INHIBITION OF FLUORESCEIN SECRETION IN THE PROXIMAL RENAL TUBULE OF THE FROG BY DIURETICS (INTRAVITAL INVESTIGATION BY CONTACT MICROSCOPY)

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Fluorescein is secreted intensively by cells of the proximal renal tubules of the frog in experiments in vivo and in vitro. Diuretics (frusemide, ethacrynic acid, triamterene, Brinaldix, Aldactone-Saltucin) in doses inhibiting sodium reabsorption, inhibit the secretion of fluorescein through the apical membrane into the lumen of the proximal tubule but do not affect its accumulation in the cells of these tubules. Inhibition of fluorescein secretion by diuretics is evidently connected with their effect on one of the elements of the sodium transport system localized in the region of the apical plasma membrane of the proximal tubule.

The mechanism and site of action of diuretics on sodium reabsorption in the nephron are highly debatable problems. Since sodium transport is coupled with the secretion of certain organic substances [5], the site of action of diuretics could perhaps be deduced from their effect on the transport of these substances.

It was decided to study whether various modern diuretics affect the secretion of organic substances by cells of the proximal tubules and whether this action is localized in the basal or apical plasma membrane of the kidney cell.

EXPERIMENTAL METHOD

Male frogs (Rana temporaria) weighing 25-35 g were used in the experiments from September to May. The frogs were kept in a refrigerator and transferred to the room on the day of the experiment. Fluorescein, made up in Ringer's solution for cold-blooded animals, was injected into the thigh muscles in a dose of 0.2 ml of the 0.01% solution. Diuretics (frusemide, ethacrynic acid, triamterene, clopamide (Brinaldix), and Aldactone-Saltucin, and also para-aminohyppurate (PAH) and tetramethylammonium bromide (TMA) were injected into the dorsal lymph sac.

Experiments in vivo. The frogs were injected with the test substance and with fluorescein 10-25 min later. The spinal cord was destroyed after 10 min. The ventral and dorsal surfaces of the kidneys were examined by contact microscopy in situ without disturbance of the blood supply or to the normal outflow of urine.

Experiments in vitro. The frog's kidneys were dissected; one of them was placed in a solution consisting of the test substance and Ringer's solution, while the other was incubated in Ringer's solution. After incubation for 10 min the two kidneys were transferred separately for the same period into fluorescein solution (1 : 250,000) and then examined by contact microscopy. Each diuretic was tested on 7-10 animals. A microscope with contact objectives [1-3] was used in these experiments, so that the superficial glomeruli and tubules could be examined without opening the kidney capsule. Observations were made with the OI-30 source of light, either in luminescence obtained with a type FS1-3 filter to the source of ex-

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citation and a ZhS-18 stop filter, or in incident light using polar filters. Photography was carried out on RF-3 film using the OLK-2 focusing camera.*

To determine the ability of the diuretics to modify the sodium excretion by the kidney, experiments were carried out on frogs with a ligated cloaca. Urine collected from the bladder was analyzed for its sodium content (Zeiss III flame photometer). Glomerular filtration was measured relative to inulin clearance.

EXPERIMENTAL RESULTS AND DISCUSSION

From 1 to 2 min after injection of fluorescein into the frogs, it was found in the blood plasma of the renal vessels, and later it appeared in the cytoplasm of the cells of the proximal tubules. Judging from the brightness of illumination of the cytoplasm, the fluorescein accumulated in it; very bright fluorescence appeared after 10-15 min in the lumen of most proximal tubules (Fig. 1). The following observations confirm that this fluorescence was due to the secretion of fluorescein. Fluorescence in the vessels and in Bowman's capsule was incomparably weaker than in the lumen of the proximal tubule. The increased intensity of fluorescence cannot be explained by an increase in the concentration of dye resulting from reabsorption of the filtrate, for judging from the inulin concentration index, the concentration of the fluid in these frogs, even in the terminal parts of the tubules, amounted to only 1.3 ± 0.13 times. Finally, the experiments on isolated frog kidneys showed that bright fluorescence appeared in the lumen of the proximal tubules after 5-10 min in fluorescein solution.

To study the action of diuretics on secretion, doses giving the maximum increase in sodium concentration in the frog's urine by inhibiting its reabsorption were used. Frusemide (10 mg/100 g) and ethacrynic acid (5 mg/100 g) completely inhibited fluorescein secretion. It did not accumulate in the lumen of the tubule. After injection of triamterene (1 ml of a 10^{-14} M solution/100 g), Brinaldix (1.25 mg/100 g) and Aldactone-Saltucin (25 mg/100 g), fluorescein accumulated in the lumen of the tubule in a much smaller amount than in the control. Of all the diuretics, only triamterene possessed spontaneous fluorescence and was detected 10-25 min after its injection in the cytoplasm of the proximal tubules. No triamterene could be found in the lumen of the proximal tubules or in the cells of the distal tubules.

The results of the experiments in vivo and in vitro show that the chief site of action of the diuretics is the apical cell membrane. Fluorescein penetrated through the membrane facing the network of blood

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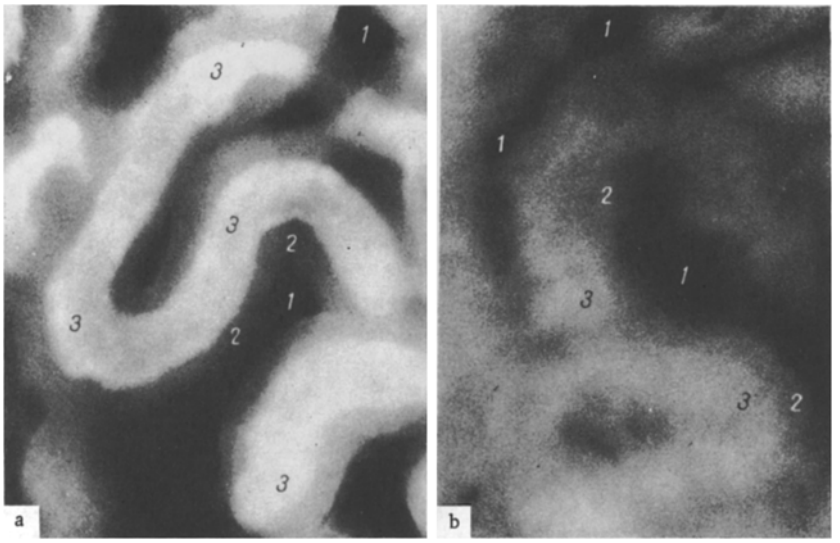

Fig. 1. Fluorescence of fluorescein in cytoplasm and lumen of renal tubules of a frog: a) control; b) after injection of frusemide (10 mg/100 g body weight). 1) intertubular capillary; 2) cell cytoplasm; 3) lumen of tubule. Contact objective 20×0.75.

vessels and accumulated in the cytoplasm of the cells of the proximal tubules but it did not pass through the apical membrane (Fig. 1). The entry of fluorescein into the cytoplasm was prevented only be the action of very high concentrations of frusemide (1 mg/ml) in the incubation medium.

These observations regarding the relative strength of secretion by the various diuretics correlate well with the sodium-excreting action of the same substances: the sodium concentration rose from 4.1 ± 1.0 meq/liter (n = 6) in the control to 74.6 ± 1.9 meq/liter (n = 9) after administration of frusemide, to 64.0 ± 4.1 meq/ liter (n = 6) after administration of ethacrynic acid, to 32.0 ± 5.0 meq/liter (n = 9) after Brinaldix, to 15 ± 1.5 meq/liter (n = 7) after triamterene, and to 18.7 ± 1.8 meq/liter (n = 13) after Aldactone-Saltucin. The diuresis increased after administration of frusemide, but in the other experiments it remained at the same level as at the control.

The decrease in fluorescein secretion produced by the diuretics could be explained in various ways. Frusemide and ethacrynic acid are organic acids and, as such, could be secreted by the cells of the proximal tubule of the nephron and could competitively inhibit the secretion of fluorescein. Another possibility is that the diuretics selectively affected the permeability of the apical membrane or certain elements of the sodium transport system, reducing both the reabsorption of sodium and the secretion of fluorescein. Experiments in which PAH was injected into the frogs in doses of 25 and 2.5 mg/100 g gave evidence of the possibility of competitive inhibition of fluorescein secretion: in the first case there was considerable, but in the second case only weak inhibition of fluorescein are thus mutually competitive, and the affinity of the cells of the proximal tubules for fluorescein is considerably higher than that of PHA, while competition was exhibited chiefly in the region of the apical membrane. Only when very high doses of PAH were used (5 mg/ml in vitro) was the accumulation of fluorescein in the cell reduced. Systems responsible for the transport of organic acids evidently exist on both membranes, and diuretics depress the activity of these systems on the apical membrane. In the modern view secretion of organic acids and bases in the proximal tubule is carried out by independent transport systems [4]. The organic base TMA in a dose of 0.5 ml of the 10^{-3} M solution/100 g did not affect fluorescein secretion. Fluorescein and TMA do not complete with each other, as the experiments in vitro clearly showed. Fluorescein and organic bases are thus evidently secreted by different systems. Meanwhile, the diuretic triamterene, which also is an organic base, reduced the secretion of fluorescein. These results suggest that the inhibition of fluorescein secretion by diuretics is connected with their influence on one of the elements of the sodium transport system in the cells of the proximal tubule, which is evidently located in the apical plasma membrane.

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