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ATP-dependent Ca^{2+} uptake and Ca^{2+}-dependent protein phosphorylation in basolateral liver plasma membranes

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ATP-dependent Ca^{2+} uptake was measured in vesicles of rat liver cell basolateral plasma membranes. Nucleotide-dependent uptake was specific for ATP and observed at pH 7.0 and 7.4/7.5 but not at pH 8.0. ATP-dependent Ca^{2+} transport was only observed in the presence of Mg^{2+}. Kinetic analysis of ATP-dependent transport revealed an apparent K_m in the submicromolar region. Addition of calmodulin and trifluoperazine had no effect on ATP-dependent uptake. A Ca^{2+}-dependent, phosphorylated intermediate with the apparent molecular weight of 135000 could be demonstrated in the basolateral plasma membranes. Phosphorylated intermediates with apparent molecular weights of 200000 and 110000 were demonstrated in microsomes and appeared to contaminate 'basolateral' membrane protein phosphorylation. The results suggest that a 135000 molecular weight protein is a Ca^{2+}-ATPase and the enzymatic expression of the liver cell basolateral membrane Ca^{2+} pump.

Introduction

It has been proposed that an energy driven Ca^{2+} pump, sited in the plasma membrane, is involved in the maintenance of low cytosolic Ca^{2+} concentration and its regulation in the hepatocyte. The pump has been identified with a high Ca^{2+} affinity, low-Mg^{2+} requiring Ca^{2+}-ATPase, but this identification has been challenged [1–9]. Ca^{2+}-dependent formation of an acyl-phosphorylated intermediate with an apparent molecular weight of 118000 has been demonstrated in liver cell plasma membrane fractions [6]; this protein has been reconstituted in phospholipid vesicles and ATP-dependent Ca^{2+} transport was observed [7]. It is noteworthy, however, that the apparent molecular weight of this compound is similar to that of the transport Ca^{2+}-ATPase of hepatic endoplasmic reticulum [10], but differs from the plasma membrane transport Ca^{2+}-ATPases of other epithelial cells and erythrocytes [11,12].

In the present study, basolateral liver cell plasma membrane vesicles were prepared [13] and ATP-driven Ca^{2+} uptake was found to be present.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol; Mops, 4-morpholinepropanesulphonic acid; HEDTA, *N*-hydroxyethylenediaminetriacetic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethyleneglycol bis(β-aminoethyl-ether)-*N*,*N*′-tetraacetic acid.

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Ca^{2+}-dependent formation of an acyl-phosphorylated intermediate with the apparent molecular weight of 135 000 was found in basolateral liver plasma membranes.

Experimental Procedures

Materials. ATP (Mg^{2+} salt), calmodulin, and the Ca^{2+} ionophore, A23187, were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), Ouabain and oligomycin were purchased from Fluka AG (Buchs, Switzerland). $^{45}CaCl_2$ (specific activity 4–50 $mCi \cdot mg^{-1}$) and [γ-^{32}P]ATP (specific activity 3000 $Ci \cdot mmol^{-1}$, 1.7 μM) were purchased from Dupont New England Nuclear Co. (Boston, MA, U.S.A.). All other chemicals were of reagent grade and were purchased from BDH Chemicals Ltd. (Poole, U.K.), Calbiochem (Lucerne, Switzerland), Fluka, (Buchs, Switzerland), Merck (Darmstadt, F.R.G.) or Sigma.

Animals. Male Sprague-Dawley rats (SUT : SDT Sueddeutsches Tierzuchtinstitut, Tuttlingen, F.R.G.) weighing 200–250 g were used for membrane preparation. The animals had free access to H_2O, and were fed ad libitum (Nafag 690 diet, Gossau, Switzerland), and were housed in a constant temperature, constant humidity environment, with alternating 12 h light (6.30 a.m. to 6.30 p.m.) and dark cycles. Fed animals were employed for study, and membrane preparations were begun between 7.30 and 8.30 a.m.

Preparation of membranes. Animals were killed by decapitation. Basolateral and canalicular liver plasma membrane vesicles were prepared by a combination of rate zonal and discontinuous sucrose density gradient centrifugation as described previously [13]. For isolation of a total microsomal subfraction, livers were perfused with ice-cold 0.9 g/l NaCl via the portal vein. The livers were homogenized in 0.25 M sucrose, and microsomes were collected from the post-mitochondrial fraction [14]. Membrane subfractions were resuspended in the appropriate buffered medium (see below), or were stored in buffer (100 mM KCl/100 mM mannitol/20 mM Hepes (pH 7.0)/5 mM $MgCl_2$/3 mM EGTA/2 mM dithiothreitol/1 mM ATP/15 $ml \cdot l^{-1}$ glycerol), and frozen in liquid N_2, at protein concentrations > 5 $mg \cdot ml^{-1}$. Membrane preparations could be stored for periods up to 4 weeks without loss of enzyme activity or Ca^{2+} transport function.

Measurement of Ca^{2+} uptake. Frozen basolateral liver plasma membrane vesicles were thawed, washed with buffer (150 mM KCl/20 mM Mops (pH 7.0)/5 mM $MgCl_2$/2 mM ouabain/1 mM dithiothreitol) and suspended. The final protein concentration was 3 to 6 $mg \cdot ml^{-1}$. In order to measure $^{45}Ca^{2+}$ uptake, 20 μl of membrane suspension was added to 160 μl of buffer (150 mM KCl/20 mM Mops (pH 7.0)/5 mM $MgCl_2$/50 μM $^{45}CaCl_2$/2 mM ouabain/10 μM oligomycin at 37 °C), and study was started by the addition of ATP (10 μl, 100 mM) or water, 10 μl, for controls. As described below, additional experiments were performed in which A23187 (5 μM) was included in the incubation medium. To test for an influence of pH on uptake rates we have also performed experiments at a pH of 8.0 by titrating the same solutions to pH 8.0 instead of 7.0. At the intervals after the start of incubation indicated in the figures and tables, 20-μl samples were obtained and subjected to rapid filtration on Sartorius cellulose nitrate filters (0.65 μm pore size). The filters were immediately washed twice with 4 ml of stop solution (150 mM KCl/20 mM Mops (pH 7.0)/2 mM $MgCl_2$/2 mM EGTA), and were then counted in a liquid scintillation counter. Initial experiments have included 5 mM $CaCl_2$ instead of EGTA in the wash solutions; the experimental data were not different for the two different procedures.

Experimental variation in Ca^{2+} uptake was noted from membrane preparation to membrane preparation. Qualitatively similar results, however, were obtained with all membrane preparations. All comparisons (e.g., Ca^{2+} uptake with and without ATP, Ca^{2+} uptake in the presence and absence of A23187) were performed simultaneously using membranes from a single preparation. Each such result was confirmed with membranes obtained from at least three separate preparations. Within each study, each uptake measurement was performed in triplicate or quadruplicate. The variations among the triplicate/quadruplicate determinations were less than 10%.

For the study of the effect of calmodulin on vesicle Ca^{2+} transport, basolateral liver plasma membranes were extracted with hypotonic EDTA

[11,15] and were resuspended in 1 ml of 160 mM KCl/20 mM Hepes (pH 7.4). Ca^{2+} uptake was measured at 37°C in media (100 mM KCl/50 mM Mops (pH 7.4)/4 mM MCl_2/1 mM HEDTA/10 μM oligomycin) in which $^{45}CaCl_2$-EGTA was adjusted to provide free Ca^{2+} concentrations in the range 68 to 786 nM [15]. Free Ca^{2+} concentrations were calculated by an iterative computerized procedure described previously [15]. In each experiment, 0.625 μg calmodulin in 2.5 μl H_2O (or H_2O alone, controls) was added to 100 μl medium. Following a 4 min preincubation of vesicles (20 μl), Ca^{2+} uptake was initiated by the addition of 5 μl 100 mM ATP.

Calcium-dependent phosphorylation of membrane proteins. Basolateral liver plasma membranes, canalicular liver plasma membranes or microsomal membranes were suspended in 0.25 M sucrose/50 mM Tris-HCl/pH 7.0 (Fig. 3) or 7.0, 7.5 and 8.0 (Fig. 4), and 100-μl samples, containing 100 μg membrane protein, were incubated for 10 s at room temperature with 10 μCi [γ-^{32}P]ATP (3000 Ci · mmol^{-1}; final ATP concentration, 17 nM). This reaction was performed in 1 mM EGTA, in 50 μM $CaCl_2$ or 50 μM $CaCl_2$ plus 20 μM $LaCl_3$. Reactions were stopped by the addition of 100 μl 10% trichloroacetic acid containing 1 mM ATP and 1 mM Na_3PO_4. The precipitated protein was collected by centrifugation, and the pellets were washed once with 0.5 ml H_2O. In certain studies, as noted below, the trichloroacetic acid pellet was treated with mild alkali or with hydroxylamine before further analysis [16]. The labeled products were separated by SDS-PAGE on acidic gels [17], and the gels were developed radioautographically using intensifying screens [18].

Other methods. Protein was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Munich, F.R.G.) or by the method of Lowry et al. [19] using γ-globulin or BSA, respectively, as a standard. Marker enzyme activities including alkaline phosphatase, ouabain-sensitive Na^+/K^+-ATPase, NADPH–cytochrome-*c* reductase, and succinate–cytochrome-*c* reductase, were determined by standard methodology [20–22].

Results

Table I shows the enrichment of specific activities of marker enzymes for the basolateral liver plasma membranes (Na^+/K^+-ATPase), for the endoplasmic reticulum (NADPH–cytochrome *c* reductase) and for mitochondria (succinate–cytochrome-*c* reductase) [13]. The basolateral liver plasma membranes were 15-fold enriched in Na^+/K^+-ATPase, 10-fold enriched in alkaline phosphatase, but had essentially unaltered mitochondrial marker, and markedly diminished endoplasmic reticulum marker as compared with the whole cell homogenate. Thus, as described by the original procedure [13], basolateral liver plasma membranes are contaminated by canalicular membranes; the contamination by endoplasmic reticulum is small and by mitochondria practically absent. On the other hand, the microsomes were little contaminated by basolateral membranes and mitochondria and not by canalicular membranes. For comparison, we have also included data on isolated canalicular membranes as obtained in the same separation procedure [13]. They are highly enriched in the canalicular marker enzyme (alkaline phosphatase) with apparently little contamination.

TABLE I

ENRICHMENT OF SPECIFIC ACTIVITIES OF MARKER ENZYMES

The specific activities are expressed as multiples of enzymatic activity in homogenate. Experimental details in text. n.d., not detectable.

	Na^+/K^+ ATPase	Alkaline phosphatase	NADPH–cyt.-*c* red.	Succinate–cyt.-*c* red.
Homogenate	1	1	1	1
Basolateral membranes	15	10	0.24	1.2
Canalicular membranes	0.05	87	0.4	0.09
Microsomes	0.12	n.d.	5.6	0.08

Fig. 1a depicts data from a representative experiment on ATP-dependent Ca^{2+} uptake by basolateral liver plasma membrane vesicles. Measured at a Ca^{2+} concentration of 50 μM and at pH 7.0, the vesicles showed rapid Ca^{2+} uptake after addition of ATP as compared to uptake in the absence of ATP. Inclusion of A23187 in the incubation medium prevented the ATP-dependent stimulation of Ca^{2+} uptake suggesting that ATP-

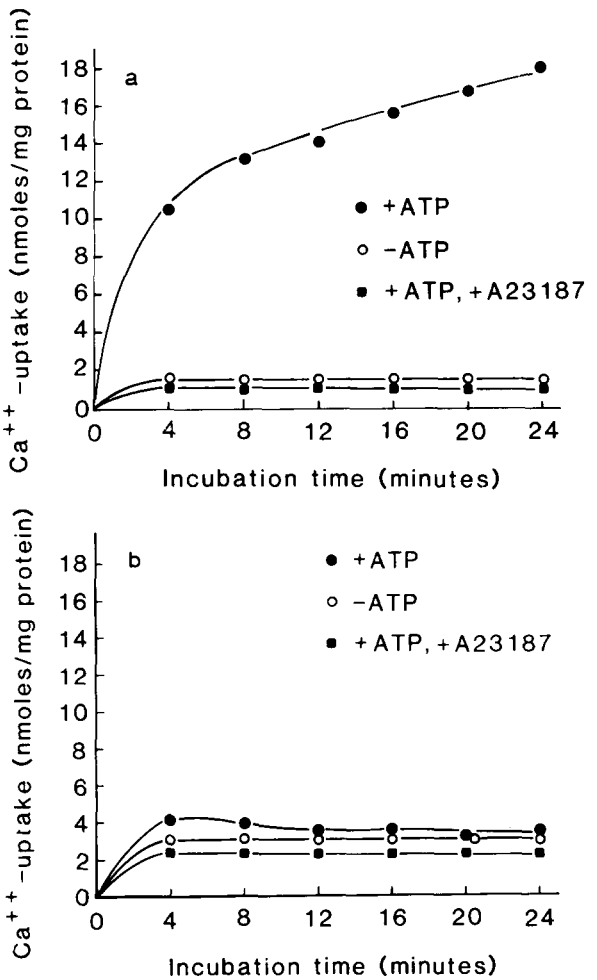

Fig. 1. Ca^{2+} uptake by basolateral (a) and canalicular (b) liver plasma membrane vesicles. Ca^{2+} uptake was studied as described in the text at a Ca^{2+} concentration of 50 μM. Representative experiments on membranes obtained within the same separation procedure are shown. Each point is the average of triplicate determinations (experimental variations less than 10%).

dependent uptake occurred against a Ca^{2+} concentration gradient. For comparison, we show an identical experiment (same separation) with canalicular membranes (Fig. 1b). As compared to the basolateral membranes, ATP-dependent transport is minimal in canalicular membranes. In most of the experiments performed, ATP-dependent Ca^{2+} uptake in canalicular liver plasma membranes was even smaller, i.e. virtually absent.

When Ca^{2+} uptake was measured at pH 7.0 and at pH 8.0, ATP-dependent uptake could only

TABLE II

pH DEPENDENCE OF ATP-STIMULATED Ca^{2+} UPTAKE BY BASOLATERAL LIVER PLASMA MEMBRANE VESICLES

ATP-dependent Ca^{2+} uptake was determined as described in the text and at a pH of 7.0 or 8.0. Ca^{2+} concentration was 100 nM. Values of a representative experiment performed in quadruplicate ($\pm$S.D.) are presented.

pH of media	Ca^{2+} uptake ($nmol \cdot mg^{-1} \cdot 4\ min^{-1}$)	
	no ATP	plus ATP
7.0	0.46 ± 0.02	1.42 ± 0.11
8.0	0.50 ± 0.06	0.47 ± 0.10

TABLE III

NUCLEOTIDE SPECIFICITY OF Ca^{2+} UPTAKE BY BASOLATERAL LIVER PLASMA MEMBRANE VESICLES

Ca^{2+} uptake in the presence of different nucleotides and of *p*-nitrophenylphosphate (pNPP) (2.22 mM each) was determined as described in the text for ATP-dependent uptake. Ca^{2+} concentration was 50 nM. Values of a representative experiment performed in quadruplicate ($\pm$S.D.) are presented.

Nucleotide	Ca^{2+} uptake ($nmol \cdot mg^{-1} \cdot 4\ min^{-1}$)
Control (no nucleotide)	0.27 ± 0.01
ATP	0.76 ± 0.04
ADP	0.29 ± 0.01
GTP	0.26 ± 0.01
ITP	0.25 ± 0.01
pNPP	0.24 ± 0.01

TABLE IV

EFFECT OF Mg^{2+} AT LOW AND ELEVATED Ca^{2+} CONCENTRATIONS ON ATP-DEPENDENT Ca^{2+} UPTAKE IN BASOLATERAL LIVER PLASMA MEMBRANE VESICLES

ATP-dependent Ca^{2+} uptake was determined as described in the text. Free concentrations of Ca^{2+} and Mg^{2+} were adjusted to the levels given in the table. Values of means $\pm$ S.D. of quadruplicate determinations.

Ion concn.	Ca^{2+} uptake ($nmol \cdot mg^{-1} \cdot 4\ min^{-1}$)	
	plus ATP	no ATP
No Mg^{2+}		
30 μM Ca^{2+}	0.38 ± 0.04	0.36 ± 0.01
73 nM Ca^{2+}	0.35 ± 0.02	0.34 ± 0.03
5 mM Mg^{2+}		
30 μM Ca^{2+}	2.01 ± 0.09	0.21 ± 0.02
73 nM Ca^{2+}	1.35 ± 0.05	0.27 ± 0.02

TABLE V

EFFECT OF CALMODULIN AND TRIFLUOPERAZINE ON ATP-DEPENDENT Ca^{2+} UPTAKE IN BASOLATERAL LIVER PLASMA MEMBRANES

ATP-dependent uptake was studied as described in the methods section at a free Ca^{2+} concentration of 100 nM. Trifluoperazine and calmodulin were added 4 min prior to initiation of uptake at the concentrations given in the tables. Values are means ± S.D. of quadruplicate determinations.

Additions	Ca^{2+} uptake ($nmol \cdot mg^{-1} \cdot 4\ min^{-1}$)	
	no ATP	plus ATP
Control	0.446 ± 0.058	1.712 ± 0.091
Trifluoperazine (60 μM)	0.411 ± 0.045	2.243 ± 0.051
Calmodulin (5 μg/ml)	0.455 ± 0.047	1.729 ± 0.075

be observed at the lower pH value (Table II). Furthermore, in agreement with the observation of Lin [6], nucleotide-dependent Ca^{2+} uptake was specific for ATP; ITP, GTP and ADP did not stimulate Ca^{2+} uptake (Table III). Finally, in the absence of Mg^{2+}, no ATP-dependent Ca^{2+} uptake was observed (Table IV).

In further experiments we have analyzed for a calmodulin dependence of the ATP-dependent Ca^{2+} uptake (Table V). Membranes have been subjected to hypotonic EDTA extraction prior to the study of uptake. The hypotonic EDTA extraction has been successfully applied in other plasma membrane preparations to magnify the effects of added calmodulin on ATP-dependent Ca^{2+} uptake [11]; this procedure leads to a removal of membrane-bound calmodulin. Furthermore, the experiment was performed at a Ca^{2+} concentration of 100 nM (below saturation, see below). Addition of calmodulin did not stimulate Ca^{2+} uptake. Trifluoperazine, an inhibitor of calmodulin activated processes, had no inhibitory effect; we have no explanation for the (small) stimulatory effect.

The kinetic properties of ATP-dependent Ca^{2+} uptake were studied at a pH 7.4 in the presence and absence of calmodulin (Fig. 2). Initial uptake rates were obtained by sampling during the linear uptake phase at 15, 45, 75 and 105 s (Fig. 2a); the corresponding saturation curve of this typical experiment is shown in Fig. 2b. In six similar

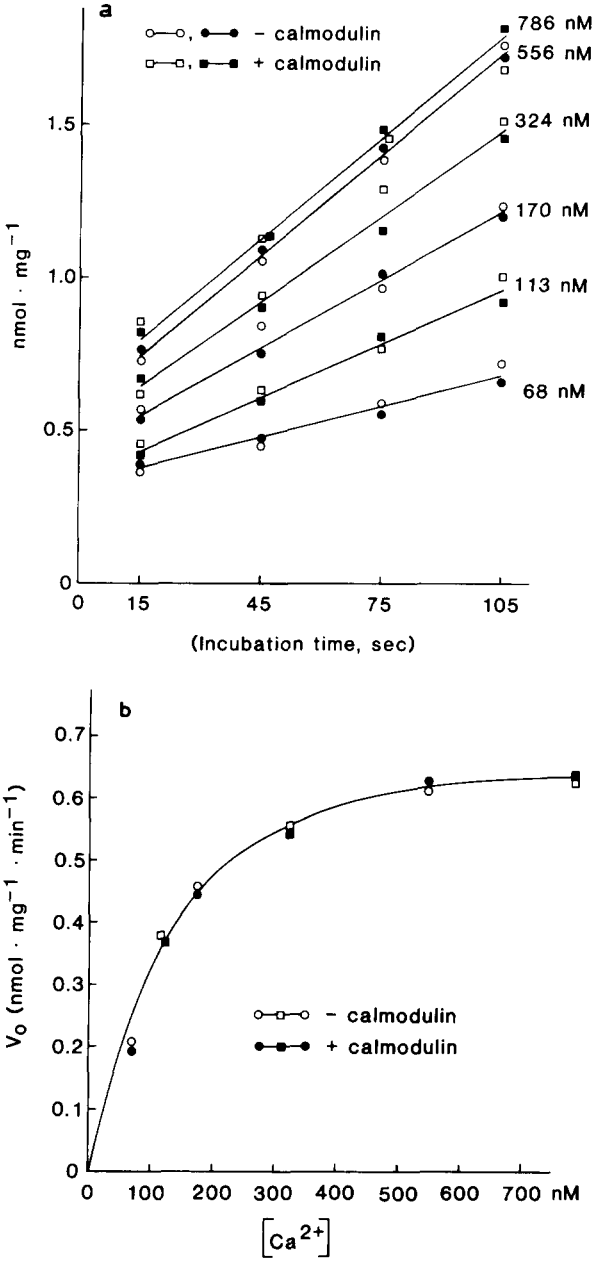

Fig. 2. Dependence of Ca^{2+} uptake in basolateral vesicles on Ca^{2+} concentration in the medium. Initial uptake rate (Fig. 2a) is plotted against Ca^{2+} concentration (Fig. 2b) in the incubation medium.

separate experiments, we obtained for apparent K_m values between 0.11 and 0.33 μM and apparent V_{max} values between 630 and 1930 $nmol \cdot mg^{-1} \cdot min^{-1}$. We have no explanation for these large variabilities. The results were not systemati-

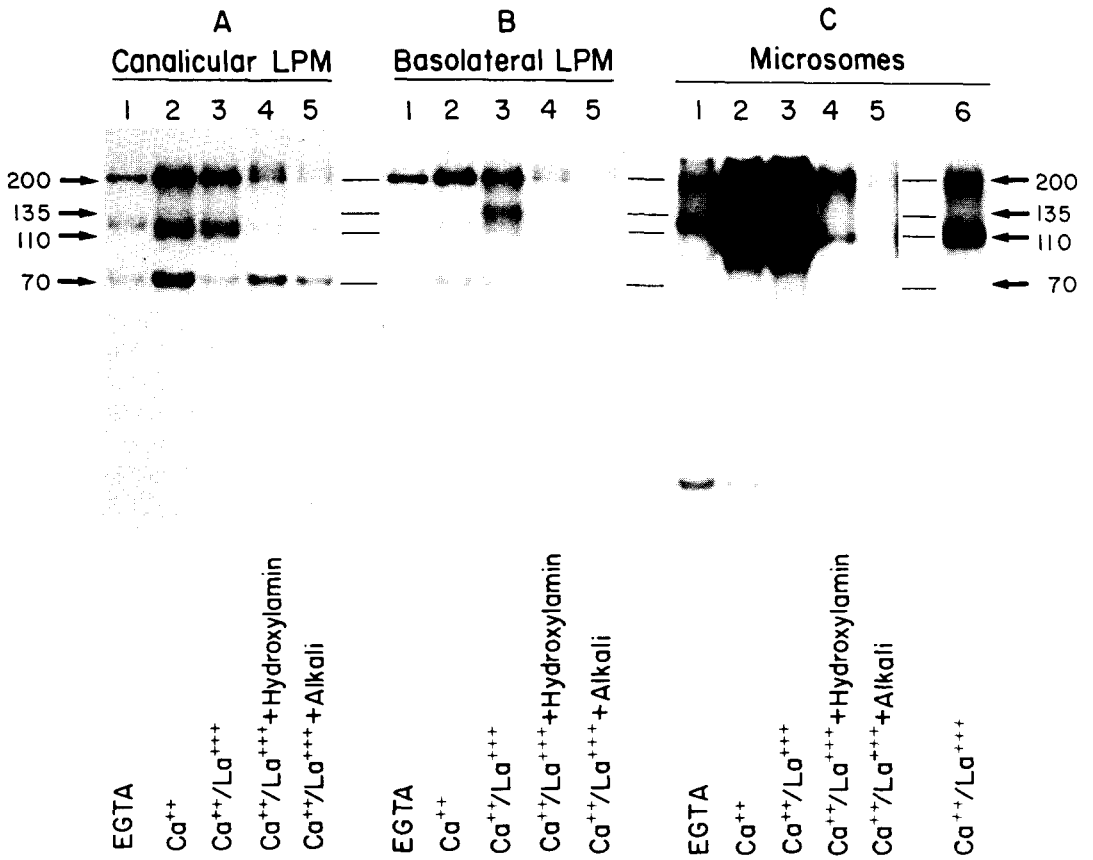

Fig. 3. SDS-PAGE and radioautography of phosphorylated intermediates of membrane fractions incubated with [γ-^{32}P]ATP. Note the presence of the 135 kDa band exclusively in basolateral liver plasma membranes incubated with Ca^{2+} and La^{3+} (lane B_3). Lane C_6 represents the same conditions as lane C_3 with the exception of a shorter exposure time of the radioautogram in order to visualize the presence of the 200 kDa and 110 kDa protein and the absence of the basolateral specific 135 kDa phosphorylated protein in microsomes. (A) Experiments with canalicular membranes; (B) experiments with basolateral membranes; (C) experiments with microsomes.

cally different whether a calmodulin depletion protocoll (see above) was used or whether membranes were directly used for the saturation experiment (results not shown). Thus, also these experiments are clearly in favour for a calmodulin-independent ATP-dependent Ca^{2+} uptake mechanism.

Ca^{2+}-dependent phosphorylation of membrane proteins in different membrane fractions incubated with [γ-^{32}P]ATP, was demonstrated by SDS-PAGE and radioautography (Fig. 3). In basolateral liver plasma membranes (Fig. 3B), and in the presence of Ca^{2+}, the phosphorylation of 200 kDa protein was dominant. A phosphorylated protein with an apparent molecular weight of 135 000 was additionally present in basolateral liver plasma membranes (Fig. 3b). Phosphorylation of this protein was measurable only in the presence of Ca^{2+} and La^{3+}, and was diminished, similar to the 110 kDa and 200 kDa proteins (see below), by exposure of the trichloroacetic acid precipitates to hydroxylamine or alkali, suggesting the presence of an acyl-phosphate bond. For comparisons, we have performed similar experiments with canalicular membranes (Fig. 3A) and microsomes (Fig. 3C). In microsomes, phosphorylation of proteins was extremely intensive and exposure time had to be reduced in order to identify phosphorylation in the 110 kDa and 200 kDa regions. In canalicular membranes (Fig. 3A),

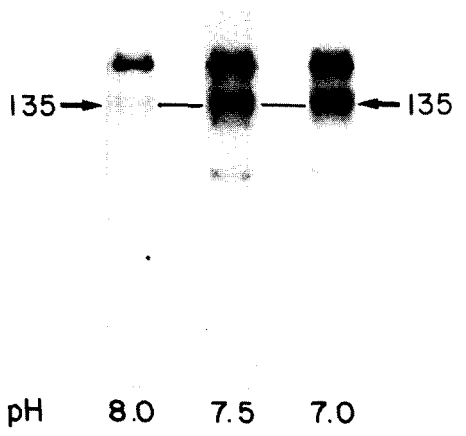

Fig. 4. SDS-PAGE and radioautography of basolateral liver plasma membranes incubated with [γ-^{32}P]ATP in the presence of Ca^{2+} and La^{3+} at pH 7.0, 7.5 and 8.0. Note the strong appearance of the 135 kDa band during incubations at pH 7.0 and 7.5, but not at pH 8.0.

Ca^{2+}-dependent phosphorylation of a 200 kDa, a 110 kDa and of a 70 kDa protein was identified. Thus, the 135 kDa protein seems to be associated preferentially with the basolateral liver plasma membrane. The 200 kDa and 110 kDa proteins are most likely of microsomal origine and it is possible that phosphorylation in other membrane fractions observed at these molecular weight ranges are due to contaminating microsomal membranes. The 70 kDa phosphorylation is preferentially found in canalicular membranes and might be present as a minor contaminant also in the basolateral membrane. As shown in Fig. 4, Ca^{2+}-dependent formation of the 135 kDa phosphorylated intermediate in basolateral membrane was easily demonstrable at pH 7.0 and 7.5, but barely or not at all at pH 8.0.

Discussion

In the present study, liver plasma membranes were isolated and separated into basolateral and canalicular subfractions. As already observed in the original description of the procedure [13], basolateral liver plasma membranes were contaminated with canalicular membranes; canalicular membranes were essentially free of basolateral contamination. ATP-dependent Ca^{2+} uptake was readily demonstrated in vesicles of basolateral liver plasma membranes, but minimally in canalicular membranes. It has been previously shown that canalicular liver plasma membranes are less than 20% 'inside-out' (inner cellular surface exposed to the medium) and therefore present a limited surface for studies of ATP-dependent Ca^{2+} uptake [23]. However, canalicular plasma membranes as obtained by this procedure are sealed and contain specific transport mechanisms, e.g. a Cl^-/HCO_3^- exchange mechanism [33]. Failure to observe uptake by canalicular membranes might thus be ascribable to vesicle 'sidedness' rather than to intrinsic properties of the membrane. Ca^{2+} uptake in liver plasma membrane subfractions might also be attributable to a contaminant (non-plasma membrane). Were the latter true, it would appear unlikely to be due to contamination with microsomes, since marker enzyme activity for the latter was low and approximately equal in both liver plasma membrane subfractions (Table I).

Ca^{2+}-dependent phosphorylation (acyl-phosphate bonds) of membrane proteins with apparent molecular weights of 200 000 and 110 000 was demonstrated in [γ-^{32}P]ATP incubations of microsomal membranes. The 110 kDa protein could refer to endoplasmic reticulum Ca^{2+}-ATPase [10]; the nature of the 200 kDa protein is unknown. The comparatively small quantities of 110 kDa and 200 kDa phosphorylated intermediates in both basolateral and canalicular liver plasma membranes are compatible with the observed low grade contamination of each subfraction with microsomal markers (Table I). The different relations of the 200 kDa and 110 kDa proteins in the canalicular and basolateral liver plasma membranes cannot be explained by a contamination with homogeneous population of microsomal membranes. The contamination containing a phosphate acceptor site at a 200 kDa protein is found preferentially in the basolateral liver plasma membrane, whereas 110 kDa and 200 kDa 'contaminations' are found at equal proportions in the canalicular membrane. A phosphorylated intermediate with an apparent molecular weight of 135 000 was exclusively observed in basolateral liver plasma membranes. Phosphorylation of this intermediate was only demonstrable by incubation with Ca^{2+} and La^{3+} and had the stability characteristics (hydroxylamine and alkali) of an acyl-phosphate. Formation was evident when incubations were

performed at pH 7.0 and 7.5, but strongly diminished at pH 8.0. This range of activity corresponds to the observed pH range of the Ca^{2+} pump activity. A 70 kDa phosphorylated intermediate was present in canalicular plasma membranes and to a small extent also in basolateral membranes. Phosphorylation of this latter protein was Ca^{2+}-dependent, was decreased by La^{3+}, and less sensitive to treatment with hydroxylamine and alkali. Thus, the 70 kDa protein might correspond to alkaline phosphatase. In conclusion, the data on phosphorylation suggests that ATP-dependent Ca^{2+} uptake in basolateral liver plasma membrane vesicles correlates with the appearance of a 135 kDa protein in phosphorylation experiments. The 200 kDa protein also presents in high amounts in canalicular and basolateral membranes is most likely not involved in ATP-dependent Ca^{2+} transport. As phosphorylation experiments and uptake studies were performed in both membranes fractions under non-permeabilizing conditions, ATP had access to this protein in both membrane fractions, but did apparently not promote Ca^{2+} uptake (only minimal transport activity in canalicular membranes).

The liver cell plasma membrane Ca^{2+} pump defined in the present study resembles in part the plasma membrane pump of other epithelia [11,15,24–29]. The apparent K_m for ATP-dependent Ca^{2+} uptake by basolateral liver plasma membrane vesicles was similar to the K_m for renal cortex basolateral vesicles [11,15]. The pH dependency of the uptake mechanism resembles that of renal cortex basolateral vesicles which is optimal at pH 7.0 and decreased at pH 8.0 [11]. The apparent molecular weight of 135000 of the Ca^{2+}-dependent phosphorylated intermediate is identical with those of renal cortical basolateral membranes [27] and of pancreatic acinar cell basolateral membranes [30], but differs from that of liver cell endoplasmic reticulum [10]. Calmodulin responsiveness, which has been demonstrated for other plasma membrane Ca^{2+} pumps [11,31,32], was not evident for basolateral liver plasma membranes. The lack of stimulatory effects of calmodulin and inhibitory effects of trifluoperazine applied under various experimental conditions strongly indicates, that the observed Ca^{2+} uptake in basolateral liver plasma membranes is a calmodulin-independent event. Furthermore, the negative results on calmodulin activation and trifluoperazine inhibition strongly argue against contamination with erythrocyte membranes as an explanation for our observations [12].

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