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Evidence for a simple Cl^- conductance pathway in nutrient membrane of frog stomach

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A decrease in nutrient Cl^- results in an increased negativity of the nutrient relative to the secretory side. The possibility emerged that Cl^- transport could be attributed to a neutral mechanism involving Cl^- in the nutrient membrane coupled to a simple Cl^- conductance pathway in the secretory membrane. The decrease in PD (potential difference) with a decrease in nutrient Cl^- could arise from a decrease in cellular Cl^- so that the ratio of Cl^- in cell to Cl^- in secretory solution was decreased. Experiments were designed to determine whether there was a need to assume a simple Cl^- conductance pathway. A 10-fold decrease in Cl^- gave in HCO_3^--containing nutrient solutions a PD decrease of 20 mV, in HCO_3^--free nutrient solutions, a PD decrease of 13.5 mV, and in HCO_3^--free and Na^+-free solutions, a PD decrease of 6.7 mV. The decrease of 6.7 mV could not be attributed to a neutral Cl^-–HCO_3^- exchanger or a NaCl symport. Also there was no evidence for a KCl symport from changes in Cl^- in presence and absence of K^+. It followed that the decrease of 6.7 mV provided evidence for a simple Cl^- conductance pathway in the nutrient membrane.

Introduction

Muallem et al. [1] reported that "no evidence was found for the presence of a Cl^- conductance in either cells or vesicles..." in the basolateral membrane of rabbit oxyntic cells. They indicated that Cl^- transport could be explained by a Cl^-–HCO_3^- exchanger. Paradiso et al. [2] explained Cl^- transport on the basis of Cl^-–OH^- or Cl^-–HCO_3^- exchange in gastric glands of rabbit stomach. In contrast, in the intact frog stomach, Cl^- transport was explained previously by a simple Cl^- conductance pathway [3] and more recently by both a simple Cl^- conductance pathway and an electrogenic NaCl symport in the nutrient (basolateral) membrane of the frog gastric mucosa [4–6]. The possibility of a neutral NaCl symport has also been considered [5,7]. Previously a neutral Cl^-–HCO_3^- exchange mechanism was postulated for the frog gastric mucosa [8] but no attempt was made to incorporate this exchange system into an explanation of the PD response due to a change in nutrient Cl^- concentration.

The question then arises whether there is a need to postulate a simple Cl^- conductance pathway in the nutrient membrane of the frog gastric mucosa. Might not the decrease in nutrient Cl^- leading to an increase in the negativity of the nutrient (basolateral) side relative to the secretory (apical) side of the frog stomach be explained by a combination of a neutral mechanism involving Cl^- in the nutrient membrane and a simple Cl^- conductance pathway in the secretory membrane? As an example, if there were a neutral Cl^-–HCO_3^- exchange in the nutrient membrane, then a decrease in Cl^- in the nutrient solution would lead to a decrease in cellular Cl^- via the Cl^-–HCO_3^- exchanger. If then there is a conductance pathway for Cl^- in the secretory membrane [9,10], the ratio of Cl^- in the cell to Cl^- in the secretory solution would be decreased. The latter decrease would then account for the increase in negativity of the nutrient relative to the secretory solution.

In the present study, we used ion substitution for Cl^- in which the decrease in Cl^- was achieved by replacement of Cl^- with the relatively impermeant ion, SO_4^{2-}. The PD response due to a 10-fold decrease in nutrient Cl^- was examined in the absence of HCO_3^-, in the absence of HCO_3^- and Na^+ and in the absence of K^+. These experiments, as reported herein, enabled us to conclude that there is evidence for a simple Cl^- conductance pathway in the nutrient membrane.

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Methods

Experiments were performed on fundi of stomachs of *Rana pipiens* by an in vitro method in which the stomachs were mounted between a pair of cylindrical chambers [11]. All experiments began with standard Cl^- solutions on both sides of the gastric mucosa. The Cl^- nutrient (serosal) solution contained (in mM): Na^+, 102; K^+, 4; Ca^{2+}, 1; Mg^{2+}, 0.8; Cl^-, 81; SO_4^{2-}, 0.8; HCO_3^-, 25; phosphate, 1; and glucose, 10; and the new Cl^- standard secretory (mucosal) solution which is hypertonic [12] contained: Na^+, 156; K^+ 4; and Cl^-, 160. In decreasing nutrient Cl^- from 81 to 8.1 mM, Cl^- was replaced with SO_4^{2-} and sucrose was added to maintain osmolality. On changing to a HCO_3^--free nutrient solution, both sides of the mucosa were always gassed with 100% O_2 and a phosphate buffer was present in the nutrient solution to maintain the pH constant at 7.2 or 7.3. In this case, the 25 mM HCO_3^- normally in the nutrient solution was replaced with 11 mM HPO_4^{2-}; 3 mM $H_2PO_4^-$ and 11 mM sucrose. In Na^+-free experiments, the Na^+ on both sides of the mucosa was replaced with choline and, in K^+-free experiments, the K^+ on both sides of the mucosa was replaced with Na^+.

In these experiments, the transmembrane resistance, the transmembrane potential difference (PD) and the H^+ secretory rate were measured. Two pairs of electrodes were used, one for sending current across the mucosa and the other for measuring the PD. The PD is considered positive when the nutrient side is positive relative to the secretory side of the stomach. The resistance was determined as the change in PD per unit of applied current. Current (20 μA per 1.3 cm^2 of tissue area) was applied for 1 or 2 s, first in one direction and 2 or 3 s later, in the other direction. The H^+ secretory rate was determined by the pH stat method of Durbin and Heinz [13]. The pH of the secretory solution during secretion was maintained generally between 4.7 and 5.0.

In the change of the concentration of Cl^- in the nutrient solution, due to the existence of a diffusion barrier between the nutrient solution and the nutrient membrane, it takes about 10 minutes (approx. five time constants) for the concentration of the ion at the cell membrane to attain the new concentration in the nutrient solution [3]. In the present experiments, the PD change at the 10-min mark was recorded.

Results

PD responses and resistance changes due to nutrient Cl^- changes in HCO_3^--free and in HCO_3^--free, Na^+-free solutions

From the Introduction, it is evidence that the two most likely neutral mechanisms which could account for the decrease in PD with a decrease in nutrient Cl^- are the Cl^-–HCO_3^- exchanger and the NaCl symport. If a PD response due to Cl^- resulted in the absence of HCO_3^- and Na^+, then this response would have to be attributed in all likelihood to a simple Cl^- conductance pathway.

Fig. 1 is a representative experiment in which, in the first part, there is no HCO_3^- and, in the second part, there is again no HCO_3^- and in addition no Na^+ in both the nutrient and secretory solutions. In zero HCO_3^-, zero Na^+ solutions, there was a further increase in resistance compared to zero HCO_3^- solutions. The decrease in Cl^- from 81 to 8.1 mM in the nutrient solution gave, 10 minutes after the change to the lower concentration, in the first part a PD decrease of about 14 mV and in the second part a PD decrease of about 6 mV. The respective increases in resistance were about 15 ohm · cm^2 and about 60 ohm · cm^2. Upon return to 81 mM Cl^-, the PD values reversed in both cases but the resistance reversed only in the first part and did not change in the second part. As to the H^+ secretory rate, there was a moderate rate of about 2 μequiv. · h^{-1} · cm^{-2} with zero HCO_3^- and 81 mM Cl^- and the rate decreased to a small extent at the lower Cl^- concentration. In zero HCO_3^- and zero Na^+ solutions, the H^+ secretory rate was zero throughout.

Table I summarizes the results for the PD and resistance in going from 81 to 8.1 and back to 81 mM Cl^- in the nutrient solution. In going from 81 to 8.1 mM nutrient Cl^-, in the first part, the PD decreased by 13.5 mV and in the second part, the PD decreased by 6.7 mV. The respective increases in resistance were 31 and 78 ohm · cm^2. Upon return to 81 mM Cl^-, the PD returned to control or near control levels in both cases and the resistance in the first part was partially reversi-

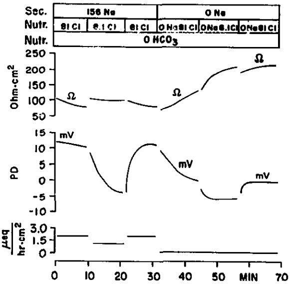

Fig. 1. Effects of changes in Cl^- concentration on the nutrient side from 81 to 8.1 mM and back to 81 mM in HCO_3^--free nutrient and secretory solutions and in HCO_3^--free, Na^+-free nutrient and secretory solutions. Resistance, PD and H^+ secretory rate are plotted vs. time. Concentrations are in mM.

TABLE I

Effect on PD and resistance of changes in Cl^- concentrations on the nutrient side in HCO_3^--free, and in HCO_3^--free, Na^+-free solutions

Values are means ± S.D. Student's *t*-test using paired observations was used to determine the level of significance. Columns labeled PD and *R* refer to the control values of transmembrane potential difference and resistance, respectively, and columns labeled ΔPD and Δ*R* refer to changes in the two parameters following the change to 8.1 mM Cl^-. *N* refers to number of experiments.

Orig. soln. $[Cl^-]$ (mM)	Final soln. $[Cl^-]$ (mM)	PD (mV)	ΔPD (mV)	*R* (ohm·cm²)	Δ*R* (ohm·cm²)
HCO_3^--free nutrient and secretory solutions ($N = 12$)					
81	8.1	14.6 ± 4.1	−13.5 ± 3.1 [a]	173 ± 9 [a]	31 ± 19 [a]
8.1	81	1.0 ± 3.6	13.2 ± 2.5 [a]	202 ± 106	−15 ± 8 [a]
HCO_3^--free, Na^+-free nutrient and secretory solutions ($N = 11$)					
81	8.1	4.2 ± 4.6	−6.7 ± 1.9 [a]	343 ± 180	78 ± 80 [a]
8.1	81	−3.2 ± 3.9	5.6 ± 2.0 [a]	426 ± 213	1 ± 48

[a] $P < 0.01$.

ble but in the second part the resistance did not change significantly.

The results pertaining to PD will be considered in the Discussion. However, we shall first consider below whether the PD decrease due to a decrease in nutrient Cl^- concentration might be accounted for by a neutral KCl symport.

PD responses and resistances changes due to nutrient Cl^- changes in K^+-containing and K^+-free solutions

In Fig. 2 in the first part of the experiment there is K^+ present in both the nutrient and secretory solutions and in the second part there is zero K^+ in both solutions. In the presence of K^+ in both solutions, there was a moderate H^+ secretory rate of about 2.2 μequiv.·h^{-1}·cm^{-2} and, in the absence of K^+ in both solutions, the H^+ secretory rate decreased to zero. Also with zero K^+ in both solutions there was a marked increase in resistance. As Fig. 2 shows, the 10-fold decrease in Cl^- gave a marked decrease in PD and the 10-fold increase in Cl^- levels brought the PD essentially back to control levels. In the presence and absence of K^+ the changes in resistance due to the changes in Cl^- were small.

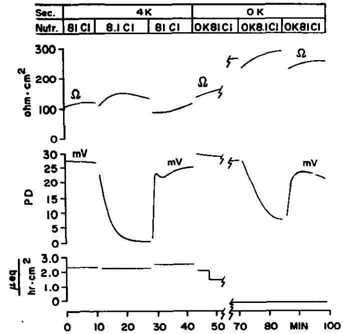

Fig. 2. Effect of changes in Cl^- concentration on the nutrient side from 81 to 8.1 mM and back to 81 mM in K^+-containing nutrient and secretory solutions and in K^+-free nutrient and secretory solutions. Resistance, PD and H^+ secretory rate are plotted vs. time. Concentrations are in mM.

Table II shows that, in going from 81 to 8.1 mM nutrient Cl^-, with K^+ present the PD decreased by 20.2 mV and with K^+ absent the PD decreased by 19.4 mV so that the average of six experiments showed no significant differences in PD responses. Upon return to 81 mM Cl^-, with K^+ present the PD returned to control levels and with K^+ absent the PD returned to near control levels. Moreover, with K^+ present there were no significant changes in resistance as previously observed [4] but with K^+ absent there were small but significant changes, an increase of 35 ohm·cm² with a 10-fold decrease in Cl^- and a decrease of 26 ohm·cm² with a 10-fold increase in Cl^-.

The differences resulting from changes of Cl^- concentration in the presence and absence of K^+ are either non-existent or of small proportions. Hence it is quite reasonable to conclude that there is no evidence of an electrogenic or neutral KCl symport.

Discussion

We now shall show that these experiments rule out the necessity of fully accounting for the PD changes with nutrient Cl^- changes on the basis of a HCO_3^-–Cl^- exchanger, a NaCl symport and a KCl symport.

With HCO_3^- present in the nutrient solution, a 10-fold decrease in nutrient Cl^- resulted in a decrease in PD of about 19 mV [3] or about 20 mV in present experiments (see Table II). In present experiments with HCO_3^--free nutrient solutions, the decrease in PD was found to be 13.5 mV. The lower value of 13.5 mV compared to 19 or 20 mV could be attributed to an increase in resistance of the Cl^- pathway in the absence

TABLE II

Effect on PD and resistance of changes in Cl^- concentrations on the nutrient side in K^+-containing and in K^+-free solutions

See Table I for details. $N = 6$.

Orig. soln. $[Cl^-]$ (mM)	Final soln. $[Cl^-]$ (mM)	PD (mV)	ΔPD (mV)	R (ohm·cm^2)	ΔR (ohm·cm^2)
K^+-containing nutrient and secretory solutions					
81	8.1	24.6 ± 6.6	−20.2 ± 3.0 [a]	135 ± 25	8 ± 15
8.1	81	2.7 ± 5.3	21.8 ± 3.6 [a]	130 ± 12	9 ± 23
K^+-free nutrient and secretory solutions					
81	8.1	25.6 ± 5.8	−19.4 ± 6.2 [a]	319 ± 83	35 ± 21 [a]
8.1	81	3.5 ± 1.0	15.4 ± 7.1 [a]	358 ± 100	−26 ± 22 [b]

[a] $P < 0.01$; [b] $P < 0.05$.

of HCO_3^- [14]. The decrease of 13.5 mV in the absence of HCO_3^- might be attributed to an electrogenic NaCl symport in the frog gastric fundus [4,6]. However, in the absence of both HCO_3^- and Na^+ in both the nutrient and secretory solutions, the 10-fold decrease in Cl^- gave a decrease in PD of 6.7 mV. This decrease cannot be attributed to the mechanisms involving HCO_3^- or Na^+ Also there is no evidence for a KCl symport. The decrease of 6.7 mV is most likely due to a simple Cl^- conductance pathway.

It is of interest to note that previously with 25 mM HCO_3^- in the nutrient solution of the frog gastric fundus, an 8-fold decrease in Cl^- gave a decrease in PD of about 16 mV in the presence of Na^+ in both solutions and about 8 mV in the absence of Na^+ in both solutions [4]. In the present experiments with HCO_3^--free solutions, a 10-fold decrease in Cl^- gave for the respective decreases in PD 13.5 and 6.7 mV. Thus, in each case in the absence of Na^+, the PD decrease was half that in the presence of Na^+. As previously [3–6], we attribute the PD responses to an electrogenic NaCl symport and a simple Cl^- conductance in the nutrient membrane.

Although these experiments do not exhaust the entire array of possible mechanisms, the simplest hypothesis is that the residual decrease in PD of 6.7 mV due to a tenfold decrease in nutrient Cl^- in the absence of HCO_3^- and Na^+ can be reasonably attributed to a simple Cl^- conductance pathway in the nutrient (basolateral) membrane.

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