Stimulation of Vascular Na-K Pump with Subpressor Angiotensin II in Rats (43376)

GEZA SIMON[1]

Department of Medicine, Veterans Administration Medical Center and University of Minnesota, Minneapolis, Minnesota 55417

Abstract. **The effect of subpressor doses of angiotensin II (ANG II) on vascular Na-K pump activity and Na-H exchange, two transmembrane signals of trophic stimulation of vascular muscle, was investigated. Male Sprague-Dawley rats (350–400 g) were given subpressor doses of ANG II by osmotic minipump intraperitoneally for 24 hr or 7–10 days. Control rats received sham procedure/vehicle infusion. Na-K pump activity (^{86}Rb uptake), total and intracellular (Li exchange at 4°C) Na content, and amiloride-sensitive and -insensitive Na uptake of aortas were measured *ex vivo*. Ouabain-sensitive ^{86}Rb uptake of aortas of rats receiving 80–100, 160–180, and 240–260 ng/kg·min^{-1} of ANG II for 24 hr was 26.6 ± 3.5, 28.8 ± 3.4, and 29.1 ± 2.6 nmol/mg dry wt·15 min^{-1} (mean ± SD, n = 7–12), respectively, compared with 25.2 ± 3.8 in controls (n = 23, $P < 0.01$). These increases were maintained at 7–10 days. After 24 hr and 7–10 days of ANG II treatment, the total Na content of aortas was increased by 9.2% ($P < 0.01$) and 7.6% ($P < 0.02$), respectively, without a change in intracellular Na content, indicating accumulation of excess extracellular Na. Total and amiloride-sensitive Na uptake of the aorta was unchanged after 24 hr or 7–10 days of ANG II administration. The dry weight of anatomically defined segments of the aorta was 40 ± 3.8 mg/kg body wt (n = 25) after 24 hr and 42 ± 4.4 (n = 20) after 7–10 days of ANG II administration, compared with 37 ± 4.8 (n = 15, $P < 0.05$) and 37 ± 4.9 (n = 17, $P < 0.01$) in appropriate controls. Increased Na-K pump activity may signal the onset of trophic stimulation of vascular muscle by ANG II.** [P.S.E.B.M. 1992, Vol 199]

According to a recent hypothesis, the initiating cause of hypertension may be trophic stimulation of vascular muscle (1). Hypertrophy, in turn, may lead to vascular hyperreactivity, pressor hyperresponsiveness, and, by positive feedback, hypertension. A number of candidate trophic factors have been proposed to be operative, such as sympathetic nervous system activation, norepinephrine, angiotensin II (ANG II), vasopressin, adrenal steroids, and insulin (1). ANG II is a prime candidate because previously it has been shown that when administered chronically in subpressor doses, it moves its own pressure-dose-response curve in an upward direction and may lead to hypertension (1–4). In tissue culture, ANG II stimulates several transmembrane signaling mechanisms involved in mitogenic stimulation and agonist-mediated contraction of vascular smooth muscle cells, including Na influx, Na-H exchange, and Na-K pump activity (5–9). Increased Na-K pump activity is associated with cell growth in many cell types (10–12). The Na-H exchange process is an important modulator of Na-K pump activity, and itself may be involved in growth regulation (12, 13). As a first step toward establishing ANG II as an endogenous growth factor of vascular muscle, we have, therefore, measured the effect of ANG II on vascular Na-K pump activity and Na-H exchange of rats. ANG II was administered short-term (24 hr) and long-term (7–10 days) to determine whether the observed effects of ANG II were sustained or not. Subpressor doses of ANG II were used to eliminate the confounding influence of arterial pressure changes on the measured parameters. In this regard, the one study that we could find that examined the long-term *in vivo* effects of ANG II on vascular Na transport did not distinguish between pressure-related and agonist-mediated effects (14).

[1] To whom requests for reprints should be addressed at 111 J2, VA Medical Center, One Veterans Drive, Minneapolis, MN 55417.

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Materials and Methods

Young adult, male Sprague-Dawley rats, weighing 350–400 g, were used throughout these studies. Syntheic ANG II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe; Sigma Chemical, St. Louis, MO) was infused into rats intraperitoneally by osmotic minipump (model 2002; Alza, Palo Alto, CA) for about 24 hr (short-term) or 7–10 days (long-term). ANG II was dissolved in saline at 4–10 mg/ml. Acetic acid (final concentration 0.01 *N*) was added to maintain the stability of ANG II. For the insertion of the minipump, rats were anesthetized with methoxyflurane. Control rats were fitted with minipump containing saline and 0.01 *N* acetic acid. The rats had free access to tap water and received standard chow (Ralston Purina No. 5002, Na 115 mmol/kg, K 250 mmol/kg). For short-term studies, systolic blood pressure (SBP) was measured in restrained awake rats by the tail cuff method (Narco Biosystems, Inc., Houston, TX) 2–3 hr prior to the experiments. For long-term studies, SBP was measured twice on different days and the results were averaged. The rats were weighed to the nearest 1 g on the day of the experiment.

For removal of the aorta, rats were anesthetized with methoxyflurane and exsanguinated from the cannulated abdominal aorta. The blood was used to measure plasma renin activity (PRA) by radioimmunoassay of angiotensin I generation (^{125}I-angiotensin I Radioimmunoassay Kit; New England Nuclear, North Billerica, MA) and plasma creatinine, Na, and K concentrations (autoanalyzer). Purposefully, we measured PRA of rats under stimulation of anesthesia and laparotomy to improve our ability to detect suppression of renin release by infused ANG II. The heart was also removed from some of the rats, cleaned of adhering tissue and blood, cut into small pieces, dried to constant weight in a 90°C oven, and weighed to the nearest 1 mg. Dry weight of heart was expressed as mg/kg body wt.

Na-K Pump Activity of Aorta

For measurement of membrane Na-K pump activity by ^{86}Rb uptake, the same anatomical segment of the aorta, from the left subclavian to the right renal artery, was removed. The aorta was cleaned of adhering tissue and cut into three equal segments. Tissue segments were cut open longitudinally, and the endothelium was removed by rubbing the luminal surface of the aorta to the bottom of a plastic dish. The two proximal segments were used for the uptake experiments and the distal segment for the cation measurements (see below). Pump activity was measured under two different Na loading conditions: in freshly excised tissue and in tissue that equilibrated in Krebs-Henseleit (K-H) solution (see below) for 90 min (low-Na tissue) (15). In freshly excised tissue, intracellular Na content is higher than under equilibrium conditions (15). Other investigators have shown that differences in pump activity between experimental groups are more readily detectable when intracellular Na content is high, and the pump is stimulated, than under equilibrium conditions (16, 17).

^{86}Rb uptake was used as an analog for active K uptake. ^{86}Rb uptake of rat aorta was measured according to the techniques of Brock and co-workers (16). The two segments of the thoracic aorta were incubated in aerated (95% O_2 and 5% CO_2) K-H solution (in m*M*: NaCl 118, $NaHCO_3$ 27, KCl 4.8, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.25, and glucose 11.1) for 5 min (fresh tissue) or 90 min (equilibrated tissue) at 37°C. One segment was then transferred to K-H solution containing ^{86}RbCl (0.5 mCi/ml) on Day 0; New England Nuclear) and 2 m*M* ouabain, and the other to K-H solution containing ^{86}RbCl but no ouabain. After 15 min, tissue segments were rinsed in four 50-ml aliquots of ice-cold buffered (Tris 10 m*M*) 0.25 *M* sucrose solution, blotted, and dried to constant weight in a heated (50°C) vacuum desiccator. The radiotracer was eluted in 0.75 *N* HNO_3 and counted (Auto-Gamma; Packard). The rate of ouabain-sensitive ^{86}Rb uptake was calculated as the difference in the rates of ^{86}Rb uptake by the tissue in the presence and absence of ouabain and was expressed as nmol/mg dry wt·15 min^{-1}. Previously, we have shown that the ^{86}Rb uptake of rat aorta smooth muscle cells in tissue culture was linear for 20 min (18). Others have demonstrated the linearity of ^{86}Rb uptake of the intact rat aorta over the same time period (19).

For each rat, the dry weight of the aorta, from the left subclavian artery to the right renal artery, was calculated by adding the dry weight of aortic segments used for the above measurements. The dry weight of the aorta was expressed as mg/kg body wt.

Total and Intracellular Cation Content of Aorta

Tissue Na, K, Ca, and Mg were eluted from freshly removed, cleaned, and dried aortic segments (see above) with 0.75 *N* HNO_3 for 7 days. Cation concentrations were measured by atomic absorption spectrophotometry, and results were expressed as mmol/kg dry wt.

Intracellular Na and K contents were estimated by the cold Li exchange method (20). The excised aorta was cut into six equal segments. Tissue segments were incubated in K-H solution at 37°C for 5 min (fresh tissue) or 90 min (equilibrated tissue), then transferred to 50 ml of ice-cold, aerated, Li-substituted salt solution (in m*M*: LiCl 115, $LiHCO_3$ 25, LiH_2PO_4 1.2, KCl 5.0, $CaCl_2$ 1.7, $MgSO_4$ 1.2, and dextrose 11.0). The pH of the Li-substituted salt solution was adjusted to 7.40–7.46 by bubbling with a 95% O_2–5% CO_2 gas mixture and was monitored with a pH meter during the entire incubation. One tissue segment was harvested at 20, 30, 40, 50, 60, and 70 min. Tissue Na and K contents were measured as outlined above. In preliminary experiments, we have determined that it requires 15–20

min to reach the "flat" portion of the Li-Na exchange curve, representing the slow exchange of intracellular Na by extracellular Li.

Na Uptake of Aorta

The excised and cleaned thoracic aorta was cut into five equal segments. Tissue segments were incubated in aerated (95% O_2 and 5% CO_2) K-H solution at 37°C for 90 min to standardize the preparation by reestablishing transmembrane cation gradients (15). At 90 min, one tissue segment, representing the initial Na and K content (Time 0) was harvested, then 2 m*M* ouabain was added to the incubation medium. The remaining tissue segments were harvested 20, 40, 60, and 90 min after the addition of ouabain. The harvested tissue segments were transferred for 30 min to ice-cold (4°C) Li-substituted salt solution to exchange extracellular Na with Li. Remaining (intracellular) tissue Na and K were measured by atomic absorption spectrophotometry after tissue digestion in 0.75 *N* HNO_4 for 1 week (see above). Tissue Na and K contents expressed as mmol/kg dry wt were plotted against the time of incubation in ouabain-containing K-H solution. In separate groups of rats, these experiments were repeated with the addition of both ouabain and 5 m*M* amiloride to the incubation medium so that the amiloride-sensitive component of Na uptake, representing Na-H exchange, could be calculated (13, 21). Finally, to validate our methodology and to estimate the maximal rate of Na uptake, in three unoperated rats, Na uptake of the aorta was measured in the presence of ouabain and 10 μM monensin, a Na ionophore.

Statistical Analysis

Results are reported as means ± SD. Group mean values of rats receiving the three different doses of ANG II and of control rats were compared by one-way analysis of variance (*F* test). Time-dependent cation washout, uptake, and efflux curves of ANG II-treated and control rat aortas were analyzed by repeated measures (one factor within, one between) analysis of variance (Superanova; ABACUS Concepts, Berkeley, CA). The dry weights and cation contents of the aorta of ANG II-treated and control rats were compared by unpaired Student's *t* test. The null hypothesis was rejected at $P < 0.05$.

Results

Forty-two rats received ANG II for 24 hr, 69 rats received ANG II for 7 to 10 days, and a similar number of rats served as short- and long-term controls. At no time were we able to detect a statistically significant difference in the tail SBP of ANG II-treated and control rats (see below). A total of 55 rats received 240–260 ng/kg·min^{-1} of ANG II, the highest dose used, for 7 to 10 days. Their SBP, 136 ± 8 mm Hg, did not differ from that of 54 long-term controls, 131 ± 6. The body weights and plasma creatinine, Na, and K concentrations of ANG II-treated and control rats also did not differ (data not shown).

SBP, PRA, and aortic ^{86}Rb uptakes of ANG II-treated and control rats are tabulated in Table I. These aortas were freshly excised and underwent only 5 min of preincubation prior to ^{86}Rb uptake measurements. The precise dose of ANG II used depended on the weight of the rat. There were no significant differences in SBP among groups of rats. The PRA of control rats after 24 hr of sham infusion was numerically lower than that of long-term controls. This difference may have been due to salt and water retention during the first day after general anesthesia and abdominal surgery. Compared with appropriate controls, there was a tendency for PRA of all ANG II-treated rats to be reduced, but this trend reached statistical significance only after 7–10 days of treatment. There was a dose-dependent increase in ouabain-sensitive ^{86}Rb uptake of the aorta of rats after 24 hr of ANG II treatment. Pump stimulation was maintained for 7 to 10 days with the highest dose of administered ANG II. There was no significant change in ouabain-insensitive ^{86}Rb uptake of the aorta of ANG II-treated rats.

In contrast to the results tabulated in Table I, there were no differences in ouabain-sensitive ^{86}Rb uptake of the aorta of ANG II-treated and control rats, when the aortas were preincubated for 90 min (low-Na tissue) prior to the addition of the radiolabel. Under these conditions, ouabain-sensitive ^{86}Rb uptake of the aorta of control rats (11.9 ± 2.2 nmol/mg dry wt·15 min^{-1}, mean ± SD, $n = 10$) was about one half of that of aortas preincubated for only 5 min (Table I). After 24 hr and 7–10 days of ANG II treatment, ouabain-sensitive ^{86}Rb uptake of aortas was 11.9 ± 0.6, $n = 10$, and 12.6 ± 1.6, $n = 6$, respectively.

In Table II, the total aortic Na, K, Ca, and Mg contents of rats receiving ANG II for 24 hr and of control rats are tabulated. The Na content of the aorta of ANG II-treated rats was increased significantly. There were no changes in the other cation contents. Nearly identical results were obtained after 7–10 days of ANG II treatment, including an increase in Na content (312 ± 15 mmol/kg dry wt, $n = 16$, vs 290 ± 19, $n = 13$, in controls, $P < 0.02$).

The results of Li washout of the aorta of rats receiving 240–260 ng/kg·min^{-1} of ANG II for 7 to 10 days and of control rats are shown in Figure 1. Like the rats represented in Table I (lower half), these ANG II-treated rats remained normotensive (135 ± 12 mm Hg, $n = 16$, vs 134 ± 9 mm Hg, $n = 17$, in controls). In both ANG II-treated and control rats, after only 5 min of preincubation, tissue levels of Na were higher and K levels were lower than after 90 min of preincubation. There were no differences in the Na content of the

Table I. Systolic BP, PRA, and ^{86}Rb Uptake of Freshly Excised Aortas of Rats after 24 Hr or 7–10 Days of Angiotensin II Treatment or Sham Procedure/Vehicle Infusion (Control)[a]

Parameters	Control ($n = 23$)	Angiotensin II (ng/kg·min^{-1}) × 24 hr			P^b
		80–100 ($n = 7$)	160–180 ($n = 12$)	240–260 ($n = 8$)	
Systolic BP (mm Hg)	126 ± 12	115 ± 13	134 ± 9	131 ± 14	NS
PRA (ng/ml·hr^{-1})	7.4 ± 3.5	4.8 ± 1.1	5.7 ± 2.8	4.4 ± 4.1	NS
^{86}Rb uptake[c]					
Ouabain sensitive	25.2 ± 3.8	26.6 ± 3.5	28.8 ± 3.4	29.1 ± 2.6	<0.01
Ouabain insensitive	7.5 ± 1.3	7.2 ± 1.8	6.9 ± 1.5	7.0 ± 1.8	NS
		Angiotensin II (ng/kg·min^{-1}) × 7–10 days			
	($n = 22$)	($n = 6$)	($n = 8$)	($n = 8$)	
Systolic BP (mm Hg)	129 ± 15	129 ± 27	120 ± 10	137 ± 12	NS
PRA (ng/ml·hr^{-1})	12.1 ± 6.7	7.5 ± 6.6	9.8 ± 1.7	4.4 ± 5.0	<0.05
^{86}Rb uptake[c]					
Ouabain sensitive	26.2 ± 4.7	24.3 ± 5.0	28.9 ± 6.0	33.3 ± 8.8	<0.05
Ouabain insensitive	7.9 ± 1.0	8.4 ± 1.1	7.6 ± 1.8	8.2 ± 1.3	NS

[a] Values are means ± SD. Freshly excised aortas were incubated for 5 min prior to the addition of radiolabel.
[b] Probability values for overall treatment effect by one-factor analysis of variance.
[c] nmol/mg dry wt·15 min^{-1}.

Table II. Electrolyte Content of Thoracic Aorta of Rats after 24 Hr of Angiotensin II Treatment or Sham Procedure/Vehicle Infusion[a]

Parameters (mmol/kg dry wt)	Control	n	ANG II × 24 hr	n
Sodium	284 ± 23	14	310 ± 14[b]	14
Potassium	114 ± 12	14	118 ± 7	14
Calcium	12.6 ± 1.6	11	12.2 ± 1.1	11
Magnesium	10.9 ± 1.3	11	11.2 ± 1.1	11

[a] Values are means ± SD. The same rats as in Table I were used.
[b] $P < 0.01$ by Student's t test for unpaired replicates.

aortas of the two groups of rats after either 5 or 90 min of preincubation. Although the K content of the aortas of ANG II-treated rats appeared to be higher than that of controls, these differences did not reach statistical significance. However, the possibility that the K content of ANG II-treated aortas is increased at the end of 90 min of preincubation was tested a priori during subsequent measurements of Na uptake (see below). The results of Li washout of aortas after 24 hr of ANG II or sham treatment showed complete overlap of the cation washout curves (data not shown).

In Figures 2 and 3, the Na uptake and K efflux curves of the aortas of rats receiving ANG II for 7 to 10 days and of control rats are shown. Confirming previous findings, the SBP of the two groups of rats did not differ, 136 ± 12 ($n = 19$) vs 131 ± 9 ($n = 22$) mm Hg. At the end of 90 min of preincubation, the K content of the aorta of ANG II-treated rats, 111 ± 13 mmol/kg, was increased compared with that of control rats, 99 ± 8 ($P < 0.01$, Fig. 3). There were no differences in the initial Na content of the aorta among groups of rats (Fig. 2). The method used for measuring Na uptake was validated by demonstrating a rapid influx of Na with monensin and by inhibiting Na uptake with amiloride (Fig. 2). The total Na uptake curves of ANG II-treated and of control rats were not significantly different. In contrast, amiloride-insensitive Na uptake of the aorta was increased in ANG II-treated rats ($P < 0.02$). Amiloride-sensitive Na uptake, the difference between total and amiloride-insensitive Na uptake, was not significantly different between the two groups. The K efflux curves of the aortas of the two groups of rats overlapped both in the presence and absence of amiloride (Fig. 3). The Na uptake and K efflux curves obtained in the presence of monensin were not statistically compared with the other curves. After only 24 hr of ANG II treatment, the Na uptake and K efflux curves of ANG II-treated ($n = 7$) and of control rats ($n = 7$) overlapped over the entire time range (data not shown).

Finally, because the same anatomic segment of the aorta was removed from all rats used for the ^{86}Rb uptake experiments (see above), we were able to calculate the dry weight of the aorta for each rat. The weight of the aorta of rats receiving ANG II for 24 hr or 7–10 days was 40 ± 3.8 ($n = 25$) and 42 ± 4.4 ($n = 20$) mg/kg body wt, respectively, compared with 37 ± 4.8 ($n = 15$, $P < 0.05$) and 37 ± 4.9 ($n = 17$, $P < 0.01$) in their appropriate controls. There were no differences in the dry heart weight of ANG II-treated and control rats after either 24 hr (data not shown) or 7–10 days of treatment (626 ± 40 mg/kg body wt, $n = 21$, vs 626 ± 25, $n = 16$).

Discussion

In the present study, subpressor doses of ANG II were given to rats. At no time were there significant

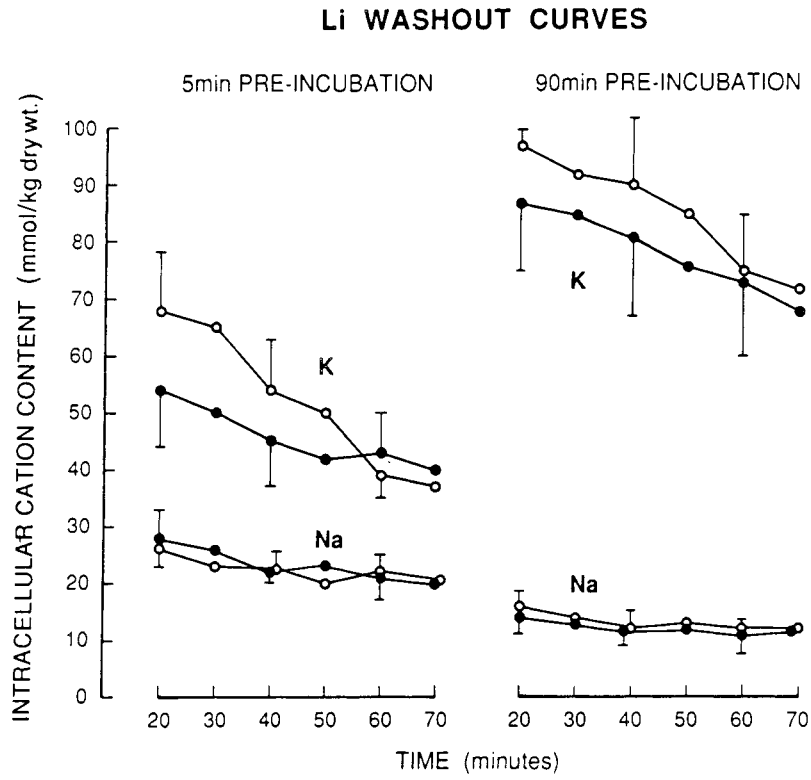

Figure 1. Intracellular Na and K contents of the aorta of rats during Li washout. Open and closed symbols represent the aorta of rats treated with ANG II for 7 to 10 days and of control rats, respectively. Symbols and vertical bars represent the mean ± SD of measurements obtained from seven to nine rat aortas.

differences in the SBP of ANG II-treated and control rats. The absence of cardiomegaly in ANG II-treated rats provided indirect evidence that a sustained rise in blood pressure did not occur. The doses of ANG II entering the systemic circulation were apparently so small that suppression of PRA was demonstrable only after 7–10 days of continuous administration of the agonist. Other investigators have used similar doses of intraperitoneal ANG II in rats and also found them to be subpressor (22, 23).

The two most significant findings in this study have been the stimulation of vascular Na-K pump activity and the accumulation of excess Na in the wall of the aorta with subpressor doses of ANG II. Both findings were detectable within 24 hr of the administration of the agonist. However, stimulation of vascular Na-K pump activity was detected only in freshly excised tissue with higher intracellular Na content, but not in equilibrated tissue with lower intracellular Na content. Other investigators have also found that vascular Na-K pump activity has to be stimulated by increased intracellular Na content to detect differences in pump stimulation between experimental groups (16, 17).

Based on this study, the cellular mechanisms responsible for pump activation of the aorta by ANG II cannot be determined. While intracellular Na is the principal regulator of pump activity, cold Li exchange did not reveal changes in the intracellular Na content of the aorta of ANG II-treated rats at either 24 hr or 7–10 days. The Li exchange method, however, does not distinguish between intracellular Na freely dissolved in cell water and bound Na. The possibility of increased intracellular Na concentration in ANG II-treated rats cannot be ruled out by these measurements. Evidence is also lacking for activation of Na-H antiporter as measured by amiloride-sensitive Na uptake. In this regard, these *in vivo* studies differ from experiments conducted in tissue culture, where ANG II is a potent activator of Na-H exchange of vascular smooth muscle cells (5, 6, 8, 9). The intracellular alkalinization resulting from the activation of Na-H exchange has been proposed as a first messenger in the cascade of events leading to cell hypertrophy and proliferation (9). It is possible that ANG II-induced activation of Na-H exchange is a transient phenomenon demonstrable only when quiescent cells in tissue culture are acutely exposed to a mitogenic stimulus. Pump activation of aortic muscle in ANG II-treated rats in our study may have been caused by more subtle alterations in the kinetic properties of internal and external cation sites (24, 25). Other possible mechanisms of pump activation include an increased number of pump sites or an increased rate of turnover of existing pump sites or both (24, 25).

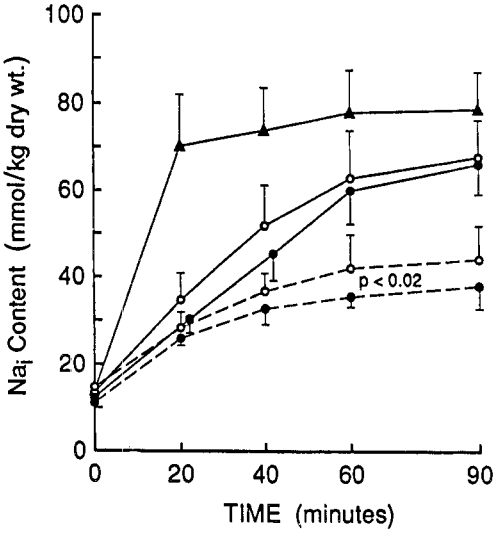

Figure 2. Na uptake curves of the aorta (means ± SD) of rats receiving ANG II for 7 to 10 days (open circles) and of control rats (solid circles) in the presence of 2 m*M* ouabain and 5 m*M* amiloride (dashed lines) and in the presence of 2 m*M* ouabain alone (lines). In the absence of amiloride, the number of observations were 13 and 16, and in the presence of amiloride, six and six in the two groups of rats. Na uptake of three unoperated rats in the presence of 10 μM monensin alone (triangles) is also shown. $P < 0.02$ by repeated measures analysis of variance for comparison of Na uptake curves in the presence of ouabain and amiloride.

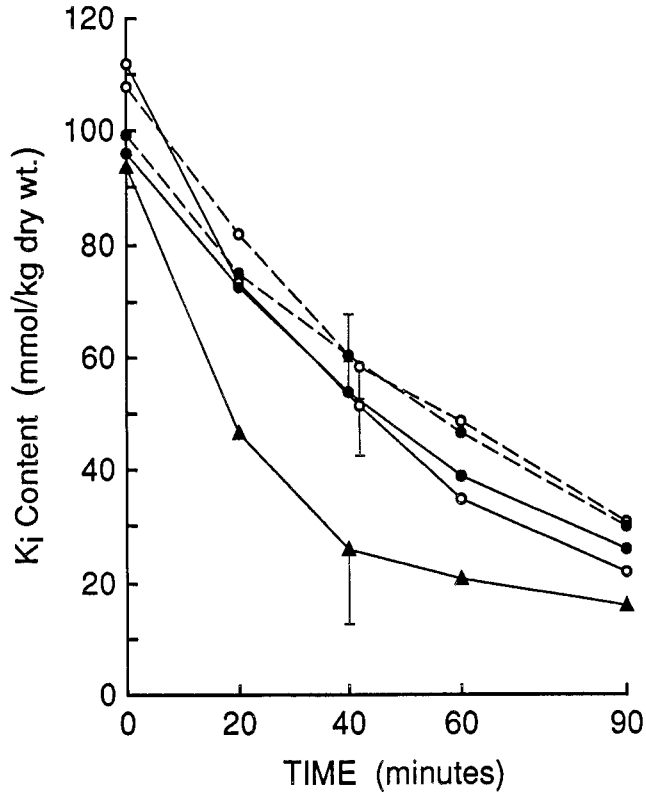

Figure 3. K efflux curves of the aorta of the same rats as in Figure 2, under the same experimental conditions.

While activation of the Na-K pump of the aorta was a feature of both short- and long-term ANG II administration, the mechanisms of pump activation may have differed under the two circumstances. In rats receiving ANG II for 7 to 10 days, additional, albeit indirect, evidence for pump activation was provided by the finding of increased K content of the aorta at the end of 90 min of preincubation. This finding suggests that under these *in vitro* conditions the aortic muscle of ANG II-treated rats was either hyperpolarized or repolarized at a faster rate than that of controls following surgical removal and incubation in an artificial salt solution. We favor the former explanation because, according to Dawkins and Bohr (15), 90 min of incubation should be sufficient for complete restoration of transmembrane cation gradients. Hyperpolarization of incubated aortic tissue may have been due to pump stimulation triggered by increased amiloride-insensitive Na uptake that was detected in these same aortas. Amiloride-insensitive or passive Na uptake is one of several ways in which Na enters cells. The others include the Na-H antiporter and Na-Ca exchange (9, 13, 21, 26). The weight of evidence, as in the present study, favors the view that ANG II stimulates the Na-K pump of vascular muscle by stimulating passive Na entry (21).

The second important finding of this study has been the accumulation of excess Na in the wall of the aorta within 24 hr of ANG II administration. The excess Na appears to be extracellularly located, because intracellular Na content was unchanged in both freshly removed and equilibrated aorta (see above). There are precedents for similar pressure-independent accumulation of vascular wall Na in the various experimental models of hypertension. In rats, Friedman and Friedman (27) detected elevations in the paracellularly bound Na fraction of arteries as early as 2–4 days after unilateral renal artery constriction or deoxycorticosterone and salt treatment, before the onset of hypertension. Rorive and Borg (28) found increased Na content of both arteries and veins 24 hr after the induction of one kidney, one clip hypertension in rats. The occurrence of similar changes on the high- and low-pressure side of the circulation suggests that the changes were not pressure related. The possible mechanisms responsible for the extracellular binding of excess Na in the present study include a direct effect of ANG II on the Na-binding properties of extracellular matrix (29). Alternatively, *de novo* synthesis and extracellular deposition of Na-binding glycosaminoglycans may be responsible (29). In this regard, ANG II has been shown to stimulate the expression of the extracellular matrix glycoprotein thrombospondin and the *de novo* synthesis of extracellular proteoglycans when vascular smooth muscle cells in tissue culture were exposed to ANG II (30). The altered matrix composition that arises as a result of ANG II administration may play a role in growth regulation and phenotypic modulation of vascular muscle (31).

The interpretation of the findings of this study is facilitated by the fact that subpressor doses of ANG II were used, and the aorta was investigated as the target organ. Subpressor doses of ANG II have been shown previously not to alter Na and water balance (22, 23, 32), to stimulate vascular prostaglandin synthesis (22), or to raise plasma aldosterone levels (23). In the present study, subpressor ANG II did not change plasma Na or K concentrations. Because the aorta is sparsely innervated (33), local interaction between ANG II and sympathetic nerves was also unlikely. However, to prove that stimulation of aortic Na-K pump activity was a direct result of ANG II, it will have to be shown that stimulation of the pump by ANG II is prevented by the co-administration of specific vascular ANG II receptor antagonists.

Stimulation of vascular smooth muscle Na-K pump activity and growth by ANG II has been demonstrated previously in tissue culture (7, 21, 34–36). In the present study, we provide evidence for stimulation of vascular Na-K pump activity *in vivo* with doses of ANG II that initially do not raise blood pressure and only minimally suppress PRA. The findings suggest that under certain pathophysiologic conditions, ANG II may promote the growth of vascular muscle also *in vivo*. Based on clinical observations (37, 38), it has been suggested previously that ANG II may act as an endogenous trophic factor, but there were few *in vivo* experimental studies to support the suggestion. In dogs, an infusion of ANG II has been shown to promote the growth of collateral vessels independently of pressure stimuli (39). In the present study, preliminary evidence for vascular growth was obtained by demonstrating an increase in the dry weight of anatomically defined segments of the aorta as early as 24 hr after the start of ANG II administration. That the increased weight of the aorta was due to hypertrophy rather than to hyperplasia of vascular muscle is suggested by the results of experiments currently being carried out in our laboratory (40). After 24 hr of treatment of rats with subpressor ANG II, we found increased protein synthesis (^{35}S-methionine incorporation) of aortic medial muscle, portal vein, and bladder wall, measured *ex vivo*. At the same time, DNA synthesis ([^{3}H]thymidine incorporation) of aortic medial muscle was inhibited. Proteolysis was unaffected by ANG II.

ANG II administered to rats in subpressor doses stimulates Na-K pump activity and increases extracellular Na content of the aorta. These changes are detectable after 24 hr and maintained at 7–10 days of ANG II administration, and are accompanied by increased dry weight of the aorta. Increased Na-K pump activity may signal the onset of trophic stimulation of vascular muscle by ANG II.

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