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VALIDITY OF THE GOLDMAN-HODGKIN-KATZ EQUATION IN PARACELLULAR IONIC PATHWAYS OF GALLBLADDER EPITHELIUM

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The Goldman-Hodgkin-Katz equation has been extensively used to determine cationic/anionic permeability ratios in the paracellular pathways of the gallbladder epithelium. Nevertheless, new experimental evidence suggests that none of the theoretical assumptions of the equation hold for these pathways. In order to assess the experimental validity of the Goldman equation the permeability ratios were calculated from zero-current diffusion potentials by means of the Goldman equation and compared with the cationic/anionic permeability ratios measured by simultaneous determinations of cation and anion tracer fluxes in the same membranes. The results indicate that the Goldman equation is empirically valid for the tested salts (KCl and RbCl) within the experimental range of concentrations (25 to 200 mM) at an electrochemical-potential difference of zero.

Since Diamond [1] introduced the use of the Goldman-Hodgkin-Katz equation [2, 3] to determine the ratio of cationic/anionic permeabilities in epithelia, it has been widely employed for studying the ion-passive transport in the gallbladder epithelium [4–13].

The Goldman equation for the electric zero-current potential may be written:

$$E = \frac{RT}{F} \ln \frac{\sum_i P_i c_{i1} \gamma_i + \sum_j P_j c_{j2} \gamma_j}{\sum_i P_i c_{i2} \gamma_i + \sum_j P_j c_{j1} \gamma_j} \qquad (1)$$

where i ions are the cations and j ions are the anions, P_i and P_j represent the permeability coefficients, c represents the ionic concentration, γ_i and γ_j the activity coefficients, 1 and 2 indicate the membrane side, F, R and T have their usual meaning and E is the potential difference at which the sum of all component ionic currents goes to zero, in other words the 'reversal potential' or 'resting potential'. Equation 1 was derived from the Nernst-Planck flux equation with at least three assumptions: (a) constant electric field within the membrane; (b) constant mobility of ions within the membrane; and (c) no competition or saturation of ions in the symmetrical boundaries of the membrane. Furthermore, the Nernst-Planck equation requires the assumption of independence among flows. Equations similar to the Goldman equation can be derived for membranes permeable only to ions of one sign and valence flowing in a common pathway, provided that diffusion coefficients for different ions remain in a constant proportion at any point within the membrane, or alternatively that the energy profiles accross the membrane for all permeant ions differ by no more than an additive constant (for a review see Refs. 14 and 15).

Barry et al. [6] and Wright et al. [7] proposed an electrically neutral thick-membrane model for the paracellular cation channels of the gallbladder epithelium. If this model were valid the use of the Goldman equation would be justified in this preparation. However, recent experimental evidence

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suggests that this model is not valid and that none of the assumptions of the Goldman equation are expected to hold for the cation channels of gallbladder epithelium. In fact, Wright et al. [7] and Moreno and Diamond [8] demonstrated that there are at least two different kinds of paracellular ionic pathways (cation channels and a non-specific free-solution shunt) and that the cation channels share at least one charged group at the selectivity site (see also Ref. 5). Recently, we have shown [13] that the independence principle [16] does not hold in the paracellular cation channels and that there is competition and saturation among the ions. Furthermore, experimental data seem to indicate that there is more than one charged site within the cation channels and that a single-file diffusion model may be appropriate for these channels. Fromm and Schultz [17] have also shown that a single-file diffusion model may apply for the paracellular ionic pathways of the descending colon epithelium of the rabbit.

As there is no theoretical support of the validity of the Goldman equation in the cation channels, this study was undertaken in an effort to resolve whether or not the Goldman equation is empirically valid in this preparation.

TABLE I

ION FLUXES (in $\mu mol \cdot h^{-1} \cdot cm^{-2}$), TRACER PERMEABILITY RATIOS ($P_X^*/P_{Cl}^* \equiv (\vec{J}_X/\vec{J}_{Cl})(c_{Cl}/c_X)$) AND PERMEABILITY RATIOS OBTAINED THROUGH THE GOLDMAN EQUATION (P_X/P_{Cl}) AT A CONCENTRATION c (mM)

Experiments No. 196, 197 and 199 were gallbladders from non-hibernated toads, with conductances 5 to 8 times greater than the other experiments on hibernated toads [13].

Expt.	Ion	c	J_X	J_{Cl}	P_X^*/P_{Cl}^*	P_X/P_{Cl}
175	K	200	2.5	1.0	2.5	1.5
181			4.3	0.6	7.4	7.0
183			8.6	2.2	3.9	3.9
184			9.8	1.3	7.4	5.4
185			3.7	1.3	2.8	3.7
186			3.9	1.9	2.0	3.3
$\bar{x}$			5.5	1.4	4.3	4.1
			±1.1	±0.2	±0.9	±0.7
168	K	100	8.6	2.7	2.9	3.4
170			3.7	0.7	5.2	4.9
$\bar{x}$			6.1	1.7	3.9	4.1
187	Rb	200	1.6	0.8	2.0	2.0
190			1.7	1.6	1.1	1.3
193			3.6	2.1	1.7	1.8
196			18.5	4.7	3.9	2.0
199			13.6	8.1	1.7	2.0
$\bar{x}$			7.8	3.5	2.1	1.8
			±3.1	±1.2	±0.4	±0.1
189	Rb	100	1.4	1.0	1.4	2.1
191			2.4	1.1	2.1	1.8
194			7.3	1.5	4.8	2.6
197			12.1	2.0	6.0	3.4
$\bar{x}$			5.6	1.4	3.6	2.5
			±1.9	±0.4	±0.9	±0.3
192	Rb	25	1.0	0.2	4.9	2.5
195			0.5	0.3	1.6	2.5
198			2.2	0.4	5.4	5.3
$\bar{x}$			1.2	0.3	4.0	3.5
			±0.4	±0.05	±1.0	±0.7

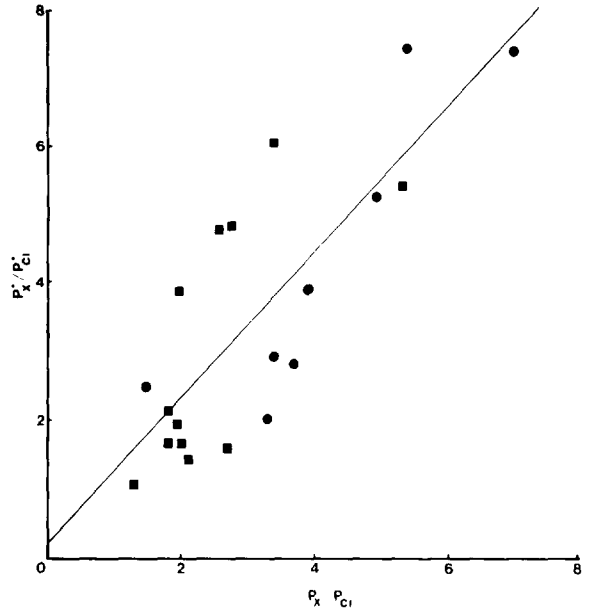

Fig. 1. P_X^*/P_{Cl}^* values are plotted against P_X/P_{Cl} obtained through the use of the Goldman equation in 20 independent experiments (Table I). ■, Rb^+ experiments; ●, K^+ experiments. Regression line: slope 1.05; ordinate 0.27; correlation coefficient 0.65; normal relative deviation of the correlation 2.83.

The results are shown in Table 1. The mean of the unidirectional ^{42}K fluxes (mucosal to serosal) (5.5 ± 1.1 $\mu mol \cdot h^{-1} \cdot cm^{-2}$) at 200 mM KCl is similar to the mean serosal to mucosal unidirectional fluxes in the same conditions (5.4 ± 0.6 $\mu mol \cdot h^{-1} \cdot cm^{-2}$) described previously [13]. The permeability ratios calculated from the Goldman equation and from tracer fluxes were compared for each ion and concentration. There is no significant difference between the variances (F tests) nor between the means (t tests).

The values of P_X^*/P_{Cl}^* (X^+ cation) calculated from tracer fluxes are plotted against P_X/P_{Cl} ratios from the Goldman equation in Fig. 1. The least-squares regression line has a slope of 1.05 and an ordinate of 0.27. The linear correlation coefficient $r = 0.65$ shows the effect of the experimental error of both isotope and electrical measurements. However, the normal relative deviation of the correlation is 2.83, thus the probability of a random correlation becomes $p < 0.001$, indicating a very significant degree of correlation.

From previous papers [6,8,11,13] there was some empirical evidence supporting the use of the Goldman equation to determine the ratio of cationic to anionic permeabilities : (a) When cations are known to be unable to permeate through cation channels, when the epithelium is severely damaged or when blockers of the cation channels are used, the P_X/P_{Cl} ratio becomes equal to μ_X/μ_{Cl} the ratio of mobilities through the free-solution shunt; (b) on the other hand some exceptionally good preparations show a $P_X/P_{Cl} > 100$ at the beginning and after an hour the ratio approaches the usual value (near 4) while the partial conductance through the cation channels remains constant (i.e. the partial conductance of Cl^- has changed); (c) in general, the partial conductances of anions and cations calculated from the total conductance and the Goldman equation behave independently of each other. Of course, this circumstantial evidence was not enough to prove the validity of the Goldman equation.

The results shown here indicate that the Goldman equation is valid for the paracellular ionic pathways of the gallbladder epithelium in KCl and RbCl within a range of concentrations between 25 and 200 mM and at an electrochemical potential difference of zero. As these pathways show a very poor selectivity among Li^+, Na^+, K^+, Rb^+ and Cs^+, the Goldman equation may be valid also for the chloride salts of the monovalent cations that were not studied here. However, unless a general demonstration of the Goldman equation (or a similar one) were available for the gallbladder paracellular pathways, the use of the Goldman equation should be experimentally supported for each ion and concentration.

A way to test the validity of Eqn. 1 is to compare its experimental results in terms of P_X/P_{Cl} (cation permeability/chloride permeability) calculated from zero-current diffusion potentials with the ratio P_X^*/P_{Cl}^* obtained by means of tracer flux measurements. In other words, the experimental validity of the identity:

$$P_i/P_j \equiv P_i^*/P_j^* \tag{2}$$

should be demonstrated for i cation and j anion. In general (see Ref. 18):

$$P^* = -J^*/\Delta c^* \tag{3}$$

where J^* is the tracer flux, c^* its concentration and $\Delta c^* = c_1^* - c_2^*$. The unidirectional flux ($\vec{J}$) is:

$$\vec{J} = -J^*/\Delta\rho \tag{4}$$

where $\Delta\rho = (c_1^*/c_1) - (c_2^*/c_2)$. Then, replacing in Eqn. 3:

$$P^* = \vec{J}\left((c_1^*/c_1) - (c_2^*/c_2)\right) / (c_1^* - c_2^*) \tag{5}$$

When c_2^* is negligible with respect to c_1^*; c_2^*/c_2 is negligible with respect to c_1^*/c_1 and both the cation i and the anion j tracers are placed at the same side of the membrane Eqn. 5 becomes simplified and can be inserted into Eqn. 2, resulting in:

$$P_i/P_j \equiv \left(\vec{J}_i/J_j\right)(c_{j1}/c_{i1}) \tag{6}$$

Eqn. 6 is the identity to be tested. In order to avoid any dependence of the measurements on the electrochemical potential difference, it should be clamped to zero during measurement of both tracer flux and diffusion potential.

Gallbladders from the South American toad *Bufo arenarum* were used. The techniques for obtaining an in vitro preparation of the open gallbladder and measuring transepithelial potential differences (p.d.) were similar to those described previously [8,13]. The bathing solutions had the following compositions (in mM): 0.25 $CaCl_2$; 1.63 K_2HPO_4; 0.73 KH_2PO_4; and 300, 200, 100, 50, 25 or 12.5 XCl (X^+ being Na^+, K^+ or Rb^+) (pH 6.75). The solutions were made isosmotic with the 300 mM salt solution by adding sucrose. The salt concentration range studied corresponds to the range employed in previous papers and includes salt concentrations below and above the physiological level.

The ratio P_X/P_{Cl} was obtained from the p.d. arising from exchanging the mucosal solution with an isosmotic solution containing diluted or concentrated XCl. The P_X/P_{Cl} value at a given concentration was obtained by linear interpolation between a dilution and a concentration diffusion potential (i.e. the P_X/P_{Cl} for 200 mM was the mean of the values obtained from a 300/200 and a 100/200 diffusion potential; mucosal concentration/serosal concentration).

Measurement of tracer fluxes: the radioactive isotopes (0.4 to 0.8 μCi/ml of $^{36}Cl^-$ in Tris chloride from The Radiochemical Centre Ltd., Amersham, U.K.; and 1 to 2 μCi/ml of carrier-free ^{42}K or ^{86}Rb from the Comisión Nacional de Energía Atómica of Argentina) were simultaneously added to the mucosal half chamber, and the flux was calculated from the rate of appearance of isotope in aliquots extracted from the serosal side. The radioactivity of the cation tracers was measured immediately in a Hewlett-Packard Spectrometer and the ^{36}Cl activity was measured after the complete decay of the tracer cation (8 to 10 mean lives) in a Tracor Analytic liquid scintillation counter. Before and after the flux measurement, the P_X/P_{Cl} ratio was calculated from diffusion potentials in order to compare the mean of both values with the ratio of tracer permeabilities. The p.d. during the tracer flux measurements was clamped to zero and the ionic concentrations were symmetrical. The actual value of the chloride concentration was calculated taking into account the isotope carrier. The tracer concentration was corrected for the unstirred layer effect as in Ref. 19 (Eqn. 2). These corrections resulted in less than 15% of the bulk solution ionic concentration in all cases.

The experiments were performed in an Na^+-free medium. Under this condition the physiological Na^+ active transport is inactivated [20], the Cl^- flux is mainly paracellular [21] and the intracellular Cl^- concentration equals or closely approaches the extracellular concentration [22]. Furthermore, there is no significant electrically silent K^+ flux [13]. Control measurements of the partial conductances and the P_X/P_{Cl} ratio in NaCl were done at the beginning and at the end of the experiments. No significant difference was found between either measurement, indicating that the use of an Na^+-free medium during the experiment does not affect the paracellular pathways. The membranes with $P_{Na}/P_{Cl} < 1.9$ at the beginning were considered as damaged and discarded.

Statistics and calculations: statistical calculations were carried out on a Hewlett-Packard 41CV calculator. Unless otherwise stated, results are expressed as mean ($\bar{x}$) $\pm$ S.E. The analysis of variance was done through Fisher's F-test and provided that there was no significant difference

between the variances, Student's t-test was used to evaluate statistical significance of the mean differences. The least squares regression for a linear model was used, and the relative variance of the correlation was calculated as r/s (r being the correlation coefficient and s the standard error of the correlation; $s = 1/\sqrt{n-1}$).

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