BIOCHEMICAL EVIDENCE THAT Na^+,K^+-ATPase IS LOCATED AT THE LATERAL REGION OF THE HEPATOCYTE SURFACE MEMBRANE

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1. Introduction

The secretion of bile by hepatocytes into the bile canaliculi is an active process accompanied by osmotic filtration of water and electrolytes [1,2]. The two major solutes are bile salts and sodium, and bile acid-dependent and bile acid-independent components of bile formation are identified experimentally. Several lines of evidence suggest that bile acid-independent bile flow is regulated by a Na^+,K^+-ATPase located at the hepatocyte plasma membrane [3–6]. The mammalian plasma membrane is, however, a complex organelle, and in hepatocytes three major domains are recognised anatomically [7] and physiologically [8,9]. It has been assumed in most studies that the Na^+,K^+-ATPase is located at the bile canalicular domain of the plasma membrane, and biochemical studies in which a single plasma membrane fraction containing mainly bile fronts was studied would appear to support this location [3–6]. A more precise location for Na^+,K^+-ATPase activity can be identified by using plasma membrane subfractions shown to originate from the sinusoidal, lateral and canalicular domains of the hepatocyte surface [12,23]. This communication shows that Na^+,K^+-ATPase is located mainly in a plasma membrane subfraction characterised by intercellular junctions originating from the hepatocyte lateral side and that activity is low in a fraction containing high specific activities of a range of enzymes characteristic for the bile canalicular plasma membrane. This predominantly lateral location of the Na^+,K^+-ATPase provides independent support for recent histochemical evidence for a baso-lateral location of the enzyme on hepatocytes [10,11].

Abbreviation: Na^+,K^+-ATPase, Na^+,K^+-adenosine triphosphatase

2. Methods

Plasma membrane subfractions were prepared from the livers of 10 male fed Sprague-Dawley rats (150–180 g) as in [12], but with the following modification. The supernatant remaining after the first low speed centrifugation step was collected, and adjusted to 8% (w/v) sucrose and this was used to prepare after removal of mitochondria and lysosomes a 'microsomal light' plasma membrane subfraction of density on sucrose gradient 1.12–1.14 g/cm^3 [12]. The plasma membranes prepared from the low speed pellet by a rate-zonal centrifugation procedure using a MSE A XIII rotor [19] were fractionated, after homogenisation in a tight-fitting Dounce, on sucrose gradients to yield the following subfractions of stated densities: zonal-light (1.12–1.13 g/cm^3) zonal-heavy A (1.15–1.16 g/cm^3) and zonal-heavy B (1.16–1.18 g/cm^3).

Membrane fractions were first hypotonically lysed by homogenisation and washing in 10 mM Tris (pH 7.6). Na^+,K^+-ATPase activity of the fractions was determined according to [13] as the proportion of ATPase activity measured in the presence of 5 mM ATP, 5 mM MgCl, 120 mM NaCl and 12.5 mM KCl

Tris–HCl buffer (pH 7.4) that was inhibited when 1 mM oubain was included in the reaction mixture. Phosphate liberated was determined colorimetrically [14], and all assays were carried out in duplicate and with the appropriate controls. The other enzymes measured were 5′-nucleotidase [15], leucylnaphthylamidase [16], alkaline phosphodiesterase [17] and γ-glutamyltranspeptidase which was determined using a Boehringer kit. Protein was determined by the method in [18] and samples of the plasma membrane fractions were prepared for electron microscopy as in [12].

3. Results

The biochemical properties of the liver plasma membrane subfractions in [12,19] are now extended to identify the hepatocyte surface region where Na^+,K^+-ATPase activity is mainly localised. When the inter-plasma membrane subfraction distribution of Na^+,K^+-ATPase was determined (table 1), specific activity was highest in the subfraction of higher density (zonal-heavy B), with lower specific activity which was highly variable measured in the less-dense zonal-heavy A subfraction. This sub-plasma membrane distribution contrasted markedly with that of Mg^{2+}-ATPase, 5′-nucleotidase, and alkaline phosphodiesterase specific activities (table 2), enzymes for which there is a body of cytochemical evidence for a predominantly bile canalicular plasma membrane location [20–22]. The results show that Na^+,K^+-ATPase activity is located mainly in a plasma membrane subfraction deriving from a non-canalicular region of the hepatocyte surface.

Morphological examination of the zonal-heavy B subfraction showed it to contain by far the largest content of membrane sheets, and many desmosomes and gap junctions can be identified (fig.1A). The zonal-heavy A subfraction (fig.1C), contained fewer mem-

Table 1
Distribution of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities and protein yield of rat liver plasma membrane subfraction

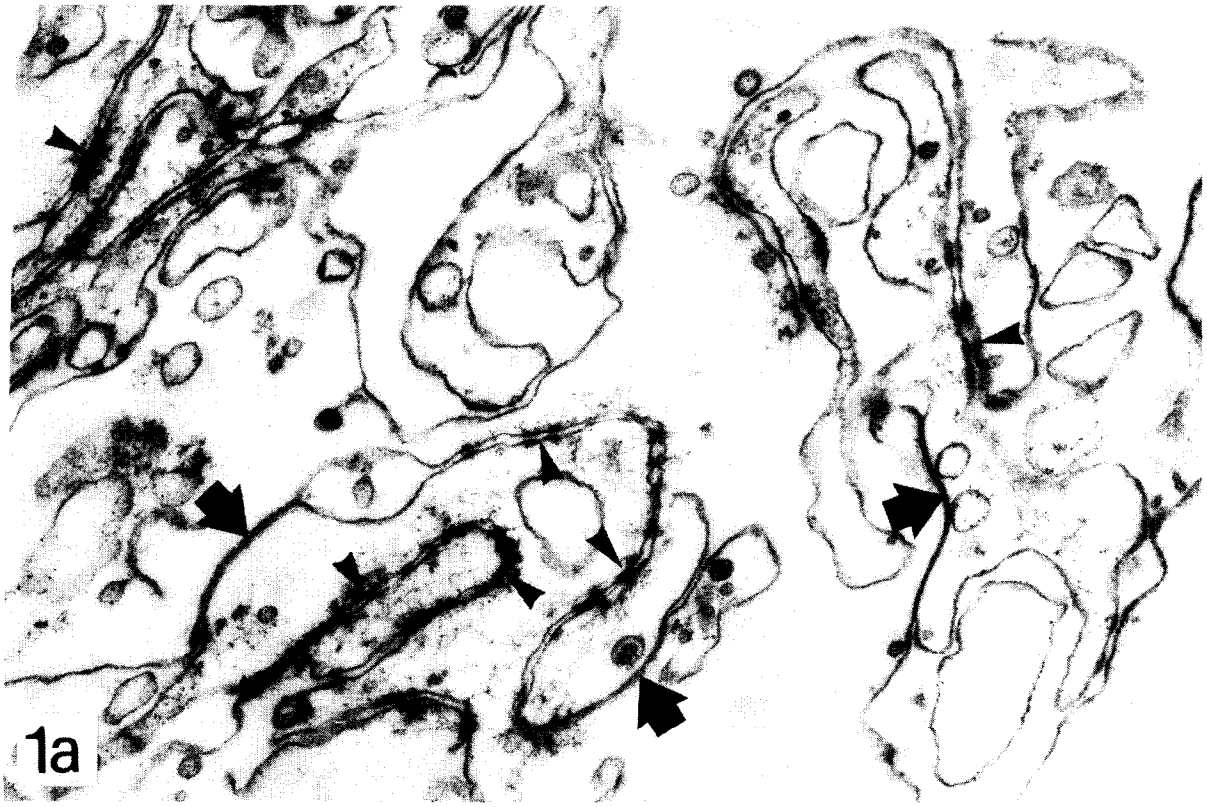
	Homogenate	Zonal-light	Zonal-heavy A	Zonal-heavy B	Microsomal-light
Na^+,K^+-ATPase	1.0 ± 0.8	5.5 ± 4.8	9.6 ± 9.6	17.3 ± 4.7	1.9 ± 2.4
Mg^{2+}-ATPase	5.6 ± 0.8	88.6 ± 17.5	45.4 ± 14.0	42.2 ± 9.5	6.8 ± 3.9
Protein yield		0.086 ± 0.028	0.103 ± 0.071	0.285 ± 0.062	0.512 ± 0.126

Values are means ± SD from 4 expt. ATPase activities are expressed as μmol P_i . mg protein^{-1} . h^{-1}. Protein yield is expressed as mg plasma membrane protein . g liver^{-1}

Table 2
Distribution of marker enzyme activities in plasma membrane subfractions from rat liver

	Homogenate	Zonal-light	Zonal-heavy A	Zonal-heavy B	Microsomal-light
5′-Nucleotidase	1.56 ± 0.40 (5)	109.5 ± 38.7 (6)	50.6 ± 19.9 (4)	28.8 ± 3.9 (5)	39.9 ± 23.5 (6)
Alkaline phosphodiesterase	2.1 ± 0.8 (5)	334 ± 114 (5)	73.2 ± 46 (4)	36.3 ± 14 (4)	45.3 ± 32 (5)
Leucylnaphthylamidase	0.29 ± 0.10 (3)	15.70 ± 6.50 (3)	2.09 ± 0.12 (3)	2.27 ± 0.43 (3)	1.51 ± 0.40 (3)
γ glutamyltranspeptidase	7.1 (2)	574 (2)	137 (2)	32 (2)	193 (2)

Values are means ± SD. Numbers in parentheses are number of preparations. Enzyme activities are expressed as μmol substrate . mg protein^{-1} . h^{-1}, except γ glutamyltranspeptidase activity which is expressed in IU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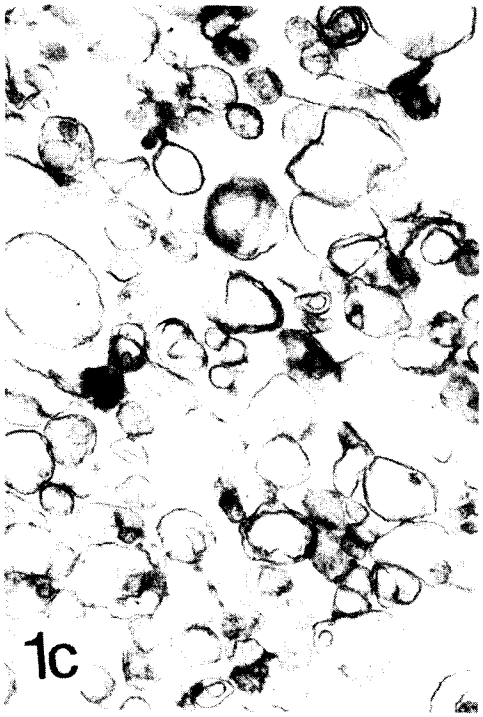
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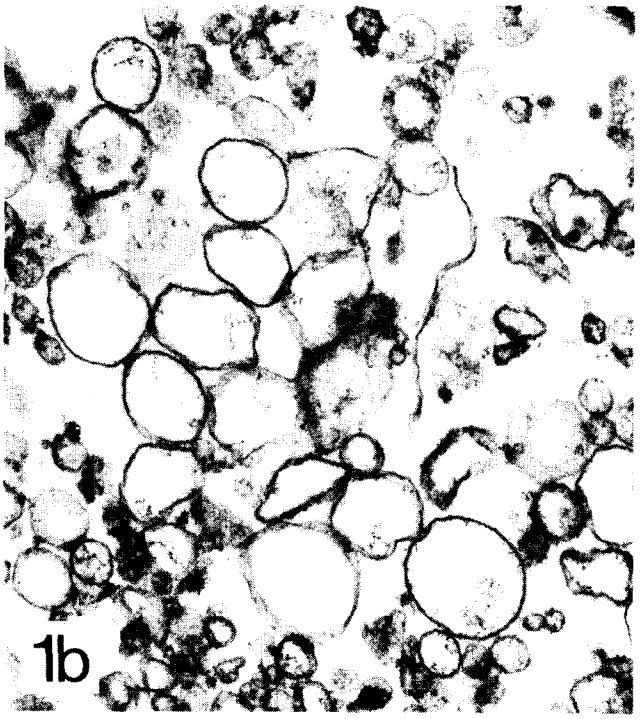
1b

brane sheets and larger numbers of vesicles of unknown surface origin, and its morphological appearance was intermediate between the zonal-heavy B and the predominantly vesicular junction-devoid microsomal-light (fig.1B) and zonal-light [12,19] plasma membrane subfractions.

The distribution of leucylnaphthylamidase and γ-glutamyltranspeptidase specific activities was similar to that of the three bile canalicular enzymes, being higher in the zonal-light subfraction (table 2). It is unlikely that these enzymic differences between the plasma membrane subfractions can be accounted for by differences in fraction purity, for it was shown [12] that contamination of these subfractions by intracellular organelles and membranes was very low with the exception of the microsomal-light subfraction which contained glycosyl transferase activities.

4. Discussion

This study using rat liver plasma membrane subfractions which have been shown to originate from each of the three major surface domains of the hepatocyte, provides compelling evidence for now assigning the location of the Na^+,K^+-ATPase activity to mainly lateral and interstitial regions of hepatocytes. Na^+,K^+-ATPase was present in those plasma membrane fractions of high density, as in [19], and is now shown to be located at highest specific activity in a subfraction containing a large number of intercellular junctions. These subfractions of high density contained low specific activities of a number of plasma membrane marker enzymes present at highest specific activities in the zonal-light subfraction that originates from the bile canalicular membrane. Low specific activities of Na^+,K^+-ATPase were measured in a microsomal-light subfraction containing adenylate cyclase activity highly activated by glucagon [12] and deriving mainly from the sinusoidal plasma membrane region [23]. These results, which extend previous work on the biochemical fractionation of the hepatocyte plasma membrane and the identification of subfractions originating from specific functional regions [12], agree with recent histochemical evidence for a non-canalicular location of the Na^+,K^+-ATPase [10,11]. However, in contrast to the histochemical data, the biochemical fractionation results show that the enzyme is present at low specific activity in a fraction originating mainly from the sinusoidal plasma membrane region. However, in view of the large variation in enzyme activity measured in the zonal-heavy A subfraction, the possibility remains that Na^+,K^+-ATPase activity may also be present in plasma membranes originating from a region intermediate between the major sinusoidal and lateral domains [7].

The present results, taken together with histochemical evidence, provide support for the paracellular pathway as a route of entry of sodium into bile [24]. The transport of sodium into the interhepatocytic cleft, catalysed by the Na^+,K^+-ATPase thus raises questions regarding the permeability properties of the hepatic tight junctions. However, the assignment of the Na^+,K^+-ATPase to the lateral side of hepatocytes now conforms with the location of the enzyme in transporting epithelia in general [25], but further work is required in liver to identify factors controlling this transepithelial pathway of sodium movement, and its entry into the bile canaliculi.

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Fig.1. (A) Electron micrograph of 'zonal-heavy B' plasma membrane subfraction. Thin arrows point to desmosomes; thick arrows point to gap junctions. Magnification × 38 750. (B) Electron micrograph of the 'microsomal-light' plasma membrane fraction. Magnification × 38 750. (C) Electron micrograph of the 'zonal-heavy A' plasma membrane subfraction. Magnification × 38 750.

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