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**BBA Report**

# Further evidence for a simple $Cl^-$ conductance pathway in nutrient membrane of frog stomach

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**A decrease in nutrient $Cl^-$ increases the negativity of the nutrient relative to the secretory side. It seemed possible that $Cl^-$ transport could result from a neutral $Cl^-$ mechanism in the nutrient membrane coupled to a simple $Cl^-$ conductance pathway in the secretory membrane. Experiments in $HCO_3^-$-free, $K^+$-free and $Na^+$-free solutions in both bathing media gave for a 10-fold change in nutrient $Cl^-$ a PD change of 9.5 mV. Similar experiments with 0.5 mM DIDS in the nutrient solution gave for a 10-fold change in nutrient $Cl^-$ a PD change of 7.9 mV. These experiments eliminated a neutral $Cl^-$-$HCO_3^-$ exchanger, a NaCl and a KCl symport. Thus the change in PD could best be explained by a simple $Cl^-$ conductance in the nutrient membrane.**

Muallem et al. [1] explained $Cl^-$ transport in the basolateral membrane of rabbit oxyntic cells by a $Cl^-$-$HCO_3^-$ exchange. Paradiso et al. [2] explained $Cl^-$ transport on the basis of $Cl^-$-$OH^-$ or $Cl^-$-$HCO_3^-$ exchange in gastric gland of rabbit stomach. In contrast, in the intact frog stomach, $Cl^-$ transport was explained previously by a simple $Cl^-$ conductance [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].

Instead of a simple $Cl^-$ conductance pathway in the nutrient membrane of the frog gastric mucosa, we speculated whether the decrease in nutrient $Cl^-$ leading to a decrease of the transmucosal potential difference (PD) of the nutrient (basolateral) side relative to the secretory (apical) side of the frog stomach might 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. Then, a decrease in nutrient $Cl^-$ would decrease the cellular $Cl^-$ via an exchanger or symport. If there were a conductance pathway for $Cl^-$ in the secretory membrane [7,8], the ratio of $Cl^-$ in the cell to $Cl^-$ in the secretory solution would decrease with a decrease in PD. The latter decrease would account for the observed decrease in PD.

Previously, experiments [9] were performed to determine whether there was a need to assume a simple $Cl^-$ conductance pathway in the nutrient membrane. A 10-fold decrease in $Cl^-$ in $HCO_3^-$ containing nutrient solutions gave a PD decrease of 20 mV; in $HCO_3^-$-free nutrient solutions, a PD decrease of 13.5 mV; and in $HCO_3^-$-free/$Na^+$-free solutions, a PD decrease of 6.7 mV. We inferred that the residual PD of 6.7 mV was not due to a neutral $Cl^-$-$HCO_3^-$ exchanger or a NaCl symport but might be due to a simple $Cl^-$ conductance pathway and/or a KCl symport in the nutrient membrane. Experiments with $K^+$-containing and with $K^+$-free nutrient and secretory solutions gave essentially the same change in PD with a 10-fold change in nutrient $Cl^-$. From this result, we thought that a KCl symport might not be involved. However, the resistance with $K^+$ present in both solutions was 135 ohm cm$^2$ and without $K^+$ in both solutions, about 320 ohm cm$^2$. Because of the difference in resistance, the same change in PD does not necessarily exclude a KCl symport.

We decided, therefore, to perform the ion substitution experiment in simultaneous $HCO_3^-$-free, $Na^+$-free and $K^+$-free nutrient and secretory solutions. In addition, the same experiments were performed with 0.5 mM DIDS (4,4′-diisothiocyanostilbene-2,2′-disulphonic acid) in the nutrient solution as an inhibitor of anion

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exchange [10]. These experiments, as reported herein, enabled us to conclude that there is evidence for a simple $Cl^-$ conductance pathway in the nutrient membrane.

The experimental methods were as follows. 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 gluconate. 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 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. For $Na^+$-free and $K^+$-free solutions, the $Na^+$ and $K^+$ were replaced with choline.

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 $\mu$A 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 was maintained generally between 4.7 and 5.0. For inhibition of the $Cl^-$-$HCO_3^-$ exchanger, 0.5 mM DIDS (4,4′-diisothiocyanostilbene-2,2′-disulphonic acid) was present in the nutrient solution.

In the change of the concentration of $Cl^-$ in the nutrient solution, due to a diffusion barrier (lamina propria, muscularis mucosa and part of submucosa) between the nutrient solution and the nutrient membrane, it takes 10 min (approximately 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.

Fig. 1 is a part of the experiment pertinent to the present considerations. Prior to this part, the nutrient solution was changed to a simultaneous $HCO_3^-$-free, $Na^+$-free and $K^+$-free solution and both sides of the mucosa were gassed with 100% $O_2$. The first portion

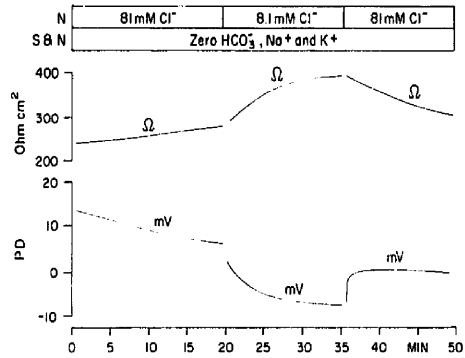


Fig. 1. Effects of changes in $Cl^-$ concentration on the nutrient side from 81 to 8.1 mM and back to 81 mM in simultaneous $HCO_3^-$-free, $Na^+$-free and $K^+$-free nutrient and secretory solutions. Resistance and PD are plotted vs. time. In the first part the secretory solution was changed to 160 mM choline chloride which remained throughout this phase of the experiment. S refers to secretory solution, N to nutrient solution.

shown here is the replacement of the regular secretory solution with a 160 mM choline chloride solution. The PD and resistance changes were followed for 20 min at which time the nutrient $Cl^-$ was changed from 81 to 8.1 mM. The decrease in PD was about 13 mV. Upon return to 81 mM $Cl^-$, the PD increased by about 8 mV. We note that, because of the diffusion barrier, the initial PD response in going from a low to a high concentration is more rapid than in going from a high to a low concentration [4].

Table I summarizes the results. In the change from 81 to 8.1 mM nutrient $Cl^-$, the PD decreased by 11.9

TABLE I

*Effect on PD and resistance of changes in $Cl^-$ concentrations on the nutrient side in simultaneous $HCO_3^-$-free, $Na^+$-free and $K^+$-free nutrient and secretory solutions without and with DIDS*

Values are mean ± S.D. The 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, in simultaneous $HCO_3^-$-free, $Na^+$-free and $K^+$-free nutrient and secretory solutions. Columns labeled $\Delta$PD and $\Delta R$ refer to changes in the two parameters following the change in nutrient $Cl^-$. Number of experiments without DIDS = 7; number of experiments with DIDS = 5. a, $P < 0.01$; b, $P < 0.05$.

| $[Cl^-]$ (mM) | | PD | $\Delta$PD | *R* | $\Delta R$ |
|---|---|---|---|---|---|
| orig. soln. | final soln. | (mV) | (mV) | (ohm $cm^2$) | (ohm $cm^2$) |
| Without DIDS in nutrient solution | | | | | |
| 81 | 8.1 | 3.3 ± 4.0 | −11.9 ± 3.6 [a] | 341 ± 120 | 66 ± 68 [b] |
| 8.1 | 81 | −8.7 ± 2.9 | 7.1 ± 1.2 [a] | 408 ± 76 | −75 ± 50 [a] |
| With DIDS in nutrient solution | | | | | |
| 81 | 8.1 | 3.4 ± 2.0 | −8.8 ± 0.7 [a] | 307 ± 56 | 78 ± 33 [a] |
| 8.1 | 81 | −5.7 ± 1.6 | 7.0 ± 0.7 [a] | 386 ± 43 | −53 ± 14 [a] |

mV and, in the change from 8.1 to 81 mM nutrient Cl⁻, the PD increased by 7.1 mV. The resistance showed a significant decrease with the increase in nutrient Cl⁻ and also a significant increase with the decrease in nutrient Cl⁻. Table I also shows the results of a 10-fold change in nutrient Cl⁻ with 0.5 mM DIDS in the nutrient solution. For the 10-fold decrease in nutrient Cl⁻, the PD decreased by 8.8 mV and, for the 10-fold increase in nutrient Cl⁻, the PD increased by 7.0 mV. Again the changes in resistance were similar to those obtained in experiments without DIDS.

We note that, in the presence of oxygen, $CO_2$ may be formed in the cell and the cell may contain $HCO_3^-$. However, the constant bubbling of both sides with 100% oxygen and the high diffusivity of $CO_2$ would result in a low intracellular $pCO_2$ and a low $HCO_3^-$. Hence, there would be at best a small PD response due to a $Cl^-$-$HCO_3^-$ exchanger with an increase in nutrient $Cl^-$. Even if $HCO_3^-$ were present in the cells, a response to a decrease in nutrient $Cl^-$ would require $HCO_3^-$ in the nutrient solution for the operation of the exchanger. The experiments with DIDS in the nutrient solution provided further evidence that the residual PD is not due to a $Cl^-$-$HCO_3^-$ exchanger. Furthermore, the experiments with DIDS eliminate the secretory membrane as responsible for the change in transepithelial PD. Despite the absence of $Na^+$ and $K^+$ from the bathing solutions, these ions may be present in the cells. In this instance, a PD response to an increase in nutrient $Cl^-$ would require $Na^+$ and $K^+$ in the nutrient solution. Therefore, this PD response cannot be explained by NaCl and KCl symports.

We can now rule out the necessity of fully accounting for the residual PD with nutrient Cl⁻ changes on the basis of a $HCO_3^-$-$Cl^-$ exchanger, a NaCl symport and a KCl symport. In solutions without $HCO_3^-$, $Na^+$ and $K^+$ and, with DIDS in the nutrient solution, there is no simple exchanger or symport available to explain completely the PD change with a change of nutrient Cl⁻. The simplest hypothesis under these circumstances is that the change in PD due to a 10-fold change in nutrient Cl⁻ can be attributed to a simple $Cl^-$ conductance pathway in the nutrient (basolateral) membrane.

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