Some Aspects of the Tubular Locus of the Natriuretic Action of Vasoactive Drugs (36931)

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(Introduced by R. H. Egdahl)

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Intrarenal infusion of acetylcholine, a potent vasodilator, increases sodium excretion both in normal (1, 2) and in edematous (3) dogs. The systemic infusion of pressor agents in addition to intrarenal vasodilators (vasoactive drugs) results in a further increment in sodium excretion (2, 3). Micropuncture studies have demonstrated that at least one site of action of acetylcholine is on the proximal tubule, in which both fractional and absolute sodium reabsorption decrease (4).

Although vasoactive drugs decrease proximal fractional sodium reabsorption (PFSR) and increase distal delivery of filtered sodium (4), it is not clear whether this proximal effect accounts for the natriuresis. In normal dogs, comparable increases in distal delivery can be induced by infusing hyperoncotic albumin, but sodium excretion increases only minimally (5). In dogs with thoracic vena caval constriction (TVC dogs), saline infusion increases distal delivery without inducing natriuresis (6). From these observations, it seemed to us unlikely that depression of PFSR alone would be an adequate mechanism to explain the natriuresis induced by vasoactive drugs in normal and TVC dogs. To test the hypothesis that inhibiton of distal reabsorption is a key factor, we attempted to block the proximal effect of vasoactive drugs. In normal dogs, prior depression of PFSR by hyperoncotic albumin infusion preempted the usual proximal effects of vasoactive drugs. Nevertheless, sodium excretion increased markedly in response to these agents. These results support the view that distal inhibition is a key factor in the natriuretic response to vasoactive drugs. Inhibition both of proximal and of distal reabsorption appeared to be factors in the natriuretic response of TVC dogs to the vasoactive agents.

Methods. Experiments were performed on 10–25 kg normal mongrel dogs and on chronic TVC dogs. TVC dogs were prepared as previously described from this unit (6, 7) and experiments were performed 4 to 7 days postoperatively. For this study, TVC dogs were defined as dogs with an inferior vena cava pressure greater than 15 cm saline, ascites and urinary sodium excretion of less than 200 μEq/min when challenged with a saline load equal to 10% of body weight. The experimental techniques for micropuncture were those previously described (6). The only additional procedure was that a size 26 needle, connected to a syringe by polyethylene tubing, was inserted in a retrograde direction into the main renal artery, usually at the division of the artery into its two main branches and fixed with a drop of adhesive. The needle was kept open with an infusion of 0.9% sodium chloride at 0.5–1.0 ml/min until acetylcholine was infused into the renal artery at 40 μg/min. In addition, each dog was infused via a femoral vein with a vasopressor, either angiotensin or metaraminol, at initial infusion rates of 1 and 10 μg/min, respectively. The dose of either agent was adjusted as necessary during an experiment to maintain a stable elevation in blood pressure of 30 to 50 mm Hg.

Group I. Eleven normal dogs were infused with 25% albumin in saline at 0.32 ml/kg/min for 20 min. When urine volume

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and the drop in hematocrit had stabilized, usually about 1 hr after completion of the infusion, tubular samples were collected. Recollections were then made during the infusion of vasoactive drugs given as described above. In eight dogs, SNGFR measurements from separate tubules were made before and during the infusion of vasoactive drugs.

Group II. Eleven TVC dogs were infused over 60–90 min with isotonic saline to 10% body weight and the rate of infusion was then decreased to exceed slightly the rate of urine flow. Initial micropuncture collections were made. Then the combination of acetylcholine via the renal artery and a pressor agent systemically were given and repuncture collections were obtained. In five dogs timed collections for measurement of superficial nephron glomerular filtration rate (SNGFR) were made in separate tubules punctured before and during the infusion of vasoactive drugs.

Some dogs in each group received angiotensin and others metaraminol as the pressor agent. Since no differences in results were noted within any group between the two pressors, the data have been pooled and the combination of either with acetylcholine is described as vasoactive drugs. Mean values for both clearance and micropuncture data were calculated for each experiment and values given in text and tables are means ± SE of the means of individual experiments. Statistical significance of the differences of means was determined by the Student's *t* test, using paired analysis wherever appropriate. Analytical methods in use in this laboratory have been described previously (6).

Results. Group I. The data from the 11 dogs in Group I are summarized in Table I. There was no significant natriuresis after albumin alone. Sodium excretion from the vasodilated kidney increased from 31 to 267 μEq/min during vasoactive drug infusion, and on the control side (not shown in Table I) increased from 46 to 108 μEq/min. There was no change in GFR, C_{PAH}, or filtration fraction on the experimental side. $(TF/P)_{In}$ (33 tubules) did not change significantly during vasoactive drugs. The results were the same for 18 tubules within this group which were identified as end-proximal with lissamine green [$(TF/P)_{In}$ 1.45 ± 0.04 to 1.42 ± 0.05]. In eight dogs in which timed collections were made, SNGFR did not change significantly during vasoactive drugs [mean of means, 70.1 before and 81.1 after drugs ($p > 0.2$)]. Kidney GFR in these dogs was 24.1 and 23.7 ml/min in the control and experimental periods. The mean ratios of SNGFR/GFR before and after drugs were 2.71 and 3.22 × 10^6, respectively; the difference was not significantly different ($p > 0.2$).

TABLE I. Effect of Vasoactive Drugs in Normal Dogs Preloaded with Hyperoncotic Albumin (Group I).[a]

	Albumin	Albumin + drugs
$U_{Na}V$ (μEq/min)	31.0 ± 5.5[b]	267 ± 40.6[b]
C_{In} (ml/min)	26.0 ± 3.1	26.3 ± 2.75
C_{PAH} (ml/min)	80.3 ± 9.1	80.2 ± 12.2
FF	0.33 ± 0.04	0.34 ± 0.04
$(TF/P)_{In}$	1.36 ± 0.04	1.33 ± 0.05
PFSR (%)	25.2 ± 1.9	23.6 ± 2.4
SNGFR (nl/min)	70.1 ± 7.2	81.1 ± 9.0
SNGFR/GFR (× 10^6)	2.71 ± 0.20	3.22 ± 0.45
P_{Na} (mEq/liter)	143.2 ± 1.0	142.8 ± 0.9
Hematocrit (%)	32.4 ± 1.5	33.6 ± 1.5
Plasma protein (g/100 ml)	6.8 ± 0.3	6.3 ± 0.3

[a] $U_{Na}V$ = urinary sodium excretion; C_{In} and GFR = inulin clearance; C_{PAH} = *p*-aminohippurate clearance; FF = filtration fraction; $(TF/P)_{In}$ = tubular fluid/plasma inulin concentration ratio; PFSR = proximal fractional sodium reabsorption; P_{Na} = plasma sodium; SNGFR = superficial nephron glomerular filtration rate.

[b] Statistically significant differences within groups, $p < .01$.

Group II. Data from the vasodilated kidneys of the eleven TVC dogs are summarized in Table II. The infusion of vasoactive drugs resulted in a brisk natriuresis from the vasodilated kidneys, excretion increasing from 28 to 391 μEq/min. In contrast, on the control side exposed only to the pressor agent (not shown in Table II), sodium excretion was 24 μEq/min during saline loading and increased only to 44 μEq/min during drug infusion. GFR on the experimental side in-

TABLE II. Effect of Vasoactive Drugs in TVC Dogs (Group II).

	Saline	Saline + drugs
$U_{Na}V$ (μEq/min)	28.0 ± 7.7[b]	391 ± 77.8[b]
C_{In} (ml/min)	29.9 ± 2.8[b]	32.4 ± 2.7[b]
C_{PAH} (ml/min)	67.8 ± 8.0	76.4 ± 7.1
FF	0.46 ± 0.04	0.45 ± 0.03
$(TF/P)_{In}$	1.36 ± 0.04[b]	1.27 ± 0.04[b]
PFSR (%)	25.05 ± 2.4[b]	19.3 ± 2.4[b]
SNGFR (nl/min)	91.3 ± 9.7	106.5 ± 18.2
SNGFR/GFR ($\times 10^6$)	3.40 ± 0.42	3.52 ± 0.46
P_{Na} (mEq/liter)	138.4 ± 1.8	138.7 ± 1.5
Hematocrit (%)	30.9 ± 1.3	31.6 ± 1.4
Plasma protein g/100 ml)	3.4 ± 0.2	3.5 ± 0.2

[a] Abbreviations and indications of statistical significance[b] of differences as in Table I.

creased from 30 to 32.4 ml/min ($p < .01$); C_{PAH} and filtration fraction did not change significantly. $(TF/P)_{In}$ ratio (mean of means) fell from 1.36 ± 0.04 in the control period to 1.27 ± 0.04 during vasoactive drugs ($p < .01$), corresponding to a 22.8% decrease in PFSR ($p < .01$). In four experiments, only end-proximal segments were punctured, as identified with lissamine green. $(TF/P)_{In}$ in 11 nephrons fell from 1.43 ± 0.05 to 1.30 ± 0.03 ($p < .05$). In five dogs in which timed collections were made, SNGFR was 91.3 (36 nephrons) and 106.5 (27 nephrons) nl/min ($p > .4$) and whole kidney GFR 27.6 and 29.7 ml/min for the control and experimental periods, respectively. The mean ratios of SNGFR/GFR were 3.40 and 3.52 $\times 10^6$, respectively, for these periods and were not significantly different ($p > .8$).

Discussion. These experiments were performed to evaluate the tubular locus of the natriuretic effect of vasoactive drugs in normal and edematous dogs. The experiments in normal dogs given hyperoncotic albumin (Table I) demonstrate that the vasoactive drug combination can cause natriuresis by purely distal actions. When hyperoncotic albumin is infused into normal dogs, PFSR decreases to the same extent as with saline loading, but there is only a small increase in sodium excretion (5). In this sense, these dogs are comparable to saline-loaded TVC dogs. In the present study, sodium excretion increased 236 μEq/min/kidney during the infusion of vasoactive drugs in normal dogs preloaded with hyperoncotic albumin. There was no change in PFSR, SNGFR or calculated distal delivery for the group as a whole. Moreover, there was no correlation between increases in excretion and in distal delivery individually in those eight dogs in which SNGFR was measured (Fig. 1). Although the ratio of SNGFR/GFR appeared to increase slightly from 2.71 to 3.22 $\times 10^6$, the difference was not significant ($p > 0.2$). In the absence of evidence for redistribution of filtrate, the lack of correlation between

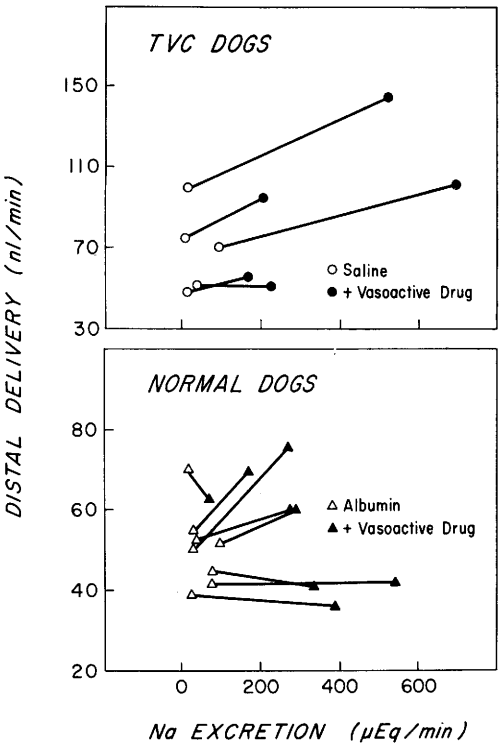

FIG. 1. Relation between change in sodium excretion per kidney and change in distal delivery of filtrate per nephron. TVC dogs infused with saline (Group II) are shown in the upper panel, normal dogs infused with hyperoncotic albumin (Group I) in the lower panel. Each pair of points connected by a line represents mean values for a single dog before and after infusion of vasoactive drugs. All dogs from each group in which both PFSR and SNGFR were measured are shown.

changes in distal delivery in single nephrons and sodium excretion would presumably apply to the kidney as a whole. Thus, we conclude that the vasoactive drugs induce natriuresis in this model by distal actions. The distal locus of this effect in normal dogs is unknown, although the possibility that these agents alter transport in the loop of Henle has been suggested by clearance studies (8, 9). This effect might be mediated by changes in blood flow to the medulla. It has been suggested (10, 11) that increases in medullary blood flow inhibit sodium reabsorption in the loop during saline loading as well. Hence, it is tempting to speculate that the actions of vasoactive drugs represent a model or even a mechanism for events in the loop of Henle during volume expansion. It must be noted, however, that there is no direct evidence that reabsorption in the loop of Henle is inhibited by saline in normal animals. Indeed, direct microperfusion studies in the rat led to the conclusion that sodium reabsorption in the loop is unaffected by saline loading (12). Additionally, it is possible that albumin enhances distal sodium transport and, as occurs in TVC dogs (13), the vasoactive drugs return transport to that of a normal, saline expanded animal. There is no experimental evidence to deny or support this possibility.

TVC dogs were used in these studies as a model of a sodium retaining state. The cause of sodium retention in this model is still unknown. However, a previous study from this laboratory (6) has shown that saline loading in chronic TVC dogs produces a depression of PFSR and an increase in distal delivery equivalent to that seen in normal dogs, but does not induce a natriuresis. This suggested that the inability of chronic TVC dogs to excrete an acute saline load is due principally to increased distal reabsorption of sodium. Direct evidence for enhanced sodium reabsorption in the loop of Henle has recently been obtained by micropuncture (13). It was shown by Friedler *et al.* (3) that a normal natriuretic response to saline can be obtained in these animals if they are infused in addition with a combination of a vasodilator intrarenally and a pressor agent systematically. Levy (13) has recently confirmed and extended these findings by obtaining micropuncture data from both proximal and distal tubules before and during infusion of vasoactive drugs. Levy did not find any decrement in PFSR while we found a significant fall of about 25% (Table II) after the infusion of vasoactive drugs in TVC dogs. We have no explanation for this difference. In our study SNGFR and distribution of filtration did not change significantly. Distal delivery (Fig. 1) increased because of the decrement in PFSR. By calculation, the increment in distal delivery for the kidney as a whole is severalfold the observed increase in sodium excretion of 360 μEq/min/kidney. Hence, it is possible that vasoactive drugs effect a natriuresis in saline-loaded TVC dogs solely by inducing increases in distal delivery beyond that which can be achieved with saline alone. Alternatively, the vasoactive drugs may inhibit distal as well as proximal sodium transport. The results from Levy's study (13) confirm the alternative possibility, that vasoactive drugs inhibit distal as well as proximal sodium transport. His data clearly indicate that vasoactive drugs can reverse abnormal sodium reabsorption in the loop of Henle and the late distal tubule-collecting ducts in TVC dogs thus producing a natriuresis. These considerations provide some support for the hypothesis that abnormal distal reabsorption in TVC dogs may be caused by a hemodynamic abnormality. However, evidence is inconclusive, since vasoactive drugs could nonspecifically reverse enhanced sodium reabsorption caused by hormonal or other nonhemodynamic mechanisms.

Summary. Experiments were performed to evaluate the relative contribution of changes in proximal and distal tubular reabsorption to the natriuresis induced by vasodilator–vasopressor combinations (vasoactive drugs). In normal dogs preloaded with hyperoncotic albumin, vasoactive drugs induced natriuresis without altering PFSR, nephron filtration rate or calculated distal delivery of filtered sodium. Thus, these agents can increase sodium excretion by inhibiting distal reabsorption in the absence of a proximal effect. In

saline-loaded dogs with thoracic vena caval constriction, the natriuresis induced by vasoactive drugs was associated with a fall in PFSR and an increase in calculated distal delivery. In these edematous dogs, proximal and distal inhibition both appear to contribute to the increase in sodium excretion.

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1. Vander, A. J., Amer. J. Physiol. **206**, 492 (1964).

2. Earley, E. A., and Friedler, R. M., J. Clin. Invest. **45**, 542 (1966).

3. Friedler, R. M., Belleau, L. J., Martino, J. A., and Earley, L. E., J. Lab. Clin. Med. **69**, 565 (1967).

4. Stein, J. H., Reineck, J. H., Osgood, R. W., and Ferris, T. F., Amer. J. Physiol. **220**, 227 (1971).

5. Howards, S. S., Davis, B. B., Knox, F. G., Wright, F. S., and Berliner, R. W., J. Clin. Invest. **47**, 1561 (1968).

6. Auld, R. B., Alexander, E. A., and Levinsky, N. G., J. Clin. Invest. **50**, 2150 (1971).

7. Levinsky, N. G., and Lalone, R. C., J. Clin. Invest. **44**, 565 (1965).

8. Daugharty, T. M., Belleau, L. J., Martino, J. A., and Earley, L. E., Amer. J. Physiol. **215**, 1442 (1968).

9. Bank, N., Aynedjian, H. S., Bansal, V. K., and Goldman, D. M., Amer. J. Physiol. **219**, 275 (1970).

10. Earley, L. E., and Friedler, R. M., J. Clin. Invest. **43**, 1928 (1964).

11. Earley, L. E., and Friedler, R. M., J. Clin. Invest. **44**, 929 (1965).

12. Morgan, T., and Berliner, R. W., Nephron **6**, 388 (1969).

13. Levy, M., J. Clin. Invest. **51**, 922 (1972).

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