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PERMEABILITY PROPERTIES AND INTRACELLULAR ION CONCENTRATIONS OF EPITHELIAL CELLS IN RAT DUODENUM

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SUMMARY

Effects of the K^+ concentration in the bathing fluid ($[K^+]_o$) on the intracellular K^+, Na^+ and Cl^- concentrations ($[K^+]_i$, $[Na^+]_i$ and $[Cl^-]_i$) as well as on the electrical potential were studied in rat duodenum. Changes in the mucosal K^+ concentration ($[K^+]_m$), bringing the sum of Na^+ and K^+ concentrations to 147.2 mM constant, had little effect on the transmural potential difference (PD_t), but did induce marked changes in the mucosal membrane potential (V_m). As $[K^+]_m$ increased, V_m was depolarized gradually and obeyed the Nernst equation for a potassium electrode in the range of $[K^+]_m$ greater than approx. 60 mM. Experiments of ion analyses were carried out on strips of duodenum to determine the effect of changing the external K^+ concentrations on $[K^+]_i$, $[Na^+]_i$ and $[Cl^-]_i$. An increase in $[K^+]_o$ resulted in increases in $[K^+]_i$ and $[Cl^-]_i$ and a decrease in $[Na^+]_i$, $[K^+]_i$ approaching its maximum at $[K^+]_o$ greater than 70 mM. Such changes in $[K^+]_i$ and $[Na^+]_i$ seem to correlate quantitatively with the changes in $[K^+]_o$ and $[Na^+]_o$. The values of the ratio of permeability coefficients, P_{Na^+}/P_{K^+} were estimated using the V_m values and intracellular ion concentrations measured in these experiments. The results suggested that there appeared a rather abrupt increase in the P_{Na^+}/P_{K^+} ratio from 0 to approx. 0.1, as $[K^+]_m$ decreased.

INTRODUCTION

Reports concerning the effect of alterations in ionic environments on membrane potential of the epithelial cell in rat and rabbit small intestine [1–4] did not include data covering intracellular ion concentrations. In order to make more accurate assessment of membrane properties, we studied the effects of ionic environments on intracellular K^+, Na^+ and Cl^- concentrations as well as on the membrane potential in rat duodenum. Based on the results obtained, certain aspects of the membrane properties are discussed in the light of the so-called ionic theory.

METHODS

Electrical potential measurements

A sheet of rat duodenum were prepared, and the transmural potential difference (PD_t) and the mucosal membrane potential (V_m) were measured at 37 °C using the technique described in our preceding paper [4]. Briefly, V_m was measured with microelectrodes (resistance, 10–35 MΩ (mean, 17 MΩ); tip potential, 0– −5.0 mV (mean, −2.1 mV) under visual control with a binocular microscope on a sheet of duodenum mounted between two halves of a Lucite chamber, while PD_t was measured with a high-input impedance voltmeter connected to mucosal and serosal fluids by calomel cells and salt bridges. In preparing the strips the blood supply was maintained intact until mounting on the chamber.

Ion analyses

A sheet of duodenum was spread out and settled on a lucite frame as described in Fig. 1. The tissue was immersed in the test solution containing [^{14}C]inulin bubbled with humidified O_2 at 37 °C for 10 min. It was shown by Esposito and Csáky [5] that the [^{14}C]inulin distribution is a suitable indicator of the extracellular space in rat small intestine, and in our preliminary experiment [^{14}C]inulin distribution was completed within 10 min. At the end of the incubation, the tissue was blotted on a filter paper. The mucosal layer was immediately scraped off with a cover glass, and the wet weight was measured at once using a torsion balance. The scraped tissue was, then, either dried at 105 °C until weight became constant (usually overnight) and reweighed to obtain the dry weight, or extracted with 0.1 M HNO_3 at 37 °C for 1–2 days. Appropriate aliquots of the extract were taken for analyses. The activity of ^{14}C was estimated by liquid scintillation counting (Horiba Liquid Scintillation Spectrometer), Na^+ and K^+ contents by flame photometry (Baird-Atomic Flame Photometer Model KY 3), and Cl^- content with a Buchler Cotlove Chloridometer. The proteins in the extract were not removed prior to the chloride analysis.

Solutions

The control mucosal and serosal fluids were phosphate-buffered saline (pH

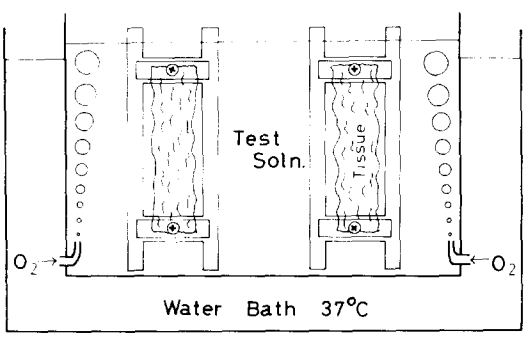

Fig. 1. Schematic view of bathing chamber for ion analysis.

7.3±0.1) containing (in mM) NaCl, 127.0; KCl, 2.7; $CaCl_2$, 0.9; $MgCl_2$, 0.5; Na_2HPO_4, 8.0; KH_2PO_4, 1.5; and mannitol, 20.0. In the present study, changes in potassium concentrations of the bathing fluid were accomplished by replacing all or a part of NaCl and Na_2HPO_4 with equimolar amounts of KCl and K_2HPO_4, respectively, and vice versa, under the fixed sum of K^+ and Na^+ concentrations (147.2 mM). Low Cl^- solutions were prepared by partially replacing Cl^- in the control medium with SO_4^{2-}, their total osmolarity being kept unchanged by the addition of an appropriate amount of mannitol.

RESULTS

Effect of reducing $[Cl^-]_m$ on the electrical potentials

In order to estimate the contribution of Cl^- to the membrane potential, V_m, the effect of reducing the Cl^- concentration in the mucosal fluid, $[Cl^-]_m$, was examined. On reducing $[Cl^-]_m$ to 5.5 mM, the membrane potential was transiently depolarized, as shown in Fig. 2. However, since the exchange of the mucosal fluid brought about the movement of villi, it was impossible in most cases to follow up potential changes due to changing the bathing solution on one and the same cell. Successful successive punctures of different cells within 1–2 min after replacing the control medium with low-Cl^- solutions always showed a transient depolarization of about 5–9 mV (Table I). Such a transient depolarization on reducing the external Cl^- concentration was already reported on single muscle fibers [6] and on cultured L-cells [7]. As noted previously [4], however, the potential drifted back to the original level within 2 min (Table I), the time constant of the potential recovery being significantly smaller in the duodenal epithelial cells than in the muscle fiber [6] and in the L-cells [7]. Such a potential recovery after a transient depolarization on exposure to a low-Cl^- solution has been regarded as evidence that Cl^- distributes passively across a cell membrane [6, 7].

On reducing $[Cl^-]_m$, the transmural potential, PD_t, also showed a transient

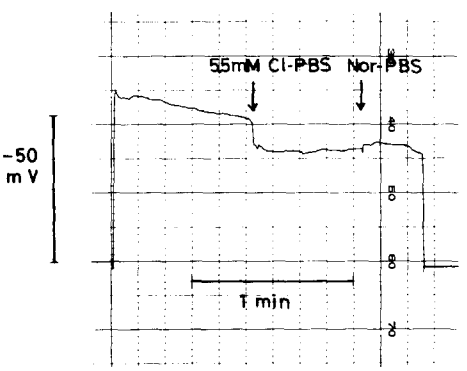

Fig. 2. Recording of the membrane potential in rat duodenum exemplifying membrane depolarization on suddenly reducing the external Cl^- concentration from 132.5 mM to 5.5 mM. PBS, phosphate-buffered saline.

TABLE I

EFFECT OF LOW Cl^- CONCENTRATION IN THE MUCOSAL FLUID ON THE MEMBRANE POTENTIAL

Conditions	Membrane potential mean ±S.E. (mV) (V_m)	Number of observations (n)	Level of significance for difference from the control
Control (132.5 mM Cl)	−55.4±0.43	110	
69 mM−Cl			
<120 s*	−49.2±1.04	29	$P < 0.005$
>120 s**	−55.1±1.35	25	$P > 0.25$
5.5 mM−Cl			
<70 s*	−47.9±1.36	19	$P < 0.005$
>70 s**	−57.3±1.14	22	$P > 0.05$

* V_m observed for 0 to 120 (or 70) s after introducing low-Cl^- solutions
** V_m observed thereafter for 8 min.

increase (up to about 4 mV at the peak) lasting about 2 min. When V_m regained its original level, however, PD_t did not always drift back to the initial level, taking a value of slightly higher (3–6 mV) than the original one (+2–+4 mV). The intestinal epithelium can be classified as a leaky tissue which has a low-resistance, transmural, extracellular pathway [2, 8] and PD_t is dominated by a diffusion potential generated across the extracellular pathways [4, 8]. Such a difference of the recovery pattern in low Cl^- solutions between PD_t and V_m appears, therefore, to be chiefly, but not solely, attributable to the difference of the permeability properties for Cl^- between the epithelial cell membrane and the transmural shunt pathway.

Effect of a K^+-Na^+ replacement in the mucosal fluid on the electrical potentials

The effect of a sudden increase in the concentration of K^+ in the mucosal fluid, $[K^+]_m$, from 4.2 to 69.7 mM by the NaCl/KCl substitution is illustrated in Fig. 3. When the high K^+ solution was applied, the mucosal membrane potentials immediately fell from their average level of approx. −52 mV to a new level of approx. −28 mV

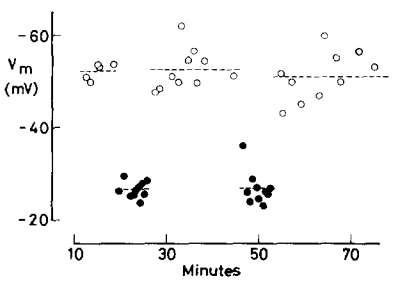

Fig. 3. Effect on V_m of changing $[K^+]_m$ from 4.2 to 69.7 mM. The broken lines indicate the mean levels of the potential observed at $[K^+]_m$ = 4.2 and 69.7 mM. ○, V_m with 4.2 mM K^+; ●, V_m with 69.7 mM K^+.

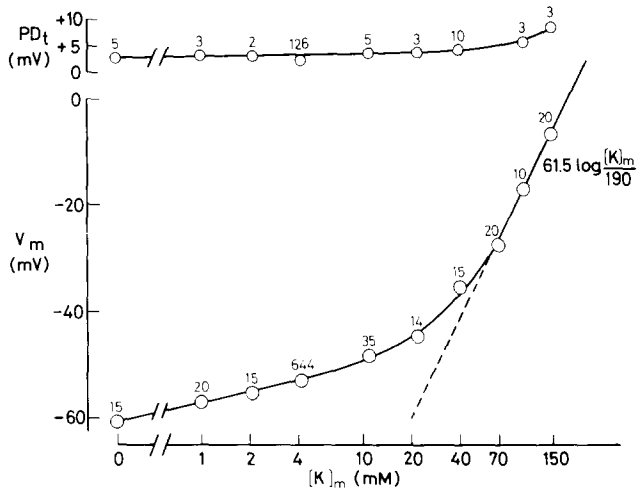

Fig. 4. Plots of PD_t and V_m against log $[K^+]_m$. The standard errors are smaller than the size of each signature. The broken line is drawn according to Eqn. 1 in the text. The number of observations are indicated in this figure.

and remained at this level. On restoring the control medium, recovery to the original level occurred immediately. Such a change in V_m induced by changing $[K^+]_m$ was reversible for 10 min at least. With other test solutions of various $[K^+]_m$ the results obtained were quite similar. The PD_t and V_m values obtained in these experiments are plotted against $\log[K^+]_m$ in Fig. 4.

As seen in this figure, the change in PD_t induced by a change in $[K^+]_m$ is quite small. The transmural permeability of K^+ appears, therefore, to be approximately equal to that of Na^+, as reported by Wright [9], Frizzell and Schultz [8] and Okada et al. [4].

Contrary to the previous observation [2, 3], the change in V_m induced by a change in $[K^+]_m$ was remarkable. In the range of $[K^+]_m$ greater than about 60 mM, a change in V_m obeyed Nernst's equation for a potassium electrode (Eqn. 1), but deviated considerably from this relation in the range of $[K^+]_m < 60$ mM, as seen in Fig. 4.

$$V_m \quad \frac{RT}{F} \ln \frac{[K^+]_m}{[K^+]_i} = 61.5 \log \frac{[K^+]_m}{190} \tag{1}$$

These results are in good agreement with our preceding report [4]. The value of 190 mM for $[K^+]_i$ thus estimated under the condition in high $[K^+]_m$ was in full accordance with the result of flame photometric determination, as described later (Fig. 5). Such behavior of membrane as a potassium electrode in high K^+ media was found in many excitable [6, 10–13] and several inexcitable membranes [14, 15].

Intracellular ion concentrations

Under the normal conditions, the total tissue water in duodenal mucosa was 81.3 ± 0.33 ($n = 5$) % of the wet weight, and [^{14}C]inulin space after 10 min was 12.2 % of the total tissue water. On the basis of these data, the intracellular K^+, Na^+

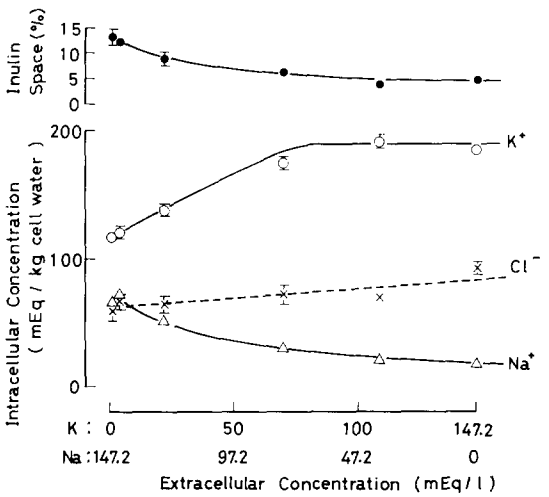

Fig. 5. Effect of changes in the external K^+ and Na^+ concentrations on the intracellular K^+, Na^+ and Cl^- concentrations and inulin space. Vertical bars represent standard errors on either side of averages. Each point represents the mean of 4–5 tissues. ○, △ and ×, Intracellular K^+, Na^+ and Cl^- concentrations, respectively (mequiv./kg cell water); ●, [^{14}C]inulin space after 10-min incubation (% of the total tissue water).

and Cl^- concentrations were calculated to be 121, 72.9 and 67.4 mequiv./kg of intracellular water, respectively. These values are in fair accord with the values of rat jejunum [3] and rabbit ileum [16–18] reported hitherto.

Experiments were carried out to determine the effects on the intracellular ion concentrations by changing the external K^+ concentrations in the range of 1.0–147.2 mM, with the sum of Na^+ and K^+ concentrations fixed to 147.2 mM. All the data obtained in these experiments are summarized in Fig. 5. As the K^+ concentration in the bathing medium, $[K^+]_o$, increased, the inulin space gradually decreased. This finding suggests that the epithelial cells swelled with an increase in the extracellular K^+ concentration. The swelling of the cells estimated from the values of inulin space is approx. 9 % in the region of high $[K^+]_o$ (100–147.2 mM). Since all cell swelling does not always show up a reduction of inulin space, the real swelling is probably greater than 9 %.

Fig. 5 shows that the cell K^+ concentration, $[K^+]_i$, varies with $[K^+]_o$ at low concentrations, but at $[K^+]_o$ greater than 70 mM the $[K^+]_i$ value approaches its maximum (approx. 190 mM) and is almost independent of the extracellular K^+ concentration. The external K^+ concentration that gives half the maximum cell K^+ concentration was found to be 38 mM. The intracellular Na^+ concentration, $[Na^+]_i$, falls as $[K^+]_o$ rises. The intracellular Cl^- concentration, $[Cl^-]_i$, rises slightly with an increase in $[K^+]_o$. Such behavior of intracellular ion concentrations with raising $[K^+]_o$ is quite similar to that reported by Wickson-Ginzburg and Solomon [19] on HeLa cells.

Permeability properties of the membrane for K^+ and Na^+

The effective e.m.f. of the duodenal epithelial cell membrane is expressed by

the following relation [4],

$$E = -V_m + \frac{1}{\mu + 1} \cdot PD_t \tag{2}$$

where $\mu = R_s/R_m$ (R_m and R_s are the resistances of mucosal and serosal membranes, respectively). The μ value can be assumed to be considerably greater than 1 [4] (for instance, $\mu \simeq 13$ in rabbit ileum estimated from the data of Frizzell and Schultz [8]). Moreover, the PD_t values were found to be considerably small compared with the V_m values under both conditions of low $[Cl^-]_m$ (as described in the foregoing section) and KCl/NaCl substitution (as seen in Fig. 4). In view of these facts, $-V_m$ obtained in the present study could be regarded as practically equal to E.

The effect of reducing $[Cl^-]_m$ on the V_m values would, therefore, indicate that the re-equilibration for Cl^- is quite rapidly attained between the intracellular and the mucosal fluids across the cell membrane, and this anion makes no significant contribution to E in the steady state.

Thus, we can safely apply, under the present experimental conditions at least, the following Goldman equation to E measured as $-V_m$,

$$E = -61.5 \log \frac{[K^+]_m + \alpha \cdot [Na^+]_m}{[K^+]_i + \alpha \cdot [Na^+]_i} \tag{3}$$

where α is the ratio of the permeability coefficients (P_{Na^+}/P_{K^+}), and the subscripts m and i denote the mucosal and intracellular fluids. Using the observed V_m values and the intracellular ion concentrations, therefore, the values of $\alpha(P_{Na^+}/P_{K^+})$ at the low K^+ concentration could be evaluated by Eqn. 3. Since the membrane potential obeyed Eqn. 1 in the range of $[K^+]_m$ greater than approx. 60 mM, the α values in this region could be regarded as practically nil. In Fig. 6, the α values thus estimated are plotted against $\log[K^+]_m$, which shows that the α values abruptly increase from 0 to approx. 0.09 at $[K^+]_m$ = 10–50 mM, a finding quite concordant with the result described in our preceding paper [4]. A similar phenomenon has already been obtained in HeLa cell membrane [15]. This finding suggests, as already discussed in our previous work

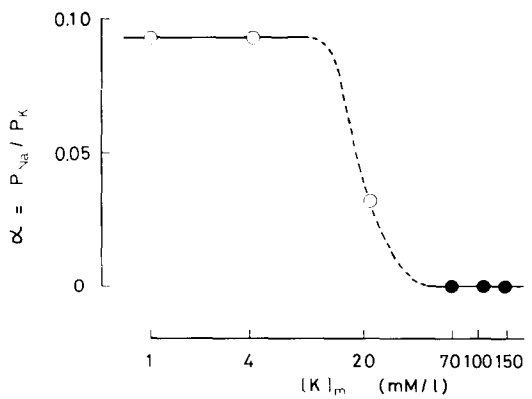

Fig. 6. Relationship between α values (P_{Na^+}/P_{K^+}) and $\log [K^+]_m$. ●, α values estimated from Eqn. 1 in the range of $[K^+]_m$ greater than 70 mM; ○, α values estimated from Eqn. 3 at lower $[K^+]_m$ region.

[4], an abrupt change in membrane properties with a change in external K^+ concentrations.

Our results presented in Fig. 5 show that ($[K^+]_i - [K^+]_o$) remains nearly constant at approx. 116 mM, and ($[Na^+]_o - [Na^+]_i$) ranges from approx. 70 to 80 mM at $[K^+]_o \leqq 22$ mM. Since α for $[K^+]_o \leqq 22$ mM is nearly constant, at approx. 0.1, the following relation can be practically applied in this range of $[K^+]_o$.

$$[K^+]_i + \alpha \cdot [Na^+]_i = [K^+]_o + \alpha \cdot [Na^+]_o + C \tag{4}$$

where C is a constant and approx. 108 mM. This relation also holds approximately in the range of 22 mM $< [K^+]_o \leqq 70$ mM, if an approx. 10 % error is allowable. Thus changes in ($[K^+]_i + \alpha \cdot [Na^+]_i$) correlate with the changes in ($[K^+]_o + \alpha \cdot [Na^+]_o$) quantitatively, and the e.m.f. given by Eqn. 3 is regarded as a function of ($[K^+]_o + \alpha \cdot [Na^+]_o$) alone in the region of low $[K^+]_o$, a fact which provides support for our previous work [4]. On the other hand, Eqn. 4 never holds for $[K^+]_o$ greater than 70 mM because of the approximately constant $[K^+]_i$. As stated above, however, the V_m value at such a high external K^+ is given by Eqn. 1 and so depends only on $[K^+]_o$ with $\alpha \simeq 0$. Thus V_m of the duodenal epithelial cells appear to be practically determined by ($[K^+]_o + \alpha \cdot [Na^+]_o$), in which α depends on $[K^+]_o$.

DISCUSSION

Our comments on the membrane potential and permeability ratio stated above are based on the assumption that the intracellular concentrations of K^+, Na^+ and Cl^- obtained by our method represent their free ionic ones in the cell fluid, their activity coefficient being essentially the same as those in simple electrolyte solution. Such a conclusion may, however, be open to question on the following grounds. Firstly, in computing the intracellular ion concentrations, it was assumed that all cell water behaves as a solvent, neglecting the presence of so-called "bound water". This might be an oversimplification [20]. But this does not affect the mutual proportionate relations between those ions and our reasoning remains qualitatively unaffected, even if some quantitative corrections, e.g. for the magnitude of α, are needed. Secondly, the activity coefficients of these ions remain unknown in the cell fluid, which contains polyvalent macromolecules in considerable amounts. Indeed, as shown in many investigations on various tissues with ion-selective electrodes [21–32], the assumption concerning the activity coefficient of such univalent ions is questionable, and the presence of a bound form of Na^+ and/or Cl^- was suggested. Thus departure from ideality in their electrochemical behavior is expected.

If the Cl^- is passively distributed across the epithelial cell membrane in small intestine as suggested not only by the previous works [2–4], but also by the present experiment (Table I), the measured membrane potential should be equal to the value calculated from the following equation.

$$V_m = 61.5 \log \frac{[Cl^-]_i}{[Cl^-]_m} \tag{5}$$

The measured V_m values were found to be in fairly good agreement with the values calculated from Eqn. 5 using the measured $[Cl^-]_i$ values at $[K^+]_m$ of 100 mM or more, but remarkably different from the values predicted from Eqn. 5 in the region

of $[K^+]_m$ below 70 mM. These facts suggest that all or at least the majority of the intracellular Cl^- behaves as free ions when the membrane is depolarized in the high $[K^+]_m$ region, but the intracellular Cl^- is, to a considerable extent, in a sequestered form at high-V_m and low-$[K^+]_m$ region. Indeed, Frizzell et al. [18] provided the evidence for compartmentalization of intracellular Cl^- in rabbit ileum. If such is also the case in rat duodenum, the compartmentalization or sequestration of intracellular Cl^- would explain our results.

In the Na^+-free medium, the intracellular free Na^+ would be quite small. The value of $[Na^+]_i$ obtained in this medium is approx. 10 mM, as shown in Fig. 5, a value which seems to point out the presence of a bound form of intracellular Na^+. Varying the activity coefficient of $[Na^+]_i$ in Eqn. 3 down to 0.5 (the value obtained by Lee and Armstrong [31] in the epithelial cell of frog intestine), however, the behavior of α value illustrated in Fig. 6 was not found to be out of due proportion. Of course, Eqn. 4 still holds fairly well for $\alpha \simeq 0.1$ even if about a half of the intracellular Na^+ is in a bound form. Granted that sequestration of Na^+ and/or Cl^- did occur in the cell fluid, therefore, our conclusions drawn from the present study appear to remain valid.

The P_{Na^+}/P_{K^+} ratio across the epithelial cell membrane (approx. 0.1) under the normal condition was remarkably different from this ratio across the epithelial tissue (approx. 1). Such a finding may be correlated to the fact that the intestinal epithelium has a low-resistance, transmural, extracellular pathway [2, 8].

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