Biochimica et Biophysica Acta, 298 (1973) 1011–1014

BBA Report

BBA 71169

Short-circuit current related to active transport of chloride in frog cornea: effects of furosemide and ethacrynic acid

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(Received February 12th, 1973)

SUMMARY

Active transport of Cl^- accounts for 90% of the short-circuit current (s.c.c.) in the isolated frog cornea. $1 \cdot 10^{-5}$ M furosemide produced a 50% reversible inhibition of this s.c.c. $1 \cdot 10^{-4}$ M ethacrynic acid reduced the corneal s.c.c. to 32% of the control. In the isolated frog skin epithelium furosemide had no effect on the s.c.c. at a concentration of $1 \cdot 10^{-4}$ M and a small stimulation at a concentration of $1 \cdot 10^{-3}$ M. The furosemide inhibitory effects seems to be specific for Cl^-, as it also inhibits Cl^- transport in the ascending limb of the loop of Henle (Burg, M.B. (1972) *Proc. 5th Int. Congr. Nephrol.*, p. 50, Abstr.).

Furosemide is a potent diuretic and it has been previously accepted that its action is mediated by an inhibition of the Na^+ reabsorption. However, using isolated perfused rabbit renal tubules, Burg[1,2] has shown an active Cl^- transport in the ascending limb of the loop of Henle that is inhibited by furosemide. Amphibian epithelium has been used as a model for the study of pharmacological agents affecting Na^+ transport, and the effect of furosemide has been tested in frog skin, toad skin and toad bladder. From the results in those epithelia no clear pattern of its action on Na^+ transport has emerged. For example, I have found that in the isolated frog skin epithelium furosemide has no effect at a concentration of $1 \cdot 10^{-4}$ M and gives a stimulation of the short-circuit current (s.c.c.) when it is added to the outside solution in concentrations higher than $1 \cdot 10^{-4}$ M. Similar results have been reported in the whole frog skin[3]. In contrast, furosemide ($0.6 \cdot 10^{-3}$ M) produced a decline in the s.c.c. of the toad skin when added to the inside solution[4].

Abbreviation: s.c.c., short-circuit current.

In the toad bladder, Ferguson[5] found no effect of furosemide on the spontaneous s.c.c. with concentrations as large as $0.76 \cdot 10^{-3}$ M, although it antagonized the stimulation of Na^+ transport produced by vasopressin. Similar results were reported by Molina *et al.*[6]. However, Sullivan *et al.*[7] reported that furosemide ($0.8 \cdot 10^{-3}$ M) reduced the s.c.c. and the mucosal to serosal Na^+ flux in the toad bladder. It is clear that high concentrations of furosemide are necessary to influence Na^+ transport in amphibian membranes and that its effect is inconsistent with its powerful diuretic action.

Active Cl^- transport occurs in a number of tissues, including the thick ascending limb of the loop of Henle[1], where furosemide ($1 \cdot 10^{-5}$ M) exerts its action; suggesting that the diuretic effect of furosemide is due to a primary depression of active Cl^- transport.

Unlike other amphibian epithelium, the isolated frog cornea actively transports

TABLE I

EFFECT OF FUROSEMIDE ADDED TO THE ENDOTHELIAL SOLUTION ON THE ELECTRICAL PARAMETERS OF THE ISOLATED FROG CORNEA

Values are mean ± S.E. Numbers in parentheses indicate the number of experiments. Control and experimental values for the same furosemide concentration are paired data. Paired differences between control and furosemide-depressed s.c.c. are significant to $P < 0.005$.

Concentration of furosemide (M)	*s.c.c. (μA/cm²)*		*PD (mV)*	
	Control	*40 min after adding furosemide*	*Control*	*40 min after addin furosemide*
$1 \cdot 10^{-6}$	12.6 ± 1.2 (8)	9.6 ± 0.9 (8)	16.7 ± 1.9 (8)	13.8 ± 2.0 (8)
$1 \cdot 10^{-5}$	11.9 ± 0.7 (10)	6.1 ± 0.6 (10)	17.4 ± 1.5 (11)	10.2 ± 1.2 (11)
$1 \cdot 10^{-4}$	12.2 ± 0.9 (15)	2.6 ± 0.3 (15)	17.3 ± 1.5 (15)	4.0 ± 0.5 (15)

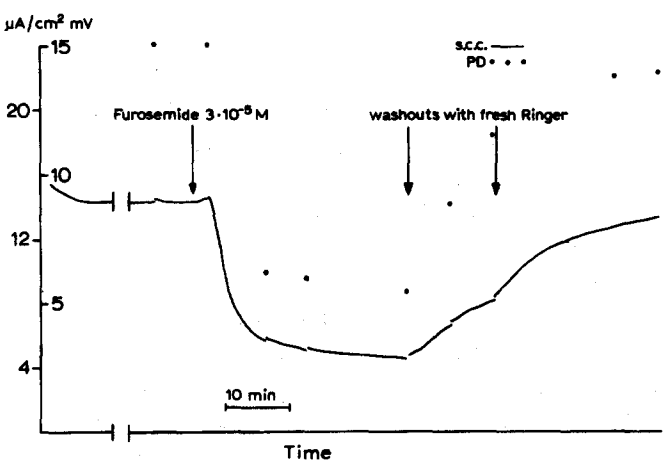

Fig. 1. Record from one experiment showing the effect of furosemide on the s.c.c. (representing Cl^- transport) and PD of the isolated bullfrog cornea.

Cl^- almost exclusively (90% of s.c.c.)[8,9]. Using methods described elsewhere[10], I have tested the action of furosemide on the s.c.c. of the isolated cornea of the frog *Rana catesbeiana* and found a 24% inhibition with concentrations as low as $1 \cdot 10^{-6}$ M. 49% and 79% inhibition were found with concentrations of $1 \cdot 10^{-5}$ and $1 \cdot 10^{-4}$ M, respectively. Table I shows average values of s.c.c. before and after the furosemide inhibition. In several corneas the s.c.c. was initially stimulated by theophylline or prostaglandin before the furosemide was added. The relative reduction in s.c.c. was similar to the non-stimulated controls. Furosemide only exerts its action when added to the endothelial (inside) bathing solution. Although the Cl^- transport system is located on the epithelial side of the cornea, most agents can reach this site only by diffusing across the more permeable endothelium and stroma. Fig. 1 shows a record from one experiment in which the time course of the action of furosemide can be seen, and demonstrates the reversibility of its effect. In Cl^--free Ringer's solution the corneal s.c.c. was considerably reduced[8,9] and furosemide had no effect. Ouabain and ethacrynic acid also reduced active Cl^- transport in kidney tubules[10]. In the frog cornea, Cl^- transport is inhibited by ouabain[11], while ethacrynic acid at $1 \cdot 10^{-4}$ M concentration in the endothelial solution produced a 70% reduction in the s.c.c. The effect of ethacrynic acid is shown in Table II.

TABLE II

EFFECT OF ETHACRYNIC ACID ADDED TO THE ENDOTHELIAL SOLUTION ON THE SHORT-CIRCUIT CURRENT OF THE ISOLATED FROG CORNEA

Values are means ± S.E.

s.c.c. ($\mu A/cm^2$)	
Control	*Ethacrynic acid ($1 \cdot 10^{-4}$ M)*
11.4 ± 0.9 (6)	3.4 ± 0.4 (6)

The inhibitory effect of furosemide seems to be rather specific for Cl^- transport, as it inhibits the transport of this ion in both kidney and cornea. The remarkable pharmacological similarity between these two tissues exhibiting active Cl^- transport suggests that a common mechanism is involved. The frog cornea may thus provide a simple *in vitro* preparation for studying this fundamental osmoregulatory process. This may have immediate practical significance for the study of diuretic and other drugs.

This work was supported by NIH Grant EY 00160 and American Heart Association Grant-in-Aid 72-846.

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