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Purification and subunit composition of atrial natriuretic peptide receptor

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ABSTRACT **A receptor for atrial natriuretic peptide (ANP) was purified 2700-fold, to apparent homogeneity, from cultured bovine aortic smooth muscle cells by affinity chromatography. The native ANP receptor has a molecular weight of 125,000 as determined by both metrizamide gradient centrifugation and nonreducing $NaDodSO_4$/polyacrylamide gel electrophoresis. With ^{125}I-labeled ANP as ligand, the purified receptor bound a maximum of 5.70 nmol of ligand per mg of protein and the dissociation constant was 4.0×10^{-10} M. Upon treatment with 10 mM dithiothreitol, the purified receptor migrated as a single band at M_r 60,500 in $NaDodSO_4$/polyacrylamide gel electrophoresis. These findings show that the holoreceptor for ANP in vascular tissue is composed of two subunits of identical apparent molecular weight, presumably linked by a disulfide bridge(s).**

Atrial natriuretic peptide (ANP) is released from mammalian atria in response to volume expansion (1) and acts as an endocrine hormone by eliciting potent biological effects in a number of target organs such as the kidney (2), the adrenal (3), and the vasculature (4). Binding sites for ^{125}I-labeled ANP (^{125}I-ANP) have been identified in all these target organs by binding studies (5–10) and, more recently, by crosslinking and photoaffinity-labeling studies (11–17). These studies have tentatively identified two classes of ANP receptor proteins of M_r 60,000–70,000 and 120,000–180,000. Crosslinking studies, however, have generated conflicting data (e.g., see refs. 12, 14, and 15). Recently, a M_r 120,000 protein has been purified from rat lung with both constitutive guanylate cyclase and ANP-binding activity (18). This protein might be related to the larger ^{125}I-ANP-crosslinked species seen previously in kidney and adrenal tissues. The data, however, are not conclusive, since ANP binding is not stoichiometric (18). To directly address these issues, purification of ANP receptor molecules with near-stoichiometric ANP-binding properties is required.

In this report we describe the purification of the ANP receptor from cultured vascular cells to apparent homogeneity that fulfills these criteria. The purified receptor, M_r 125,000, is composed of two M_r 60,500 subunits.

MATERIALS AND METHODS

Peptide Synthesis. ANP analogs were synthesized by manual or automated solid-phase techniques as described (5, 20). ^{125}I-ANP was prepared and purified by HPLC as described (5). Angiotensin II, insulin, and glucagon were obtained from Sigma. γ-Melanotropin was purchased from Peninsula Laboratories (Belmont, CA).

Cultured Cells. Cultured bovine aortic smooth muscle (BASM) cells were derived from tissue explants as described (5, 19). Cultured cells were grown in Dulbecco's modified Eagle's medium supplemented with 15% calf serum in 850-cm^2 roller bottles. BASM cultures were used after 3–10 passages. No detectable differences in cell morphology or maximal ^{125}I-ANP binding parameters were detectable among cultures of these passage numbers.

Binding Studies with ^{125}I-ANP. Standard ^{125}I-ANP binding assays were performed as follows. To measure "total" ^{125}I-ANP receptor binding, various concentrations of ^{125}I-ANP (0.01–20 nM; specific activity 100 cpm/fmol of peptide) were added to one set of 12 × 75-mm glass tubes in 0.5 ml of binding buffer containing 100 mM Tris (pH 7.5), 100 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 2 mg of octaethyleneglycol dodecyl ether ($C_{12}E_8$; Calbiochem) per ml. For measurement of nonspecific binding, an identical set of tubes was prepared with the addition of 100-fold molar excesses of unlabeled ANP. Binding reactions were initiated by the addition of receptor protein (1 ng–200 μg) in 0.05 ml of binding buffer, and the tubes were incubated for 30 min at 21°C. The binding reactions were terminated by the addition of acetone (40%, vol/vol). These conditions reversibly precipitate ≥98% of the specific ^{125}I-ANP receptor binding activity. The tubes were centrifuged at 3000 × *g* for 10 min at 4°C and unbound ^{125}I-ANP was removed by aspiration. Radioactivity in the samples was measured with a model 5500 Beckman gamma counter (efficiency 88%). The amount (fmol) of ^{125}I-ANP specifically bound to receptor protein was calculated as the difference between total and nonspecific binding (cpm), divided by the specific activity of the ligand (100 cpm/fmol of ^{125}I-ANP). Analysis of the binding data was performed as described by Scatchard (21).

Association and dissociation experiments were performed as described (5, 22). Values for k_1 were calculated from the slopes of lines generated from plots of $\ln(B_{eq}/B_{eq} - B_t)$ vs. time, where B_{eq} and B_t are the amounts of ^{125}I-ANP bound at equilibrium and time t, respectively. Values for k_2 were calculated from the slopes of lines generated from plots of $\ln(B_t/B_{eq})$ vs. time.

Sedimentation Analyses. Metrizamide (Accurate Chemicals, Westbury, NY) gradients (3.5–14.0% wt/vol) in binding buffer (10 ml) were prepared in H_2O and ^2H_2O. Solubilized BASM membranes (1.0 ml, 2 mg of protein) were layered on top of the gradients. In parallel tubes, 2 mg each of bovine serum albumin (M_r 68,000; $s_{20,w}$ = 4.2), aldolase (M_r 158,000; $s_{20,w}$ = 7.7), and catalase (M_r 222,000; $s_{20,w}$ = 11.3) were dissolved in 1.0 ml of binding buffer and layered on top of the metrizamide gradients. The protein standards were obtained from Pharmacia. Samples were centrifuged at 80,000 × *g* for 20 hr at 4°C. After centrifugation, the tubes were punctured at the bottom and 0.3-ml fractions were collected. An ^{125}I-ANP binding assay or protein content assay (23) was performed on 0.05-ml aliquots containing either receptor protein or marker proteins, respectively.

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Abbreviations: ANP, atrial natriuretic peptide; ^{125}I-ANP, ^{125}I-labeled ANP; BASM, bovine aortic smooth muscle; $C_{12}E_8$, octaethyleneglycol dodecyl ether.

Purification of the ANP Receptor. *Preparation of cell membranes.* BASM cell monolayer cultures (10^{10} cells per purification; 100 roller bottles) were washed twice with wash buffer [phosphate-buffered saline (50 mM Na_2HPO_4/10 mM NaH_2PO_4/150 mM NaCl) containing 5.0 mM EDTA]. The third addition of wash buffer contained elastase (10 μg/ml; Sigma type IV) and collagenase (25 μg/ml; Sigma type VII). The roller bottles were incubated with the proteases for 10 min at 21°C with continuous rolling. Under these conditions, no loss of ^{125}I-ANP binding activity occurred (data not shown). The released cells were pooled and adjusted to 5% (vol/vol) calf serum. The cells were centrifuged at 5000 × *g* for 10 min at 4°C and resuspended in wash buffer (1 ml per 10^8 cells), and the centrifugation step was repeated. The cell pellet was resuspended in 30 ml of homogenization buffer [50 mM Tris·HCl, pH 7.5/0.25 M sucrose/100 mM NaCl/5 mM EDTA containing leupeptin (25 μg/ml; Sigma), aprotinin (25 μg/ml; Sigma), and phenylmethylsulfonyl fluoride (0.2 mM; Sigma)] and homogenized on ice with 10 strokes of a 40-ml ground-glass homogenizer (Wheaton). The disrupted cells were centrifuged at 100,000 × *g* for 30 min at 4°C. The membrane pellet was resuspended in 30 ml of homogenization buffer, and the homogenization and centrifugation steps were repeated. The final membrane pellet was resuspended and homogenized in 10 ml of homogenization buffer, and the protein concentration was determined. The membrane fraction was adjusted to 5.0 mg of protein per ml with homogenization buffer prior to solubilization.

Solubilization of the ANP receptor. Cell membranes were diluted 1:1 with wash buffer at 21°C. $C_{12}E_8$ (20 mg/ml in distilled H_2O) was added to the membranes over a 10-min period to a final concentration of 4 mg/ml. The $C_{12}E_8$-solubilized membranes were centrifuged for 1 hr at 100,000 × *g* and the supernatant was retained. The solubilized receptor was stored at 4°C or −70°C and used within 1 week for ANP affinity chromatography. ANP specific binding remained unchanged for ≥2 weeks at 4°C or ≥4 months at −70°C (data not shown).

ANP-agarose affinity chromatography. Both CNBr-activated Sepharose 4B (Pharmacia) and Affi-Gel 10 (Bio-Rad) were used as matrices for ANP. Affi-Gel 10 gave better yields of receptor and was used to obtain the results reported here. The agarose (2 g) was rinsed with 100 volumes of 1 mM HCl and incubated for 40 hr at 4°C in 0.1 M $NaHCO_3$ (pH 8.3) containing 40 mg of pro-ANP-(102–126)-peptide (residues 4–28 of human ANP). The agarose was rinsed exhaustively with alternate washes of 0.1 M sodium acetate (pH 4.0) and 0.1 M $NaHCO_3$ (pH 8.3). The final wash was with binding buffer. The ANP-agarose column was found to be unstable and therefore was prepared fresh and discarded after each use. The solubilized receptor preparation was adjusted to 10 mM $CaCl_2$ and 10 mM $MgCl_2$ and filtered (Millipore type GS, 0.2-μm pore size) prior to affinity chromatography. The filtered preparation was chromatographed at a flow rate of 20 ml/hr on the ANP-agarose. The affinity matrix was washed with binding buffer until the A_{280} = 0.000. Elution buffer (0.1 M sodium acetate, pH 5.0/100 mM NaCl/10 mM $CaCl_2$/10 mM $MgCl_2$ containing 2 mg of $C_{12}E_8$ per ml) was added to the ANP-agarose to release bound receptor. The eluate was placed on ice, adjusted to 40% (vol/vol) acetone, and then centrifuged at 3000 × *g* for 10 min at 4°C. The supernatant was aspirated, and the purified receptor was resuspended in 0.5 ml of binding buffer.

Gel Electrophoresis. $NaDodSO_4$/PAGE was performed as described by Laemmli (24). After electrophoresis, protein was visualized with either Coomassie blue or silver (25).

RESULTS

Characterization of the Solubilized ANP Receptor. A number of detergents were used in an attempt to solubilize specific ^{125}I-ANP-binding activity from the membrane preparations. Of the 12 nonionic detergents tested, only $C_{12}E_8$ solubilized >20% of the original ANP-binding activity in a highly stable form. The solubilized activity was characterized with regard to its ^{125}I-ANP-binding parameters (Fig. 1). The binding of ^{125}I-ANP was concentration-dependent and saturable. Assays at higher concentrations of ^{125}I-ANP (3–12 nM) showed that binding was fully saturable (data not shown). Scatchard analysis (Fig. 1 *Inset*) of the data gave a linear plot, suggesting a single class of binding sites with maximal binding (B_{max}) = 3.5 pmol of ^{125}I-ANP per mg of protein and dissociation constant (K_d) = 1.77 × 10^{-10} M.

To obtain an estimate of the molecular weight of the native ANP receptor, sedimentation analysis of the solubilized ANP receptor in metrizamide gradients was performed (Fig. 2*A*). A single peak of binding activity corresponding to $s_{20,w}$ = 5.6 is evident in the gradient. This value can be used to estimate the molecular weight of the ANP receptor–detergent complex according to the equation (26)

$$M_r = \frac{6\pi N\eta}{1 - \nu\rho}\,\alpha s_{20,w},$$

where N is Avogadro's number, η is the viscosity of water at 20°C, ρ is the density of water at 20°C, ν is the partial specific volume, α is the Stokes radius, and $s_{20,w}$ is the sedimentation coefficient. In separate experiments, the Stokes radius of the ANP receptor complex was found to be 56 ± 3 Å by agarose (Bio-Gel A-1.5m) gel filtration. The value for ν can be estimated by the relative rates of sedimentation of the ANP receptor in H_2O and ^2H_2O (27). Fig. 2*B* shows that in ^2H_2O the ANP receptor has $s_{20,w}$ = 5.2. Thus, for the ANP receptor–detergent complex, ν = (5.2/5.6) × 0.72 = 0.67 (see ref. 27). These values give an estimate of 112,000 ± 13,000 for the molecular weight of the ANP holoreceptor–$C_{12}E_8$ complex.

Purification of the ANP Receptor by Affinity Chromatography. Prior to affinity chromatography, the rates of association and dissociation for ^{125}I-ANP and the solubilized ANP receptor from BASM cells were investigated to identify appropriate purification conditions. Table 1 shows that maximal association rates (2-fold greater than control) required the presence of $C_{12}E_8$ and divalent cations and neutral pH

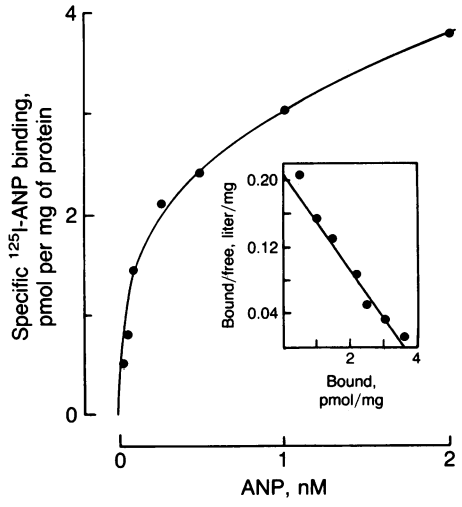

FIG. 1. Concentration-dependent binding of ^{125}I-ANP to solubilized BASM cell membranes. Solubilized membranes (20 μg of protein per tube) were incubated with the indicated concentrations of ^{125}I-ANP as described in *Materials and Methods*. (*Inset*) Scatchard analysis. Binding parameters obtained from the negative inverse of the slope and the *x*-intercept, respectively, are K_d = 1.77 × 10^{-10} M and B_{max} = 3.5 pmol per mg of protein. Each data point represents the mean of triplicate determinations.

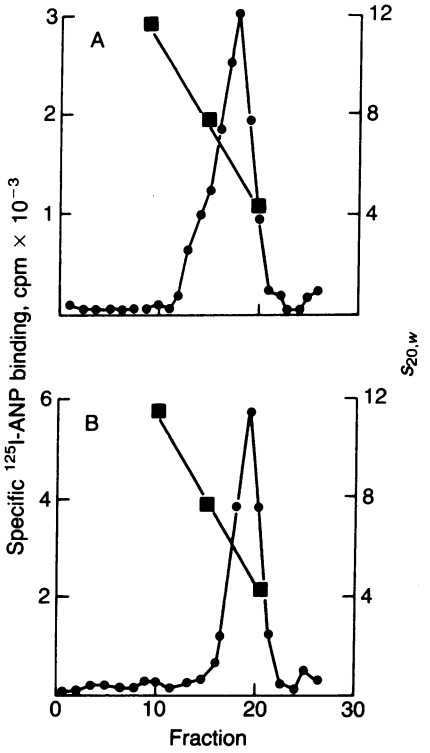

FIG. 2. Metrizamide density gradient analysis of crude solubilized ANP receptor. Solubilized ANP receptor and marker proteins were layered onto separate linear gradients of 3.5–14.0% (wt/vol) metrizamide in either H_2O (*A*) or ^2H_2O (*B*). Samples were centrifuged at 80,000 × *g* for 20 hr at 4°C. The tubes were fractionated from the bottom and assayed for either ^{125}I-ANP-binding activity (●) or protein content (23) as described in *Materials and Methods*. The locations of marker proteins, as determined by protein assays, were plotted according to their $s_{20,w}$ values (■, from upper left to lower right in *A* and *B*, catalase, aldolase, and albumin, respectively). Each data point represents the mean of triplicate determinations (±12% standard error in all cases).

(7.5). The requirement for divalent cations was apparent only in the presence of $C_{12}E_8$. In the absence of detergent, their presence had little if any effect on the association rate (Table 1). Two conditions abolished the association of ^{125}I-ANP with its receptor—dithiothreitol and low pH (5.5). The association rate was only marginally affected at pH 6.5 and 8.5 (83% and 78% of control association rates, respectively). Dissociation studies, however, showed that the only condition that increased the dissociation rate was pH 5.5 buffer (Table 1). Dithiothreitol (10 mM) had no effect on the dissociation of the ligand–receptor complex (Table 1).

Table 1. Effects of various conditions on the rates of association and dissociation of ^{125}I-ANP and solubilized ANP receptor

Condition*	Rate constant,† % control	
	Association	Dissociation
Control	100	100
EDTA	103	102
$MgCl_2$	94	98
$CaCl_2$	115	83
$C_{12}E_8$ + EDTA	0	102
$C_{12}E_8$ + $MgCl_2$ + $CaCl_2$	200	91
Dithiothreitol	0	104
pH 6.5	83	108
pH 5.5	0	>1000
pH 8.5	78	120

*All compounds listed were used at a concentration of 10 mM. The buffer used for these determinations was 100 mM Tris·HCl (pH 7.5), except where pH was varied. Buffers with pH values other than 7.5 were prepared using appropriate ratios of 100 mM Na_2HPO_4 and 100 mM NaH_2PO_4.

†Association (k_1) and dissociation (k_2) rate constants were measured and calculated as described in *Materials and Methods*. Control values (100%) were $k_1 = 8.2 \times 10^5$ liter·M^{-1}·sec^{-1} and $k_2 = 6.4 \times 10^{-5}$ liter·sec^{-1}. All values are the mean of linear regression curves with standard error ≤17% in all cases.

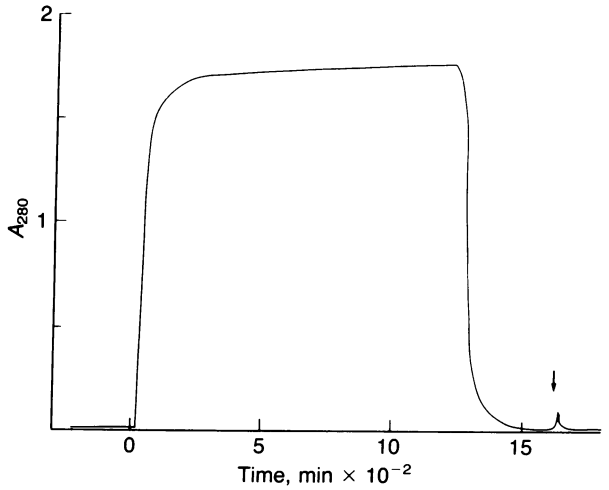

FIG. 3. Purification of the ANP receptor by affinity chromatography. Solubilized membranes were supplemented with 10 mM $CaCl_2$ and 10 mM $MgCl_2$ and chromatographed on ANP-Affi-Gel 10. The solubilized ANP receptor was applied to the column, followed by wash buffer until A_{280} = 0.0; then 6.0 ml of elution buffer was added (arrow). The eluate was acetone-precipitated and analyzed by ^{125}I-ANP binding assay and $NaDodSO_4$/PAGE.

Based on the association and dissociation results, affinity chromatography of the solubilized receptor on ANP-agarose was investigated. Solubilized ANP receptor was supplemented with 10 mM $CaCl_2$ and 10 mM $MgCl_2$ (to optimize the association rate) and chromatographed on ANP-agarose (Fig. 3). Pooled fractions containing the eluted material were analyzed by $NaDodSO_4$/PAGE under nonreducing and reducing conditions. Under nonreducing conditions, a single protein of M_r 125,000 was visible after staining with silver (Fig. 4, lane 1) or Coomassie blue (data not shown). Incubation of the sample with 10 mM dithiothreitol prior to electrophoresis resulted in the appearance of a single protein of M_r 60,500 after electrophoresis and staining (Fig. 4, lane 2).

Specific binding of ^{125}I-ANP to the purified receptor was concentration-dependent and saturable (Fig. 5*A*). This find-

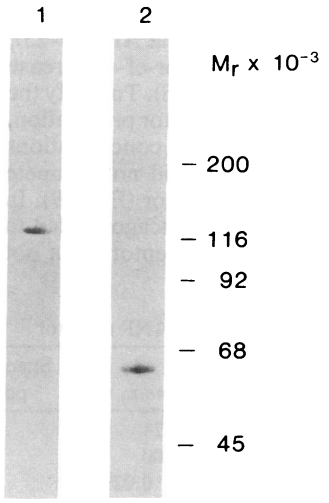

FIG. 4. $NaDodSO_4$/PAGE of purified ANP receptor. Material eluted from the affinity column was analyzed by $NaDodSO_4$/PAGE. Before electrophoresis, purified ANP receptor (1 μg) was suspended in 30 μl of sample buffer containing either no dithiothreitol (lane 1) or 10 mM dithiothreitol (lane 2). Proteins were visualized by silver staining. The migration positions of standard proteins are shown at right.

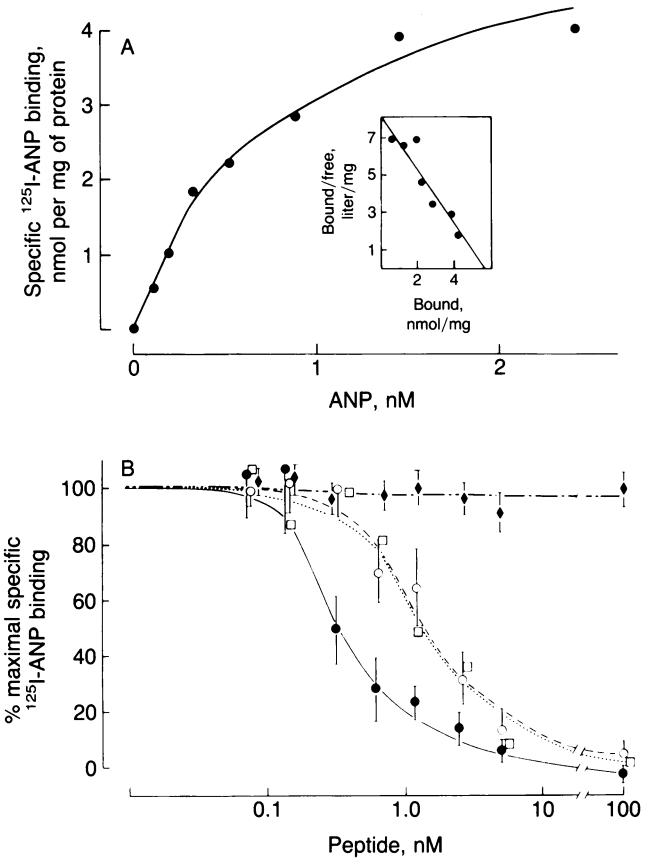

FIG. 5. (*A*) Concentration-dependent binding of ^{125}I-ANP to purified ANP receptor. Purified receptor (10 ng per tube) was incubated with various concentrations of ^{125}I-ANP. The binding parameters obtained by Scatchard analysis (*Inset*) were $K_d = 7.0 \times 10^{-10}$ M and $B_{max} = 5.7$ nmol per mg of protein. Each data point represents the mean of triplicate determinations. (*B*) Inhibition of ^{125}I-ANP binding to purified ANP receptor by various ANP analogs. Purified ANP receptor (5 ng per tube) was incubated with 10^{-10} M ^{125}I-ANP in the presence of the indicated concentrations of human ANP-(4–28) (●), human ANP-(7–28) (○), or rat ANP-(5–25) (□). As a control, γ-melanotropin was also tested (◆). Maximal (100%) binding (at 10^{-10} M ^{125}I-ANP) was 17,100 ± 2000 cpm (mean ± SEM).

ing extends to the highest concentration of ^{125}I-ANP tested, 20 nM (data not shown). Scatchard analysis (Fig. 5*A Inset*) of the data suggests a single class of binding sites ($K_d = 7.0 \times 10^{-10}$ M) with maximal binding (B_{max}) of 5.7 nmol per mg of protein. The binding specificity of the ANP receptor was investigated by competition studies (Fig. 5*B*). Human ANP-(4–28)-peptide had an apparent $K_i = 3.2 \times 10^{-10}$ M. Two other ANP analogs, human ANP-(7–28) and rat ANP-(5–25), had $K_i = 1.2 \times 10^{-9}$ M, thus showing a factor-of-4 decrease in affinity relative to that of human ANP-(4–28). To verify the specificity of binding of ^{125}I-ANP to the receptor preparation, γ-melanotropin was used as competitor. At all concentrations examined (0.01–100 nM), γ-melanotropin did not compete with ^{125}I-ANP for binding to the ANP receptor (Fig. 5*B*). In addition, 1 μM angiotensin II, insulin, or glucagon failed to inhibit ^{125}I-ANP binding to the purified receptor (data not shown).

A summary of the purification fractions and their binding parameters is given in Table 2. Relative to the initial cell membrane preparation, the ANP receptor was purified 2700-fold with a recovery of 11% of the original binding activity. The ratio of moles of ANP bound per mol of purified ANP receptor (M_r 125,000 form) is 0.7:1.0.

DISCUSSION

Based on a knowledge of the association and dissociation kinetics of ^{125}I-ANP for its receptor, affinity chromatography was used to purify the ANP receptor from vascular smooth muscle cells 2700-fold, to apparent homogeneity.

The binding properties of the crude solubilized ANP receptor ($K_d = 1.77 \times 10^{-10}$ M, $B_{max} = 3.5$ pmol per mg of protein, Fig. 1) are remarkably similar to the binding properties of intact BASM cells (5–7). Further characterization of the solubilized ANP receptor by centrifugation in metrizamide gradients suggested the presence of only a single ^{125}I-ANP-binding species, with M_r of 112,000 ± 13,000. These results are similar, though not identical, to those previously reported for the solubilized bovine adrenal cortex ANP receptor (12), whose apparent M_r (as estimated by size-exclusion chromatography) is 140,000.

To fully optimize the affinity-chromatography purification step, the ^{125}I-ANP association and dissociation kinetics of the ANP receptor were investigated. Association rate experiments identified several unusual properties of the solubilized ANP receptor (Table 1). In the absence of $C_{12}E_8$ (i.e., ≤0.005% wt/vol), no divalent cation dependence of ^{125}I-ANP binding was evident. In the presence of $C_{12}E_8$, however, there is an absolute requirement for divalent cations. A requirement for Ca^{2+} has also been observed for ligand binding to the low density lipoprotein and insulin receptors (28, 29). Unlike the various requirements for increased association rate, only low pH (5.5) caused a rapid dissociation of the receptor–ANP complex (Table 1). The simplest explanation for these results is that upon ANP binding to the receptor, a thermodynamically favored conformational state occurs in the receptor that renders it insensitive to the effects of $C_{12}E_8$, divalent cations, or reducing agents. Further experiments (data not shown) showed that pH 5.0 was optimal for complete and reversible dissociation of the ligand–receptor complex. Thus, the results of the kinetic experiments clearly defined conditions appropriate for affinity chromatography. It is interesting that successful purifications of the insulin receptor (29) and the lutropin/human chorionic gonadotropin receptor (30) utilized a similar low-pH elution step.

Results of NaDodSO$_4$/PAGE suggest that the purified ANP receptor consists of a homogeneous population of M_r 60,500 subunits that form disulfide-bridged dimeric structures in their native, nonreduced state. This interpretation is supported by both nonreducing NaDodSO$_4$/PAGE (Fig. 4, lane 1) and metrizamide gradient analysis (Fig. 2), which provide evidence for a M_r 112,000 ± 13,000 active ANP receptor. Further, the finding that the affinity and specificity of the ANP receptor for ^{125}I-ANP and various ANP analogs (Fig. 5*A*) are equivalent to those of intact tissue and cells (5) demonstrates that the purified M_r 125,000 protein alone is sufficient to bind ^{125}I-ANP. The calculated stoichiometry of mol of ANP bound per mol of ANP receptor [5.7 nmol/8.0

Table 2. Purification of the ANP receptor

Fraction	Protein, mg	Specific activity, pmol per mg of protein	Purification factor	% recovery
Membranes	724	2.1	1.0	100
Solubilized membranes	300	3.5	1.7	69
ANP-agarose eluate	0.029	5700	2715	11

nmol (= 1 mg of a M_r 125,000 protein)] is 0.70 (Table 2). This finding essentially excludes the possibility that a contaminant protein is responsible for the ANP-binding activity, since it would have to represent a majority of the mass of the purified receptor preparation yet be undetectable by silver or Coomassie blue staining.

In all tissues examined, ^{125}I-ANP crosslinking or photoaffinity-labeling studies have identified proteins of M_r 60,000–70,000 and/or 120,000–180,000 (9–18). Data presented in this report identify the M_r 60,000–70,000 species as the ANP receptor. The identity of the M_r 120,000–180,000 species remains unclear. Recently, Kuno *et al.* (18) reported characterization of a purified M_r 120,000 protein with ANP-binding properties and constitutive guanylate cyclase activity. However, Hirose and coworkers (31), working with bovine lung, have demonstrated apparent separation of these two activities by chromatography on ANP-agarose. We have found that BASM cells might have two subpopulations of ANP binding sites because, although they bind human ANP-(4–28) and show an increase in cGMP, they also bind several truncated ANP analogues that compete with ^{125}I-labeled human ANP-(4–28) for binding but not for stimulation of guanylate cyclase (32). These findings suggest the predominant form of the ANP receptor in BASM cells is not coupled to guanylate cyclase. Consistent with this possibility, and perhaps the findings of Hirose and coworkers, the receptor we have isolated has no guanylate cyclase activity when assayed in its purified form (data not shown). These findings suggest that there might be a single receptor that, depending on the presence of guanylate cyclase, exhibits different binding specificities; alternatively, there might be multiple ANP receptors.

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1. Lang, R. E., Tholken, H., Ganten, D., Luft, F. C., Ruskoaho, H. & Unger, T. (1985) *Nature (London)* **314,** 264–266.
2. Atlas, S. A., Kleinert, H. D., Camargo, M. S., Januszewicz, A., Sealey, J. E., Laragh, J. H., Schilling, J. W., Lewicki, J. A., Johnson, L. K. & Maack, T. (1984) *Nature (London)* **309,** 717–719.
3. Kudo, T. & Baird, A. (1984) *Nature (London)* **312,** 756–757.
4. Currie, M. G., Geller, D. M., Cole, B. R., Boylon, J. G., Wu, Y. S., Holmberg, S. W. & Needleman, P. (1983) *Science* **221,** 71–73.
5. Schenk, D. B., Johnson, L. K., Schwartz, K., Sista, H., Scarborough, R. M. & Lewicki, J. A. (1985) *Biochem. Biophys. Res. Commun.* **127,** 433–442.
6. Hirata, Y., Tomita, M., Yoshimi, H. & Ikeda, L. (1984) *Biochem. Biophys. Res. Commun.* **125,** 562–568.
7. Hirata, Y., Tomita, M., Takada, S. & Yoshimi, H. (1985) *Biochem. Biophys. Res. Commun.* **128,** 538–546.
8. Napier, M. A., Vandlen, R. L., Albers-Schonberg, G., Nutt, R. F., Brady, S., Lyle, T., Winquist, R., Faison, E. P., Heinel, L. A. & Blaine, E. H. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5946–5950.
9. Inui, K., Saito, H., Matsukawa, Y., Nakao, K., Morii, N., Imura, H., Shimokura, M., Kiso, Y. & Hori, R. (1985) *Biochem. Biophys. Res. Commun.* **132,** 253–260.
10. De Lean, A., Gutowska, J., McNicoll, N., Schiller, P. W., Cantin, M. & Genest, J. (1984) *Life Sci.* **35,** 2311–2318.
11. Schenk, D. B., Phelps, M., Porter, J. G., Scarborough, R. M., McEnroe, G. A. & Lewicki, J. (1985) *J. Biol. Chem.* **260,** 14887–14890.
12. Hirose, S., Akiyama, F., Shinjo, M., Ohno, H. & Murakami, K. (1985) *Biochem. Biophys. Res. Commun.* **130,** 574–579.
13. Vandlen, K. L., Arcuri, K. E. & Napier, M. A. (1985) *J. Biol. Chem.* **260,** 10889–10922.
14. Misono, K. S., Grammer, R. T., Rigby, J. W. & Inigami, T. (1985) *Biochem. Biophys. Res. Commun.* **130,** 994–1001.
15. Meloche, S., Ong, H., Cantin, M. & De Lean, A. (1986) *J. Biol. Chem.* **261,** 1525–1528.
16. Yip, C. C., Liang, L. P. & Flynn, T. G. (1985) *J. Biol. Chem.* **260,** 8229–8232.
17. Koseki, C., Hayashi, Y., Ohnuma, N. & Imai, M. (1986) *Biochem. Biophys. Res. Commun.* **136,** 200–207.
18. Kuno, T., Andresen, J., Kamisaki, Y., Waldman, S., Chang, L. Y., Saheki, S., Leitman, D. C., Nakane, M. & Murad, F. (1986) *J. Biol. Chem.* **261,** 5817–5823.
19. Longenecker, J., Kilty, L. A. & Johnson, L. K. (1982) *J. Cell. Physiol.* **113,** 197–202.
20. Merrifield, R. B. (1964) *Biochemistry* **3,** 1385–1390.
21. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51,** 660–672.
22. Schenk, D. B., Hubert, J. J. & Leffert, H. L. (1984) *J. Biol. Chem.* **259,** 14941–14951.
23. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Morrissey, J. H. (1981) *Anal. Biochem.* **117,** 307–310.
26. Clarke, S. (1975) *J. Biol. Chem.* **250,** 5459–5469.
27. Edsall, J. T. (1953) in *The Proteins*, eds. Neurath, H. & Bailey, K. (Academic, New York), Vol. 1, pp. 549–726.
28. Schneider, W. J., Goldstein, J. L. & Brown, M. S. (1980) *J. Biol. Chem.* **255,** 11442–11447.
29. Petruzzelli, L., Herrera, R. & Rosen, O. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3327–3331.
30. Wimalasena, J., Moore, P., Wiebe, J. P., Abel, J. & Chen, T. T. (1985) *J. Biol. Chem.* **260,** 10689–10697.
31. Ishido, M., Fujita, T., Hagiwara, H., Shimonaka, M., Saheki, T., Hirata, Y. & Hirose, S. (1986) *Biochem. Biophys. Res. Commun.* **140,** 101–106.
32. Scarborough, R. M., Schenk, D. B., McEnroe, G. A., Arfsten, A., Kang, L.-L., Schwartz, K. & Lewicki, J. A. (1986) *J. Biol. Chem.* **261,** 12960–12964.