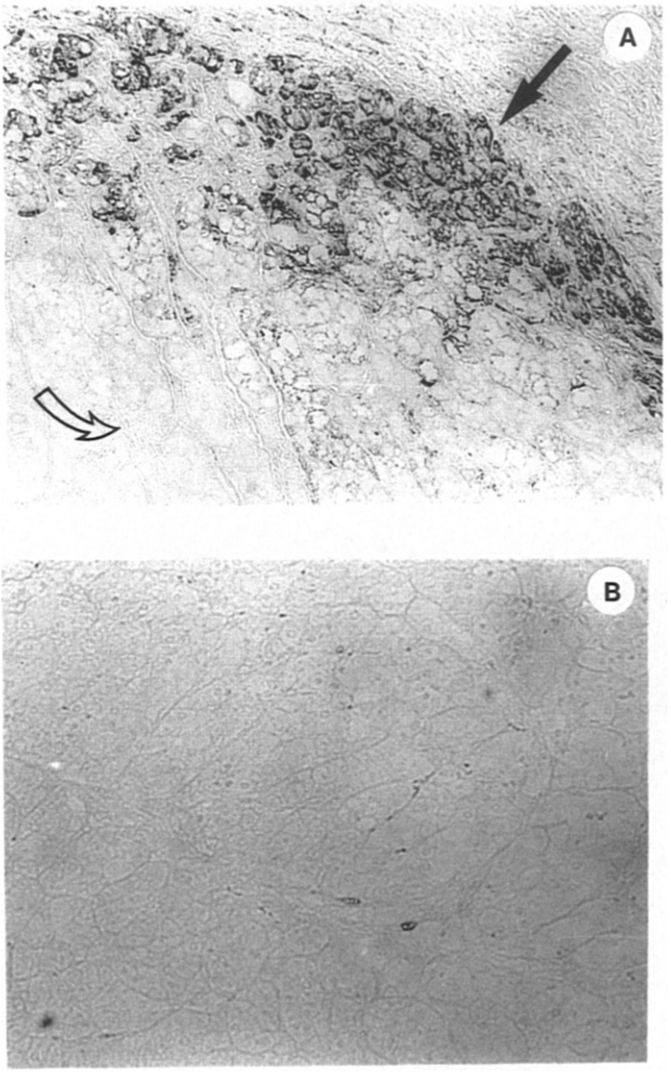

Figure 2. Section of human adrenocortical tissue stained for atrial natriuretic peptide(ANP) receptor. The intense staining is demonstrated in the normal(A) zona glomerulosa cells(↑), however, the staining is absent in the aldosteronoma(B) and in the normal zona fasciculata cells(A,⇑). Paraffin sections of the tissues were stained by the avidin-biotin-peroxidase complex method for localization of ANP receptor using anti-ANP-receptor antiserum which was raised in rabbit by immunizing ANP receptor purified from bovine lung.

indicates that the defect is at the level of receptor. Consistent with these results, immunohistochemical examination revealed no staining in the aldosteronoma, and, as expected, normal zona glomerulosa cells are densely labeled demonstrating the presence of ANP receptors.

Other investigators have shown that ANP decreased aldosterone release from rat zona glomerulosa cells in a dose-dependent manner(21,22). In our study those observations are recognized in human adrenocortical tissue, while h-ANP had no effect on aldosterone release from human aldosteronoma which lacks h-ANP receptor. Our results confirmed the other report(14) which has described that h-ANP did not inhibit basal and ACTH-stimulated aldosterone secretion in aldosteronoma cells. Furthermore, dense staining of the glomerulosa cells surrounding the aldosteronoma with the anti-ANP-receptor antiserum suggests upward-regulation of the ANP receptor by excessive aldosterone. In support of this even the homologous bovine glomerulosa cells gave moderate-to-weak staining. Although definitive proof for the upward-regulation should be obtained using, for example, cultured cells, it seems to be an effective, and therefore, likely, compensatory mechanism to suppress aldosterone secretion from the normal zona glomerulosa cells.

The results reported here will form an important basis for understanding the pathophysiology of primary aldosteronism.

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