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Aldosterone Response to Angiotensin Blockade in the Conscious Sodium-Depleted Rabbit (40732)

B. P. ROWE, J. O. DAVIS, R. H. FREEMAN, G. A. STEPHENS, J. M. DEFORREST, A. A. SEYMOUR, AND G. M. WILLIAMS

Department of Physiology, School of Medicine, University of Missouri, Columbia, Missouri 65212

The important role of the renin–angiotensin system in the control of the aldosterone response to sodium depletion is well established in several species. The advent of specific peptide inhibitors of the renin–angiotensin system proved an enormous asset in confirming the primacy of the mechanism. These inhibitors reduced the plasma aldosterone level in sodium-depleted man (1), dog (2–4) and rat (5, 6) but the situation in the sheep (7) and the rabbit (8) remains controversial. In the sodium-depleted, conscious rabbit, Steele and Lowenstein (8) failed to show a decrease in plasma aldosterone concentration (PAC) with [Sar^1, Ala^8] angiotensin II; instead they reported an agonism with low doses of the blocking agent. Experiments presented here with two specific inhibitors of the renin–angiotensin system, [Sar^1, Ala^8] angiotensin II and SQ 20881, were designed to reinvestigate the role of angiotensin II in the contral of aldosterone in the sodium-depleted, conscious rabbit.

Methods and materials. Animals. Fourteen male New Zealand rabbits (2.4–2.9 kg) were used in this study. Blood pressure was monitored continuously throughout the acute study from a chronically indwelling right carotid artery catheter (Tygon 40 × 70) by use of a pressure transducer (Statham P23DB) connected to a recorder (Hewlett–Packard 7702B). Two catheters were passed into the right jugular vein from a superficial branch. Dexamethasone sodium phosphate (Merck, Sharpe and Dohme) and inhibitory analogs of the renin–angiotensin system were administered via one catheter (Tygon 40 × 70) while angiotensin I (U.S. Biochemical Corporation) and angiotensin II (Hypertensin CIBA) were given via the second catheter (PE50, Clay Adams). All catheters were implanted under thiopental anesthesia, exteriorized between the shoulder blades and patency was maintained by daily withdrawal of blood and reinfusion of bovine heparin (200 U/ml) in 0.9% saline. After surgery, the animals were housed in stainless steel metabolic cages and allowed 15 mEq of sodium and 69 mEq of potassium per day (Purina rabbit chow) and water *ad libitum*. Daily food intake was measured and urine was analyzed daily; when normal sodium balance was established, control blood samples were taken, the bladder was catheterized and Mercuhydrin (0.5 ml im) was given on two successive days. After the first injection of Mercuhydrin, the animals were changed to a sodium-deficient diet (Teklad, Na deficient diet for rabbits) and maximum electrolyte intake was 1.5 meq of sodium and 30 meq of potassium per day. During 4–6 days of sodium restriction, cumulative net negative sodium balance was determined by balance studies and the conscious animal was placed in an open box but not restrained. Subsequently, the animals were subjected to one of the experimental procedures described below.

Infusion of [Sar^1 Ala^8] angiontensin II. Hypophyseal influences on steroidogenesis were minimized with Dexamethasone; 10 mg was given (im) 60 min before collection of the first control blood sample and thereafter infused at 1 mg/hr iv throughout the acute experiment. All infusions were given in a 0.9% sodium chloride vehicle (1.6 ml/hr). After a 60-min preinfusion control period, [Sar^1, Ala^8] angiontensin II (Morton-Norwich) was given (1 μg/kg/min, iv) for 60 min and followed by 50 μg/kg/min during the next 60 min; a 120-min recovery period was allowed. Blood samples were taken to determine plasma renin activity (PRA) and PAC at the end of each of the

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four periods in all seven animals. Blood pressure was monitored throughout and blockade of angiotensin vascular receptors was determined from the pressor response to angiontensin II (1 μg). Plasma electrolytes were measured at the end of the preinfusion control period and after antagonist infusion.

Infusion of SQ 20881. Dexamethasone was administered as described above. After an identical control period an iv bolus of 7 mg/kg SQ 20881 was followed by a constant infusion (60 μg/kg/min, iv) for 30 min in two animals and for 60 min in the remaining five. Blood samples were collected to determine PRA and PAC at the end of the control period and after 30 and 60 min of infusion of SQ 20881. Inhibition of angiotensin converting enzyme was ascertained by the pressor response to angiotensin I (2 μg) but the protocol was otherwise similar to that described in the previous section.

Assays. Plasma and urine electrolytes were determined by flame photometry. The plasma was obtained from 1 ml of blood collected over 1 drop of bovine heparin (1000 U/ml). PRA and PAC were determined from plasma samples obtained by collecting 5 ml blood over 0.1 ml of a 10% solution of disodium ethylenediaminetetraacetate (EDTA) and the red cells were resuspended in 0.9% NaCl and returned to the animal. Normal sodium replete values for PRA, PAC, and electrolytes were determined from samples taken before sodium restriction. PRA and PAC were determined by radioimmunoassay modified from methods previously described (9, 10).

Statistical analysis. Student's paired *t* test was used for statistical evaluation of the data and the values are presented as mean ± standard error of the mean.

Results. Effects of Infusion of [Sar^1, Ala^8] angiotensin II. The mean cumulative net negative sodium balance in seven animals was 32.2 ± 2.4 meq; no value below 25 meq was recorded. Many of the animals found the sodium-deficient diet unpalatable and rarely consumed all food offered. Plasma potassium and sodium concentrations were unaltered during the acute experiment but both were reduced after altering the dietary regimen. The plasma sodium concentration fell from 140.7 ± 4.4 to 135.3 ± 3.0 meq/liter ($P < 0.005$) and plasma potassium concentration fell from 4.4 ± 0.1 to 3.0 ± 0.2 meq/l ($P < 0.0005$) (Table I).

The blood pressure response is also presented in Table I. Vascular angiotensin receptor blockade with [Sar^1, Ala^8] angiotensin II was demonstrated by almost total abolition of the acute pressor response to

TABLE I. PLASMA ELECTROLYTE AND BLOOD PRESSURE CHANGES DURING INFUSION OF [Sar^1, Ala^8] ANGIOTENSIN II IN SODIUM-DEPLETED RABBITS ($N = 7$)

	Plasma sodium (meq/liter)	Plasma potassium (meq/liter)	Pressor response to AII (mm Hg)	Mean arterial pressure (mm Hg)
Preinfusion control	135.3 ± 1.4[a]	3.0 ± 0.2	29 ± 3	81 ± 4
[Sar^1, Ala^8] Angiotensin II (1 μg/kg/min)	—	—	2 ± 1	64 ± 7*
[Sar^1, Ala^8] Angiotensin II (50 μg/kg/min)	136.0 ± 1.0	3.1 ± 0.2	0 ± 0	60 ± 6*
Recovery	—	—	10 ± 3	61 ± 5*
Sodium replete condition	140.7 ± 0.6*	4.4 ± 0.1*	—	—

[a] Values are means ± SE. Figures in columns 1, 2, and 4 are compared with preinfusion controls (paired Student's *t* test).
* $P < 0.005$.

angiotensin II (1 μg) with the 1 μg/kg/min dose of the antagonist. Only partial recovery of the pressor response occurred after 120 min; this analogue is particularly resistant to angiotensinase activity. Infusion of [Sar^1, Ala^8] angiotensin II (1 μg/kg/min) reduced arterial pressure from 81 ± 4 to 64 ± 7 mm Hg ($P < 0.005$) and further to 60 ± 6 mm Hg ($P < 0.005$) when the dose was increased to 50 μg/kg/min. Arterial pressure remained suppressed (61 ± 5 mm Hg) 120 min later.

PRA and PAC changes are summarized in Fig. 1. With the exception of the isolated sodium replete values, all remaining values were obtained from Dexamethasone-treated, sodium-depleted animals; all experimental values are compared with preinfusion controls. PRA increased with sodium depletion from 1.2 ± 0.2 to 10.3 ± 2.0 ng angiotensin I/ml/hr ($P < 0.025$). Infusion of [Sar^1, Ala^8] angiontensin II (1 μg/kg/min) for 60 min increased PRA further to 20.4 ± 3.0 ng angiontensin I/ml/hr ($P < 0.0005$) and when the dose was increased to 50 μg/kg/min PRA was elevated to 23.0 ± 3.8 ng angiotensin I/ml/hr ($P < 0.0005$). The recovery value (19.4 ± 3.6 ng angiontensin I/ml/hr) was also higher than preinfusion controls ($P < 0.01$). During sodium restriction PAC increased from 10.6 ± 4.0 to 41.1 ± 8.2 ng % ($P < 0.01$) and after 60 min infusion of [Sar^1, Ala^8] angiotensin II (1 μg/kg/min) this value was not significantly reduced (33.3 ± 7.4 ng%); when the dose was increased to 50 μg/kg/min, PAC decreased to 15.4 ± 3.7 ng% ($P < 0.025$ compared to the preinfusion control of 41.1 ± 8.2 ng%). This value of 15.4 ± 3.7 ng% did not differ significantly from that (10.6 ± 4.0 ng%) in the normal sodium replete rabbits ($P > 0.05$, paired t test). After 120 min of recovery, PAC returned to 57.2 ± 14.0 ng% which was not significantly different from the preinfusion control value.

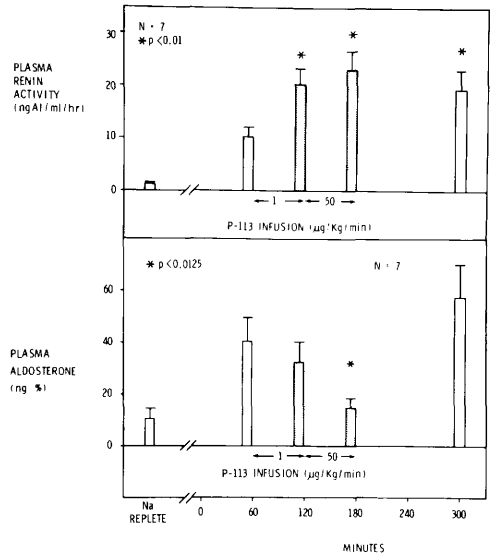

FIG. 1. Effects of [Sar^1, Ala^8] angiotensin II on plasma renin activity and plasma aldosterone concentration. Stippled columns represent values obtained during infusion of [Sar^1, Ala^8] angiotensin II at 1 and 50 μg/kg/min in the sodium-depleted animals. Error bars are SE of the mean.

Effects of infusion of SQ 20881. Mean cumulative net negative sodium balance for this series of seven animals was 30.2 ± 0.8 meq. Nearly all sodium was excreted during the first 2 days of depletion and in one animal where the value failed to reach 25 meq, a further dose of Mercuhydrin was administered (0.3 ml, im) to ensure adequate depletion. Plasma electrolytes are presented in Table II; values from sodium replete animals and values obtained at the end of the SQ 20881 infusion were compared with those obtained at the end of the preinfusion control period. No changes in either plasma sodium or potassium occurred during the acute experiment. Dietary manipulation produced a small fall in plasma sodium concentration from 138.7 ± 1.2 to 136.4 ± 0.9 meq/liter ($P < 0.05$) but the plasma potassium level was unchanged.

Blood pressure data are also presented in Table II. The pressor response to angiotensin I (2 μg) indicated inhibition of angiotensin converting enzyme but even with this large dose of SQ 20881, complete blockade did not occur. Partial recovery of the response was demonstrated 150 min after termination of infusion. Infusion and post-infusion mean arterial blood pressure values were compared with preinfusion control values; during infusion SQ 20881 reduced arterial pressure from 74 ± 4 to 61 ± 5 mm Hg ($P < 0.0005$). The recovery value (68 ± 4 mm Hg) was also significantly re-

TABLE II. PLASMA ELECTROLYTE AND BLOOD PRESSURE CHANGES DURING INFUSION OF SQ 20881 IN SODIUM-DEPLETED RABBITS

	Plasma sodium (meq/liter)	Plasma potassium (meq/liter)	Pressor response to AI (mm Hg)	Mean arterial pressure (mm Hg)
Preinfusion control	136.4 ± 0.9[a]	4.0 ± 0.1	22 ± 3	74 ± 4
After infusion of SQ 20881	136.8 ± 0.9	4.0 ± 0.5	8 ± 2(t60)[b]	61 ± 5**
Recovery	—	—	16 ± 3	68 ± 4*
Sodium deplete condition	138.7 ± 1.2*	4.4 ± 0.5	—	—

[a] Values are means ± SE. Figures in columns 1, 2, and 4 are compared with preinfusion controls (paired Student's *t* test).

[b] SQ 20881 infusion started with a bolus of 7 mg/kg followed by 60 μg/kg/min. The infusion was terminated 30 min later in two animals (t30) but extended to 60 min (t60) in the remaining five.

* $P < 0.05$.

** $P < 0.0005$.

duced from the preinfusion control ($P < 0.05$) but was significantly higher than the infusion value ($P < 0.05$) which indicated that recovery was incomplete.

Changes in PRA and PAC are presented in Fig. 2. As before, sodium-depleted animals were treated with Dexamethasone and all values were compared with preinfusion controls. Sodium depletion produced an increase in PRA from the normal replete value of 1.6 ± 0.4 to 6.7 ± 1.0 ng angiotensin I/ml/hr ($P < 0.005$). During infusion of SQ 20881, PRA increased further from 6.7 ± 1.0 to 14.3 ± 2.3 ng angiotensin I/ml/hr ($P < 0.0025$) at 30 min and to 13.9 ± 2.8 angiotensin I/ml/hr ($P < 0.01$) at 60 min. The recovery value (12.1 ± 1.4 ng angiotensin I/ml/hr) was also significantly elevated ($P < 0.001$). Sodium restriction induced a rise in PAC from 11.9 ± 2.6 to 86.6 ± 21.0 ng% ($P < 0.01$) and during infusion of SQ 20881 PAC fell from 86.6 ± 21.0 to 63.9 ± 18.2 ng% ($P < 0.025$) at 30 min and 63.3 ± 16.2 ng% ($P < 0.01$; $n = 5$) at 60 min. PAC showed an excellent recovery value of 100.0 ± 24.0 ng% which was not significantly different from the preinfusion control value.

Discussion. Earlier studies have demonstrated that the aldosterone response to sodium depletion is mediated by the renin–angiotensin system in the rat (5, 6), man (1) and the dog (2–4) but the situation in the sheep remains unclear (7). The rabbit has been less widely studied and although exogenous angiotensin has been shown to elevate PAC in normal conscious or anesthetized animals (11, 12) the renin–angiotensin system has not been implicated in

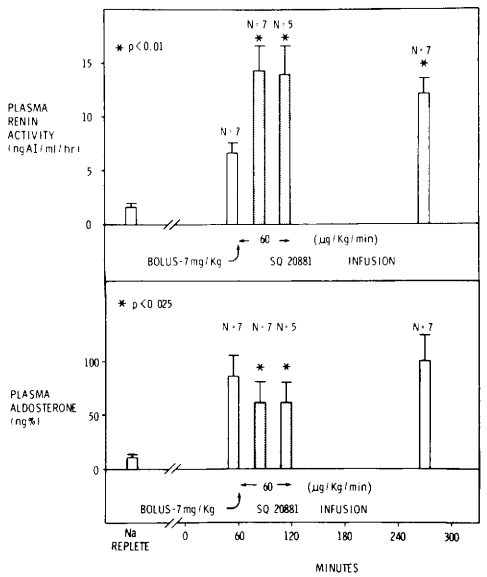

FIG. 2. Effects of SQ 20881 on plasma renin activity and plasma aldosterone concentration. Stippled columns show values obtained during infusion of SQ 20881 (60 μg/kg/min) into sodium-depleted animals. The infusion was initiated after a bolus of 7 mg/kg (iv) and was terminated at 30 min in two animals and 60 min in the remaining five. Error bars are SE of the mean.

the aldosterone response to sodium depletion.

In 1972, Lowenstein and associates (13) found that PAC increased during sodium depletion in angiotensin II-immune rabbits similar to that which occurred in nonimmune animals. Similar experiments (8) also failed to show a reduction of PAC when [Sar^1, Ala^8] angiotensin II (5 μg/kg/min, iv) was given to sodium-depleted rabbits but a lower dose (1 μg/kg/min) showed an agonistic action at the adrenal cortex. Some analogs of angiotensin II with an amino acid substitution in the 8-position are excellent inhibitors of vascular angiotensin receptors but an agonistic action has been reported for the adrenal cortex (3, 4). It seems probable that his phenomenon is associated with physiological conditions in which endogenous angiotensin is normal or only slightly elevated (3, 4) and this is consistent with the concept of a partial agonist.

In the present study both [Sar^1, Ala^8] angiotensin II and the converting enzyme inhibitor, SQ 20881, reduced PAC in the conscious, sodium-depleted rabbit; these observations lend support to the concept of renin–angiotensin involvement in this model. Despite almost total blockade with [Sar^1, Ala^8] angiotensin II of vascular responses to bolus injections of angiotensin II and a substantial decrease in arterial pressure with 1 μg/kg/min of the antagonist, no change in PAC was observed at this dose level. It is of interest that an excellent response in PRA occurred with the 1 μg/kg/min level of infusion of the angiotensin II antagonist. Also, preliminary experiments in the present study showed no reduction of PAC with infusions of [Sar^1, Ala^8] angiotensin II between 1 and 10 μg/kg/min which closely parallels Steele and Lowenstein's data and confirms their view that adrenal and vascular receptors show distinctly different characteristics in this model. The present data indicate that the response in PRA is similar to that observed from blockade of the vascular receptors. A dose of 50 μg/kg/min [Sar^1, Ala^8] angiotensin II reduced PAC to within the normal sodium replete levels in the present study whereas 6 μg/kg/min in the chronically, sodium-depleted conscious dog (4), and 10 μg/kg/min in the anesthetized hypophysectomized, sodium-depleted rat (5) were sufficient to effect the same response. These findings suggest important species differences in the responses in PAC.

The converting enzyme inhibitor, SQ 20881, was also given in very high doses in the present study and, despite this, blockade of the vascular response to angiotensin I remained incomplete. Relatively poor efficacy of converting enzyme inhibitors appears to be peculiar to the rabbit and this conclusion is supported by others (14). In the present group of animals the reduction in PAC after SQ 20881 was significant ($P < 0.025$) but less substantial than the response to [Sar^1, Ala^8] angiotensin II; this difference might be related to incomplete inhibition of converting enzyme activity.

Sodium depletion produced an elevation in PRA and PAC in both groups of animals. Both inhibitors induced a further rise of PRA due to a fall in blood pressure and interruption of the negative feedback loop of angiotensin II on renin release. During infusion of these inhibitors plasma sodium and potassium concentrations were unchanged and a fall in PAC in both groups of animals indicates that the renin–angiotensin system exerted direct control on aldosterone production.

In the present study, hypophyseal influences on the adrenal cortex were eliminated and an action of plasma potassium was excluded since hyperkalemia was not present. Failure of PAC to return to normal sodium replete levels during blockade of the renin–angiotensin system might be attributed to incomplete blockade but we cannot exclude the possibility of other unknown factors. The observations show that the renin–angiotensin system does control, at least in part, the aldosterone response to sodium depletion in the conscious rabbit.

Summary. To evaluate the role of the renin–angiotensin system in the aldosterone response to sodium depletion in the conscious rabbit, angiotensin blockade was achieved with [Sar^1, Ala^8] angiotensin II and SQ 20881. After infusion of [Sar^1, Ala^8] angiotensin II (1 μg/kg/min) for 60 min into sodium-depleted animals, PAC was unchanged (33.3 ± 7.4 ng% compared with a

control sodium-deplete value of 41.1 ± 8.2 ng%). When the dose was increased to 50 μg/kg/min for an additional 60 min, PAC fell to 15.4 ± 3.7 ng% ($P < 0.0125$), a value similar to the sodium replete value. In a second group of animals, SQ 20881 was given initially as a 7 mg/kg bolus and followed by an infusion of 60 μg/kg/min for 30 min; PAC fell from a sodium-deplete level of 86.6 ± 21.0 to 63.9 ± 18.2 ng% ($P < 0.025$). The infusion was extended to 60 min in five of the seven animals and PAC remained significantly reduced (63.3 ± 16.2 ng%, $P < 0.01$). These studies demonstrate that attenuation of the aldosterone response to sodium depletion occurred with both [Sar[1], Ala[8]] angiotensin II and SQ 20881 in the conscious rabbit indicating a role for the renin–angiotensin system in this model.

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