Separate roles of sodium ion concentration and fluid volumes in salt-loading hypertension in sheep

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IN SEVERAL PREVIOUS STUDIES from our laboratory our findings have suggested that hypertension following sodium loading results from the effect sodium has in increasing the extracellular fluid volume, which in turn causes the hypertension (4, 7, 9, 12). We did not find, in those studies, any indication that sodium has a direct effect on the arterioles in increasing their resistance and in this way cause the hypertension. However, our impressions were not entirely in accord with the views of other investigators who have suggested a direct effect of sodium ions on peripheral vascular resistance. For instance, Tobian and Redleaf (20) and Friedman and Friedman (8) have both proposed mechanisms by which increased intracellular penetration by sodium ions could lead to increased vascular resistance and consequent hypertension, and other authors have shown that administration of deoxycorticosterone acetate (DOCA) with concomitant sodium loading causes either a disturbance in norepinephrine storage (6) or an increased vascular reactivity to norepinephrine (2), either of which could cause increased vascular resistance and elevated arterial pressure. Yet, in opposition to these findings and in support of our impressions, Haddy and Scott (10) showed that direct perfusion of tissues with blood containing excess plasma sodium not only fails to increase the vascular resistance but actually decreases it.

Nevertheless, there still remained the question whether sodium retention causes hypertension entirely as a result of salt-induced volume increase or whether increased body sodium can also elevate arterial pressure by a direct effect of sodium ions on vascular resistance. Therefore, the object of the present study was to determine in one group of animals the chronic effect on arterial pressure caused by increased extracellular fluid volume but with no change in plasma sodium concentration, and to determine in another group the effect of increasing the plasma sodium concentration with no change in extracellular fluid volume. For study of the effect of increased extracellular fluid volume, sheep were bilaterally nephrectomized and maintained on chronic hemodialysis; then the parameters of dialysis and fluid intake were changed to increase the extracellular fluid volume while keeping the plasma sodium concentration constant. To study the effect of increased plasma sodium concentration without concomitant change in extracellular fluid volume, we treated unilaterally nephrectomized sheep with DOCA to reduce renal loss of sodium; then the plasma sodium was elevated by dialyzing the sheep against a dialysate with high sodium concentration, while extracellular fluid volume was maintained at a normal level by simultaneous ultrafiltration through the dialyzing membrane.

METHODS

Eleven sheep were used in this study—six bilaterally nephrectomized and five unilaterally nephrectomized. The six anephric sheep were used to study the effects of high extracellular fluid volume on arterial pressure. The five unilaterally nephrectomized sheep were used to study the effect of high plasma sodium concentration (high [Na]) on arterial pressure.

Surgical Preparation

The anephric sheep were initially prepared by right nephrectomy under pentobarbital anesthesia and by implantation of a chronic arteriovenous shunt to be used later for chronic hemodialysis. The shunt, Silastic tubing of 4.88 mm OD x 2.65 mm ID, was inserted into the right renal
artery and vein, and then about 15 cm into the aorta and inferior vena cava, respectively. The shunt was exteriorized through the abdominal wall, and Dacron wings were used to anchor the tubing to the abdominal muscle. The left kidney was excised after a minimum recovery period of 2 wk. Surgical preparation of the high-[Na] sheep differed only in that the left kidney was not removed.

**Experimental Protocol**

**Bilaterally nephrectomized, high-extracellular fluid volume sheep.** After surgical preparation, all the anephric sheep were maintained on a regime of chronic hemodialysis, with 4-h dialysis sessions either every day or every other day throughout the experiment. Arterial pressure, cardiac output, sodium concentration, and sodium space were measured periodically, as reported in the subsequent data.

Beginning 2 days after all surgery had been completed, we recorded base-line data for 3 days. During this time, the plasma sodium concentration was maintained as close to normal as possible by appropriate hemodialysis, and the extracellular fluid volume was maintained at its normal level by adjusting the degree of ultrafiltration during each dialysis session while measuring the animal’s weight continuously on a scale with 1-oz graduations.

After the base-line period, the sheep were divided equally into two groups. One of these groups was continued as controls, while in the other group the extracellular volumes were acutely elevated to levels equal to the base-line values plus 20%. This elevation of extracellular fluid volume was achieved by intravenous infusion of saline during the dialysis session on the last day of the base-line period. Thereafter, for a 7-day experimental period, dialysis was used to maintain as closely as possible a) normal extracellular fluid volume and sodium concentration in the control sheep, and b) 20% expansion of extracellular fluid volume but normal sodium concentration in the high-volume sheep. These conditions were achieved by precise adjustment of the parameters of dialysis and ultrafiltration during each dialysis session.

**Unilaterally nephrectomized, high-sodium concentration sheep.** The protocol for the unilaterally nephrectomized sheep consisted of an 8-day base-line period, begun a minimum of 2 wk after the right nephrectomy, and this being followed by a 7-day experimental period. Arterial pressure, sodium concentration, sodium space, and blood volume were measured periodically, as presented in the subsequent data. During this entire sequence each animal was given 25 mg/day of DOCA oil administered intramuscularly.

On the last day of the base-line period, each sheep was subjected to a single 4-h hemodialysis period during which the animal was dialyzed against dialysate with high salt concentration. This caused an acute increase in plasma sodium concentration averaging 18 meq/liter. Once the sodium concentration had been increased to its elevated value, it was kept at this level throughout the entire experimental period by restricting the sheep’s fluid intake. We found that so long as the sheep did not receive enough fluid to elevate their extracellular fluid volumes, DOCA escape did not occur, and the animals continued to maintain their elevated sodium concentrations.

**Instrumentation**

The sheep were dialyzed using a standard Kiil artificial kidney. They were prepared with 200 U of sodium heparin per kilogram body weight and were then given 100 U/kg per h for the duration of the dialysis period. During dialysis, the sheep were restrained in a stanchion mounted on a scale. In this way continuous monitoring of body weight could be used to determine fluid volume change consequent to ultrafiltration during the hemodialysis period.

Arterial pressure, cardiac output, and blood samples were all obtained by way of the animal’s shunt. Cardiac output was determined by dye dilution (Cardio-green, supplied by Hynson, Westcott, and Dunning, Inc., Baltimore, Md.), with use of a Gilford model 103-IR cuvette densitometer. Plasma sodium concentration was determined by flame photometry.

The sodium-22 space was determined from 150- and 180-min plasma samples. Plasma volume was determined in the unilaterally nephrectomized sheep with use of Evans blue dye and extrapolation to zero time; blood volume was determined from these data and packed red cell volume.

**Analysis**

The paired-<i>t</i> test was used to compare the data collected during the experimental period with the corresponding data of the base-line period.

**Results**

**Effects of Increased Extracellular Fluid Volume on Arterial Pressure and Other Circulatory Variables**

Following the second nephrectomy, the protocol for the two groups of anephric sheep consisted of a 2-day recovery period followed by a 3-day base-line period and a 7-day experimental period. The hemodialysis protocol after the 3rd base-line day differed according to the experimental group. Animals in the control group continued to be dialyzed in a manner that maintained base-line sodium and fluid volume values. Sheep in the high-volume group were given a 20% step increase in extracellular fluid volume (as determined by sodium space measurements), while plasma sodium concentrations were maintained at base-line levels.

Arterial pressure, plasma sodium, and sodium-space data obtained from the bilaterally nephrectomized sheep are presented in Fig. 1. Vertical arrows separate the 3-day base-line period from the 7-day experimental period. Table 1 gives average values for all the data during the base-line and experimental periods and results of the statistical analysis.

In sheep of the control group there were no statistically significant differences in arterial pressure, plasma sodium, or sodium space between the base-line and experimental periods.

In sheep with 20% increase in extracellular fluid volume, the arterial pressure increased steadily following the step increase in extracellular volume, and rose to 150% of base line by the last 2 days of the experimental week (arterial pressure averaged 141% of base line during the entire experimental week). The rise in arterial pressure in each of the high-volume sheep was well sustained.

Average cardiac output on the last day of the base-line
period was 137 ± 8.4 ml/min per kg for the control sheep and 132 ± 25 ml/min per kg for the high-volume sheep. Cardiac output of the control sheep remained between 100 and 110% of the base line without statistically significant changes between the base-line and experimental periods. However, following the step increase in extracellular fluid volume in the high volume sheep, cardiac output increased to an average of 115% of the base line during the experimental week (P < .05). The calculated total peripheral resistance also increased steadily after the step increase in extracellular volume in the high-volume sheep; it averaged 121% of base line (P < .01) during the experimental week.

Effects of Increased Plasma Sodium Concentration on Arterial Pressure and Other Circulatory Variables

In the unilaterally nephrectomized sheep plasma sodium concentration was increased suddenly an average of 18 meq/liter on the last day of the base-line period and was maintained at this new level thereafter, as explained in METHODS. However, extracellular fluid volume was kept nearly normal, with only a small increase in sodium space, as given in Table 1. The average base-line blood volume was 77 ± 6.9 ml/kg. During the experimental week blood volume averaged 84 ± 5.4 ml/kg, which was not significantly different from base line. The resulting changes in plasma sodium concentration and mean arterial pressure are illustrated in Fig. 2.

Note the very significant increases in plasma sodium concentration on all days of the experiment, but note also that the mean arterial pressure on any single day did not rise enough to be significant. Yet, as shown in Table 1, when the average arterial pressure during the entire experimental period is compared with the average arterial pressure during the entire base line period there is an average rise in arterial pressure of 4 mmHg, a value that is very slight but significant. Thus these experiments show that there was little or no effect on arterial pressure caused by marked increases in sodium ion concentration when the extracellular fluid volume was maintained near the normal level.

Effect of Increased Exchangeable Sodium on Arterial Pressure

In both the high-volume and the high-[Na] experiments, the exchangeable sodium was increased in each type of animal during the experimental period—an average increase of 20% in the high-sodium concentration sheep and an average increase of 21% in the high-volume sheep. Despite this almost exactly equal increase in exchangeable sodium, the arterial pressure responses were entirely different, as summarized in Fig. 3. Pressure rose 47 mmHg in the high-volume animals during the final 2 days of the experimental wk and only 7 mmHg in the high-[Na] animals. Since it was not possible to prevent a slight increase in the extracellular fluid volume in the high-sodium animals (as explained

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<th>TABLE 1. Arterial pressure, plasma sodium, and sodium space during base-line and experimental periods in 3 groups of sheep</th>
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<tr>
<td><strong>Arterial Pressure, mmHg</strong></td>
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<td>Base line</td>
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Values are means ± SF. Statistics given are paired-t test comparisons of average experimental period values to corresponding data of the base-line period.
SALT VS. FLUID VOLUMES IN HYPERTENSION

FIG. 2. Mean arterial pressure and plasma sodium concentration of uninephrectomized sheep given a step increase in plasma sodium. Solid lines are average values, and symbols represent individual data for 5 high-[Na] sheep. Arrows separate the base-line and experimental periods.

FIG. 3. Mean arterial pressure versus exchangeable sodium in high-volume sheep and high-[Na] sheep. Left-hand points are base-line values, while right-hand points are average values during final 2 days of experimental period. Vertical and horizontal bars represent ±1 SE.

above), it is likely that the slight pressure increase that did occur in these animals was caused by increased volume rather than by a direct effect of the sodium on the peripheral resistance.

DISCUSSION

Both in experimental animals and in human beings a prolonged increase in total exchangeable sodium is usually associated with hypertension. Though most research workers have recognized that the increase in extracellular fluid volume that is usually associated with increase in exchangeable sodium is one of the factors that causes hypertension, there has been a strong belief as well that the increased sodium might also have the direct effect of increasing total peripheral resistance and that this might be the important factor in hypertension (3, 18).

Therefore, the goal of the present studies was to determine the relative importance of sodium-induced volume changes versus nonvolume-dependent effects of sodium in the causation of hypertension. The results were very clear, especially as illustrated in Fig. 3. An increase in exchangeable sodium in animals with elevated plasma sodium concentration and only a minor increase in extracellular fluid volume caused an average rise in pressure of only 7 mmHg during the final 2 days of the experimental period, while an almost equal increase in exchangeable sodium in animals in which the extracellular fluid volume increased an equivalent amount caused an average rise of 47 mmHg on the last 2 days of the week.

These results are not surprising in view of clinical experience, for patients with essential hypernatremia but without evidence of expanded extracellular fluid volume do not develop hypertension despite a greatly elevated plasma sodium concentration (1, 11). Also, experience in essentially all clinical-dialysis wards has shown that proper maintenance of fluid balance in anephric human patients produces normotension, while overhydration results in hypertension (3, 21). On the other hand, inadvertent abnormalities of sodium ion concentration without concomitant changes in volume have little effect on the arterial pressure.

A possible criticism of these studies is that the animal models are not exactly comparable—the hypervolemia was achieved in totally nephrectomized animals while the hypernatremia was achieved in unilaterally nephrectomized animals. In view of the fact that many investigators have suggested that kidneys exert nonexcretory, hypotensive functions (5, 14, 17, 19), and since sodium seems to play a role in the release of prostaglandins and other renal hormonal substances (13, 15, 16), it is possible that the failure of the pressure to rise in the hypernatremic animals was due to the presence of the remaining single kidney. However, there are several reasons to doubt this. The most important is that numerous studies have shown that hypertension of approximately the same magnitude as that produced in the present experiments occurred in partially nephrectomized dogs that were salt loaded and in which the volume increased without increase in sodium ion concentration (4, 7, 12). In other words, the presence of normal renal tissue did not prevent the hypertension in these animals when the volume increased. On the other hand, in the present studies the pressure did not increase when there was increased sodium load without increased volume. Therefore, it is our belief that the presence of kidney tissue did not play a significant role in these experiments.

We conclude from these experiments that increased sodium ion concentration has little, if any, nonvolume-related effect on arterial pressure, while sodium-induced fluid-volume expansion has the very marked effect of elevating arterial pressure.

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