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EFFECT OF FUROSEMIDE ON UNIDIRECTIONAL FLUXES OF SODIUM AND CHLORIDE ACROSS THE SKIN OF THE FROG, *RANA PIPIENS*

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SUMMARY

1. The diuretic furosemide, when added to the outside solution at a concentration of $5 \cdot 10^{-4}$ M, increases the electrical potential difference (PD) across the isolated frog skin, but the short-circuit current (I_{sc}) is unchanged. Lower concentrations had no significant effect on these electrical parameters.

2. When SO_4^{2-} or NO_3^- are substituted for Cl^- in the Ringer's solution furosemide has no effect on the PD or I_{sc}.

3. Simultaneous unidirectional fluxes of Na^+ and Cl^- show that furosemide ($5 \cdot 10^{-4}$ M outside) reduces both the influx and outflux of Cl^-, while the Na^+ fluxes are not altered.

4. Furosemide ($5 \cdot 10^{-4}$ M) on the corium side of the frog skin had no significant effect on either PD, I_{sc} or unidirectional fluxes of Cl^-.

5. It is suggested that furosemide reduces passive Cl^- transfer, possibly by interacting with the Cl^-/Cl^- exchange diffusion mechanism which has been observed in this tissue. These observations further suggest that perhaps the diuretic action of furosemide may be mediated by such an effect on passive Cl^- permeability which is linked to the active Cl^- transport mechanism in the renal tubule.

INTRODUCTION

The effect of the potent diuretic drug furosemide on ion transport across epithelial membranes has received considerable attention. However, its mode of action is controversial. It was initially suggested that furosemide reduces Na reabsorption in the renal tubule by an action on the active Na^+ transport mechanism [1], but subsequent observations showed that its primary effect is an inhibition of active Cl^-

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transport in the ascending loop of Henle [2]. The frog skin and toad urinary bladder have been extensively used as "models" for studying the mechanism of action of furosemide on active Na^+ and Cl^- transport [3–10]. However, as with the observations in the kidney, similar contradictions appear. Fülgraff et al. [3] have shown that furosemide increases the electrical potential difference (PD) and short-circuit current (I_{sc}) across the isolated frog skin (*Rana esculenta*) and suggested that it increases Na^+ permeability by an action at the mucosal surface of the cell. The earlier observations of Karger [4] implied that the primary effect of this diuretic in frog skin was a reduction in chloride permeability. No ion flux measurements were included in either of these investigations. Karger's hypothesis is supported by the findings that Cl^- fluxes across frog skin are decreased by furosemide [5, 6]. These observations however disagreed as Bevis et al. [5] concluded that furosemide inhibited both influx and outflux of Cl^-, whereas Lote [6] found only an effect on influx. No measurements of Na^+ fluxes have been made. The present study was therefore undertaken in the hope that it could clarify and extend these observations. The effects of furosemide on PD, I_{sc} and unidirectional fluxes of both Na^+ and Cl^- (measured simultaneously) were investigated.

METHODS

Frogs, *R. pipiens*, were obtained from a commercial supply house (Lake Champlain Frog Farm, Vermont) and kept in terraria in the laboratory at 23 °C. Ventral abdominal skins of frogs were mounted as diaphragms in Ussing-type divided chambers (surface area of 3 cm^2) for the determination of $^{24}Na^+$ and $^{36}Cl^-$ unidirectional fluxes and for recording PD and I_{SC}. Unidirectional fluxes of $^{24}Na^+$ and $^{36}Cl^-$ were measured simultaneously in the same skin preparation (four 15-min control periods and four 15-min periods following the addition of either furosemide or thiocyanate). The data presented represent the mean values $\pm$S.E. for two periods before and two periods following the addition of furosemide or thiocyanate. The methods used for evaluating transepithelial PD, I_{sc} and flux data have been described previously [11]. The composition of the bathing solutions was: NaCl-Ringer, 111 mM NaCl, 4 mM $NaHCO_3$, 2.54 mM $CaCl_2$, 3.35 mM KCl and 5 mM glucose, final pH, 8.0. SO_4^{2-}-Ringer, 57.0 mM Na_2SO_4, 3.35 mM $KHCO_3$, 2.58 mM Ca^- gluconate, 5.0 mM glucose and 57.0 mM sucrose (to make the solution iso-osmolar), final pH 8.0. NO_3^--Ringer, 115 mM $NaNO_3$, 3.35 mM $KHCO_3$, 2.58 mM Ca^- gluconate and 5.0 glucose, final pH 8.0.

RESULTS AND DISCUSSION

Table I shows that when furosemide ($5 \cdot 10^{-4}$ M) is added to the outside solution bathing the frog skin, the PD promptly increases but the I_{sc} was not significantly changed, although it did slightly increase on some occasions. The addition of furosemide ($5 \cdot 10^{-4}$ M) to the corium side of the frog skin resulted in no appreciable change in either PD or I_{sc} (Table I). This observation differs from previous studies [5]; however this may reflect differences in the species of frog or the different concentrations of the drug. A concentration of 10^{-5} M furosemide added to the outside solution was without an observable effect on these electrical parameters. Thus the

TABLE I

EFFECTS OF FUROSEMIDE ON PD (mV) AND I_{sc} (μA cm^{-2}) ACROSS FROG SKIN IN THE PRESENCE AND ABSENCE OF CHLORIDE

SO_4^{2-} and NO_3^- replaced Cl^- in both bathing solutions, the SO_4^{2-}-Ringers was made iso-osmotic with the addition of sucrose. The number of experiments are in parenthesis.

		Cl^- present		+ Furosemide (15 min)
I. $5 \cdot 10^{-4}$ M furosemide	PD	28±4		47±8*
Outside solution (9)	I_{sc}	21±4		26±4
II. $5 \cdot 10^{-4}$ M furosemide	PD	25±2		22±2
Inside solution (10)	I_{sc}	19±2		15±2
		Cl^- present	Cl^--free	+ Furosemide ($5 \cdot 10^{-4}$ M outside)
III. SO_4^{2-}-Ringer (10)	PD	28±3	65±5	62±4
	I_{sc}	25±3	11±2	11±2
IV. NO_3^--Ringer (10)	PD	26±3	67±8	67±8
	I_{sc}	25±3	28±4	28±4

* $p < 0.05$ from pre-furosemide values.

PD and I_{sc} was 30±2 mV and 26±3 μA cm^{-2} respectively prior to the addition of the drug and 30±3 mV and 28±3.1 μA cm^{-2} after 10^{-5} M furosemide (means ±S.E. of 10 experiments).

The electrical resistance (PD/I_{sc}) across the frog skin in the presence of furosemide ($5 \cdot 10^{-4}$ M outside) increased, which may be reflected as a decrease in ion permeability. Substitution of the divalent anion SO_4^{2-} for Cl^-, in the bathing solution resulted in an increase in the PD (Table I) which was probably due to the lower permeability of the frog skin to SO_4^{2-} than Cl^-. When furosemide was subsequently added ($5 \cdot 10^{-4}$ M outside) under these conditions, there was no further increase in PD. If a monovalent anion, NO_3^-, was substituted for Cl^- in the bathing solutions, the PD across the frog skin also increased (Table I), as NO_3^- permeates the frog skin less readily than Cl^-. Subsequent addition of furosemide ($5 \cdot 10^{-4}$ M outside) had no effect on the PD or I_{sc}. These observations suggest that furosemide increases the electrical PD and resistance across frog skin by reducing Cl^- conductance. However, these results are equivocal since it has also been suggested that such an effect is due to changes in Na^+ conductance [3], and only flux data would support either hypothesis. Therefore, unidirectional fluxes of both Na^+ and Cl^- were measured simultaneously under short-circuited conditions. Tabel II shows that Cl^- influx and outflux are decreased in the presence of furosemide ($5 \cdot 10^{-4}$ M outside), whereas Na^+ fluxes were unchanged. In addition, no changes in Cl^- fluxes were observed when furosemide at an equal concentration was added to the corium side or when this diuretic was present at a lower concentration in the outside solution (10^{-5} M) (Table II). These results are consistent with the earlier observations that the electrical parameters, PD and I_{sc}, were not affected under these conditions.

Chloride transfer in frog skin is considered to be a passive phenomenon, though active Cl^- transport has been observed in another species of frog [12] and

TABLE II

EFFECT OF FUROSEMIDE ON PD (mV), I_{sc} (μA cm^{-2}) AND UNIDIRECTIONAL FLUXES (μequiv. · cm^{-2} · h^{-1}) ACROSS ISOLATED FROG SKIN

The values presented represent the mean ±S.E. of two periods before and two periods after the addition of furosemide.

		Initial	Furosemide
A. $5 \cdot 10^{-4}$ M furosemide			
Outside solution			
Influx (8)	Na^+	1.35±0.1	1.24±0.09
	Cl^-	0.82±0.06	0.40±0.06*
	PD	23 ±1.2	40 ±2.8*
	I_{sc}	22 ±2.1	23 ±1.9
Outflux (11)	Na^+	0.59±0.06	0.59±0.05
	Cl^-	0.76±0.08	0.49±0.08*
	PD	28 ±5.1	47 ±7.1*
	I_{sc}	22 ±2.6	23 ±3.0
B. $5 \cdot 10^{-4}$ M furosemide			
Inside solution			
Influx (6)	Cl^-	0.67±0.06	0.71±0.11
Outflux (6)	Cl^-	0.67±0.08	0.62±0.11
C. 10^{-5} M furosemide			
Outside solution			
Influx (6)	Cl^-	0.66±0.17	0.61±0.17
Outflux (6)	Cl^-	0.64±0.11	0.63±0.09

* $p < 0.01$ from pre-furosemide values.

under special ionic conditions [13]. However, in the present study no active Cl^- transport was apparent, since there was no net Cl^- flux. Therefore, furosemide appears to be acting on passive Cl^- movements in this frog skin preparation. As furosemide has been shown to alter active Cl^- transport in other epithelial tissues [2, 14] it is interesting that it can also alter passive transfer. It has been suggested that Cl^- movements across frog skin involves a Cl^-/Cl^- exchange [15] and a "carrier" which may be responsible for this behavior has also been described [16, 17]. It is therefore possible that furosemide, being an anion, may interfere with this exchange by interacting with this proposed "carrier", perhaps at a halide-binding site. These observations further suggest that the means whereby furosemide produces its diuretic action in reducing active Cl^- transfer in the renal tubule may be mediated by such an effect on passive Cl^- permeability which could be linked to the active Cl^- transport mechanism.

Another anion, thiocyanate, has also been shown to alter Cl^- transfer across epithelial membranes [18, 19], including frog skin [17], and therefore its action was compared to that of furosemide. Table III shows that thiocyanate (added to the outside), in much higher concentrations than furosemide, produces a similar increase in PD. The unidirectional fluxes show that thiocyanate reduces both the influx and outflux of Cl^-. Furosemide and thiocyanate thus appear to be acting in a similar way in reducing passive Cl^- transfer in either direction across this membrane.

TABLE III

EFFECT OF THIOCYANATE ON PD (mV), I_{sc} (μA cm^{-2}) AND UNIDIRECTIONAL FLUXES OF Cl^- (μequiv. · cm^{-2} · h^{-1}) ACROSS ISOLATED FROG SKIN

Thiocyanate (20 mM) was added to the outside bathing solution (isoosmolar conditions were maintained). The protocol for flux measurements was the same as described in Table II.

	Initial	+ Thiocyanate
Influx 8		
Cl^-	0.62 ± 0.05	0.20 ± 0.03*
PD	21 ± 1.3	52 ± 1.3*
I_{sc}	22 ± 2.1	24 ± 2.4
Outflux (6)		
Cl^-	0.61 ± 0.06	0.21 ± 0.02*
PD	26 ± 1.9	55 ± 3.8*
I_{sc}	22 ± 3.6	23 ± 4.1

* $p < 0.01$ for difference from initial value.

TABLE IV

UNIDIRECTIONAL FLUXES OF CHLORIDE (μequiv. · cm^{-2} · h^{-1}) ACROSS FROG SKIN IN THE PRESENCE OF 9 % ETHANOL. EFFECTS OF FUROSEMIDE AND THIOCYANATE

The ethanol was added to the external bathing solution as was furosemide ($5 \cdot 10^{-4}$ M) and thiocyanate (20 mM). 12–15 min periods were measured, 4 before the addition of the ethanol, 4 after and 4 following the addition of furosemide or thiocyanate. The values presented represent the means ± S.E. of flux measurements 30 min before ethanol, 30 min after ethanol, and 30 min after the addition of the anion. The number of experiments are in parenthesis.

Anion	Initial	+ Ethanol	+ Ethanol and anion
Furosemide			
Cl^- Influx (6)	0.69 ± 0.03	1.21 ± 0.17*	1.38 ± 0.24
Cl^- Outflux (6)	0.66 ± 0.04	1.19 ± 0.10*	1.24 ± 0.18
Thiocyanate			
Cl^- Influx (6)	0.59 ± 0.06	1.16 ± 0.25*	0.40 ± 0.11*
Cl^- Outflux (6)	0.61 ± 0.08	1.12 ± 0.15*	0.46 ± 0.09*

* $p < 0.01$ for difference from the previous value.

We have observed that passive Cl^- movements in frog skin and toad urinary bladder increase in the presence of ethanol [20, 21]. It was therefore of interest to see if furosemide or thiocyanate could reduce this increased Cl^- permeability as it may provide some insight into their mechanism of action. As can be seen in Table IV thiocyanate reduces the increase in Cl^- movements produced by the ethanol, whereas furosemide has no effect. Thus ethanol may be altering the membrane characteristics in such a way so as to prevent furosemide from acting. This result also suggests that furosemide and thiocyanate are working via different mechanisms in reducing transepithelial Cl^- movements. This may reflect their relative abilities to interact with specific components of the membrane, perhaps the "carrier" of the Cl^-/Cl^- exchange mechanism.

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