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Membrane potential measurements in isolated rat liver plasma membrane vesicles: Effect of transmembrane ion concentration gradients

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In isolated basolateral and canalicular rat liver plasma membrane vesicles the membrane potential (measured with DiS-C_2 (5)) varied with transmembrane concentration gradients of Na^+, K^+ and Cl^- revealing the following ion permeabilities: basolateral vesicles: P_{Na}/P_K: 0.76, P_{Cl}/P_K: 0.45 and canalicular vesicles: P_{Na}/P_K: 0.69, P_{Cl}/P_K: 0.56. The data indicate a permselectivity of $P_K > P_{Na} > P_{Cl}$ for both membranes.

The hepatobiliary transport of various organic compounds is regulated by the membrane potential. At the basolateral membrane the intracellular negative potential favours electrogenic sodium-coupled uptake (e.g. amino acids) and diffusion of cations into the cell whereas, at the luminal membrane, the intracellular negative electric potential promotes the biliary excretion of anions (lumen −5 mV, cell −40 mV), both with reference to the vascular space [6,7].

Many transport processes were studied in isolated plasma membrane vesicles of the basolateral and canalicular domain of the liver cell by measuring radioactive tracer fluxes [10]. Although of considerable impact, estimates of the membrane potential have been obtained only by indirect approaches (cf. Ref. 4). In membrane vesicles from various other cells and organs the use of voltage-sensitive optical probes proved to be a useful tool in studying electrogenic transport processes and ion permeabilities (for reviews see Refs. 14 and 16).

It was therefore the aim of this study (i) to use the voltage-sensitive carbocyanine dye 3,3′-diethylthiadicarbocyanine iodide (DiS-C_2(5)) [1,11,16] in order to determine the membrane potential in membrane vesicles of rat liver parenchymal cells and (ii) to measure relative ion permeabilities by analyzing diffusion potentials generated by various transmembrane ion concentration gradients.

The data show (i) that fluorescence quenching of the cationic dye DiS-C_2(5) in vesicle suspensions is linearly related to the intravesicular negative membrane potential and (ii) that both domains of the hepatocyte plasma membrane exhibit a permeability sequence of $P_K > P_{Na} > P_{Cl}$. The results are discussed with respect to measurements in intact liver and to their possible significance for hepatic electrolyte transport.

Plasma membrane vesicles of basolateral and canalicular origin of rat liver were prepared according to the methods of Wisher and Evans [15] with small modifications as described elsewhere [13]. In brief: from a nuclear pellet prepared from a rat liver homogenate a mixed (canalicular and basolateral) membrane fraction was isolated on a sucrose gradient in a zonal rotor at a density range between 1.13 and 1.18. After vigorous homogenization of the mixed fraction basolateral and canalicular domains were separated on a discontinuous sucrose gradient at 1.13 and 1.18, respectively. The degree of purification was determined by the enzyme markers glucagon-stimulated adenylate cyclase and Na^+/K^+-ATPase (10–18-fold enrichment over the initial homogenate in basolateral membranes) and alkaline phosphatase and Mg^{2+}-ATPase (30–50-fold enrichment over the initial homogenate in canalicular membranes). Both membrane fractions exhibited only minor cross contamination (for details see Ref. 12). Protein was measured according to the method of Bradford [2] with bovine serum albumin as a standard. pH and osmolarity of the incubation media were controlled on a pH M 62 Standard pH-Meter (Radiometer, Kopenhagen) and on a Gonotec osmometer (Berlin, Germany), respectively.

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For membrane potential measurements membrane vesicles were preloaded with 150 mM potassium gluconate, 1 mM NaCl, 10 mM Hepes-KOH (pH 7.4) for at least 6 h at 0°C.

Transmembrane potential differences were measured using the fluorescent dye DiS-C_2(5) as described by Burckhardt and Murer [1]: Briefly, under vigorous stirring 20 μl of the basolateral or canalicular membrane suspension (protein concentration 10 mg/ml) were diluted into 2 ml of external medium (composed as described in the legends to Table I and Fig. 1) containing 20 μl of an ethanolic stock solution of DiS-C_2(5) (300 μM). Fluorescence was measured with a Shimadzu RF 510 spectrofluorometer at 669 nm (excitation 662 nm) at 22–23°C. The dependence of fluorescence quenching (ΔF) on the magnitude of the intravesicular negative potential was studied by applying outward-directed K^+ gradients in the presence of 2 μM of gramicidin (5 μl of an ethanolic stock solution). Addition of 5 μl ethanol without gramicidin did not influence DiS-C_2(5) fluorescence quenching. Outward-directed K^+ gradients were obtained by diluting preloaded vesicles into 'external' medium containing graded concentrations of potassium gluconate. K^+ was either replaced by tetramethylammonium or by *N*-methyl-D-glucosamine in order to provide constant ionic strength (compare Ref. 17). Under these conditions the membrane potential (V_m) is assumed to be identical with the K^+-diffusion potential (Nernst potential difference, E_K). A calibration of ΔF vs. V_m was obtained for each vesicle preparation. The dependence of V_m on transmembrane concentration gradients of K^+, Na^+ and Cl^- was studied by diluting membrane vesicles into media of various ionic composition (see Table I, Fig. 2). Relative ion permeabilities (P_{Na}/P_K, P_{Cl}/P_K) were calculated by the Goldman-Hodgkin-Katz equation [5,9].

All experiments were repeated several times (n) with fresh membrane preparations. In each individual experiment measurements were done in triplicate. Values are expressed as mean ± S.D. (n).

Fig. 1 shows the correlation between fluorescence quenching and membrane potential (interior negative) for basolateral and canalicular rat liver plasma membrane vesicles. The relationship between the membrane potential (E_K) in the range of −9.75 to −83 mV and the fluorescence was linear ($r > 0.995$) with slopes ranging from 0.19 to 0.42% ΔF/mV in individual vesicle preparations. Canalicular membrane vesicles had a tendency to exhibit smaller slopes (0.25 ± 0.05, $n = 9$) than basolateral vesicles (0.31 ± 0.06, $n = 10$) ($0.05 > p > 0.025$), possibly indicating that canalicular membranes are permeable to gluconate to some degree.

Ionic diffusion potentials were generated by diluting K^+-loaded vesicles into a medium containing as the predominant permeant ions, Na^+, K^+ and Cl^-, respectively. Dilution resulted in a rapid (< 1 s) maximal

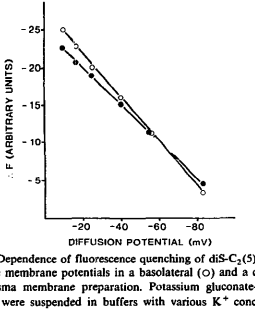

Fig. 1. Dependence of fluorescence quenching of diS-C_2(5) on inside negative membrane potentials in a basolateral (○) and a canalicular (●) plasma membrane preparation. Potassium gluconate-preloaded vesicles were suspended in buffers with various K^+ concentrations followed by an addition of gramicidin. Diffusion potentials were calculated from the Nernst equation. K^+ was replaced by *N*-methyl-D-glucosamine.

quenching of fluorescence which slowly recovered as the imposed ion gradients dissipated. The potential amplitude (ΔmV) was obtained from the peak fluorescence quenching and the calibration line for the individual vesicle preparation. Data from individual experiments are summarized in Table I.

Relative ion permeabilities were calculated from the Goldman-Hodgkin-Katz equation using the linear correlation between P_{Na}/P_K and P_{Cl}/P_K in form of:

$$P_{Cl}/P_K = \frac{P_{Na}/P_K(Na_o - e^{VF/RT}Na_i) + K_o - e^{VF/RT}K_i}{e^{VF/RT}Cl_o - Cl_i}$$

where F, R, and T are the Faraday, gas constant and absolute temperature, respectively. As shown in Fig. 2 each experimental result of Table I is represented by a straight line, their intercepts giving the common solution for the relative permeabilities P_{Na}/P_K and P_{Cl}/P_K.

Basolateral vesicles: $P_{Na}/P_K = 0.76$, $P_{Cl}/P_K = 0.45$. Canalicular vesicles: $P_{Na}/P_K = 0.69$, $P_{Cl}/P_K = 0.56$. This calculation shows that both the basolateral and the canalicular plasma membrane vesicles exhibit a permeabilities sequence of $P_K > P_{Na} > P_{Cl}$.

The present study shows that the carbocyanine dye DiS-C_2(5) can be used to measure changes of the electric membrane potential in isolated rat liver plasma membrane vesicles. Effects of various transmembrane ion concentration gradients on the membrane potential show that membrane vesicles derived from both the basolateral and canalicular domain of the plasma membrane, exhibit a permselectivity of $P_K > P_{Na} > P_{Cl}$. For the basolateral membrane vesicles this sequence is in accordance with earlier indirect estimates [4,12], but in the intact tissue [3,8] and in isolated cells [6] a selectiv-

TABLE I

Ionic diffusion potentials in isolated basolateral and canalicular plasma membrane vesicles

Vesicles were preloaded with 150 mM potassium gluconate, 1 mM NaCl, 10 mM Hepes-KOH (pH 7.4) for at least 6 h at 0°C and suspended in the following 'external media' at 25°C.
'NaCl': 150 mM NaCl, 1 mM potassium gluconate, 10 mM Hepes-KOH (pH 7.4).
'KCl': 150 mM KCl, 1 mM sodium gluconate, 10 mM Hepes-KOH (pH 7.4).
'Sodium gluconate': 150 mM sodium gluconate, 1 mM KCl, 10 mM Hepes-KOH (pH 7.4).

External medium	Ion movements	Voltage change (Δ mV)	
		basolateral vesicles	canalicular vesicles
'NaCl'	K^+ →, Na^+ ←, Cl^- ←	−14.9 ± 5.7 (14)	−18.3 ± 5.0 (16)
'KCl'	K^+ →, K^+ ←, Cl^- ←	−9.4 ± 3.8 (18)	−12.2 ± 4.4 (12)
'Sodium'	K^+ ←, Na^+ →	−6.9 ± 3.7 (17)	−9.1 ± 4.5 (12)

ity sequence of $P_K > P_{Cl} > P_{Na}$ has been found. Conductive transport of Cl^- in canalicular membrane vesicles has been reported [10] but comparable data on relative ion permeabilitics of this membrane domain are not available.

Assuming passive distribution of Cl^- [3,6] and application of the high values of the relative Na^+ permeability (P_{Na}/P_K) measured in this study to intact cells would result in a membrane potential of near zero whereas values of −40 mV have been reported (−40 mV' at 37°C [3,6] and −30 mV at room temperature [16]).

This disparity together with the discrepancy in the permselectivity of intact cells and isolated membrane vesicles make it unfeasible to draw conclusions on the presence or absence of polar properties of ion permeabilities in the intact cell that might result in transcellular electrolyte transport.

It appears that membrane vesicle suspensions exhibit quantitatively different permeability properties than membranes of intact cells. This difference may result from the density and conductance of individual ion channels in the intact cell membrane (e.g., a small number of K^+ channels with a high conductance) and their random distribution to individual membrane vesicles. A fraction of membrane vesicles could lack K^+ channels and fluorescence quenching of these vesicles would not be altered by K^+ gradients. In addition factors with regulate ion permeabilities in the intact cell may be absent or modified in the isolated membrane preparation (compare Ref. 17).

In conclusion electrogenic transport of K^+, Na^+ and Cl^- has been demonstrated in isolated rat liver plasma membrane vesicles by use of the fluorescence quenching of the carbocyanine dye DiS-C_2(5). The data indicated that channels for all three ions are present in both membrane domains but inferences on their quantitative role in the intact cell appear unfeasible from our vesicle data.

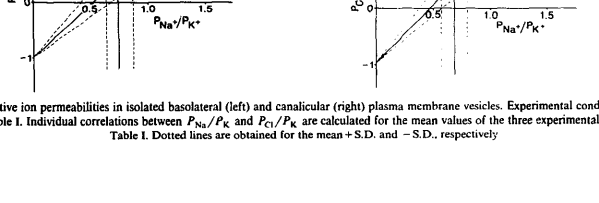

Fig. 2. Relative ion permeabilities in isolated basolateral (left) and canalicular (right) plasma membrane vesicles. Experimental conditions were as given in Table I. Individual correlations between P_{Na}/P_K and P_{Cl}/P_K are calculated for the mean values of the three experimental conditions of Table I. Dotted lines are obtained for the mean + S.D. and − S.D., respectively

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