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Furosemide Inhibits Chloride Influx into the Intestine of the Freshwater Turtle *Mauremys caspica*

Previous studies using the intestine of freshwater turtle *Mauremys caspica* have confirmed a transepithelial potential difference (PD) reversal in presence of HCO_3^- in the bathing media. Subsequent addition of 1 mM furosemide to HCO_3^- medium blocked, at least partially, the PD sign reversal induced by the presence of HCO_3^- (2). These results suggest the possibility of a Cl^-/HCO_3^- exchange process in the intestinal cells of *M. caspica*. Experiments were undertaken to test this hypothesis. The Cl^- influx into the intestine was studied under experimental conditions in which the effect of HCO_3^- on Cl^- influx into the intestine could be assessed.

Freshwater turtles *M. caspica* obtained from a local dealer were used. Animals were doubly pithed. The shell was opened and a segment of intestine was excised. The segment was opened along the mesenteric border and rinsed in Ringer's solution. The composition of the bathing medium was (in mM): NaCl, 91; HCO_3Na, 9; HCO_3K, 5.4; Ca-gluconate, 1.8; Tris-Cl was added to bring the Cl^- concentration to 100 mM. This solution was gassed with 95 % O_2 and 5 % CO_2. The pH was 7.2. In one set of experiments there was not HCO_3^- in the bathing medium. In this case the bathing medium composition was (in mM): NaCl, 100; KH_2PO_4, 0.8; K_2HPO_4, 2.3; Ca-gluconate, 1.8; mannitol, 21. This solution was gassed with O_2. The pH was 7.2. Furosemide or piretanide were added at a final concentration of 1 mM.

The unidirectional influx of chloride from the mucosal solution into the intestinal epithelium was estimated by measuring the uptake of ^{36}Cl (New England Nuclear) using the technique of SCHULTZ *et al.* (6).

Chloride uptake was studied as a function of time of exposure to the test solution (fig. 1). Chloride uptake was lin-

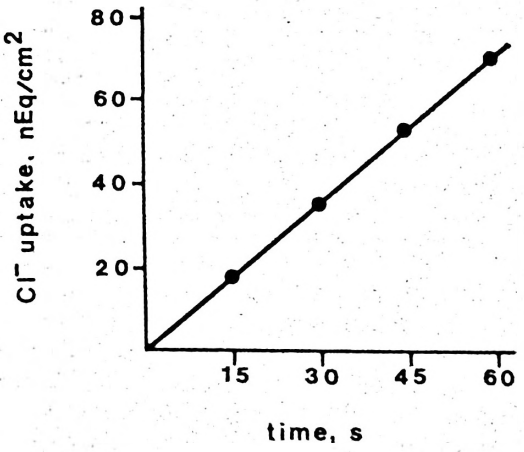

Fig. 1. *Uptake of chloride from mucosal solution as a function of time of exposure to test solution.*

ear, within experimental error, for at least 60 s. This suggests that over a 60 s period chloride uptake is essentially unidirectional, and that this method offers a reliable estimate of the unidirectional influx of chloride from the mucosal solution into the intestinal epithelium. The fact that the origin intercept of the uptake was not significantly different from zero lends support to the reliability of this technique and also suggests that the process of chloride uptake is suddenly stopped by the cold mannitol wash.

Table I shows the values obtained for chloride influx. The time of exposition to test solution for the data presented in table I was 30 s. Removal of HCO_3^- from bathing medium induced a significant decrease in the chloride influx. The addition of 1 mM furosemide inhibited the chloride influx to values close to those observed when HCO_3^- was absent from bathing media. Piretanide, a new loop diuretic, at 1 mM concentration had no effect on chloride influx into the intestine of *M. caspica.*

Table I. *Chloride influx into the intestine of* Mauremys caspica.
Mean values ± SEM are given. N is the number of tissues studied. Unpaired t-test was used (* $p < 0.05$).

	N	Cl^- influx $\mu Eq/cm^2\ h$
Control	28	5.0±0.3
HCO_3^--free	15	4.3±0.2 *
Control + 1 mM furosemide	20	4.4±0.1 *
Control + 1 mM piretanide	20	5.0±0.4

The results presented in this report are consistent with the existence of a fraction in the chloride influx that is HCO_3^- dependent and which, in addition, can be inhibited by furosemide. These results lend support to the possibility of the existence of a Cl^-/HCO_3^- exchange process located in the mucosal membrane of the intestinal cells of *M. caspica.*

The action of furosemide on chloride transport by different epithelia has been widely studied. In this way, furosemide has been reported to inhibit the Na-coupled electrogenic chloride secretion described in several epithelial tissues (3). In the flounder intestine, furosemide inhibits NaCl coupled entry by reducing unidirectional chloride influx across the mucosal membrane (4). In the present report, an inhibition of the Cl^- influx by furosemide is described. This finding agrees well with the reported action of furosemide on guinea pig gallbladder, where HEINTZE *et al.* (5) have described an inhibition of Cl^-/HCO_3^- exchange process by 1 mM furosemide. Piretanide was without effect on chloride influx. Preliminary experiments have shown that the reversal of PD sign induced by HCO_3^- is blocked by 1 mM furosemide, but not by 1 mM piretanide (1). These findings, together with the lack of action of piretanide on chloride influx suggest that the action of furosemide and piretanide are of different character.

It has been shown in a previous paper (2) that the presence of HCO_3^- in the bathing medium did not induce any change in the ionic intracellular concentrations of sodium, potassium and chloride. In the present report we have shown that the presence of HCO_3^- in the bathing medium increases chloride influx into the intestine. The lack of action of HCO_3^- on Cl^- intracellular concentration suggests that the exit of chloride from the intestinal cells might be stimulated by the presence of HCO_3^-.

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