EFFECTS OF ALDOSTERONE AND CORTISOL ON HENLE'S LOOP IN THE ADRENALECTOMIZED RAT'S KIDNEY

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It is generally accepted that urine is concentrated by the mammalian kidney through the operation of a countercurrent mechanism in the renal medulla and that active sodium transport out of the ascending limb of Henle's loop plays an important role in this mechanism. In addition, it has been reported that in the adrenalectomized rat urine volume is decreased and the corticomedullary osmotic gradient lowered (1, 2).

Experiments studying the effects of aldosterone on free water clearance (3) and the attainment of maximum urinary osmolality (4), have suggested that aldosterone may cause an increased rate of transport of sodium from the inside of the ascending limb of Henle's loop to the surrounding tissue. On the other hand, the action of glucocorticoids, particularly their effects on the loop of Henle have not yet been precisely defined. Experiments dealing with free water reabsorption (T^0H_2O) indicated, that glucocorticoids may enhance sodium transport at the ascending limb (5, 6). Hierholzer's experiments (7) suggested that cortisol affects the water permeability of distal tubular segments. In the present experiments we have attempted, by microperfusion of Henle's loop in the kidneys of adrenalectomized rats, to clarify the mode of action of corticosteroids on these tubular segments.

METHODS

Male albino rats weighing 150–230 g were adrenalectomized and given free access to 0.9% saline and a dry pellet diet. Intact rats were kept on tap water. The following groups of rats were studied (a) intact animals on controls, (b) adrenalectomized animals 18–24 hours, 4 days and 7–9 days after operation, (c) animals adrenalectomized 7–9 days previously and given 12.5 micrograms of d-aldosterone monoacetate (Aldocorten, Ciba) subcutaneously prior to anesthesia. A further dose of 3.1 micrograms was given intravenously when the kidney had been prepared for micropuncture, (d) animals adrenalectomized 7–9 days previously and given a subcutaneous injection of 2.5 mg cortisol (alcohol free, Merck) for 2 days. In some animals of this group, 5 mg of cortisol was given daily for 3 days prior to micropuncture. (e) The final group of animals were given both aldosterone and cortisol similarly to those of group c and d.

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The animals were anesthetized with intraperitoneal Nembutal (30 mg/kg, Abbott) and placed on a heated table. After tracheotomy, the femoral vein and the left ureter were catheterized, and the left kidney was exposed. During these surgical procedures, 1 ml of Ringer's solution was infused intravenously to replace surgical losses.

The loop of Henle was perfused according to the method of Cortney et al. (8) as has previously described (9). The perfusion pipet was placed in a late segment of a proximal tubule and a collection was made from the early segment of the distal tubule of the same nephron. The early proximal segment was filled with oil to prevent contamination with glomerular filtrate. One percent NaCl solution colored with 0.05% of lissamine green (315 mOsm/kg H_2O) was used as perfusate and the perfusion rate was 19 nl/min.

The transit time of tubular fluid to the distal tubule was measured after intravenous injection of 0.05 ml of 10% lissamine green and was the time from the initial blush of dye on the surface of the kidney to the earliest appearance of dye in the distal tubules.

The transit time through Henle's loop was calculated from the difference between the time of complete disappearance of the pigment from the late proximal tubules and the time of appearance of dye in distal tubular segments on the surface of the kidney.

Left ureteral urine was collected at appropriate intervals for 2 to 4 hours. Blood sample was collected by heart puncture after the experiment.

The osmolalities of the tubular samples and ureteral urine were estimated with a micro-osmometer similar to that developed by Ramsay and Brown (10). The sodium concentration of the tubular fluid was determined with an ultramicro-flamephotometer (Erma, Model 677). The sodium and potassium concentrations in serum and urine were determined with a flamephotometer (Evans, Model A). The results were expressed as the ratio of the osmolality or sodium concentration in the distal tubule sample to the those of the perfusion solution (TF/PS). In the experiments where free flow collections were obtained the denominator of the fraction was the plasma osmolality or sodium concentration (TF/P).

The corticomedullary concentration gradients of electrolyte and urea were examined in additional rats under conditions similar to those pertaining during the microperfusion experiments. Two hours after anesthesia and abdominal incision, both kidneys were quickly excised and divided into three parts: papilla with inner medulla; outer medulla; cortex. Left kidney slices were used for the determination of sodium, potassium and urea, and slices of the other kidney for the determination of water content. Urea concentration was determined by the phenol-hypochlorite method (11) using Conway's micro-diffusion apparatus.

In order to calculate the permeability of the tubular segments to water, tubules were also perfused with Ringer's solution containing inulin (0.15 to 0.20%) and lissamine green (0.05%). The osmolality of this solution was 302 mOsm/kg H_2O . The inulin concentration of collected samples was determined with a micro-fluorophotometer (Aminco) according to the method of Vurek and Pegram (12). The lissamine green concentration used in the present experiments did not affect the photometric estimation of inulin (13).

After each experiment perfused tubules were filled with latex solutions. Following maceration of the kidney, the puncture sites were localized by microdissection of the tubules.

RESULTS

1. Effects of adrenalectomy

Perfusion experiment

In a series of experiments tubules were perfused at various time intervals after adrenalectomy. The results in Table 1 indicate that the typical effects of adrenalectomy were not seen within 24 hours but appeared to be present at the fourth day. However the transit time was markedly increased at 24 hours.

TABLE 1. The changing of osmolality and solute concentration following adrenalectomy.

Times after adrenalectomy	18-24 hr	4 days	7-9 days	Intact
No. of rats	3	3	7	4
(TF/PS) _{osm}	0.58 (6) ± 0.06	0.71 (5)*** ± 0.05	0.76* (13) ± 0.03	0.57 ± 0.03 (8)
(TF/PS) _{Na}	0.50 (6) ± 0.06	0.64 (5)* ± 0.05	0.60* (13) ± 0.03	0.40 ± 0.03 (7)
Transit time (sec)	71	74	82	41
Serum Na (mEq/L)	147 ± 1.5	147#	141*** ± 1.1	147 ± 2.0
Serum K (mEq/L)	6.25 ± 0.74	6.12#	7.75 ± 0.96	4.77 ± 0.62
Urine Na (mEq/L)	37.5 ± 12.6	64.0#	83.0 ± 15.4	46.8 ± 21.2
Urine K (mEq/L)	214 ± 68.4	246#	154 ± 43.5	285 ± 61.6
Urine Na/K	0.17 ± 0.01	0.26#	0.67 ± 0.25	0.16 ± 0.05
Urine osmolality (mOsm/kg H ₂ O)	1138 ± 299	746#	893* ± 147	2098 ± 154
Urine flow (μl/min)	0.80 ± 0.26	—	0.66** ± 0.13	3.36 ± 1.20

* $p < 0.01$

** $p < 0.02$

*** $p < 0.05$

one rat only

± standard error

Number in parentheses represents the number of samples.

The mean TF/PS osmolality ratio of 0.76 ± 0.03 in adrenalectomized rats was significantly different from that of 0.57 ± 0.03 in intact animals ($p < 0.01$). The mean TF/PS sodium concentration ratio after adrenalectomy (0.60 ± 0.03) was significantly higher than a control level of 0.40 ± 0.03 ($p < 0.01$). The table indicates further that adrenalectomy elevated serum concentration of potassium, increased urinary Na/K ratios, and lowered the urine osmolality from a control level of 2098 to 893 mOsm/kg H₂O ($p < 0.01$).

Free flow experiment

Distal tubular fluid (TF) to plasma (P) ratios were also measured in free flow 7-9th day after the adrenalectomy. The results are summarized in Table 2. The mean value of the TF/P osmolality ratios was elevated from a value of 0.55 ± 0.07 in intact rats to 0.75

TABLE 2. Summary of distal transtubular ratio of osmolality and sodium concentration in free flow experiments.

Treatment	No. of rats	(TF/P) _{osm}	(TF/P) _{Na}
Intact	4	0.55±0.07 (9)	0.27±0.02 (7)
Adrex	7	0.75±0.06 (11)	0.48±0.05 (11)
Aldosterone	5	0.68±0.10 (8)	0.33±0.05 (8)
Cortisol	4	0.55±0.06 (8)	0.37±0.03 (8)
Aldosterone+Cortisol	4	0.54±0.06 (7)	0.34±0.04 (7)

± standard error

Aldosterone 12.5 µg (s.c.)+3.1 µg (i.v.)

Cortisol 2.5 mg (s.c.) for 2 days.

Number in parentheses represents the number of samples

±0.06 in adrenalectomized rats and the mean TF/P Na concentration ratio was elevated in parallel with the osmolality ratio, from 0.27±0.02 to 0.48±0.05.

The transit time of lissamine green was prolonged from 41 to 82 seconds in the adrenalectomized rats ($P<0.01$).

2. Effect of aldosterone

In the microperfusion experiments, the TF/PS ratios of osmolality and Na concentration did not return to control levels with aldosterone treatment and were not significantly different from those of the adrenalectomized group (Table 3). However, serum concentration of sodium and urinary Na/K ratio reverted to those seen in the controls. In free flow experiments (Table 2), however, the TF/P Na concentration ratio fell from 0.48±0.05 to 0.33±0.05 after the administration of aldosterone. This result agrees with data obtained by Hierholzer et al. (7). The mean TF/P osmolality ratio also fell from 0.75±0.06 to 0.68±0.10, but this was not significant.

TABLE 3. Effects of corticosteroids on the distal tubular fluid/perfusate ratios of osmolality and sodium concentration.

	No. of rats	(TF/PS) _{osm}	(TF/PS) _{Na}
Intact	4	0.57±0.03 (8)	0.40±0.03 (7)
Adrenalectomy	7	0.76±0.03 (13)	0.60±0.03 (13)
Adrenalectomy+ Aldosterone (15.6 µg)	4	0.75±0.01 (15)	0.57±0.04 (10)
Adrenalectomy+ Cortisol (5 mg)	4	0.68±0.04 (11)	0.53±0.04 (11)
Adrenalectomy+ Aldosterone (15.6 µg) Cortisol (5 mg)	4	0.65**±0.03 (13)	0.43*±0.02 (13)
Adrenalectomy+ Aldosterone (15.6 µg) Cortisol (1 mg)	2	0.70±0.05 (5)	0.51±0.06 (5)

* Significantly different from the adrenalectomized rats $p<0.01$ ** $p<0.05$

± standard error

Number in parentheses represents the number of samples.

3. Effect of cortisol

The results obtained after 2 daily injection of 2.5 mg cortisol were similar to those obtained with 3 daily injections of 5 mg cortisol. In Table 4, the results after 3 daily injections of cortisol are presented.

In microperfusion experiment cortisol caused a fall in the mean TF/PS osmolality ratio from 0.76 ± 0.03 to 0.68 ± 0.04 and the mean TF/PS Na concentration ratio from 0.60 ± 0.03 to 0.53 ± 0.04 . However, these differences of the osmolality and Na concentration were not statistically significant (Table 3).

In free flow experiment, the administration of cortisol caused a fall in the TF/P osmolality ratio from 0.75 ± 0.06 to 0.55 ± 0.06 ($P < 0.01$) which was the same as in intact animals, but the change in the TF/P Na concentration ratio was not as great as that of TF/P osmolality ratio (see Table 2). Cortisol shortened transit time through the loop from 82 to 50 seconds similar to that in intact animals.

TABLE 4. Effects of cortisol on early distal tubular fluid in adrenalectomized rat kidney (microperfusion of Henle's loop).

	Adrenalectomy	Adrenalectomy + Cortisol (5 mg $\times$ 3 days)
No. of rats	10	5
(TF/PS) _{osm}	0.80 ± 0.01 (24)	0.66 ± 0.01 (19)*
(TF/PS) _{Na}	0.69 ± 0.01 (20)	0.50 ± 0.01 (18)*
(TF/PS) _{in}	1.49 ± 0.07 (18)	1.51 ± 0.06 (18)
Sodium reabsorption* (nanEq/min)	1.48 ± 0.09 (16)	1.88 ± 0.06 (17)
Urine Na/K	0.33 ± 0.04	$1.21 \pm 0.09^*$
Urine osmolality (mOsm/kg H ₂ O)	1048 ± 57	$1672 \pm 94^*$
Urine flow (μ l/min)	0.92 ± 0.14	$2.72 \pm 0.31^*$
Serum sodium (mEq/L)	146 ± 1.3	144 ± 2.8
Serum potassium (mEq/L)	6.43 ± 0.21	5.57 ± 0.29

* $p < 0.01$

$\pm$ standard error

Number in parentheses represents the number of samples.

* Sodium reabsorption was calculated from an equation.

$$V_p \cdot (\text{Na})_p \left(1 - \frac{(\text{TF/PS})_{\text{Na}}}{(\text{TF/PS})_{\text{in}}} \right) \quad (\text{see text})$$

In the experiments with 3 daily injections of cortisol, inulin was present in the perfusate and thus the net absorption of water and sodium could be measured. The mean TF/PS ratio of inulin concentration at the early distal tubule did not change after cortisol (Table 4).

Net sodium reabsorption, which was calculated from the equation:

$$\text{Na}_{\text{reabs}} = V_p \cdot \text{Na}_p \left(1 - \frac{(\text{TF/PS})_{\text{Na}}}{(\text{TF/PS})_{\text{in}}} \right)$$

was significantly increased with cortisol, from 1.48 ± 0.09 to 1.88 ± 0.06 nanoEq/min ($P < 0.01$). This fact indicated that cortisol increased net sodium reabsorption without chang-

ing net water absorption in the Henle's loop of the kidney of adrenalectomized rats.

V_p = perfused volume/min

Na_p = sodium concentration of perfusate

4. Effect of simultaneous treatment of cortisol and aldosterone

In the microperfusion experiments the mean TF/PS Na concentration ratio fell from 0.60 ± 0.03 to 0.43 ± 0.02 ($P < 0.01$) with the simultaneous administration of aldosterone and cortisol (Table 3). The TF/PS osmolality ratio also fell significantly ($P < 0.05$). The change in distal transtubular gradient for sodium resulting from the simultaneous administration of both corticosteroids was greater than that with cortisol treatment alone. The ureteral urine osmolality was increased from 893 to 1896 mOsm/kg H_2O ($P < 0.01$).

In free flow experiments, the mean TF/P ratio for osmolality and Na concentration were not different from those obtained after treatment with aldosterone or cortisol alone under the experimental conditions which were shown in Table 2.

As described earlier, ureteral urine osmolality was markedly increased with cortisol or with simultaneous treatment with cortisol and aldosterone. Therefore the experiments were performed to determine the electrolyte or solute concentration in tissue, in order to calculate the changing of the tissue osmolality.

TABLE 5. Effects of corticosteroids on the gradients of Na, K as well as urea concentration in tissue slices of rat kidney.

	Intact (4)			Adrex (3)			Adrex + Aldo. (4)			Adrex + Cortisol (4)		
	I.M.	O.M.	Cort.	I.M.	O.M.	Cort.	I.M.	O.M.	Cort.	I.M.	O.M.	Cort.
Na (mEq/L. tissue water)	309	150	67.5	171	106	57.3	209	106	59.4	293*	127	60.4
K (mEq/L. tissue water)	70.8	72.6	72.8	75.4	79.4	86.2	63.3	79.3	76.4	75.4	70.3	74.7
Urea (mMol/L. tissue water)	899	238	42.6	241	118	31.3	473*	157	33.2	532*	158	33.8
Total osmolality (mOsm/L. tissue water)	1659	683	323	735	488	315	1017	526	305	1268*	577	305
Water content (%)	84.1	83.1	76.1	89.1	83.5	76.8	85.0	82.2	77.4	83.2	83.1	77.9

I.M.; Inner medulla + papilla, O.M.; outer medulla, Cort.; cortex, Aldo.; d-aldosterone monoacetate 15.6 μ g, Cortisol; alcohol free, 5 mg

*; significantly different from the adrenalectomized rat, $p < 0.02$

Number in parentheses represents the number of rats.

The results are presented in Table 5. The Na concentration of inner medulla was increased slightly after aldosterone administration from 171 to 209 mEq/L (tissue water). Urea concentration and total osmolality (which was calculated as the sum of Urea + 2 [Na + K]) were also increased significantly after cortisol.

DISCUSSION

It has been found that in unanesthetized rat, urinary Na/K ratios are significantly elevated 18–24 hours after adrenalectomy (14). In the present experiments, no differ-

ences were observed between the urinary Na/K ratios in intact rats and in rats 18-24 hours after adrenalectomy (Table 1). The failure to find a change at this time may be due to a direct or indirect influence of anesthesia and operative stress. Microperfusion experiments were performed 7-9 days after adrenalectomy. At this time the deviations from the TF/PS ratios of osmolality and Na concentration in the intact controls were moderately developed, and serum concentration of potassium and sodium and urinary Na/K ratios also exhibited the characteristic pattern of adrenalectomized animals.

The distal transtubular gradients for osmolality and sodium in adrenalectomized animals shifted towards the normal values after the administration of adrenal hormones (Table 2). These results could be explained in two ways. In the adrenalectomized rats, active reabsorption of Na ions in the ascending limb could be inhibited or water permeability in Henle's loop may be increased and these are reversed by steroid administration.

Eigler and Peterson (15) reported that aldosterone is secreted into the adrenal veins at a maximum rate of 10 $\mu\text{g/day/gland}$ in anesthetized rats. Thus, it appears probable that the dose employed in the present experiment was slightly higher than the quantity secreted daily by the adrenals. Although the TF/PS ratios of osmolality and sodium concentration in microperfusion experiments were not clearly changed with aldosterone, they did fall in the free flow experiments. This dissociation could arise in several ways.

Lassiter et al. (16) indicated that the permeability to water in distal tubules is affected by calcium ions. Therefore the effects of calcium and potassium ions on the TF/PS ratios of osmolality and Na concentration in early distal tubules was examined. There was no difference between the results obtained in microperfusion with 0.9% NaCl (171 mEq/L, 315 mOsm/kg H_2O) or Ringer's solution (NaCl: 147, KCl: 4.02, CaCl_2 : 2.7 mMol/L, 302 mOsm/kg H_2O).

From these results, it may be concluded that the difference between the results of microperfusion and free flow is not due to an effect of calcium and potassium ions in the perfusion fluid.

A different cause of this dissociation may be the rate of volume flow. The determination of transit time through Henle's loop by dye injection showed that the flow rate during microperfusion was over 2 times faster in comparison with the free flow in a similar group of rats which were adrenalectomized and injected with aldosterone. The difference in these flow rates may cause the different results or in other words, the increased contact time may be important as has been considered by several authors (8, 17).

The collection sites in these distal tubules were between 15 and 50% of the distal tubule length (18). Thus the dissociation may be explained by aldosterone acting on the distal segment rather than on the ascending limb. Since the change in transtubular osmolal gradients in early distal tubules depends on the acceleration of active transport of sodium ions in the ascending limb of Henle's loop, the result implies that aldosterone stimulates the exchange of Na or other ions in the early distal tubules.

The dose of cortisol employed in the present experiments, 5 mg/rat, is about three times greater than the maximum secretion rate of corticosterone in anesthetized rats (15)

(19).

In contrast to the results with aldosterone, the effects with the cortisol were observed clearly both under experiments of free flow and microperfusion. The following modes of action of cortisol might be suggested.

1. Cortisol increases reabsorption of sodium ions in the ascending limb of Henle's loop.

2. Cortisol causes a decrease of water permeability in Henle's loop. Stolte et al. (20) have indicated that an increase of water permeability in the distal tubules of adrenalectomized rats is inhibited by cortisone treatment.

To determine which of these two possibilities is more likely, we measured net water flux in perfused Henle's loops by the determination of inulin. The TF/PS ratios of inulin concentration at early distal tubules were not changed by cortisol, but the net Na reabsorption was significantly increased. This result indicates that cortisol increased the transtubular gradients of osmolality and Na concentration at the ascending limb by increasing the active reabsorption of sodium ions. An inhibition of water permeability by glucocorticoid may take place in the more distal portions of the tubules, but in Henle's loop it may be not of prime importance.

In the present experiments, glomerular filtration rate and renal blood flow were not measured. Many authors (20-23) have already demonstrated that mineralcorticoid has no effect on GFR and RBF, but glucocorticoid increases their values. In the present perfusion experiments, the effects of changes in GFR were excluded because the proximal part of the perfused nephron was blocked with oil. An increased RBF, however, may change medullary blood flow. It has been suggested by Giebisch and Windhager (24) that the concentration gradients of sodium ions in early distal tubules may be determined by changes in medullary blood flow (25).

In addition, an increased blood flow may lead to the reestablishments of enzyme system of energy metabolism and supply of substrates, which are responsible for the improvement of transtubular movement of sodium ions (26-30). The results obtained in the present study do not allow us to conclude whether cortisol acts directly on the transport mechanism at the cellular level or indirectly via changes in renal blood flow.

Stolte et al. (20) reported that in adrenalectomized rats, there is an increased water permeability in the distal convoluted tubules and that the administration of glucocorticoid returns this to control values. Since in our experiments the collection sites, from which samples were collected, were 15 to 55% along the distal tubule, an inhibition of water permeability by cortisol might take place at a more distal segment.

SUMMARY

Male albino rats weighing 150-230 g were adrenalectomized. Aldosterone or cortisol was given under various conditions and 7-9 days after adrenalectomy the TF/P ratios of osmolality and Na-concentration at the early distal tubule were measured under the free flow as well as the microperfusions experiment of Henle's loop. The effects of cortisol

on the Henle's loop were clearly demonstrated than those of aldosterone under the perfusions experiments. The mechanisms of these actions were discussed.

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