Relationship between Plasma Sodium Concentration and Vascular Reactivity in Man

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ABSTRACT Experiments were done to study the effects of acute changes in plasma sodium concentration [Na] on vasoconstrictor responses to norepinephrine and to reflex sympathetic stimulation in man. [Na] in the venous return from the forearm of each of 21 normal subjects was reduced (to an average of 118 mEq/liter), increased (147 mEq/liter), and maintained within the normal range (140 mEq/liter) by means of infusions into the brachial artery of three solutions containing different [Na]. Mannitol or sucrose and disodium sulfate were substituted for sodium chloride to produce the desired changes in [Na] without changing blood osmolarity.

Blood flow to the forearm (plethysmograph) increased during infusion of the three solutions, but the increase in flow was not related to [Na]. Vasoconstrictor responses to injections of norepinephrine and angiotensin into the brachial artery were reduced at low [Na] and augmented at high [Na]. Reflex vasoconstriction, activated by lower body negative pressure, was similarly affected by changes in [Na]. In the isolated, perfused gracilis muscle of dog vasoconstrictor responses to norepinephrine and to nerve stimulation were attenuated, and the extraction of norepinephrine by this muscle was smaller when plasma [Na] was reduced.

INTRODUCTION

The relationship between sodium and vascular responsiveness to catecholamines has important implications concerning the pathophysiology and treatment of hypertension and of certain hypotensive states. Numerous investigations have been carried out to clarify this relationship using in vitro preparations of arterial strips or perfused vascular beds in animals. Bohr, Brodie, and Cheu (2) found that the responsiveness of rabbit aortic strips to epinephrine was increased in a low sodium medium. Friedman, Friedman, and Nakashima (3), using the perfused rat tail, found that low sodium in the perfusate decreased the constrictor effect of norepinephrine. Tobian (4), referring to observations made by Martin and himself on rat aortic strips, reported that responsiveness to norepinephrine is not different at various sodium concentrations. The differences in conclusions probably reflect the differences in preparations and the rapidity of changes in sodium concentration in the extracellular environment (2–7).

Indirect evidence in man suggests an important role of sodium in vascular responsiveness. The increased pressor activity of catecholamines after treatment with adrenocorticoids (8–10) implicates sodium in vascular responsiveness. There have been no observations in man which relate directly vasoconstrictor responses to sodium concentration.

In the present study the responses of forearm resistance vessels in man to norepinephrine, angiotensin, and to lower body negative pressure were studied during intra-arterial infusion of solutions containing low, normal, and high sodium concentrations. Because changes in tonicity are known to affect vascular tone (11), the osmolarity of the three solutions was maintained equivalent. The vasoconstrictor effect of lower body negative pressure was studied to see if sodium altered responsiveness to the released neurotransmitter as well as responses to circulating norepinephrine. The possibility that changes in sodium concentration may alter responsiveness to norepinephrine by altering the rate of extraction of the circulating catecholamine was explored by measuring the extraction of norepinephrine in the gracilis muscle of dog perfused with blood containing low, normal, and high sodium concentrations.

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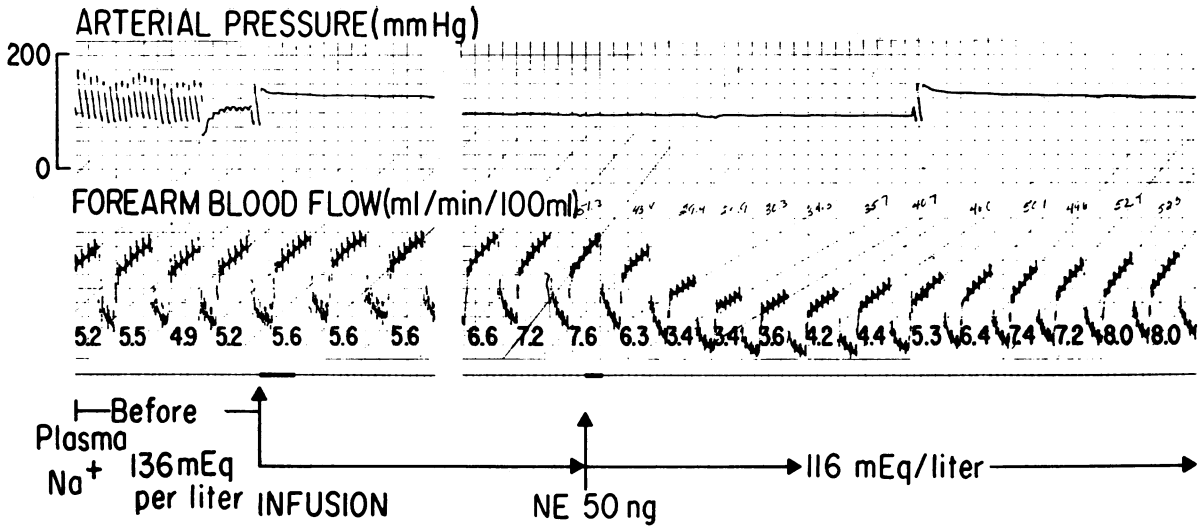

FIGURE 1 Arterial pressure and forearm blood flow before and during an infusion of low sodium solution into the brachial artery. Phasic arterial pressure was changed to mean arterial pressure before the infusion. During the infusion there was no record of arterial pressure since the infusion was made through the catheter used to record pressure; the infusion was intermittently interrupted to record 2–3 sec of arterial pressure. The plethysmographic tracings of changes in forearm volume during intermittent venous occlusion indicate forearm blood flow; the number under each slope represents flow in milliliters per minute per 100 milliliters of forearm. The distance between the vertical lines represents 2 sec. The sodium concentration in the venous blood from the forearm decreased from 136 mEq/liter before to 116 mEq/liter during the infusion of low sodium solution. Norepinephrine was injected at the arrow (NE 50 ng), causing a decrease in forearm blood flow without a change in systemic arterial pressure.

METHODS

Studies in man. Studies were carried out on 21 healthy male students 21–35 yr of age. The subjects were supine and comfortable with room temperature maintained at approximately 82°F.

The left brachial artery was cannulated with a polyethylene cannula (PE 60) after minimal superficial infiltration of the antecubital area over the artery with 1% procaine. The cannula was introduced approximately 2 in. into the artery and connected to a syringe for infusions of electrolyte solutions to change sodium concentration in the blood perfusing the forearm. The cannula was also connected to a pressure transducer. A No. 17 intracath was inserted about 2 in. into the left basilic vein for sampling of venous blood during the procedure.

Forearm blood flow was measured with a water plethysmograph (12). A pneumatic cuff placed around the left wrist was inflated to suprasystolic pressure during the measurements to exclude the contribution of venous return from the hand to changes in volume of the forearm. Another cuff was placed proximal to the plethysmograph and inflated intermittently for 4–8 sec to pressures sufficient to produce venous occlusion. The rapid increase in volume during the short successive periods of venous occlusion is directly proportional to the rate of forearm blood flow (Fig. 1). The increase in volume of the forearm during venous occlusion displaces water out of the plethysmograph into a narrow chimney and is measured as an increase in pressure.

Three different electrolyte solutions were infused in each subject. Their compositions are shown in Table I. One solution contained a normal sodium concentration. A second

TABLE I
Solutions Infused into Brachial Artery

	Low sodium	Normal sodium	High sodium
Sodium, *mEq/liter*	0	145	193
Potassium, *mEq/liter*	4.3	4.3	4.3
Calcium, *mEq/liter*	1.2	1.2	1.2
Magnesium, *mEq/liter*	0.8	0.8	0.8
Chloride, *mEq/liter*	8.4	152	8.4
Sulfate, *mEq/liter*	0	0	96.7
Mannitol or sucrose, *g/liter*	52.8 or 99.8	0	0
Osmolarity, *mOsm/liter*	305	305	305
pH	7.0–7.5	7.0–7.5	7.0–7.5

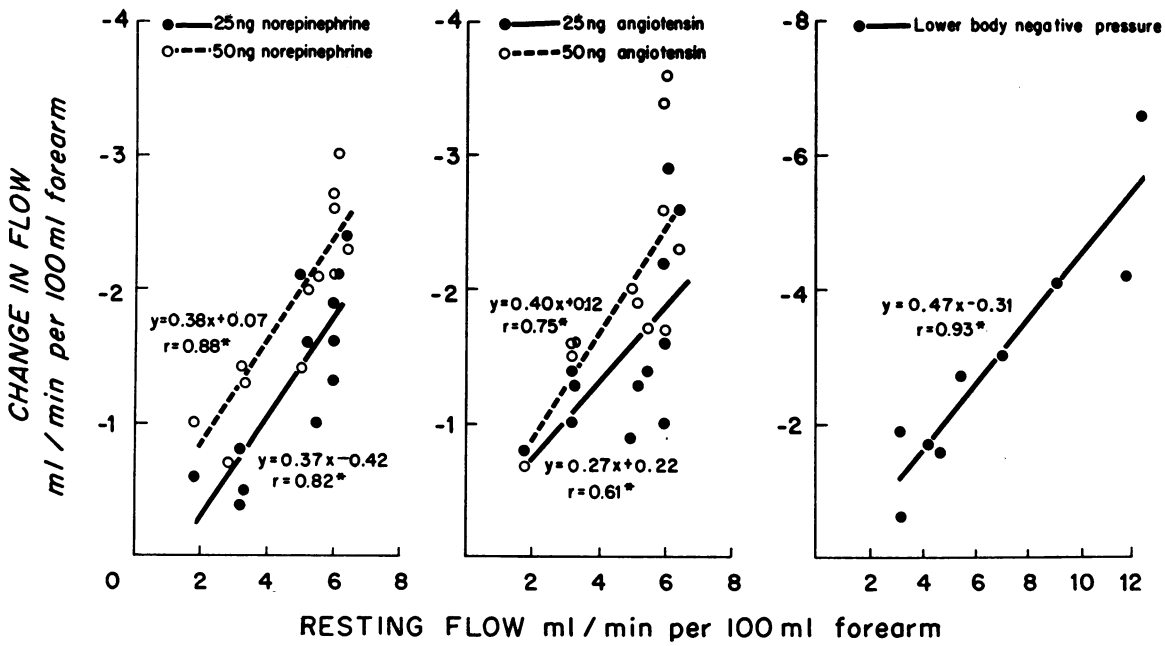

FIGURE 2 Relationship between resting forearm blood flow (after glucose injection) and the decrease in forearm blood flow in response to an injection of two doses of norepinephrine (left panel), angiotensin (middle panel), and to lower body negative pressure (right panel). Values were obtained during infusion of normal sodium solution. The asterisk indicates significant ($P < 0.05$) correlation coefficient.

solution did not contain sodium, but sucrose (in six subjects) or mannitol (in six subjects) was added to that solution in amounts calculated to maintain normal osmolarity. In the third solution disodium sulfate was substituted for sodium chloride to increase the concentration of sodium without changing osmolarity. Concentrations of potassium, calcium, and magnesium were similar in the three solutions, and the pH was adjusted to a level between 7.0 and 7.5. The small variations in pH of these unbuffered solutions could easily be compensated for by the buffering capacity of the blood in vivo.

The three solutions could be given in six possible sequences, and a different sequence was given to each of six consecutive subjects. The solutions were infused with a constant infusion pump at a rate of 5.7 ml/min for a period of 12 min, and a 25 min rest period was allowed between infusions.

In 12 subjects we studied the effects of changes in plasma sodium concentration on responses to norepinephrine and angiotensin. Beginning 2 min after the start of each infusion of electrolyte solution and while the infusion was continued, two doses of norepinephrine bitartrate (25 and 50 mμg as the base), two doses of angiotensin (25 and 50 mμg), and 1 ml of 5% dextrose in water were injected into the arterial cannula through which the solutions were infused. Injections were made at 2-min intervals in random order. The drugs were diluted in 1 ml of 5% dextrose in water.

In nine other subjects the effects of changes in sodium concentration on reflex vasoconstriction induced in the forearm were studied by exposure of the lower part of the body to subatmospheric pressure during the intra-arterial infusions. Subatmospheric pressures were achieved by enclosing the body below the iliac crests in an airtight box and lowering the pressure within the box to a pressure 40 mm Hg below ambient barometric pressure for 1½–2 min (13).

A venous blood sample was obtained before, at a variable time during, and just before the end of each infusion. 3–5 ml of blood were heparinized with 20 μl (400 U) of heparin. Plasma sodium and potassium concentrations were measured by flame photometry. Hematocrit and serum osmolarity were determined.

Analysis of data. Blood flow to the forearm was calculated from the rate of increase of volume of the forearm during venous occlusion and expressed in milliliters per minute per 100 milliliters of forearm.

The effects of norepinephrine and angiotensin on forearm blood flow were determined by comparing the values of blood flow measured immediately after the injections of the drugs with those measured immediately after the injection of 5% dextrose in water, which was used as the diluent for the drugs. The values of flow after glucose are referred to as "resting flow." The responses to the drugs were expressed as the ratio of the "change of flow" to the "resting flow." For example the response to norepinephrine was

$$\frac{\text{resting flow} - \text{flow after norepinephrine}}{\text{resting flow}}.$$

The reason for expressing the responses as ratios is the positive correlation between the resting flow and the magnitude of the reduction in flow in response to the vasoconstrictor intervention (Fig. 2); i.e., the higher the resting flow the greater the change in flow (resting flow − flow after the intervention). Similarly responses to lower body

negative pressure were expressed as a ratio of the "change in flow" (i.e. flow before − flow during negative pressure) to the resting flow (flow before negative pressure). Statistical comparisons were made by analysis of variance of these ratios during low, normal, and high sodium (14). Since changes in arterial pressure were negligible during the interventions, the changes in flow reflected changes in vascular resistance.

Animal experiments. Male mongrel dogs were anesthetized with chloralose (500 mg/kg) and urethane (50 mg/kg), treated with decamethonium bromide (0.3 mg/kg), and ventilated artificially. The gracilis muscle was dissected free from all surrounding tissue except for the artery and vein. The branch of the obturator nerve to the gracilis was cut, and its distal end was secured for subsequent nerve stimulations. After injecting 5 mg/kg heparin intravenously, a large cannula was advanced into the abdominal aorta via a femoral artery. The cannula was connected to tubing, which was secured in a variable speed peristaltic pump, and the distal end was inserted into the gracilis artery. Blood flow was adjusted at the beginning of each experiment so that perfusion pressure approximated systemic arterial pressure. Flow was maintained constant throughout the experiment and averaged 15.2 ml/min in these experiments. At a constant flow, changes in perfusion pressure reflected changes in resistance.

Responses in the gracilis to injections of norepinephrine (0.1, 0.3, and 1.0 μg, as the base, given in random order) and to 15 sec of nerve stimulation with a bipolar electrode (10 v, 10 msec, 1.5, 3, and 6 Hz in random order) were studied during infusions containing low, normal, and high

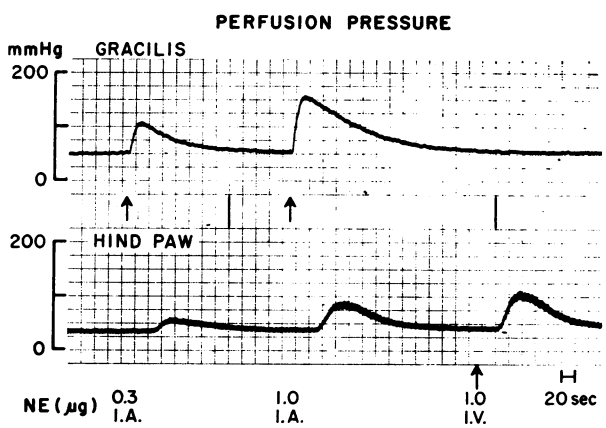

FIGURE 3 Autobioassay of norepinephrine present in venous effluent from the gracilis muscle. The first two arrows (upper tracing) indicate injections of norepinephrine into the tubing perfusing the gracilis muscle (I.A. = intra-arterial injections); the norepinephrine caused an increase in perfusion pressure (indicating vasoconstriction) in the gracilis and a delayed increase in perfusion pressure in the hind paw (the assay organ). The third arrow (lower tracing) indicates injection of norepinephrine into the tubing coming from the gracilis vein (I.V. = intravenous injection) to the hind paw. The response in the hind paw to an injection of 1 μg norepinephrine into the gracilis artery (second arrow) was smaller than the response in the hind paw to the same dose injected into the gracilis vein (third arrow), indicating that injected norepinephrine was taken up during transit through the gracilis muscle.

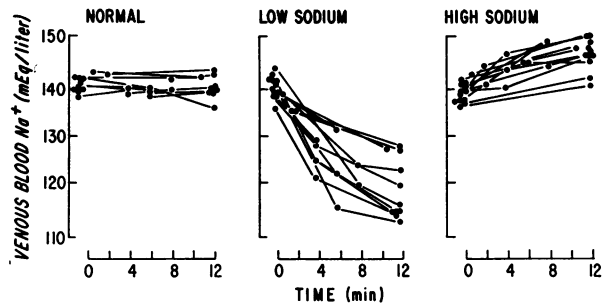

FIGURE 4 Concentration of sodium in the basilic vein in each of 12 subjects before and during infusion into the ipsilateral brachial artery of solutions containing normal (145 mEq/liter), low (0 mEq/liter), and high (193 mEq/liter) sodium concentration.

concentrations of sodium, following the same design used in man. Infusions were made upstream from the perfusion pump and ranged from 1.9 to 5.7 ml/min, depending on the rate of blood flow.

Autobioassay. In order to determine the extraction of norepinephrine by the perfused gracilis, the venous effluent from the muscle was perfused at a constant rate of flow into the isolated denervated hind paw via its cranial tibial artery. The hind paw served as an "assay organ," as changes in perfusion pressure in the paw occurred in response to varying concentrations of vasoactive substances in the venous blood from the gracilis (15). Injections of norepinephrine into the tubing perfusing the gracilis at a site upstream from the pump elicited vasoconstrictor responses in the muscle followed by vasoconstrictor responses in the perfused "assay paw." The delay of the response in the assay paw was caused by the transit time between the muscle and the paw. Responses in the assay paw to injections of norepinephrine upstream from the gracilis muscle were compared with responses in the paw to injections of norepinephrine made on the venous side of the gracilis. Responses of the assay paw to graded doses of norepinephrine injected downstream from the gracilis were used to plot a "standard curve" of the response for bioassay (Fig. 3).

RESULTS

Studies in man

Sodium and potassium concentrations in venous blood from the forearm. During the intra-arterial infusion of the solution containing 145 mEq/liter of sodium, the concentration of sodium in venous blood from the forearm remained at an average value of 140 mEq/liter (Fig. 4). During infusion of the solution which did not contain sodium, there was a progressive drop in sodium in venous blood to an average of 118 mEq/liter. Infusion of 193 mEq/liter of sodium intra-arterially caused a rise in sodium concentration in venous blood to an average level of 147 mEq/liter. The potassium concentration in the venous blood from the forearm was 4.1 ±0.1 (SE) mEq/liter during low sodium, 4.0 ±0.1 mEq/liter during the normal sodium, and rose significantly ($P < 0.001$) to 4.6 ±0.2 mEq/liter during the high sodium infusion.

TABLE II

*Average Changes in Hematocrit and Osmolarity in the Venous Effluent from the Forearm during the Intra-Arterial Infusions**

	Low sodium			Normal sodium			High sodium		
	C	2.5 min	6.5 min	C	2.5 min	6.5 min	C	2.5 min	6.5 min
Hematocrit									
Mean	48.12	41.85	42.50	46.55	44.85	43.60	47.90	44.35	42.70
SE	0.74	1.02	1.23	1.04	1.23	0.86	0.12	1.74	1.10
n	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Osmolarity									
Mean	316.5	312.7	310.9	310.5	311.3	313.5	309.8	305.5	307.4
SE	14.21	6.02	7.05	10.58	8.22	10.04	7.82	7.86	8.85
n	(6)	(6)	(8)	(6)	(6)	(8)	(6)	(6)	(8)

* Samples were obtained before (C) and after 2.5 and 6.5 min of infusion.

Effect of hematocrit and osmolarity. Decreases in hematocrit ranged from 5 to 20% (Table II) during the infusions, and they were not related to sodium concentration. Changes in plasma osmolarity were small during the infusions, and they also were not related to changes in sodium concentration. Thus the osmolarity and hematocrit were similar during infusion of the three solutions.

Effect of sodium concentration on resting forearm blood flow. The three infusions caused significant increases in resting blood flow. There was no correlation between sodium concentration in the solution and the magnitude of increase in flow (Fig. 5).

Relation of sodium concentration to responsiveness to norepinephrine and angiotensin. The intra-arterial injections of norepinephrine and angiotensin were insufficient to cause detectable changes in arterial blood pressure. In the forearm, responses to norepinephrine and angiotensin were reduced during infusion of low sodium solution and were augmented during infusion of high sodium solution (Tables III and V).

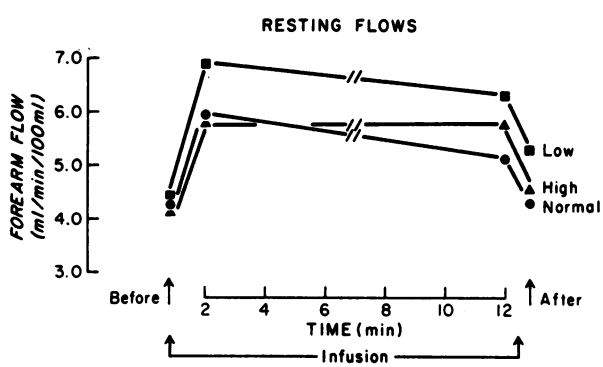

FIGURE 5 Average forearm blood flow in 21 subjects before, during, and after the infusion of low, normal, and high sodium solutions into the brachial artery. Values were obtained before the infusions, during the 2nd and 11th min of infusions, and during the 2nd min after the infusions were stopped. All three solutions increased resting forearm blood flow; there was no direct correlation between flow and sodium concentration in the infusate.

Responses to lower body negative pressure. The magnitude of the decreases in forearm blood flow in response to lower body negative pressure was also directly related to the concentration of sodium in the infusion (Tables IV and V).

Animal experiments

Effects of changes in sodium concentration in the perfused gracilis. Sodium concentration in the venous effluent from the gracilis was 109 ±5.1 (SE) mEq/liter during low sodium infusion, 149 ±2.2 during normal sodium, and 164 ±2.8 during high sodium. The infusion of the three solutions decreased perfusion pressure from an average of 110.1 ±10.4 (SE) mm Hg to an average of 95.4 ±8.4, from 106.3 ±8.0 to 83.9 ±5.8, and from 107.3 ±8.3 to 95.3 ±8.6 during the low, normal, and high sodium solutions, respectively. There was no correlation between the sodium concentration in the perfusate and vascular resistance. Venous hematocrit averaged 32.8 ±1.9 (SE)%, 37.3 ±1.7%, and 37.5 ±1.8% during infusion of the low, normal, and high sodium solutions.

There was a positive correlation between sodium concentration in the perfusate and the responses to norepinephrine and to nerve stimulation (Tables VI and VII).

Extraction of norepinephrine. The extraction of norepinephrine by the perfused gracilis was decreased in seven of nine animals during the low sodium infusions and rose again during the high sodium infusions in six of seven experiments (Fig. 6).

DISCUSSION

These experiments indicate that responses of forearm resistance vessels to hormonal and neurogenic vasocon-

TABLE III

*Forearm Blood Flow in Response to Norepinephrine and Angiotensin during Infusion of Low, Normal, and High Sodium Solutions**

	Low sodium solution					Normal sodium solution					High sodium solution				
Subject	Glu	NE_1	NE_2	A_1	A_1	Glu	NE_1	NE_2	A_1	A_2	Glu	NE_1	NE_2	A_1	A_2
D. P.	5.5	5.7	3.3	5.2	2.1	5.0	2.9	3.6	4.1	3.0	5.4	2.8	1.9	2.7	1.8
		(−0.04)‡	(0.40)	(0.05)	(0.62)		(0.42)	(0.28)	(0.18)	(0.40)		(0.48)	(0.65)	(0.50)	(0.67)
R. S.	4.1	5.5	5.6	3.9	3.2	3.3	2.8	2.0	2.0	1.7	5.2	2.9	2.2	2.7	1.7
		(−0.34)	(−0.36)	(0.05)	(0.22)		(0.15)	(0.39)	(0.39)	(0.48)		(0.44)	(0.58)	(0.48)	(0.67)
C. R.	8.9	6.2	5.5	6.1	6.8	6.0	4.4	3.4	5.0	4.3	5.2	3.5	3.1	4.7	3.2
		(0.30)	(0.38)	(0.31)	(0.24)		(0.27)	(0.43)	(0.17)	(0.28)		(0.33)	(0.40)	(0.10)	(0.38)
H. S.	5.7	4.6	4.2	4.9	5.8	6.0	4.1	3.3	3.8	2.6	5.4	3.4	2.6	3.0	2.6
		(0.19)	(0.26)	(0.14)	(0.02)		(0.32)	(0.45)	(0.37)	(0.57)		(0.37)	(0.52)	(0.44)	(0.52)
G. L.	3.2	3.6	2.5	2.4	2.3	3.2	2.8	2.1	2.2	1.6	3.2	2.3	1.8	2.0	2.3
		(−0.12)	(0.22)	(0.25)	(0.28)		(0.12)	(0.34)	(0.31)	(0.50)		(0.28)	(0.44)	(0.38)	(0.28)
J. B.	7.1	5.0	4.2	5.8	3.7	6.1	4.0	3.1	3.2	2.5	8.1	4.8	3.4	3.5	3.1
		(0.30)	(0.41)	(0.18)	(0.48)		(0.34)	(0.49)	(0.48)	(0.59)		(0.41)	(0.58)	(0.57)	(0.62)
J. A.	7.6	5.4	6.1	5.3	4.9	6.0	4.7	3.9	4.4	3.4	5.2	3.5	3.3	3.6	2.5
		(0.29)	(0.20)	(0.30)	(0.36)		(0.22)	(0.35)	(0.27)	(0.43)		(0.33)	(0.36)	(0.31)	(0.52)
W. T.	6.2	4.9	5.8	6.0	4.8	5.2	3.6	3.2	3.9	3.3	5.9	4.5	3.4	4.2	2.6
		(0.21)	(0.06)	(0.03)	(0.22)		(0.31)	(0.38)	(0.25)	(0.36)		(0.24)	(0.42)	(0.29)	(0.56)
J. K.	4.1	3.4	2.2	2.4	2.1	3.2	2.4	1.8	1.8	1.7	5.0	3.2	4.0	2.3	1.9
		(0.17)	(0.46)	(0.41)	(0.49)		(0.25)	(0.44)	(0.44)	(0.47)		(0.36)	(0.20)	(0.54)	(0.62)
R. A.	4.1	3.2	2.9	3.9	3.6	1.8	1.2	0.8	1.0	1.1	2.7	2.0	1.5	2.1	1.6
		(0.22)	(0.29)	(0.05)	(0.12)		(0.33)	(0.56)	(0.44)	(0.39)		(0.26)	(0.44)	(0.22)	(0.41)
W. J.	7.2	6.1	5.0	7.0	4.8	5.5	4.5	3.4	4.1	3.8	7.2	5.0	3.8	4.0	3.6
		(0.15)	(0.30)	(0.03)	(0.06)		(0.18)	(0.38)	(0.25)	(0.31)		(0.30)	(0.47)	(0.44)	(0.50)
J. G.	7.9	8.2	7.4	7.1	6.0	6.4	4.0	4.1	3.8	4.1	6.0	5.1	4.4	4.9	5.9
		(−0.04)	(0.06)	(0.10)	(0.24)		(0.38)	(0.36)	(0.41)	(0.36)		(0.15)	(0.27)	(0.18)	(0.02)
Mean	5.96	5.14	4.56	4.99	4.18	4.81	3.45	2.89	3.28	2.77	5.38	3.58	2.95	3.30	2.74
SE	0.53	0.40	0.47	0.45	0.47	0.33	0.30	0.28	0.35	0.32	0.42	0.30	0.28	0.28	0.35
Mean of Δ flow/ resting flow		(0.108)	(0.223)	(0.158)	(0.279)		(0.274)	(0.404)	(0.330)	(0.428)		(0.329)	(0.444)	(0.371)	(0.481)

* Values are average blood flow during 1 min after injection of glucose (Glu) or 25 or 50 mμg of norepinephrine (NE_1 and NE_2) or angiotensin (A_1 and A_2).

‡ The values in parentheses are ratios of changes in flow after norepinephrine or angiotensin to the resting flow; e.g. the ratio for $NE_1 = \frac{Glu - NE_1}{Glu}$ (see text).

TABLE IV

*Forearm Blood Flow in Response to Lower Body Negative Pressure during Infusion of Low, Normal, and High Sodium Solutions**

	Low sodium solution		Normal sodium solution		High sodium solution	
Subject	Resting	LBNP	Resting	LBNP	Resting	LBNP
D. L.	11.6	6.3	11.8	7.6	11.0	5.4
		(0.46)		(0.36)		(0.51)
J. S.	5.2	2.5	3.2	1.3	3.6	1.9
		(0.52)		(0.59)		(0.47)
D. C.	4.4	5.1	5.5	2.8	4.2	3.2
		(−0.16)		(0.49)		(0.24)
D. B.	10.8	6.4	12.5	5.9	10.0	4.3
		(0.41)		(0.53)		(0.57)
D. S.	6.2	4.2	7.1	4.1	7.8	3.3
		(0.32)		(0.42)		(0.58)
M. R.	5.8	4.4	3.2	2.6)	6.6	3.8
		(0.24)		(0.19)		(0.42)
M. T.	11.2	5.1	9.2	5.1	9.9	4.7
		(0.54)		(0.44)		(0.52)
L. O.	5.9	6.3	4.2	2.5	3.0	1.5
		(−0.07)		(0.40)		(0.50)
V. V.	5.1	3.8	4.7	3.1	5.0	2.6
		(0.25)		(0.34)		(0.48)
Mean	7.36	4.90	6.82	3.89	6.79	3.41
SE	0.98	0.44	1.19	0.66	1.01	0.43
Mean of Δ flow/ resting flow		(0.279)		(0.417)		(0.477)

* Values are average blood flow during 3 min of control (resting) and during the last 1 min of lower body negative pressure (LBNP). In parentheses are ratios of response to resting flow $\frac{(\text{resting} - \text{LBNP})}{\text{resting}}$.

strictor stimuli are reduced when the concentration of sodium in the plasma is reduced and are augmented when the sodium concentration is increased. Indirect evidence in man had sugggested an effect of sodium on vascular responsiveness (8–10). The present study provides direct evidence relating vasoconstrictor responses to sodium concentration in man.

Injections of norepinephrine and angiotensin were begun 2 min after starting the infusions of low, normal, and high sodium solutions at a time when sodium concentration in the venous effluent was changing. Therefore some of the injections during the early period of the low, or high, sodium infusion were made at a time when sodium concentration had not yet changed maximally. Thus we may have underestimated the effect of the change in sodium concentration on responsiveness. The reason for not waiting until the concentration of sodium in the venous effluent had reached a plateau at the lowest or highest level was that we did not want to prolong the period of arterial occlusion at the wrist with the pneumatic cuff for more than 15 min. Longer periods of occlusion would have caused discomfort and introduced a variable in our observations.

In addition to the comparison of responses to norepinephrine and angiotensin during the three infusions of sodium, the responses were compared based on the venous sodium concentration estimated to be present at the exact time of injection of norepinephrine or angiotensin (Fig. 7). The sodium concentration at the time of each injection was estimated for each subject from the slope of the concentration of sodium in the venous effluent obtained from that subject (Fig. 4). There was again evident a relationship between sodium concentration and vasoconstrictor responses.

In contrast with the effect of sodium on vascular responsiveness, we found no relationship between sodium concentration and resting blood flow. This finding is in agreement with previous studies (11, 16). The increase in resting blood flow appears to be due, at least in part, to diminished blood viscosity during the infusion (11).

In these experiments sodium concentration in the forearm was altered without changing osmolarity, which is

TABLE V

*Analysis of Variance of Forearm Response to Norepinephrine, Angiotensin, and Lower Body Negative Pressure during Infusion of Low, Normal, and High Sodium Solutions**

Source of variation	df	ms	*F*	*P*
Response to norepinephrine				
Subjects	11	0.0385	2.1154	
Norepinephine	1	0.2604	14.3077	<0.01‡
Sodium	2	0.3256	17.8901	<0.01§
Sodium × norepinephrine	2	0.0004	0.0220	>0.05
Error	55	0.0182		
Response to angiotensin				
Subjects	11	0.0481	3.0063	
Angiotensin	1	0.2167	13.5438	<0.01‡
Sodium	2	0.2832	17.7000	<0.01§
Sodium × angiotensin	2	0.0008	0.0500	>0.05
Error	55	0.0160		
Response to lower body negative pressure				
Subjects	8	0.0429	1.9953	
Sodium	2	0.0929	4.3209	<0.05§
Error	16	0.0215		

* Values analyzed are ratios in Tables III and IV.

‡ Indicates significant dose-response effect of norepinephrine and angiotensin.

§ Indicates significant effect of sodium concentration on responses to norpinephrine, angiotensin, and lower body negative pressure.

known to have important vascular effects (11, 17). Because it is not possible to change sodium concentration without also changing anion concentration and because osmolarity cannot be maintained without substituting another osmotically active material, the experimental design required that, in addition to altered sodium concentration, chloride, sulfate, sucrose, and mannitol concentrations would change. These experiments were not designed to evaluate the role of these substances, but it is unlikely that they play an important role. Mannitol and sucrose are inert sugars which do not enter the cell, and it is unlikely that they are active when osmolarity is not changed. Previous experiments suggest that chloride and sulfate are not active (11). There is indirect evidence in this study that chloride and sulfate did not alter vasoconstrictor responses. The chloride concentration was the same in the high and low sodium solutions, but the vasoconstrictor responses were altered; similarly the sulfate concentration in the normal and low sodium solutions was the same, but the responses were altered.

The finding that the potassium concentration increased in the venous effluent from the forearm during the infusion of high sodium solution was not anticipated. The reason for this increase is not clear. In view of the efflux of potassium from arterial walls in response to norepinephrine (18), one might postulate that the increased response to norepinephrine during the high sodium infusion was associated with a greater effect of norepinephrine on membrance potential of vascular smooth muscle and consequently a greater potassium efflux. The high potassium would not explain the augmented reactivity during the high sodium infusion, however. Studies of the effect of intra-arterial infusions of potassium chloride on vascular reactivity in the hand indicate that increased potassium concentration does not alter vascular responsiveness (19).

There are several mechanisms by which sodium may affect vascular responses to norepinephrine. Sodium may influence the water content of vessel walls. Sodium and water retention in the vessel wall would increase wall thickness (20), perhaps altering vascular reactivity. This mechanism requires a change in osmolarity, as well as sodium content, and is therefore not applicable to the present experiment. Another possible mechanism is an effect on membrane potential (21). Sodium may also compete with calcium for a carrier in the cell membrane (22). Sodium has also been shown to facilitate the superprecipitation of actomyosin by calcium (23), suggesting an important interaction of sodium and calcium in contraction of smooth muscle. It is also possible that sodium may alter the rate of metabolism of norepinephrine in the vessel wall.

Another mechanism by which sodium may alter vascular responses may be an effect on norepinephrine trans-

TABLE VI

*Changes in Gracilis Perfusion Pressure in Response to Norepinephrine and Nerve Stimulation during Infusion of Low, Normal, and High Sodium Solutions**

	Low sodium solution			Normal sodium solution			High sodium solution		
Dog	NE_1*	NE_2	NE_3	NE_1	NE_2	NE_3	NE_1	NE_2	NE_3
Norepinephrine									
1	56	78	106	73	109	132	41	95	130
2	55	85	130	52	90	132	45	82	135
3	32	47	65	40	65	78	32	47	82
4	7	17	27	20	24	41	15	32	52
5	25	40	75	45	57	89	37	68	96
6	37	66	108	37	74	115	33	60	98
7	12	28	58	18	23	58	42	55	85
8	50	93	125	60	92	134	75	95	135
9	32	40	78	37	60	68	40	80	115
10	17	30	57	22	35	62	25	48	87
Mean	32.3	52.4	82.9	40.4	62.9	90.9	38.5	66.2	101.5
SE	5.5	8.3	10.5	5.7	9.3	11.0	5.0	6.8	8.6

	Low sodium solution			Normal sodium solution			High sodium solution		
Dog	1.5‡	3	6	1.5	3	6	1.5	3	6
Nerve stimulation									
11	20	37	52	37	55	75	39	55	72
12	36	70	72	55	100	174	56	93	145
13	35	38	51	32	46	50	60	90	133
14	10	15	30	25	40	64	28	40	59
15	36	52	67	40	53	60	23	36	45
16	23	32	52	20	37	55	33	57	100
7	20	38	45	28	50	65	35	57	70
8	13	18	38	20	37	60	32	50	77
17	35	44	60	72	106	145	67	99	132
Mean	25.3	38.2	51.9	36.6	58.2	83.1	41.4	64.1	92.6
SE	3.5	5.6	4.4	5.8	8.8	14.8	5.2	7.9	12.1

* Pressure measurements were recorded at constant flow, and the changes in pressure reflect changes in resistance. NE_1 NE_2, and NE_3 indicate injections into the gracilis artery of 0.1, 0.3, and 1.0 μg norepinephrine. Entries are the maximum changes in perfusion pressure after each injection.
‡ See preceding footnote. Nerve stimulation at 1.5, 3, and 6 Hz was maintained for 15 sec.

port in the vessel wall. Sodium is known to be essential for several carrier transport systems of nonelectrolytes (24, 25), and observations on perfused rat hearts indicate that the rate of uptake of norepinephrine is dependent on the concentration of sodium (26, 27). The present study indicates that a decrease in extracellular sodium concentration is associated with decreased extraction of injected norepinephrine as well as decreased vasoconstrictor responses. If these observations are related, the results suggest that the reduction of extraction involves the free fraction of norepinephrine available for receptor sites. When infused norepinephrine is extracted by tissues, a fraction of it is bound to neurons and possibly to extraneuronal binding sites (28, 29) and thus "inactivated" or metabolized, but a fraction of it apparently remains in a free form for approximately 5–10 min (30) and may be accessible to smooth muscle receptors for that short period of time. A reduction in the "bound" fraction would increase responsiveness as is seen after cocaine, but a reduction in the fraction which remains transiently free from binding would decrease responsiveness. Sodium may influence the retention of "free" norepinephrine by the vessel wall.

The effect of sodium on the availability of free norepinephrine for the receptors need not be specific for norepinephrine and could explain also the decreased responsiveness to angiotension which we have observed. In six dogs we attempted to determine the effect of sodium on extraction of angiotension by the bioassay method used for norepinephrine. We were unable to de-

TABLE VII

*Analysis of Variance of Response of Dog Gracilis to Norepinephrine and Nerve Stimulation during Infusion of Low, Normal, and High Sodium Solutions**

Source of variation	df	ms	F	P
Response to norepinephrine				
Dogs	9	4,904.7	37.8	
Norepinephrine	2	22,594.1	174.2	<0.005‡
Sodium	2	1,300.9	10.0	<0.005§
Error	76	129.7		
Response to nerve stimulation				
Dogs	8	3,421.8	10.89	
Nerve stimulation	2	11,597.3	36.9	<0.005‡
Sodium	2	5,571.1	17.7	<0.005§
Error	68	314.2		

* Values analyzed are changes in perfusion pressure in Table VI.

‡ Indicates significant dose-response effect of norepinephrine and frequency-response effect of nerve stimulation.

§ Indicates significant effect of sodium concentration on responses to norepinephrine and nerve stimulation.

termine the extraction because of tachyphylaxis to this agent which prevented us from establishing the reproducible dose-response curve necessary for the bioassay.

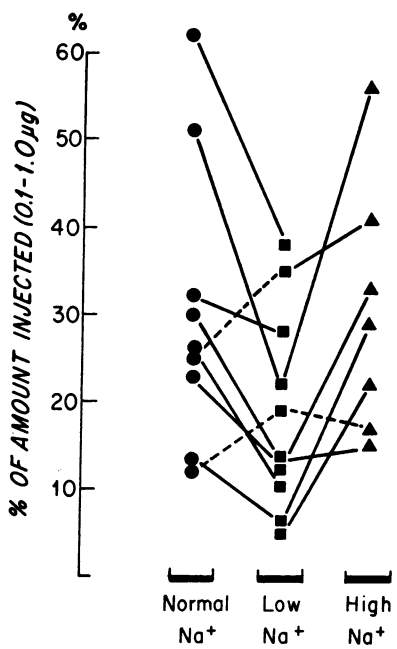

FIGURE 6 Extraction of norepinephrine by the gracilis was calculated by assaying the amount of norepinephrine in the venous effluent and subtracting it from the amount injected (see text and Fig. 3). Each entry represents an average of three values obtained with three different doses of norepinephrine. The extraction of norepinephrine was decreased ($P < 0.05$ by Wilcoxon signed ranks test) during the low sodium infusions.

Decreasing sodium concentration caused a decrease in extraction of norepinephrine, but increasing sodium did not consistently increase the extraction. One possible explanation is that the degree of hyponatremia was greater than the degree of hypernatremia. We were able to eliminate sodium from the infusion and cause a marked fall in sodium concentration in the venous effluent, but it was not possible to increase sodium concentration in the plasma to the same extent without changing osmolarity. It is also possible that maximal extraction of norepinephrine occurs at sodium concentrations that are within the physiologic range. In support of this

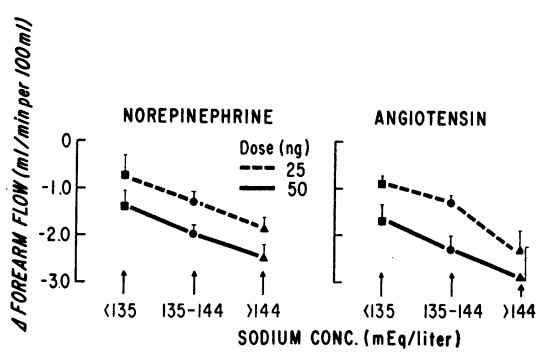

FIGURE 7 Changes in forearm blood flow in response to norepinephrine and angiotensin plotted against the concentration of sodium estimated to be present in the effluent at the time of injection of the constrictor drug. The middle values (135–144 mEq/liter) include some responses seen during infusion of the low sodium or the high sodium solution, but at the time of injection of the vasoconstrictor drug the sodium concentration had not fallen below 135 nor risen above 144 mEq/liter.

possibility, the studies reported by Horst, Kopin, and Ramey on rat hearts (*27*) indicate that uptake of norepinephrine was diminished in the presence of low sodium concentration in the bathing medium and was not augmented when sodium concentration was increased above normal levels.

One should consider also the possibility that the reduction in extraction of norepinephrine, which occurs when sodium concentration is decreased, may not be causally related to the decrease in vascular reactivity. Sodium concentration may not influence reactivity of all vessels equally so that reduction of sodium concentration may shunt norepinephrine into arteriovenous anastamoses causing decreased responses to norepinephrine and a decreased extraction of the amine. In light of the limited role of arteriovenous anastamoses in skeletal muscle, this possibility is unlikely but cannot be completely excluded.

The relationship between sodium concentration and the presence of free norepinephrine in the vessel wall, which we have proposed in this study, will remain speculative until more direct observations become available.

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