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EVIDENCE FOR ELECTROGENIC BICARBONATE TRANSPORT IN *AMPHIUMA* SMALL INTESTINE

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Summary

Isolated segments of small intestine of *Amphiuma* were short-circuited in buffer containing bicarbonate. Theophylline (10 mM) increased short circuit current (I_{sc}) in proportion to the bicarbonate concentration in the bath. The theophylline-stimulated I_{sc} was rapidly reduced, though not abolished, in the presence of acetazolamide at concentrations as low as 10^{-6} M. Unidirectional fluxes of ^{22}Na and ^{24}Na in paired intestinal segments in Cl-free buffer reveal that the increase in I_{sc} produced by theophylline is not accounted for by an increase in net sodium flux. These results suggest that theophylline stimulates an electrogenic secretion of bicarbonate.

When challenged with cholera toxin or an agent such as theophylline, which elevates tissue cyclic AMP [1,2], mammalian small intestine in vivo responds with alterations in electrolyte transport including enhanced bicarbonate secretion [3—5]. However, in vitro mammalian small intestinal segments do not apparently secrete HCO_3^- as judged by the equivalency between short circuit current (I_{sc}) and the sum of the net Na^+ and Cl^- fluxes [6—8]. In contrast, isolated segments of proximal small intestine from *Amphiuma*, an amphibian, appear to secrete HCO_3^- (or absorb H^+) by an electrogenic mechanism when exposed to theophylline.

Intestinal segments stripped of their smooth muscle layers [9] were clamped between two halves of a lucite chamber and bathed in a chloride-based or sulfate-based buffer containing 10 mM HCO_3^-. In chloride-based buffer when gassed with 95% O_2/5% CO_2 the tissue generated a transmural potential difference serosa negative to mucosa. As seen in Fig. 1a, addition of 10 mM theophylline (equimolar substitution for mannitol) induced a rapid reversal of potential difference and I_{sc}, the extent of the stimulation de-

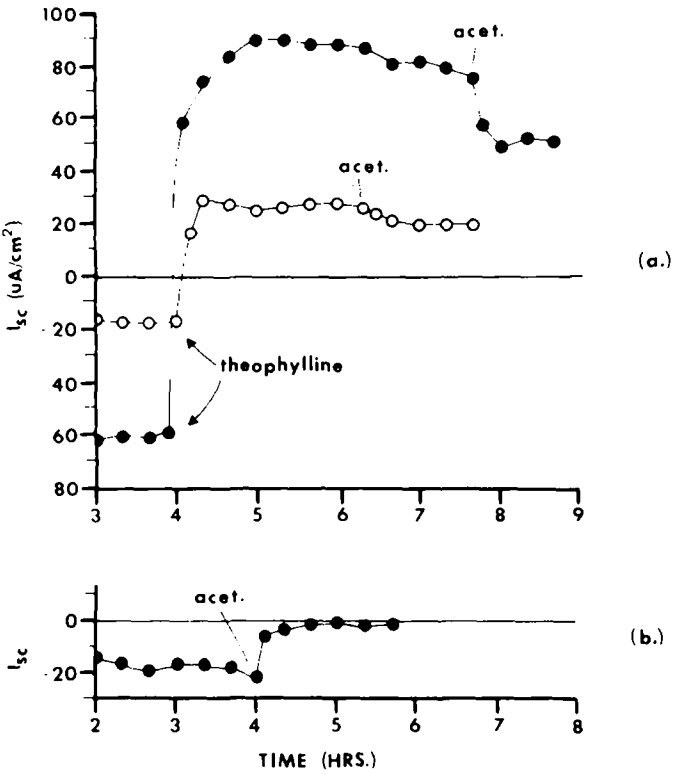

Fig. 1. (a) Time course of short-circuit current (I_{sc}) response to theophylline (10 mM) in isolated intestinal segments bathed in chloride-based buffer containing in mequiv./l: Na^+ 95, K^+ 2.5, HPO_4^{2-} 1.25, Ca^{2+} 0.9, Mg^{2+} 1.0, mannitol 20 mM and either 88.8 mequiv./l Cl^- and 10 mM HCO_3^- (○) or 48.8 mequiv./l Cl^- and 50 mM HCO_3^- (●). At the time indicated acetazolamide (acet.) was introduced to a final concentration of 10^{-4} M. In other experiments maximal inhibition was observed with 10^{-6} M acetazolamide. (b) Effect of acetazolamide (10^{-4} M) on the basal I_{sc}, i.e., in the absence of theophylline.

pending on the concentration of HCO_3^- in the buffer (bicarbonate was substituted for chloride). The maximum stimulation was observed in 50 mM HCO_3^-; the response was reduced in 100 mM HCO_3^-. This sensitivity to the bicarbonate concentration was not due to the concomitant change in buffer pH since the stimulation in 25 mM HCO_3^- gassed with 5% CO_2 (pH = 7.4) was 96.2±12.7 (S.E.M.) $\mu A/cm^2$ (n=4) and not significantly different ($P>0.10$) from the stimulation (ΔI_{sc} = 77.1±7.6) when gassed with 10% CO_2 (pH = 7.1).

The theophylline-stimulated I_{sc} could be partially inhibited by the subsequent introduction of the carbonic anhydrase inhibitor acetazolamide at concentrations as low as 10^{-6} M. The inhibition is illustrated in Fig. 1a. Acetazolamide should be very specific for carbonic anhydrase at this concentration [10]. The extent of the inhibition was again dependent on the bath HCO_3^- concentration and was maximal at 50 mM HCO_3^-. Also, acetazolamide at a very low concentration (10^{-4} M) virtually abolished the basal (unstimulated) I_{sc} (Fig. 1b).

Similar electrical events were observed in chloride-free (sulfate) buffer. Since, under these conditions, it is likely that only Na^+ and HCO_3^- (or H^+) are

TABLE I

SODIUM FLUXES IN RESPONSE TO THEOPHYLLINE

Simultaneous unidirectional fluxes of ^{22}Na and ^{24}Na from mucosa to serosa (m→s) or serosa to mucosa (s→m) were measured on adjacent segments of small intestine under short-circuit conditions. One segment was incubated in sulfate-based buffer (control), the other in buffer containing 10 mM theophylline. The unidirectional fluxes obtained from 20-min flux periods were subtracted to obtain the net sodium flux (J^{Na}_{Net}) for each flux period. This allowed a comparison between the net sodium flux and the short-circuit current (I_{sc}) for each flux period. All of the units are nequiv./min per cm^2. Errors are expressed as the standard error of the mean. In parentheses is given the number of tissues: number of flux periods.

	$J^{Na}_{m\rightarrow s}$	$J^{Na}_{s\rightarrow m}$	J^{Na}_{Net}	I_{sc}
Control	75.1±2.2 (14:58)	64.4±2.7 (14:58)	10.7±3.2 (14:58)	1.9±0.4 (14:58)
Theophylline	58.9±2.0 (15:69)	54.1±2.7 (15:69)	4.8±2.8 (15:69)	18.9±0.4 (15:69)

moving in response to theophylline, simultaneous unidirectional fluxes of ^{22}Na and ^{24}Na were measured in sulfate-based buffer containing 25 mM HCO_3^-. Preliminary results given in Table I show a large difference between the flux of Na from mucosa to serosa ($J^{Na}_{m\rightarrow s}$) and the serosa to mucosa flux ($J^{Na}_{s\rightarrow m}$) under short circuit conditions. The net sodium flux (J^{Na}_{Net}) in the control tissue was significant ($P<0.01$) and in the direction of absorption. Since J^{Na}_{Net} significantly exceeds I_{sc} ($P<0.02$) the movement of at least one additional ion, possibly absorption of HCO_3^- is indicated under these conditions. In contrast, in the presence of theophylline the I_{sc} significantly exceeds J^{Na}_{Net} ($P<0.01$). This observation is consistent with enhanced secretion of an anion such as HCO_3^- following exposure to theophylline.

Since the theophylline-induced increase in I_{sc} is proportional to the bath bicarbonate concentration, is sensitive to very low concentrations of acetazolamide and is not due to increased Na^+ transport in Cl-free buffer we feel the evidence is consistent with stimulation of an electrogenic HCO_3^- secretory mechanism. The response of *Amphiuma* small intestine in vitro appears to be quite similar to in vivo mammalian intestine and promises to be an excellent preparation for further examination of the bicarbonate transport mechanism and intestinal secretory phenomena.

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