Mechanism for the Positive Inotropic Effect of Angiotensin II on Isolated Cardiac Muscle

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SUMMARY Angiotensin II (A II) and analogues were tested for their ability to restore electrical and mechanical activity to cardiac muscle preparations in which the fast Na+ channels had been inactivated by partial depolarization (22–27 mM K+) or by tetrodotoxin (TTX). The partially depolarized or TTX-blocked preparations were chosen because under these conditions electrical and mechanical responses are primarily Ca2+-dependent. In depolarized rabbit right atria, A II restored spontaneous mechanical and electrical activity (measured by both intracellular and extracellular recording techniques). The frequency of action potential discharge was concentration-dependent; the threshold concentration of A II was 10⁻¹⁰ M, the ED₅₀ was 8 x 10⁻⁷ M, and the maximum effect was observed at 5 x 10⁻⁵ M. In contrast, depolarized guinea pig atria were insensitive to A II, Sar'-angiotensin II, and des-Asp'-angiotensin II, even at concentrations as high as 10⁻⁴ M. Rabbit papillary muscle (TTX-blocked), embryonic (18-day) chick heart (partially depolarized) and chick heart reaggregates (TTX-blocked) responded similarly to rabbit atria in that A II (9.6 x 10⁻⁷ M) restored both electrical and mechanical activity. We found that in these preparations the action of A II was unaffected by propranolol (5.0 x 10⁻⁷ M to 5.0 x 10⁻⁴ M) but was blocked by Mn²⁺ (10⁻⁷ M), D-600 (1 x 10⁻⁷ g/ml) and the specific A II antagonists Sar'-Ala'-angiotensin II (P-I13) (5.0 x 10⁻⁷ M) and Sar'-Ile'-angiotensin II (5.28 x 10⁻⁷ M). We conclude that the positive inotropic effect of A II on the myocardium is due to its ability to increase transmembrane Ca²⁺ movements in or through the cell membrane. The ability of Mn²⁺ and D-600 to block this effect suggests that this ion movement is via the so-called “slow channels.”

THE CARDIAC ACTION potential is thought to result from the inward movement of both Na⁺ and Ca²⁺ ions through two distinct membrane channels.¹ These have been designated as “fast channels” and “slow channels” on the basis of their kinetics and other specific properties. Under normal circumstances the fast channels carry primarily, if not exclusively, Na⁺ and these channels can be blocked by tetrodotoxin (TTX) or inactivated by elevated [K⁺]. The slow channels, on the other hand, carry primarily Ca²⁺, or Ca²⁺ and Na⁺, and can be blocked by Mn²⁺ as well as by verapamil and its analogue D-600. Because the slow channels are inactivated at a less negative membrane potential than the fast channels, moderate elevations in extracellular K⁺ can inactivate the fast channels while not affecting the slow channels.

It has been known for many years that angiotensin II (A II) exerts a positive inotropic action when applied to mammalian cardiac muscle in vitro.²,³ In that regard, Bonnardeaux and Regoli⁴ recently suggested that the positive inotropic action of A II is the result of a prolongation of the Ca²⁺ permeability of the cardiac cell during electrical excitation. They reported an increase in action potential duration coincident with the inotropic response as evidence in support of this hypothesis. Furthermore, in the smooth muscle of the rat uterus,⁵ A II also appears to have strong interactions with the Ca²⁺ pool required for maintenance of membrane excitability.

Because the inotropic action of the catecholamines and methylxanthines⁶ on the myocardium depends on the activation of the slow channels, it is possible that the same might be true for A II. Therefore, experiments were designed to investigate the effects of A II on transmembrane Ca²⁺ ion currents in cardiac muscle. Partially depolarized (22–27 mM K⁺) and TTX-blocked cardiac muscles were studied. Under these conditions, spontaneous electrical activity and responses to electrical stimulation are abolished, but drug-induced action potentials are easily demonstrable.¹⁻¹⁰ More important, however, is the fact that the drug-induced action potentials generated in the presence of elevated [K⁺]o or TTX primarily result from transmembrane Ca²⁺ currents carried through the slow channel.

Methods

We used hearts isolated from mammals and birds. Rabbits were killed by injection of air into an ear vein and guinea pigs were killed by cervical dislocation. Chick embryos were decapitated. Sinoatrial preparations were isolated from guinea pig and rabbit hearts; papillary muscles also were removed from rabbit hearts. These tissues were placed in chambers and were superfused with Tyrode’s solution. Hearts removed from chick embryos on the 18th day of incubation were perfused through the coronary vessels. The brachiocephalic arteries were ligated and a polyethylene cannula was inserted into the aortic stump. The cannula was connected to reservoirs that contained control (Tyrode’s) and test solutions heated to 37°C. The coronary vessels were perfused at a flow rate of about 0.014 ml/min.

Chick embryo hearts also were used to prepare cell cultures. Spherical reaggregate cultures were prepared by methods previously described in detail.¹¹ They have been

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shown to retain highly differentiated membrane electrical properties.\textsuperscript{11} Ventricles from 18-day embryonic chicks were washed free of blood and dispersed into single cells by stirring in Ca\textsuperscript{2+}-free and Mg\textsuperscript{2+}-free Tyrode's solution containing 0.05% trypsin, glucose (1 mg/ml), and 5 mM ATP. At 15-minute intervals, the cloudy cell-containing supernatant fluid was decanted into chilled culture medium (100% horse serum, 40% Puck's N-16 nutrient medium, 50% Tyrode's balanced salt solution, and penicillin/streptomycin, 50 U/ml), and fresh trypsinizing solution was added; this procedure was repeated four times. The harvested cells were diluted with culture medium to a concentration of about 10\textsuperscript{4} cells/ml, and plated in culture dishes containing Cellophane squares. Within 3–5 days the cells reaggregated to form small spheres (0.1–2.0 mm in diameter). For experiments, the spheres were transferred to a heated bath (37°C).

The control Tyrode's solution contained (millimolar concentrations): K\textsuperscript{+}, 2.7; Na\textsuperscript{+}, 149; Ca\textsuperscript{2+}, 1.8; Mg\textsuperscript{2+}, 1.0; Cl\textsuperscript{-}, 145; HCO\textsubscript{3}\textsuperscript{-}, 11.9; H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, 0.4; and glucose, 5.5. This solution was gassed with 95% O\textsubscript{2}-5% CO\textsubscript{2} and had a pH of 7.2–7.3. Solutions containing elevated concentrations of K\textsuperscript{+} (22–27 mM) were prepared by mixing an appropriate volume of the control Tyrode's solution (see above) with a volume of a high K\textsuperscript{+} stock solution that had the following composition (mM): K\textsuperscript{+}, 140; Na\textsuperscript{+}, 12; Ca\textsuperscript{2+}, 1.8; Mg\textsuperscript{2+}, 1.0; Cl\textsuperscript{-}, 145; HCO\textsubscript{3}\textsuperscript{-}, 11.9; and H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}, 0.4. Ion concentrations (Na\textsuperscript{+}, K\textsuperscript{+}, and Mg\textsuperscript{2+}) in the solution were measured routinely. Measurements of Na\textsuperscript{+} and K\textsuperscript{+} were made with a modified flame photometer having filters (Baird Atomic) that minimized interference between Na\textsuperscript{+} and K\textsuperscript{+}; Ca\textsuperscript{2+} and Mg\textsuperscript{2+} were measured with an atomic absorption spectrophotometer (Perkin-Elmer 290B).

Contractions of rabbit papillary muscle and perfused chick embryo hearts were recorded with a force-displacement transducer (Statham FT-03) and displayed on a polygraph. In the case of the rabbit, the muscle was attached to the chorda tendinea; in the experiments with the chick embryo, a suture was placed through the apex of the heart. Contractions were evoked by 5-msec rectangular current pulses applied to the tissues through platinum electrodes. Stimulus frequency was 1/sec for these two preparations. Atrial preparations were not stimulated. Transmembrane potentials and electrograms were recorded from the sinoatrial preparations and from chick heart cells. Glass capillary microelectrodes, filled with 3 M KCl and having tip resistances of 10–30 M\textOmega, were used to impale cells that were visualized under the microscope. The microelectrode was connected to an Ag-AgCl half cell; the reference electrode was a chloridized Ag wire. Potentials were led through an electrometer amplifier (WPI, model 7) that had capacitance neutralization for optimal recording of membrane potential transients, and the signals were displayed on an oscilloscope. Extracellular records of sinoatrial activity were obtained with platinum wires (insulated except at the tips) that touched the tissue in the region of the crista terminals. The signals were led through a preamplifier (Grass, 7P1B) and displayed on a polygraph.

Peptides were synthesized by the Merrifield solid phase method essentially as described by Stewart and Young.\textsuperscript{12} The completed peptides were removed from the resin by cleavage for 45 minutes in anhydrous HF at 0°C. Crude peptides were purified by countercurrent distribution (100 transfers) in n-butanol-acetic acid-water (4:1:5) or preparative high voltage electrophoresis in pyridine-acetate buffer. Final purity was determined by paper high voltage electrophoresis at pH 5.0 and in at least two thin layer chromatography systems.

Other drugs used in this study included the angiotensin antagonists, Sar\textsuperscript{1}-Ala\textsuperscript{2}-angiotensin II (P-113) (Norwich Pharmacal), Sar\textsuperscript{1}-Ile\textsuperscript{2}-angiotensin II, epinephrine bitartrate, propranolol hydrochloride (Ayerst), tetrodotoxin, verapamil, and D-600 (the last two compounds were supplied by Knoll Pharmaceuticals).

**Results**

**ISOLATED RIGHT ATRIAL PREPARATIONS**

A II, 10\textsuperscript{-8} M, rapidly restored both electrical and mechanical activity to partially depolarized (22 mM K\textsuperscript{+}) rabbit right atria (Fig. 1, upper panel, and Fig. 2). The effect usually was observed within 1–3 minutes after addition of A

![Figure 1](http://circres.ahajournals.org/)

**Figure 1** Effect of angiotensin on spontaneous electrical activity in partially depolarized rabbit and guinea pig right atria. Rabbit (A–C) and guinea pig (D–F) right atria were placed in Tyrode's solution containing K\textsuperscript{+} = 22 mM for 30–40 minutes at 30°C. A + D: control resting potential as measured by intracellular electrode. B + E: effect of angiotensin (10\textsuperscript{-8} M) on rabbit (B) and guinea pig (E) atria 10 minutes after addition of the polypeptide. C: records from the same cell shown in E, 10 minutes after addition of epinephrine (10\textsuperscript{-4} M).

\[\text{[Ref. text]}\]
Figure 2 Effect of angiotensin on pacemaker frequency in partially depolarized rabbit atria. Atria were placed in Tyrode’s solution containing K⁺ 22 mM for 30-40 minutes at 30°C. Pacemaker frequency was measured 5 minutes after addition of the polypeptide. Each point represents the average ± SEM of results from three to five experiments.

II and persisted for as long as the polypeptide remained in the bathing solution (up to 30 minutes). As can be seen in Figure 1C there was little effect on action potential frequency after addition of propranolol, 3 x 10⁻⁸ M. This concentration of propranolol completely inhibited the response of this preparation to norepinephrine at the mean effective dose (ED₈₀). The sensitivity of this system to A II then was determined by a standard concentration-effect curve with the frequency of action potential discharge used as end point. Clearly, the ability of A II to increase frequency of action potential discharge (in the presence of propranolol, 3 x 10⁻⁸ M), was concentration-dependent, with 8 x 10⁻⁸ M giving 50% maximum response and 5 x 10⁻⁸ M giving maximal stimulation (Fig. 2). Further increments in A II concentration up to 10⁻⁷ M did not increase frequency; instead there was a tendency toward a decrease in frequency (not statistically significant).

The guinea pig atria offers an interesting contrast to the rabbit atria in that A II, at concentrations as high as 10⁻⁶ M and in the absence of propranolol, did not restore electrical activity (Fig. 1E). The same preparations, however, responded to epinephrine (1 x 10⁻⁸ M) with action potentials (74 mV) occurring at a regular frequency (Fig. 1F). Since it is known that some effector systems are more sensitive to the heptapeptide metabolite of A II (i.e., des-Asp'–angiotensin II) than to A II itself, this compound was synthesized and, when tested on the guinea pig atrium, yielded the same negative result as did A II (Table I). Furthermore, to minimize the effects of degradation of A II by aminopeptidases, the N-terminal-modified analogue, Sar'-angiotensin II, was synthesized and tested. However, like A II and the heptapeptide, it was without effect at concentrations as high as 10⁻⁶ M (Table I). As before, epinephrine (1 x 10⁻⁸ M) was tested as a positive control and it restored mechanical activity to 27.2 ± 6.1% of the control tension observed in normal Tyrode’s solution.

### Table I Effect of Angiotensin and Epinephrine on Mechanical Activity of Depolarized Guinea Pig Atria

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>Control tension (%)</th>
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<tbody>
<tr>
<td>K⁺ (mM)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.4</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>22.0</td>
</tr>
<tr>
<td>des-Asp'-angiotensin II</td>
<td>22.0</td>
</tr>
<tr>
<td>Sar'-angiotensin II</td>
<td>22.0</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>22.0</td>
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Tension generated by spontaneously beating atria was taken as 100%. Drugs were added directly to bath fluid 30 minutes after placing the preparation in 22.0 mM K⁺ Tyrode’s solution. If no mechanical response was observed after 5 minutes of contact, the drug was washed out and 20 minutes were allowed to pass before making further additions to the preparation. Data represent the average ± SEM of three to five experiments.

### ISOLATED RABBIT PAPILLARY MUSCLE

A II (5.9 x 10⁻⁴ M) exerted a positive inotropic action on rabbit papillary muscle in normal Ringer’s solution (Fig. 3A and B). This effect was rapid in onset (maximum effect in 2 minutes) and readily reversible by washout of the A II (Fig. 3C). The same effect was observed in each of four experiments, and is consistent with the time course of action found for the partially depolarized preparations. Similarly, in preparations in which electrically evoked contractions had been abolished by TTX (6.2 x 10⁻⁸ M), A II (5.5 x 10⁻⁸ M) restored the ability of the tissue to respond to such stimulation (Fig. 3F). The return of the contractile events was not due to release of endogenous catecholamine, because it persisted in the presence of propranolol (5.0 x 10⁻⁸ M). Under these circumstances, however, the rate of stimulation was 60/min compared to a control spontaneous rate of about 150/min. The slow rate of stimulation was necessary because the TTX-treated preparation was incapable of following more rapid stimulus rates. The reduced amplitude of the contractions at this rate may result from a reverse positive staircase effect or the local anesthetic effect of propranolol. As before, the A II effect would appear to be Ca²⁺ dependent because it was abolished in the presence of 1.0 mM Mn²⁺ (Fig. 3G).

### EMBRYONIC CHICK HEARTS

Embryonic chick heart* and embryonic chick heart reaggregate cultures in which the fast channels are blocked by TTX exhibit slow responses in the presence of catechola-
propranolol still are present.

contractions after addition of Mii 1* (1 mM); angiotensin, TTX, and stimulated rabbit papillary muscles. A-C: positive inotropic action muscle blocked by tetrodotoxin (TTX). D: control contractions in normal Tyrode’s solution. E: absence of contractions after addition of A II. A: control contractions in normal Tyrode’s solution. B: addition of A II (5.9 x 10^-8 M); C: washout of A II. D-G: induction by A II of contractile responses in papillary muscle blocked by tetrodotoxin (TTX). D: control contractions in normal Tyrode’s solution. E: absence of contractions after addition of TTX (3.1 x 10^-1 M); F: contractions after addition of A II (5.5 x 10^-8 M) in the presence of TTX and propranolol (5.0 x 10^-8 M); the rate of stimulation was decreased below the control because the muscle could not follow the more rapid stimulation. G: absence of contractions after addition of Mn^2+ (1 mM); angiotensin, TTX, and propranolol still are present.

mines. It was of interest, therefore, to determine whether these preparations also would respond to A II. In seven experiments, addition of A II (9.7 x 10^-7 M) rapidly (within 2 minutes) produced slowly rising (about 20 V/sec) action potentials in reaggregated embryonic chick ventricular cells in the presence of TTX (3.1 x 10^-1 M) (Fig. 4). Contractions, which had been abolished along with the action potentials by TTX, also were restored and accompanied the slow response induced by A II. Addition of propranolol (1.9 x 10^-9 M) did not affect the control action potential (Fig. 4A), nor did it prevent the induction of the slow response by A II (Fig. 4G), indicating that the A II did not exert its effect via the β-adrenergic receptor. In contrast, the slow response to A II could be blocked by either of the specific A II antagonists Sar'-Ile'-angiotensin II (5.3 x 10^-8 M) (Fig. 4D) or Sar'-Ala'-angiotensin II (P-113) (5.0 x 10^-8 M). Furthermore, the addition of Mn^2+ (1 mM) or D-600 (1.0 x 10^-7 g/ml), an analogue of verapamil, also eliminated the electrical activity in response to A II, indicating that the inward current observed is at least partly carried by Ca^2+.

Because contractions could not be recorded from the cultured chick heart reaggregates, the effect of A II on developed tension in embryonic myocardial cells was determined on isolated perfused embryonic hearts. In such hearts, in which excitability had been blocked by increasing [K^+]o to 27 mM and inactivating the fast Na^+ channels (Fig. 5B), addition of A II (9.7 x 10^-7 M) rapidly (within 2 minutes) restored evoked contractions which persisted in the presence of propranolol (5 x 10^-8 M) (Fig. 5C). As in the reaggregate cultures, the A II-induced contractions were abolished by the A II antagonist Sar'-Ile'-angiotensin II (5.3 x 10^-8 M) (Fig. 5D).

Discussion

These experiments provide good evidence that the positive inotropic response of isolated cardiac muscle to A II is mediated by an increase in the availability of slow channels. More specifically, the evidence suggests that the inotropic response is a result of an A II-mediated increase in the permeability of the cell membrane to Ca^2+ during the action potential. Most convincing is the observation that A II restored both electrical and mechanical activity to a variety of cardiac preparations in which the fast Na^+ channels were inactivated either by TTX or elevated K+. Under these conditions, there is no spontaneous electrical activity, but the ability of drugs like the catecholamines and the methylxanthines to restore electrical and mechanical activity clearly are Ca^2+-dependent (see introduction). Qualitatively,

![Figure 3](https://example.com/figure3.png)

**Figure 3** Effect of angiotensin II on the mechanical responses of stimulated rabbit papillary muscles. A-C: positive inotropic action of A II. A: control contractions in normal Tyrode’s solution. B: contractions after addition of A II (5.9 x 10^-8 M); C: washout of A II. D-G: induction by A II of contractile responses in papillary muscle blocked by tetrodotoxin (TTX). D: control contractions in normal Tyrode’s solution. E: absence of contractions after addition of TTX. F: contractions after addition of A II (5.5 x 10^-8 M) in the presence of TTX and propranolol (5.0 x 10^-8 M); the rate of stimulation was decreased below the control because the muscle could not follow the more rapid stimulation. G: absence of contractions after addition of Mn^2+ (1 mM); angiotensin, TTX, and propranolol still are present.

![Figure 4](https://example.com/figure4.png)

**Figure 4** Induction by angiotensin II of slow electrical responses in cultured reaggregates of embryonic (18-day-old) chick ventricular cells blocked by tetrodotoxin (TTX). A: control action potential in the presence of propranolol (1.9 x 10^-9 M); addition of propranolol did not affect the response. B: addition of TTX (3.1 x 10^-1 M). C: addition of angiotensin II (9.6 x 10^-7 M) in the presence of TTX. D: effect of Sar'-Ile'-angiotensin II (5.3 x 10^-8 M) on angiotensin II-induced electrical response. Calibrations in D apply to all panels. Field stimulation was applied throughout.
the effect of AII on the rabbit and chick embryo hearts is indistinguishable from that of the catecholamines. Additional support for the slow channel hypothesis of AII action comes from the observation that the ability of the peptide to restore electrical activity can be eliminated by known inhibitors of the slow response (e.g., D-600 and Mn**).

The exact mechanism by which AII activates the slow response has not yet been determined. It is clear, however, that it does so by interaction of the polypeptide with its receptor. This was shown by the ability of the AII inhibitors, Sar1-Ala8-angiotensin II (P-113) and Sar1-Ile8-angiotensin II, to prevent the AII-induced slow responses in both rabbit and chick hearts. In this connection, it is interesting to note that in other tissues (i.e., rat uterus and guinea pig ileum), AII has been postulated to interact with a membrane-bound Ca** pool involved in the maintenance of membrane excitability. Furthermore, the electrical response of rat uterine smooth muscle is mediated primarily by Ca** and is blocked by Mn** and D-600. Further study of the interaction of AII with these two Ca**-dependent systems should be interesting.

Angiotensin also is known to interact with the autonomic nervous system in a variety of ways, including an action on cardiac sympathetic nerve terminals to increase release of norepinephrine. The reaggregated chick heart cells are denervated and circumvent this possibility. In the case of the rabbit and chick hearts, the inotropic effect of AII appears to result from a direct action on the cardiac cell membrane because even high concentrations of propranolol did not prevent or reduce the ability of the peptide to restore electrical and mechanical activity.

The negative data obtained with AII in studies of the guinea pig are consistent with results of previous studies in which the peptide was found to have little or no inotropic action in isolated atria bathed in normal Tyrode’s solution. From this, it would be expected that AII (10^{-8} M) would not be able to restore either electrical or mechanical activity to partially depolarized guinea pig atria, and this prediction was verified. Epinephrine, on the other hand, has a positive inotropic action in the chick, rabbit, and guinea pig hearts bathed in normal Tyrode’s solution, and it restored electrical and mechanical activity to all three preparations when they had been partially depolarized by elevated [K1]. The reason for the lack of response of guinea pig atria is not clear. Several possibilities include the absence of AII receptors in this tissue. We have no data to support or refute this possibility. Clearly, there are sufficient slow channels to allow for the action of catecholamines and therefore it is not likely that a defect in or absence of the slow channel mechanism is the reason for the lack of an AII effect. This assumes, of course, that the final common path in the slow channel mechanism is utilized by all effective agonists. An alternative possibility is that one of the membrane mechanisms coupling hormone-receptor interaction and the slow channel conductance is absent or inoperative. This possibility is especially intriguing because previous studies have suggested that in rat uterus the coupling mechanism is intimately associated with AII receptor occupation and depends upon Ca**. Whatever the explanation, the results clearly illustrate that AII has neither a direct nor an indirect agonist action on the isolated guinea pig heart. This feature of the pharmacological properties of AII can be advantageous in the study of the cardiovascular effects of the peptide. Because in guinea pigs changes in the rate and force of cardiac contractions accompany the intravenous administration of AII, these effects must be attributed to neurogenic involvement.

One final point worthy of note is that when extracellular K** is elevated there is a concentration-dependent positive chronotropic action of AII on rabbit right atrial preparations (Fig. 1). Although we have no direct evidence from our systems to implicate Ca**, in this phenomenon, it is consistent with a recent report in which the positive chronotropic action of epinephrine on frog atria was associated with an increase in inward Ca** current.

The maximum rate of the partially depolarized atria was only 13.6 ± 2.3 beats/min, a value that is only 14% of the spontaneous rate (97 ± 4.1 beats/min) observed in normal Tyrode’s solution. This rather weak chronotropic action may account for the lack of a clear positive chronotropic action of the peptide in vivo.

In conclusion, we believe the data provide evidence that the inotropic action of AII on cardiac muscle is due to an increase in the activity or availability of calcium current through the slow channels as a consequence of an interaction of the peptide with its receptor.

Acknowledgments

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Response of Aldosterone and Blood Pressure to Angiotensin II Infusion in Anephric Man

Effect of Sodium Deprivation


SUMMARY: Angiotensin II, infused intravenously, increased plasma aldosterone concentration in two of six anephric subjects taking their usual dietary quantities of sodium. After 3 days of dietary sodium restriction and weight-reducing hemodialysis, the aldosterone response to infused angiotensin II in the two previously reactive subjects was enhanced, while the four previously unreactive subjects also showed a rise in plasma aldosterone. Before and after sodium depletions the anephric subjects were less responsive than normal subjects. Even when sodium-depleted, the anephrics showed an aldosterone response with plasma angiotensin II concentrations up to at least 370 pg/ml. Before the infusion of angiotensin II, arterial plasma renin, angiotensin II, and aldosterone were detectable in the anephrics, but were unchanged by dietary sodium restriction or weight-reducing hemodialysis. Sodium depletions caused significant falls in weight, plasma sodium, and blood pressure, but no changes in plasma potassium or cortisol. Increases in blood pressure in relation to increments of arterial plasma angiotensin II were unaffected by sodium depletion, as might be expected in the absence of a rise in endogenous angiotensin II.

ALTHOUGH previously disputed,1-3 several studies recently have shown that in man the adrenal cortex is sensitized by sodium depletion in that the dose-response curve relating plasma aldosterone to angiotensin II is steepened significantly.4-7 Sodium depletion in man causes a rise in the circulating concentrations of renin and of angiotensin II,4-7 and recent evidence8 supports the suggestion that the sensitization in part may be the consequence of a trophic effect of angiotensin II on the adrenocortical zona glomerulosa. However, this seems not to be the full explanation, because the dose-response curves following prolonged angiotensin II infusion and following sodium depletion are similar in slope, but not identical.8

We felt that study of anephric subjects might clarify this problem because, although significant amounts of renin and angiotensin II may remain in the circulation after bilateral nephrectomy,11 the levels are said to be unchanged by shifts in sodium balance.13 Thus anephric man provides an opportunity to study possible alterations in the sensitivity of the adrenal cortex to angiotensin II in the presence of sodium depletion and in the absence of variations in endogenous renin or angiotensin II.

Several previous authors have suggested that, following bilateral nephrectomy, despite a normal rise in plasma cortisol after ACTH administration, the response of plasma aldosterone to angiotensin II is reduced16-18 or abolished.14,18,20 Such phenomena might represent the converse of the trophic effect of prolonged angiotensin II administration. Interpretation of these reports is difficult for several reasons. Angiotensin II usually has been given at a single dose rate and, as we have discussed,3 such experiments do not permit the distinction between changes in threshold and changes in slope of the dose-response curve. In none of these early studies have plasma angiotensin II concentrations been measured. Moreover, the state of sodium and potassium balance often has been unclear.

In the present study we examined the angiotensin II/aldosterone and angiotensin II/pressor dose-response relationships by giving a series of incremental infusions of angiotensin II before and after sodium depletion in anephric subjects. We compared the results to those of similar experiments performed under varying conditions of controlled sodium and potassium balance in normal man.4,8,10
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