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Brain natriuretic peptide interacts with atrial natriuretic peptide receptor in cultured rat vascular smooth muscle cells

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The effect of synthetic porcine brain natriuretic peptide (pBNP), a novel brain peptide with sequence homology to α-human atrial natriuretic peptide (hANP), on receptor binding and cGMP generation, was studied in cultured rat vascular smooth muscle cells (VSMC) and compared with that of α-hANP. ^{125}I-pBNP bound to the cells in a time-dependent manner similar to that of ^{125}I-α-hANP. Scatchard analysis indicated a single class of binding sites for pBNP with affinity and capacity identical to those of α-hANP. pBNP and α-hANP were almost equipotent in inhibiting the binding of either radioligand and stimulating intracellular cGMP generation. These data indicate that BNP and ANP interact with the same receptor sites to activate guanylate cyclase in rat VSMC.

cyclic GMP; Brain natriuretic peptide; Atrial natriuretic peptide; Receptor binding; (Vascular smooth muscle cell)

1. INTRODUCTION

Atrial natriuretic peptide (ANP) is a potent diuretic and vasodilatory peptide hormone originally isolated from the mammalian heart [1,2] and subsequently from the brain [3]. A novel natriuretic peptide has recently been identified in the porcine (p) brain, designated as brain natriuretic peptide (BNP) [4]. pBNP comprises 26 amino acid residues with a remarkable homology to, but definitely distinct from, the known sequence of ANPs. pBNP induces diuretic-natriuretic and hypotensive effects in rats and chick rectum relaxant activity in a manner similar to those of α-human (h) ANP. Therefore, it is suggested that BNP may function in concert with ANP to regulate the homeostatic balance of body fluid and blood pressure through a dual mechanism [4].

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However, whether BNP acts on the target cells via the mechanism identical to or distinct from that of ANP remains obscure. To address this intriguing issue, we have studied the effect of synthetic pBNP on receptor binding and cGMP generation in cultured rat vascular smooth muscle cells (VSMC) which have abundant ANP receptors functionally coupled to the guanylate cyclase system [5] and compared its effect with that of α-hANP.

2. MATERIALS AND METHODS

2.1. *Materials*

pBNP synthesized by means of a solution method possesses diuretic-natriuretic and hypotensive effects comparable to those of natural pBNP [4]. Synthetic α-hANP was obtained from the Peptide Institute (Osaka). Iodination of pBNP and α-hANP was performed by the lactoperoxidase method [5] and resulted in activities of ~100 and ~150 μCi/μg, respectively.

2.2. *Binding experiment*

VSMCs isolated from aorta of adult Wistar rats were subcultured (10–15th passages) and used in binding experiments as reported [5]. Briefly, confluent (2×10^5) cells were usually in-

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cubated at 24°C for 60 min with ^{125}I-pBNP or ^{125}I-α-hANP in Hanks' balanced salt solution (pH 7.4), containing 0.1% bovine serum albumin essentially according to [5]. Specific binding was determined by subtracting nonspecific binding in the presence of excess (10^{-6} M) unlabeled pBNP or α-hANP from total binding.

2.3. *Down-regulation of ANP receptors*

ANP receptors were down-regulated by incubating VSMC (10^5 cells) with 10^{-8} M pBNP or α-hANP in serum-free medium at 37°C for 24 h. After incubation, cells were washed extensively with ice-cold medium to remove unbound ligand and reincubated in fresh medium at 37°C for 2 h to permit degradation of the bound ligand as described [6]. Cells were then subjected to saturable binding studies using ^{125}I-α-hANP.

2.4. *Determination of intracellular cGMP*

VSMCs were incubated with or without pBNP or α-hANP at 37°C for 10 min in the presence of 0.5 mM methylisobutylxanthine and the content of intracellular cGMP determined by radioimmunoassay (RIA) as in [5,6].

3. RESULTS

The time courses for binding of ^{125}I-pBNP and ^{125}I-α-hANP to rat VSMC at 24°C were essentially similar, attaining apparent equilibrium after 60 min; nonspecific binding of ^{125}I-pBNP and ^{125}I-α-hANP was 10–15% of total binding (not shown). Equilibrium binding of ^{125}I-pBNP and ^{125}I-α-hANP was a saturable process (fig.1). Scatchard plots (fig.1, inset) revealed the presence of a single class of binding sites for both pBNP and α-hANP: their apparent dissociation constants (K_d 2.5×10^{-10} M) and maximal binding capacities (B_{max} 70000 sites/cell) were equal. Competitive binding of ^{125}I-α-hANP (fig.2A) and ^{125}I-pBNP (fig.2B) by unlabeled α-hANP and pBNP was studied; whereas hANP was about 2-fold more potent than pBNP in displacing the binding of ^{125}I-

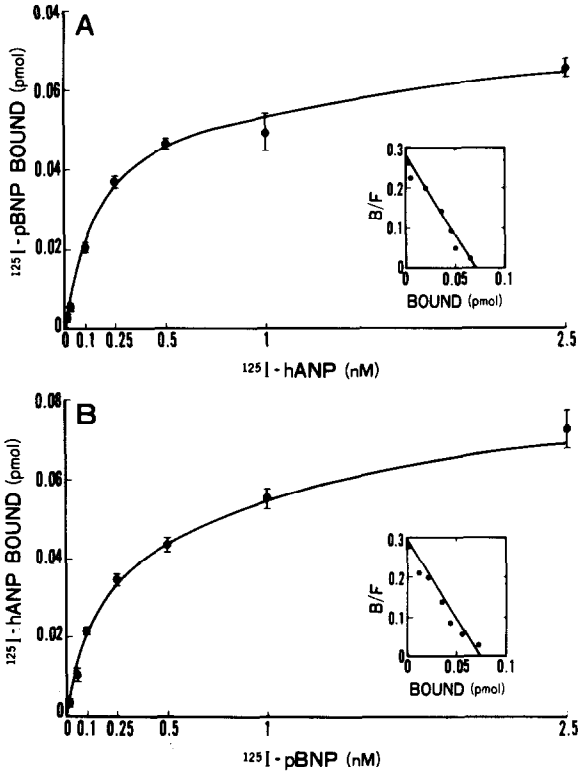

Fig.1. Saturable binding of ^{125}I-α-hANP and ^{125}I-pBNP to cultured rat VSMC. Confluent (2×10^5) cells were incubated with various doses of ^{125}I-α-hANP (A) and ^{125}I-pBNP (B) at 24°C for 60 min. Specific binding of ^{125}I-α-hANP and ^{125}I-pBNP was 85–90 and 90–94% of total binding, respectively. Each point is the mean of three experiments; bar shows SE (inset) Scatchard plot of binding data.

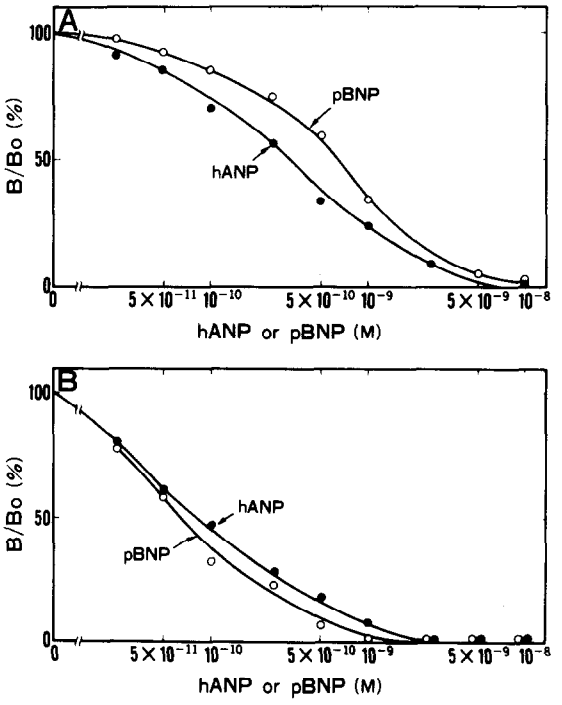

Fig.2. Competitive binding of ^{125}I-α-hANP and ^{125}I-pBNP to cultured rat VSMC. Confluent (2×10^5) cells were incubated at 24°C for 60 min with 1 nM ^{125}I-α-hANP (A) and ^{125}I-pBNP (B) in the absence and presence of various doses of α-hANP (●) and pBNP (○). Results are expressed as the percentage of specific binding in the absence of peptides (B_0); each point is the mean of duplicate dishes. Specific binding of ^{125}I-α-hANP and ^{125}I-pBNP was 81 and 82%, respectively.

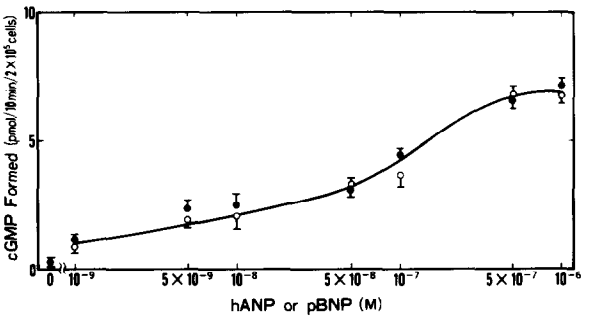

Fig.3. Effects of α-hANP and pBNP on formation of intracellular cGMP in cultured rat VSMCs. Confluent (2×10^5) cells were incubated at 37°C for 10 min in the absence (⊙) and presence of various doses of α-hANP (●) and pBNP (○). Concentrations of intracellular cGMP were determined by RIA. Each point is the mean of triplicate dishes: bar shows SE.

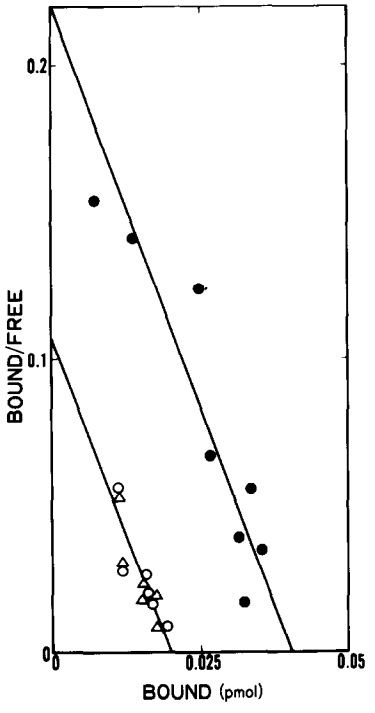

Fig.4. Scatchard plots of saturable binding of ^{125}I-α-hANP to cultured rat VSMC after ANP receptor down-regulation. VSMCs (10^5 cells) were preincubated at 37°C for 24 h in the absence (●) and presence of 10^{-8} M unlabeled α-hANP (○) or pBNP (△), after which the cells were subjected to binding experiments as described in section 2. Each point is the mean of two experiments.

α-hANP from its receptor sites, hANP and pBNP equally inhibited the binding of ^{125}I-pBNP.

As shown in fig.3, pBNP and α-hANP similarly stimulated cGMP formation with an approximate ED_{50} of 5×10^{-8} M. No additive effect was induced on simultaneous addition of pBNP and α-hANP at maximal doses (not shown).

Pretreatment with either pBNP or α-hANP at an equimolar concentration (10^{-8} M) induced a marked and similar reduction in B_{max} (40000 sites/cell) without changing its affinity (K_d 2×10^{-10} M) compared to B_{max} (80000 sites/cell) in untreated cells (fig.4).

4. DISCUSSION

BNP, a novel natriuretic peptide comprising 26 amino acid residues isolated from porcine brain, shows a remarkable homology to, but is distinct from, α-hANP, and possesses biological activity comparable to that of α-hANP as determined from the in vivo diuretic-natriuretic and hypotensive effects in rats [4].

Using radioligand-binding experiments, the present study clearly demonstrates that synthetic pBNP binds to ANP receptors in cultured rat VSMC essentially in the manner as α-hANP; Scatchard analysis of binding data shows that the binding capacity and affinity of BNP appear to be identical to those of ANP, suggesting that BNP and ANP interact with the same binding sites. Our results that unlabeled pBNP and α-hANP are almost equipotent in competing with either radioligand to its binding sites are also compatible with this notion.

We have previously shown that cultured rat VSMCs have ANP receptors functionally coupled to guanylate cyclase [5,6]. The present study also shows that pBNP is equipotent to hANP in stimulating cGMP formation in rat VSMC. The ring structure formed by the intramolecular disulfide linkage in the ANP molecule that is essential for its biological activity [7] as well as for stimulating guanylate cyclase [6] is highly conserved in the BNP molecule, although four out of 17 amino acid residues in this region are replaced [4]. Therefore, it seems most likely that BNP-induced cGMP formation is mediated through the same receptors as for ANP.

Previously, we have demonstrated that vascular ANP receptors are under the control of a homologous down-regulation mechanism [6]. The present result that long-term exposure of pBNP leads to a substantial reduction in total vascular ANP receptor number without changing its affinity in a similar fashion to that of α-hANP also lends further support to the contention that BNP and ANP interact with the same receptor sites to induce homologous receptor down-regulation.

The questions as to whether BNP acts on the unique receptor distinct from ANP receptors in other target tissues including the central nervous system, and whether BNP circulates in the blood to act in harmony with ANP remain to be clarified.

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