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THE EFFECT OF THEOPHYLLINE ON INTESTINAL BICARBONATE TRANSPORT MEASURED BY pH STAT IN *AMPHIUMA*

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SUMMARY

1. The influence of theophylline on the mucosa to serosa and serosa to mucosa fluxes of HCO_3^- were measured by the pH stat technique in isolated segments of proximal small intestine from *Amphiuma* maintained under short-circuited conditions. The mucosal or serosal fluid was exposed to media containing 25 mM-HCO_3^- (pH 7·4) while the pH of unbuffered media in the opposite compartment was maintained by addition of acid.

2. Theophylline significantly increased the secretory flux of HCO_3^- and significantly reduced the absorptive flux when measured in Cl^--free (SO_4^{2-}) media.

3. In normal media theophylline did not alter the secretory flux but significantly lowered the absorptive flux of HCO_3^-.

4. Acetazolamide (0·1 mM) inhibited the theophylline-stimulated secretory flux of HCO_3^- and reduced the effect of theophylline on the absorptive flux.

5. In normal intestine there was an inequality between the secretory or absorptive HCO_3^- flux and the short-circuit current (I_{sc}) consistent with the presence of Cl^- absorption. After addition of theophylline the I_{sc} was more nearly equal to the net secretory or absorptive HCO_3^- flux.

6. Exogenous cyclic AMP had effects identical with theophylline.

7. The results provide strong evidence that elevation of cyclic AMP stimulates net HCO_3^- secretion in urodele small intestine and provide indirect evidence that Cl^- absorption is simultaneously reduced.

INTRODUCTION

Certain pathophysiological conditions cause a severe alteration in the electrolyte transport capabilities of the small intestinal mucosa resulting in fluid loss, diarrhoea and metabolic acidosis. Most notable among these pathological states is cholera. In this disease a toxin secreted by the *Vibrio* organism attaches to the mucosal surfaces of the absorptive cells and stimulates adenyl cyclase. The resulting elevation of intracellular 3′,5′-cyclic adenosine monophosphate (AMP) is followed by alterations in electrolyte transport which have been examined *in vivo* and *in vitro* either using cholera toxin or other agents which elevate intracellular cyclic AMP. While these studies have consistently shown that Na^+ and Cl^- absorption are blocked and net

Cl^- secretion ensues the secretion of Na^+ has not been a constant finding (Field, 1974). More important only a few *in vitro* studies (Powell, Farris & Carbonetto, 1974; Sheerin & Field, 1975) have been able to offer some support for the observation in *in vivo* studies that the secretion of HCO_3^- into the intestinal lumen is actually increased above normal (Carpenter, Sack, Feeley & Steenberg, 1968; Leitch & Burrows, 1968; Leitch, Iwert & Burrows, 1966; Moore, Bieberdorf, Morawski, Finkelstein & Fordtran, 1971; Norris, Curran & Schultz, 1969). The sensitivity of the cholera-enhanced HCO_3^- secretion to acetazolamide reported in two *in vivo* studies (Leitch, Iwert & Burrows, 1966; Norris, Curran & Schultz, 1969) has not been confirmed *in vitro*.

Recent observations of the effect of theophylline on amphibian small intestine indicated that theophylline, the inhibitor of phosphodiesterase accelerates acetazolamide-sensitive HCO_3^- secretion (Gunter-Smith & White, 1979). Theophylline stimulated the short circuit current (I_{sc}) proportional to media HCO_3^- in the presence or absence of media Cl^-. Labelled Na^+ flux measurements revealed that the increase in I_{sc} was accompanied by inhibition of net Na^+ transport in Cl^--free media providing evidence that a residual flux, most likely net HCO_3^- secretion, was occurring. The reduction of the I_{sc} and the residual flux by acetazolamide supported this proposal. In the previous paper it was also shown that theophylline stimulated the residual flux in Cl^--containing media as well (White, 1981).

In order to test this proposal more carefully and examine the characteristics of this reputed secretory process a direct measure of HCO_3^- transport was required. We have recently adapted the pH stat technique for this purpose after it became clear that HCO_3^- transport in the urodele intestine is quite large, even capable of generating pH gradients across the mucosa (Imon & White, 1981). In the present study the effect of theophylline on the absorptive and secretory flux of HCO_3^- is examined. We show that in the urodele intestine theophylline stimulates HCO_3^- secretion and reduces HCO_3^- absorption. These effects are moderated by pre-exposure to acetazolamide. Further indirect evidence for simultaneous inhibition of Cl^- absorption is also presented (White, 1981).

METHODS

Animals and chambers. Adult *Amphiuma* were stored and anaesthetized as described in the previous publication (White, 1981). Segments of proximal small intestine were stripped of their outer muscle layers opened and mounted as a sheet in a Plexiglas chamber that allowed measurement of the rate of HCO_3^- transport while the tissue was voltage clamped to eliminate the transepithelial electrical potential. For these measurements the chamber used accommodated 1·77 cm^2 of mucosa in a circular opening. The chamber was identical in most other respects with the Ussing-type chambers used previously (White, 1980) except for a minor modification to allow placement of a pH electrode and titration pipette into the bathing media. In addition a wide-bore segment of tubing was inserted in series with the tubing comprising the O_2 lift in one half-chamber (containing buffered media) to double the volume capacity. The mucosa was exposed on one surface to 5 ml. unbuffered media and on the other surface to 10 ml. buffered media. Many initial measurements were performed using a chamber with an oval opening which accommodated 10·8 cm^2 of epithelium. The transepithelial electrical potential was sensed with agar- saturated KCl bridges. Current was passed normal to the epithelial surface through bridges formed from agar (4 %) in the appropriate unbuffered media.

Bathing media. The normal bathing media contained (m.equiv/l.): 95 Na^+, 2·5 K^+, 76·3 Cl^-, 25 HCO_3^-, 0·9 Ca^{2+}, 1·0 Mg^2 and 20 mM-mannitol and was buffered to pH 7·4 by gassing with humidified 95 % O_2 + 5 % CO_2. In the companion, unbuffered media, HCO_3^- was replaced with SO_4^{2-}

and the osmolarity maintained by elevation of the mannitol to 32·5 mM. The unbuffered media was gassed with 100% O_2 which was passed through a solution of NaOH to remove CO_2. Chloride-free media were prepared by substitution with sulphate, osmolarity being maintained by increasing mannitol to 55 mM. The HCO_3^- concentration was varied from 5 to 25 m-equiv/l. in one study by substitution with SO_4^{2-}, the Cl^- concentration being maintained constant at 76·3 m-equiv/l. Acetazolamide, theophylline, adenosine 3′,5′-cyclic monophosphoric acid (cyclic AMP) and dibutyryl cyclic 3′,5′-monophosphoric acid (dBcAMP) were obtained from Sigma (St. Louis, MO) and usually added simultaneously to both sides of the tissue.

Electrical measurements. The transmural potential difference and the current required to reduce the potential to zero, here referred to as the short-circuit current (I_{sc}), were usually measured with an automatic voltage clamp device which compensates automatically for the resistance of the solution between the potential-sensing electrodes and the tissue. The tissue resistance was calculated from the open-circuit electrical potential and the I_{sc}. All measurements were performed under short-circuit conditions.

Measurement of HCO_3^- absorption and secretion. Except for some early studies in which titration was performed manually a Radiometer automatic pH stat assembly was used to measure either HCO_3^- absorption or secretion. The HCO_3^-secretory flux was measured by titrating the unbuffered media bathing the mucosal surface of the intestine with 0·2–0·4 N-H_2SO_4 and recording the time and volume of acid added automatically. The mucosal pH was held constant at the pH of the serosal media (7·35–7·45). The rate of titration also referred to as the rate of HCO_3^- secretion ($J_{s\to m}^{HCO_3^-}$) was expressed in μequiv/hr . cm^2.

The mucosal fluid pH was measured with a Radiometer GK 2321C pH electrode (London Company, Cleveland, Ohio) and Radiometer PHM 61 meter calibrated with Metrepack pHydron buffers. The titrant was standardized by titration with K hydrogen phthalate (National Bureau of Standards, Washington, DC). The total volume of titrant added over 5 hr was maximally about 0·20 ml.

The HCO_3^- absorptive flux ($J_{m\to s}^{HCO_3^-}$) also referred to as the rate of HCO_3^- absorption was measured by titrating the unbuffered media bathing the serosal surface with H_2SO_4 while the mucosal surface was bathed with normal buffered media. The serosal pH was held constant at the pH of the mucosal media (7·35–7·45).

The term '*net* HCO_3^- secretion' is reserved to describe the difference between the absorptive and secretory HCO_3^- fluxes.

Protocol. Before killing the animal the tissue chambers were assembled and pre-equilibrated with the gas mixture. With the tissue in place the bathing media was frequently exchanged with fresh media for the first 20–60 min. During this period the pH of the unbuffered media rarely was less than 5. At 1 hr titration usually commenced.

Statistical tests. Statistical tests of significance were conducted using Student's *t* test for paired or unpaired comparisons. All errors are expressed as the standard error of the mean.

RESULTS

The effect of theophylline on the secretory HCO_3^- flux

Chloride-free media. HCO_3^- secretion by short-circuited intestinal segments bathed in Cl^--free media was markedly elevated by theophylline in two separate series different only in their basal values of HCO_3^- secretion and I_{sc}. The results of both series were pooled. The response of a single tissue to addition of 1 mM-theophylline is seen in Fig. 1. The secretory HCO_3^- flux ($J_{s\to m}^{HCO_3^-}$) was constant at about 1 μequiv/hr . cm^2 before addition and, after a delay of about 10 min, increased over the next hour. The short-circuit current (I_{sc}) was initially lower, indicating that other ions are moving as well, and was rapidly elevated by theophylline. As seen in Table 1 for eleven tissues the increase in I_{sc} after 1 hr was largely accounted for by an increase in $J_{s\to m}^{HCO_3^-}$. The difference between the I_{sc} and the rate of HCO_3^- secretion, I_{sc}-$J^{HCO_3^-}$, was small, significant ($P < 0·01$) and unchanged by theophylline. In Fig.

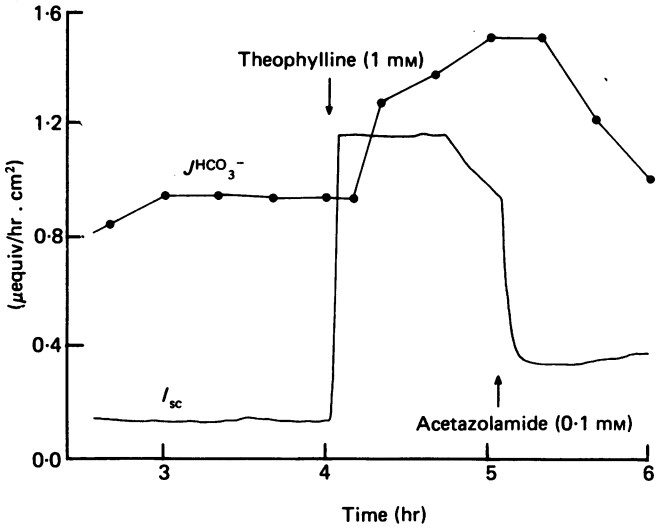

Fig. 1. Time course of rate of HCO_3^- secretion ($J^{HCO_3^-}$) and short-circuit current (I_{sc}) before and after exposure to 1 mM-theophylline followed by 0·1 mM-acetazolamide in Cl^--free media. The I_{sc} was retraced from the strip chart record.

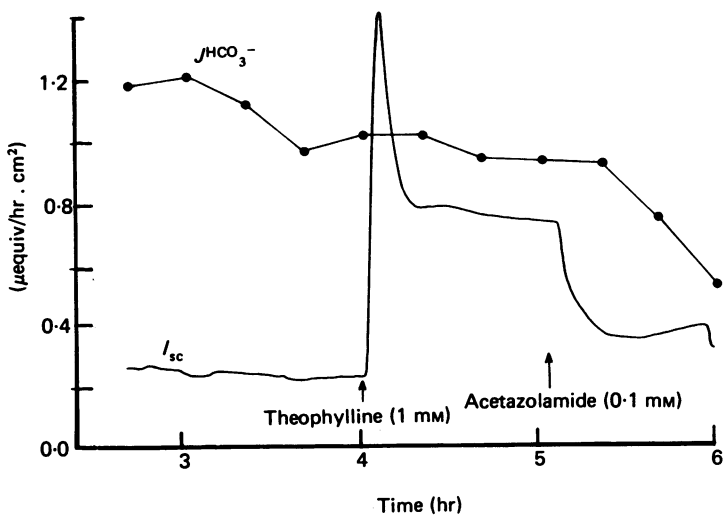

Fig. 2. Time course of rate of HCO_3^- secretion ($J^{HCO_3^-}$) and short-circuit current (I_{sc}) in normal media before and after exposure to 1 mM-theophylline followed by 0·1 mM-acetazolamide.

TABLE 1. Effects of theophylline on HCO_3^- secretion

	n	$J^{HCO_3^-}$	I_{sc}	I_{sc}-$J^{HCO_3^-}$	R_t
Cl^--free media	(11)	0·62±0·08	0·27±0·04	−0·35±0·08	267±26
+Theophylline		0·94±0·10*	0·69±0·06***	−0·25±0·07	313±36
Normal media	(8)	1·10±0·08	0·23±0·08	−0·87±0·10	203±21
+Theophylline		0·96±0·07	0·70±0·09*	−0·26±0·09*	243±20

$J^{HCO_3^-}$ and I_{sc} in μequiv/hr . cm². R_t is in Ω . cm². n is the number of tissues studied. After at least 3 hr the rates were recorded over a 20 min interval before theophylline addition and 1 hr after addition. The asterisks indicate significant difference from controls at $*P < 0·05$, $***P < 0·001$.

1 the carbonic anhydrase inhibitor acetazolamide (0·1 mM) added after theophylline reduced the I_{sc} rapidly and, after about 15 min, began to reduce $J_{s\to m}^{HCO_3^-}$. This effect is documented more fully below.

TABLE 2. Effect of theophylline on HCO_3^- secretion after acetazolamide

	n	$J^{HCO_3^-}$	I_{sc}	I_{sc}-$J^{HCO_3^-}$	R_t
Cl^--free media	(4)	0·62±0·03	0·24±0·03	−0·38±0·05	271±12
+Acetazolamide		0·56±0·05	0·18±0·03*	−0·39±0·05	266±20
+Theophylline		0·72±0·10*	0·42±0·08*	−0·30±0·06	261±25

After at least 3 hr the control rate was measured over a 20 min interval. 1 hr after acetazolamide and 1 hr later after theophylline rates were also recorded. The asterisks indicate significant difference from the pre-addition value at $*P < 0·05$.

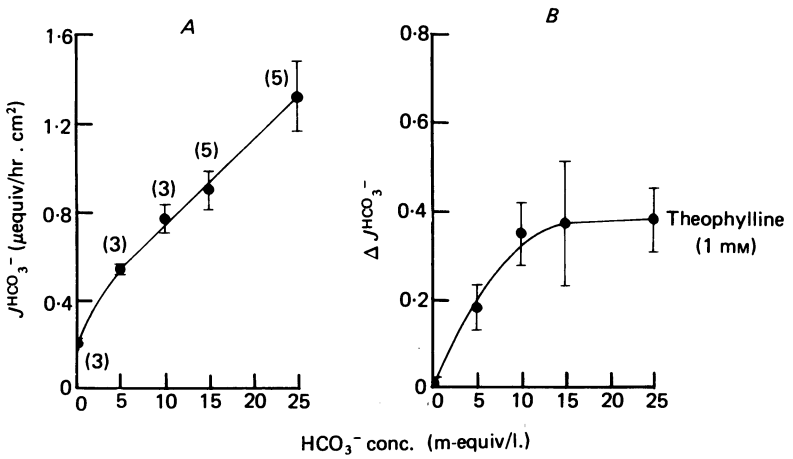

Fig. 3. Isolated segments were exposed on their serosal surface to media containing HCO_3^- at 0–25 mM. Rates were measured under open circuit conditions. Curves were drawn by inspection. Media was Cl-free.
A, the basal rate of mucosal alkalinization ($J^{HCO_3^-}$) as a function of serosal [HCO_3^-] at the end of 4 hr. The number of tissues examined is in parentheses.
B, the change in the rate of mucosa alkalinization ($\Delta J^{HCO_3^-}$) 1 hr after addition of theophylline.

Normal media. HCO_3^- secretion was not altered in segments bathed in normal media. As seen in Fig. 2 $J^{HCO_3^-}$, which declines slowly under these conditions (Imon & White, 1981) was unaffected by theophylline. In contrast the I_{sc} was rapidly increased before declining to a stable value. (The polarity of I_{sc} is not serosa negative as in the previous manuscript (White, 1981) due to the absence of mucosal HCO_3^-.) Identical results from eight tissues are averaged in Table 1. It is also shown that I_{sc}-$J_{s\to m}^{HCO_3^-}$ was significantly higher than in the Cl^--free media ($P < 0·01$) consistent with the absorption of Cl^-. Theophylline significantly reduced I_{sc}-$J_{s\to m}^{HCO_3^-}$ to a value similar to that in Cl^--free media. Also seen in Fig. 2 the addition of acetazolamide in the presence of theophylline reduced the I_{sc} and $J_{s\to m}^{HCO_3^-}$.

Influence of acetazolamide on stimulated secretion. Exposure of intestinal segments to the carbonic anhydrase inhibitor acetazolamide reduced the effect of theophylline in Cl^--free media. As seen in Table 2 acetazolamide itself did not significantly alter

$J^{HCO_3^-}_{s\to m}$. Subsequent addition of theophylline caused a smaller but significant increase in $J^{HCO_3^-}_{s\to m}$ ($P < 0{\cdot}05$) and I_{sc} ($P < 0{\cdot}05$). That the stimulatory effect of theophylline was smaller after the pre-dose of acetazolamide, seen from a comparison of Tables 1 and 2, was established statistically. Thus pre-addition of acetazolamide significantly reduced the stimulation of $J^{HCO_3^-}_{s\to m}$ ($P < 0{\cdot}02$) and I_{sc} ($P < 0{\cdot}05$). Further evidence of the ability of acetazolamide to attenuate the secretory response to theophylline is given below.

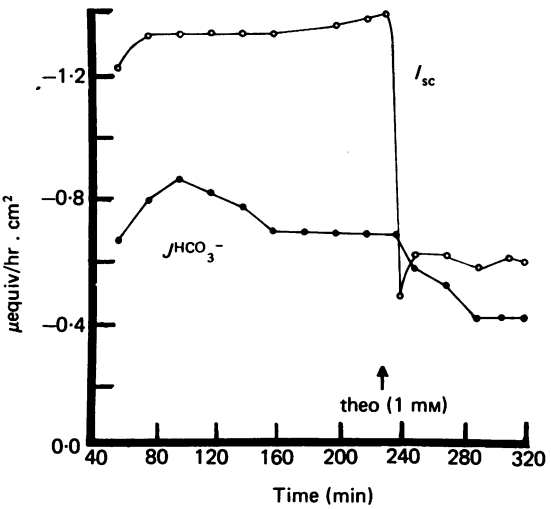

Fig. 4. Time course of rate of HCO_3^- absorption ($J^{HCO_3^-}$) and short-circuit current (I_{sc}) before and after exposure to 1 mM-theophylline (theo) in normal media.

Relationship to serosal [HCO_3^-]. The ability of theophylline to stimulate $J^{HCO_3^-}_{s\to m}$ was dependent on the serosal [HCO_3^-]. In Fig. 3*A* the control value of $J^{HCO_3^-}_{s\to m}$ is seen to increase linearly as HCO_3^- was raised from 5 to 25 mM. Addition of theophylline after 4 hr caused a significant stimulation of $J^{HCO_3^-}_{s\to m}$ ($\Delta J^{HCO_3^-}_{s\to m}$) at all levels of HCO_3^- studied (Fig. 3*B*). The degree of stimulation was constant above 10 mM-HCO_3^-. In the absence of exogenous HCO_3^- and CO_2 (phosphate buffered media, pH 7·4) no stimulation of $J^{HCO_3^-}_{s\to m}$ was observed.

Addition of acetazolamide after theophylline caused a significant decline in $J^{HCO_3^-}_{s\to m}$ at every level of serosal HCO_3^- studied ranging from 44% (5 mM-HCO_3^-) to 56% (25 mM-HCO_3^-). There remained however a significant theophylline-dependent HCO_3^- flux. This effect was not on the theophylline-independent HCO_3^- flux since the control $J^{HCO_3^-}_{s\to m}$ was not influenced by acetazolamide (Table 2). Thus acetazolamide specifically inhibits the theophylline-dependent component of $J^{HCO_3^-}_{s\to m}$.

The effect of theophylline on the absorptive HCO_3^- flux

Normal media. In addition to stimulating the HCO_3^- secretory flux theophylline reduced the HCO_3^- absorptive flux as well. As seen in Fig. 4 for a single tissue bathed in normal media the absorptive HCO_3^- flux, measured by titrating unbuffered serosal media, was reduced from a steady value of 0·70–0·42 μequiv/hr . cm² over 50 min.

The I_{sc}, initially at a higher value rapidly dropped to a value nearer that of $J^{HCO_3^-}_{m\to s}$.

The tissue resistance was initially low, characteristic of segments exposed to mucosal HCO_3^- alone (Gunter-Smith & White, 1979), and was unaltered by theophylline. The average of four tissues is seen in Table 3. Theophylline decreased the absorptive flux by 34% and produced a larger decline in the I_{sc}. The effects were both significant (Table 3). The difference, I_{sc}-$J^{HCO_3^-}$, which was significant in controls ($P < 0.02$) and consistent with Cl^- absorption was not different from zero ($P > 0.10$) after theophylline.

TABLE 3. Effect of theophylline on HCO_3^- absorption

	n	$J^{HCO_3^-}_{m\to s}$	I_{sc}	I_{sc}-$J^{HCO_3^-}$	R_t
Normal media	(4)	-0.79 ± 0.07	-1.28 ± 0.15	-0.50 ± 0.13	98.7 ± 8.0
+Theophylline		-0.52 ± 0.09*	-0.55 ± 0.23**	-0.03 ± 0.15*	90.8 ± 13.7
Cl^--free media	(6)	-0.98 ± 0.11	-0.64 ± 0.12	$+0.33\pm0.10$	95.6 ± 7.0
+Theophylline		-0.71 ± 0.10**	-0.35 ± 0.06*	$+0.36\pm0.10$	94.4 ± 8.9

The control rates were measured at 210–240 min. The rates in the presence of theophylline were recorded 90 min after addition. Asterisk indicates significant change from control at $*P < 0.05$, $**P < 0.02$.

TABLE 4. Effect of theophylline on HCO_3^- absorption after acetazolamide

	n	$J^{HCO_3^-}$	I_{sc}	I_{sc}-$J^{HCO_3^-}$	R_t
Cl^--free media	4	-1.17 ± 0.09	-0.96 ± 0.11	$+0.22\pm0.11$	89.9 ± 10.4
+Acetazolamide		-0.71 ± 0.10**	-0.64 ± 0.06*	$+0.07\pm0.08$	105.8 ± 6.4
+Theophylline		-0.67 ± 0.06	-0.57 ± 0.07	$+0.10\pm0.12$	100.5 ± 6.7

Control rates were measured at the end of 3 hr. The rates after exposure to acetazolamide for 1 hr and then acetazolamide plus theophylline were obtained at 4 and 5 hr respectively. The asterisks indicates a significant difference from the pre-addition value at $*P < 0.02$, $**P < 0.01$.

Cl^--free media. In Table 4 it is seen that theophylline also lowered the absorptive HCO_3^- flux in Cl^--free media. In all six tissues $J^{HCO_3^-}_{m\to s}$ was lowered, the decline averaging 0·27 μequiv/hr . cm^2. The I_{sc} which was smaller than in normal media was reduced an equal amount. Thus I_{sc}-$J^{HCO_3^-}$ was unchanged.

Influence of acetazolamide. Addition of acetazolamide (0·1 mM) before theophylline reduced HCO_3^- absorption and blocked the effect of theophylline added subsequently on $J^{HCO_3^-}_{m\to s}$. In Table 4 acetazolamide is seen to reduce $J^{HCO_3^-}$ significantly ($P < 0.01$) as well as I_{sc} ($P < 0.02$) without significantly influencing I_{sc}-$J^{HCO_3^-}$ ($P > 0.20$). Subsequent addition of theophylline was without effect on $J^{HCO_3^-}$ or I_{sc}. Clearly theophylline and acetazolamide act similarly on the process responsible for HCO_3^- absorption.

Effect of cyclic AMP on HCO_3^- fluxes

Absorptive HCO_3^- flux. Exogenous cyclic AMP (1 mM) added to the buffered mucosal media reduced the absorptive flux of HCO_3^- and the I_{sc} similar to theophylline. The average reduction in three tissues seen in Table 5 was 26%. In two additional tissues from the middle region of the urodele small intestine inhibition of $J^{HCO_3^-}_{m\to s}$ by cyclic AMP was seen in both. In the first cyclic AMP reduced $J^{HCO_3^-}_{m\to s}$ by 36% in 1 hr; in the second cyclic AMP reduced $J^{HCO_3^-}_{m\to s}$ by only 9% in 90 min but subsequent exposure to 1 mM-dibutyryl cyclic AMP (dBcAMP) reduced $J^{HCO_3^-}_{m\to s}$ by 34% from the control value in 1 hr.

Secretory HCO_3^- flux. Neither exogenous cyclic AMP or dBcAMP stimulated $J^{HCO_3^-}$ when added to the serosal fluid even though theophylline added subsequently produced a detectable stimulation. In contrast mucosally applied dBcAMP consistently stimulated $J^{HCO_3^-}_{m\rightarrow s}$ but by variable amounts as seen in Fig. 5. In this series performed in summer animals the elevation of $J^{HCO_3^-}$ following addition of dBcAMP was not sustained. Subsequent addition of theophylline produced sizeable but again transient increases in $J^{HCO_3^-}$ only in those tissues not markedly stimulated by the pre-dose of dBcAMP (Fig. 5).

TABLE 5. Effect of cyclic AMP on absorptive HCO_3^- flux

	n	$J^{HCO_3^-}$	I_{sc}	I_{sc}-$J^{HCO_3^-}$	R_t
Cl^--free media	3	$-0{\cdot}83\pm0{\cdot}11$	$-0{\cdot}68\pm0{\cdot}13$	$0{\cdot}14\pm0{\cdot}05$	$101{\cdot}8\pm6{\cdot}7$
+Cyclic AMP		$-0{\cdot}61\pm0{\cdot}06$	$-0{\cdot}52\pm0{\cdot}15$	$0{\cdot}10\pm0{\cdot}17$	$107{\cdot}5\pm5{\cdot}3$

Control rates were measured at the end of 4 hr. The rate after exposure to cyclic AMP was obtained at 5–6½ hr. Cyclic AMP was added at 1 mM by complete replacement of the mucosal media.

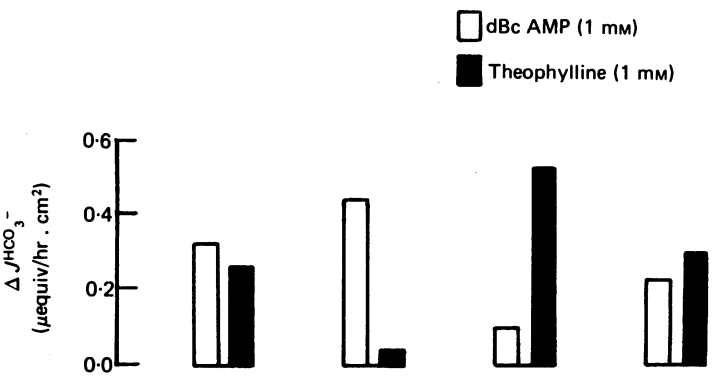

Fig. 5. Stimulation of secretory HCO_3^- flux ($\Delta J^{HCO_3^-}$) first by mucosally applied exogenous dibutyryl cyclic AMP (dBcAMP) at 1 mM and subsequently by serosally applied theophylline. The control rate of $J^{HCO_3^-}$ in these four tissues averaged $0{\cdot}79\pm0{\cdot}05$ μequiv/hr . cm².

DISCUSSION

This study demonstrates that theophylline, the inhibitor of phosphodiesterase, elevates the secretory flux of HCO_3^- and also lowers the absorptive flux. Since net transport capacity is the sum of absorptive and secretory fluxes both of these changes will promote net secretion of HCO_3^-.

This assumes that the unidirectional HCO_3^- fluxes are independent and thus additive. HCO_3^- secretion, but not HCO_3^- absorption is dependent on media Cl^- (compare Tables 1 and 3) and media Na^+ (Imon & White, 1981 and unpublished observations). These observations and the agreement between the reported residual fluxes and the calculated net HCO_3^- flux provide evidence the unidirectional fluxes are indeed independent.

In the absence of Cl^- theophylline elevated the HCO_3^- secretory flux and reduced the HCO_3^- absorptive flux. In contrast in the presence of Cl^- this agent reduced the absorptive flux but did not change the HCO_3^- secretory flux. Therefore greater stimulation of net HCO_3^- secretion would be expected in the absence of Cl^-.

It was previously reported that in the absence of Cl^- theophylline stimulates the residual flux, that portion of the short-circuit current not due to net Na^+ transport (Gunter-Smith & White, 1979). The sign of the residual flux was consistent with secretion of HCO_3^-. As seen in the preceding report however the increased residual flux in the presence of Cl^- was smaller and of borderline significance (White, 1982). The present results provide direct evidence for enhanced net HCO_3^- secretion in both the presence and absence of Cl^- and provide an explanation for the difficulty in earlier studies in detecting enhanced HCO_3^- secretion in other than Cl^--free conditions (Powell *et al.* 1974; Sheerin & Field, 1975), namely that net HCO_3^- secretion is greater in the absence of Cl^- because of simultaneous stimulation of the HCO_3^- secretory flux. Clearly a comprehensive understanding of the effect of theophylline on net HCO_3^- transport would be incomplete without measurement of both unidirectional HCO_3^- fluxes.

The effect of theophylline on the HCO_3^- absorptive flux has a parallel in the kidney since McKinney & Myers (1980) reported that dibutyryl cyclic AMP inhibits bicarbonate absorption in rabbit proximal tubules.

The ion transport events underlying the stimulation of the HCO_3^- secretory flux are not clear. After exposure to theophylline it appears the principal ion moving in a conductive, electrogenic manner is HCO_3^- or its equivalent. Therefore there is no need to invoke the operation of neutral ion exchange. The proportionality between the serosal $[HCO_3^-]$ and $J_{s\to m}^{HCO_3^-}$ in Cl^--free media lends credence to the notion that the bicarbonate ion itself is transported. However this conclusion is equivocal since elevation of the $[HCO_3^-]$ in isobaric CO_2 is accompanied by an increase in media pH. Thus it cannot be excluded that the OH^- ion is secreted or the relationship is a consequence of change in intracellular pH. Nevertheless a working model which envisions a HCO_3^- transport process receiving a fraction of substrate directly from the serosal media is consistent with the data. Flemstrom & Garner (1980) recently reported that gastric inhibitory peptide stimulates HCO_3^- secretion in bullfrog duodenum.

The effect of theophylline on the absorptive HCO_3^- flux may be due to inhibition through cyclic AMP of H^+ secretion. A secretory H^+ transport mechanism operating in normal tisue to absorb HCO_3^- is suggested, in part, by the observation that serosal alkalinization is reduced by the carbonic anhydrase inhibitor acetazolamide (Table 4). H^+ secretion would normally generate intracellular HCO_3^- via carbonic anhydrase for eventual entry into and alkalinization of the serosal media. Blair, Lucas & Matty (1975) observed that aminophylline reduced H^+ secretion in rat jejunum. H^+ transport linked to Na^+ was observed by Murer, Hopfer & Kinne (1976) in isolated brush border vesicles from rat intestine. Irrespective of the mechanism, theophylline and exogenous cyclic AMP have an identical effect as acetazolamide to reduce HCO_3^- absorption. This similarity of effect undoubtedly accounts for the fact that pre-exposure to acetazolamide reduced the impact on the absorptive HCO_3^- flux of theophylline added subsequently.

In contrast the effect of acetazolamide on the opposite, secretory HCO_3^- flux was antagonistic to that produced by theophylline. Pre-addition of acetazolamide at concentrations specific for carbonic anhydrase moderated the theophylline-induced HCO_3^- secretion (Table 2). Added after theophylline, acetazolamide halved the

secretory response. Clearly these agents have antagonistic effects on the transport process. One interpretation of these results is that elevation of intracellular cyclic AMP stimulates carbonic anhydrase activity possibly by activation of the enzyme (Narumi & Miyamoto, 1974) thereby supplying additional HCO_3^- for transport.

In summary elevation of intracellular cyclic AMP inhibits carbonic anhydrase-dependent HCO_3^- absorption and simultaneously stimulates carbonic anhydrase-dependent HCO_3^- secretion. These are not incompatible if cyclic AMP inhibits the H^+ secretory process beyond the point of carbonic anhydrase involvement. Perhaps more likely though two populations of carbonic anhydrase are present in the mucosa each influenced differently by cyclic AMP. Some evidence consistent with the presence of two populations of carbonic anhydrase in the mucosa was described in the previous paper (White, 1982). The observations using cyclic AMP are also suggestive of the presence of two populations of cells. Cyclic AMP usually reduced the HCO_3^- absorptive flux as completely as theophylline but neither cyclic AMP nor the more permeable dibutyryl cyclic AMP produced maximal stimulation of the HCO_3^- secretory flux (Fig. 5). This result would not be expected if the level of cyclic AMP in a single cell type influenced the rate of both processes. While not proven in this study it is more likely that a population of cells highly permeable to cyclic AMP absorb HCO_3^- while cells less permeable to cyclic AMP control HCO_3^- secretion.

Indirect evidence is provided in this study that theophylline inhibits Cl^- absorption. The difference between the control absorptive or secretory HCO_3^- flux and the I_{sc} i.e. I_{sc}-$J^{HCO_3^-}$ was consistent in sign with electrogenic Cl^- absorption (Tables 1, 3) and was greatly reduced by replacement of Cl^- with SO_4^{2-} in both series. The significant reduction of I_{sc}-$J^{HCO_3^-}$ in the presence of theophylline (Fig. 2) supports the contention of reduced Cl^- absorption already demonstrated directly in the previous paper using isotopically labelled Cl^- (White, 1982). Coupled with the effects on Cl^- transport, the effects of theophylline on HCO_3^- transport, although moderate, serve to convert an absorbing intestine into a secreting epithelium, the major secreted ion being HCO_3^-. This response to elevation of cyclic AMP has physiological significance as well as pathophysiological significance in the fluid and electrolyte secretion from the intestine following exposure to bacterial pathogens.

Theophylline significantly elevated the secretory HCO_3^- flux in Cl^--free media (Fig. 4) but left unaltered the same flux in Cl^--containing media although the I_{sc} was stimulated (Fig. 2). There are two possible explanations for this. First, in normal media net secretion of Cl^- may occur instead of HCO_3^-. In fact in the previous paper stimulation of the secretory Cl^- flux in the presence of theophylline was reported (White, 1982). However, there was no evidence that *net* Cl^- secretion is initiated. Second, theophylline may stimulate HCO_3^- secretion but simultaneously inhibit a parallel Cl^--linked HCO_3^- secretory process. In normal intestine Cl^- absorption is linked to HCO_3^- secretion. We have recently shown that the HCO_3^- secretory flux is greatly reduced by removal of Cl^- from the bathing media (Imon & White, 1981). This was confirmed in the present study (Table 1). In parallel with this observation Cl^- absorption requires the presence of HCO_3^- in the bathing media (White, 1980). These observations pointed to active Cl^- absorption linked to HCO_3^- secretion in the urodele intestine. The inhibition of both processes by the serosal application of the stilbene SITS (White, 1980; Imon & White, 1981) indicated the operation of a serosal

Cl^--HCO_3^- exchange mechanism like that in erythrocytes (Cabantchik & Rothstein, 1972). If the anion fluxes are linked in this way then inhibition of HCO_3^--dependent Cl^- absorption by theophylline (Table 1, 3) should be accompanied by a reduction in the HCO_3^- secretory flux. The absence of a significant effect of theophylline on this flux may well be due to simultaneous stimulation of the Cl^--independent HCO_3^- secretory flux. In Cl^--free media then only the latter effect of theophylline would be expected. Although the present data do not allow a quantitative analysis of this hypothesis, from a qualitative standpoint this explanation could account for the near constancy of the secretory HCO_3^- flux after theophylline in normal media.

Mammalian small intestine responds to elevation of intracellular cyclic AMP by increased secretion of HCO_3^- (Carpenter *et al.* 1968; Leitch & Burrows, 1968; Norris *et al.* 1969; Moore *et al.* 1971) which is reduced by administration of acetazolamide (Leitch, Iwert & Burrows, 1966; Norris *et al.* 1969). This was not demonstrable in *in vitro* mammalian small intestine (Field, 1974). The isolated urodele intestine, in contrast, responds similarly to *in vivo* mammalian intestine and should prove an excellent preparation for further investigation of intestinal HCO_3^- secretion.

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