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Influence of Altering Total Body Sodium on Angiotensin I Systemic Converting Activity[1] (38573)

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While it is generally accepted that the renin–angiotensin system plays a major role in controlling aldosterone secretion, the complex control mechanisms that regulate the circulating levels of angiotensin II are not completely understood. There are several possible control points; of these, regulation of renin output is probably the most important with respect to angiotensin II production, and considerable work has been done in this area. Less is known concerning the factors which may alter converting enzyme activity. Franklin *et al.* (1) working with the perfused *in situ* canine kidney observed a decrease in converting enzyme activity in this organ following prolonged deoxycorticosterone acetate (DOCA)-salt treatment, while Merrill *et al.* (2), using the same technique reported a decrease in renal converting enzyme activity following a low sodium diet and mercuhydrin treatment. These findings prompted us to study the effects of both a high salt diet with DOCA and a low salt diet with mercuhydrin on the systemic conversion of angiotensin I to angiotensin II.

Methods. Ten female pedigreed American foxhounds were used. Each dog was studied twice while on a normal diet. The ten animals were then divided into two groups of five, one group being placed on a low sodium diet (Hartroft formula, General Biochemicals) while receiving 2 ml mercuhydrin on alternate days, and the other group receiving 6 g of salt twice, and 25 mg DOCA once daily. After being on the special diets for at least 2 wk, two more studies were done on each dog. All animals were then returned to normal diets and after two weeks two more studies were done on each.

For the experiments the animals were anesthetized with pentobarbital sodium (30 mg/kg) via a catheter in the cephalic vein, which was also used for subsequent injections. The trachea was intubated and the saphenous artery cannulated as far distally as the artery could be detected by palpation. Later cannulations were performed successively proximal to the first to reach the still patent region of the artery, until each hind leg had been cannulated three times. Blood pressure was monitored directly by means of a Statham strain gauge transducer and recorded on a Beckman recorder.

Solutions of angiotensin I and angiotensin II (Schwarz-Mann Biochemicals) and norepinephrine were made up at concentrations of 20 μg/ml in physiological saline and stored frozen. Injections of angiotensin were made in pairs: an injection of angiotensin I was followed by an injection of one-half the amount of angiotensin II as soon as the blood pressure had returned to baseline levels. Angiotensin I was injected in amounts of 4, 5, 6 or 8 μg. Following the series of angiotensin injections, 5 and 10 mμ injections of norepinephrine were made. All injections were in saline, 1 ml total volume, and were followed quickly by 1 ml of saline. Following the norepinephrine injections, plasma volume was measured by the dye-dilution technique of Chinard and Eder (1948). Plasma samples were frozen and analyzed later for sodium and potassium by flamephotometer, and for calcium and magnesium by atomic absorption spectrophotometry.

Vascular reactivity to angiotensin I and II and norepinephrine was calculated as the maximal increase in blood pressure divided by the number of micrograms of compound injected, to yield the mean blood pressure change per microgram of drug. Angiotensin I to angiotensin II conversion was calculated by dividing angiotensin I vascular reactivity by angiotensin II vascular reactivity and

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then multiplying by 125. The latter takes into account that 1.0 μg of the nonvasoactive decapeptide angiotensin I, if completely converted, will yield only 0.8 μg of the vasoactive octapeptide angiotensin II. The same injection pair of the two peptides was used for the calculation. A paired-value t test was used to determine statistical significance.

Results. Blood pressure showed a similar pattern of response following the injection of either angiotensin I or II. A latent period of 10–15 sec followed injection into the vein, with blood pressure reaching a peak value 0.5–1.5 min later. Blood pressure usually returned to the baseline level within 4 min, the duration of the response being roughly proportional to the magnitude of the pressure change. Often, an early peak was followed by a second peak approximately a minute later, perhaps related to the recirculation of the peptide. Norepinephrine produced a more rapid response, with a latent period of approximately ten seconds, reaching a peak value in 0.5–1 min, and then returning to the baseline in about 4 min. The response to norepinephrine was not biphasic. The results of studies carried out on the high salt diet with DOCA are shown in Table I. Prior to sodium loading, angiotensin I to angiotensin II conversion was 59%, during sodium loading 71% ($P < 0.01$) and when the animals were returned to a normal salt intake conversion decreased again to 62% ($P < 0.02$). The increase in conversion during sodium loading was not associated with significant changes in resting arterial blood pressure, plasma volume, plasma sodium or plasma magnesium. However, there was a significant decrease in plasma potassium from 3.64 mEq/l to 1.68 mEq/l ($P < 0.001$) and plasma calcium from 6.02 mEq/l to 5.29 mEq/l ($P < 0.02$). Vascular reactivity to angiotensin II showed an increase from 11 to 14 mmHg/μg ($P < 0.01$) while reactivity to norepinephrine did not change significantly. Return to a normal diet was not associated with significant changes in plasma volume, plasma sodium, plasma calcium or vascular reactivity to either angiotensin II or norepinephrine. Resting arterial pressure decreased slightly from 137 to 128 mmHg ($P < 0.05$). Plasma potassium increased from 1.68 to 3.60 mEq/l ($P < 0.001$) and plasma magnesium increased from 1.45 to 1.72 mEq/l ($P < 0.001$).

The results of the studies carried out on the low salt diet and mercuhydrin are shown in Table II. Prior to the low sodium diet conversion was 74%, during the low sodium diet 62% ($P < 0.02$) and when returned to a normal diet 65% ($P < 0.05$). During sodium depletion neither resting arterial pressure nor plasma calcium changed significantly. However, plasma volume decreased from 1223 ml to 992 ml ($P < 0.001$), plasma sodium from 151 to 140 mEq/l ($P < 0.02$). Plasma potassium increased from 3.46 to 4.26 mEq/l ($P < 0.05$), and plasma magnesium from 1.45 to 1.95 mEq/l ($P < 0.001$). Vascular reactivity to angiotensin II decreased from 6.9 to 4.3 mmHg/μg ($P < 0.001$) while norepinephrine reactivity increased from 3.94 to 4.89 mmHg/μg ($P < 0.05$).

When the animals were returned from the low to a normal sodium diet, there was no significant change in resting arterial pressure or plasma sodium. Plasma volume increased from 922 to 1262 ml ($P < 0.001$), plasma potassium decreased from 4.26 to 3.38 mEq/l ($P < 0.01$) and plasma magnesium from 1.95 to 1.57 mEq/l ($P < 0.001$). Plasma calcium decreased from 5.84 to 5.46 mEq/l ($P < 0.001$). Vascular reactivity to norepinephrine did not change significantly while reactivity to angiotensin II increased from 4.3 to 13.0 mmHg/μg ($P < 0.001$).

Discussion. The data presented above indicate that alterations in sodium intake can alter systemic angiotensin I converting activity. The results from the animals that received a high salt diet and DOCA are clear in that A-I–A-II conversion during sodium loading was significantly higher than during either the pre- or postcontrol periods. However, the results from the animals on a low sodium diet are not as definitive. Although converting activity decreased significantly when the animals were maintained on a low sodium diet and mercuhydrin, converting activity did not increase again when the animals were returned to a normal salt intake. The reason for this is not clear. It is possible that sufficient time was not allowed for body sodium stores to be replenished after the animals were returned to a normal diet. How-

TABLE I. Dog Nos. 67, 69, 70, 71, 74. Treatment with High Salt Diet.[a]

		Mean ± SE	N[d]	$\bar{\Delta}$[e]	n[f]	P[g]	P_1[i]
AI–AII Conversion (%)	C[a]	59 ± 2.9	43				
				+13	41	<0.01	
	E[b]	71 ± 2.5	48				NS*h*
				−8	48	<0.02	
	R[c]	62 ± 1.6	48				
Vascular reactivity to AII (mmHg/μg AII)	C	11 ± 0.58	42				
				+2	42	<0.01	
	E	14 ± 0.59	48				<0.01
				0	48	NS	
	R	13 ± 0.67	48				
Vascular reactivity to norepinephrine (mmHg/μg Norepi.)	C	6.6 ± 0.87	16				
				−0.7	16	NS	
	E	5.6 ± 0.50	17				NS
				+0.5	14	NS	
	R	5.9 ± 0.81	14				
Plasma volume (ml)	C	1008 ± 80	5				
				+50	5	NS	
	E	1059 ± 59	10				NS
				−12	10	NS	
	R	1047 ± 59	10				
Mean baseline blood pressure (mmHg)	C	136 ± 4	10				
				+1	10	NS	
	E	137 ± 4	10				NS
				−9	10	<0.05	
	R	128 ± 3	10				
Plasma Na^+ (mEq/l)	C	151 ± 1.6	15				
				−2.93	15	NS	
	E	145 ± 2.3	20				NS
				+2.15	20	NS	
	R	147 ± 1.9	20				
Plasma K^+ (mEq/l)	C	3.64 ± 0.08	15				
				−1.90	15	<0.001	
	E	1.68 ± 0.04	20				NS
				+1.92	20	<0.001	
	R	3.60 ± 0.06	20				
Plasma Mg^{2+} (mEq/l)	C	1.54 ± 0.05	15				
				−0.09	15	NS	
	E	1.45 ± 0.03	20				<0.01
				+0.28	20	<0.001	
	R	1.72 ± 0.03	20				
Plasma Ca^{2+} (mEq/l)	C	6.02 ± 0.25	15				
				−0.74	15	<0.02	
	E	5.29 ± 0.07	20				NS
				+0.26	20	NS	
	R	5.54 ± 0.09	20				

[a] C = preexperimental control value. E = experimental value, during altered-sodium regimen. R = recovery control value, after return to normal diet. N = number of values obtained and used to calculate the Mean. $\bar{\Delta}$ = mean change between paired values as a result of altering sodium intake or of return to normal diet. n = Number of paired values used for testing statistical significance (paired t test). P = statistical probability of error. NS = no statistically significant change. P_1 = statistical probability of a significant difference between pre- and postexperimental control values.

TABLE II. Dog Nos. 68, 72, 73, 75, 76. Treatment with Low Sodium Diet.[a]

		Mean ± SE	N	$\bar{\Delta}$	n	P	P_1
AI-AII Conversion	C	74 ± 4.7	41				
				−13	34	<0·02	
(%)	E	62 ± 2.9	49				NS
				+3	46	NS	
	R	65 ± 2.0	48				
Vascular reactivity to AII	C	6.9 ± 0.64	43				
(mmHg/μg AII)				−2.5	36	<0.001	
	E	4.3 ± 0.30	49				<0.001
				+8	44	<0.001	
	R	13.0 ± 0.59	47				
Vascular reactivity to Norepi-	C	3.94 ± 0.50	14				
nephrine (mmHg/μg Norepi.)				+0.94	14	<0.05	
	E	4.89 ± 0.50	14				<0.05
				+0.30	14	NS	
	R	5.19 ± 0.31	14				
Plasma volume (ml)	C	1223 ± 97	4				
				−285	4	<0.001	
	E	922 ± 40	10				NS
				+341	10	<0.001	
	R	1262 ± 54	10				
Mean baseline blood pressure	C	126 ± 4	10				
(mmHg)				+1	10	NS	
	E	127 ± 4	10				NS
				−7	10	NS	
	R	120 ± 3	10				
Plasma Na^+ (mEq/l)	C	151 ± 2.7	14				
				−10.6	14	<0.02	
	E	140 ± 1.5	20				NS
				+1.5	20	NS	
	R	142 ± 3	20				
Plasma K^+ (mEq/l)	C	3.46 ± 0.11	14				
				+0.80	14	<0.05	
	E	4.26 ± 0.22	20				NS
				−0.88	20	<0.01	
	R	3.38 ± 0.10	20				
Plasma Mg^{2+} (mEq/l)	C	1.49 ± 0.06	14				
				+0.40	14	<0.001	
	E	1.95 ± 0.05	20				NS
				−0.41	20	<0.001	
	R	1.57 ± 0.03	20				
Plasma Ca^{2+}	C	6.11 ± 0.37	14				
				−0.35	14	NS	
	E	5.84 ± 0.05	20				<0.05
				−0.38	20	<0.001	
	R	5.46 ± 0.08	20				

[a] See footnote of Table I.

ever, against this possibility is the observation that plasma volume and plasma potassium had returned to the control levels.

The finding that alterations in sodium intake are associated with changes in the vascular reactivity to angiotensin II is consistent with the work of others. It has been shown that a high salt diet increases (3), while sodium depletion decreases (4) vascular reactivity to angiotensin II. Davis *et al.* have suggested that the decreased vascular reactivity to angiotensin II during sodium depletion

may be due to high circulating levels of angiotensin II (4). It is possible that these high angiotensin II levels or those of renin also inhibit systemic converting enzyme activity. The possibility that the anesthetic employed may have modified resting vascular reactivity is to be acknowledged. However, since the anesthetic was used in both groups of animals, conclusions concerning at least the directional changes in reactivity would appear to be valid even if the anesthetic did have an effect.

The alterations in angiotensin I systemic converting activity do not appear to be related to circulating levels of steroids per se since with the high salt diet, DOCA was administered while the animals on the low salt diet and mercuhydrin presumably had high circulating levels of aldosterone. These findings are not consistent with the suggestion of Merrill *et al.* (2), that mineralcorticoids may inhibit converting enzyme activity.

The variable response of plasma electrolytes during either high or low sodium intake precludes any conclusion concerning their possible contribution to the changes observed in systemic converting activity.

It has been shown in the dog that both sodium loading and DOCA (1), and sodium depletion (2), decrease the renal conversion of angiotensin I to angiotensin II. Since the present experiments suggest a direct relationship between dietary sodium and systemic angiotensin I converting activity it appears that changes in local conversion cannot be considered to reflect changes in systemic conversion. In addition, since most converting enzyme activity is located in the lung (5, 6), this organ would be the likely site where changes in systemic converting activity occurs.

Summary. Studies were carried out in the dog to determine the extent to which alterations in total body sodium influence the systemic conversion of angiotensni I. When the animals were maintained on a high salt diet and DOCA, conversion of angiotensin I increased significantly and returned to control levels when a normal salt diet was provided. When another group of animals were provided a low salt diet and mercuhydrin, angiotensin I conversion decreased significantly, although returning the animals to a normal salt diet had no further influence upon conversion. These experiments indicate that there is a direct relationship between total body sodium and the systemic conversion of angiotensin I.

1. Franklin, W. G., Peach, M. J., and Gilmore, J. P., Circ. Res. **27,** 321 (1970).
2. Merrill, J. E., Peach, M. J., and Gilmore, J. P., Amer. J. Physiol. **224,** 1104 (1973).
3. Lefer, A. M., Manwaring, J. L., and Verrier, R. L., J. Pharmacol. Exp. Ther. **154,** 83 (1966).
4. Davis, J. O., Hartroft, P. M., Titus, E. O., Carpenter, C. C. J., Ayers, C. R., and Spiegel, H. E., J. Clin. Invest. **41,** 378 (1962).
5. Ng, K. K. F., and Vane, J. R., Nature (London) **216,** 762 (1967).
6. Turker, R. K., Yamamoto, M., Bumpus, F. M., and Khairallah, P. A. Circ. Res. **28,** 559 (1971).

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