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Effect of Bicarbonate and Furosemide on Chloride Accumulation by the Intestine of the Freshwater Turtle *Mauremys caspica*

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The influence of HCO_3^- on Cl^- absorption by the intestine of the freshwater turtle *Mauremys caspica* has been studied. Na^+, K^+ and Cl^- intracellular concentrations together with transepithelial potential difference were measured. In HCO_3^--free medium, transepithelial potential difference was serosal positive. A reversal in the sign of the transepithelial potential difference was observed in HCO_3^- medium. A subsequent addition of 10^{-3} M furosemide to HCO_3^- medium blocked, at least partially, this response. However, neither the presence of HCO_3^- nor addition of 10^{-3} M furosemide to HCO_3^+ medium had any effect on Na^+, K^+ or Cl^- intracellular concentrations. These results are discussed in relation to the presence of a Cl^-/HCO_3^- exchange process.

Human ileum (12), mammalian and amphibian small intestine (2, 4, 6, 9) and mammalian colon (5, 8) secrete HCO_3^- into the luminal or mucosal solution. There is experimental evidence that HCO_3^- secretion is related to intestinal absorption. A Cl^-/HCO_3^- exchange process has been proposed to account for this interrelation between Cl^- absorption and HCO_3^- secretion (2, 8, 10, 12, 13).

The purpose of the present investigation was to test the influence of HCO_3^- on Cl^- absorption by the intestine of the freshwater turtle *Mauremys caspica*. Na^+, K^+ and Cl^- intracellular concentrations together with transepithelial potential difference and transepithelial electrical resistance were measured under experimental conditions in which the influence of HCO_3^- on Cl^- absorption could be tested.

Materials and Methods

Methods related to animals, control solutions and determination of intracellular concentrations have been described in a previous work (1). When HCO_3^- was

present in the bathing medium, the composition of the solution (in mM) was: NaCl, 91; HCO_3K, 5.4; HCO_3Na, 9; 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. pH was 7.2.

In order to measure transepithelial potential difference, the stripped intestine was mounted as a plane sheet between two Ussing half-chambers at room temperature (11). Transepithelial potential difference (p.d.) was measured by means of two calomel electrodes connected to the bathing solution through saturated KCl agar bridges. Two other calomel electrodes were used to pass current across the tissue. A device based on the characteristics of the 555 integrated circuit (Fairchild) was used to pass a repetitive current pulse (12.5 $\mu A/cm^2$, 2 s) at 8 s intervals. In this way transepithelial resistance (R_t) was continously monitored.

Furosemide was a generous gift from Hoechst.

Results

Table I shows the obtained values for p.d. and R_t both in the HCO_3^--free medium and in the HCO_3^- medium. In the HCO_3^--free medium p.d. was low, but it was always serosal positive. Furosemide (10^{-3} M) had no significant action either on p.d. or on R_t in the absence of HCO_3^- from the bathing medium. When HCO_3^- was added to the bathing medium p.d. remained low, but its sign was reversed, i.e.: p.d. was serosal negative. Addition of furosemide (10^{-3} M) to the serosal or mucosal bathing medium elicited a significant increase in p.d. without inducing any change on R_t. These results suggest that furosemide blocks, at least partially,

Table I. *Effect of 10^{-3} M furosemide on transepithelial potential difference (p.d.) and transepithelial electrical resistance (R_t).*
Mean values ± S.E.M. are given. N is the number of experiments. Paired t-test was used.

	N	p.d. (mV)	R_t (Ω cm²)
HCO_3^--free medium			
Control	5	0.1±0.1	114±10
+furosemide (both sides)	5	0.1±0.1	118±11
HCO_3^- medium			
Control	6	—0.2±0.2	103±13
+furosemide (serosal side)	6	0.2±0.3*	104±13
Control	6	—0.7±0.2	71±16
+furosemide (mucosal side)	6	—0.4±0.2*	75±18

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table II. *Effect of 10^{-3} M furosemide on cell water, C_{Na}, C_K and C_{Cl} in HCO_3^--free and HCO_3^- media.*
Mean values ± S.E.M. are given. N is the number of experiments. Paired t-test was used.

	N	Cell water (per cent wet weight)	C_{Na} (mM)	C_K (mM)	C_{Cl} (mM)
Group 1: HCO_3^--free					
Control	9	73 ± 2	63 ± 5	88 ± 5	57 ± 8
+ furosemide	9	73 ± 2	60 ± 5	82 ± 5	62 ± 7
Group 2:					
Control (HCO_3^--free)	14	71 ± 1	46 ± 1	96 ± 1	64 ± 4
+HCO_3^-	14	71 ± 1	49 ± 2	97 ± 2	64 ± 3
Group 3: HCO_3^- medium					
Control	8	71 ± 1	51 ± 3	94 ± 2	63 ± 3
+ furosemide	8	71 ± 1	48 ± 2	93 ± 3	63 ± 4

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

the transport mechanism that makes p.d. to be serosal negative in HCO_3^- medium (table I).

The action of 10^{-3} M furosemide on C_{Na}, C_K and C_{Cl} is shown in table II (group 1). No significant action of furosemide on any of these concentrations was observed. Table II (group 2) shows Na^+, K^+ and Cl^- intracellular concentrations when HCO_3^- was present in the bathing medium. When these values were compared to control values obtained in HCO_3^--free medium, no significant changes were obtained. No significant action of 10^{-3} M furosemide on C_{Na}, C_K or C_{Cl} was obtained when this agent was added to HCO_3^- medium (table II, group 3).

Discussion

GUNTHER-SMITH and WHITE (9) in *Amphiuma* small intestine and ARMSTRONG and YOUMANS (2) in bullfrog small intestine have reported that the presence of HCO_3^- in the bathing medium reverses the sign of the normally serosal positive p.d. and short circuit current. The observations are in good agreement with the serosal negative p.d. presented in table I when HCO_3^- was added to the bathing medium.

Since the early studies of TURNBERG *et al.* (12), Cl^- absorption and HCO_3^- secretion have been considered to be linked by means of a Cl^-/HCO_3^- exchange process. Later studies have suggested that the Cl^-/HCO_3^- exchange process is located at the basolateral membrane of the epithelial cell (2, 13). The precise origin of the serosal negative p.d. found in the presence of HCO_3^- although related to coupled HCO_3^- secretion and Cl^- absorption is by no means entirely clear. Different theories have been proposed to explain it: Cl^-/HCO_3^- exchange is an electrogenic process (2); Cl^-/HCO_3^- exchange is an electroneutral process but there is a rheogenic entry of Cl^- at the apical membrane (13) and the serosal negative p.d. arises from a salt diffusion potential across tight junctions (7). This proposal, however, does not provide a role for HCO_3^- which is essential for the appearance of a serosal negative p.d.

HEINTZE *et al.* (10) have reported that 10^{-3} M furosemide inhibits Cl^-/HCO_3^- process in guinea pig gallbladder without altering Na^+ net flux and net fluid absorption. This result is consistent with our findings. Furosemide (10^{-3} M) was without effect on p.d. in absence of HCO_3^- in the bathing medium. But addition of 10^{-3} M furosemide to HCO_3^- medium reversed the serosal negative sign of p.d. observed in HCO_3^- medium. These results are consistent with an inhibition of Cl^-/HCO_3^- process by 10^{-3} M furosemide in the intestine of *Mauremys caspica*.

In HCO_3^--free medium furosemide was without effect on sodium, potassium and chloride intracellular concentrations. These results together with the lack of action of furosemide on transepithelial potential difference suggest that furosemide does not exert any inhibiting action on the intestine of *Mauremys caspica* when HCO_3^- is absent from the bathing medium.

The presence of HCO_3^- in the bathing medium did not induce any change in the ionic intracellular concentrations of sodium, potassium and chloride, although it induced the reversal of the normal sign of the transepithelial potential difference. However, an increase in chloride intracellular concentration as a consequence of the presence of a Cl^-/HCO_3^- exchange would be expected. Nevertheless, intracellular concentrations depend both on entry and exit processes. Results presented in this paper are consistent with an increase in chloride entry into the cell and another increase in chloride exit from the cell, in such a way that Cl^- intracellular concentration remains unchanged. In regard to this question, the present results are in good agreement with a re-

cent report (3) in wich chloride intracellular activity in *Necturus* gallbladder was measured by means of ion-selective microelectrodes. In this preparation, the presence of HCO_3^- in the bathing medium did not induce any change in chloride intracellular activity.

In bathing medium containing HCO_3^- furosemide did not alter sodium, potassium and chloride intracellular concentrations, despite of the fact that furosemide blocked, at least partially, the effect of HCO_3^- on transepithelial potential difference. This lack of action must be related to the absence of changes induced by the presence of HCO_3^- in sodium, potassium and chloride intracellular concentrations. The presence of HCO_3^- in the bathing medium did not elicit changes in the ionic intracellular concentrations and therefore, furosemide could not inhibit them.

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Resumen

Se estudia la influencia del ion bicarbonato sobre la absorción de cloro en el epitelio intestinal de la tortuga *Mauremys caspica*. Se miden las concentraciones intracelulares de sodio, potasio y cloro, junto con la diferencia de potencial y resistencia eléctrica transepitelial.

Cuando la solución de incubación no contiene bicarbonato, la diferencia de potencial es serosal positiva. En solución con bicarbonato, se observa una inversión en la diferencia de potencial transepitelial. La adición de furosemida (10^{-3} M) bloquea, al menos en parte, esta respuesta. Sin embargo, ni la presencia de bicarbonato ni la adición de furosemida tienen ningún efecto sobre la concentración intracelular de sodio, potasio y cloro. Se discuten estos resultados en relación con la presencia de un proceso de intercambio Cl/HCO_3^-.

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