Increased Renal Secretion of Norepinephrine and Prostaglandin E_2 during Sodium Depletion in the Dog

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ABSTRACT To determine whether vasoactive renal hormones modulate renal blood flow during alterations of sodium balance, simultaneous measurements of arterial and renal venous concentrations of norepinephrine and prostaglandin E_2 (PGE_2) and of plasma renin activity, as well as renal blood flow and systemic hemodynamics were carried out in 24 sodium-depleted and 28 sodium-replete anesthetized dogs. The mean arterial blood pressure of the sodium depleted dogs was not significantly different from that of the animals fed a normal sodium diet, but cardiac output was significantly lower (3.07±0.18 vs. 3.77±0.17 liters/min, mean±SEM; $P < 0.01$). Despite the higher total peripheral vascular resistance in the sodium-depleted dogs (46.1±2.9 vs. 37.0±2.1 arbitrary resistance U; $P < 0.02$), the renal blood flow and renal vascular resistance were not significantly different in the two groups. The arterial plasma renin activity and concentration of norepinephrine were higher in the sodium-depleted animals than in the controls; the arterial concentration of PGE_2 was equal in both groups. The renal venous plasma renin activity was higher in the sodium-depleted dogs. Similarly, the renal venous norepinephrine concentration was higher in the sodium-depleted dogs than in the controls (457±44 vs. 196±25 pg/ml; $P < 0.01$); renal venous PGE_2 concentration was also higher in the sodium depleted dogs (92±22 vs. 48±11 pg/ml; $P < 0.01$). Administration of indomethacin to five sodium-replete dogs had no effect on renal blood flow. In five sodium-depleted dogs indomethacin lowered renal blood flow from 243±19 to 189±30 ml/min ($P < 0.05$) and PGE_2 in renal venous blood from 71±14 to 15±2 pg/ml ($P < 0.02$). The results indicate that moderate chronic sodium depletion, in addition to enhancing the activity of the renin-angiotensin system, also increases the activity of the renal adrenergic nervous system and increases renal PGE_2 synthesis. In sodium-depleted dogs, inhibition of prostaglandin synthesis was associated with a significant decrease in renal blood flow. The results suggest that the renal blood flow is maintained during moderate sodium depletion by an effect of the prostaglandins to oppose the vasoconstrictor effects of angiotensin II and the renal sympathetic nervous system.

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INTRODUCTION

The role of the vasoactive hormone systems in the regulation of the renal blood flow during alterations of sodium balance is incompletely understood. Angiotensin II, a potent renal vasoconstrictor, is thought to be involved in renal blood flow regulation during chronic sodium depletion because the administration of angiotensin II antagonists induces striking increases in renal blood flow in sodium-depleted animals but fails to do so during sodium repletion (1–3). Whether the renal sympathetic nerves also modulate renal blood flow during sodium depletion is not known. However, the renal blood vessels receive abundant adrenergic innervation (4) and constrict in response to exogenous norepinephrine or to nerve stimulation (2, 5). Moreover, plasma and urinary norepinephrine concentrations have been found to be elevated in sodium-depleted animals (6).

Recently it has become apparent that the hormonal regulation of renal blood flow in a variety of situations is determined by an interplay of vasoconstrictor and vasodilator hormonal influences. For example, administration of exogenous angiotensin II or of norepinephrine reduces renal blood flow and stimulates the release of prostaglandins from the kidney (5, 7); prior administration of an inhibitor of prostaglandin synthesis increases the renal vasoconstriction produced by both agents (8). Similarly, inhibition of prostaglandin synthesis enhances the renal ischemia mediated by angiotensin II and the sympathetic nerves during hemorrhagic hypotension in dogs (9). Furthermore, it is of interest that the renal blood

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flow is not invariably reduced by sodium depletion despite activation of the renin angiotensin system (10–12).

This study was designed to explore whether the renal sympathetic nervous system and the renin-angiotensin system interact with the renal prostaglandins to modulate the renal blood flow during chronic sodium depletion. Accordingly, simultaneous measurements of arterial and renal venous concentrations of norepinephrine and prostaglandin E_2 (PGE_2)[1] and of the plasma renin activity, as well as renal blood flow and systemic hemodynamics were carried out in sodium-depleted and sodium-replete anesthetized dogs.

METHODS

The experiments were performed in 52 mongrel dogs of either sex, weighing an average of 24.7±0.7 kg (mean±SEM) that were maintained in two different states of sodium balance: (*a*) 28 animals were fed a normal chow diet (Respond 2,000 Agway Inc., Syracuse, N. Y.) that provided a sodium intake of 80–100 meq/d. (*b*) Dietary sodium depletion was induced in 24 dogs by the injection of 10 mg i.m. of furosemide 9 and 10 d before study followed by administration of a diet (h/d diet, Riviana Foods Inc., New York) that provided about 10 meq/d of sodium. The mean urinary sodium concentration of these animals was 12±3 meq/liter (mean±SEM) on the day of study.

Anesthesia was induced with sodium pentobarbital (30 mg/kg i.v.) and maintained with periodic additional administration. After anesthesia, the dogs fed a normal chow diet received an infusion of Ringer's lactate equal to 4% of body weight through a catheter followed by a sustained infusion of 0.2 ml/kg per min for the remainder of the experiment. After cannulation of the trachea, the dogs were ventilated with a Harvard respirator (Harvard Apparatus Co, Millis, Mass.). Through a femoral artery, a catheter was introduced into the aorta for blood pressure measurements [P23Db Statham transducer (Statham Instruments, Inc., Oxnard, Calif.) and Grass polygraph recorder (Grass Instrument Co., Quincy, Mass.)] and arterial blood collections. Via the right jugular vein, a catheter was introduced into the right atrium for the administration of indocyanine dye solution. Cardiac output was determined as described (3). Through a left flank incision and retroperitoneal dissection the left renal artery was dissected and fitted with a flow probe of appropriate diameter (Carolina Medical Electronics Inc., King, N. C.). Renal blood flow was measured with an electromagnetic flowmeter as described (13). Through the left ovarian/testicular vein, a small catheter was retrogradely introduced into the left renal vein for blood collections. In all instances, aortic and renal vein bloods were taken simultaneously. At least 1 h was allowed to elapse after surgery before making control measurements. In five of the sodium-depleted dogs and five of the sodium-replete dogs, 5 mg/kg of indomethacin was administered as a bolus after completion of the control determinations (3). Hemodynamic measurements and blood collections were performed 20–30 min later.

Plasma norepinephrine was measured by the procedure described by Passon and Peuler (14). Both aortic and renal vein blood (2 ml) were placed in chilled tubes containing EGTA and reduced glutathione (3.6 mg and 2.4 mg, respectively) and centrifuged under refrigeration to separate plasma from the cells. Plasma samples (500 μl) were mixed with 20 μl of 60% perchloric acid and 50-μl aliquots of the deproteinized supernatant fraction were assayed for norepinephrine. Norepinephrine (100 pg) was added to one aliquot from each sample to serve as an internal standard. Blanks, prepared with 0.001 N HCl, were treated in a fashion similar to that of plasma. A reaction mixture was prepared and 100 μl of this mixture was pipetted into each sample to begin enzymatic *O*-methylation. The final concentrations of reactants in each sample were as follows: 0.5 μM, reduced glutathione; 133 mM, $MgCl_2 \cdot 6H_2O$; 0.4 M, Tris; 18 mM EGTA; 10 mM, dithiothreitol; 2.5 μCi, *S*-adenosyl-L-methionine-([^{3}H]methyl) ([^{3}H]SAM) (9–11 Ci/mmol); and catechol-*O*-methyl transferase, 0.5 mg of standardized enzyme preparation (20.5 mg/ml) (15). The pH of the reaction mixture was 8.9. At the end of 60 min incubation at 38°C, the reaction was terminated by the addition of 100-μl aliquots of 1 M sodium borate, pH 11.0, containing 1 mM normetanephrine. The [^{3}H]-*O*-methylated products of the reaction were extracted into 3 ml of toluene-isoamyl alcohol (3:2, vol/vol). At this and each extraction step, the aqueous and organic phases were separated by centrifugation. After freezing the aqueous phase in a mixture of dry-ice-acetone, the organic epiphase was decanted into 150 μl of 0.1 M acetic acid solution which partitioned the normetanephrine back into the aqueous phase. This phase was washed once with 2.5 ml of toluene-isoamyl alcohol (3:2, vol/vol) and the organic layer was aspirated and discarded. Absolute ethanol (100 μl) was added to the acetic acid extract and separation of [^{3}H]-normetanephrine from other *O*-methylated products was accomplished by thin-layer chromotography on 250-μm thickness silica gel plates (Analtech, Inc., Newark, Del.). Plates were developed in tertiary amyl alcohol/toluene/40% methylamine solution (21:7:10). After drying, each plate was scanned under UV light (254 nm) to visualize the normetanephrine and the corresponding zone was scraped into a scintillation vial. To each scintillation vial was added in succession the following solutions: 1 ml of 50 mM ammonium hydroxide solution to elute the methylated derivative from the silica gel, 50 μl of 4% sodium periodate to convert [^{3}H]normetanephrine to [^{3}H]vanillin and, after 5 min, 50 μl of glycerol (10% vol/vol) to eliminate unreacted periodate. The resulting solution was then acidified with 1 ml of 0.1 N acetic acid and the [^{3}H]vanillin was partitioned into 10 ml of toluene/Liquifluor (New England Nuclear, Boston, Mass.) (20:1) with vigorous mixing. Radioactivity in these samples was determined by counting in Packard Tri-carb scintillation spectrometer, model 3330 (Packard Instrument Co., Inc., Downers Grove, Ill.). The accuracy of the assay was assessed by the recovery of standard amounts (25–755 pg) of norepinephrine added to a dog plasma pool. Linear regression analysis of the recovered standard gave $Y = 1.04X - 1.3$ ($r = 0.998$; $n = 14$), where Y is the amount of standard recovered (corrected for endogenous concentration) and X is the amount of norepinephrine added. Reproducibility of the assay was determined by the measurement of aliquots of the sample plasma pool. When aliquots were measured in eight different assay runs, the mean±SD concentration was 440±20 pg/ml. The interassay coefficient of variation was 5%.

PGE_2 was measured by radioimmunoassay. [^{3}H]PGE_2 was obtained from Amersham Corp., (Arlington Heights, Ill.) (160 Ci/mmol) and PGE_2 antisera was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). The percentage

[1] *Abbreviations used in this paper*: PGE_2, prostaglandin E_2; PRA, plasma renin activity; RU, arbitrary resistance units.

of cross-reactivity of various prostaglandins with the antibody is <0.3% for prostaglandins A_1, A_2, B_1, B_2, and $F_{2\alpha}$ and <3.4% for prostaglandins E_1 and 6-keto-$F_{1\alpha}$ (16). Blood was collected in heparinized chilled tubes containing 10 μg indomethacin/ml blood. After separation, the plasma was stored at −20°C until assay time. To 2 ml of plasma, about 1,000 cpm of [^{3}H]PGE_2 were added for recovery estimation. The samples were then acidified to pH 3.5 with formic acid and extracted with 5 ml of ethylacetate. The organic layer was then collected and dried under N_2. The dried extracted samples were redissolved in 0.5 ml of 0.1 M phosphate buffer (pH 7.40) containing 0.1 mg/ml Brij 35 (ICI United States, Inc. Wilmington, Mass.) to facilitate PGE_2 recovery. The mean±SD overall recovery of [^{3}H]PGE_2 from plasma after extraction was 95±7% (n = 19). After taking aliquots for recovery estimation, 100-μl aliquots of sample were added to assay tubes containing ~8,000 cpm of [^{3}H]PGE_2 (equivalent to 12 pg) dissolved in 100 μl of redistilled water and 100 μl of diluted antisera in phosphate buffer. Standards containing 3–708 pg of unlabeled PGE_2 were treated in a similar manner. After incubation for 2 h at 4°C, the bound and free PGE_2 were separated by a charcoal-dextran mixture. The final antisera dilution resulted in an initial binding of about 40%. Nonspecific binding was 2.1±1.2% (mean±SD; n = 10). Standard curves were analyzed by the method of Rodbard and Hutt (17), which is a nonlinear transformation of the logit-log relationship. A 10% decrease in bound counts was observed at 3.4±0.1 pg (mean±SD; n = 5). The mean±SD amount of PGE_2 required to displace zero point binding by 50% was 28±12 pg (n = 10). Extraction blanks averaged <2 pg. The accuracy of the assay was assessed by the recovery of standard amounts of PGE_2 (9.4–944 pg) added to a plasma pool. Linear regression analysis of the recovery standards gave $Y = 0.94X + 17$ (r = 0.96; n = 28), where Y is the amount of standard recovered and X is the amount of PGE_2 added. The reproducibility of the assay was determined by measurements of aliquots of the same plasma pool. When eight aliquots were measured in the same assay run, the mean±SD concentration was 81±11 pg/ml; the intrassay coefficient or variation was 13%. When aliquots of three separate plasma pools were measured in different assay runs, the mean±SD concentrations were: 15±3 (n = 8); 43±8 (n = 7); 95±20 pg/ml (n = 7). The interassay coefficients of variation were 20, 19, and 21%, respectively.

Plasma renin activity (PRA) was measured as described (2).

All values are expressed as mean±SEM. Due to the marked nonnormality of many of the measured variables, nonparametric statistical procedures were applied for hypothesis testing. Differences in paired data were analyzed by the sign test; differences in unpaired data were analyzed by the rank-sum test (18). A 5% significance level was used for all statistical tests.

RESULTS

Table I summarizes the body and kidney weights and the hemodynamic measurements in the two groups of animals. Although the administration of furosemide and a low sodium diet to 24 dogs produced a mean weight loss from 24.7±1.0 to 23.6±1.1 kg ($P < 0.01$), at the time of study neither the mean body weight nor the mean kidney weight of the sodium-depleted animals was significantly lower than that of the sodium-replete animals. Arterial hematocrit was significantly higher (44 vs. 36%, $P < 0.01$) in the sodium-depleted dogs.

Table I also shows that the mean arterial blood pressure of the sodium-depleted dogs was not significantly different from that of the animals fed a normal diet; however, cardiac output was significantly lower (3.07 vs. 3.77 liters/min, $P < 0.01$). Despite the higher total peripheral vascular resistance in the sodium-depleted animals (46.1 vs. 37.0 arbitrary resistance units (RU), $P < 0.02$), the renal blood flow and renal vascular resistance were not significantly different in the two groups.

Table II depicts the PRA in the arterial and renal venous bloods in the two groups of animals. In both the sodium-replete and the sodium-depleted animals, PRA in the renal vein blood was significantly higher than the arterial PRA. Renal venous PRA in the normal sodium group ranged from 0.5 to 7.4 and averaged 2.5 $ng \cdot ml^{-1} \cdot h^{-1}$; whereas renal venous PRA in the sodium-depleted dogs ranged from 7.0 to 164.3 and averaged 58.2 $ng \cdot ml \cdot h^{-1}$. The levels of PRA in both the arterial and renal venous blood were significantly

TABLE I

Body and Kidney Weights, Hematocrit and Systemic and Renal Hemodynamics in Normal and Negative Sodium Balances

	Normal Na^+ (n = 28)	Na^+ depletion (n = 24)	P
Body weight, *kg*	25.7±0.8	23.6±1.1	NS
Kidney weight, *g*	68.3±4.0	58.1±3.1*	NS
Hematocrit, %	36±1	44±1	<0.01
Mean arterial pressure, *mmHg*	133±3	131±3	NS
Cardiac output, *liters/min*	3.77±0.17*	3.07±0.18	<0.01
Total peripheral vascular resistance, *RU*	37.0±2.1*	46.1±2.9	<0.02
Renal blood flow, *ml/min*	235±16	226±12	NS
Renal vascular resistance, *RU*	0.64±0.04	0.63±0.04	NS

Values are mean±SEM.
* n = 22.

TABLE II

PRA in Arterial and Renal Vein Bloods and Renal Renin Secretion Rate in Normal and Negative Sodium Balances

	Arterial	Renal vein	Δ	*P*	Renal secretion rate
	ng·ml⁻¹·h⁻¹				*ng/min*
Normal Na^+ (n = 28)	1.9±0.2	2.5±0.3	0.6±0.1	<0.001	96±27
Na^+ depletion (n = 24)	35.4±5.3	58.2±8.7	22.8±4.0	<0.01	2,854±551
P	<0.01	<0.01			<0.01

Values are mean±SEM. Renal secretion rate = (V-A)[RBF(1-Hct)], where V is the venous concentration, A is the arterial concentration, RBF is the renal blood flow, and Hct is the hematocrit.

higher in the sodium-depleted animals. The calculated secretion rate of renin by sodium-depleted animals was significantly higher than that of the normal sodium dogs (2,854 vs. 96 ng/min, $P < 0.01$).

Table III depicts the plasma concentrations of norepinephrine in the renal vein and arterial bloods. In both groups of dogs, the norepinrephrine concentration in the renal venous plasma was significantly greater than that of the arterial plasma. In the normal sodium dogs, norepinephrine concentration in renal venous plasma ranged from 7 to 496 pg/ml and averaged 196 pg/ml; whereas in the sodium-depleted dogs it ranged from 172 to 996 pg/ml and averaged 457 pg/ml. Both arterial and renal venous norepinephrine concentrations were significantly higher in the sodium-depleted animals than in the sodium-replete control group. The calculated rate of renal norepinephrine overflow was significantly higher in the sodium-depleted animals than in the sodium-replete dogs (32,565 vs. 12,758 pg/min, $P < 0.01$).

Table IV depicts the arterial and renal vein plasma PGE_2 concentration in the two groups of dogs. In both, there was net addition of PGE_2 into the renal venous blood. The renal venous plasma concentration of PGE_2 ranged from 14 to 197 pg/ml and averaged 48 pg/ml in the normal sodium dogs; it ranged from 27 to 378 pg/ml and averaged 92 pg/ml in the sodium-depleted animals. Although the PGE_2 concentrations in the arterial plasma were not different (28 vs. 27 pg/ml) in the two groups of dogs, renal vein plasma PGE_2 was significantly higher in the sodium-depleted group. The calculated secretion rate of PGE_2 into the renal venous blood was also significantly increased in the sodium-depleted animals (8,047 vs. 2,448 pg/min, $P < 0.01$).

Table V depicts the changes in systemic and renal hemodynamics induced by indomethacin in five sodium-replete and five sodium-depleted dogs. Indomethacin had no significant effect on mean arterial blood pressure and cardiac output of both groups of dogs. The renal blood flow of the normal sodium dogs was also unaffected by indomethacin. In marked contrast, indomethacin reduced blood flow in the sodium-depleted dogs from 243 to 189 ml/min ($P < 0.05$). Concomitant with this reduction in renal blood flow there was a decrease in the plasma concentration of PGE_2 in arterial blood from 19±3 to 14±1 pg/ml ($P < 0.05$) and in renal venous blood from 71±14 to 15±2 pg/ml ($P < 0.02$); the calculated rate of renal PGE_2 secretion was lowered by indomethacin from 7,351±1,871 to 96±285 pg/min ($P < 0.02$).

DISCUSSION

The data of the present study confirm the hemodynamic effects of moderate chronic sodium depletion. The animals subjected to sodium depletion underwent a modest decrease in weight. When compared to the sodium-replete dogs, they had a lower cardiac output and a higher hematocrit, presumably as a consequence of a decreased plasma volume (6, 19). Although the sodium-depleted dogs had a significantly higher total peripheral vascular resistance than the

TABLE III

Plasma Norepinephrine Concentration in Arterial and Renal Vein Bloods and Renal Norepinephrine Secretion Rate in Normal and Negative Sodium Balances

	Arterial	Renal vein	Δ±SEM	*P*	Renal secretion rate
	pg/ml				*pg/min*
Normal Na^+ (n = 26)	102±10	196±25	94±20	<0.001	12,758±2,715
Na^+ depletion (n = 23)	213±24	457±44	244±38	<0.001	32,565±5,016
P	<0.01	<0.01			<0.01

Values are mean±SEM. The number of determinations is given in parenthesis.

TABLE IV

Plasma PGE_2 Concentration in Arterial and Renal Vein Bloods and Renal PGE_2 Secretion Rate in Normal and Negative Sodium Balances

	Arterial	Renal vein	Δ	*P*	Renal secretion rate
	pg/ml				*pg/min*
Normal Na^+ (n = 17)	28±4	48±11	20±10	<0.05	2,448±1,221
Na^+ depletion (n = 17)	27±4	92±22	65±19	<0.001	8,047±2,227
P	NS	<0.01			<0.01

Values are mean±SEM. The number of determinations is given in parenthesis.

normal sodium animals, arterial blood pressure, renal blood flow, and renal vascular resistance did not differ significantly in the two groups.

The finding that arterial blood pressure was not significantly lower in the sodium-depleted than the sodium-replete animals despite a significantly lower cardiac output, is in agreement with previous work (2, 3, 20). Arterial PRA was significantly higher in the sodium-depleted dogs. It is now firmly established that angiotensin II directly participates in the increased peripheral vascular resistance of chronic sodium depletion. Administration of angiotensin II antagonists or of inhibitors of the converting enzyme (2, 3) induces hypotension and a fall in the total peripheral vascular resistance in sodium depletion, whereas no change is observed in sodium repletion. Arterial plasma norepinephrine concentration was also higher in the dogs with sodium depletion, which is consistent with observations by others (6, 21, 22). It is likely that the enhanced activity of the sympathetic nervous system is involved along with angiotensin II in the maintenance of blood pressure during sodium depletion because the hypotensive effect of pharmacologic sympathectomy is greatly enhanced by extracellular volume depletion (23).

The finding that renal blood flow in the sodium-depleted dogs was not lower than that in the sodium-replete dogs is at variance with some studies in animals (2) and in normal volunteers (19) who had undergone dietary sodium deprivation. However, it is consistent with a number of studies in conscious animals (11, 12) and in man (10), in which renal blood flow was found to be unaffected by sodium depletion. It is possible, in part, that the conflicting results are related to the different methods used by the investigators to measure renal blood flow. However, it is likely that the response of the renal vasculature to sodium depletion depends upon the magnitude and the rapidity of depletion. This is known to be the case for the response of arterial blood pressure (20, 24) and of proximal tubular reabsorption (25). The observation that renal blood flow and renal vascular resistance in the sodium-depleted animals were not different from sodium-replete controls despite a diminished cardiac output and increased total peripheral vascular resistance is similar to data obtained by Vatner and his associates (26, 27). In a series of elegant studies in conscious dogs these workers demonstrated little or no renal vasoconstriction during conditions associated with reduced blood pressure or cardiac output and significantly increased total peripheral vascular resistance.

In both groups of dogs, the renal vein PRA was significantly higher than arterial PRA, indicating net renin secretion by the kidney. In the sodium-depleted dogs the renal vein PRA and the calculated secretion rate of renin by the kidney were markedly higher than

TABLE V

Effect of Indomethacin on Mean Arterial Pressure, Cardiac Output and Renal Blood Flow in Normal and Negative Sodium Balances

		Mean arterial pressure	Cardiac output	Renal blood flow
		mmHg	*liters/min*	*ml/min*
Normal Na^+ (n = 5)	Control	130±2	4.32±0.40	243±13
	Indomethacin	127±2	4.10±0.59	240±42
	P	NS	NS	NS
Na^+ depletion (n = 5)	Control	131±8	3.18±0.30	243±19
	Indomethacin	135±10	2.82±0.39	189±30
	P	NS	NS	<0.05

Values are mean±SEM. The number of experiments is given in parenthesis.

in the sodium-replete animals. Although the precise stimulus for renin secretion during sodium depletion remains to be fully defined, it is known that renal sympathetic nerves are involved; renal denervation reduces the rise in PRA in response to deprivation of dietary salt (11).

While the roles of the renin angiotensin system in maintaining arterial blood pressure and in stimulating aldosterone secretion are well established, the role of the system in the regulation of the renal blood flow is less well defined. Administration of angiotensin II antagonists (1, 2) or of inhibitors of angiotensin II generation (2, 3) consistently elicits renal vasodilation in sodium-depleted dogs but fails to do so in sodium repletion. This observation has been taken as evidence that angiotensin II-induced renal vasoconstriction is a characteristic of sodium depletion (1, 28). However, in as much as renal blood flow has been found to be unchanged by sodium depletion in conscious animals, and in the present study no difference in blood flow was found between the two groups of dogs, the physiological interpretation of the renal vasodilation elicited by angiotensin II inhibition in sodium depletion remains to be clarified.

In the sodium-depleted dogs the renal vein norepinephrine concentration and the calculated overflow of norepinephrine from the kidney was more than twofold higher than in the normal sodium dogs. It is likely that the elevated renal vein plasma norepinephrine concentration was due to an enhanced level of norepinephrine release from the sympathetic terminals rather than to a change in its elimination from plasma since the plasma concentration of dopamine-β-hydroxylase (21), released from nerve terminals, and the urinary excretion of normetanephrine (22) are also increased during sodium depletion. In a previous study the overflow of norepinephrine from the kidneys was found to vary with the frequency of electrical stimulation of the renal nerves (29). Accordingly, the present data suggest that the activity of the renal sympathetic nerves is enhanced by chronic sodium depletion.

The precise stimulus responsible for enhanced renal overflow of norepinephrine during sodium depletion is unknown. Plasma volume changes have an inverse effect on renal sympathetic activity (30), and sodium depletion is known to decrease plasma volume. It is reasonable to postulate that the decrease in plasma volume (or some function of it) acts as a stimulus to enhance activity of the renal sympathetic nervous system during sodium depletion.

Since the renal nerves participate in the renin response to sodium depletion, they also contribute indirectly to the renal sodium conservation mediated by the renin-angiotensin-aldosterone system (11). Whether the renal adrenergic nerves facilitate urinary sodium conservation during sodium depletion by influencing renal hemodynamics is unknown. Despite enhanced norepinephrine outflow from the kidney, the renal blood flow in the sodium-depleted animals was not reduced below that of controls. Recent data suggest that the renal adrenergic nervous system may be directly involved in renal tubular reabsorption. Changes in efferent renal sympathetic nerve activity parallel changes in renal tubular sodium reabsorption without altered hemodynamics (31, 32). In addition, norepinephrine has been shown to stimulate active sodium reabsorption across isolated epithelial membranes (33). The observation that the release of norepinephrine from the kidney was markedly enhanced in antinatriuretic sodium depleted dogs supports the hypothesis that increased intrarenal norepinephrine may be a factor mediating the enhanced tubular reabsorption of sodium during chronic sodium depletion.

In both the normal sodium and the sodium-depleted animals the concentration of PGE_2 in renal vein blood was significantly higher than in arterial blood. The values in the normal sodium animals are similar to those reported by Terragno et al. (34) in unanesthetized dogs and by Dunn et al. (35) in anesthetized dogs that underwent retroperitoneal renal dissection. In the sodium-depleted animals renal venous PGE_2 concentration was significantly higher than in the sodium-replete animals and the calculated renal secretion rate of PGE_2 from the kidney into the renal venous blood was increased threefold. The finding that arterial PGE_2 concentration was not different in the two groups of animals despite increased overflow of PGE_2 from the kidney during sodium depletion is consistent with the observation that PGE_2 is largely inactivated during a single passage through the lungs (36). As the release of prostaglandins reflects *de novo* biosynthesis (37), the data provide new evidence that renal synthesis of PGE_2 is significantly increased during chronic sodium depletion.

Other workers have studied renal prostaglandin synthesis during sodium depletion by measuring the levels of PGE_2 in the renal tissue or excreted in the urine. Whereas Lifschitz et al. found no difference in urinary excretion of PGE_2 in rabbits on high or low sodium diets (38), Weber et al. (39) and Stahl et al. (40) reported that urinary PGE_2 excretion rates were higher in rabbits during sodium depletion. The latter workers also found that in vitro PGE_2 synthesis by papillary and medullary kidney slices was enhanced in tissue taken from sodium-depleted rabbits (40).

The cellular site of origin of the PGE_2 released from the kidney into urine or blood is unknown. It is likely both the medullary collecting duct (41) and papillary interstitial cells (42) contribute to urinary levels of PGE_2. Biosynthesis of PGE_2 has also been documented in vascular endothelial cells (43) and smooth muscle

cells (44). Accordingly, it is reasonable to speculate that the increased overflow of PGE_2 into renal venous blood observed in the sodium-depleted animals results, at least in part, from synthesis of PGE_2 in the renal cortical vasculature. Whether biosynthesis of other prostaglandins (such as PGD_2 or PGI_2) is also enhanced in chronic sodium depletion cannot be answered from the present data. Oliw et al. (45) have recently reported that urinary excretion of 6-keto-prostaglandin $F_{1\alpha}$ in sodium-depleted rabbits was unchanged from that of controls (45).

Although a variety of stimuli that are known to enhance prostaglandin biosynthesis might have been responsible for the elevated overflow of PGE_2 into renal venous blood in the sodium-depleted dogs, it is likely that both the markedly increased renal output of renin (angiotensin II) and of renal norepinephrine were involved. Angiotensin II stimulates in vitro PGE_2 synthesis in endothelial (43), vascular smooth muscle (44) and renal interstitial cells (42), and intrarenal infusions of angiotensin II causes increased biosynthesis of PGE_2 in the dog kidney (7, 35). Evidence for increased synthesis of renal prostaglandins has also been obtained in several conditions in which there is increased activity of renin-angiotensin system: hemorrhage (9), renal artery stenosis (35), cirrhosis with ascites (46) and Bartter's syndrome (46). Intrarenal infusions of norepinephrine and renal nerve stimulation (5) also enhance the release of PGE-like substances from the kidney and norepinephrine stimulates prostaglandin synthesis is isolated canine kidney cells (47). Whether other hormones, known to stimulate PGE_2 synthesis (antidiuretic hormone and bradykinin) also contribute to enhanced prostaglandin synthesis in the sodium-depleted animals is not known.

What is the physiological significance of the increased secetion of PGE_2 during chronic sodium depletion? Since the cellular origin and the stimulus responsible for the increased renal venous PGE_2 in sodium depletion are unknown, an answer to this question must remain inferential. In this study, administration of indomethacin to sodium-depleted dogs decreased the concentration of PGE_2 in renal venous plasma and abolished the secretion of this prostaglandin by the kidney, suggesting effective inhibition of prostaglandin synthesis. Concomitant with these changes there was a significant reduction in renal blood flow. Similar findings in sodium-depleted animals have been reported from this laboratory (3). On the other hand and in agreement with observations by others (48, 49), indomethacin had no significant effect on renal blood flow in sodium-replete dogs. These data suggest that the increased renal synthesis of prostaglandins attenuates the vasoconstrictor effect of increased angiotensin II and renal norepinephrine overflow (enhanced renal nerve stimulation) during chronic sodium depletion. Support for this interpretation is also found in pharmacological studies indicating that prostaglandin synthesis inhibition potentiates the renal vasoconstrictor effect of exogenous angiotensin II (8), of norepinephrine (8), and renal nerve stimulation (50). Further, administration of inhibitors of prostaglandin synthesis in a variety of conditions with enhanced activity of the renin-angiotensin system such as hemorrhagic hypotension (9, 26), renal artery constriction (35, 49) and liver cirrhosis with ascites (51) reduces renal blood flow.

In summary, this study demonstrates that dogs with moderate chronic sodium depletion have diminished cardiac output, increased total peripheral vascular resistance, and normal arterial pressure in association with increased arterial PRA and norepinephrine concentration. Despite increased peripheral vascular resistance, neither renal blood flow nor renal vascular resistance was different from that obtained in sodium-replete controls. The data also indicate that the renal vein PRA and the renal venous concentrations of norepinephrine and of PGE_2 as well as the renal secretion rates of PRA, norepinephrine and PGE_2 were higher in the sodium-depleted animals. Moreover, inhibition of renal prostaglandin synthesis reduced the renal blood flow in sodium-depleted animals. These findings suggest that renal blood flow and renal vascular resistance are maintained during chronic sodium depletion by an effect of prostaglandins to oppose the vasoconstrictor effects of angiotensin II and the renal sympathetic nervous system.

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