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SCN^- AND HSCN TRANSPORT THROUGH LIPID BILAYER MEMBRANES

A MODEL FOR SCN^- INHIBITION OF GASTRIC ACID SECRETION

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Diffusion of thiocyanate (SCN^-) and thiocyanic acid (HSCN) (p$K = -1.8$) through lipid bilayer membranes was studied as a function of pH. Membranes were made of egg phosphatidylcholine or phosphatidylcholine plus cholesterol (1:1 mol ratio) dissolved in decane or tetradecane. Tracer fluxes and electrical conductances were used to estimate the permeabilities to HSCN and SCN^-. Over the pH range 1.0 to 3.3 only HSCN crosses the membrane at a significant rate. The relation between the total SCN flux (J_A), concentrations and permeabilities is:

$$1/J_A = 1/P^{ul}([A^-]+[HA]) + 1/P^m_{HA}[HA],$$

where $[A^-]$ and [HA] are the concentrations of SCN^- and HSCN, P^{ul} is permeability coefficient of the unstirred layer, and P^m_{HA} is the membrane permeability to HSCN. By fitting this equation to the data we find that $P^m_{HA} = 2.6$ cm·s^{-1} and $P^{ul} = 9.0 \cdot 10^{-4}$ cm·s^{-1}. Conductance measurements indicate that $P^m_{A^-}$ is $5 \cdot 10^{-9}$ cm·s^{-1}. Addition of cholesterol to phosphatidylcholine (1:1 mol ratio) reduces P^m_{HA} by a factor of 0.4 but has no effect on $P^m_{A^-}$. SCN^- is potent inhibitor of acid secretion in gastric mucosa, but the mechanism of SCN^- action is unknown. Our results suggest that SCN^- acts by combining with H^+ in the mucosal unstirred layer (secretory pits) and diffusing back into the cells as HSCN, thus dissipating the proton gradient across the secretory membrane. A similar mechanism of action is proposed for some other inhibitors of gastric acid secretion, e.g. nitrite (NO_2^-), cyanate (CNO^-) and NH_4^+.

Introduction

Thiocyanate is a potent inhibitor of gastric acid secretion [1], but the mechanism of SCN^- inhibition remains unknown. Hersey et al. [2] recently reviewed and tested various hypotheses for the mechanism of SCN^- action in isolated gastric glands. Their major conclusion was that SCN^- acts primarily at a site dital to the energy consuming step in proton transport, possibly by dissipating a preformed proton gradient.

One possible mechanism of SCN^- action that has not been recently considered is that the active form of SCN^- is actually HSCN (thiocyanic acid), which has a pK of about -1.8 [3]. Although under physiological conditions HSCN exists in extremely low concentrations, its permeability might be sufficiently high to allow HSCN to function as a proton carrier in the gastric mucosa where the primary secretion has a pH < 1. In order to evaluate this hypothesis, it is necessary to know the membrane permeabilities to HSCN and SCN^-.

In this study we measured the permeabilities of planar lipid bilayer membranes to HSCN and SCN^- using tracer and electrical conductance techniques. We found that the permeability of egg phosphatidylcholine-decane bilayers is about 2.6 cm·s^{-1} for HSCN and $5 \cdot 10^{-9}$ cm·s^{-1} for

SCN^-. Our calculations suggest that HSCN formation in the luminal unstirred layer and subsequent diffusion through the luminal (secretory) membrane can explain the inhibition of gastric HCl secretion by SCN^-.

Theory

A membrane and associated unstirred layers are analogous to conductances in series. The equation relating the one-way flux (J_A) of a permeant acid to the concentrations of the nonionic (HA) and ionic (A^-) forms is [4–6]:

$$\frac{1}{J_A} = \frac{1}{P^{ul}([A^-] + [HA])} + \frac{1}{P^m_{HA}[HA]} \quad (1)$$

where P^{ul} is the unstirred layer permeability to A^- and HA, and P^m_{HA} is the membrane permeability to HA. This model assumes that (1) only HA crosses the membrane at a significant rate, (2) chemical reactions between A^-, HA, H^+ and buffer are at equilibrium throughout the unstirred layer, (3) the unstirred layer permeabilities to HA and A^- are similar and (4) aqueous solutions are symmetrical or sufficiently buffered so that no pH gradients exist in the unstirred layers.

Eqn. 1 can be rearranged to give

$$\frac{1}{P^t} = \frac{[A^t]}{[HA]P^m_{HA}} + \frac{1}{P^{ul}} \quad (2)$$

where $[A^t]$ is the total acid concentration, i.e., $[A^-] + [HA]$, and P^t is the total permeability coefficient, i.e., $J_A/[A^t]$. If the assumptions listed above are correct, a plot of $1/P^t$ vs. $[A^t]/[HA]$ will yield a straight line with a slope if $1/P^m_{HA}$ and an intercept of $1/P^{ul}$. Thus, Eqn. 2 provides a convenient way to measure both the membrane and unstirred layer permeabilities.

Materials and Methods

Lipid bilayer membranes were made by the brush technique of Mueller et al. [7]. Membranes were formed from a mixture of egg phosphatidylcholine in decane (25 $mg \cdot ml^{-1}$) or egg phosphatidylcholine (30 $mg \cdot ml^{-1}$) plus cholesterol (15 $mg \cdot ml^{-1}$) (1:1 mol ratio) in tetradecane. Membranes were formed on a 1.8 mm^2 hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each. The aqueous solutions contained NaCl, HCl, NaSCN, and, in some experiments, sodium citrate buffer. Unless otherwise stated, the ionic strength was 0.1 and the NaSCN concentration was 1 mM. Experiments were conducted over a pH range of 1.0 to 5.6. In order to vary the ratio of $[SCN^-]$ to [HSCN], we varied the pH as described by the Henderson-Hasselbalch equation. The pK of HSCN was assumed to be -1.8 [3]. The temperature was 24 ± 2°C.

After a stable membrane was formed, about 5 μCi of ^{14}C-labeled NaSCN were injected into the rear compartment. The rate of appearance of radioactivity in the front compartment was measured by continuous perfusion (1–2 $ml \cdot min^{-1}$) and collection of samples at 3-min intervals. The samples were collected by aspiration into a vacuum trap. During the flux experiment the rear compartment was sampled with a microsyringe. Radioactvity was measured in a liquid scintillation counter.

The one-way flux of solute was calculated by the equation:

$$J_A = \frac{^{14}C^F}{t\,A\,SA^R} \quad (3)$$

where J_A is the flux ($mol \cdot cm^{-2} \cdot s^{-1}$), $^{14}C^F$ is the total amount of tracer (cpm) entering the front compartment during the time interval t (s), A is the surface area of the membrane (cm^2) and SA^R is the specific activity of tracer in the rear compartment ($cpm \cdot mol^{-1}$).

We measured the membrane resistance at 3-min intervals by applying a known voltage pulse across the membrane in series with a known resistance (voltage divider circuit). The membrane potential was recorded as the potential difference between two calomel-KCl electrodes which made contact with the front and rear solutions.

The partition coefficient of thiocyanic acid was determined by repeated extractions of ^{14}C-labeled HSCN into hexadecane. The hexadecane was passed through an alumina column to remove polar impurities. The aqueous phase was HCl (pH = 0.98). The aqueous and hexadecane phases were allowed to equilibrate for 1–3 days with gentle

shaking at 22–23°C. Then the hexadecane was removed and centrifuged to remove traces of the bulk aqueous solution. Aliquots of the hexadecane and aqueous phases were analyzed for ^{14}C in a liquid scintillation counter. The partition coefficient was expressed as the ratio of [HSCN] in hexadecane to [HSCN] in the aqueous phase, which was calculated from the Henderson-Hasselbalch equation.

^{14}C-labeled NaSCN was obtained from ICN (Irvine, CA). Egg phosphatidylcholine was obtained from Avanti (Birmingham, AL) or Lipid Products (Surrey, U.K.). Cholesterol, *n*-decane, *n*-tetradecane and citric acid were obtained from Sigma Chemical Co. (St. Louis, MO). Hexadecane was obtained from Eastman Kodak Co. (Rochester, NY).

Results

SCN^- conductance and permeability

SCN^- produced a moderate (14–31-fold) increase in the membrane conductance (G_m). In 100 mM NaCl G_m ranged from (1.4 to 2.3) $\cdot 10^{-8}$ $S \cdot cm^{-2}$. In 100 mM NaCl plus 20 mM NaSCN, G_m ranged from (3.3 to 4.3) $\cdot 10^{-7}$ $S \cdot cm^{-2}$. SCN^- (20 mM) produced the same G_m regardless of whether the aqueous solution contained 100 mM NaCl, 100 mM HCl, or no electrolyte other than NaSCN. Similar results were obtained with membranes made from either phosphatidylcholine in decane or phosphatidylcholine plus cholesterol (1:1 mol ratio) in tetradecane. The SCN^- permeability coefficient (P_{A^-}) can be calculated from the relation, $P_{A^-} = G_{A^-}RT/cF^2$, where G_{A^-} is the SCN^- conductance, R is the gas constant, T is the absolute temperature, c is the SCN^- concentration and F is the Faraday [8,9]. This calculation yields values of P_{A^-} ranging from (4.4 to 5.8) $\cdot 10^{-9}$ $cm \cdot s^{-1}$.

Our estimated SCN^- permeability falls within the range of values reported recently by Dilger et al. [9], who studied the electrical effects of SCN^- on three types of lipid bilayers. Our value is slightly higher than P_{A^-} in diphytanoylphosphatidylcholine-decane bilayers but about two orders of magnitude lower than P_{A^-} in diphytanoylphosphatidylcholine-chlorodecane bilayers. Dilger et al. [9] also found that G_m was proportional to the first power of the SCN^- concentration, and our measurements (data not shown) agree with their findings. The fact that 20 mM SCN^- produces the same conductance in either 100 mM NaCl or 100 mM HCl rules out the possibility that the conductibe species is a dimer (HA_2^-) formed from SCN^- plus HSCN.

HSCN permeability

HSCN fluxes were measured over a pH range of 1.0 to 3.2. In this series of experiments the solutions were symmetrical except for the addition of tracer to the rear (*cis*) compartment. The total SCN concentration was 1 mM. The total permeability (P^t) is thus equal to $J_A/[A^t]$, where $[A^t] = [A^-] + [HA]$. Fig. 1 shows the permeability data plotted according to Eqn. 2. The linear relationship between $1/P^t$ and $[A^t]/[HA]$ indicates that only HA is crossing the membrane at a significant rate. This conclusion is substantiated by the fact that the maximum values of P_{A^-}, estimated from the membrane conductances, were always less than 0.1% of the observed values of P^t.

Linear regression analysis of the data (solid line in Fig. 1) yields values of $P^m_{HA} = 2.56 \pm 0.06$ $cm \cdot s^{-1}$ and $P^{ul} = 8.98 \cdot 10^{-4}$ $cm \cdot s^{-1}$. This HSCN permeability is very high, roughly similar to the

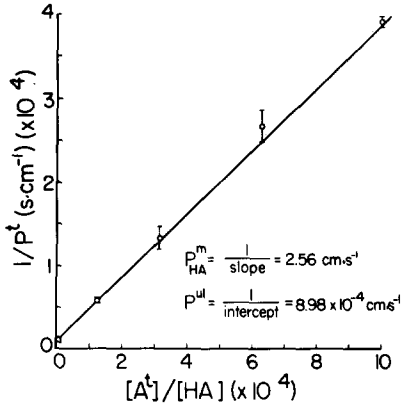

Fig. 1. Reciprocal of total SCN permeability ($1/P^t$) as a function of $[A^t]/[HA]$, plotted according to Eqn. 2. The ratio, $[A^t]/[HA]$, was calculated assuming $pK = -1.8$ [3]. Solutions were symmetrical and buffered with either HCl or citric acid (pH range 1.0 to 3.2). the SCN concentration was 1 mM and ionic strength was 0.1. Error bars indicate the standard deviations of permeability measurements on two or three membranes at each pH. Solid line is calculated by least squares linear regression.

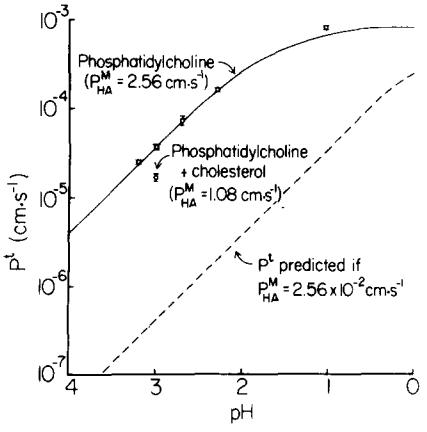

Fig. 2. Total SCN permeability (P^t) as a function of pH. P^t is defined as $J_A/[A^t]$, where J_A is the total flux and $[A^t]=[HA]+[A^-]$. Error bars indicate the standard deviations of two or three membranes at each pH. The solid line is calculated from Eqn. 2, assuming $P^m_{HA}=2.56$ cm·s^{-1} and $P^{ul}=8.98\cdot10^{-4}$ cm·s^{-1}. Dashed line is calculated from Eqn. 2, assuming $P^m_{HA}=2.56\cdot10^{-2}$ cm·s^{-1} and $P^{ul}=8.98\cdot10^{-4}$cm·s^{-1}.

permeabilities to HCl, CO_2 and salicylic acid [5,6,10]. The value of P^{ul} can be used to estimate the unstirred layer thickness, which is defined operationally as D/P^{ul}, where D is the HSCN diffusion coefficient in water. Assuming that $D=1.3\cdot10^{-5}$ cm$^2\cdot$s^{-1}, we estimate a combined unstirred layer thickness of 145 μm, similar to the value obtained by ourselves and others in planar lipid bilayer systems [5,11].

Fig. 2 shows P^t as a function of pH. the solid line is calculated from Eqn. 2, assuming that $P^m=2.56$ cm·s^{-1} and $P^{ul}=8.98\cdot10^{-4}$ cm·s^{-1}. At pH > 2, the total permeability is controlled primarily by the membrane permeability to HSCN. Thus, P^t is proportional to $[H^+]$ because [HSCN] is proportional to $[H^+]$. At pH < 1, the total permeability is controlled primarily by P^{ul}. Thus, P^t 'saturates' at a value equal to P^{ul}, approx. 10^{-3} cm·s^{-1}.

Membranes made from cholesterol plus phosphatidylcholine (1:1 mol ratio) had a P^t value of $(1.72\pm0.58)\cdot10^{-5}$ cm·s^{-1} at pH 3.0 (Fig. 2). Since this P^t value is $\ll P^{ul}$, no unstirred layer correction is necessary. Thus, we can estimate P^m_{HA} by dividing P^t by the fraction of nonionized HSCN, i.e., $1.58\cdot10^{-5}$. This yields a value of $P^m_{HA}=1.09$ cm·s^{-1}, about 41% of P^m_{HA} for phosphatidylcholine bilayers. The reduction in HSCN permeability caused by cholesterol is similar to the effect of cholesterol (1:1 mol ratio) on the permeability to other small molecules, e.g., water [12], HCl [10], HNO_3 and HF [13].

The greatest source of error in our values of P^m_{HA} is the uncertainty in the value of pK, which was assumed to be -1.8 [3], although values ranging from -0.7 to -2.0 have been reported [14]. However, the measured values of P^t shown in Fig. 2 are accurate regardless of the assumed pK, because P^t is based on the total concentration, i.e., $[SCN^-]+[HSCN]$. Thus, the values of P^t can be used to predict the total SCN flux as a function of pH in biological situations, e.g., gastric mucosa, assuming the lipid bilayer to be a reasonable model of the lipid barrier in biological membranes.

HSCN partition coefficient and predicted permeability

The partition coefficient (K_p) of HSCN into hexadecane was 0.464 ± 0.037. Orbach and Finkelstein [15] have shown that the permeability of a variety of small nonelectrolytes through egg phosphatidylcholine-decane bilayers is proportional to the first power of $K_p\cdot D$, where K_p is the hexadecane/water partition coefficient and D is the diffusion coefficient of the solute in water. Using the empiracle relationship established by Orbach and Finkelstein [15] and assuming that $D_{HSCN}=1.3\cdot10^{-5}$ cm$^2\cdot$s^{-1} (similar to acetamide), we predict the HSCN permeability to be 2.4 cm·s^{-1}, similar to our measured value (Fig. 1). The predicted relationship between P^m_{HA} and K_pD is not affected by the assumed value of pK, provided that the same pK is used for calculating both P^m_{HA} and K_p.

Effect of unstirred layer pH on HSCN flux

Fig. 2 shows that the total SCN permeability is proportional to $[H^+]$ at pH > 2. Since we wish to relate these results to HSCN fluxes in localized regions of gastric mucosa, i.e., the unstirred layer, we did one experiment to show, in a crude way, how H^+ 'secretion' into an unstirred layer can stimulate the HSCN flux in the opposite direction. In this experiment the *cis* side of the membrane was bathed by a solution containing unbuffered NaCl (150 mM) plus NaSCN (5 mM), pH 5–6. The *trans* solution contained sodium citrate buffer (150 mM, pH 5.0) with or without sodium salicy-

late (40 mM). We have shown previously [5] that under similar conditions a large net flux of salicylic acid ($pK = 2.9$) produces pH gradients in the unbuffered unstirred layer. In the present experiment we found that the presence of salicylic acid in the trans solution caused a roughly 10-fold increase in the oppositely directed net SCN flux, i.e., the flux increased from 61 ± 6 to 721 ± 115 pmol $\cdot$ cm$^{-2} \cdot$ s^{-1}. This increase in net SCN flux is caused by a decrease in pH in the cis unstirred layer which results in HSCN formation, some of which diffuses through the membrane into the *trans* solution. Although we cannot relate these results quantitatively to the gastric mucosa, we note that the anatomy of the secretory surface and the presence of a mucous layer would result in unstirred layers thicker than those existing in a planar lipid bilayer system [16,17].

Discussion

Our data show that HSCN is a highly permeant species with permeability coefficients ranging from 1.1 to 2.6 cm $\cdot$ s^{-1} in two types of lipid bilayer membranes. Assuming that the lipid bilayer is a reasonable model for the lipoid region of a biological membrane, we can make some predictions about the ability of SCN^- to act as a proton carrier in the gastric mucosa. In experiments on isolated gastric mucosa and gastric glands, SCN^- is usually applied at a concentration of about 10 mM [2,17–19]. Thus, from a knowledge of P^t (Fig. 2) and [SCN^-] we estimate that over a pH range of 4 to 1 the total HSCN flux will range from about $4 \cdot 10^{-11}$ to $1 \cdot 10^{-8}$ mol $\cdot$ cm$^{-2} \cdot$ s^{-1}.

In order for SCN^- to function as an effective proton carrier across the secretory surface of gastric mucosa, the pH near the membrane must be much lower than the pH of the bulk mucosal solution, which is usually above 4 in experiments on isolated gastric mucosa or gastric glands [2,17–19]. In other words, the reaction between secreted H^+ and SCN^- must occur in an unstirred layer adjacent to the secretory surface. The anatomy of the secretory pits plus the presence of a mucous layer ensures that a large unstirred layer exists at the secretory surface [16,17]. Thus, the luminal surface of the oxyntic cells in secretory pits is actually bathed by an HCl solution of pH approx. 1.0 [17].

If HSCN can dissipate the H^+ gradient across the secretory membrane of gastric mucosa, then the predicted HSCN flux must be equal to or greater than the maximum rate of H^+ secretion. According to Kidder [17], this rate is about $1.4 \cdot 10^{-9}$ mol $\cdot$ cm$^{-2} \cdot$ s^{-1}, which should be corrected by a factor of about 130 to allow for the highly invaginated secretory surface [17,20]. Thus, the maximum rate of H^+ secretion is about $1.1 \cdot 10^{-11}$ mol $\cdot$ cm$^{-2} \cdot$ s^{-1}, which is much smaller than the predicted HSCN fluxes over the pH range 1–4.

The HSCN permeability of the secretory membrane is not known, but it may be substantially lower than the permeability of a phosphatidylcholine-decane bilayer. In pig gastric microsomes the cholesterol:phospholipid mol ratio is about 2:1 [21], which suggests a rather low permeability to small nonelectrolytes. The addition of cholesterol to phosphatidylcholine (1:1 mol ratio) reduces the HSCN permeability by a factor of 0.41 (Fig. 2). Furthermore, when the cholesterol:phosphatidylcholine ratio is 4:1, the permeabilities to a variety of small nonelectrolytes are reduced by a

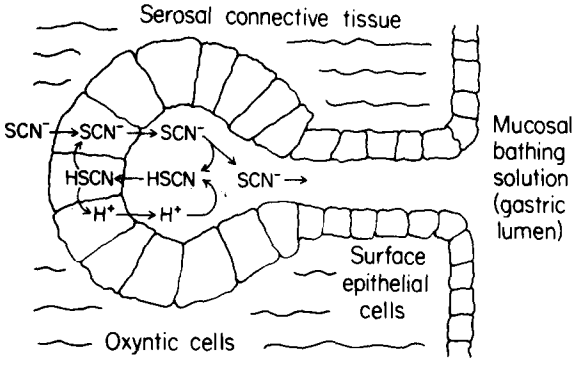

Fig. 3. Model for SCN^- inhibition of gastric H^+ secretion. SCN^- added to serosal (nutrient) solution enters cell via Cl^- pathway (Cl^-/HCO_3^- countertransport of NaCl cotransport). SCN^- crosses secretory membrane via Cl^- pathway (conductive diffusion, active transport or KCl cotransport). In the luminal solution (low pH), some of the SCN^- combines with secreted H^+ to form HSCN. Since the path length for diffusion into the cell is much shorter than the distance to the mucosal bathing solution, most of the HSCN diffuses down its concentration gradient into the cell, thus dissipating the H^+ gradient. The schematic diagram of gastric mucosa is redrawn from Kidder [17].

factor of about 0.12 [11]. Sachs et al. [22] estimated the water permeability of isolated gastric vesicles to be 10^{-5} cm · s^{-1}, two orders of magnitude lower than the water permeability of a phosphatidylcholine-decane bilayer [15]. If the HSCN permeability of the secretory membrane is also 100 times lower than our value for phosphatidylcholine bilayers, then the solid line in Fig. 2 will be shifted to the right by 2 pH units (dashed line in Fig. 2). In this case, the predicted HSCN flux will exceed the maximum rate of H^+ secretion if the pH at the secretory surface is in the range of 1–2.5, assuming a total SCN concentration of 10 mM.

Hersey et al. [2] have shown that SCN^- acts at a site distal to the energy consuming step in acid secretion, and they raised the possibility that SCN^- acts by dissipating a preformed proton gradient across the secretory membrane. Our results support this 'protonophore' model of SCN^- action and suggest further that HSCN formation and diffusion are essential steps in H^+ gradient dissipation. Our 'protonophore' model of SCN^- action is shown schematically in Fig. 3.

Since SCN^- inhibits acid secretion when added to the serosal (nutrient) solution, we must postulate that SCN^- moves rapidly through both the serosal and mucosal membranes. In order to function as an effective 'protonophore', SCN^- must be secreted into the luminal solution where it combines with a secreted H^+ and then diffuses back into the oxyntic cell as HSCN. The lipid bilayer permeability to SCN^- is much too low ($5 \cdot 10^{-9}$ cm · s^{-1}) to allow SCN^- to diffuse across the tissue by simple diffusion. However, SCN^- movement from serosal to mucosal solutions probably occurs by the same mechanism as Cl^- secretion, which involves Cl^-/HCO_3^- countertransport and/or NaCl cotransport through the serosal membrane and active transport, conductive diffusion or KCl cotransport through the mucosal membrane [17,22–25]. SCN^- is known to be actively transported in the gastric mucosa [27], which contains a relatively nonspecific anion transport system [28]. Furthermore, a recent survey indicates that in most biological membranes $P_{SCN^-} > P_{Cl^-}$ [26].

In addition to the requirement for rapid SCN^- movement from serosal to mucosal solutions, we would also expect that the effectiveness of SCN would be increased by adding it to the mucosal solution where it has direct access to the secretory surface. In fact, Kidder [17] has shown that SCN^- is much more effective when added to both serosal and mucosal solutions.

Our model also explains why imidazole (and other permeant weak bases) prevent SCN^- from inhibiting acid secretion [19]. Since imidazole has a pK of 7.0, it buffers the secreted H^+ and largely prevents the formation of HSCN in the mucosal unstirred layer. Our model can also explain the effects of SCN^- and imidazole on intracellular pH, which increases from about 7.4 in the resting state to 8.0 during acid secretion [29]. We suggest that HSCN forms in the secretory pits and diffuses back into the cells, thus lowering intracellular pH to about 7.1 [29]. We believe that imidazole prevents HSCN formation in the secretory pits and thus prevents the acid shift in intracellular pH [30].

SCN^- has variable effects on H^+ accumulation in isolated gastric vesicles, which contain an ATP-driven H^+/K^+ exchange pump [22,25]. Although SCN^- (20 mM) almost totally inhibits H^+ accumulation in frog gastric vesicles [31], the inhibition is usually about 40% in hog gastric vesicles [32,33]. We believe that the variable effectiveness of SCN^- is related to the fact that the minimum internal pH in gastric vesicles is about 2.5 [25,33], much higher than the minimum pH of about 1.0 in the secretory pits of gastric mucosa. Thus, the maximum [HSCN] in vesicles is only about 3% of [HSCN] in the secretory pits, which could account for the reduced effectiveness of SCN^- in the vesicle system. Another important factor is the surprisingly low anion permeability of the vesicle membranes [22]. SCN^- must be added 10 min prior to ATP in order to produce maximum inhibition of H^+ accumulation [31,33]. The preincubation period allows SCN^- to equilibrate with the vesicle interior before H^+ uptake begins. Considering the several basic differences between gastric vesicles and the intact secretory membrane, we believe the mechanism of SCN^- inhibition is the same in both systems, i.e., dissipation of the H^+ gradient by HSCN diffusion.

One argument cited as evidence against the 'protonophore' model of SCN^- action is that

SCN^- does not increase the electrical conductance of gastric mucosa [2]. However, the expected increase in tissue conductance is based on analogy with classical weak acid uncouplers which are characterized by anionic permeabilities several orders of magnitude higher than the SCN^- permeability [34]. Although SCN^- does increase the conductance of lipid bilayers, the absolute conductance is too low to produce a significant increase in the conductance of gastric mucosa. For example, at 20 mM SCN^- the lipid bilayer conductance is about $4 \cdot 10^{-7}$ S $\cdot$ cm^{-2}, compared to the gastric mucosa conductance of about $5 \cdot 10^{-5}$ S $\cdot$ cm^{-2}. (This value includes a 130-fold correction factor for the invaginated secretory surface [17].)

Our model does not explain why SCN^- usually causes a decrease in the conductance of isolated gastric mucosa [18,19]. Our results show that HSCN transport is electrically silent and SCN^- conductance is relatively small. Hersey et al. [2] have pointed out that SCN^- has more than one effect on gastric mucosa. For example, SCN^- (10–30 mM) inhibits respiration in isolated mitochondria and gastric glands [2,35]. SCN^- is also known to adsorb to lipid bilayer membranes and produce negative surface potentials [36]. Thus SCN^- may alter the ionic permeability of the serosal and/or mucosal membranes. Whatever the mechanism of the conductance decrease, we postulate that this is a secondary effect not directly related to the 'protonophore' action of SCN^-. In support of this idea is the observation that permeant weak bases can reverse SCN^- inhibition of H^+ secretion without altering the effect of SCN^- on tissue conductance and potential difference [19].

In 1964 LeFevre et al. [18] considered the possibility that SCN^-, NO_2^- and CNO^- inhibit acid secretion by neutralizing secreted H^+ and then diffusing back into the oxyntic cells in the nonionic form. However, they rejected this hypothesis on the grounds that HSCN is a strong acid which is 'completely' dissociated in the luminal solution. HNO_2 and HCNO are weak acids with pK values of 3.2 and 3.5, respectively [14]. Thus, these acids would be largely nonionized in the tubular lumina of gastric mucosa. Although the membrane permeabilities to HNO_2 and HCNO are unknown, they are probably higher than the permeability to HNO_3, which is about 10^{-3} cm $\cdot$ s^{-1} [13], similar to the water permeability. Thus, HNO_2 and HCNO might function as proton carriers in the same manner we have proposed for HSCN. Since our results show that HSCN produces large proton fluxes even when $>99.9\%$ dissociated, we believe that the hypothesis rejected by LeFevre et al. [18] is, in fact, the correct explanation for the effects of SCN^-, NO_2^- and CNO^- on gastric mucosa.

LeFevre et al. [18] also found that NH_4^+ (10 mM) completely inhibits acid secretion when added to the serosal solution. We suggest the following explanation for the NH_4^+ effect. At pH 7.5 and 10 mM NH_4^+, the NH_3 concentration is about 0.2 mM (p$K = 9.2$). The permeability of serosal and mucosal membranes is assumed to be about 10^{-2} cm $\cdot$ s^{-1}, similar to the NH_3 permeability of mammalian red cell membranes [37] and phosphatidylcholine bilayers (Walter, A., unpublished data). Thus, NH_3 crosses the gastric mucosa at a rate of about (10^{-2} cm $\cdot$ s^{-1}) ($2 \cdot 10^{-7}$ mol $\cdot$ cm^{-3}) $= 2 \cdot 10^{-9}$ mol $\cdot$ cm^{-2} $\cdot$ s^{-1}, substantially faster than the rate of H^+ secretion. In the secretory pits NH_3 combines with H^+ to form NH_4^+. As long as the pH in the luminal solution is more acidic than the cytoplasmic pH, NH_3 diffuses rapidly into the secretory pits, which act as a leaky 'trap' for NH_4^+. Some of the NH_4^+ then reenters the cells in competition with K^+ via the H^+/K^+ exchange pump [25,32]. Thus, cyclic movements of NH_3 and NH_4^+ dissipate the H^+ gradient across the secretory membrane in a manner analogous to the proposed mechanism for SCN^- and HSCN (Fig. 3). If NH_4^+ is added to the mucosal solution, significant trapping of NH_4^+ does not occur because the pH gradient is in the wrong direction to cause NH_4^+ accumulation in the secretory pits. Thus, mucosal NH_4^+ is a less effective inhibitor than serosal NH_4^+ [18].

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