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PRESENCE OF FUNCTIONAL RECEPTORS FOR ATRIAL NATRIURETIC PEPTIDE IN HUMAN PHEOCHROMOCYTOMA

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Pheochromocytoma, a catecholamine-secreting adrenomedullary tumor, has been shown to contain the functional receptor for human atrial natriuretic peptide(h-ANP). Release of catecholamines from tissue slices of pheochromocytoma was inhibited by h-ANP in a dose-dependent manner. Binding assays using ^{125}I-ANP revealed a single class of high affinity binding sites for ANP. When covalently tagged with ^{125}I-ANP and electrophoresed under non-reducing and reducing conditions, the receptor migrated as a 140-kDa band and a 70-kDa band, respectively, reflecting its disulfide-linked subunit structure. The presence of ANP receptor in pheochromocytoma was further demonstrated by immunohistochemistry; the tumor was positively stained with an anti-receptor antiserum. The antiserum was also useful to establish the zona glomerulosa localization of ANP receptor in the normal human adrenal gland.

Pheochromocytoma is a rare adrenal medullary tumor composed of chromaffin cells. The tumor causes hypertension by producing and releasing excessive catecholamines; surgical removal of the tumor usually cures the hypertension. Normal chromaffin cells release catecholamines by exocytosis, but in pheochromocytoma catecholamines are released into the circulation mainly by diffusion(1). In addition to this diffusion process, pheochromocytoma has also been shown to release catecholamines in response to various stimuli such as hypovolemia, muscular

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exercise, and hormones. In the present study, therefore, we examined the presence of cell surface receptors regulating the release of catecholamines from pheochromocytoma and found that atrial natriuretic peptide(ANP) receptor is one of such receptors. ANP is a peptide hormone secreted from the atria(2) and capable of producing potent effects on blood pressure and fluid and electrolyte balance. As its name implies, ANP increases salt and water output from the kidney; it also relaxes vascular smooth muscle, inhibits aldosterone secretion from the zona glomerulosa, and suppresses vasopressin release from the posterior pituitary(3). These processes, culminating in reduced fluid volume and blood pressure, and initiated by binding of ANP to its specific receptor on various target tissues.

We have recently purified the ANP receptor from the bovine lung and raised an antiserum against it(4). Using the antiserum, radioligand(^{125}I-ANP), and assays which measure catecholamines and cGMP we demonstrate here the presence of functional ANP receptor in pheochromocytoma.

Materials and Methods

Alpha-human ANP(h-ANP)(5) was obtained from the Peptide Institute, Japan; ^{125}I-ANP(rat, 2000Ci/mmol) was from Amersham; avidin-biotin peroxidase complex(ABC) kits were from Vecterstain. Surgically removed pheochromocytoma was sectioned into sliced preparations and used to examine the effect of h-ANP on basal epinephrine and norepinephrine release. The sliced tissues with average weight of 32mg was washed repeatedly with medium 199 containing 5mM EDTA and incubated in triplicate with various amount of h-ANP(10^{-9} to 10^{-7}M) in 1ml of medium 199 containing 5mM EDTA, 0.5mM phenylmethylsulfonyl fluoride and 2mg/ml bovine serum albumin. The amounts of epinephrine and norepinephrine released into the medium were measured by fluorophotometry following high performance liquid chromatographic separation. The sensitivity of epinephrine and norepinephrine assay was 0.01ng/ml, and the intraassay and interassay coefficients of variations were 4.5% and 5.8%, respectively.

Preparation of membranes, Scatchard analysis, and affinity labeling of ANP receptor with ^{125}I-ANP and disuccinimidyl suberate were carried out as previously described(6).

Immunohistochemistry was performed using ABC kits and antiserum raised against purified bovine ANP receptor(4).

Results and Discussion

When incubated in medium 199, tissue slices of pheochromocytoma released epinephrine and norepinephrine at a rate of 0.50±0.01(mean±SE) and 0.62±0.01ng/mg tissue/40min, respectively. This basal release of catecholamines was inhibited by ANP in a dose-dependent manner(Fig.1), indicating the presence of ANP receptor on the pheochromocytoma, through which ANP suppresses the release of catecholamines. There is a report(6) that secretion of norepinephrine from cultured pheochromocytoma cells was decreased by treatment with ANP and ANP may inhibit the activity of dopamine-β-hydroxylase. Thus our results confirmed their result that ANP suppresses catecholamine synthesis in human pheochromocytoma tissue in vitro.

Binding assays using membrane preparations and ^{125}I-ANP in the absence and presence of excess of unlabeled ANP also indicated the presence of ANP receptor; Scatchard analysis of the binding data revealed a single class of binding sites for ANP with a Kd of 1nM and a Bmax of 0.4pmol/mg protein (Fig. 2).

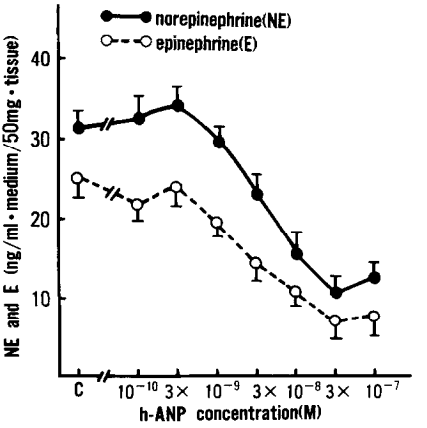
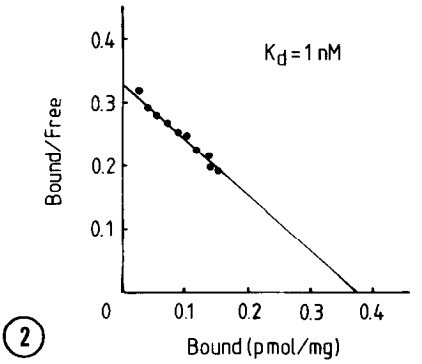


Figure 1. Effects of synthetic alpha human-atrial natriuretic peptide(h-ANP) on secretion of norepinephrine(NE)(●) and epinephrine(E)(○) in human pheochromocytoma tissue-slice. Basal secretions of NE and E from sliced pheochromocytoma-tissue were inhibited by h-ANP in a concentration-dependent manner. 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.



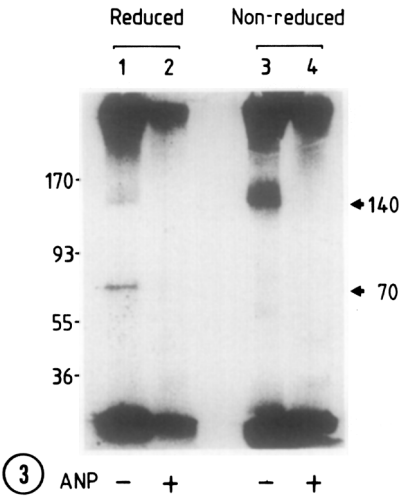


Figure 2. Scatchard analysis of the binding assay for membrane preparations obtained from human pheochromocytoma.
A single class of binding sites for ANP with a Kd of 1nM and Bmax of 0.4pmol/mg protein were observed.

Figure 3. Identification of ANP receptor band on SDS-polyacrylamide gel.
ANP-binding site in pheochromocytoma visualized by affinity labeling followed by SDS-PAGE and autoradiography. When covalently tagged with ^{125}I-ANP and electrophoresed under non-reducing conditions, the receptor from pheochromocytoma migrated as a 140-kDa band; on reduction, the band shifted to a position corresponding to 70-kDa.

The size of ANP receptor in pheochromocytoma was estimated by affinity labeling in which ^{125}I-ANP was cross-linked to the receptor with disuccinimidyl suberate and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The results are shown in Fig. 3. When covalently tagged with ^{125}I-ANP and electrophoresed under non-reducing conditions, the receptor from pheochromocytoma migrated as a 140-kDa band; on reduction, the band shifted to a position corresponding to 70kDa. This electrophoretic behavior is very similar to that of bovine(4,7,8,9) and rat(8) ANP receptors and indicates that the human ANP receptor is also a 140-kDa dimer composed of two apparently identical disulfide-linked 70-kDa subunits.

Membranes were prepared from pheochromocytoma and assayed for guanylate cyclase activity in the absence and presence of

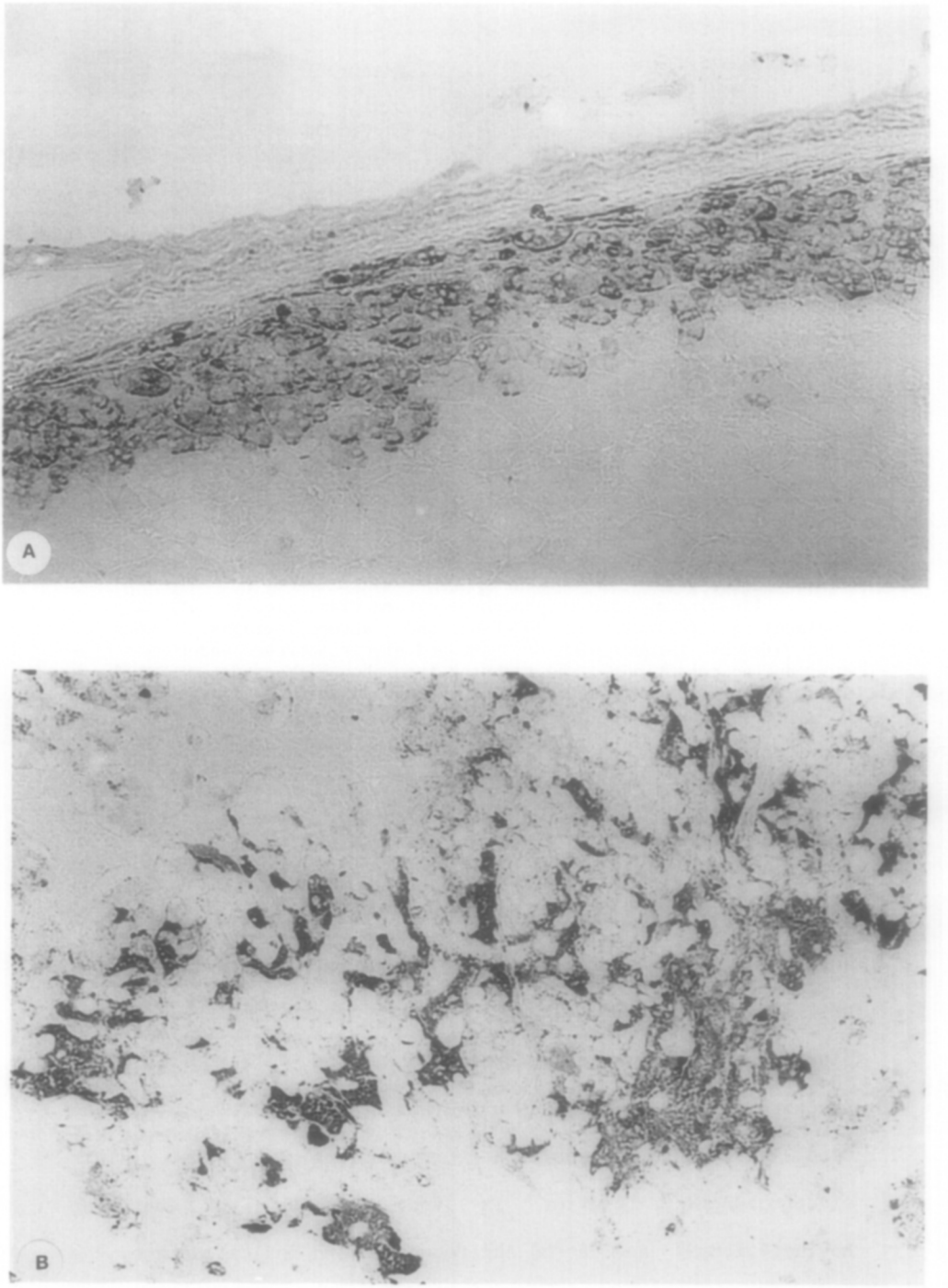


Figure 4. Section of human adrenal tissue stained for atrial natriuretic peptide(ANP) receptor.
The surrounding normal adrenocortical tissues demonstrated excellently the glomerulosa localization of the ANP receptor(Fig. 4A); pheochromocytoma was also positively(Fig. 4B).

10^{-7}M ANP. Addition of ANP resulted in a 2-fold increase(from 88 to 155 pmol cGMP/min/mg protein) in cGMP accumulation, indicating the coupling of ANP receptor and particulate guanylate cyclase.

Immunohistochemically stained sections of the pheochromocytoma and the surrounding normal tissues demonstrated excellently the glomerulosa localization of the ANP receptor(Fig. 4A); pheochromocytoma was also stained positively(Fig. 4B), reinforcing the above conclusion that the catecholamine-producing adrenomedullary tumor contains functional ANP receptor.

In summary, we identified the functional ANP receptor in pheochromocytoma. Through its characterization, we established that 1) catecholamine release is under the control of ANP, 2) human ANP receptor has a disulfide-linked subunit structure like the receptors from other species, and 3) the zona glomerulosa is the site of receptor localization as visualized for the first time using antireceptor antiserum.

REFERENCES

1. Louis, W. J., Jaorott, B., and Doyle, A. E. (1975) The role of the autonomic nervous system in human hypertension. Studies in essential hypertension and in pheochromocytoma. In Pathophysiology and management of arterial hypertension (Berglund, G., Hansson, L., Werkoe, L. eds.) pp.16-21, Lindgren & Soener, Sweden.
2. De Bold, A. J., Borenstein, H. B., Veress, A. T., and Sonnenberg, H. (1981) Life Sci. 28, 89-94.
3. Richards, A. M. (1986) Current Opinion in Cardiology 1, 614-621.
4. Shimonaka, M., Saheki, T., Hagiwara, H., Ishido, M., Nogi, A., Fujita, T., Wakita, K., Inada, Y., Kondo, J., and Hirose, S. (1987) J. Biol. Chem. 262, 5510-5514.
5. Kangawa, K., and Matsuo, H. (1984) Biochem. Biophys. Res. Commun. 118, 131-139.
6. Racz, K., Kuchel, O., Buu, N. T., Debinski, W., Cantin, M., and Genet, J. (1986) New Engl. J. Med. 314, 321-322.
7. Hirose, S., Akiyama, F., Shinjo, M., Ohno, H., and Murakami, K. (1985) Biochem. Biophys. Res. Commun. 130, 574-579.
8. Shinjo, M., Hirata, Y., Hagiwara, H., Akiyama, F., Murakami, K., Kojima, S., Shimonaka, M., Inada, Y., and Hirose, S. (1986) Biomedical Res. 7, 35-38.
9. Schenk, D. B., Phelps, M. N., Porter, G., Fuller, F., Cordell, B., and Lewicki, J. A. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 1521-1525.