THE ACTION OF DIURETICS ON SODIUM TRANSPORT IN THE FROG KIDNEY AND SKIN

Yu. V. Natochin and E. A. Lavrova

UDC 615.254.1.015.45:612.464.1

Frusemide, ethacrynic acid, aldactone, hypothiazide, triamterene, acetazolamide, and clopamide depress sodium reabsorption in the renal tubules of the frog. Mersalyl, aldactone, hypothiazide, and ethacrynic acid inhibit active Na transport by the cells of the frog's skin if added to the solution on the side of the apical membranes of the cells. Frusemide and acetazolamide have no effect on Na transport by frog skin cells. The reasons for the differences between the reactions of the cells of the skin and renal tubules to these diuretics are discussed.

Diuretics act specifically on different components of the Na transport system in the cells of the nephron. To understand the mechanism of action of the diuretics it is important to discover whether they affect Na transport in the cells of the nephron only or whether they also inhibit it in other cells, and also to discover on which of the cell membranes they act, for the apical and basement plasma membranes of the nephron cells have different properties relative to Na transport. In experiments on isolated membranes of the skin and urinary bladder of amphibians, analogues of the nephron cells, it is possible to determine on which cell membrane the diuretic acts. Data on the effects of certain diuretics on the urinary bladder and skin of the toad and frog are contradictory: some workers observed activation of Na transport by diuretics [2, 3, 7] while others observed inhibition [8-10].

The object of this investigation was to study the effects of all the principal types of modern diuretics on Na excretion by the kidney and on active Na transport by the cells of the frog's skin.

EXPERIMENTAL METHOD

Male winter frogs (Rana temporaria) were used. To investigate the action of the diuretics on the frog kidney under standard conditions of the water balance, during the experiment the animals were kept in water. All preparations were injected subcutaneously in the dorsal region, after which the cloaca was ligated. The

TABLE 1. Effect of Diuretics on Sodium Excretion by the Frog Kidney (M ± m)

Diuretic	Number of experiments	Dose of prep. (in mg/100 g body weight)	Diuresis (in l/min/100 g body weight)	Na concentration in urine (in μeq/ml)
Control	16		53±3	14±1,3
Frusemide	10	2,5	56±4	78±2,9‡
Ethacrynic acid	10	1,2	46±4	51±2,5‡
Aldactone	4	2,5	75±4†	24±5,3*
Hypothiazide	4	2,5	64±8	31±3,5‡
Acetazolamide	9	2,0	40±3†	35±2,5‡
Diaphylline	9	10,0	40±4†	21±2,1†
Clopamide	17	0,5	30±5‡	51±3,3‡
Triamterene	8	0,015	39±3*	28±3,1‡

Note: Here and in Table 2: *P < 0.05, †P < 0.001, ‡P < 0.001.

Laboratory of Development of Excretory Function, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 77, No. 4, pp. 63-66, April, 1974. Original article submitted February 20, 1973.

TABLE 2. Effect of Diuretics on Active Sodium Transport in the Frog Skin (M ± m)

Diuretic	Dose of preparation (in mole/liter)	Number of experiments	(in μA/0.5 cm²) initial	after 60 min
Frusemide	$3 \cdot 10^{-3}$	8	24,6±3,4	26,0±3,8
	$5 \cdot 10^{-4}$	4	31,5±7,0	29,5±6,2
Ethacrynic acid	$1,4 \cdot 10^{-3}$	4	44,6±5,1	0‡
	$1,2 \cdot 10^{-3}$	5	26,6±4,6	17,8±1,5
	$5,4 \cdot 10^{-4}$	3	43,3±4,6	43,3±3,4
	$5,4 \cdot 10^{-5}$	5	18,4±2,7	22,2±3,0
Acetazolamide	$9 \cdot 10^{-4}$	8	23,1±4,5	33,6±6,3
	$1 \cdot 10^{-4}$	5	30,6±3,8	27,4±4,9
Triamterene	$1 \cdot 10^{-4}$	5[1]	40,2±6,8	0‡
	$1 \cdot 10^{-5}$	10[1]	33,2	19,0 (14,1±3,6)†
	$5 \cdot 10^{-6}$	4[1]	33,0±4,5	27,7±2,5
	$1 \cdot 10^{-6}$	10[1]	31,8±5,3	33,6±5,3
Diaphylline	$1 \cdot 10^{-2}$	9	55,3	74,6 (20,0±5,6)‡
Hypothiazide	$1 \cdot 10^{-2}$	5	23,2	15,0 (8,2±2,3)*
	$5 \cdot 10^{-3}$	8	28,0	19,2 (8,8±2,2‡)
	$1 \cdot 10^{-3}$	5	31,8±4,0	35,8±5,8
	$1 \cdot 10^{-4}$	5	24,2±2,1	26,6±3,5
	$5 \cdot 10^{-3}$	5[1]	45,8	32,0 (13,8±4,1)*
Mersalyl	$1 \cdot 10^{-3}$	4	26,5±1,5	0‡
	$1 \cdot 10^{-4}$	5	37,3±3,6	0‡
	$1 \cdot 10^{-5}$	5	47,2±4,5	0‡
	$1,5 \cdot 10^{-5}$	4	37,5	33,0 (3,5±0,86)*
	$5 \cdot 10^{-6}$	5	38,0±3,1	42,0±3,4
Aldactone	$6,2 \cdot 10^{-4}$	10	34,4	9,3 (25,1±3,8) ‡
	$3,1 \cdot 10^{-4}$	10	39,3	19,7 (19,6±4,1) ‡
	$1,5 \cdot 10^{-4}$	8	29,7±2,8	29,2±3,3

[1]Preparation added to solution on outer surface of frog's skin.

Note: The results of experiments in which the changes under the influence of the diuretic were in the same direction were subjected to statistical analysis by the method of paired variables; in these cases the first figure in the column headed "after 60 min" signifies the mean, and the figures in parentheses signify Δ ± m.

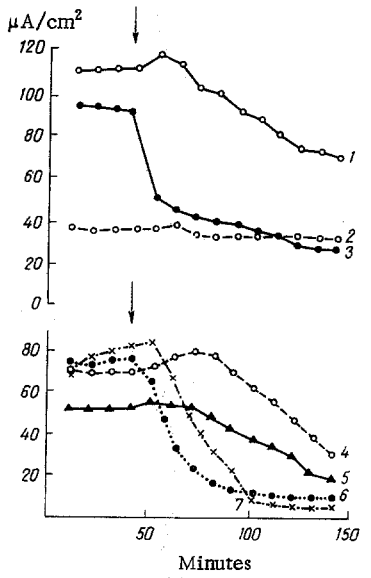

Fig. 1. Dynamics of changes in active Na transport by frog skin cells under the influence of various diuretics. Arrow marks time of addition of diuretic to Ringer's solution: 1) hypothiazide $5 \cdot 10^{-3}$ mole/liter; 2) frusemide $3 \cdot 10^{-3}$ mole/liter; 3) triamterene $1 \cdot 10^{-5}$ mole/liter; 4) mersalyl $1.5 \cdot 10^{-5}$ mole/liter; 5) hypothiazide $5 \cdot 10^{-3}$ mole/liter; 6) aldactone $6.2 \cdot 10^{-4}$ mole/liter; 7) ethacrynic acid $1.4 \cdot 10^{-3}$ mole/liter (1 and 3 – addition to the solution on the outer surface of the skin; 2, 4, 5, 6, 7 – addition to the solution on the inner surface of the skin). Abscissa, time (in min); ordinate, short-circuiting current (in $\mu A/cm^2$).

urine accumulating during the experiment in the urinary bladder was collected after 90-120 min. The Na concentration in the urine was determined with the Zeiss-III flame photometer. Active Na transport by the frog skin cells was measured as the magnitude of the short-circuiting current [1]. The action of the following diuretics was studied: frusemide, ethacrynic acid, clopamide, acetazolamide, diaphylline, aldactone, hypothiazide, triamterene, and mersalyl.

EXPERIMENTAL RESULTS AND DISCUSSION

By using the diuretics in doses producing maximal inhibition of Na reabsorption it was possible to estimate the relative strength of their sodium-excreting action. In these experiments frusemide and ethacrynic acid were most effective (Table 1). The action of these drugs depended mainly on a decrease in Na reabsorption and not on the change in diuresis. No effect of mersalyl on the kidney could be detected: injection of a dose of 100 mg/kg led to anuria, whereas smaller doses did not change the diuresis, but no sodium-excreting action could be observed.

The Na reabsorption by the kidney cell cannot be reduced simply to the operation of the sodium pump, but the transport system includes the passage of Na from the lumen of the tubule into the cell, its transport through the cell to the pump, and its active secretion against the gradient into the blood. A decrease in Na reabsorption could be due to activity directed against any of the elements of this system. The experiments on the frog's skin showed that after the addition of ethacrynic acid, mersalyl, hypothiazide, and aldactone to to the Ringer's solution on the inner side of the skin the Na transport was reduced; frusemide, acetazolamide and triamterene were ineffective (Table 2). The kinetics of inhibition differed with these compounds. In some cases initially there was a small increase in active transport, followed by a gradual and considerable decrease (Fig. 1). Triamterene and hypothiazide were effective when added to the solution on the outer side of the skin (Fig. 1). Triamterene was known to immobilize Na transport without affecting the work of the pump [6]. The effectiveness of hypothiazide on the outer surface of the skin is probably attributable to a different cause, for the inhibition of transport was different in character (Fig. 1) and unlike hypothiazide, triamterene had no effect on the inner surface of the skin (Table 2). Clopamide, which increases Na excretion by the kidney, does not affect its transport in the skin [5]. Unlike the other diuretics, diaphylline activates Na transport; it is a synergist of antidiuretic hormone and its action is due to the inhibition of phosphodiesterase [4].

The change in Na transport in the kidney took place after the administration of much smaller doses of the diuretics than in the experiments on the skin. The most effective diuretic – frusemide – could not change Na transport in the frog's skin. This could result from several causes: in the experiments in vivo the conditions for the action of the diuretics were different; in the frog skin cells those elements of the sodium transport system on which the "ineffective" diuretics act are absent; the diuretics penetrate with difficulty into the cells of the frog's skin, whereas in the cells of the tubules there are specialized systems for the transport of organic acids and bases which facilitate the entry of these substances inside the cell.

Consequently, the frog's skin cell reacts differently from the kidney cell to the action of certain diuretics and caution must be exercised with the extrapolation. All diuretics except diaphylline either inhibited Na transport after various time intervals or did not affect it; in no case was prolonged activation of Na transport observed. The action of triamterene is based on inactivation of the Na-permeability of the apical membrane whereas the other preparations evidently affect Na transport during its penetration into the cell.

LITERATURE CITED

1. V. V. Ivanov and Yu. V. Natochin, Fiziol. Zh. SSSR, 54, No. 1, 122 (1968).
2. A. A. Lebedev, in: The Kidney and Electrolytes [in Russian], Kuibyshev (1967), p. 115.
3. A. A. Lebedev, Kardiologiya, No. 1, 89 (1971).
4. Yu. V. Natochin, Probl. Endokrinol., No. 4, 118 (1968).
5. Yu. V. Natochin, Farmakol. i Toksikol., No. 1, 49 (1972).
6. J. Crabbe, Arch. Internat. Pharmacodyn., 173, 474 (1968).
7. W. Herms and K. E. Hofmann, Arch. Exp. Path. Pharmak., 251, 355 (1965).
8. R. L. Jamison, J. Pharmacol. Exp. Ther., 133, 1 (1961).
9. S. Lipson and R. M. Hays, J. Clin. Invest., 45, 1042 (1966).
10. L. P. Sullivan, J. M. Tucker, and M. J. Scherbenske, Am. J. Physiol., 220, 1316 (1971).