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ACTION OF THIOCYANATE ON GASTRIC MUCOSA *IN VITRO*

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SUMMARY

Thiocyanate in addition to its well-known action in inhibiting HCl secretion by the amphibian gastric mucosa has been shown to inhibit ^{42}K flux and the potential difference change due to K^+ or Rb^+ concentration changes in the bathing solutions. Cs^+ does not substitute for K^+ in the stomach. Other secretory inhibitors have similar effects. These data may be interpreted by regarding the mucosa as consisting of two parallel circuits residing in the parietal and surface cells, with the parietal cell being responsible for the major fraction of the ^{42}K movement through the mucosa, which is associated with HCl secretion, and the surface cell possessing the K^+ perm-selective membrane.

INTRODUCTION

Thiocyanate has been intensively studied as an inhibitor specifically of acid secretion by the gastric mucosa[1]. At one time it was thought that its action could be explained by an effect on carbonic anhydrase[2], but since that time it has been shown repeatedly[3] that inhibition of this enzyme affects the Cl^- rather than the H^+ transport mechanism in the secreting mucosa. More recently, an ATPase which has been isolated from various species shows sensitivity to SCN^-, occurs in the microsomal fraction obtained from gastric mucosal homogenates and thus by inference has been implicated in the acid transporting mechanism[4,5].

In addition to its gastric actions, SCN^- has multiple other effects including effects on iodide transport in the thyroid, central nervous system disturbances and generalized effects on histochemical reactivity of membrane enzymes[6].

In efforts to obtain some insight into SCN^- action, O_2 consumption studies of gastric mucosa either in an Ussing chamber, or in the Warburg respirometer have shown that SCN^-, while completely inhibiting acid secretion, has only a gradual effect on O_2 consumption reaching a level of about 10–15 % inhibition at 10 mM and about 40 % at 30 mM final concentration[7]. This observation has raised serious difficulties for any simple redox theory of acid secretion, since ratios of changes in acid secretion rate to changes in O_2 consumption are obtained which greatly exceed the maximum $\Delta H/\Delta O_2$ ratio of 4 predicted by redox theory. However, if one considers that SCN^-

Abbreviation: PD, potential difference.

acts by inhibition of an acid transport ATP-dependent system in the tissue by inhibiting ATP breakdown[4,5], the lack of an effect on O_2 consumption is not explained. One possible explanation is that along with inhibition of membrane ATPase, there is mitochondrial uncoupling. That this is not the case is suggested by the finding that mitochondrial ATPase may also be inhibited by this ion[5], and in direct mitochondrial studies no such uncoupling was observed[8]. Since SCN^- not only does not inhibit Cl^- flux in gastric mucosa (*i.e.*, short circuit chloride) but in fact may be transported by the Cl^- or anion pump[9], we must localize its action to the H^+ transport mechanism, distal to the energy-consuming reaction. This action is equivalent in part to the action of uncouplers in mitochondrial systems whereby the substrate-consuming reactions are uncoupled from the energy utilization pathway of phosphorylation.

The action of SCN^- on the apparent passive permeability of the gastric mucosa to K^+ demonstrated in this paper is reflected in a like action on mitochondrial K^+ movement which is discussed in a subsequent paper.

METHODS

Rana pipiens or Necturus gastric fundic or esophageal mucosa were mounted in an Ussing flux chamber modified as previously described[10]. In a lucite chamber, measurements were routinely made of potential difference (PD), resistance, short-circuit current (I_{sc}) and acid secretion rate using the pH stat technique[11].

When O_2 consumption studies were to be performed, a Kel-F chamber was used, with Clark O_2 electrodes and Radiometer amplifiers either with direct recording, or by an oxystat method[12]. Solution compositions (frog Ringer nutrient, isotonic NaCl secretory) were varied by K^+ substitution for Na^+, SO_4^{2-} or SCN^- substitution for Cl^- and choline substitution for Na^+. In some experiments Rb^+ or Cs^+ replaced K^+. Flux measurements were carried out by standard techniques using ^{42}K (high specific activity) ^{22}Na or ^{36}Cl, and counting was performed in a liquid scintillation counter.

RESULTS

Fig. 1 illustrates the general action of SCN^- on the gastric mucosa. Thus there is a rapid fall of the H^+ rate to zero, at 10 mM SCN^-. With the fall in acid secretion rate there is a maintained rise in resistance and a rise in the PD which may be maintained or may be transient. O_2 consumption falls with the onset of secretory inhibition, by about 20 % in this particular experiment. Although it has been previously reported that addition of SCN^- results in oxidation of cytochrome *c*, in a system where 10 % CO was used to eliminate possible interference by hemoglobin[13], this observation could not be confirmed with any consistency in well-perfused gastric mucosae when CO was not used.

Table I summarizes the results of 20 experiments where H^+ rate, PD and I_{sc} were measured. Additionally Table I summarizes the data obtained for $^{36}Cl^-$ flux measurements in 20 experiments (10 in either direction) and $S^{14}CN^-$ flux measurements (N→S) in 10 experiments. It can be seen that the effect of SCN^- is to reduce the H^+ rate and Cl^- rate by an equivalent amount, leaving the short-circuit chloride unaffected. In addition the exchange diffusion component is also uninfluenced by the presence of thiocyanate. The flux of SCN^- is only a small fraction of the Cl^- flux in the

presence of chloride. When Cl^- is omitted from the bathing solutions and is replaced by SCN^-, there is a marked reduction in I_{sc}, and the flux of SCN^- is much increased, but still falls short of the Cl^- flux under Cl^- conditions. This suggests that the exchange diffusion component (or Cl^- carrier) handles SCN^- much less efficiently than Cl^-.

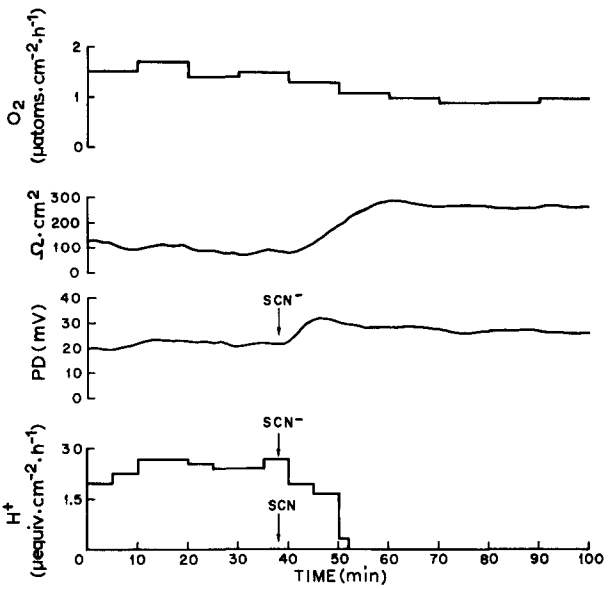

Fig. 1. Effect of 10 mM SCN^- on nutrient side of frog gastric mucosa on H^+, PD, resistance and O_2 consumption.

TABLE I

EFFECT OF SCN^- ON VARIOUS PROPERTIES OF GASTRIC MUCOSA

Values are ± S.E. and except where noted expressed as μequiv. cm^{-2}·h^{-1}.

	Control	*10 mM SCN⁻*	*109 mM SCN⁻*
H^+	3.12 ± 0.28	0	0
I_{sc}	2.51 ± 0.19	2.11 ± 0.13	1.43 ± 0.20
$^{36}Cl^-$ N→S	10.43 ± 0.41	7.10 ± 0.39	—
$^{36}Cl^-$ S→N	5.51 ± 0.29	5.77 ± 0.27	—
$S^{14}CN^-$ N→S	—	0.24 ± 0.03	2.14 ± 0.33
PD (mV)	32 ± 3	41 ± 5	21 ± 2

The change in PD and resistance has been explained as being due to the inhibition of the H^+ transport mechanism. In fact, these changes have been regarded as strong evidence for the electrogenicity of H^+ transport[14].

However, since some workers have regarded the gastric PD as being a result of K^+ and/or Cl^- gradients transmucosally[15], it is also possible that SCN^- had some action on Cl^- or K^+ permeability, measured either chemically or by the PD change resulting from concentration changes in the bathing solutions. In Cl^- solutions it

appears that for Nectures or for *Rana pipiens* the charge permeability of the nutrient membrane can be described by the equation

$$E = \frac{RT}{nF} \ln \frac{[K_i^+] + r\,[Cl_0^-]}{[K_0^+ + r\,[Cl_i^-]}$$

where $r = P_K/P_{Cl}$ (ref. 15).

This is confirmed by the fact that altering the $[K^+]$ and $[Cl^-]$ in the nutrient solutions, product constant, results in a 53 mV change in potential.

The effect of a K^+ and a Rb^+ change from 4 to 20 mM in the nutrient bathing solution is shown in Fig. 2. While the effects of K^+ or Rb^+ were very similar, Cs^+ had little if any effect. Additionally Cs^+ replacing K^+ in the bathing solutions did not support secretion of Necturus or of *Rana pipiens* mucosa. Addition of Cs^+ to K^+-containing solutions did not inhibit secretion.

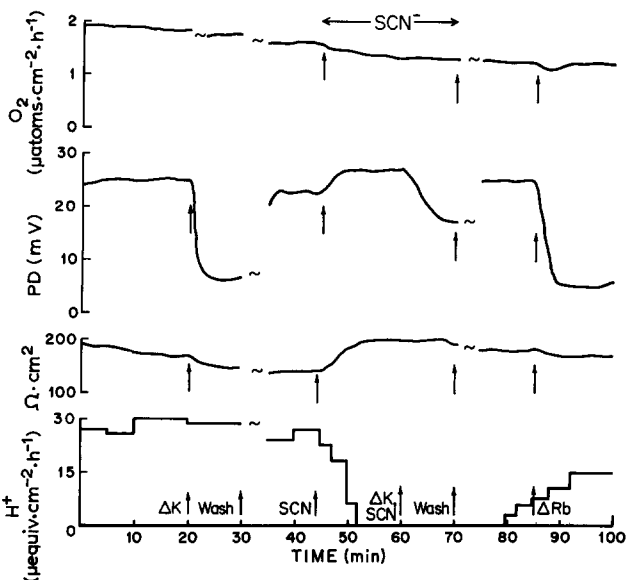

Fig. 2. Effect of changes of K^+ and Rb^+ on the PD, resistance and O_2 consumption of the gastric mucosa and the effect of 10 mM SCN^- on the K^+ induced PD changes.

With SCN^- there appears to be a drastic reduction in both the rate and magnitude of the PD changes. Addition of 10 mM SCN^- on the secretory side has a much lesser effect on the PD change induced by K^+ changes on the nutrient side of the mucosa, which would localize the PD effect to the nutrient membrane of the cells of the gastric mucosa, although the acid mechanism is located on the secretory side.

Since 10 mM SCN^- abolishes the acid secretion rate within 10 min, an attempt was made to distinguish between the effects of SCN^- on K^+ and on H^+ either by using only 1 mM SCN^-, or by K^+ change induced within 1 min of 10 mM SCN^- addition. It was found that even 1 mM SCN^- induced profound changes in the PD response to K^+ addition (30 % suppression).

When Cl^- changes were carried out under similar conditions again there was a significant reduction in the PD response. Table II summarizes the effects of addition

TABLE II

EFFECT OF 5-FOLD CONCENTRATION CHANGES OF K^+ AND 4.5-FOLD CHANGES OF Cl^- ON THE PD OF NECTURUS GASTRIC MUCOSA

Values are mean ± S.E. (number of determinations).

Conditions	*ΔPD (mV)*			
	K^+ 4–20 mM	*Rb^+ 4–20 mM*	*Cs^+ 4–20 mM*	*Cl^- 20–89 mM*
Control	23 ± 4 (10)	25 ± 2 (10)	3 ± 3 (5)	16 ± 3 (5)
ΔPD, mV/min	1.3 ± 0.20	—	—	—
SCN^- (10 mM)	12 ± 2 (10)	11 ± 2 (10)	4 ± 2 (5)	7 ± 4 (5)
ΔPD, mV/min	0.6 ± 0.22	—	—	—
Amytal (10 mM)	15 ± 3 (5)	14 ± 4 (5)	1 ± 3 (5)	—

of 10 mM SCN^- on the PD response to concentration changes of K^+, Rb^+, Cs^+ and Cl^- in Necturus gastric mucosa.

Since it appeared that there might be some relationship between the presence of an H^+ inhibitor and K^+ charge permeability, a series of other inhibitors was tested, such as amytal, dinitrophenol, anoxia, *m*-chlorocyanocarbonylphenylhydrazone. With this class of inhibitors there is also a reduction in the magnitude of the PD changes, although not as drastic as with SCN^-.

Changes of K^+ and Cl^- concentrations, at product constant, result in a mean change of 53 mV for a 10-fold concentration change. In the presence of SCN^- or amytal, for example, significant differences in rate and/or magnitude of PD change are found, with values ranging from 33 to 45 mV.

To amplify the above observations, measurement of K^+ flux across the *Rana pipiens* gastric mucosa was performed using ^{42}K. The conditions chosen were with K^+ gradients across the mucosa of 4–0 mM, 20–0 mM or 89–0 mM K^+, N→S, since it was found that the presence of K^+ on the secretory side had little effect on the K^+ flux.

Table III summarizes the findings on the N→S movement of K^+ under a variety of conditions. Increasing the K^+ gradient results in a marked increase in K^+ flux, as might be expected. Under these conditions simultaneous measurement of N→S $^{36}Cl^-$ flux showed little change, but after 1 h there was an increase in the S→N $^{36}Cl^-$ flux.

TABLE III

N→S MOVEMENT OF K^+

Values are mean K^+ fluxes ± S.E. (number of experiments).

Conditions	*ΔK^+, N→S ($\mu equiv \cdot cm^{-2} \cdot h^{-1}$) [$K^+$] nutrient (mM):*		
	4	*20*	*80*
Control	0.203 ± 0.046 (8)	0.471 ± 0.055 (6)	1.43 ± 0.17 (5)
SCN^-	0.031 ± 0.022 (6)	0.083 ± 0.06 (4)	0.21 ± 0.22 (6)
Amytal	0.094 ± 0.041 (4)	—	0.50 ± 0.38 (4)
Control*	0.100 ± 0.050 (4)*	—	—

* ΔK^+, S→N.

Also included in the table are measurements of the effect of SCN^- at the different K^+ concentrations, and the effect of amytal at the 4 and 80 mM K^+ levels.

Accordingly SCN^- reduces the N→S flux of K^+ almost to zero under all conditions. Amytal and other inhibitors also reduce K^+ flux but not as significantly as SCN^-. An interesting relationship between resistance, inhibitor and K^+ flux may be seen in Fig. 3, which shows the effect of 10^{-6} M *m*-chlorocyanocarbonylphenylhydrazone on the standard parameters of the gastric mucosa, and additionally on N→S K^+ flux. In the phase of increased mucosal resistance, there is diminution of K^+ flux, whereas when the resistance starts to fall, K^+ flux increases.

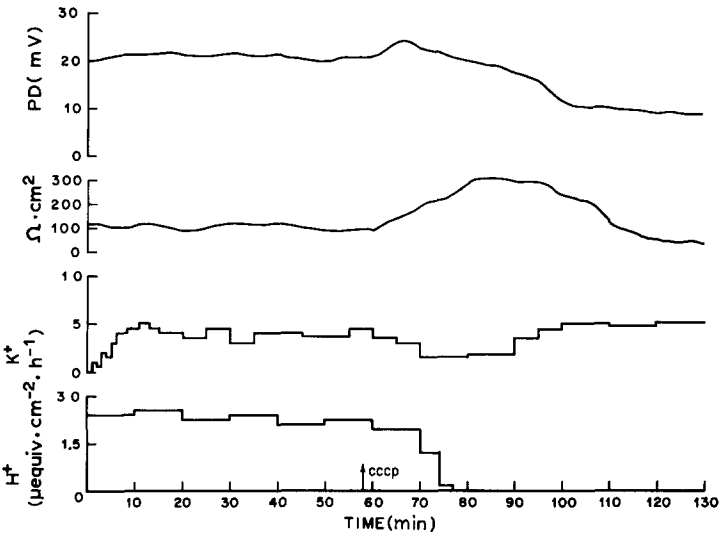

Fig. 3. Effect of *m*-chlorocyanocarbonylphenylhydrazone (CCCP) on H^+, PD, resistance and K^+ flux N→S of gastric mucosa.

Although the above data appear to substantiate a relationship between resistance and K^+ flux, this is not invariably the case, as is illustrated in experiments carried out in collaboration with Dr. W. S. Rehm. It had been previously shown that addition of Ba^{2+} (1 mM) increased resistance approx. 2-fold[16]. This occurs in the absence of any significant change in the H^+ rate, and may be reversed by increase of nutrient K^+ concentration. In spite of this, addition of Ba^{2+} has no effect on measured K^+ flux, although there is reduction of the PD changes in K^+ concentration.

DISCUSSION

The inhibition of HCl transport in the gastric mucosa by SCN^-, as well as the rise of resistance and lack of change of I_{sc} in the chambered preparation, has been well documented[7].

From the data in Table I, there is no significant change in I_{sc}, which reflects nonacidic Cl^- transport, confirmed by measurements of net Cl^- flux. It must be concluded, therefore, that the action of SCN^- is specifically on the HCl component of transport in this tissue, and that SCN^- is without effect on the nonacidic Cl^-

component. Although net SCN^- transport does occur, it is only a small fraction of the Cl^- transport. When Cl^- was omitted from the bathing solutions, and completely replaced by SCN^-, transport of SCN^- increased, but remained at less than 20% of the unidirectional Cl^- movement, showing that both the "active" Cl^- mechanism and the "carrier" presumably responsible for the high calculated Cl^- conductance[17] have relatively low affinity for SCN^-.

Since, therefore, with inhibition of HCl secretion SCN^- does not alter the current produced at zero external potential difference the action of SCN^- does not appear to result in any change in the electrogenicity of gastric mucosal transport. This conclusion, however, does not take into account the transients involved in reaching the final steady state, namely an increase in PD and resistance. One interpretation of this finding has been that these changes are due to inhibition of a primary electrogenic H^+ transport mechanism, but, according to the data presented, this does not contribute to current flow in the secretory steady state. An alternative interpretation is based on the possibility that the potential gradient across the gastric mucosa is due to K^+ and/or Cl^- gradients established across the nutrient surface of the gastric cells. According to this view, alterations in the passive permeability characteristics of the cell membrane to either of these ions would result in a lowering of the tissue conductance, or a rise in the resistance.

Measurement of alterations of PD when appropriate bathing solution concentrations are changed is the simplest method of determining the relative permeability of a membrane to the various ions. This, however, does not give information as to the relative chemical flux rates, since it may not involve passage of charged species across the serosal or mucosal boundary. When K^+ or Cl^- induced potential difference changes were measured in the presence or absence of SCN^-, significant differences were observed. Thus, in particular, the response to K^+ substitution for Na^+ was much reduced, suggesting that the selectivity of the membrane to K^+ with respect to Na^+ had been altered by SCN^-. Measurements of the $^{42}K^+$ flux showed that SCN^-, while inhibiting H^+, also inhibited $^{41}K^+$ flux in the gastric mucosa in the open-circuit condition. Thus SCN^- reduces both "chemical" and "charge" permeability of the gastric mucosa to potassium. This is in contrast to conclusions reached by others using chemical methods to measure K^+ flux[18].

The relationship between the degree of inhibition of acid secretion and K^+ flux was also striking, and the use of other inhibitors of acid secretion, such as amytal, also concomitantly reduced H^+ and K^+ flux. This confirms what has been found in the intact mammalian stomach, namely that there is an association between acid and K^+ secretion[19]. Hence the larger fraction of N→S K^+ movement observed in frog mucosa *in vitro* is associated with parietal cell function. *Necturus esophagus*, which is readily mounted in the Ussing chamber and generates an I_{sc} of between 10 and 25 μA, shows a PD response to change of K^+ which is similar to that observed in the fundus, which contains both surface and parietal cells[20]. Thus at least part of the potential response to K^+ changes is probably a function of the surface epithelial cell.

Accordingly it should be possible to dissociate the K^+ transport properties of the mucosa by selective inhibition of one or the other of the cell types. Ba^{2+}, which has been shown to increase transmucosal resistance, and decrease the K^+ response[16], does not alter the $^{42}K^+$ flux transmucosally. Thus this ion may be affecting primarily the surface cell properties whereas amytal and other metabolic inhibitors affect primarily

the parietal cell. SCN^- would then be unique, since it drastically inhibits both the PD response and $^{42}K^+$ movement, and would thus affect the relative permeability characteristics of both cell types. This is further brought out by the inhibitory effects of low (non-acid inhibitory) concentrations of SCN^- on K^+ potential response when added to the nutrient side.

An alternative interpretation of PD changes due to alterations of K^+ concentrations has been advanced by HOGBEN[21]. He showed that 45 min following a change in K^+ concentration, the S→N Cl^- flux was significantly increased. However, since in SO_4^{2-} solutions K^+ changes also alter the PD, the short-term potential and current changes across the mucosa resulting from changes in K^+ concentration are due to alterations in K^+ transport, not Cl^-. Over longer periods, however, it appears that Cl^- back flux is increased. The current across the tissue, therefore, in the presence of a K^+ gradient is carried by Cl^- and K^+, as our flux data showed.

REHM[16] has shown by the use of equivalent circuits, that alteration of resistance across the secretory membrane would be predicted to alter the PD response to a change in the nutrient membrane electromotive force, hence alteration in PD response to K^+ changes does not necessarily reflect the passive charge permeability of the gastric mucosa. However, measurements of $^{42}K^+$ movement in the case of SCN^- or amytal show that it is reduced. To reconcile these data, it is only necessary to postulate two pathways for K^+ movement across the mucosa, the surface cell and the parietal cell[22]. The surface cell pathway has a high resistance relative to the parietal cell. Alterations of K^+ affect the PD across the nutrient membrane of the surface cell, and the PD change would be given by

$$\Delta \text{ PD} = \frac{-R_{\text{SEC}}}{R_{\text{PC}} + R_{\text{SEC}}} \cdot \Delta E_{\text{SEC}}$$

where R_{SEC} is resistance through surface cell, R_{PC} is parietal cell resistance, ΔE_{SEC} is actual change in surface cell nutrient membrane PD due to K^+ changes and Δ PD is transmucosal PD change. If R_{PC} is altered by SCN^- or amytal and is then of the same order of magnitude as R_{SEC}, evidently Δ PD will decrease, for a given ΔE_{SEC}. Thus agents which alter the electrical resistance of the parietal cell would be expected to alter the PD change across the mucosa in response to K^+ changes. Amytal would be such an agent, and significantly depresses K^+ flux as well as the PD response. SCN^- depresses the K^+ flux even more dramatically and may therefore also affect the surface cell membrane and hence ΔE_{SEC} directly.

From these observations, the resistance changes due to SCN^- action may be due to a more general action of SCN^- on biological membranes, whereby the selective permeability properties are altered. This is likely to be due to the chemical property of SCN^- of being able to participate in halide reactions. Thus if there are anionic binding sites on the nutrient and secretory surfaces of the gastric mucosa such that in Cl^- solutions they normally bind Cl^-, yet have a high affinity for SCN^-, the membrane lattice will be significantly modified by the presence of SCN^-.

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