Sodium Intake and Vascular Smooth Muscle Responsiveness to Norepinephrine and Angiotensin in the Rabbit

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ABSTRACT
The effect of dietary sodium restriction on the responses to norepinephrine and angiotensin was assessed in two vascular smooth muscle preparations in the rabbit. Sodium restriction reduced the response to angiotensin of both the limb vessels in vivo and the aorta in vitro. The response to norepinephrine was potentiated by sodium restriction in both preparations. The effects on angiotensin responsiveness did not appear to be related to tachyphylaxis, changes in extracellular electrolyte composition, or nonspecific depression of smooth muscle function. Probably, the change in sodium intake induced a change in the smooth muscle cell membranes which modified the affinity of their receptors for angiotensin and norepinephrine.

KEY WORDS
leg blood flow vascular reactivity tachyphylaxis aorta dietary sodium

The therapeutic efficacy of sodium restriction in treating hypertension has focused attention on the role of the sodium ion in control of smooth muscle responsiveness (1-5). But, in view of the potential importance of vascular reactivity in hypertension, there is surprisingly little direct information available on the influence of dietary sodium intake on vascular smooth muscle responsiveness. The majority of studies have assessed the effects of either acute changes in sodium concentration in vitro or agents such as diuretics and adrenal mineralocorticosteroids in vivo (6-18). When diet has been controlled, pressor responses have usually been used as the index of vascular reactivity (17-19).

The relevance or specificity of each type of study is open to question. Dietary sodium restriction results in a reduction in the total amount of sodium in the body, but changes in the sodium concentration of the body fluids are minimal (20). Diuretics and salt-retaining steroids may well have effects beyond their influence on the sodium content of the body (16, 21, 22), and the blood pressure response to an agent is the integral of a large number of factors beyond the reactivity of the arterioles (23). For these reasons, we have explored the effects of dietary sodium restriction on the responses of two vascular preparations in the rabbit to the two most active endogenous vasoconstrictor agents, angiotensin and norepinephrine. Blood flow to the isoperfused rabbit leg was studied in vivo. The rabbit aortic strip, studied in vitro, provided insight into the effects of dietary sodium intake on vascular smooth muscle reactivity without the complicating effects of changes in blood volume, resting blood flow, and arteriolar diameter in a setting in which the characteristics of the milieu could be controlled.

Methods
Studies were carried out in 30 New Zealand white rabbits weighing 2.5-3.5 kg. Until 5-7 days
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before the study, the rabbits were fed a standard rabbit diet ad libitum with tap water. Sodium restriction was achieved in 12 rabbits by replacing the standard diet with a sodium-deficient test diet for rabbits (General Biochemicals, no. 170940). Tap water was replaced with distilled water. A high-sodium intake was achieved in the other rabbits with the same diet by supplementing it with 7.3 g sodium/kg chow. The effectiveness of the diets was assessed at 5–7 days by collecting a bladder urine sample for the determination of sodium and creatinine concentrations. The addition of sodium resulted in a sodium intake of approximately 5 mEq/kg body weight/day.

Anesthesia for the blood flow studies was induced with sodium thiopental, about 30 mg/kg, administered into an ear vein and sustained with intravenously administered chloralose, 80 mg/kg, and urethane, 900 mg/kg. The anesthetic regimen was supplemented with infiltration of lidocaine subcutaneously for skin incisions and into the left femoral nerve proximal to the dissection of the artery. The rabbits underwent tracheostomy; respiration was controlled with a Harvard Instruments small-animal respirator. Body temperature was monitored with a rectal probe (Yellow Springs Instrument Company) and was maintained at 36–37°C with an infrared heat lamp.

Blood flow to the left leg was measured with a drop-counting system similar in principle to that described by Lindgren (24). The blood was rendered incoagulable prior to arterial catheterization by the intravenous administration of heparin, 5 mg/kg. The largest polyethylene catheters possible, generally PE 205, were inserted into the right carotid and left femoral arteries. The catheters and drop-counting system were siliconized prior to use. The arterial inflow from the carotid artery was directed into the translucent drop chamber of a disposable clinical intravenous administration set (Travenol Labs, Plexitron, R 41). The chamber was filled with liquid silicone of low viscosity (Dow Corning 200 series, viscosity 2.0 centistokes at 25°C), which the blood traversed as a series of relatively constant-sized drops. The outflow from the chamber was directed to the left femoral artery and, thus, to the vascular bed of the leg. The active dead space of the system was approximately 3 ml, less than 2% of the rabbit’s estimated blood volume.

The drop rate was registered with a photocell (Grass P TTI photoelectric transducer) placed around the drop chamber, and the photocell actuated an ordinate recorder (Black Lion Productions). The photocell signal cleared an active integrator whose ramp voltage immediately prior to clearing was a linear function of the signal interval. The interval between drops determined excursion of the ordinate recorder. The system was calibrated with heparinized rabbit blood: drop size was essentially constant over the physiological ranges of hematocrit and flow rates registered in this study. There were approximately 3 drops/ml. Arterial perfusion pressure was measured with a Statham P23DC transducer, and hormone injections were made by way of the rubber flashball of the Travenol set in the outflow portion of the system. Drugs were administered as a 0.1-ml bolus controlled by a Hamilton micrometer syringe and injected through a PE 10 coaxial catheter whose tip lay near the femoral arterial insertion site. Pressure and flow were recorded continuously on a Grass Instruments polygraph.

Dose-response curves were obtained for noradrenaline bitartrate (Levophed, Winthrop) and for angiotensin amide (Hypertensin, Ciba) in random order in 14 rabbits on controlled sodium intake. The agents were administered in log-dose increments into the arterial inflow circuit. Doses ranged from 1 to 1,000 ng, calculated as the salt, for each agent. The responses were assessed independently by two individuals on a coded basis without reference to the dietary protocol.

In an additional seven rabbits, attempts were made to induce tachyphylaxis to angiotensin in the limb vascular bed. High doses (300–1,000 ng) of angiotensin were injected into the artery serially at 5–10-minute intervals for 80–90 minutes. The possibility that the contact of angiotensin with blood and circulating angiotensinase in the inflow circuit prior to reaching the arteriolar bed had influenced the final free concentration and the responses was also assessed in two sodium-restricted rabbits. Angiotensin was diluted in saline or in the rabbit’s whole blood. After 1–3 minutes of incubation, the diluted angiotensin was injected in random order into the inflow circuit, and the resultant response was used to assess the contribution of circulating angiotensinase activity. The possibility that arterial catheterization and the drop-counting system had altered resting skeletal muscle perfusion was assessed in two rabbits by injecting radioactive microspheres (3µm, 35µ) into the left ventricle and counting samples of skeletal muscle taken from the gastrocnemius of each leg.

The rabbit aortic strip preparation used in this laboratory has already been described in detail (25); it is based on the original description of Furchgott and Bhadrakom (26). In brief, four strips from each rabbit aorta were mounted with 4 g of tension in muscle chambers with a 10-ml working volume containing a modified Krebs-bicarbonate medium (25). The same medium was used for aortas from rabbits on the high-
the low-sodium diets. The solution was maintained at $37 \pm 0.5^\circ C$ and aerated constantly with a gas mixture containing $95\% O_2-5\% CO_2$. Isotonic contractions were monitored with a force transducer. The tissues were allowed to equilibrate for 60 minutes prior to drug administration. The total interval in Krebs medium from removal of the tissue to the first exposure to a vasoactive agent was at least 120 minutes, of which about 60 minutes were at room temperature. Norepinephrine and angiotensin were used on the aorta from every rabbit, but individual strips were exposed to only one agent. A cumulative dose-response curve was obtained starting at $10^{-11} g/ml$ with logarithmic increments until a maximum contraction was achieved. The results were expressed as a percent of the peak response achieved for each strip.

All mean values have been expressed with the standard error of the mean as the index of dispersion. Tests of statistical significance were made with the Wilcoxon rank sum test for nonparametric data, and the null hypothesis was rejected when a $P$ value of less than 0.05 was achieved.

**Results**

The seven rabbits on the high-sodium diet had a mean arterial blood pressure of $84.4 \pm 4.5$ mm Hg and a leg blood flow of $13.5 \pm 0.53$ ml/min. Urine sodium concentration normalized to the concentration of creatinine was $0.69 \pm 0.17$ mEq/mg creatinine. Sodium restriction in seven rabbits reduced mean arterial blood pressure to

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**FIGURE 1**

Recording of a dose-response curve to angiotensin in the limb of a rabbit on the low-sodium diet. Note that the ordinate recording scale is logarithmic and arranged so that a reduction in blood flow is represented by a larger downward excursion. At the lowest rates of blood flow, the individual drops are easily evident.

**FIGURE 2**

Effect of sodium restriction on the vascular responses of the limb to angiotensin. Each point represents the mean response at that dose, and the standard error of the mean is represented as the index of dispersion. Note the parallel shift in the dose-response curve induced by the low-sodium diet, reflecting a reduction in sensitivity.
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68.2 ± 1.7 mm Hg (P < 0.01) and the normalized urine sodium concentration to 0.10 ± 0.17 mEq/mg creatinine (P < 0.01). Sodium restriction did not change resting blood flow to the leg. The well-maintained blood flow (12.6 ± 0.77 ml/min) despite a significant reduction in arterial blood pressure must have reflected a decrease in vascular resistance. Urine sodium, blood pressure, and resting blood flow did not differ in rabbits on the usual pellet diet and in those on the high-sodium diet.

An example of a blood flow recording is shown in Figure 1 and the full dose-response curves in Figures 2 and 3. In all rabbits on the high-sodium diet, 1 ng of angiotensin induced a response, with an average flow reduction of approximately 8%. Progressively larger flow reductions occurred with increasing doses. Higher doses also increased blood pressure, presumably through recirculation, but the maximal flow reduction usually preceded the pressor response. Norepinephrine did not induce a response until a threshold dose of approximately 10 ng was given in the rabbits on the high-sodium diet. Increasing doses of norepinephrine also induced progressively larger flow reductions. But, on a weight basis, angiotensin was approximately tenfold more active than norepinephrine.

Sodium restriction resulted in a striking reduction in the sensitivity of the limb vessels to angiotensin (Fig. 2). Only one of six rabbits tested responded to doses below 10 ng. There was a significant parallel shift of the dose-response curve (P < 0.001), reflecting approximately a threefold reduction in sensitivity to angiotensin. Sodium restriction resulted in a directionally opposite effect on the responsiveness to norepinephrine (Fig. 3). The responses to low doses were especially potentiated (P < 0.001), but the responses became progressively more similar in sodium-restricted and sodium-loaded rabbits with increasing norepinephrine doses. The responses were identical at a dose of 1,000 ng.

Introduction of the drop-counting system in the arterial supply of the left leg did not have a demonstrable effect on resting flow as assessed with radioactive microspheres. The delivery of the indicator to the skeletal muscle vascular bed of the experimental (364 counts/min g⁻¹) and the control (338 counts/min g⁻¹) limb was similar.

Angiotensin tachyphylaxis could not be induced acutely in this vascular bed with the protocol used. The largest reduction in response is shown in Figure 4. Attempts to demonstrate a significant destructive effect of blood and tissue angiotensinase during a period of exposure exceeding the time required for the hormone to reach the arterioles were also unsuccessful.

The aortas taken from rabbits on the high-sodium diet showed essentially identical dose-response relationships to norepinephrine and angiotensin. The threshold dose for each agent was below 1 ng/ml (Fig. 5). Sodium restriction again resulted in opposite effects on the responsiveness to angiotensin and norepinephrine. Sodium restriction reduced the sensitivity to angiotensin significantly (P < 0.001); the dose inducing a response which was 50% of the maximum (ED₅₀) rose from approximately 8 to 15 ng/ml, a 50% reduction in sensitivity.
Responses of the limb vessels to serial doses of angiotensin. Ten injections of 300 ng were made at 5-10-minute intervals over 90 minutes. The first, second, third, sixth, and tenth responses are shown. The very modest reduction in response evident in the tenth injection was the largest desensitisation recognized. This system is resistant to the development of angiotensin tachyphylaxis.

The responses to norepinephrine were potentiated ($P<0.001$), with a reduction in $ED_{50}$ from approximately 8 to 3 ng/ml. The effect of sodium restriction on norepinephrine responsiveness differed in vivo and in vitro: the potentiation was apparent primarily at threshold doses in the limb, whereas an approximately parallel shift occurred in the dose-response curve of the aorta.

Discussion

A reduction in pressor responsiveness to angiotensin by sodium restriction is well documented (17-19). A priori, this effect could be due to any one of a number of influences of sodium restriction on the cardiovascular system such as a change in reflex responsiveness (23) or a reduction in plasma volume and cardiac output (16). The present study, however, clearly suggests a direct effect of sodium restriction on arterial smooth muscle or its receptors. The studies with norepinephrine were included to assess the specificity of changes in responsiveness to angiotensin.

Potentiation of responses to norepinephrine by sodium restriction was not expected when the study was initiated in view of the effects of sodium restriction on hypertension. However, a similar effect of sodium restriction on the responses of the canine renal vascular bed to norepinephrine has been reported by Kilcoyne and Cannon (27), and we have noted a similar, but smaller, effect on the renal vasculature in man (28).

The directionally opposite effect of sodium restriction on the responsiveness to angiotensin II and to norepinephrine suggests that different mechanisms are operative. It is often assumed, generally tacitly, that unresponsiveness to angiotensin with sodium restriction represents a form of angiotensin tachyphylaxis due to the increase in circulating angiotensin levels. However, the two tissues studied, the rabbit leg and aorta, are both resistant to the acute development of angiotensin tachyphylaxis (12, 25). It is likely, therefore, either that acute and chronic angiotensin tachyphylaxis are fundamentally different—a concept not
Easily accounted for at the receptor level—or that a mechanism unrelated to angiotensin tachyphylaxis is involved. The observations with norepinephrine make it clear that the decrease in responsiveness to angiotensin does not reflect a nonspecific reduction in smooth muscle reactivity. This study also indicates that the reduction in sensitivity with sodium restriction could not have been only apparent, reflecting an increase in local and circulating angiotensinases. Such an increase would reduce the angiotensin concentration at the receptor level and, thus, result in an apparent reduction in sensitivity. Sodium restriction does potentiate the angiotensinase activity of the rat kidney (29), but exposure of angiotensin to blood from sodium-restricted rabbits for an interval which exceeded the exposure in vivo did not reduce the response. The suggestion that the effects of sodium intake on angiotensin response reflect changes in the affinity of the receptor for angiotensin is attractive (30) but requires more direct evidence before a rigorous conclusion can be drawn.

The effects of sodium restriction on the response to either agent are probably not due directly to changes in the extracellular electrolyte composition. The in vitro experiments with the aortic strips allowed precise control of the milieu. In fact it is surprising that a difference was demonstrable: the treatment included 2 hours of exposure to an identical solution, part of the exposure being at room temperature. This maneuver has been carefully studied (31). Such profound effects are exerted on the intracellular electrolyte composition that it seems equally unlikely that sodium restriction exerted its effect there. The in vitro studies also provided ample opportunity for both diffusible substances to leave the aortic smooth muscle and for the angiotensinase content of the aorta to destroy angiotensin accumulated during the period of sodium restriction (25). Sodium restriction must have exerted its effect on the angiotensin response through other less-apparent mechanisms, perhaps through a relatively persistent effect on the cell membrane or its receptors. This concept is consistent with the hypothesis of Brunner et al. (30). Perhaps the mechanism involves the relationship between the paracellular matrix responsible for sodium binding (32, 33), sodium intake, and the angiotensin receptor. The presence of a number of mucopolysaccharides with an exceptional...
affinity for sodium distinguishes arteries from other tissues containing smooth muscle. The concentration of sodium at this locus may be three times that in the extracellular fluid. The effect of dietary sodium intake on this system and its relationship to vascular receptors is not known.

The potentiation of the effects of norepinephrine is equally difficult to explain. One possibility is that in the in vivo studies the heightened responses reflect the differences in the resting tone of the vessels. Arterial perfusion pressure was reduced significantly, but blood flow was essentially unchanged by sodium restriction. Thus, a reduction in arteriolar resistance and an increase in arteriolar diameter could account for the potentiation. Clearly, such geometric factors could not account for the potentiation in the rabbit aortic strip.

An extensive literature exists on the relationship between the sodium ion and smooth muscle responsiveness. As was pointed out, difficulties in the interpretation of most studies can be defined in three broad categories. First, many concerned themselves with changes in response with manipulation of sodium concentration in the milieu. Even for this relatively simple maneuver, a uniform influence on responses to catecholamines of changes in sodium concentration has not been reported. A reduction in sodium concentration results in an increase in tone (7) and potentiation of the responsiveness of the rabbit aortic strip (6); similarly an increase in sodium concentration decreases the responsiveness of this preparation (12). On the other hand, a reduction in sodium concentration reduces the responsiveness of the perfused rat tail (8, 9, 13), rat colon (9), and human and dog limb vascular bed (10, 11). Finally, the responses of the rabbit ear are not significantly influenced by the sodium concentration of the perfusate (14). Thus, it is difficult to define a central theme in the effects of changes in sodium concentration. One must recognize, moreover, that dietary intake influences sodium concentration very little: the relevance of these studies to the effects on vascular reactivity of changes in sodium intake are thus equally unclear.

In the second broad group of studies, the total sodium content of the organism was manipulated by the administration of agents which influence salt balance through an action on the kidney (15, 16). The implicit assumption is that such agents act only, or primarily, through their effects on sodium balance. This class of agent may have a considerably more complex action (21, 22).

When sodium intake has been controlled directly, vascular reactivity has generally been assessed on the basis of blood pressure responses (17–19, 30). This approach provides a crude and potentially misleading index of arteriolar responsiveness. Pressor responses are the net result of a complex series of reactions: a variable amount of constriction occurs in different vascular beds, venous constriction increases venous return to the heart as a function of venous volume, and both direct and reflex effects influence cardiac output. More direct indexes will be necessary to achieve insight into the surprisingly large effects of a low-sodium diet: excretion of no more than 4% of the total body sodium exerts profound effects on so many systems.

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References


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