Identification and Characterization of Guinea Pig Angiotensin II Ventricular and Atrial Receptors: Coupling to Inositol Phosphate Production

Kenneth M. Baker and Harold A. Singer

Angiotensin II (All) elicits a receptor-mediated positive inotropic response in cardiac tissue from most mammalian species. The data strongly suggest that the positive inotropic response to All is mediated by activation of the voltage-sensitive slow Ca\(^{2+}\) channels. Angiotensin II binding sites that have the characteristics of a membrane receptor were identified in ventricular (myocardial) and atrial membrane preparations from guinea pigs. In ventricles, saturation-binding data yielded an optimal fit to a two-site model with a high-affinity site \(K_d = 3.6 \pm 0.7\) nM and a low-affinity site \(K_d = 433 \pm 126\) nM and binding capacities of 66 \pm 10 and 821 \pm 49 fmol/mg protein, respectively. In atria, saturation binding data yielded an optimal fit to a two-site model with a high-affinity site \(K_d = 1.6\) nM and a low-affinity site \(K_d = 300\) nM and capacities of 145 and 752 fmol/mg protein, respectively. The ventricular binding of \(^{125}\)I-angiotensin II was stimulated twofold in the presence of the divalent cations calcium and magnesium (10 mM). Nonhydrolyzable analogues of guanosine triphosphate increased the dissociation rate of the bound \(^{125}\)I-angiotensin II and decreased hormone binding to the receptor at equilibrium. Competition for \(^{125}\)I-angiotensin II binding by an agonist-antagonist analogue series correlated with previous studies obtained in the rabbit, a mammal in which inotropic responses to angiotensin II were demonstrated. The data indicate the presence of angiotensin II myocardial and atrial receptors and a G-type coupling protein in guinea pig. Although this species lacks an inotropic response to angiotensin peptides, there is a dose-dependent increase in inositol-1-phosphate production in response to angiotensin II, and this response is blocked by the selective angiotensin II antagonist [Sar\(^{1}\),Ile\(^{8}\)]angiotensin II. This species may provide the opportunity to study the angiotensin II-induced phosphoinositide response and potential physiological sequelae (i.e., hypertrophy) in isolation from Ca\(^{2+}\) channel activation and resultant mechanical responses. (Circulation Research 1988;62:896–904)

From the Division of Research, Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania.

Presented in part at the Seventy-first Annual Sessions of the Federation of American Societies for Experimental Biology.

Supported by research grants from the American Heart Association, Northeastern Pennsylvania Affiliate, and the Geisinger Research Foundation.

Address for correspondence and reprints: Kenneth M. Baker, MD, Weis Center for Research, Geisinger Clinic, Danville, PA 17822.

Received March 26, 1987; accepted November 19, 1987.
and of phosphatidylinositol metabolites in guinea pig atrial and ventricular myocytes. 27

The purposes of the present study were to determine if AII-binding sites were present in guinea pig ventricle and atrium, a species that does not exhibit an inotropic response to the peptide, and to determine if AII elicits other myocardial responses in this species, specifically activation of phosphoinositide breakdown and inositol phosphate accumulation. We report the identification of 125I-AII-binding sites in ventricular and atrial membrane preparations from guinea pigs that have the characteristics of a membrane receptor. We confirm the apparent lack of coupling between the ventricular and atrial binding sites and inotropic response in this species. However, AII was found to stimulate accumulation of water-soluble phosphoinositides in a dose-dependent manner that was antagonized by specific AII-receptor antagonists. These findings indicate that the lack of inotropic response to AII in guinea pig hearts in vitro is not due to absence of AII-recognition sites but apparently reflects a lack of coupling to membrane calcium channels. Although in guinea pig the AII ventricular and atrial receptors do not couple to inotropic activities, they do couple to phosphoinositide turnover.

Materials and Methods

Angiotensin I (AI), AII, and angiotensin III (AIII) were obtained from the United States Biochemical Corporation, Cleveland, Ohio. 125I-AII (specific activity 1,880 μCi/μg) and myo[2-3H]inositol (specific activity 17.1 μCi/mmole) were obtained from New England Nuclear, Boston, Massachusetts. Other chemicals and reagents were obtained from Fischer Scientific Corporation, King of Prussia, Pennsylvania, and Sigma Chemical, St. Louis, Missouri. Number 30 glass fiber filters were purchased from Schleicher and Schuell, Keene, New Hampshire. The anion-exchange resin (formate form) and column were obtained from Bio-Rad, Richmond, California, and Kontes, Vineland, New Jersey, respectively.

Tissues were obtained from male Dunkin-Hartley guinea pigs (300-350 g). The animals were anesthetized with sodium pentobarbital (30 mg/kg i.p.). The heart was rapidly removed, and the left atrium dissected free. Left atria were prepared for inotropic studies by the method of Neely and Rovetto. 29 Guinea pigs were killed as described above, and the hearts were rapidly excised and placed in ice-cold modified Krebs-Henseleit buffer containing (mM) NaCl 119, CaCl2 1.9 plus 0.5 to balance EDTA; NaH2PO4 1.18, MgCl2 1.18, NaHCO3 25, KCl 5, and glucose 11.1. The aorta was cannulated and back-perfused with buffer at 80 mm Hg at a flow rate of 30 ml/min. The buffer was maintained at 37° C and equilibrated with 95% O2-5% CO2. A balloon-tipped catheter coupled to a P23 pressure transducer (Gould, Cleveland, Ohio) was placed in the left ventricular cavity via the mitral orifice, and left ventricular pressure was displayed on a Model 7 Grass polygraph. Following a 30-minute equilibration period, agonist compounds were infused into the spontaneously beating hearts (via the aortic cannula) for 5 minutes at each concentration.

Guinea pig ventricular (myocardial) membranes were prepared from the left ventricles and atria by previously described methods. 31 The methods are a modification of the original protocol of Harigaya and Schwartz 28 as described by Jones et al. 32 The left ventricles or atria were removed from the hearts and cut into 2-3-mm pieces. A homogenate of the tissue (20% w/vol) was prepared in 0.25 M sucrose, 25 mM Tris, at pH 7.5 (on ice), with a Brinkman Polytron (Westbury, New York) using a PT-20 probe at a setting of 5.5 for two 30-second periods. Following centrifugation of the homogenate at 10,000g for 20 minutes, the supernatant was decanted and sedimented at 45,000g for 30 minutes. The 45,000g pellet was resuspended in 10 ml of 0.6 M KCl and 30 mM histidine, pH 7.0, and resedimented at 45,000g for 30 minutes to yield a fraction of crude membrane vesicles. All centrifugations were performed at 5° C. The pellets obtained from the final centrifugation were washed three times and resuspended in binding buffer of 50 mM Tris, 10 mM MgCl2, 10 μg/ml bacitracin, 5 x 10-4 M dithiothreitol, and 0.2% crystalline bovine serum albumin (BSA), pH 7.5. The membranes were used immediately in binding assays. Protein determinations were performed by the protein dye binding method of Bradford. 32

The concentration of 125I-AII used in the binding experiments was ~0.30 nM. The binding reaction was initiated by the addition of the ventricular or atrial membrane fraction to AII (40 μl, 1.5 mg of protein/ml, final concentration) and incubated in a total volume of 0.1 ml of assay buffer for 45 minutes at 18° C in 12 x 75 mm polypropylene tubes. Following incubation, 4 ml ice-cold 50 mM Tris solution, pH 7.5, was added to each assay tube. Bound hormone was trapped by vacuum filtration using glass fiber filters that had been presoaked in 0.2% solution of fraction V BSA. This was followed by a 4-ml rinse of the incubation tube and 2 x 4 ml rinse of the filters with Tris (50 mM, 4° C). The filters were dried in room air, and bound radioactivity was determined by counting in a Beckman Biogamma Counter (Fullerton, California) with an efficiency for 125I-AII of 70%. Saturable binding is defined as that portion of total binding displaced by...
10^{-5} M unlabeled ligand. Experimental results for the competition studies by the peptides are expressed as$^{125}$I-AII bound (B/B_o × 100) where B and B_o are the binding of labeled All in the presence and absence, respectively, of the unlabeled peptides.

Metabolism of$^{125}$I-AII during the binding assays was assessed by thin layer chromatography (TLC).$^{125}$I-AII (0.35 nM) was incubated with membranes (total volume 100 μL, membranes 40 μg) at a concentration of 1.5 mg protein/ml for 60 minutes at 18°C. Bound and free ligand were separated by centrifugation. The bound hormone was solubilized by adding 100 μL of 50% glacial acetic acid to the pellet, followed by incubation in a boiling water bath for 5 minutes. The free and bound hormones were spotted on Eastman Kodak plastic-backed cellulose TLC plates and developed in a solvent of n-butyl alcohol: acetic acid: pyridine: water (15:3:10:12). The plates were cut into 1-cm² strips and counted in a Beckman Biogamma Counter. The TLC standard on each chromatogram was the native$^{125}$I-AII.

Incubation of the tissue and analysis of inositol-1-phosphate was performed by modifications of the procedures of Berridge et al^{33} and Brown and Brown.<sup>34</sup> The guinea pigs were anesthetized with sodium pentobarbital (30 mg/kg i.p.). Hearts were quickly excised, the left ventricles or atria isolated within 30 seconds, blotted to remove excess blood, washed in oxygenated (95% O_2-5% CO_2) Krebs-Henseleit buffer (4°C), and wet weights determined. The tissues were transferred to polystyrene tubes containing continuously oxygenated buffer (37°C) and equilibrated for 20 minutes with one change of buffer (10 minutes). Fresh buffer containing 7.5 μCi/ml myo[2-3H]inositol was added for 45 minutes. This was replaced with another volume containing 5 μCi/ml myo[2-3H]inositol. Five minutes following this buffer change, LiCl (10 mM) was added; 10 minutes following the addition of LiCl, either drug or control solutions were added. Antagonist compounds were added 5 minutes prior to agonist compounds. Following 30 minutes of incubation with the drug or control solutions, the tissues were blotted and rinsed with ice-cold buffer. The tissues were transferred to a Pyrex tissue grinder (13 x 100 mm) containing 1.3 ml ice-cold CHCl₃: MeOH: H₂O (5:10:4) and homogenized for 1 minute. Two phases were obtained with the solution. The water-soluble extract (upper aqueous phase, 650 μL) was removed and transferred to a column containing 180 mg anion exchange resin (Bio-Rad, AG 1-x 8, 100-200 mesh, formate form). The column was washed with 20 ml H₂O to remove myo[2-3H]inositol. Labeled myo-inositol-1-phosphate was eluted with 10 ml of 0.1 M formic acid, 0.2 M ammonium formate and counted in an equal volume of Scinti Verse E (Fisher) in an LKB Rackbeta LS counter (Gaithersberg, Maryland).

The affinities and number of binding sites for the saturation binding isotherms were determined using a nonlinear least-squares curve-fitting program to fit the data to a one- or two-site model.<sup>35</sup> Data were also transformed to a Scatchard plot.<sup>36</sup> Data are expressed as mean ± SEM. When comparing two groups, statistical significance was determined using an unpaired Student’s t test. More than two means were compared by ANOVA and the Newman-Keuls test. In both cases, p<0.05 was considered statistically significant.

Results

The binding of$^{125}$I-AII to the 45,000g myocardial particulate fraction of guinea pig left ventricle increased linearly with protein concentrations between 25 and 150 μg/100 μL. The rate of association and maximal binding of$^{125}$I-AII were dependent on time and temperature. At 18°C, binding remained stable from 45 to 90 minutes. Chromatography of$^{125}$I-AII bound and labeled peptide(s) in the medium demonstrated that degradation of the radioligand was prevented or attenuated in the binding buffer used for the experiments (data not shown).

The concentration-dependent binding of$^{125}$I-AII to the guinea pig ventricular particulate fraction is shown in Figure 1A. The binding isotherm spans more than four orders of magnitude with a threshold concentration approximating 10^{-9} M All and saturation of sites occurring at 10^{-5} M All. When the data were transformed according to Scatchard,<sup>36</sup> the plot suggested the
presence of two classes of binding sites (Figure 1B). Computer analysis of the saturation-binding data yielded an optimal fit to a two-site model with a high-affinity site $K_a = 3.6 \pm 0.7 \text{ nM}$ and a low-affinity site $K_a = 433.4 \pm 126 \text{ nM}$ and capacities of $66 \pm 10$ and $821 \pm 49 \text{ fmol/mg protein}$, respectively. Competition by angiotensin peptides with $^{125}$I-AII binding to the guinea pig ventricular membrane fraction is shown in Figure 2. The displacement curve for [Sar'$,Ile$'$]AII was shifted to the left of [Ile$]AII by approximately fivefold. [Sar'$,Thr$']AII was equipotent with [Ile$]AII in displacing $^{125}$I-AII. Angiotensin I and III were 1% and 2%, respectively, as potent as [Ile$]AII in inhibiting the binding of $^{125}$I-AII.

The binding of $^{125}$I-AII to the guinea pig ventricular membrane fraction was determined in the presence of various cations. These experiments were performed with a particulate fraction prepared and washed several times in binding buffer that contained 50 mM Tris, $10^{-4}$ M EDTA, $10 \mu$g/ml bacitracin, $5 \times 10^{-4}$ M diithiothreitol, and 0.2% crystalline BSA, pH 7.5. At concentrations of 10 mM free ion, the divalent cations Mg$^{2+}$ and Ca$^{2+}$ stimulated maximal $^{125}$I-AII binding approximately twofold over control levels ($-270\%$ stimulation in the presence of Mg$^{2+}$). The binding of $^{125}$I-AII was decreased ($-80\%$) in the presence of monovalent cations, K$^+$ and Na$^+$ (Figure 3).

Guanine nucleotides accelerate the dissociation rate of many agonists from their membrane receptors. As shown in Figure 4, $10 \text{ mM Gpp(NH)p}$ increased the dissociation rate of $^{125}$I-AII by unlabeled AII from the binding sites in a preparation of guinea pig ventricular membranes. The divalent cation dependence of the GTP effect is shown in the inset (Figure 4). In the absence of added Mg$^{2+}$, Gpp(NH)p does not increase the dissociation rate of the $^{125}$I-AII by unlabeled AII from the ventricular binding sites. The GTP effects on $^{125}$I-AII binding in ventricle at equilibrium are shown in Figure 5A. Gpp(NH)p shifts the competition displacement curve of $^{125}$I-AII by [Ile$]AII$ to the right in the ventricular membrane fraction. Competition by

![Figure 2](https://example.com/f2.png)

**Figure 2.** Displacement of $^{125}$I-AII bound to guinea pig ventricular membranes by [Ile$]AII (○), AII (●), [Sar'$,Ile$'$]AII (×), and [Sar'$,Thr$']AII (Δ). $^{125}$I-AII (0.35 nM) was incubated for 45 minutes at 18°C with 1.5 mg/ml protein. $B$ and $B_0$ are binding in presence and absence, respectively, of unlabeled peptides. Results are mean of three separate experiments, each performed in triplicate.

![Figure 3](https://example.com/f3.png)

**Figure 3.** $^{125}$I-AII (0.35 nM) was incubated with guinea pig ventricular membranes in presence of varying concentrations of Ca$^{2+}$ (○), Mg$^{2+}$ (□), K$^+$ (■), and Na$^+$ (●). After 45 minutes' incubation at 18°C, saturable binding was determined as described in "Materials and Methods." Data are expressed as percentage of control binding, control being EDTA-containing buffer with no added ions. Data points represent pooled results from three experiments, each performed in triplicate.

[Ile$]AII$ with $^{125}$I-AII binding to the guinea pig atrial particulate fraction is shown in Figure 5B. Computer analysis of the saturation-binding data from a representative experiment yielded an optimal fit to a two-site model with a high-affinity site $K_a = 1.6 \text{ nM}$ and a low-affinity site $K_a = 300 \text{ nM}$ and capacities of 145 and 752 fmol/mg protein, respectively. The [Ile$]AII$ competition curve in the atrial particulate fraction is shifted $>1 \text{ log}$ to the right in the presence of 10 mM Gpp(NH)p (Figure 5B).

In an isolated perfused heart preparation, no significant inotropic responses were demonstrated with AII (Figure 6). The β-adrenergic agonist isoproterenol and the muscarinic agonist carbachol produced significant positive ($-70\%$ increase) and negative ($-35\%$ decrease) inotropic responses, respectively, demonstrating the viability of these preparations. Biological activities for AII and the β-adrenergic agonist isoproterenol on atrial contractions are shown in Figure 7. The responsiveness to isoproterenol was used as an indicator of the integrity of the tissues. We were unable to demonstrate positive inotropic responses to AII in the guinea pig atria over the expected dose range ($10^{-10}$ to $10^{-4}$ M) estimated from the binding data. In addition, angiotensin pretreatment had no significant effect on the isoproterenol cumulative concentration-response relation (data not shown). No inotropic responses were obtained with any angiotensin analogues studied, including AII, AIII, and [Sar'$,Ile$'$]AII (10$^{-10}$ to $10^{-8}$ M). It should be noted that in vascular preparations (aortic rings) from the guinea pig, AII elicits dose-dependent contractile responses, indicating intact vascular smooth muscle AII receptors and coupling mechanisms in this species (data not shown).

The concentration-response for AII stimulation of phosphoinositide hydrolysis in guinea pig ventricle is shown in Figure 8A. Threshold for the AII-mediated response in ventricle approximates $10^{-8}$ M in this species. The AII responses were significantly greater than control but only 17% of the response observed.
with 100 μM carbachol (Figure 8B). The carbachol (100 μM)-stimulated increase in inositol-1-phosphate (IP₁) production in guinea pig ventricle (Figure 8B) confirms previous observations in rat and chicken heart.¹⁶,²⁸,²⁹ Both the All- and cholinergic-induced IP₁ responses were blocked by the specific receptor antagonists [Sar¹, Ile⁸]All (Figure 8A) and atropine (Figure 8B), respectively. The All-induced IP₁ response was not blocked by the α-adrenergic antagonist phenoxybenzamine or the β-adrenergic antagonist nadolol (Figure 8B).

Carbachol and All produced a similar stimulation (when compared with the ventricle) of phospholipid hydrolysis in guinea pig atria (Figure 9). These responses were also blocked by the specific receptor antagonists (Figures 9A and 9B). The All-induced IP₁ response was not blocked by phenoxybenzamine or nadolol (Figure 9B). As in ventricle, the threshold for the All-mediated response in atria approximates 10⁻⁹ M in this species.

**Discussion**

In this study, we have characterized the binding of ¹²⁵I-All in a particulate fraction of plasma membrane and sarcoplasmic reticulum of guinea pig left ventricle and atria and provided evidence that these binding sites are the putative receptors that mediate the All-stimulated production of inositol-1-phosphate in this species. The binding of ¹²⁵I-All in this preparation was saturable (Figure 1), reversible (Figure 4), specific
FIGURE 7. Effects of All (C) and isoproterenol with (D) and without (E) propranolol (10^{-7} M) on guinea pig atrial contractility. Isolated left atria were maintained at 37°C and paced by point stimulation at 60 beats/min with twice-threshold voltage. Isometric contractions were recorded from base of about 3.0 g resting tension (maximum length-tension). Results are mean of eight experiments.

(Figure 2), and of both high- and low-affinity (Figure 1B). Two classes of All-binding sites have previously been demonstrated in rabbit heart,^18^ rat cardiocytes,^19^ adrenal gland,^20^ liver,^21^ and kidney. A single class of All-binding sites has previously been identified in rabbit^12^ and bovine heart,^43^ vascular smooth muscle,^44^ and brain. Some variability with respect to the one- versus two-site models of All binding reported for cardiac tissue may be expected based on methodological and species differences. In the guinea pig, using the present methodology, the best computer fit was with the two-site model in both the ventricular and atrial particulate fractions. The two-component exponential displacement curve of 125I-AII in the presence of All is consistent with this model (Figure 4) as are the GTP effects on agonist binding (Figure 5).

The high-affinity binding site for 125I-AII that we have identified in the guinea pig is very similar to that identified in rabbit myocardium. The major distinction between rabbit heart compared with the guinea pig is the difference in physiological responsiveness. Specifically, guinea pigs lack the mechanical changes (contractility) in response to All in both ventricles and atria (Figures 6 and 7). These results confirm previous studies. Using isolated guinea pig papillary muscles, Iven and Zetler^13^ and Iven et al. were unable to demonstrate any effect of All on action potential duration, dV/dt^max_, half-life of recovery of dV/dt^max_, or refractory period. In a Langendorff preparation of guinea pig heart, All produced a small decrease in mechanical (contractile) activity, which was most likely secondary to the vasoconstrictive effects of All on the coronary arteries. In field-stimulated guinea pig left atria, All produced a slight increase in contractility, while in point-stimulated atria, there was no response to All up to 1 μmol/L. The slight increase in contractility in the field-stimulated preparation was most likely secondary to noradrenaline release from intramural neurons.

In cardiac tissue, the inotropic response to All when present is related to an increase in the permeability of the cell membrane to Ca^{2+} during the action potential. This receptor-mediated coupling with the voltage-sensitive Ca^{2+} channel is present in rabbit and avian cardiac muscle. In rabbit right atria, All (10^{-10} M) rapidly restored both electrical and mechanical activity during K^+ (22 mM) depolarization. Consistent with the lack of a mechanical response, All at concentrations as high as 10^{-5} M did not restore electrical activity in guinea pig atria. [Sar^1]All and [des-Asp^1]All yielded the same negative result as did All in the guinea pig atrium. These results suggest that in guinea pig, the angiotensin receptor is uncoupled from the voltage-sensitive slow Ca^{2+} channel. There is evidence that in guinea pig ventricles, phosphorylation of phospholamban occurred predominantly by cyclic AMP (cAMP)-dependent phosphorylation. Interventions that increased Ca^{2+} concentrations by non-cAMP-dependent mechanisms did not stimulate 32P incorporation into phospholamban. Because in cardiac tissue All-mediated actions are via cAMP-independent mechanisms, it is possible that in this species it is the failure...
Consistent with recently reported effects in heart and other tissues, we have demonstrated a significant increase in inositol-1-phosphate production in guinea pig myocardium (Figure 8A) and atrial tissue (Figure 9A). Similar All-induced effects on phosphoinositide metabolism in ventricular and atrial tissue are consistent with recently reported effects in heart and other tissues. A principal finding in the present study is the demonstration of a significant All-stimulated increase in inositol-1-phosphate production in isolated guinea pig atrial tissue (Figure 8A) and atrial (Figure 9A) tissue. The response to All was not blocked by α- or β-adrenergic receptor antagonists in either ventricular (Figure 8B) or atrial (Figure 9B) tissue. Similar All responses were observed in rat, rabbit, and chick myocardium (data not shown). This response to All in the heart appears to be specific in that it is blocked by the All-receptor antagonist [Sar^1, Ile^8]All. Percent changes were calculated from mean basal value of 7 cpm/mg tissue wet wt (average weight 100–150 mg). Data are expressed as mean ± SEM of three to nine determinations. **p < 0.05 compared with All (10⁻⁷ M).

**Figure 9.** Panel A: Dose-related responses to All stimulation of inositol-1-phosphate production in guinea pig atrial tissue. Response to All is significantly blocked in presence of the selective antagonist [Sar^1, Ile^8]All. Percent changes were calculated from mean basal value of 7 cpm/mg tissue wet wt (average weight 100–150 mg). Data are expressed as mean ± SEM of three to nine determinations. *p < 0.05 compared with All (10⁻⁷ M). Panel B: Effect of All in presence and absence of phenoxybenzamine (PBZ) and nadolol (Nad) and of carbachol (Carb) in presence and absence of atropine (Atr) on percent change in inositol-1-phosphate production in guinea pig atrial tissue. Data are mean ± SEM of three to nine determinations. **p < 0.05 compared with carbachol.

to phosphorylate phospholamban that is responsible for the lack of Ca²⁺ transport by cardiac sarcoplasmic reticulum.

A principal finding in the present study is the demonstration of a significant All-stimulated increase in inositol-1-phosphate production in isolated guinea pig ventricular (Figure 8A) and atrial (Figure 9A) tissue. The response to All was not blocked by α- or β-adrenergic receptor antagonists in either ventricular or atrial tissue. Similar All responses were observed in rat, rabbit, and chick myocardium (data not shown). This response to All in the heart appears to be specific in that it is blocked by the All-receptor antagonist [Sar^1, Ile^8]All (Figures 8A and 9A). These All-induced effects on phosphoinositide metabolism in ventricular and atrial tissue are consistent with recently reported effects in heart and other tissues. In this species, we have shown a magnesium-dependent acceleration of the rate of dissociation of [³²P]-All from the receptor in the presence of guanine nucleotides (Figure 4 inset). In preliminary studies in rat heart, we have also demonstrated a GTP effect on the dissociation of [³²P]-All from the receptor (i.e., increased acceleration of the labeled agonist [K.M. Baker and H.A. Singer, unpublished data]). There is evidence that GTP-binding proteins are involved in the coupling of various Ca²⁺-mobilizing receptors to phosphatidylinositol 4,5-bisphosphate hydrolysis and Ca²⁺ mobilization and that GTP analogues potentiate hormonal stimulation of phospholipase C.¹³ The specific G-protein that couples to the All myocardial receptor has not been identified; however, hepatic All receptors have been shown to negatively couple to adenylate cyclase through N₅. It should be noted that in cardiac tissue, for all species studied, there is no evidence that All affects adenylate cyclase activity or the accumulation of cAMP, either positively or negatively. There is recently published evidence suggesting that G₅₅ is involved in the functional coupling of opiate receptors to neuronal voltage-dependent calcium channels. Direct activation of mammalian atrial muscarinic potassium channels by a guanine nucleotide regulatory protein was recently reported. These channels were in isolated inside-out patches of membranes from atrial cells and were activated by a G-protein that was a substrate for pertussis toxin, with an α-subunit of 40 kDa. In several mammalian or avian species, muscarinic stimulation of inositol phospholipid hydrolysis has been demonstrated in atrial and ventricular tissue and in isolated cardiocytes. In this study, we have demonstrated the All and also the muscarinic stimulation of inositol phospholipid hydrolysis in guinea pig ventricular and atrial tissue (Figures 8 and 9). We have observed similar responses in rat and avian heart (K.M. Baker and H.A. Singer, unpublished data). However, the physiological consequences of All or muscarinic stimulation of phosphoinositide hydrolysis in cardiac tissue are unclear. In skinned guinea pig myocardium, IP₃ was reported to have no direct effect on calcium sensitivity or maximum force generated by the contractile apparatus. But IP₃ did appear to modulate excitation-contraction coupling by enhancing the calcium-induced release of calcium from the sarcoplasmic reticulum. The All-induced phosphoinositide hydrolysis demonstrated in the guinea pig would appear not to be coupled to Ca²⁺ release based on the lack of a contractile effect to the peptide.

Observations have been made regarding the involvement of phosphoinositides in cell growth and proliferation. Phosphorylation of a 40-s ribosomal protein in chicken embryo fibroblasts or C127 murine cells was temporally correlated with protein synthesis. In rat heart, there is a reported effect of All on myocardial protein synthesis, including localization of [³H]All in the nuclei of cardiac muscle cells. Chronic (6 days) infusion of All into male Sprague-Dawley rats increased protein content, heart weight, and RNA concentration and content. The β-adrenergic agonist propranolol did not prevent the increase in heart weight following administration of All, suggesting that the response was not mediated by catecholamines. It seems possible that the All myocardial receptors
described here may be linked to phosphoinositide hydrolysis, which ultimately may account for the stimulation of protein synthesis and cardiac hypertrophy that has been described in response to All.

In conclusion, data describing the binding of 125I-AII to guinea pig ventricular and atrial membranes have been presented. These binding sites exhibited the properties expected of a hormonal receptor and likely represent the receptors that interact with All to mediate the stimulation of inositol phospholipid hydrolysis. The physiological implications of this response are unknown but may be related to protein synthesis. The guinea pig is remarkably different from other mammals in that there is an absence of a cardiac contractile response to All in ventricular and atrial tissue, which is not due to an absence of All ventricular or atrial receptors. Perhaps the absence of a contractile response is related to the uncoupling of the receptor(s) from the Ca2+ channel or the absence of the G-protein that may couple the All receptor to the voltage-sensitive slow Ca2+ channels. This species may provide a unique model in which to study the coupling of the All myocardial receptor to inositol phospholipid hydrolysis independent of coupling to the voltage-sensitive slow Ca2+ channels.

Acknowledgments
We thank Donna Wood, Beth Letcher, Kathleen Aurand, and Hui-Quan Han for their excellent technical assistance, Dr. Michael Peach for critical discussion, and Debbie McCaffery for typing the manuscript.

References
36. Bockaert J: Angiotensin II Cardiac Receptors 903
39. Bockaert J: Angiotensin II Cardiac Receptors 903


KEY WORDS • angiotensin II • hormone receptors • guanine nucleotide • polyphosphoinositides • myocardium • ventricle • atrium • cell membrane
Identification and characterization of guinea pig angiotensin II ventricular and atrial receptors: coupling to inositol phosphate production.

K M Baker and H A Singer

doi: 10.1161/01.RES.62.5.896

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/62/5/896

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/