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INFLUENCE OF SODIUM DEPRIVATION AND LOADING ON THE PLASMA-RENIN IN MAN

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The mechanisms controlling the secretion of aldosterone are incompletely understood (Gross, 1958; Farrell, 1959; Bartter, Mills, Biglieri & Delea, 1959; Coghlen, Denton, Goding & Wright, 1960; Davis, 1962; Wright, 1962), although the immediate stimulus to the adrenal gland is probably hormonal, at least in certain circumstances (Denton, Goding & Wright, 1959; Yankopoulos, Davis, Kliman & Peterson, 1959). There is evidence that this hormone comes from the kidney (Deane & Masson, 1951; Davis, Ayers & Carpenter, 1961; Bartter, Casper, Delea & Slater, 1961; Mulrow & Ganong, 1962) and that it might be the enzyme renin (Gross, 1958, 1960; Davis, Carpenter, Ayers, Holman & Bahn, 1961; Davis, Harcroft, Titus, Carpenter, Ayers & Spiegel, 1962). Renin reacts with a plasma substrate to form the peptide angiotensin, which, when infused intravenously, increases the urinary excretion (Genest, Biron, Koiw, Nowaczynski, Chrétien & Boucher, 1961) and secretion (Laragh, Angers, Kelly & Lieberman, 1960) of aldosterone. Infusion of small amounts of angiotensin directly into the artery leading to the transplanted adrenal gland also stimulates aldosterone production (Blair-West, Coghlen, Denton, Goding, Munro, Peterson & Wintour, 1962). The cells responsible for the secretion of renin are found in or close to the glomerulus (Goormaghtigh, 1939; Cook, Gordon & Peart, 1956; Bing & Wiberg, 1958; Cook & Pickering, 1959); the most favoured are the juxta-glomerular granular cells or the macula densa (Bing & Kazimierczak, 1960; Edelman & Hartroft, 1961). When sodium intake is restricted, the juxta-glomerular cells increase in size and granularity (Hartroft & Hartroft, 1953; Tobian, Thompson, Twedt & Janecek, 1958; Pitcock & Hartroft, 1958; Newmark, Hartroft & Edelman, 1959), the extractable renin of the whole kidney is elevated (Gross & Sulser, 1956, 1957; Gross, Loustalot & Sulser, 1956; Pitcock, Hartroft & Newmark, 1959) and aldosterone secretion is augmented (Ulick, Laragh & Lieberman, 1958; Denton *et al.* 1959; Davis, Ayers & Carpenter, 1961; Blair-West *et al.* 1962; Mills, 1962). A diet rich in sodium produces

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the reverse effects. However, as far as we are aware, measurements of circulating renin or angiotensin during alterations in dietary sodium have not been reported. The experiments described in this paper were undertaken to study changes in plasma renin, in normal subjects, during conditions of sodium deprivation and loading. A preliminary account of this work has been published previously (Brown, Davies, Lever & Robertson, 1963).

METHODS

Six healthy medical students (two females), aged 21–27 years, took part in the two groups of experiments. Normal activities were permitted throughout and the diet was unmodified except where stated. In the sodium deprivation experiments, 100 ml. blood samples were taken from an arm vein, between 5 and 6 p.m. on the days indicated (Table 1). During venesection, the subjects were supine and blood pressure was measured with a sphygmomanometer atintervals of one minute. In the sodium loading experiments, 50 ml. blood samples were taken from an arm vein between 1 and 2 p.m. on the days shown (Table 2). During this procedure the subjects were sitting.

Sodium deprivation. During days 5–10 each subject ate a fixed diet which contained 10 m-equiv Na, 56 m-equiv K and 2100 kcal daily.

Sodium loading. During days 7–11, the daily diet of each subject contained 2300 kcal, 65 m-equiv Na and 74 m-equiv K, supplemented with known amounts of sodium chloride, sprinkled on the food and swallowed in gelatin capsules.

Plasma renin was measured by an enzyme kinetic technique originally developed for use with rabbit plasma by Lever, Robertson & Tree (1963, 1964). Minor modifications in this method permit the estimation of renin in human plasma (Brown, Davies, Lever & Robertson, 1964; Brown, Davies, Lever, Robertson & Tree (1964). The mean recovery of human renin added to plasma was 37 % (S.D. 6·7 % recovery.) Aliquots of plasma samples estimated on different occasions gave results which varied with S.D. 18 % about the mean of the replicates for each sample. The results are expressed in terms of arbitrary units of standard human renin, measured by the velocity of angiotensin formation under standard conditions of incubation (Lever *et al.* 1964). The urinary concentrations of Na and K were measured by flame photometry.

RESULTS

Sodium deprivation

The subjects remained symptom-free throughout the investigation and no change in blood pressure was observed either during venesection or at other times. While the diet was uncontrolled, the sodium excretion in the urine varied from 138 to 224 m-equiv/24 hr (Table 1). When sodium intake was restricted, the urinary sodium excretion of each subject fell progressively to reach 8 m-equiv or less during the last 24 hr. There was a striking decrease in Na/K ratio in the urine in each subject. During sodium deprivation, the total excretion of Na in the urine exceeded the cumulative intake by amounts varying from 29 to 148 m-equiv (Table 1). During Na restriction the plasma renin increased in all six subjects (Table 1). The rise in plasma renin was progressive in five subjects, and was sufficient to exceed the normal range (Brown *et al.* 1964 *a*, *b*) in all except one (subject 5). The increase in plasma renin varied from 49 to 648 %, with a mean increase

of 277 %. The difference between the values of twelve samples before and twelve samples during sodium deprivation was highly significant ($t = 5{\cdot}13$ $P < 0{\cdot}001$). After resuming a normal diet the plasma renin fell to the previous range in each subject.

Sodium loading

One subject vomited after the first meal containing salt supplements and withdrew from the experiment. Severe nausea prevented three others continuing with the high salt intake after the third day. The two remaining subjects continued for 4 days.

During the last 2 days of increased sodium intake, three subjects complained of slight dyspnoea while climbing stairs and of peri-orbital swelling on waking. Their faces looked fatter but pitting oedema was not observed.

During salt loading, the total sodium intake varied from 116 to 560 m-equiv/24 hr (Table 2). The urinary excretion of sodium increased in this period, but during the first 3 days of sodium loading, the total sodium intake exceeded the cumulative loss in the urine by 113–555 m-equiv (Table 2). The urinary Na/K ratio increased in each subject.

Following the large increase in sodium and water excretion during the second day of sodium loading in two subjects (2 and 3, Table 2), the blood pressure fell from 125/65 to 108/60 and from 125/75 to 90/50 respectively. There was no striking change in blood pressure in the other cases.

The plasma renin decreased during sodium loading in all cases. The decrease varied from 33 to 62 % of control levels and in subject 4, the plasma renin during the last day of sodium loading fell below the normal range (Brown *et al.* 1964*a*, *b*). The difference between the mean levels before and during treatment was highly significant ($t = 4{\cdot}15$, $P < 0{\cdot}01$). After returning to a normal diet, the plasma renin increased to the normal range in all subjects (Table 2).

DISCUSSION

The present finding of a striking elevation of plasma renin in conditions of sodium deprivation, and of a consistent decrease in plasma renin during sodium loading, supports, but does not prove, the concept that the renin-angiotensin system is a humoral regulator of aldosterone secretion. More detailed quantitative studies, including the simultaneous measurement of plasma renin and aldosterone production, in various circumstances, are required before this issue can be settled. Meanwhile, an increase in circulating renin has been demonstrated in normal pregnancy (Brown, Davies, Doak, Lever & Robertson, 1963), hepatic cirrhosis with ascites, and malignant hypertension (Brown *et al.* 1964*a*). Increased aldosterone production has been reported in each of these circumstances (Ulick *et al.*

TABLE 1. The effect of sodium deprivation upon the urinary Na and K excretion, blood pressure and plasma renin

Subject	Day	Urine: Volume (ml./24 hr)	Urine: Na (m-equiv/24 hr)	Urine: K (m-equiv/24 hr)	Na/K	Na intake minus urine Na (m-equiv/24 hr)	K intake minus urine K (m-equiv/24 hr)	Plasma renin (units/l.)
1. B.R.	1	—	—	—	—	—	—	5·9
	2	1180	224	126	1·8	—	—	7·0
	3	1170	211	91	2·3			
	6	1110	84	87	0·97	−74	−19	17·3
	7	910	23	64	0·36	−13	+ 4	
	8	670	10	63	0·12	0	+ 5	30·0
	9	940	8	74	0·10	+ 2	− 6	
						Cum. −85	−16	
	15	—	—	—	—	—	—	9·0
2. S.B. ♀	1	—	—	—	—	—	—	4·0
	2	1010	186	84	2·2	—	—	3·0
	3	995	166	58	2·9			
	6	1040	58	75	0·77	−48	− 7	7·4
	7	1200	56	61	0·92	−46	+ 7	
	8	430	9	41	0·22	+ 1	+27	26·2
	9	540	5	26	0·19	+ 5	+42	
						Cum. −88	+69	
	15	—	—	—	—	—	—	10·5
3. C.E.	1	—	—	—	—	—	—	7·4
	2	1050	206	73	2·8	—	—	5·9
	3	1020	152	49	3·1			
	6	1030	53	47	1·1	−43	+21	20·9
	7	940	29	70	0·4	−19	− 2	
	8	1070	15	79	0·19	− 9	−11	16·8
	9	760	8	78	0·12	+ 2	−10	
						Cum. −65	− 2	
	15	—	—	—	—	—	—	4·0

TABLE 1 (*cont.*)

Subject	Day	Urine: Volume (ml./24 hr)	Urine: Na (m-equiv/24 hr)	Urine: K (m-equiv/24 hr)	Na/K	Na intake minus urine Na (m-equiv/24 hr)	K intake minus urine K (m-equiv/24 hr)	Plasma renin (units/l.)
4. A.P.	1	—	—	—	—	—	—	5·1
	2	1280	142	55	2·6	—	—	4·0
	3	1590	138	41	3·4			
	6	1275	33	56	0·59	−23	+12	11·7
	7	800	22	60	0·37	−12	+ 8	
	8	1120	9	65	0·14	+1	+ 3	15·0
	9	1600	5	96	0·05	+5	−28	
						Cum. −29	−5	
	15	—	—	—	—	—	—	3·0
5. A.G.	1	—	—	—	—	—	—	11·0
	2	1070	157	72	2·2	—	—	7·2
	3	880	143	72	2·0			
	6	1310	124	66	1·9	−114	+2	11·7
	7	1230	47	65	0·72	−37	+3	
	8	680	12	65	0·18	−2	+3	13·9
	9	980	5	64	0·08	+5	+4	
						Cum. −148	+12	
	15	—	—	—	—	—	—	5·1
6. R.H. ♀	1	—	—	—	—	—	—	7·8
	2	1450	144	68	2·1	—	—	8·6
	3	1280	215	64	3·4			
	6	1280	77	56	1·38	−67	+12	10·5
	7	1600	35	72	0·49	−25	−4	
	8	740	20	73	0·14	0	−5	20·6
	9	740	5	65	0·08	+5	+3	
						Cum. −87	+6	
	15	—	—	—	—	—	—	4·0

Cum. = cumulative.

TABLE 2. The effect of sodium loading upon the urinary excretion of Na and K, blood pressure and plasma renin

Subject	Day	Urine			Na/K	Plasma renin (units/l.)	Total Na intake (m-equiv/ 24 hr)	Na intake minus urine Na (m-equiv/ 24 hr)	K intake minus urine K (m-equiv/ 24 hr)
		Volume (ml./24 hr)	Na (m-equiv/ 24 hr)	K (m-equiv/ 24 hr)					
1. B.R.	1	1460	268	96	2·8	7·0	—	—	—
	2	1660	133	53	2·5	8·4	—	—	—
	7	1390	320	59	5·4	4·7	560	+240	+15
	8	1980	490	89	5·5	5·9	440	−50	−15
	9	1560	330	72	4·6	5·1	470	+140	+2
	10	—	—	—	—	5·1	65	Cum. +330	Cum. +2
	14	1094	177	96	1·8	8·6	—	—	—
2. P.V.A. ♀	1	2090	178	96	1·8	—	—	—	—
	2	2095	220	60	3·7	10·2	—	—	—
	7	1575	295	88	3·3	9·0	560	+265	−14
	8	2950	350	82	4·3	6·6	490	+140	−8
	9	2860	390	74	5·3	—	540	+150	0
	10	—	—	—	—	5·1	135	Cum. +555	Cum. −22
	14	910	56	73	0·8	9·5	—	—	—
3. A.P.	1	1300	180	49	3·7	6·6	—	—	—
	2	1650	192	60	3·2	8·2	—	—	—
	7	1800	290	90	3·2	5·5	470	+180	−16
	8	4000	550	108	5·1	4·4	470	−80	−34
	9	2910	154	29	5·3	4·0	167	+13	+45
								Cum. +113	Cum. −5
	17	1900	106	65	1·6	5·5	—	—	—

TABLE 2 (*cont.*)

Subject	Day	Urine			Na/K	Plasma renin (units/l.)	Total Na intake (m-equiv/24 hr)	Na intake minus urine Na (m-equiv/24 hr)	K-intake minus urine K (m-equiv/24 hr)
		Volume (ml./24 hr)	Na (m-equiv/24 hr)	K (m-equiv/24 hr)					
4. A.G.	1	—	—	—	—	7·8	—	—	—
	2	930	145	58	2·5	7·8	—	—	—
	7	1190	305	67	4·6	7·8	540	+235	+7
	8	1870	610	79	7·7	4·4	525	−85	−5
	9	1320	425	62	6·9	3·4	475	+50	+12
	10	—	—	—	—	3·0	—	Cum. +205	Cum. +14
	14	940	77	32	2·4	7·8	—	—	—
5. R.H. ♀	1	1170	156	72	2·2	9·0	—	—	—
	2	695	106	41	2·6	7·4	—	—	—
	7	1960	290	98	3·0	7·8	420	+130	−24
	8	1430	275	66	4·2	5·5	460	+185	+8
	9	1260	189	38	5·0	5·9	116	−73	+36
	10							Cum. +242	Cum. −20
	14	1025	164	65	2·5	18·0	—	—	—

Cum. = cumulative.

1958; Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook & Greenwood, 1959; Laragh, Ulick, Januszewicz, Deming, Kelly & Lieberman, 1960; Cope, Harwood & Pearson, 1962). However, aldosterone secretion might well be controlled by more than one mechanism, and the relative importance of the separate factors could vary in different circumstances. Blair-West *et al.* (1962) produced changes in aldosterone secretion in the sheep by altering locally the Na and K concentration of the blood perfusing the adrenal gland. A considerable rise in aldosterone production occurred when the adrenal venous Na was decreased by 5–10 m-equiv/l. and the K concentration raised by 0·5–1·0 m-equiv/l. Similar observations were made in the dog by Davis, Urquhart & Higgins (1963).

The changes in aldosterone production in these experiments were presumably due to a direct effect of the ionic changes upon the adrenal cells. Electrolyte changes of this order occur in some of the clinical conditions associated with a rise in aldosterone secretion. However, in other pathological circumstances (Davis *et al.* 1963), and in the more physiological conditions of sodium deprivation or loading, changes in aldosterone production are not accompanied by adequate alterations in plasma Na or K concentrations (McCance, 1935; Black, Platt & Stanbury, 1950; Wiggins, Manry, Lyons & Pitts, 1951; Leaf & Couter, 1949). In these instances, angiotensin might be the immediate stimulus to the adrenal cortex, if the changes in plasma renin recorded in this paper lead to similar alterations in circulating angiotensin.

The mechanisms controlling the secretion of renin are also undetermined. Braun-Menendez, Fasciolo, Leloir & Muñoz (1940) and Kohlstaedt & Page (1940) claimed that experimental constriction of a renal artery stimulates renin secretion. Tobian (1960) suggested that the juxta-glomerular cells might act as stretch receptors; a decrease in local arteriolar pressure might then stimulate these cells and augment renin secretion. Conversely increased stretching of the arteriolar wall might suppress the juxta-glomerular cells and inhibit the secretion of renin. Indirect evidence supporting this concept was recently obtained by measuring the renin content of individual glomeruli arising from a single interlobular artery. In normal rabbits glomeruli situated near the origin of an interlobular artery contained little or no renin while glomeruli closer to the capsule of the kidney were rich in renin (Brown, Davies, Lever, Parker & Robertson, 1963*a*). This distribution of renin within the normal kidney might be related to regional variations in renal arteriolar pressure. The application of a constricting clip to the renal artery, which presumably decreased the perfusion pressure, led to an increase in the renin content of separate glomeruli, particularly those adjacent to the renal medulla (Brown, Davies, Lever, Parker & Robertson 1963*b*).

These findings support Tobian's theory, if changes in the renin content of glomeruli indicate parallel alterations in renin secretion. In more physiological circumstances, the juxta-glomerular cells might detect changes of intravascular volume.

Plasma volume falls during sodium deprivation (McCance, 1937), while the juxta-glomerular cells hypertrophy (Hartroft & Hartroft, 1953; Tobian *et al.* 1958; Newmark, Hartoff & Edelman, 1959) and circulating renin is here shown to increase. Conversely sodium loading leads to increased plasma volume (Lyons, Jacobson & Avery, 1944; Grant & Reischsman, 1946), decreased size and granularity of the juxta-glomerular cells and a fall in plasma renin. Bartter, Liddle, Duncan, Barber & Delea (1956) produced evidence suggesting that experimental alterations of intravascular volume were followed by converse changes in aldosterone production.

Another way in which renin secretion might respond to changes in sodium balance arises from the work of Goormaghtigh (1944), Oberling & Hatt (1960), Latta, Maunsbach & Cook (1962) and others, who have suggested that the juxta-glomerular granular cells might be affected by the adjacent macula densa in some way. Amongst the possible stimuli considered were changes in the sodium content or osmolality of fluid in the early distal tubule.

Micropuncture studies have shown that the tubular fluid in contact with the macula densa is hypotonic to plasma. This hypotonicity is unaffected by dehydration or by the diureses produced by water, glucose, mannitol or urea (Wirz, 1957; Gottschalk & Mylle, 1959; Lassiter, Gottschalk & Mylle, 1961; Ullrich, Schmidt-Nielsen, O'Dell, Pehling, Gottschalk, Lassiter & Mylle, 1963). In contrast, the diuresis produced by hypertonic saline is associated with a further decrease in the osmolality of fluid in this site (Gottschalk & Mylle, 1959).

If the macula densa were sensitive to changes in osmolality in the same way as the hypothalamic-pituitary-ADH system, a mechanism could exist in which changes in sodium balance were converted into changes in the molality of the distal tubular fluid and appreciated as such by osmoreceptors in the macula densa. These in turn could provide the stimulus for renin release. The regional distribution of renin within the kidney might then be related to differences in the molality of the early tubular fluid of nephrons with superficial compared with juxta-medullary glomeruli. As far as we are aware this aspect has not been studied, but the renin-poor juxta-medullary nephrons are known to have several distinct anatomical and histological features (Barclay, Daniel, Franklin & Prichard, 1947; Sperber 1944).

Whatever the stimulus to renin release, the present results indicate that the renin angiotensin system might play an important role in the maintenance of sodium balance.

SUMMARY

1. In each of six healthy subjects, the plasma renin increased during a 5-day period of sodium deprivation.

2. Conversely, sodium loading decreased the circulating renin in each of five subjects.

3. These findings are discussed in relation to the possible role of the renin-angiotensin system in the control of aldosterone secretion and extracellular fluid volume.

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