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CHLORIDE-BICARBONATE EXCHANGE IN THE URINARY BLADDER OF THE TURTLE

INDEPENDENCE FROM SODIUM ION

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Summary

The rates of Cl^- absorption and HCO_3^- secretion were not different in turtle urinary bladders bathed in Na^+-containing and Na^+-free solutions.

These results in turtle bladder are inconsistent with Na^+-anion cotransport but can be accounted for by a Cl^-/HCO_3^- exchange system.

Transport of Cl^- occurs by three mechanisms in epithelial tissues: passively down electrochemical gradients, by Na^+-Cl^- cotransport and by Cl^-/HCO_3^- exchange [1]. The urinary bladder of the turtle (*Pseudemys scripta*) transport Cl^- from the mucosal solution to the serosal solution [2, 3] and HCO_3^- from serosal→mucosal [3, 4]. Earlier studies have shown that the absorption of Cl^- is independent of Na^+ [2]. In the presence of Na^+, Cl^- absorption and HCO_3^- secretion occur by a coupled anion exchange system [3]. The present study was designed to determine whether the stoichiometry of the exchange process is altered in the absence of Na^+.

The experimental methods are described in detail in previous publications [3, 5]. In brief, hemibladders were mounted on lucite chambers and bathed on both surfaces by a HCO_3^--free Ringer's solution containing 116 mM Na^+ or a HCO_3^--free Ringer's solution with the Na^+ replaced by Cs^+ [5]. HCO_3^- secretion was measured by pH-stat titration and mucosal→serosal movement of Cl^- by flux of radioactive Cl^- (^{36}Cl).

The mucosal pH was lowered until H^+ secretion was abolished [6] and the rate of unidirectional mucosal→serosal Cl^- flux was determined. In the absence of exogenous HCO_3^- in the bathing solutions the mucosal→serosal Cl^- flux is equivalent to the serosal→mucosal Cl^- flux [7]. The addition of

HCO_3^- to the serosal solution causes an increase in the mucosal→serosal Cl^- flux but has no effect on the serosal→mucosal flux [7]. Therefore, the mucosal→serosal Cl^- flux in the absence of HCO_3^- has been used as an estimate of passive flux and the increment in the mucosal→serosal flux of Cl^- after HCO_3^- addition is an estimate of the exchange flux.

After measurement of mucosal→serosal Cl^- flux in the absence of HCO_3^-, the serosal solution was replaced with a Ringer's solution containing 20 mM HCO_3^-. HCO_3^- secretion (serosal→mucosal) and mucosal→serosal Cl^- flux were determined following a 1 h equilibration period. At the completion of the flux measurements, 10^{-4} M amiloride was added to the mucosal solution. Under such conditions (i.e. complete inhibition of electrogenic Na^+ and H^+ transport), the remaining current is unaffected by cyanide or deoxyglycose (Cohen, L.H. personal communication). This current is probably due to passive diffusion of ions down their chemical gradients. Chemical gradients exist only for HCO_3^- and SO_4^{2-} in the present experiments. The SO_4^{2-} gradient (10 mM) would account for less than 10% of the diffusion current based on data for SO_4^{2-} fluxes in turtle bladder [8]. Therefore, the post-amiloride current has been used as an estimate of the passive HCO_3^- flow.

The mucosal pH at which net acid secretion was abolished was 4.58 ± 0.10 in Na^+-containing Ringer's and 4.55 ± 0.12 in Na^+-free Ringer's. Table I shows that the total HCO_3^- movement from serosal→mucosal was similar in Na^+-containing and Na^+-free Ringer's. The total Cl^- flux from mucosal→serosal also was unaffected by the absence of Na^+. The total HCO_3^- movement was equal to the total Cl^- flux during both Na^+ and Na^+-free conditions.

The total anion fluxes can be dissected into a passive component and an exchange component. Table II shows that the passive portion of the Cl^-

TABLE I

EFFECT OF Na^+ ON UNIDIRECTIONAL ANION MOVEMENTS

Mean values ± S.E. of anion movements. 20 mM HCO_3^- and 1% CO_2 were present in the serosal solution.
The area of exposed tissue was 8 cm^2

	Bathing media	
	Na (n = 5)	Na-free (n = 7)
mucosal→serosal Cl^- (μmol/h)	0.53 ± 0.12	0.56 ± 0.08
serosal→mucosal HCO_3^- (μmol/h)	0.58 ± 0.08	0.55 ± 0.05

TABLE II

EFFECT OF Na^+ ON PASSIVE ANION MOVEMENTS

Mean values ± S.E. of passive anion movements. Passive Cl^- movement was estimated as the mucosal→serosal Cl^- flux in the absence of serosal HCO_3^-. Passive HCO_3^- movement was estimated as the current remaining after blockade of Na^+ transport (10^{-4} M amiloride to the mucosal solution) and acidification (by lowering mucosal pH). Same tissues as in Table I.

	Bathing media	
	Na (n =5)	Na-free (n = 7)
mucosal→serosal Cl^- (μmol/h)	0.30 ± 0.06	0.28 ± 0.04
serosal→mucosal HCO_3^- (μmol/h)	0.33 ± 0.09	0.23 ± 0.04

flux and the diffusional HCO_3^- flow were similar in the presence and absence of Na^+. The diffusional flow of HCO_3^- was estimated after inhibition of Na^+ transport by amiloride. Amiloride addition to the Na^+-containing Ringer's caused a large, rapid decrease in the short-circuit current while there was no change in short-circuit current in Na^+-free Ringer's after amiloride addition.

The exchange flows of HCO_3^- and Cl^- were calculated as the difference between the measured total and passive movements of the individual ions. The calculated rate of Cl^- exchange was 0.23 ± 0.06 μmol/h in Na^+-containing media and 0.28 ± 0.08 μmol/h in Na^+-free media. Calculated HCO_3^- exchange rates were 0.25 ± 0.12 and 0.31 ± 0.05 μmol/h in Na^+-containing and Na^+-free Ringer's, respectively. The rate of HCO_3^- secretion was not statistically different from the rate of Cl^- absorption regardless of the Na^+ concentration and the Cl^-/HCO_3^- exchange rate was the same in the absence of Na^+ as in its presence.

A previous investigation with the turtle urinary bladder has shown that in the presence of Na^+ in the bathing solutions, Cl^- transport occurred by a Cl^-/HCO_3^- exchange process and was not affected by ouabain [3]. HCO_3^- secretion by the turtle bladder was also unaffected by amiloride [9]. The cortical collecting tubule of the rabbit is also capable of HCO_3^- secretion [10, 11]. Net HCO_3^- secretion by the collecting tubule is not altered by addition of ouabain and is increased after amiloride [11]. These results suggest that secretion of HCO_3^- by these tissues is independent of transepithelial Na^+ transport. However, removal of Na^+ from the solutions bathing the isolated collecting tubule abolishes net HCO_3^- secretion [11].

As similar experiments had not been previously attempted in turtle bladder, the present study was undertaken. In this study, as in a previous investigation from another laboratory [2], Cl^- absorption was not a function of Na^+ concentration. The equality of Cl^- absorption and HCO_3^- secretion in the presence of Na^+ also confirms previous results [3]. However, unlike the results in collecting tubule [11], we have demonstrated that the rate of HCO_3^- secretion is independent of Na^+. In addition, the rates of Cl^- absorption and HCO_3^- secretion by the turtle bladder are equal in Na^+-free media.

Two of the three modes of transepithelial Cl^- transport that have been shown to occur in epithelial tissues require Na^+ [1]. Cl^- transport across tissues such as frog skin [12] and toad bladder [13] occurs passively down the electrical gradient created by transepithelial Na^+ transport. In a wide variety of epithelia [1], Cl^- movement against an electrochemical gradient is coupled to Na^+ movement down its electrochemical gradient. The input of metabolic energy maintains the electrochemical gradient for Na^+, and Cl- transport occurs as a 'secondary active' process [1]. Clearly, Cl^- transport across the turtle urinary bladder is not due to either of these processes. The results presented in this paper are most consistent with Cl^- transport across the urinary bladder of the turtle occurring via a Cl^-/HCO_3^- exchange system. The driving force for this system remains to be defined.

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