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EFFECT OF CARBON DIOXIDE UPON THE THIOCYANATE INHIBITION OF HYDROCHLORIC ACID SECRETION IN FROG GASTRIC MUCOSA

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SUMMARY

1. The action of thiocyanate on chloride fluxes, acid secretion rate, and short-circuit current was studied in the isolated gastric mucosa of the frog. SCN^- inhibits the H^+ secretion and the Cl^- flux from the nutrient to the secretory side but not the Cl^- flux from the secretory to the nutrient side. The inhibition is stronger if SCN^- is present on the nutrient rather than on the secretory side.

2. The inhibition of the Cl^- flux from the nutrient to the secretory side becomes more powerful upon raising the partial pressure of CO_2 on the secretory side, but seems to be weakened by raising the partial pressure of CO_2 on the nutrient side.

3. The SCN^- flux from the nutrient to the secretory side during the inhibition has been measured and found too small to be compatible with the view that SCN^- simply competes with Cl^-.

4. The stimulative and preventive effects of CO_2 on the two sides on the SCN^- action have been tentatively interpreted, assuming that carbonic anhydrase participates in the Cl^- transport.

INTRODUCTION

The observations that carbonic anhydrase (EC 4.2.1.1) occurs in the parietal cells, and that acid secretion is stimulated by CO_2, have led to the assumption that CO_2 or HCO_3^-—or both—are in an essential way involved in gastric acid secretion[1,2]. In accordance with such an hypothesis, Diamox, a powerful inhibitor of carbonic anhydrase, is reported to inhibit gastric acid secretion and, more strongly, the electrical activity of the gastric mucosa *in vitro*[3]. On the other hand, a weaker inhibitor of carbonic anhydrase, SCN^-, also inhibits gastric acid secretion very strongly, but without a major effect on the mucosal electromotive force. Since it does so even at concentrations below those required for the inhibition of carbonic anhydrase, its

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effect, though as yet completely unclear, is considered to be fundamentally different from that of carbonic anhydrase inhibitors. DURBIN assumed that SCN^- competes with Cl^- in a reaction leading to the formation of HCl (refs. 4,2). The effect of SCN^- upon Cl^- flux through the gastric mucosa, however, has not yet been precisely examined and observations concerning the effect of SCN^- upon the short-circuit current are somewhat conflicting[5,6]. In the present work, therefore, the effect of SCN^- upon Cl^- flux across the isolated mucosa and its own flux during the period of inhibition were studied. Since it was noticed that the action of SCN^- varies greatly with the partial pressure of CO_2 in the oxygenating gas, the dependence of the Cl^- fluxes on the partial pressure of CO_2 on both sides of the membrane has also been investigated.

METHODS

The isolated gastric mucosa of the frog (*Rana esculenta*) was mounted between two lucite chambers and bathed with oxygenated physiological solutions (Table I). The CO_2 content (P_{CO_2}) of the oxygenating gas varied between 0 and 30%. The nutrient solutions were similar to the secretory ones, except that in order to obtain a pH of 7.3, an appropriate portion of Cl^- was equivalently substituted by HCO_3^- and $H_2PO_4^-$. If the nutrient solution was aerated by pure O_2 its pH was maintained near 7.3 by automatic titration with 0.1 M HCl using a pH-stat device analogous to that used to measure acid secretion as described below.

The temperature of the solution was maintained at 25±0.5°. Acid secretion rate was measured by means of the pH-stat method[3] using a Radiometer titrator TTTIc, Titrigraph SBR2c and Autoburette ABUIa. The pH of the secretory solution was maintained constant at 4.5 throughout the experiments.

The potential difference was measured by two Ingold calomel electrodes and a Knick millivoltmeter MV2U. The mucosae were short-circuited during the experiments by an external current, keeping the potential difference across the mucosa at zero by adjusting the resistance of the Ringer solutions between two electrodes.

TABLE I

PHYSIOLOGICAL SOLUTIONS

The concentrations of the various components are given in mM.

Compound	*Secretory solution*	*Nutrient solutions for various* $P^n_{CO_2}$ *(%)*			
		0	*5*	*20*	*30*
NaCl	102.3	102.3	84.3	30.3	—
KCl	4.0	4.0	3.2	3.2	3.2
$CaCl_2$	1.8	1.8	1.8	1.8	1.8
KH_2PO_4	—	—	0.8	0.8	0.8
$MgSO_4$	0.8	0.8	0.8	0.8	0.8
$NaHCO_3$	—	—	18.0	72.0	102.3
Glucose	—	10.0	10.0	10.0	10.0
Histamine phosphate	0.04	0.04	0.04	0.04	0.04
Total Cl^- concentration	109.9	109.9	91.1	37.1	6.8

The Cl^- flux across the mucosae was measured by labelling Cl^- in the *"cis"* solution with $^{36}Cl^-$. At 1-h intervals, the samples were withdrawn from the *"trans"* solution for counting, using equipment of Frieseke and Höpfner, Erlangen. After a control period of 1 h, NaSCN was added to one of the solutions to give a final concentration of 10 mM. For the determination of SCN^- flux, the SCN^- was labelled with $S^{14}CN^-$. At 1-h intervals, 1-ml samples of the opposite *"trans"* solution were transferred into glass vials, to which 10 ml of ethanol and 10 ml of a phosphor solution (made up of 4 g PPO and 0.1 g POPOP in 1 l of toluene) were added. The ^{14}C radioactivity was counted with a Packard scintillation spectrometer.

TABLE II

EFFECT OF SCN^- ON Cl^- FLUX FROM NUTRIENT TO SECRETORY SIDE, ACID SECRETION AND SHORT-CIRCUIT CURRENT

Fluxes are given as μequiv·cm^{-2}·h^{-1} ($\pm$ standard deviation). $\Phi_{ns}^{Cl^-}$ means Cl^- flux from the nutrient to the secretory side; σ_{H^+}, H^+ secretion rate and $I_{s.c}$ short-circuit current. The figures in parentheses give the number of frogs used. Each value is the average of a 1-h period. The secretory solution was aerated either with 5% CO_2 and 95% O_2, or with 100% O_2. The concentration of SCN^- was 10^{-2} M throughout.

Aerating gas on the secretory side	*Addition of SCN^- to*	*Period*	$\Phi_{ns}^{Cl^-}$	σ_{H^+}	$I_{s.c.}$
5% CO_2 + 95% O_2	Nutrient side	Control	12.6 ± 1.2 (12)	3.4 ± 1.1 (18)	4.3 ± 1.5 (18)
		SCN^-	7.9 ± 1.5	0.1 ± 0.1	5.1 ± 1.0
5% CO_2 + 95% O_2	Secretory side	Control	13.3 ± 1.8 (5)	3.9 ± 1.1 (9)	2.8 ± 1.3 (9)
		SCN^-	10.1 ± 2.0	0.3 ± 0.3	4.8 ± 1.0
100% O_2	Nutrient side	Control	11.1 ± 1.1 (6)	2.3 ± 1.1 (12)	3.2 ± 1.2 (12)
		SCN^-	9.2 ± 1.7	0.2 ± 0.2	5.3 ± 1.6
100% O_2	Secretory side	Control	11.5 ± 0.6 (6)	3.6 ± 1.3 (6)	2.7 ± 1.2 (6)
		SCN^-	12.3 ± 2.0	0.8 ± 0.9	4.7 ± 1.2

RESULTS

Table II shows the effect of SCN^- on the Cl^- flux from the nutrient (n) to the secretory (s) surface ($\Phi_{ns}^{Cl^-}$), the H^+ secretion rate (σ_{H^+}), and the short-circuit current ($I_{s.c.}$). In either case the SCN^-, if added to the nutrient solution, inhibits $\Phi_{ns}^{Cl^-}$ and σ_{H^+} more strongly than if added to the secretory solution. $I_{s.c.}$ is always increased by SCN^-.

The effect of SCN^- upon the Cl^- flux from the secretory to the nutrient surface ($\Phi_{sn}^{Cl^-}$) is shown in Table III. The nutrient side was aerated by a mixture of 5% CO_2 and 95% O_2 throughout, the secretory side by gas mixtures with varying P_{CO_2}. SCN^- was added to the nutrient solution, except in the experiments with 5% CO_2 in which the effect of SCN^- from the secretory solution was studied. In no case was $\Phi_{sn}^{Cl^-}$ appreciably affected by the addition of SCN^-.

TABLE III

THE EFFECT OF SCN^- ON Cl^- FLUX FROM SECRETORY TO NUTRIENT SIDE

The nutrient side was aerated by 95% O_2 and 5% CO_2, the secretory side by a gas mixture with varying CO_2 content. 10^{-2} M SCN^- was added to the nutrient solution, except for the experiments with 5% P_{CO_2} in which the effect of SCN^- from the secretory solution was also studied. Fluxes are given as μequiv $\cdot cm^{-2} \cdot h^{-1}$ ($\pm$ standard deviation). The figures in parentheses give the numbers of frogs used. ${}_sP^s_{CO_2}$ is the partial pressure of CO_2 in per cent of the pressure of the aerating gas mixture on the secretory side.

Period	*Cl^- flux from the secretory to the nutrient side ($\Phi^{Cl^-}_{sn}$)*				
	$P^s_{CO_2} = 0\%$		$P^s_{CO_2} = 5\%$	$P^s_{CO_2} = 20\%$	$P^s_{CO_2} = 30\%$
Control	6.0 $\pm$ 1.5 (6)	5.6 $\pm$ 1.1 (6)	5.3 $\pm$ 1.4* (4)	6.1 $\pm$ 1.5 (4)	6.6 $\pm$ 2.2 (5)
SCN^-	5.8 $\pm$ 1.0	5.4 $\pm$ 1.5	5.6 $\pm$ 0.9*	5.7 $\pm$ 1.5	5.1 $\pm$ 1.5

* SCN^- added to the secretory side.

Table IV shows how varying P_{CO_2} of the aerating gas affects the inhibition of $\Phi^{Cl^-}_{ns}$, σ_{H^+} and $I_{s.c.}$ by SCN^- added to the nutrient solution. When P_{CO_2} is increased up to 20% on either side, $\Phi^{Cl^-}_{ns}$ is also increased except for the 20% column of $P^s_{CO_2}$. When P_{CO_2} is increased to 30%, $\Phi^{Cl^-}_{ns}$ is decreased, suggesting an over-supply of CO_2. The very small values of $\Phi^{Cl^-}_{ns}$ in the fourth rows, where $P^s_{CO_2} = 30\%$, are due to the

TABLE IV

THE EFFECT OF CO_2 PRESSURE ON SCN^- ACTION

The effect of varying the P_{CO_2} of the aerating gas on the inhibition of $\Phi^{Cl^-}_{ns}$, σ_{H^+} and $I_{s.c}$ by SCN^- added to the nutrient solution. Each column refers to a given P_{CO_2} on the secretory side, which increases from left to right; each row refers to a given P_{CO_2} on the nutrient side. In order to avoid numerical confusion, only the mean values of the experiments without the errors are shown. Fluxes are given as μequiv $\cdot cm^{-2} \cdot h^{-1}$. $P^n_{CO_2}$ is the partial pressure of CO_2 in % of the pressure of the aerating gas on the nutrient side. The concentration of SCN^- was 10^{-2} M throughout.

$P^n_{CO_2}$ (%)	*Period*	$P^s_{CO_2}$ (%)											
		0			*5*			*20*			*30*		
		$\Phi^{Cl^-}_{ns}$	σ_{H^+}	$I_{s.c.}$	$\Phi^{Cl^-}_{ns}$	σ_{H^+}	$I_{s.c.}$	$\Phi^{Cl^-}_{ns}$	σ_{H^+}	$I_{s.c.}$	$\Phi^{Cl^-}_{ns}$	σ_{H^+}	$I_{s.c.}$
0	Control	9.7	0.8 (5)	4.0	10.2	2.8 (5)	3.0	14.4	5.5 (1)	3.7	12.3	6.3 (1)	2.0
	SCN^-	6.3	0.1	2.6	5.9	0.3	2.3	2.4	0.1	1.2	0.1	0.2	0.6
5	Control	11.1 (6)	2.3 (12)	3.2 (12)	12.6 (12)	3.4 (18)	4.3 (18)	13.1 (8)	2.7 (12)	4.2 (12)	12.8 (5)	4.1 (10)	3.7 (10)
	SCN^-	9.2	0.2	5.3	7.9	0.1	5.1	7.1	0.1	4.0	6.4	0.2	2.9
20	Control	11.2	4.7 (4)	1.5	14.5	6.9 (1)	1.8	9.6	4.5 (2)	1.9			
	SCN^-	6.5	0.2	4.2	6.8	0.3	4.5	5.4	0.1	2.8			
30	Control	3.0	4.3 (1)	−2.9	2.8 (1)	2.7	−1.5				1.8	3.0 (1)	−1.0
	SCN^-	0.5	0	0.6	0.8	0	0.2				0.6	0.1	0.3

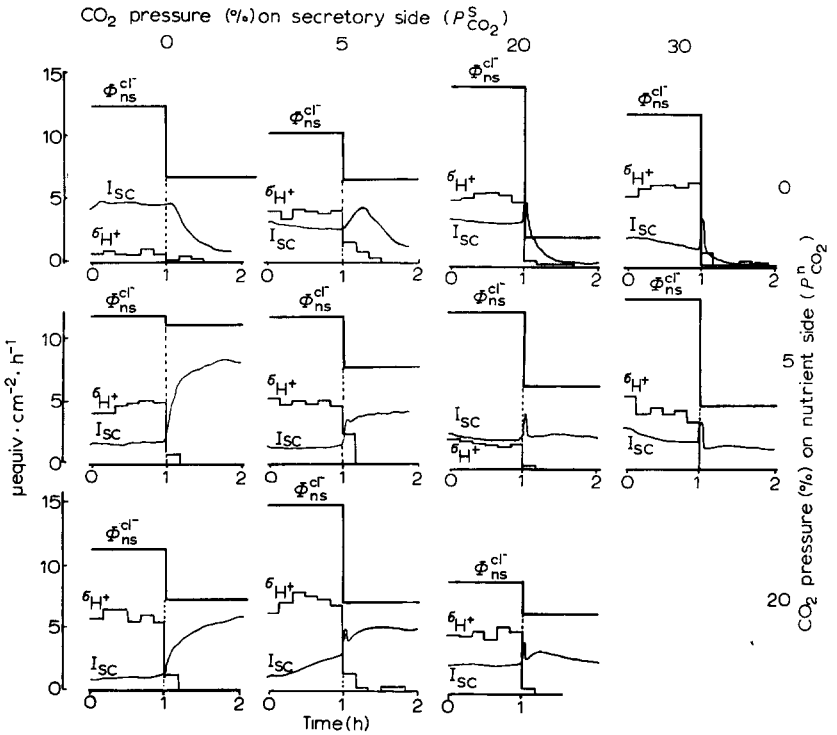

Fig. 1. Time courses of typical experiments, showing Cl^- flux from nutrient to secretory side ($\Phi^{Cl^-}_{ns}$), H^+ secretion rate (σ_{H^+}) and short-circuit current ($I_{s.c.}$) in μequiv·cm^{-2}·h^{-1}. From left to right, the partial pressure of CO_2 on the secretory side ($P^s_{CO_2}$) increases, and from top to bottom that on the nutrient side ($P^n_{CO_2}$) increases. After 1 h SCN^- was added to the nutrient solution to give a final concentration of 10 mM.

low Cl^- concentration in the nutrient solution. The rate of H^+ secretion (σ_{H^+}) also tends to rise with increasing P_{CO_2} on either side, suggesting the necessity of CO_2 supply for the H^+-secretion system. When $P^n_{CO_2} = 30\%$, σ_{H^+} is fairly large, in spite of the low Cl^- concentration, and the sign of $I_{s.c.}$ is reversed. These observations resemble those made with sulfate solutions[7].

The inhibition of $\Phi^{Cl^-}_{ns}$ by SCN^- is greatly enhanced by increasing $P^s_{CO_2}$. This remarkable feature is particularly pronounced in the upper two rows ($P^n_{CO_2} = 0\%, 5\%$). In the first row, $I_{s.c.}$ is always depressed by SCN^-. In the second row, however, $I_{s.c.}$ is increased by SCN^- at low $P^s_{CO_2}$, and decreased at high $P^s_{CO_2}$. In the third row, where $P^n_{CO_2}$ is 20%, $I_{s.c.}$ is stimulated by SCN^- at all $P^s_{CO_2}$ values.

These phenomena are also shown in the figure which gives time courses of typical experiments*. When $P^n_{CO_2} = 0\%$, $I_{s.c.}$ rises transiently after the addition of SCN^- but drops afterwards. This transient rise becomes more prominent with increasing $P^s_{CO_2}$ (from left to right), as is also true for the experimental series where $P^n_{CO_2}$ are 5 and 20%.

It is assumed that during the action of SCN^- some ions other than Cl^- and H^+

* For the sake of convenience, the experiment, where $P^n_{CO_2} = x\%$ and $P^s_{CO_2} = y\%$, was denoted as experiment (x, y).

TABLE V

SCN⁻ FLUX FROM THE NUTRIENT TO THE SECRETORY SIDE

$\Phi_{ns}^{SCN^-}$ is given in μequiv·cm^{-2}·h.

Expt.	*Period (1 h)*		
	1		*2*
1	0.35		0.40
2	0.42		0.45
3	0.42		0.27
	mean	0.38	

—possibly SCN^- or HCO_3^-—might carry the extra current. SCN^- can probably be ruled out because the fluxes of SCN^-, measured with $S^{14}CN^-$ as shown in Table V, are not large enough to account for the extra $I_{s.c.}$ at high $P_{CO_2}^n$. Hence it is supposed that an enhanced flux of HCO_3^- may be responsible for the rise of $I_{s.c.}$, according to the equation:

$$I_{s.c.} = \Phi_{net}^{Cl^-} + \Phi_{ns}^{HCO_3^-} - \Phi_{sn}^{HCO_3^-} - \sigma_{H^+}$$

In the control, both unidirectional bicarbonate fluxes may be balanced, contributing nothing to $I_{s.c.}$ (ref. 2), probably because the HCO_3^- leaking from the nutrient to the secretory side is actively transported back to the nutrient side. However, the addition of SCN^- will disturb this balance, possibly by inhibiting the s→n flux of HCO_3^- while leaving the opposing flux unchanged. Due to the higher concentration of nutrient HCO_3^-, especially in the experiment with higher $P_{CO_2}^n$, $\Phi_{ns}^{HCO_3^-}$ should be large so that $I_{s.c.}$ after inhibition of $\Phi_{sn}^{HCO_3^-}$ should increase appreciably. Accordingly a gradual rise of the secretory pH was occasionally observed after interrupting the H^+ secretion by SCN^-.

DISCUSSION

The fact that SCN^- acts upon $\Phi_{ns}^{Cl^-}$ and σ_{H^+} more strongly from the nutrient than from the secretory side, suggests that the site of the Cl^- transport is more easily accessible from the serosa than from the mucosa. VILLEGAS[8] reported that the barrier sensitive to histamine is close to the mucosal surface whereas another barrier permeable to Cl^- exists near the serosal face of the cells. SCN^- may penetrate the serosal barrier as easily as does Cl^-. But on which of the two cell surfaces the hypothetical pump is located, on which SCN^- may act, is not clear. It was also reported by DURBIN[4] that the rate of HCl production depends critically on the supply of Cl^- to the serosal surface. Thus, although its histological location is still unknown, the "Cl^- pump" may be accessible more easily from the serosal side.

The present results show that secretory CO_2 enhances the SCN^- inhibition of $\Phi_{ns}^{Cl^-}$ and that nutrient CO_2 up to 5% tends to counteract this inhibition. If, however, $P_{CO_2}^n$ is 20%, $\Phi_{ns}^{Cl^-}$ is appreciably inhibited, in seeming contrast to the above conclusion. In this case the nutrient CO_2 may be carried to the secretory side where it augments the inhibitory action of SCN^-.

The potentiating effect upon the SCN^- action of the secretory CO_2 and the preventive one of the nutrient CO_2 would be consistent with the following hypothetical mechanism. On the secretory side CO_2 combines with an OH^- bound by an enzyme. The resulting enzyme–HCO_3^- complex moves to the nutrient side, there exchanging its HCO_3^- for Cl^-. The enzyme–Cl^- complex thus formed returns to the secretory side and exchanges its Cl^- for OH^- from water, yielding HCl. SCN^- would replace HCO_3^- and, less effectively, OH^- on the enzyme*. On the nutrient side the inhibited enzyme does not exchange its SCN^- with Cl^- but rather with HCO_3^- (provided the concentration of HCO_3^- is large enough on that side) thereby regaining its activity to transport Cl^-. In other words, it is supposed that both SCN^- and Cl^- have a strong affinity for the HCO_3^-–enzyme complex, but their replacing HCO_3^- on the enzyme leads to different products, namely the inhibited enzyme and the Cl^--transporting carrier, respectively. In the view of the low values of $\Phi_{ns}^{SCN^-}$, however, the inhibitory action of SCN^- upon the Cl^- flux cannot be simply attributed to a competition between the two ion species. It can only be said that the enzyme has a strong affinity for SCN^-, which is greatly enhanced by the presence of CO_2.

The transient rise in $I_{s.c.}$ by SCN^- suggests that σ_{H^+} is inhibited more rapidly than is $\Phi_{ns}^{Cl^-}$. But which of the two processes, Cl^- transport or H^+ secretion, is the primary one, cannot be decided on the basis of the present study.

The strong influence of P_{CO_2} on the inhibitive action of SCN^- is consistent with an intimate relationship between carbonic anhydrase and the chloride-transport system. Accordingly it has been observed recently in this Institute that $\Phi_{ns}^{Cl^-}$ increases with rising carbonic anhydrase activity.

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* The appreciable inhibition by SCN^- in experiment (o, o), where no exogeneous CO_2 was supplied, would probably be due to the metabolic CO_2.