Effect of amiloride, ouabain, and furosemide on distal tubular function in the rat

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Am. J. Physiol. 221(2) : 632-639. 1971.- Free-flow micropuncture experiments were carried out on male Long-Evans rats ranging from 295 to 415 g and anesthetized by the intraperitoneal injection of Inactin (80-100 g/kg body wt). The methods used for micropuncture and localization of the tubular collection site have been described previously (30, 31, 33). A flank approach for the exposure of the kidney was used.

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The effects of the three diuretic agents, amiloride, ouabain, and furosemide, were studied. Amiloride completely suppressed K secretion at the distal tubular level with only small modifications of distal tubular Na reabsorption. Ouabain induced distal kaliuresis and natriuresis in animals kept on a low-potassium diet. The effects of ouabain upon distal tubular electrolyte transport consisted in depression of both tubular Na and K reabsorption. The distal potassium effects of ouabain were independent of any increase in distal sodium delivery and are thought to be due to depression of active potassium uptake across the luminal cell membrane of distal tubule cells. The main kaliuretic effect of furosemide on distal potassium transport is related to the delivery to the distal tubule of a much greater than normal fraction of fluid. After furosemide administration, despite a very significant elevation of distal tubular sodium concentration, fractional Na reabsorption rate along this tubular segment greatly exceeded control values.

 METHODS

Free-flow micropuncture experiments were carried out on male Long-Evans rats ranging from 295 to 415 g and anesthetized by the intraperitoneal injection of Inactin (80-100 g/kg body wt). The methods used for micropuncture and localization of the tubular collection site have been described previously (30, 31, 33). A flank approach for the exposure of the kidney was used.

Since collection of fluid from the distal tubule may be subject to possible error by the aspiration of fluid from the collecting ducts, special care was taken to avoid contamination by reflux of fluid from downstream nephron segments. These precautions consisted in the careful placement and observation of a large oil block having a length corresponding to several tubular diameters or extending into the downstream nephron loop. Its position was carefully controlled during the collection. Furthermore, the direction of tubular fluid movement was noted (as judged by the displacement of the oil column) and compared to the anatomy of the tubule after filling with latex and microdissection. In addition, in about one-half of the collections, single-nephron GFR was estimated from timed collections and inulin TF/P ratios. Exclusion of abnormally high values (values in excess of those expected from total GFR, assuming a total of 30,000 nephrons per kidney and no major shifts in the distribution of GFR between superficial and deep nephrons) provided an additional safeguard against contamination with fluid from more distally located nephron segments. Although none of these methods provides an absolute proof against downstream contamination, we feel that the method of distal tubular micropuncture is adequate to detect modifications of distal tubular function of the magnitude to be described in this paper.

 Four groups of rats were investigated: 1) control rats kept on a control diet of Purina laboratory chow (0.46% Na, 0.79% K); 2) rats kept on a similar diet but receiving during the experiment amiloride (Merck MK 870) intravenously at a rate of 0.04 mg/kg-min after a single priming dose of 1 mg/kg; 3) rats kept on a low K diet (Nutritional Biochemicals Corp., Cleveland, Ohio) for several weeks
prior to the experiment receiving either the inulin prime and sustaining infusion described below or, in addition, a single injection of 5 mg/kg ouabain (Lilly) intravenously, at least 60 min before tubular collections were started; and 4) rats kept on a control diet and given different amounts of furosemide. Since the results obtained within each of the following furosemide-treated groups were not sufficiently different to warrant separate presentation, they were pooled. The following three groups have been studied: a) high dose: a priming dose of 40 or 25 mg/kg and a sustaining infusion containing 48 or 24 mg/kg·hr; b) medium dose: a priming dose of 2.5 mg/kg and a sustaining infusion of 12 mg/kg·hr, or a sustaining infusion containing 6.6 mg/kg·hr without priming infusion; c) low dose: a priming infusion of 1 mg/kg without sustaining infusion of solely a sustaining infusion of 3.3 mg/kg·hr.

All rats received an initial priming infusion of 60 μC inulin-14C in a volume of 0.5–0.7 ml of isotonic saline. Two to four milliliters of the latter were also given during the operative procedure to replace fluid loss. The priming dose of the diuretics was added to the inulin-14C priming infusion. The different diuretic agents were dissolved in a 5% mannitol-saline solution delivered at a rate of 0.1 ml/min. Control animals received the same infusion but without addition of the diuretic agent. A period of 30–45 min was allowed to elapse after administration of the priming infusion before beginning the micropuncture experiments.

Sodium and potassium concentrations were measured in samples of distal tubular fluid by ultramicro flame photometry (29), and in urine and plasma with a Baird K2 flame photometer. Inulin-14C activity in urine and plasma samples was measured by liquid scintillation spectrophotometry (30). Electrical potential differences across the distal tubular epithelium were measured in control and amiloride-treated rats with glass microelectrodes according to methods described previously (16, 29, 30).

RESULTS

Table 1 contains a summary of the effects of the three diuretics, amiloride, ouabain, and furosemide, on urine flow rate, glomerular filtration rate (GFR), and fractional excretion rates of water, sodium, and potassium. It is apparent that, with the exception of furosemide, none of the compounds exerted a major effect upon GFR. The effects of amiloride consisted in only a moderate increase of fractional water and sodium excretion, whereas both U/P potassium ratios as well as fractional potassium excretion were greatly depressed. The latter observation is illustrated by the fall of fractional potassium excretion rates from 43 % in control to 8.4 % in amiloride-treated rats. Mean absolute excretion rate of sodium was increased from a control value of 37 to 67 μEq/kg·min, whereas that of potassium was reduced from a value of 16 to 1.2 μEq/kg·min.

The demonstration of consistent effects of ouabain upon distal tubular potassium transport is complicated by the relative insensitivity of the rat to cardiac glycosides (1). Although a significant effect of ouabain upon distal potassium and sodium transport can be demonstrated in recollection micropuncture studies in rats on a control diet (unpublished observations), since the difficulties of comparing different nephron populations are avoided, it proved easier in the present experiments to detect effects of ouabain on distal tubular K transport in animals fed a low-K diet. The latter has been shown to accentuate the renal effects of cardiac glycosides (6). Compared to such control animals, the diuretic effects of ouabain manifested themselves in an increment of fractional water excretion, an increase in U/P Na and K ratios, as well as in enhanced fractional Na and K excretion rates. Mean absolute excretion rate of sodium was increased from the control value in low-K animals of 1.0 to 53 μEq/kg·min and that of potassium from 0.3 to 2.0 μEq/kg·min.

It is apparent that administration of furosemide, particularly in the higher dose range, led to a significant fall in GFR. At the same time fractional water, sodium and potassium excretion were augmented. This is demonstrated by the enhancement of fractional sodium excretion from 14.7 to 23 % of the filtered load (compared to some 3 % in controls). With respect to potassium which, in control animals, was excreted at a rate of 43 % of the filtered load, a very dramatic enhancement of fractional potassium excretion from 87 % at the lowest furosemide dose to a peak

<table>
<thead>
<tr>
<th>Expt Conditions</th>
<th>Vol, ml/kg·min</th>
<th>GFR, ml/kg·min</th>
<th>Inulin U/P</th>
<th>Na U/P</th>
<th>Na/In U/P</th>
<th>K U/P</th>
<th>K/In U/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>221 ± 22.3</td>
<td>9.02 ± 0.49</td>
<td>43.15 ± 5.2</td>
<td>0.00 ± 0.06</td>
<td>0.029 ± 0.003</td>
<td>14.94 ± 1.33</td>
<td>0.434 ± 0.026</td>
</tr>
<tr>
<td>Amiloride, MK 870</td>
<td>320 ± 13.9</td>
<td>8.83 ± 0.52</td>
<td>25.36 ± 3.8</td>
<td>0.75 ± 0.05</td>
<td>0.053 ± 0.009</td>
<td>1.847 ± 0.40</td>
<td>0.084 ± 0.008</td>
</tr>
<tr>
<td>Low-K diet control</td>
<td>65 ± 11.6</td>
<td>6.68 ± 0.73</td>
<td>114.64 ± 25.1</td>
<td>0.478 ± 0.05</td>
<td>0.002 ± 0.005</td>
<td>1.445 ± 0.16</td>
<td>0.021 ± 0.004</td>
</tr>
<tr>
<td>Low-K diet + ouabain</td>
<td>269 ± 16.1</td>
<td>8.11 ± 0.64</td>
<td>31.87 ± 2.1</td>
<td>0.98 ± 0.04</td>
<td>0.047 ± 0.005</td>
<td>2.987 ± 0.26</td>
<td>0.061 ± 0.005</td>
</tr>
<tr>
<td>Furosemide, low dose</td>
<td>692 ± 54.8</td>
<td>5.17 ± 0.62</td>
<td>10.25 ± 2.6</td>
<td>0.029 ± 0.03</td>
<td>0.147 ± 0.018</td>
<td>6.069 ± 0.70</td>
<td>0.060 ± 0.070</td>
</tr>
<tr>
<td>Furosemide, medium dose</td>
<td>752 ± 111.8</td>
<td>3.93 ± 0.12</td>
<td>5.13 ± 0.7</td>
<td>0.725 ± 0.02</td>
<td>0.17 ± 0.022</td>
<td>4.766 ± 0.52</td>
<td>0.065 ± 0.025</td>
</tr>
<tr>
<td>Furosemide, high dose</td>
<td>902 ± 91.7</td>
<td>3.31 ± 0.22</td>
<td>3.65 ± 0.2</td>
<td>0.81 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>4.64 ± 0.107</td>
<td>1.33 ± 0.065</td>
</tr>
</tbody>
</table>

Values are means ± se. Number in parentheses gives number of observations.
completely blocking net secretion of this ion species at the distal tubular level.

Figure 2 demonstrates the effects of amiloride upon distal tubular sodium reabsorption. It can be seen from the distribution of sodium TF/P ratios that 11 out of the 12 highest values occurred after amiloride administration. A trend toward higher sodium TF/P ratios is also present in amiloride-treated rats. It can be seen that at most points along the distal tubule and the collecting ducts, the lowest sodium TF/P ratios are controls. It should also be noted...
that the urinary excretion rate of sodium was slightly elevated as evidenced from the increase of fractional excretion rates from 2.9 to 5.3% (0.01 < P < 0.02). It is clear, however, that despite its very powerful action to completely abolish net potassium secretion at the distal tubular level, amiloride produces at best only a small depressing effect upon distal tubular sodium reabsorption.

The distal tubular effects of the cardiac glycoside ouabain upon potassium transport in low-potassium animals are graphically summarized in Fig. 3. It is apparent that in control low-K animals the concentration of potassium fails to increase along the distal tubule. Mean plasma potassium level in these animals was 2.06 ± 0.07 mEq/liter. Compared to control animals on a normal K intake (see Fig. 1) final U/P potassium ratios were markedly depressed to a level of 1.44. Similarly, fractional excretion rate of potassium averaged only some 2% of the filtered load, whereas the corresponding value in rats on a normal K intake was 43%. In essence, these data on low-K animals confirm previous results in that dietary K deprivation suppresses distal tubular potassium secretion (30). The effects of ouabain manifested themselves in a moderate elevation of the distal TF/P potassium ratios along the second half of the distal tubule and a moderate but significant (P < 0.001) increase in U/P potassium ratios. The mean plasma level of potassium was 4.03 ± 0.12 mEq/liter. Fractional excretion rate of potassium increased threefold to a mean level of 6% of the filtered load, and it is apparent that depressed reabsorption along the distal tubule partakes in this effect. The scatter of distal tubular potassium data is considerable. A recent distal tubular recollection study combined with injection of ouabain directly into the renal artery of animals on a low or normal K intake (unpublished observations) has confirmed the suggestive evidence of the present study that ouabain 1) raises distal tubular K concentrations, 2') elevates the fraction of K remaining in the tubule, and 3) effects significant kaliuresis.

The effects of ouabain on distal tubular sodium transport are shown in Fig. 4. A distal tubular effect of the cardiac glycoside is evident. Ouabain prevented the development of steep transepithelial concentration differences of Na which normally develop across the second half of the distal tubule (30). Final Na U/P ratios were significantly elevated. Inspection of the lower part of Fig. 4 underscores the distal effects of this drug. Fractional reabsorption of Na is diminished, and a significantly higher amount of sodium is excreted into the final urine. It can also be seen that fractional sodium reabsorption along the collecting ducts has been depressed to insignificant levels. On the other hand, the comparison of early distal sodium/inulin TF/P ratios does not indicate that nephron segments prior to the distal tubule contributed to the natriuresis.

Figure 5 graphically summarizes the effects of three different doses of furosemide upon distal tubular potassium transport. Two points deserve mention: 1) it is apparent that, compared to control rats on a normal K intake (see Fig. 1), early distal tubular TF/P potassium ratios are elevated. Control values of less than 0.5 are regularly observed at the early distal tubular level in controls, whereas such low ratios were not observed in furosemide-treated animals. In these, most TF/P potassium ratios at the beginning of the distal tubule were unity or above unity; 2) the progression of TF/P potassium ratios along the remainder of the distal tubule appears similar in the three groups of drug-treated rats. Late distal concentration ratios, although demonstrating considerable scatter, reach the same magnitude as similar ratios in control rats (Fig. 1). Fractional urinary excretion rates differed in the three groups of animals: net secretion of potassium (mean K/inulin U/P ratio: 1.33) was present in animals treated with the highest dose, whereas in animals treated with the medium dose the amount of K in the final urine was equal to that filtered, and in those receiving the lowest dose was equivalent only to some 87% of the filtered load. A similar trend toward higher apparent secretion rates with increasing doses of furosemide can also be observed at the distal tubular level. Clearly, at the lowest dose given, net addition of K to the tubular fluid is less than that at the higher dose range. Although, as stated above, the late distal transepithelial concentration ratios of potassium were not greatly affected by furosemide, tubular fluid reabsorption along the distal tubule was progressively diminished as the dose of furosemide was increased. This is reflected by the progressive decline of U/P inulin ratios from about 10 at the lowest to 3.6 at the highest dose. Accordingly, a larger fraction of K was excreted into the final urine as volume flow rate and fractional fluid excretion rate rose. Thus, at very similar distal and urinary potassium TF/P or U/P ratios, respectively, overall net secretion of potassium occurred at the highest furosemide dose, whereas net reabsorption was
FIG. 5. Summary of potassium and potassium-to-inulin concentration ratios as function of distal tubular length from animals given high (△), medium (●) and low (○) doses of furosemide.

FIG. 6. Summary of sodium and sodium-to-inulin concentration ratios as function of distal tubular length from rats infused with increasing doses of furosemide. "Control" overlay indicates range of control values observed in this and similar experimental situations (31).

evident in rats receiving the lowest dose. It is concluded that furosemide affects the distal tubular potassium transport system indirectly by increasing tubular fluid delivery.

Figure 6 shows the results dealing with distal tubular sodium transfer during furosemide administration. A dramatic elevation of the tubular TF/P sodium ratios is apparent and indicates that the ability of this nephron segment to lower the tubular sodium concentration was severely compromised. Inspection of the lower section of Fig 6 also indicates that a much larger fraction of Na than normally enters the distal tubule. As the furosemide dose is increased, a progressively greater fraction of sodium reabsorption is inhibited and results in urinary excretion of a larger fraction of filtered sodium. A further point concerns the collecting duct transport pattern of sodium. Whereas at the low dose of furosemide significant net reabsorption of sodium persists at this nephron level, it is virtually absent at the highest dose. It should be noted that, on a fractional basis, a much larger fraction of the filtered sodium is reabsorbed along the distal tubule, particularly at the lower and medium dose. Usually, some 8–10% of the filtered Na is reabsorbed along the distal tubule (5, 17), a value markedly below the fraction of filtered sodium subject to reabsorption in almost all furosemide-treated animals in which corresponding values of 20–30% were observed.

In view of this very significant increase in fractional reabsorption rate, it is safe to conclude that despite the considerable fall in GFR in furosemide-treated animals the absolute amount of sodium reabsorbed along the distal tubule did either increase (low furosemide dose) or remain within the range of the amount of sodium normally transferred from lumen to peritubular fluid.

DISCUSSION

The experiments described indicate that the three diuretics studied have widely varying effects upon distal tubular sodium and potassium transport. Whereas amiloride has a powerful action on potassium transport and its effect upon sodium reabsorption is quite small, ouabain depresses both sodium and potassium reabsorption. In contrast, the effects of furosemide upon distal tubular sodium and potassium transport appear to be due largely to the greatly increased fluid and sodium delivery to this nephron segment.

Amiloride is a weak natriuretic agent which decreases urinary excretion of potassium (2, 3, 20). In addition, it also inhibits hydrogen ion secretion (3, 20). Our results clearly show that the potassium sparing effect of amiloride is localized at the distal tubular level. These results confirm similar conclusions regarding the site of action of this compound reached by stop-flow experiments (3).

In contrast to the very dramatic effect upon distal tubular potassium transport, the effect of amiloride upon sodium reabsorption at this nephron level is of small magnitude at best. Fractional urinary excretion rate rose only very moderately, and the effect upon distal tubular sodium reabsorption, although suggested by a trend toward higher TF/P sodium and Na/In TF/P ratios in amiloride-treated rats, is not striking. Since recollection studies which minimize errors inherent in the comparison of different nephron populations were not carried out, larger effects upon sodium reabsorption might have escaped detection. Nevertheless, the conclusion appears justified that the very profound effect of the drug in completely suppressing potassium secretion is associated with at best only a small interference with distal tubular sodium transport.

The mechanism of action of amiloride at the nephron level, particularly with respect to its action upon potassium...
transport, is not clear. In the course of the present study
distal transtubular potential differences were found to be
depressed by the drug. Compared to control rats in which
the mean transepithelial potential difference was $-45.6 \pm 1.57$ mV, that in amiloride-treated rats was only $-25.8 \pm 0.35$ mV (lumen negative). A reduction of the transepithelial
potential difference after amiloride treatment, accompanied
by a reduction of the short-circuit current and of active
sodium transport, was observed in the toad bladder prepara-
tion (4, 12). It was proposed that amiloride reduces the
$Na^+$ permeability of the apical cell membrane of these iso-
lated epithelial structures. This would result in reduction
of the entrance of sodium into the cell prior to its active
extrusion across the serosal cell boundary. A similar mecha-
nism could underly the mode of action of amiloride with
respect to its effect upon distal tubular potassium transport
(20). Accordingly, a reduced passive permeability of the
luminal cell membrane could impede entry of potassium
from cell into the tubular lumen and thus be responsible
for the suppression of distal tubular potassium secretion.
However, acceptance of this hypothesis (20) is complicated
by a number of considerations. Selective reduction of the
potassium permeability of the luminal cell membrane
would, in view of the direction of the diffusion gradient
(cell potassium $>$ luminal potassium), decrease electrical
polarization of the luminal cell membrane. Such a reduc-
tion of electrical polarization of the luminal cell boundary
would be expected to result in an increase, not a decrease,
of the overall transepithelial potential difference. If a reduc-
tion in potassium permeability is indeed present after
amiloride treatment, it would have to be accompanied by a
disproportionately larger fall in the sodium permeability of
the luminal cell membrane to account for the observed fall
in the transepithelial potential difference. Other possibili-
ties to explain the electrogenic effects of amiloride should
be considered. In the frog skin amiloride blocks Na uptake
across the outer cell boundary (5). If this compound were
to reduce electrogenic sodium transport from lumen into
the tubule cell, it would by this mechanism also decrease the
electrical polarization of the luminal membrane. This
would result in the reduction of the transepithelial potential
difference and lower the electrochemical potential gradient
favoring entry of potassium into the distal tubular lumen.
According to such a view, the effect of amiloride upon
potassium transport would be secondary to its effect upon
sodium transport and mediated solely by changes in the
electrical driving force. It might be argued that if this were
the case a larger inhibitory effect upon distal tubular sodium
transport might be expected. Also, it should be noted that
net potassium secretion into the distal tubule is completely
suppressed at a time when electrical polarization of the
distal tubular epithelium is reduced, but by no means com-
pletely abolished. Clearly, more experimental work is
necessary to evaluate more directly the contribution of
changes in the electrical potential difference and a number
of other processes known to participate in distal tubular
potassium transport. While we consider it unlikely that
amiloride stimulates active uptake of potassium across the
luminal cell membrane and thereby lowers the luminal
potassium concentration, the possibility of an inhibitory
effect of this compound upon peritubular potassium uptake
should be considered. Such an action could lower the po-
tassium content of a critical cellular potassium transport
pool (43), thereby reducing both the electrochemical potent-
tial gradient of potassium across the luminal membrane
and secretion. Such an unproven mode of action would con-
istute a direct action of amiloride on the potassium trans-
port system and be independent of an effect upon sodium
transport.

Data presented within the body of this paper provide
strong evidence that ouabain exerts an effect upon distal
tubular sodium transport. The data also show—and similar
studies in rats utilizing recollection techniques (unpub-
lished observations) as well as studies in perfused Amphiuma
kidneys (43) support this view—that ouabain induces
kaliuresis by inhibiting potassium reabsorption at the distal
tubular level. The effect of cardiac glycosides of promoting
significant urinary loss of sodium and potassium confirms
reports by others (8, 35–37, 44), although a fall in potassium
excretion has also been described, particularly in potassium-
loaded animals (35). It should be noted that the natriuresis
and kaliuresis of even high doses of ouabain are considera-
nably smaller in the rat kidney than in other species, such as
the dog (8, 21) and the chicken (35). It has recently been
suggested that this difference is due to a lower sensitivity in
the rat of its renal Na-K-stimulated ATPase to the inhibi-
tory effect of cardiac glycosides (1).

It has been proposed that cardiac glycosides promote
renal potassium loss by the delivery of larger-than-usual
quantities of sodium to the distal tubule, thereby permitting
accelerated sodium for potassium exchange (35). This thesis
demands that 1) ouabain acts on sodium transport at
nephron sites more proximal than the distal tubule and 2) that
the increased distal sodium load is more potent in
stimulating such carrier-linked cation exchange across the
luminal membrane than the inhibitory effect of ouabain
upon a peritubular sodium for potassium exchange pump
which is thought to effect net sodium reabsorption. Accord-
ing to such a mode of ouabain action on distal potassium
transport, a larger-than-normal fraction of sodium should
enter the distal tubule, and distal tubular sodium reabsorp-
tion should be augmented commensurate with increased
potassium secretion. The present experiments permit a test
of this hypothesis. Inspection of Fig. 4 shows that the frac-
tion of sodium entering the distal tubule during ouabain
treatment is not different from that in control animals since
early distal sodium/inulin TF/P ratios are quite similar. In
addition, sodium reabsorption along the distal tubule is
reduced in ouabain-treated rats. Nevertheless, despite such
reduced sodium reabsorption, potassium excretion along
the distal tubule and along the collecting duct is enhanced.
Thus, kaliuresis can develop 1) in the absence of an in-
creased delivery of sodium to the main site of tubular po-
tassium secretion and 2) during diminished net sodium
reabsorption along the distal tubule. Similarly, in Amphiuma
an elevation of distal tubular potassium concentration and
diminished potassium reabsorption have been observed
after ouabain administration. These alterations in tubular
potassium transport are not associated with changes in
fractional reabsorption of sodium at this site (43). Obviously,
delivery of an increased sodium load to the distal tubule
may occur in species more sensitive to ouabain (8, 21, 35)
and contribute to the kaliuresis by increasing distal tubular flow rate (23), enhancing intratubular negativity (16) or providing more sodium to the cortical collecting ducts where active sodium-potassium exchange has been shown to occur (19). However, in the rat enhanced sodium delivery and reabsorption are not a prerequisite for the effects of ouabain upon distal tubular potassium transport.

In view of the findings presented in this study and those obtained in distal recollection studies in the rat (unpublished observations), and in Amphiuma distal tubules (43), it is suggested that ouabain acts directly on distal potassium transport by inhibiting active uptake of this ion at the luminal cell membrane. Evidence presented elsewhere argues in favor of an active reabsorptive potassium pump at this site (14, 15, 17, 30, 32). Reduction of the pumping rate in the distal tubule would explain both elevated potassium concentrations within the distal tubular lumen, particularly at the late distal tubular level, as well as excretion of increased quantities of potassium into the urine. It is of interest that, as shown by distal recollection techniques, the kaliuretic effect of ouabain is also present in rats on a normal control diet in which potassium secretion occurs along the distal tubule (unpublished observations). Reduction of a component of unilateral active K flux from lumen to tubule cell would in this situation similarly increase the concentration of potassium and elevate urinary excretion rates.

Although we feel that the available evidence strongly suggests a direct effect of ouabain on potassium transport at the distal tubular level, particularly in view of its clear-cut distal tubular depression of sodium reabsorption, the elevation of the plasma potassium level after ouabain in the present study suggests that, in addition to its renal effects, the drug also has an extrarenal effect. It is presently not possible to assess the extent to which this extratubular effect of an elevated plasma potassium level after ouabain administration influences the distal tubular potassium transport system. However, since the effects of the present study are qualitatively similar to those observed in a series of experiments in which ouabain was only injected into one renal artery and in which the changes in the plasma potassium level were much smaller (unpublished observations) it is certain that the drug induced kaliuresis in the present experiments is not exclusively due to extrarenal effects of the elevated plasma potassium level.

It is also apparent that in addition to the proposed inhibition of luminal potassium reabsorption ouabain also inhibited distal tubular sodium transport. According to presently held views, sodium is extruded from the cell across the peritubular cell membrane, a process involving some exchange for potassium (13, 40-42). Incomplete inhibition of this sodium for potassium exchange is the likely explanation for the observed inhibition of net sodium transport along the distal tubule. However, the reduction of intracellular potassium concentration, which may occur subsequent to such peritubular action of ouabain, is apparently insufficient to compromise the attainment of higher intratubular potassium concentrations.

Different sensitivities of tubular potassium transport to cardiac glycosides could be related to variations in distal tubular potassium concentrations. It is well established that a low external potassium concentration increases and a high external potassium concentration decreases the ouabain sensitivity of sodium-for-potassium exchange pumps (18). In animals on a low-K intake the intratubular potassium concentrations are low and increase little along the distal tubule (Fig. 3). This would favor a proportionately stronger inhibitory ouabain action on luminal K uptake resulting in kaliuresis. During situations in which distal K secretion is stimulated, the range of distal tubular potassium concentrations consistently exceeds that of plasma K levels, particularly along the latter part of the distal tubule (30, 31, 33). This would make relatively more sensitive the peritubular sodium-for-potassium exchange to the inhibitory action of ouabain and account for the decrease in potassium secretion which may occur when K secretion had been stimulated by previous potassium loading (35).

Available evidence indicates that one of the main sites of furosemide action within the nephron is the ascending limb of Henle's loop (11, 28, 34). The fact that fluid, sodium, and potassium reabsorption along this nephron segment are inhibited is evident from the finding that the fractions of sodium and potassium entering the distal tubule are dramatically elevated (see Figs. 5 and 6).

With respect to potassium, the concentration of this ion in the fluid entering the distal tubule is elevated compared to control conditions. However, it should be noted that the progressively increased fractional excretion rates of potassium from 87 to 133% of the filtered load during administration of increasing doses of furosemide are not associated with elevated potassium concentrations along the distal tubule. It is also of interest that Malnic et al. (28) did not detect any changes in the magnitude of the transepithelial distal electrical potential difference after furosemide. These ratios do not exceed, but are frequently even lower than, those observed in control animals. Rather, it appears that rate of fractional distal tubular potassium secretion is flow dependent. Accordingly, with the delivery of increasingly larger fractions of fluid into the distal tubule the apparent secretion rate increases, because the time course of establishing transepithelial concentration differences is rapid enough to make these independent of variations of tubular flow rate. It is consistent with this interpretation that rate of fractional fluid excretion should determine final fractional excretion rates. Thus, net overall secretion of potassium is present at the lowest U/P inulin ratios (high furosemide dose), whereas net potassium reabsorption obtains at the lowest fractional fluid excretion rates (high U/P inulins, low furosemide dose). These observations strongly suggest that furosemide affects distal tubular potassium transport not directly but by virtue of alterations of fluid delivery into this nephron segment. It is of interest that the pattern of the distal tubular TF/P potassium and K/In TF/P ratios after furosemide treatment is similar to that seen after hypertonic sodium chloride loading (31). Thus, at comparable fractional urinary excretion rates of sodium in sodium chloride-loaded animals, the fractional urinary potassium excretion approaches that of furosemide-treated rats. However, it should be noted that the absolute rate of urinary potassium loss after furosemide is curtailed by the reduction in GFR after furosemide treatment. Although the cause of the fall in GFR is not entirely clear, it...
is likely to be related to the increase in free flow intratubular hydrostatic pressure which develops after the drug-induced reduction in tubular sodium and fluid reabsorption (25) and which is likely to reduce filtration pressure at the glomerular level. Essentially, similar conclusions with respect to the absence of a direct effect of furosemide upon distal potassium transport have also been reached by Suki et al. (38) by clearance experiments in the dog.

Our studies provide evidence that distal tubular sodium transport undergoes significant modifications in furosemide-treated animals. Most conspicuously the sodium concentration fails to decline along the distal tubule and remains elevated at high levels throughout this nephron segment. Similar findings have been reported with respect to chloride (28) and osmolality (9). Although the persistence of high intratubular concentrations of sodium along the distal tubule suggests some inhibition of sodium transport, it should be noted that, nevertheless, considerably higher fractions of filtered sodium (some 20–30%) are reabsorbed along distal tubules of furosemide-treated animals than in control animals in which the corresponding fractional reabsorption along the distal tubule is about 7–10% (17, 31).

We have observed a similar phenomenon, i.e., elevated distal tubular sodium concentration and an unchanged or increased fractional sodium reabsorption rate in dichlorphenamide-treated rats (unpublished observations) after ouabain and acetazolamide treatment in the perfused Amphiuma kidney (43) and during progressive expansion of the extracellular fluid volume by isotonic saline infusions in rats (22). Such observations indicate that the larger than normal load of sodium entering the distal tubule provides additional sodium for an unsaturated distal tubular reabsorptive process, despite the fact that the latter may have been subject to partial inhibition as evidenced by the higher intraluminal sodium concentrations which are frequently obtained after the administration of these diuretics.

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