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SPECIFIC RECEPTORS FOR ATRIOPEPTIN III IN RABBIT LUNG

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Binding studies revealed the presence of a single class of high affinity binding sites for atriopeptin III on rabbit lung membranes. An apparent dissociation constant (Kd) of 0.32 nM and a binding capacity of 166 fmol/mg protein was determined. Binding was time-dependent and saturable. The relative binding affinities of atrial peptide analogs correlated well with their potencies in eliciting relaxation of norepinephrine-contracted rabbit aorta strips. Unrelated peptide hormones did not compete for the atriopeptin binding site on rabbit lung membranes. The atrial peptide binding data are similar to those obtained for other tissues and indicate the presence of a physiologically relevant atrial peptide receptor in lung.

Heart atria contain specific granules from which atrial natriuretic factors are derived (1). These peptides have been shown to elicit natriuresis, diuresis, vasorelaxation and to inhibit aldosterone secretion (2-5). Specific receptors for atrial peptides have been located in the kidney, adrenal cortex, and aortic smooth muscle and endothelial cells (6-8). We have screened a number of different tissues and found that the lung had a high capacity for binding ^{125}I-labelled atriopeptin (AP) III . In addition, Waldman et al. (9) have observed activation of guanylate cyclase by atrial natriuretic factor in the homogenates of a number of tissues including lung. O'Donnell and coworkers (10) obtained data from studies on the relaxation of guinea pig airway and vascular smooth muscle which indicated that atrial peptides may be important in the regulation of pulmonary vascular tone.

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Abbreviations: Nomenclature for atriopeptins (AP) is as described by Katsube et el. (18).

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We wish to report our data from binding studies which have led us to conclude that rabbit lung contains a single class of high affinity receptors for atrial peptides.

MATERIALS AND METHODS

Bradykinin, arginine-vasopressin, angiotensin I and angiotensin II were purchased from Sigma Chemical Co., St. Louis, MO.

Radioiodination of Atriopeptin (AP) III. Synthetic APIII (H_2N-Ser^1-Ser-Cys-Phe-Gly-Gly -Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr^{24}-COOH) was purchased from Peninsula Labs (Belmont, Ca), and was iodinated by the method of Markwell (11) with modifications. Briefly, AP III (15 ug in 0.015 ml deionized water) was incubated for 30 s at room temperature with 0.01 ml ^{125}INa (1 mCi) in 0.025 ml 0.5 M phosphate buffer pH 7.6 with one iodobead (Pierce Chemical Co., Rockford, IL). The reaction was terminated by removing the iodobead. The monoiodinated AP III was purified by reverse-phase high performance liquid chromatography using an acetonitrile/water gradient. The radiolabelled peptide was stored at -20°C and was stable for up to 4 weeks.

Preparation of rabbit lung membranes. The procedure was carried out at 4°C. Frozen rabbit lung lobes (Pel-Freez, Rogers, AR) were minced and then homogenized for 30 s with a Brinkmann Polytron in 5 volumes of a solution containing 0.25 M sucrose, 3 mM $MgCl_2$, 1 mM EDTA and 5 mM Tris pH 7.5. The homogenate was filtered through cheesecloth to remove some fat and connective tissue and the filtrate was centrifuged at 5,000 x g for 20 min. The supernatant was centrifuged at 100,000 x g for 90 min and the membrane pellet was collected. The pellet was resuspended in 50 mM Tris pH 7.5 and the suspension was recentrifuged at 100,000 x g for 90 min. The washed pellet was suspended in 50 mM Tris pH 7.5 at a final concentration of 4 mg/ml. The Biorad protein assay kit was used to assay membrane protein. The rabbit lung membrane preparation was stored in aliquots at -80°C and was stable for at least 2 months.

^{125}I-AP III Binding Assay. The binding assay consisted of 0.25 ml of a solution containing 50 mM Tris pH 7.5, 0.1 % BSA, 100 ug rabbit lung membranes and ^{125}I-AP III (2-3 x 10^4 cpm; specific activity of ca. 430 Ci/mmol) in the absence or presence of unlabelled AP III. The reaction was started by the addition of membranes and the mixture was incubated at 25°C for 30 min. The incubation was terminated with 4 ml ice-cold 50 mM Tris pH 7.5 and the mixture was filtered to separate membrane-bound labelled peptide from free ligand using a Skatron (Sterling, VA) filtration device. The incubation tube and filter were washed twice with an additional 4 ml of cold buffer. Filters were assayed for radioactivity in a Beckman gamma counter. Specific binding was defined as total binding minus nonspecific binding in the presence of 10^{-6} M unlabelled AP III.

Atrial peptide bioassay. Atrial peptides were assayed for vasorelaxant activity using norepinephrine-contracted rabbit thoracic aortic strips (3).

RESULTS

Our studies showed that the binding of ^{125}I-AP III to rabbit lung membranes was specific and constituted more than 80% of total binding. Fig. 1

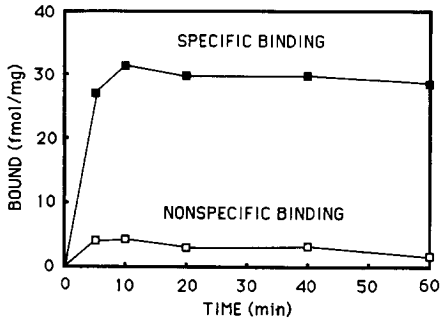

Fig. 1. Binding kinetics of ^{125}I-AP III to rabbit lung membranes. The radiolabelled peptide was incubated at a concentration of 135 pM. Specific binding (■) was defined as total binding minus nonspecific binding (□) in the presence of 1 uM unlabelled AP III. Each data point is the mean of two determinations.

shows that binding was rapid and reached an apparent equilibrium by 20 min. ^{125}I-AP III binding was linear in the range of 5 - 100 ug membrane protein. Equilibrium binding of ^{125}I-AP III to rabbit lung membranes was saturable, and Scatchard analysis of the binding data revealed the presence of a single class of high affinity binding sites for AP III (Fig. 2). An apparent dissociation constant (Kd) of 0.32 nM, and a binding capacity of 166 fmol/mg protein was determined. Non-radioactive monoiodinated AP III was indistinguishable from unlabelled AP III in competing for binding sites on rabbit lung membranes and in its vasorelaxant activity in a rabbit aorta bioassay (data not shown). Furthermore, unrelated peptide hormones such as vasopressin, bradykinin,

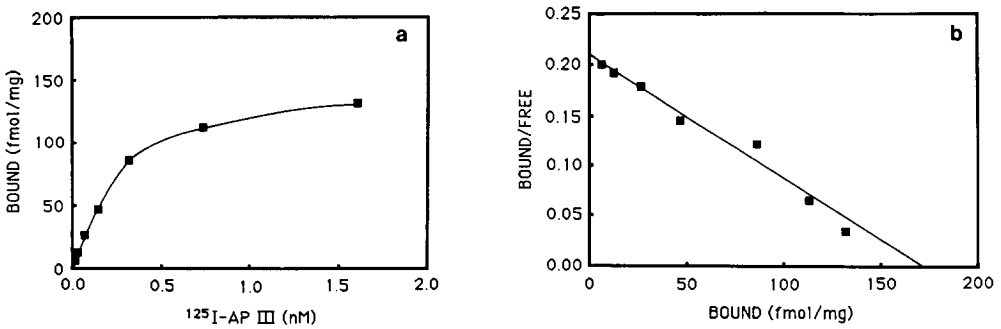

Fig. 2. (a) Saturable binding of ^{125}I-AP III to rabbit lung membranes. Various concentrations of ^{125}I-AP III were incubated with rabbit lung membranes (100 ug) for 30 min. Specific binding was calculated as total binding minus nonspecific binding in the presence of 1 uM unlabelled AP III. Each point is the mean of two determinations. (b) Scatchard analysis of the binding data. The ratio of bound to free ^{125}I-AP III is plotted against the amount of bound labelled peptide.

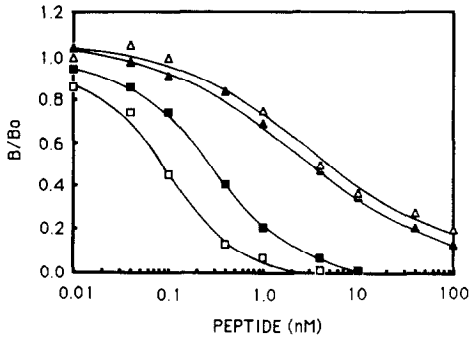

Fig. 3. Competitive binding of ^{125}I-AP III to rabbit lung membranes. The membranes were equilibrated with 135 pM ^{125}I-AP III and varying concentrations of unlabelled atrial peptides: SLRR-AP III (■), AP III (□), AP I (▲), and AP III (3-19) (Δ). Values of specific binding measured in the presence of unlabelled peptide (B) were expressed as a fraction of the specific binding measured in the absence of competitor (Bo). Data points represent the mean of two determinations.

angiotensin I and angiotensin II, did not compete for binding at concentrations of 0.1 uM. As shown in Fig. 3, binding of ^{125}I-AP III to rabbit lung membranes was competitively inhibited by increasing concentrations of unlabelled AP III. As can be seen, various atrial peptide analogs competed for rabbit lung AP III binding sites. The relative binding affinities (IC_{50}) of these peptides, using AP III as standard, correlated well with their vasorelaxant activities on norepinephrine-contracted rabbit aorta strips (Table I).

Table I

Relative potencies of atrial peptides using AP III as standard (given an arbitrary value of 1.0) in receptor binding affinity (IC50) to rabbit lung membranes and vasorelaxant activity on norepinephrine-contracted rabbit aorta strips

	Receptor Binding	Vasorelaxant Activity
SLRR-AP III	3.0	2.7
RR-AP III	3.3	2.1
R-AP III	2.0	2.4
AP III	1.0	1.0
(Met^{8}) AP III	1.0	1.0
AP II	1.6	1.5
AP I	0.08	0.01
AP III (3-19)	0.05	0.004

Correlation coefficient between the relative potencies for receptor binding and vasorelaxant activity was 0.93 (n = 8).

DISCUSSION

The binding data obtained in this study is consistent with there being a single class of high affinity receptors for atriopeptin III in rabbit lung. The value obtained for the apparent dissociation constant of the receptor is similar to those reported for other tissues (6-8), and is consistent with atrial peptide levels measured in plasma (12,13). The relative binding affinities of a number of atrial peptide analogs also correlated well with their relaxant activities on contracted vascular tissue strips. The location of atrial peptide receptors in lung is not known at present, although it has been reported that these factors appear to act on the vasculature rather than on airway smooth muscle (10).

It is known that atrial natriuretic factors are released from the atria of the heart, and that the right atrium contains a higher concentration of atrial peptides than the left (14). Atriopeptins can survive passage through the lung (15), as would be required of a circulating hormone (16,17). Thus, when atrial peptides are released from the right atrium, they would first encounter the pulmonary circulation before being transported to other tissues. It is not unreasonable, therefore, to speculate that receptors for atrial peptides exist in the lung vasculature and that atrial peptides, via their vasorelaxant, diuretic and natriuretic activities, could be of use in the therapeutic treatment of pulmonary edema or hypertension. Further studies will be required, however, to determine whether atrial peptides do play a role in the regulation of pulmonary function.

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