BBA 75362

ACETAZOLAMIDE-SENSITIVE SHORT-CIRCUITING CURRENT *VERSUS* MUCOSAL HCO_3^- CONCENTRATION IN TURTLE BLADDERS

C. F. GONZALEZ AND T. P. SCHILB

Institute for Medical Research and Studies and Departments of Biophysics and Physiology, Mount Sinai Medical and Graduate Schools, City University of New York, New York, N.Y. (U.S.A.)

(Received July 15th, 1969)

SUMMARY

Isolated bladders of Pseudemys turtles, interposed between choline sulfate Ringer solution on the mucosa and choline sulfate Ringer solution with HCO_3^- (20 mM) on the serosa were short-circuited in a chamber. Mucosal concentration of HCO_3^- was varied from 0.2 to 20 mM in the presence and in the absence of acetazolamide.

Both in the presence and in the absence of acetazolamide, spontaneous transbladder potential and short-circuiting current (I_{sc}) increased with increasing mucosal HCO_3^- concentration. When I_{sc} was plotted as a function of mucosal HCO_3^- concentration, it exhibited Michaelis–Menten-like characteristics.

In the absence of acetazolamide, the saturation current and half maximal concentration of mucosal HCO_3^- were 1.98 mA/g dry tissue and 2.4 mM HCO_3^-, respectively. In the presence of 0.1 mM acetazolamide, they were 0.37 mA/g dry tissue and 3.15 mM HCO_3^-, and in the presence of 0.2 mM acetazolamide they were 0.24 mA/g dry tissue and 3.89 mM HCO_3^-.

The changes in the half maximal concentration of mucosal HCO_3^- were not significant. Hence, acetazolamide appears to inhibit HCO_3^- transport in a manner analogous to noncompetitive inhibibition. Data further indicate that the inhibition of HCO_3^- transport by acetazolamide is reversible.

Analysis of data from experiments carried out in the absence of acetazolamide suggest the existence of a second HCO_3^- transport process having a saturation current of 1.13 mA/g dry tissue and a half maximal concentration of 0.17 mM HCO_3^- in the mucosal fluid.

INTRODUCTION

It has been established by this laboratory that the urinary bladder of the turtle, interposed between HCO_3^--containing Ringer solutions, transport HCO_3^- *per se* from mucosal to serosal fluid. This transport process results in acidification of the mucosal fluid and alkalinization of serosal fluid[1,2].

This laboratory and others[1,3] have reported the acidification of HCO_3^--free mucosal fluids. When bladder sacs were filled with HCO_3^--free mucosal fluid, the

mucosal fluid was acidified by accumulation of CO_2 and an unidentified non-volatile acid[1]. STEINMETZ and co-workers[3,4] have reported an acetazolamide-inhibitable acidification of mucosal fluid in HCO_3^--free bathing fluids and have attributed it to H^+ secretion.

The object of the present work is to examine the kinetics of the HCO_3^- transport process. The relationship between short-circuiting current (I_{sc}) and mucosal HCO_3^- concentration and the effect of acetazolamide on this relationship are described.

METHODS

All experiments were performed, during the fall and early winter, on excised bladders of the fresh water turtle, *Pseudemys scripta*. Bladders were excised, washed free of Na^+ and mounted in a modified Ussing chamber. The mucosal and serosal surfaces of the bladder were bathed by 10–12 ml of various Ringer solutions. The temperature of the bathing solutions was maintained between 24 and 25°.

Elution, mounting and electrical measurements

Excised bladders were stretched into flat sheets[5] and eluted for 10 min in each of four successive 200-ml portions of oxygenated NaCl-free Ringer solution. Tissues were mounted in a modified Ussing chamber having an area of 5 cm^2. Electrical measurements made, by techniques described previously[5], included I_{sc}, spontaneous transbladder potential (PD) and transbladder resistance (R). Values of I_{sc} were recorded continuously, except when the external circuit was opened, for 1 sec at 5-min intervals, to obtain values for PD. During the 1-sec period of open circuiting, R was determined by sending calibrated pulses of current through the bladder[5].

Experimental design

In all the experiments the serosal surface of the bladder was bathed by choline sulfate Ringer solution containing 20 mM HCO_3^- and gassed with CO_2–O_2 (1–2:99–98, v/v). The pH of the serosal fluid remained between 7.5 and 7.8 throughout the experiment. No changes were made in the serosal fluid during the course of the experiment. The mucosal surface was initially bathed by HCO_3^--free choline sulfate Ringer solution gassed with 100% O_2 (pH 7.2–7.4). After 60–100 min of incubation under the above-stated conditions, increments of HCO_3^-, as choline bicarbonate, were added isohydrically to the mucosal fluid, and the CO_2 content of the gas perfusing the mucosal fluid was adjusted accordingly. The pH of the mucosal fluid was monitored continuously and oscillated between pH 7.2 and 7.8. These variations in mucosal pH had no apparent effect on the electrical parameters. Each bladder was exposed successively to nominal mucosal concentrations of 0.2, 1.0, 2.5, 5.0, 15.0 and 20 mM HCO_3^-. After each addition of HCO_3^- to the mucosal fluid, the PD, R and I_{sc} were observed for periods of 15–30 min. This interval was sufficient for the bladder to attain quasi steady-state values for I_{sc} and PD. This experimental design was carried out in the presence and in the absence of acetazolamide. Acetazolamide was used at two final concentrations in the serosal fluid 0.1 and 0.2 mM.

When acetazolamide was used, the tissue was incubated with the acetazolamide-containing Ringer solution for at least 120 min before the serial increases in mucosal concentration of HCO_3^- were made.

Sampling and analysis

At the end of each experimental period, a 6-ml sample of mucosal fluid was removed for analysis. The samples were replaced by adding 4–5 ml of choline sulfate Ringer solution and 1–2 ml of an appropriate choline bicarbonate solution.

The composition of solution used herein in terms of final mM concentrations was as follows.

Ringer solutions: (a) Bicarbonate-free choline sulfate: choline$^+$, 118.1; SO_4^{2-}, 61.8; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; Mg^{2+}, 0.8; and glucose 11. Sucrose was added to achieve a final osmolality of 220 mosmoles. The final pH was kept between 7.4 and 7.6 pH units. (b) HCO_3^--rich choline sulfate Ringer solution: choline$^+$, 118.1; SO_4^{2-}, 53.3; Ca^{2+}, 2.0; $H_2PO_4^-$, 0.07; HPO_4^{2-}, 0.73; HCO_3^-, 17; Mg^{2+}, 0.8; CO_2, 0.33; and glucose 11. Sucrose was added to achieve a final osmolality of 220 mosmoles. The final pH was kept between 7.4 and 7.6 pH units.

Choline bicarbonate solutions: (a) choline$^+$, 9.2; HCO_3^-, 9.2; sucrose was added to achieve a final osmolality of 220 mosmoles. (b) Choline$^+$, 22; HCO_3^-, 22; sucrose was added to achieve a final osmolality of 220 mosmoles. (c) Choline$^+$, 230; HCO_3^-, 230. Prior to use, choline bicarbonate solutions were gassed with 100 % CO_2 in order to bring the pH to 7.4–7.6.

Solutions were analyzed for Na^+, Cl^-, HCO_3^-, osmolality and pH prior to use. Samples taken from solutions in contact with the tissue as described above were subjected to the same analysis.

Na^+ determinations were done in an Eppendorf flame photometer, Cl^- on a Cotlove chloridometer and CO_2 in the Van Slyke apparatus.

Normalization of data

The area of tissue exposed to the bathing fluid in the chamber was 5 cm^2. However, due to the elasticity of the bladder tissue, it was necessary to report I_{sc} per g dry mass of tissue rather than I_{sc}/cm^2. It was found that this procedure reduced the standard deviation of the saturation currents by a factor of 2.

The average dry weight ($\pm$S.E.) of the tissue exposed in the chamber was 66.2 $\pm$ 8.2 mg.

RESULTS

Relationship of I_{sc} to mucosal HCO_3^- concentration

The urinary bladder of the turtle transports HCO_3^- from mucosal to serosal fluid and acidifies its mucosal fluid. The acidification process occurs in the presence[1,2] and in the absence of exogenous mucosal HCO_3^- (ref. 3). In both cases, negative PD and I_{sc} are associated with the acidification process.

The electrical parameters of the bladder were examined under two sets of conditions.

In one set of eight experiments, the bladders were bathed on the mucosal and serosal surface by a choline sulfate Ringer solution containing HCO_3^- and gassed with O_2–CO_2 (99:1, v/v). In a second set of eight experiments, bladders were bathed with the same Ringer solution except that HCO_3^- was omitted from the Ringer solution on the mucosal surface. In both sets of experiments, the pH of the Ringer solution remained about 7.4. The PD, I_{sc} and R were measured over a period of 2 h. The dry

mass of the tissue and the HCO_3^- concentration of the bathing fluids were measured at the end of the experiments. The results are summarized in Table I.

Table I shows statistical parameters and mean values for mucosal HCO_3^- concentrations, PD, I_{sc}, normalized I_{sc}—*i.e.* I_{sc} per g dry mass of tissue—and R for the two sets of experiments described above.

TABLE I

EFFECT OF MUCOSAL HCO_3^- ON ELECTRICAL PARAMETERS OF BLADDERS

Mean values for mucosal HCO_3^- concentration, PD, I_{sc}, normalized I_{sc} (*i.e.*, I_{sc} per g dry wt. of tissue), and R for two groups of eight bladders. The mucosal fluid of the first groups contained no exogenous HCO_3^-. The mucosal fluid of the second groups contained 20 mM HCO_3^-. The serosal fluid of both groups of bladders was a choline sulfate Ringer solution containing 20 mM HCO_3^-.

Statistical parameters	*Mucosal* $[HCO_3^-]$ *(mM)*	*PD (mV)*	I_{sc} *(μA)*	I_{sc}*/g dry wt. (mA/dry wt.)*	R *(Ω)*
Mean	17.2	−34.1	−82.6	−1.14	413
S.E.	0.5	4.7	5.3	0.07	33
Mean	0.24	−14.6	−37.5	−0.56	389
S.E.	0.06	1.9	5.3	0.08	35
Difference of the mean		19.5	45.1	0.58	24.0
Probability		$P < 0.01$	$P < 0.001$	$P < 0.001$	$P > 0.6$

The data indicate that the mean values for PD, I_{sc} and I_{sc} per g dry mass of the bladders bathed on the mucosal surface by 20 mM HCO_3^- were significantly greater than those of the bladders bathed by 0.2 mM HCO_3^-. However, the mean resistances of the two groups of bladders were not significantly different.

The results indicate that the magnitudes of PD and I_{sc} are strong functions of the mucosal HCO_3^- concentration in the absence of transportable ions other than HCO_3^-.

In order to investigate further the relationship of I_{sc} to mucosal HCO_3^- concentration, sequential additions of HCO_3^- were made to initially HCO_3^--free mucosal fluids in the Ussing chamber. Additions of HCO_3^- were made to the mucosal fluid at 20-min intervals, and the concentration of CO_2 in the perfusing gas was changed accordingly to maintain the pH of the mucosal fluid at 7.4. In order to eliminate favorable diffusion gradients for HCO_3^-, the serosal concentration was maintained at 20 mmoles/l during the course of the experiment. Values for PD and I_{sc} increased in a roughly stepwise manner subsequent to each HCO_3^- addition. At the end of each 20-min period, the mucosal fluid was sampled for analysis in the Van Slyke apparatus.

Fig. 1 shows PD and I_{sc} *versus* time at various mucosal HCO_3^- concentrations for one of the experiments of Table II. Values of PD and I_{sc} are plotted below their respective abscissae to emphasize that they are negative quantities.

The data of Fig. 1 indicate a finite negative PD and I_{sc} in the absence of exogenously added HCO_3^-, at the beginning of the experiment. These data also indicate that the PD and I_{sc} increased in magnitude subsequent to each addition of HCO_3^-

to the mucosal fluid and that the responses of the PD were somewhat sharper than those of the I_{sc}. The time-course of the experiment depicted in the figure is typical of the experiments to be presented in Table II.

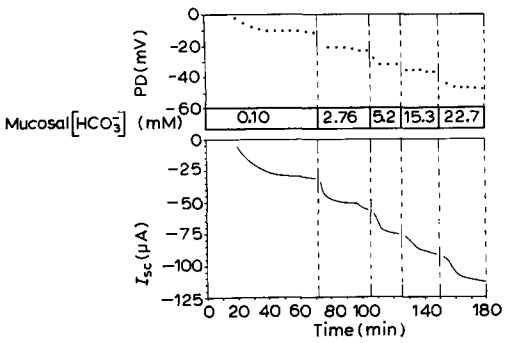

Fig. 1. Plot of PD and I_{sc} *vs.* time for a bladder bathed on the serosal surface by choline sulfate-Ringer solution containing 20 mM HCO_3^- and on the mucosal surface by initially bicarbonate-free choline sulfate Ringer solution. The concentration of mucosal HCO_3^- was raised to the values shown in the figure at the times indicated by the vertical dashed lines. HCO_3^- was added as choline bicarbonate.

TABLE II

EFFECT OF INCREMENTS IN MUCOSAL HCO_3^- ON ELECTRICAL PARAMETERS

Mean values and S.E. for electrical parameters at various mucosal HCO_3 concentrations for seven bladders. HCO_3 was added to mucosal fluid as choline bicarbonate and was the only transportable ion in the mucosal fluid. Serosal surfaces were bathed by choline sulfate Ringer solution containing 20 mM HCO_3^-.

n	*Mucosal* $[HCO_3^-]$ *(mM)*	*PD (mV)*	I_{sc} *(μA)*	I_{sc}*/g dry wt. (mA/g dry wt.)*	*R* (Ω)
6	0.20 ± 0.02	−15.6 ± 2.0	− 44.7 ± 3.3	−0.61 ± 0.09	463 ± 37
5	0.93 ± 0.06	−25.2 ± 0.9	− 61.0 ± 9.6	−0.94 ± 0.09	462 ± 83
7	2.35 ± 0.15	−28.4 ± 2.1	− 73.0 ± 8.6	−1.07 ± 0.11	454 ± 31
6	5.76 ± 0.69	−37.2 ± 3.5	− 88.3 ± 10.7	−1.40 ± 0.09	454 ± 25
7	14.3 ± 0.9	−48.6 ± 4.8	−123 ± 18	−1.75 ± 0.09	408 ± 26
5	26.2 ± 1.9	−49.0 ± 5.4	−134 ± 5.3	−1.85 ± 0.19	380 ± 38

Table II presents values for mucosal HCO_3^- concentration, PD, I_{sc}, I_{sc} per g dry mass and *R*, for a set of seven experiments similar to those represented by Fig. 1.

The data of Table II indicate that the PD, I_{sc} and I_{sc} per g dry mass increased in value with each increment of mucosal HCO_3^- over the range of HCO_3^- concentrations studied. These data also indicate that the resistance of the bladder tended to decrease somewhat as mucosal HCO_3^- was added.

Effect of acetazolamide on HCO_3^- transport

STEINMETZ and co-workers[3,4] have previously reported an inhibitory effect of acetazolamide on the acidification process of the urinary bladder of the turtle. It therefore seemed appropriate to investigate the effects of this drug on the kinetics of

HCO_3^- transport. To this end, a series of experiments, having a format similar to that described for Fig. 1 and the experiments of Table II, was performed in the presence of two different serosal concentrations of acetazolamide.

Table III, similar in format to Table II, presents values for PD, I_{sc}, I_{sc} per g dry mass and R for a group of five bladders treated with 0.1 mM acetazolamide.

TABLE III

EFFECT OF INCREMENTS IN MUCOSAL HCO_3^- ON ELECTRICAL PARAMETERS IN THE PRESENCE OF 0.1 mM ACETAZOLAMIDE IN THE SEROSAL FLUID

Mean values and S.E. for electrical parameters at various mucosal HCO_3^- concentrations for five bladders. HCO_3^- was added to mucosal fluid as choline bicarbonate and was the only transportable ion in the mucosal fluid. Serosal surfaces were bathed by choline sulfate Ringer containing 20 mM HCO_3^- and 0.1 mM acetazolamide.

n	*Mucosal* $[HCO_3^-]$ *(mM)*	*PD (mV)*	I_{sc} *(μA)*	I_{sc}*/g dry wt. (mA/g dry wt.)*	R *(Ω)*
5	5.79 ± 0.38	−17.0 ± 1.1	−24.7 ± 5.1	−0.26 ± 0.07	762 ± 114
5	14.6 ± 0.8	−20.6 ± 2.9	−32.6 ± 7.0	−0.40 ± 0.09	750 ± 119
4	24.3 ± 1.5	−24.5 ± 3.8	−14.9 ± 7.7	−0.43 ± 0.13	780 ± 125

These tissues were preincubated for 60 min in acetazolamide-containing Ringer solution and bathed on the serosal side by acetazolamide-containing Ringer solution during the experiment. Increments of HCO_3^- were added to the mucosal fluid at 20-min intervals as in the experiments of Table II.

The data of Table III indicate that the PD and I_{sc} increased subsequent to each increment of mucosal HCO_3^-. However, the increments in PD and I_{sc} were smaller than those observed in the absence of acetazolamide.

Table IV presents data for a group of four bladders treated in all respects the same as those reported in Table III, except that the concentration of acetazolamide was increased to 0.2 mM.

The results in Table IV indicate that the increased concentration of acetazolamide caused further inhibition of HCO_3^- transport.

TABLE IV

EFFECT OF INCREMENTS IN MUCOSAL HCO_3^- ON ELECTRICAL PARAMETERS IN THE PRESENCE OF 0.2 mM ACETAZOLAMIDE IN THE SEROSAL FLUID

Mean values and S.E. for electrical parameters at various mucosal HCO_3^- concentrations for four bladders. HCO_3^- was added to mucosal fluid as choline bicarbonate and was the only transportable ion in the mucosal fluid. Serosal surfaces were bathed by choline sulfate Ringer solution containing 20 mM HCO_3^- and 0.2 mM acetazolamide.

n	*Mucosal* $[HCO_3^-]$ *(mM)*	*PD (mV)*	I_{sc} *(μA)*	I_{sc}*/g dry wt. (mA/g dry wt.)*	R *(Ω)*
4	4.61 ± 0.37	−6.25 ± 2.10	−16.6 ± 5.9	−0.14 ± 0.04	402 ± 50
4	13.9 ± 0.4	−7.63 ± 2.51	−20.7 ± 6.1	−0.18 ± 0.04	388 ± 45
4	25.6 ± 2.8	−9.50 ± 3.48	−27.8 ± 9.7	−0.23 ± 0.08	435 ± 61

When the values of I_{sc} per g dry mass of tissue reported in Tables II, III and IV were plotted against the respective mucosal HCO_3^- concentrations, the results were hyperbolic curves similar to those of Michaelis–Menten-type kinetics.

The results in Tables II, III and IV are further summarized as Lineweaver–Burk plots in Fig. 2. The points corresponding to the 0.2- and 1-mM mucosal HCO_3^- concentrations were deleted from this figure and from the calculations of the regression lines for the Lineweaver–Burk plots and will be discussed later.

Fig. 2 presents plots of the reciprocal of the I_{sc} per g dry mass *versus* the reciprocal of the mucosal HCO_3^- concentration for the experiments presented in Tables II, III and IV.

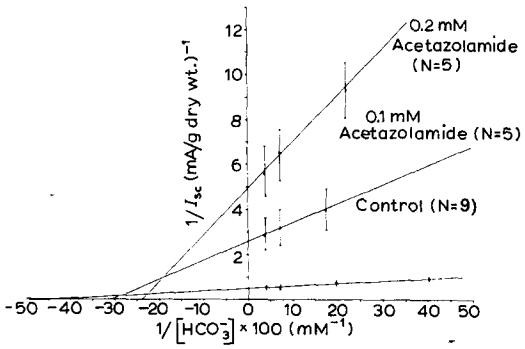

Fig. 2. Lineweaver–Burk plots of I_{sc} per g dry mass of tissue *vs.* mucosal HCO_3^- concentration for the experiments presented in each of the Tables II, III and IV.

TABLE V

EFFECT OF ACETAZOLAMIDE ON KINETIC PARAMETERS OF HCO_3^- TRANSPORT

Acetazolamide (mM)	*Saturation current (μA/g)*	*Half maximal mucosal [HCO_3^-] (mM)*
None	1.98 ± 0.11	2.40 ± 0.55
0.1	0.39 ± 0.13	3.15 ± 1.42
0.2	0.24 ± 0.05	3.89 ± 0.75

These data indicate that acetazolamide significantly changed the slope of the lines but did not significantly change the intercept at the abcissa. The saturation currents and half maximal mucosal HCO_3^- concentrations corresponding to the lines plotted in Fig. 2 are presented in Table V.

The data in Table V show a roughly 10-fold change in the value of the saturation current with increasing concentrations of acetazolamide. Although the values for the half maximal mucosal HCO_3^- concentration increased with increasing concentrations of acetazolamide, differences between the values of apparent. K_m in the three groups of experiments were not statistically significant. These data are consistent with the notion that acetazolamide inhibits HCO_3^- transport in a manner analogous to noncompetitive inhibition.

In the experiments in which 0.1 or 0.2 mM acetazolamide were used, 1–2 h

were required for the drug to have its full effect and inhibition of the I_{sc} did not reach 100 %.

In order to determine whether higher concentrations of acetazolamide would act to inhibit I_{sc} more rapidly and more completely, four additional experiments were performed. In these experiments bladders were bathed on both sides by choline sulfate-Ringer solution containing 20 mM HCO_3^-, and acetazolamide was added to the serosal fluid to a final concentration of 1 mM. In these four experiments, the mean percentage of inhibition of I_{sc} was 64 $\pm$ 5.7; the half time for the inhibition was 22 min, and the time required for the complete effect was 59 min. The inhibition of the PD followed a similar course, and the resistance did not change significantly. Thus, there was no substantial difference in the time-course and magnitude of the inhibition produced by 0.2 or 1 mM acetazolamide. In these four experiments, there was considerable variation in the time-course of the inhibition. The shortest time required for the full effect was 20 min, and the longest was 90 min.

Fig. 3 presents values for PD and I_{sc} *versus* time for a bladder bathed on both surfaces by choline sulfate Ringer solution containing 20 mM HCO_3^-. Acetazolamide (final concn. 1 mM) was added to the serosal fluid at 40 min and washed out at 150 min.

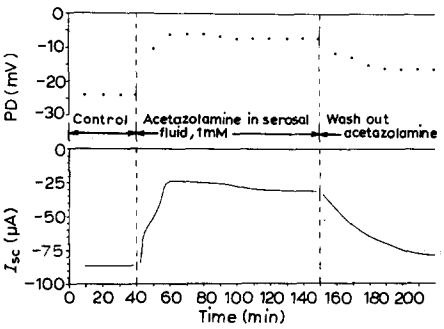

Fig. 3. Plots of PD and I_{sc} *vs.* time for a bladder bathed on both surfaces by choline sulfate Ringer solution containing 20 mM HCO_3^-. Acetazolamide (final concn. 1 mM in serosal fluid) was added at 40 min and washed out of the serosal fluid at 150 min.

The effect of acetazolamide was complete within 20 min and persisted until the drug was removed. The response shown in Fig. 3 was the most rapid one observed. At 150 min, acetazolamide was washed out of the serosal fluid and the I_{sc} recovered almost to the control levels during the next hour.

After replacement of the acetazolamide-containing Ringer solution with acetazolamide-free Ringer solution, the I_{sc} increased to its pretreatment level in two experiments and increased half way toward its pretreatment level in two other experiments.

The data suggest that the inhibition of HCO_3^- transport by acetazolamide is reversible.

Kinetic parameters for low and high mucosal HCO_3^- concentrations

Points corresponding to mucosal HCO_3^- levels below 2.5 mM were deleted from the data presented in the Lineweaver–Burk plot of Fig. 2 because they did not fall

on the curve. Moreover, data derived from the entire range of mucosal HCO_3^- concentrations studied (0.2–20 mM) could not be conveniently displayed on the same Lineweaver–Burk plot. Hence, a Hofstee plot of all the data in Table II is presented in Fig. 4. In this plot, the points divide into two groups, each group falling along a straight line. The points corresponding to HCO_3^- levels above 2.5 mM fall along Line I, and those corresponding to HCO_3^- levels below 2.5 mM fall along Line II.

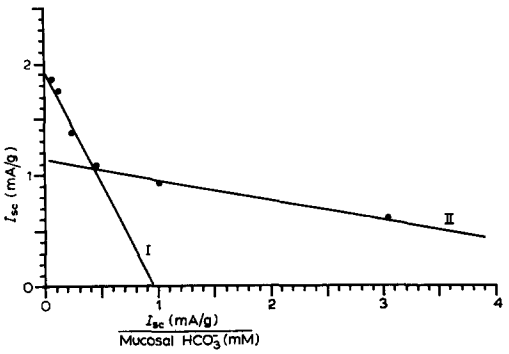

Fig. 4. Shows a Hofstee plot of I_{sc} per dry mass of tissue *vs.* the mucosal HCO_3^- concentration for all of the mucosal HCO_3^- concentrations reported in Table II. Points corresponding to HCO_3^- concentrations above 2.5 mM fall along Line I, and those corresponding to HCO_3^- levels below 2.5 mM fall along Line II. The two lines suggest two different transport processes.

From Line I, the half maximal concentration of mucosal HCO_3^- and the saturation current, in close agreement with the values estimated from the Lineweaver–Burk plot, were 2.09 mM and 1.98 mA/g, respectively. From Line II, the half-maximal concentration and saturation current, not considered in the Lineweaver–Burk plot, were 0.17 mM and 1.13 mA/g, respectively.

These data suggest that there are two HCO_3^- transport mechanisms. The mechanism corresponding to Curve II appeared to have a high affinity for HCO_3^- and could presumably remove HCO_3^- from very dilute HCO_3^- solutions.

The mechanism corresponding to Curve I appeared to have about one tenth the affinity and twice the transport capacity of the mechanism corresponding to Curve II.

It is pertinent to examine the data when the mucosal HCO_3^- concentration was was low, (*i.e.*, near zero to approx. 0.2 mM), as shown in Curve II. This mechanism apparently reaches its half-maximal capacity for HCO_3^- transport (40 μA per 5 cm^2 of bladder surface) when the concentration of mucosal HCO_3^- is no more than 0.2 mM. Moreover, the current density of 40 μA at 0.2 mM HCO_3^- is around twice the range (11–25 μA per 7 cm^2) reported by others in allegedly "HCO_3^--free" Ringer solutions wherein no determinations of HCO_3^- or CO_2 content were reported[3,4].

When bladders were exposed to 0.2 mM acetazolamide and 26 mM mucosal HCO_3^-, I_{sc} observed was far below the saturation current for both Mechanisms I and II. This indicates that both transport processes are sensitive to acetazolamide.

Present data do not permit a quantitative determination of the individual sensitivities of the two processes to acetazolamide.

The tendency for I_{sc} to return when acetazolamide is washed off the bladder

indicates that the effect of acetazolamide is reversible on at least one of the HCO_3^- transport processes.

Amendment of previous claims

Apart from any interpretation, data described in this report do not match previous claims of this laboratory[1,2]. SCHILB AND BRODSKY[1,2] suggested that "acetazolamide did not affect the lowering of mucosal pH in bladder sacs bathed by CO_2-rich media" (ref. 1) and, in addition, it was claimed that "the inhibitor had no effect on a negative I_{sc} of about 40 A/cm^2 in short-circuited bladders bathed by Na^+-free (choline) Ringer solution containing HCO_3^-" (ref. 2). In the light of the data reported herein, these claims were clearly in error.

DISCUSSION

HOGBEN and co-workers[6,7] have reported (1) that acetazolamide and other sulfonamides significantly reduced the active transport of Cl^- and the PD in frog stomach; (2) that the relative potency of these inhibitors towards the transport followed a similar sequence to their activities as inhibitors of carbonic anhydrase; (3) that required concentrations of inhibitor were more than two orders of magnitude greater than those required for inhibition of carbonic anhydrase; and (4) that similar effects were reported for the Cl^- transport in frog cornea, which contains no carbonic anhydrase activity. On the basis of these findings, HOGBEN and co-workers have postulated that there is a Cl^- transport receptor in these tissues with some structural similarity to carbonic anhydrase.

STEINMETZ[3] has shown that acetazolamide added to the serosal fluid at a concentration of 2 mM markedly reduced the acidification process in the turtle bladder. This effect was attributed to the inhibition of carbonic anhydrase. However, MAREN[8] has reported that there is no carbonic anhydrase activity in the turtle bladder. The present findings indicate that acetazolamide inhibits HCO_3^- transport in the turtle bladder in a manner analogous to noncompetitive inhibition. Thus, one could suppos- that some essential constituent of the HCO_3^- transport system has some structural resemblance to carbonic anhydrase and is attacked by acetazolamide, even though it lacks enzymatic activity.

The two transport mechanisms suggested in Fig. 4 could be explained by either of two models. There could be two independent transport systems or there could be one transport system with two kinds of sites for HCO_3^- attachment.

ACKNOWLEDGMENTS

This study was supported in part by National Institutes of Health Research Grants: A-461; AM 10659; AM 13138; in part by National Science Foundation Research Grant GB-5183; in part by National Aeronautics and Space Administration Grant NGR 18–002–015. The authors thank Miss Neal Linker, Mrs. Cecilia Gonzalez, and Mrs. Mercedes Serrano for their technical assistance.

REFERENCES

1 T. P. SCHILB AND W. A. BRODSKY, *Am. J. Physiol.*, 210 (1966) 997.
2 W. A. BRODSKY AND T. P. SCHILB, *Federation Proc.*, 26 (1967) 1314.
3 P. R. STEINMETZ, *J. Clin. Invest.*, 46 (1967) 1531.
4 P. R. STEINMETZ, R. S. OMACHI AND H. S. FRAZIER, *J. Clin. Invest.*, 46 (1967) 1541.
5 C. F. GONZALEZ, Y. E. SHAMOO AND W. A. BRODSKY, *Am. J. Physiol.*, 212 (1967) 641.
6 C. A. M. HOGBEN, *Mol. Pharmacol.*, 3 (1967) 318.
7 S. KITAHARA, K. R. FOX AND C. A. M. HOGBEN, *Nature*, 214 (1967) 836.
8 T. H. MAREN, *Physiol. Rev.*, 47 (1967) 595.