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SOMATOSTATIN STIMULATES COUPLED SODIUM CHLORIDE INFLUX ACROSS THE BRUSH BORDER OF THE RABBIT ILEUM

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SUMMARY

Somatostatin has been previously shown to stimulate Na and Cl absorption in the rabbit ileum in vitro, but the transport pathways involved have not been determined. Therefore, we examined the effect of somatostatin on Na and Cl influx across the brush border of the rabbit ileum. Somatostatin increased Na influx from 3.25 to 4.72 μmoles/cm^2·hr ($p<0.001$) and increased Cl influx from 2.90 to 3.90 μmoles/cm^2·hr ($p<0.001$). In the absence of Na, somatostatin had no effect on Cl influx and in the absence of Cl, somatostatin had no effect on Na influx. These findings indicate that somatostatin stimulates the coupled influx of Na and Cl across the brush border of the rabbit ileum.

INTRODUCTION

Somatostatin (SRIF) stimulates Na and Cl absorption in the rabbit ileum and rat colon and blocks diarrhea in patients with the carcinoid syndrome (1-4). Because SRIF is widely distributed throughout the intestine, present in both endocrine cells and nerve tissue, it is of potential physiologic as well as pharmacologic importance in the control of intestinal ion transport. The mechanism by which SRIF stimulates ion transport in the rabbit ileum has not been determined; however, SRIF does not affect cyclic AMP content in the mucosa, nor does it stimulate glucose coupled sodium absorption (1,2,5). Since Na and Cl can be absorbed by a neutral, coupled influx mechanism at the brush border, we proposed, and have demonstrated in the following studies, that SRIF stimulates this influx mechanism (6). These studies represent the first demonstration of stimulation of coupled Na and Cl influx by a hormone or other agent.

Abbreviations used: SRIF = somatostatin; J_{mc} is the unidirectional influx rate from the mucosal solution into the cell; PEG = polyethylene glycol.

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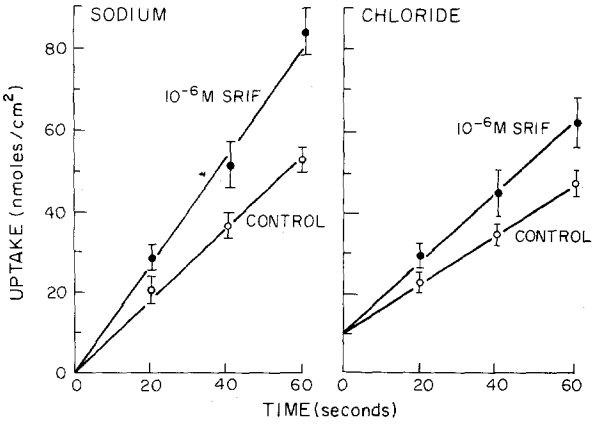

Figure 1. The effect of 10^{-6}M SRIF on Na and Cl influx into isolated rabbit ileum. Rabbit ileum was mounted in Lucite chambers allowing the mucosal and serosal surface to be bathed with oxygenated Ringer solution (in mM, Na, 140; Cl, 119.8; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.4; Mg, 1.2; and Ca 1.2) pH, 7.4 at 37°C. SRIF was added to the serosal solution only, before mounting the tissue. After 20 minutes, the mucosal solution was replaced with a test solution of identical composition containing ^{22}Na, ^{36}Cl and ^{3}H PEG. Uptake was determined over 20-60 sec. time intervals according to the method of Schultz et al (7). Each point represents the mean ± S.E. of 14 tissues from 14 animals.

METHODS

New Zealand white male rabbits were sacrificed with an intravenous air bolus, a section of terminal ileum removed, stripped of its muscle layer and mounted mucosal surface-up in a lucite chamber so that the mucosal and serosal surfaces were bathed with identical oxygenated Ringer solution (in mM, Na, 140; Cl, 119.8; HCO_3, 25; K, 5; HPO_4, 2.4; H_2PO_4, 0.4; Mg, 1.2; and Ca, 1.2) pH, 7.4 at 37°C. SRIF was added to the serosal solution only, before mounting the tissue. After 20 minutes, the mucosal solution was replaced with a test solution of identical composition containing ^{22}Na, ^{36}Cl and ^{3}H PEG. Uptake was determined over 20-60 sec. time intervals according to the method of Schultz et al (7), except that influxes of Na and Cl into the mucosa were determined simultaneously. Na isethionate replaced NaCl in the Cl-free experiments and choline chloride and choline HCO_3 replaced NaCl and $NaHCO_3$ in the Na-free experiments.

RESULTS AND DISCUSSION

Figure 1 shows the effect of SRIF on uptake of Na and Cl into isolated rabbit ileum determined over a period of 60 seconds. Uptake is a linear function of time, indicating that only unidirectional influx is being measured. The unidirectional influx rate from the

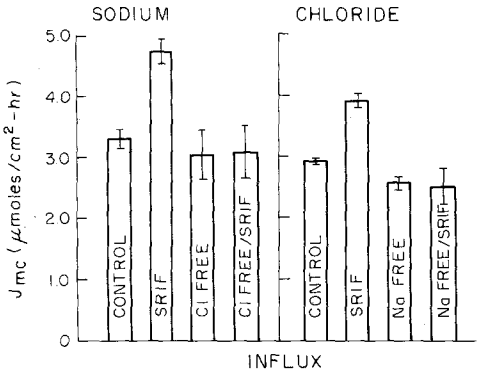

Figure 2. Effect of Cl-free and Na-free media on Na and Cl influx. Experiments were performed in an identical manner as described in Figure 1, except that Na isethionate replaced NaCl in the Cl-free experiments and choline chloride and choline HCO_3 replaced NaCl and $NaHCO_3$ in the Na-free experiments. In the Na and Cl-free experiments, uptake was determined only at 60 seconds and each bar represents the mean ± S.E. of 6 tissues from 6 animals. The control and SRIF bars are derived from the data in Figure 1.

mucosal solution into the cell, J_{mc}, was calculated from the slope. 10^{-6}M SRIF increased Na influx from 3.25 to 4.72 μmoles/cm^2·hr. ($p < .001$), and increased Cl influx from 2.90 to 3.90 μmoles/cm^2·hr. ($p < .001$). The absolute increases in J_{mc}^{Na} and J_{mc}^{Cl} are not statistically different, consistent with the hypothesis that SRIF stimulates coupled influx.

If SRIF stimulates coupled Na and Cl influx, its effect should be abolished by the removal of either ion. Replacement of Cl with isethionate abolished the effect of SRIF on Na influx and replacement of Na with choline abolished the effect of SRIF on Cl influx (Fig. 2).

These findings indicate that the increase in Na and Cl absorption induced by SRIF in rabbit ileum can be partially attributed to the stimulation of a coupled, neutral Na-Cl influx process at the brush border. In rabbit ileum, SRIF decreases short-circuit current and increases transmural tissue conductance (1). The decrease in short-circuit current has been shown to be attributable to an excess of Cl over Na absorption. Thus, one hypothesis for the mechanism by

which SRIF increases electrolyte absorption involves stimulation of coupled Na-Cl influx with back diffusion of Na through cation-selective paracellular channels. Such a mechanism has been proposed to explain electrogenic Cl absorption in the flounder intestine (10). In these experiments we have isolated this influx step and shown that it is indeed critical for the SRIF effect.

The coupled Na-Cl influx mechanism has been shown to be inhibited by both cyclic AMP (theophylline) and non-cyclic AMP mediated (serotonin) secretogogues as well as acetazolamide (8,9). SRIF has been shown to block the effect of theophylline and serotonin on transmural ion transport in the rat colon and thus may do so in part by blocking the inhibitory effect of these agents on neutral Na and Cl influx (2).

The precise mechanism by which SRIF stimulates the coupled Na-Cl influx process is not known. Since SRIF blocks both cyclic AMP and non-cyclic AMP mediated secretogogues, its site of action is probably independent of, or distal to cyclic AMP formation. There is indirect evidence in non-intestinal tissue that somatostatin may modulate intracellular calcium ion concentrations. Studies are currently in progress to determine if SRIF is affecting calcium transport in enterocytes.

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