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EFFECT OF THIOCYANATE ON IONS TRANSPORT ACROSS THE RAT GASTRIC MUCOSA[1]

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ABSTRACT Stripped gastric mucosa of rats was mounted in Ussing chambers and bathed in NaCl, Na_2SO_4 or choline-Cl Ringer solution. There was a potential difference (PD) across the mucosa with the serosa being positive. Addition of D-glucose increased the PD and short circuit current (Isc) with a slight decrease in the resistance. Isotopic ion flux measurement demonstrated that SCN^- inhibited the Cl^- secretion and had very little effect on Na^+ flux. A model was presented to illustrate the proposed site of action of SCN^-.

KEY WORDS thiocyanates; ion transport;

gastric mucosa; sodium (^{22}Na); chlorides(^{36}Cl); D-glucose

Thiocyanate (SCN^-), a complex lipid-soluble anion, is a potent inhibitor of gastric acid secretion. In the resting mammalian stomach, Na^+ is actively absorbed and Cl^- is secreted[1]. The addition of SCN^- to a histamine-Mecholyl-stimulated stomach of dog caused an increase in resistance and a decrease in potential difference to the resting level[2]. SCN^- inhibits active transfer of Cl^- in teleost gills[3] and in frog cornea[4]. Is there a similar mechanism in the resting stomach? The present investigation is to study the effect of SCN^- on resting rat gastric mucosa: a) How does SCN^- affect the active ions transport and electrical properties of the rat gastric mucosa and b) What is the mechanism of inhibition of gastric secretion by SCN^-?

METHOD

Adult male Sprague-Dawley rats were decapitated and the stomach opened along the lesser curvature. The serosal and muscular layers were stripped away and the remaining mucosal epithelium layer (with the muscularis mucosae intact) was divided equally and mounted into 2 identical Ussing chambers. A buffered bathing solution was placed in each side of the chamber and continuously stirred and gassed with 95% O_2 + 5% CO_2. The bathing solution was either NaCl, Na_2SO_4 or choline-Cl Ringer solution. at 37℃. Their compositions were described in our previous intestinal studies[5]. The potential difference (PD), short circuit current (Isc) and resistance (R) across the mucosal membrane were recorded continuously.

^{22}Na and ^{36}Cl were used to measure Na^+ and Cl^- fluxes simultaneously across the short-circuited mucosa membrane. Samples were collected at 30-min intervals and counted in an automatic well-scintillation counter and an automatic liquid scintillation counter.

RESULTS

Electrical properties across the gastric mucosa Using NaCl Ringer solution as the bathing media, a steady PD with the serosa electropositive was observed. Totally 50 rats gastric mucosa were performed. The PD averaged 5.7 ± (SD) 6.2 mV, the Isc averaged 33±39 μA cm^{-2}, and the transmucosal resistance averaged 232 ± 86 ohm·cm^2. When the NaCl solution was replaced by Na_2SO_4 Ringer solution, both the PD and Isc decreased and the resistance increased. But replacement by choline-Cl Ringer solution caused the PD and Isc to drop to a level approaching zero. These results are shown in Fig 1.

Addition of D-glucose to the NaCl Ringer solution in both sides at a concentration of 5.5 m*M* increased the PD and Isc to 10.0±4.7 mV and 45 ± 16 μA·cm^{-2}, respectively. The resistance dropped to 221±69 ohm·cm^2. These effects were proportional to the concentrations of D-glucose added. A typical experiment, in which 22 m*M* D-glucose were added in the

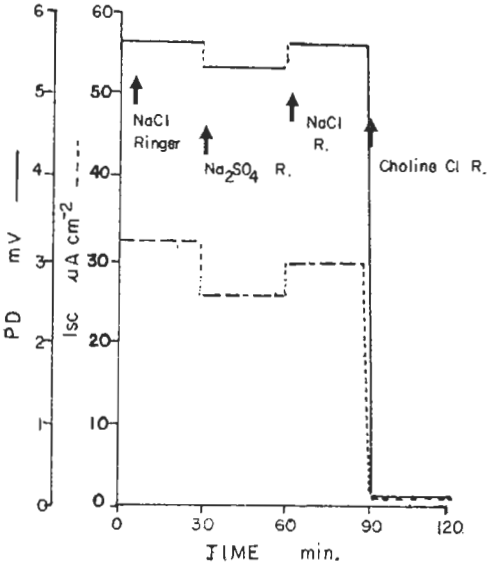

Fig 1. Electrical properties of rat's gastric mucosa. Ordinate is either the potential difference(PD) or the short-circuit current (Isc) oriented with respect to serosal side.

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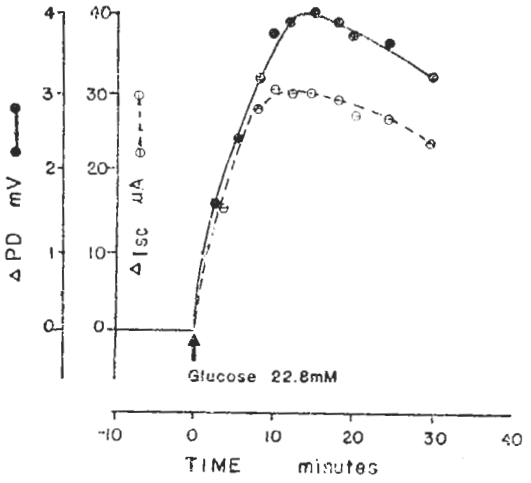

Fig 2. Effect of D-glucose on PD and Isc across the stripped gastric mucosa.

NaCl Ringer solution, is shown in Fig 2. The stimulating effect reached a peak after 10 min and then gradually leveled off.

D-glucose in the bathing solution not only exerted a stimulating effect on the ion transport, but also served as an energy source and increased the lividity of the tissue as much as 2 h or longer. Thus all of the following experiments were conducted in bathing solutions containing 5.5 m*M* D-glucose.

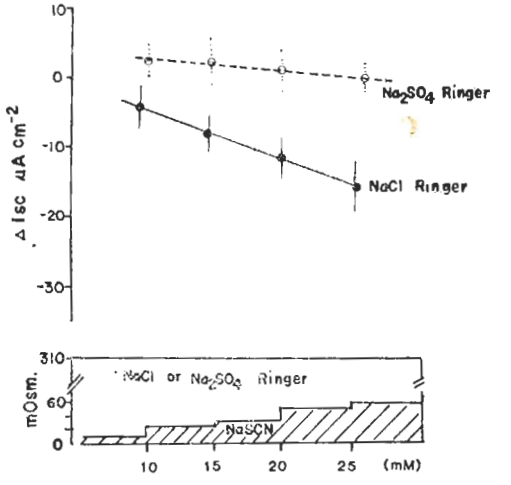

Fig 3. Effect of SCN^- under iso-osmotic condition on Isc across the rat's gastric mucosa. $\bar{x} \pm SD$ of 6–8 rats. Solid line: effect of SCN^- by replacing NaCl in NaCl Ringer solution. Dotted line: in Na_2SO_4 Ringer solution. Abscissa is molar concentration of SCN^- which replaced either NaCl or Na_2SO_4.

Effect of SCN^- In the NaCl Ringer bath, when NaSCN was used to replace NaCl on a mole to mole basis (maintaining an iso-osmolarity at 310 mOsm), a decrease in both PD and Isc was seen with little change in resistance. Such reduction in PD and Isc was linearly proportional to the NaSCN concentration. However, similar replacement of Na_2SO_4 by NaSCN, using the Na_2SO_4 Ringer as bathing solution, produced no reduction in either PD or Isc. The results are summarized in Fig 3.

Sixteen pair experiments were performed to measure the ion fluxes under short-circuited condition. The results are presented in Tab 1. There were a net Na^+ absorption plus a net Cl^- secretion across the rat gastric mucosa. By subtracting the Isc value with these 2 net ion fluxes a negative residual flux, J^R_{net}, was obtained. It was suggested that there was either a H^+ secretion or a HCO_3^- absorption across the gastric mucosa. When SCN^- (20 m*M*) was added to the bathing NaCl Ringer solution a reduction of the Isc and a significant decrease in Cl^- secretion were seen, and the negative J^R_{net} was switched from negative to positive, indicating that there was no H^+ secretion.

DISCUSSION

Data presented here demonstrate that the rat's stripped gastric mucosa exhibits a serosa-electropositive PD and Isc which are similar to that reported in dog [2], guinea pig [6] and monkey [8] as well as that of mice intestine [5]. The PD and Isc were reduced by replacement of the NaCl Ringer bathing solution with Na_2SO_4 Ringer solution and dropped practically to 0 after replacement with choline-Cl Ringer solution (Fig 1). The probable explanation, a combination of active Na^+ absorption and Cl^- secretion, was verified by isotopic measurements. As shown in Tab 1, the negative residual flux, J^R_{net}, also suggests a small quantity of H^+ secretion in the resting stomach. Such findings were also reported by others [6, 7]. The augmentation of both PD and Isc by the addition of D-glucose in the bathing solution

implies a Na^+-glucose co-transport system as seen in the intestine[3].

Replacement of NaCl in the NaCl Ringer bathing solution by NaSCN resulted in a reduction of the PD and Isc across the mucosa. The reduction is linearly proportional to the NaSCN concentration. But the NaSCN had little effect on transmucosal resistance. An increase in transmucosal resistance was proposed[5] to be the cause of the SCN inhibitory action on the histamine-Mecholyl-stimulated stomach. A difference in response between the resting and stimulated stomach cannot be ascertained from these experiments.

As seen in Fig 3, SCN^- reduced neither the PD nor the Isc when the mucosa was bathed in Na_2SO_4 solution. This suggests that the reduction of the PD and Isc is mainly due to a decrease in Cl^- fluxes rather than due to a decrease in Na^+ absorption. Such conclusions are supported by the studies using radioisotopes. As shown in Tab 1, SCN^- 20 m*M* shows little influence on both $J^{Na}_{m\to S}$ and $J^{Na}_{S\to m}$ fluxes or $J^{Cl}_{m\to S}$, but markedly reduced the serosa-to-mucosa Cl^- flux, $J^{Cl}_{m\to S}$. This results in a reduction of net Cl^- secretion from 6.12 to 3.93 μEq h^{-1} cm^{-2} and a reduction in the Isc from 1.51 to 0.75 μEq h^{-1} cm^{-2}.

There are many examples of SCN^- inhibition on halide anion transport, such as Cl^- efflux in the teleost gills[3], I^- uptake in the thyroid gland[9]. Hersey *et al*[10] studying isolated gastric glands reported an inhibition of respiration of the histamine-stimulated glands by SCN^-. They claimed that SCN^- is not acting as competitive antagonist of histamine.

A working model for gastric secretion and

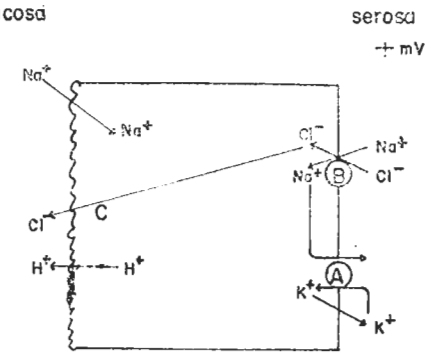

Fig 4. Working model for Na^+ absorption and Cl^- secretion by resting gastric mucosa. Site A is the Na^+–K^+ exchange pump; site B is Na^+–coupled entry of Cl^- from serosa into the cell; site C is the exit of Cl^- at the mucosal side.

SCN^- effect is diagramed in Fig 4. During the short-circuited condition, Na^+ diffuses intracellularly from mucosal side by gradient. In the presence of D-glucose, the rate of Na^+ influx will be increased by the Na^+ glucose co-transport system and by an increase of Na^+–K^+-ATPase pump at the basolateral side (Site A). Cl^- is coupled with Na^+ into the cell at basolateral side (Site B) and then secreted into the mucosal site (Site C). Probably the external K^+ is required for the exchange of Na^+ or Na^+-coupled Cl^- entry at the basolateral (serosal) membrane. Epstein *et al*[3] proposed that SCN^- blocked the K^+-stimulated effect on Na^+ and Cl^- efflux in teleost gills, namely at site B, because they found that SCN^- did not have inhibitory effect on Mg-sensitive ATPase activity. But our results demonstrated that SCN^- did not reduce the PD or the Isc when added to the Na_2SO_4 Ringer bathing solution.

Tab 1. Effect of SCN^- 20 m*M* on ion fluxes across the stripped gastric mucosa of rat. 8 rats/group. $\bar{x} \pm SD$

		$J_{m\to s}$	$J_{s\to m}$ $\mu Eq \cdot h^{-1}\ cm^{-2}$	J_{net}	Isc	J^R_{net}
Control:	^{22}Na	3.3±0.8	2.4±0.7	0.9±0.8	1.5±0.6	−0.82
	^{36}Cl	4.8±0.7	6.1±0.7	−1.3±0.6		
SCN^-:	^{22}Na	3.6±0.4*	2.9±1.0*	0.7±0.9*	0.8±0.4***	1.19***
	^{36}Cl	5.1±0.9*	3.9±0.7***	1.2±0.8***		

J^R_{net} residual flux = Isc − $(J^{Na}_{net} - J^{Cl}_{net})$. Compared to control * $p > 0.05$, *** $p < 0.01$

Under these conditions there is no Na^+-coupled entry of Cl^- at the serosal membrane but still a Na^+-K^+-ATPase exchange pump (Site A). This suggests that SCN^- is not acting at ATPase pump, but rather at site B by blocking the Na^+-coupled Cl^- entry, secondary to reduce the Cl^- exit (at site C) and the H^+ secretion. Isotopic measurements support this conclusion since there is clearly no inhibitory effect on Na^+ flux by SCN^-.

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