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Detection of Different Adenosine Triphosphatases in Human Placental Brush Border Membranes

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Abstract. The microvillous membrane of human placental syncytiotrophoblast cells contains a high ATPase activity. The purpose of this study was to characterize this activity and to investigate the presence of vacuolar type H^+ ATPase in this membrane. Intact brush border membrane vesicles strongly hydrolyzed ATP, reflecting the presence of ATPase on the external side of the membrane. The ATPase activity was entirely Mg^{2+} dependent and increased with pH. At pH 7.5, Vmax was 31.0 ± 1.7 μmol/mg/20 min and Km 0.18 ± 0.03 mM ATP. Hydrolysis of ATP was not influenced by the presence of bicarbonate or alkaline phosphatase inhibitors, but at pH 8 it decreased by half following addition of 100 μM dicyclohexylcarbodiimide (DCCD). At pH 7.5, 1 mM N-ethylmaleimide (NEM) depressed this activity by less than 5%. Opening the membrane vesicles with 0.1% desoxycholate (DOC) or Triton-X neither revealed any additional ATPase activity nor altered the low sensitivity to NEM. Treatment of these membranes with 1% cholate decreased the ATPase activity by more than 70% and did not enhance the sensitivity of ATP hydrolysis to NEM. 10^{-7} M Bafilomycin, which reduced by 56 ± 9% the ATPase activity in dog kidney brush border membranes treated with 0.1% DOC, had no effect on placental brush border membranes subjected to the same procedure. Finally, neither immunocytochemical staining using monoclonal antibody to the Mr 31000 subunit of V-type H^+ ATPase, nor electron microscopic examination detected the presence of H^+ ATPase in placental membranes.

In conclusion, the placental brush border membrane is the site of a strong "ecto" ATPase activity which is partially DCCD sensitive. However, our results did not detect the presence of any vacuolar type H^+ ATPase activity in these membranes.

Key words: ATPase — Human placenta — Brush border membrane

Introduction

In mammalian systems, the metabolism of organic molecules generates protons and the fetus is no exception. During extrauterine life, the kidney is the main scavenger of protons. During fetal life, protons are excreted via the placenta. Syncytiotrophoblast cells have the task to transport toward the maternal compartment the acid load produced by the fetus, in addition to the protons generated by its own metabolism. This task appears to be extremely efficient since from the 18th to 35.9th week of gestation, the fetal blood pH remains around 7.42, bicarbonate at 23 mEq/L and base-excess between 0 and −1 mEq/L, despite a relative hypoxia: pO_2 remains approximately at 40 mmHg (Weiner, 1990*a,b,* Chestnut et al., 1990).

The human syncytial brush border membrane is the site of a Na^+/H^+ exchanger (Balkovetz et al., 1986; Chipperfield, Langridge-Smith & Steele, 1988; M.G. Brunette and M. Leclerc, submitted). In addition to this mechanism of proton transport, Kulanthaivel et al. (1990) have reported the presence of an ATP driven H^+ pump in these membranes. These authors showed that transient exposure of the brush border vesicles to cholate reorients the H^+ pump so that an acidification of intravesicular space is observed upon addition of ATP to the extravesicular medium.

The main goal of the present study was to charac-

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Table 1. Membrane marker activities of placenta homogenates and brush border membranes (BBM)

	UNITS	HOMO	BBM	x	n
Alkaline Phosphatase	µmol/mg/15′	8.42 ± 1.1	193.60 ± 9.4	23.0	8
Na-K ATPase	µmol/mg/60′	0.30 ± 0.33	1.25 ± 0.23	4.2	8
^{3}H dihydro-alprenolol binding	pmol/mg	3.10 ± 0.37	7.05 ± 0.76	2.3	5
Succinate dehydrogenase	nmol/µg/15 min	15.43 ± 0.7	5.28 ± 2.00	0.34	3
Glucose-6-phosphatase	µmol/mg/min	0.55 ± 0.01	0.14 ± 0.02	0.25	3

n = number of experiments

terize the ATPase activity in human placental brush border membranes in both their native state and after membrane solubilization, by using various inhibitors. In addition, fluorescent immunocytochemistry studies using an antibody to a subunit of mammalian kidney vacuolar H^+ ATPase and electron microscopy examinations were also performed in order to further confirm the presence of H^+ pumps in these membranes.

The results indicate that the placental brush border membranes are the site of a strong ATPase activity. This activity increased with pH but was not inhibited by alkaline phosphatase inhibitors. Increasing bicarbonate concentration while keeping the pH constant did not modify the ATPase activity. In intact vesicles, ATP hydrolysis decreased by half with 100 µM DCCD. At pH 7.5 and above, a very small (less than 5%) sensitivity to 1 mM NEM was also observed. Preincubation of the membranes with DOC did not increase this sensitivity to NEM and did not reveal any Bafilomycin sensitive activity either. Finally, examination of fragments of placental tissue either with an antibody to mammalian kidney vacuolar H^+ ATPase or by electron microscopy failed to detect the presence of H^+ ATPase in the syncytial brush border membrane.

Material and Methods

Membrane Preparation

Brush border membrane vesicles were prepared from human term placentas. Fresh placentas were perfused with 0.9% NaCl solution through the umbilical artery ramifications. Following removal of the chorionic surface and decidua, the villous tissue was collected, cut into small fragments, suspended in 280 mM mannitol, 20 mM TRIS HEPES pH 7.4 and gently stirred with a magnetic bar at 4°C for 30 min. Then the suspension was filtered through a coarse cotton gauze, and the filtrate was centrifuged at 100000 × *g* for 40 min. The red blood cell layer was discarded, and the whitish pellet was homogenized with a Potter homogenizer at 2000 rpm, resuspended in 280 mM mannitol, 20 mM TRIS-HEPES pH 7.4 containing 10 mM $MgCl_2$, and again stirred with a magnetic bar for 20 min at 4°C. The suspension was then centrifuged at 3000 × *g* for 10 min to remove the Mg^{2+} aggregated nonbrush border membranes. Brush border membranes were collected from the supernatant by another centrifugation at 27000 × *g* at 4°C for 15 min. The pellet was washed and finally suspended in mannitol TRIS-HEPES buffer at a final protein concentration of approximately 12 µg/µl. Whole placental homogenates were prepared directly from the crude villous tissue.

Characterization of the Membranes

Alkaline phosphatase activity was measured according to the technique of Kelly and Hamilton (1970) using p-nitrophenol as the chromogenic substate, Na^+/K^+ ATPase with the technique of Post and Sen (1967) and succinate dehydrogenase with the technique of Pennington (1961). Protein concentration was determined by the method of Lowry et al. The vesicle preparations used in this study were enriched 30-fold in alkaline phosphatase (the specific enzyme for brush border membranes), 4.2-fold in Na/K ATPase, 2.3-fold in ^{3}H dihydroalprenolol binding, 0.34-fold in succinate dehydrogenase and 0.25-fold in glucose-6-phosphatase (Table). The orientation of the vesicles was determined by measuring the alkaline phosphatase activities in the membranes prior to and following solubilisation with DSD 0.1%. The mean ratio of the two measurements was 80.0% ± 7.1% indicating that most of the vesicles were right-side out.

ATPase Assays

ATPase assays were always performed in the presence of ouabain and oligomycin to eliminate any contaminating activities from basolateral or mitochondrial membranes. 500 µl of a diluted membrane suspension (10 µg protein/assay) were added to 500 µl of incubation medium containing (in mM): 120 NaCl, 5 $MgCl_2$, 0.8 to 5 ATP, 20 TRIS-HEPES at the indicated pH, 10 µM oligomycin and 5 mM ouabain, with or without the ATPase inhibitors to be tested. In the experiments with NEM and Bafilomycin, the medium contained (in mM): 10 ATP, 25 imidazol-histidine buffer pH 7.0, 100 NaCl, 5 KCl, 5 $MgCl_2$, phospho(enol)pyruvate, 15 U/ml pyruvate kinase, 1 vanadate, 5 ouabain and 10 µM oligomycin. In experiments with Schering 28080, 20 mM NaCl was replaced by 20 mM KCl. The mixture was incubated for 20 min at 35°C and the reaction was stopped by addition of 1 ml ice-cold 10% trichloroacetic acid. The liberated phosphate was determined using the reagent of Atkinson (1973). Unless otherwise indicated, the final concentrations of ATPase inhibitors in the incubation medium were 10^{-4} M vanadate, 10^{-4} M DCCD, 10^{-3} M NEM, 10^{-8} M Schering 28080 and 10^{-8} M Bafilomycin. In some experiments, alkaline phosphatase activity was also inhibited by addition of 25 mM phenylalanine to the incubation medium.

Exposure to DOC or Triton

In an attempt to detect an ATPase activity located at the cytosolic side of the membranes, vesicle suspensions (3 µg protein/µl) were prein-

cubated at room temperature with various concentrations of either DOC for 30 min, or Triton for 15 min. The treatment was abruptly terminated by a dilution of 1/150 in cold mannitol buffer.

Exposure of the Membrane Vesicles to Cholate

The brush border membranes were suspended in 280 mM mannitol, 20 mM TRIS HEPES pH 7.5 (3 mg protein/ml). As proposed by Simon & Burckhardt (1990), a 10% Na cholate (w/v) stock solution was added to obtain a final concentration of 1%, unless otherwise indicated. The suspension was incubated at 37°C for 1 min, immediately cooled to 4°C and dialysed, also at 4°C, overnight against a 100-fold volume of 150 mM KCl, 20 mM Tris Hepes pH 7.0, using "spectrapor" dialysis tubing (membrane cut off 6000–8000). ATPase activity was subsequently measured as described above.

Electron Microscopy

Small fragments of two fresh human and three rat placentas obtained from dams at 21 days of gestation were fixed by immersion in Karnovsky's fixative (Karnovsky, 1965) at 4°C for at least 24 hr. Fixed tissues were washed in 0.1 M cacodylate buffer, pH 7.4 in 0.2 M sucrose. They were post-fixed with 2% osmium tetroxide for 1 hr, dehydrated in solutions of ethyl alcohol at increasing concentrations, and embedded in Araldite. 1 μm slices were stained with toluidine blue, and thin sections with uranyl acetate and lead citrate according to the method of Reynolds et al. (1963). Examination was conducted with a Philips EM300 electron microscope. Using similar processing protocols, studs are regularly, although not constantly found, in the plasma membranes of kidney intercalated cells (Brown, Gluck & Hartwig, 1987; Narbaitz Vandorpe & Levine 1991; Narbaitz, Kapal & Levine, 1993).

Immunofluorescence

Small fragments of villous tissue from freshly delivered placentas were preserved in B_5 fixative ($HgCl_2$ 0.22 mM, Na acetate 90 mM, formaldehyde 3.7%), and embedded in paraffin. Slices of 4 μm thickness were serially incubated in xylene-ethanol in decreasing concentrations, Lugol's iodine solution, sodium thiosulfate and phosphate buffered saline. To block nonspecific binding of the primary antibody, sections were incubated in the "blocking solution" (10% calf serum, 10% goat serum, 1% polyethylene glycol (Mr 20000) in PBS) for 30 min. Sections were then incubated for 2 hr with a monoclonal antibody to the Mr 31000 subunit of the bovine kidney vacuolar H^+ ATPase (Bastani 1991 et al.; Hemken et al., 1992). For comparison, a fragment of human kidney was treated in the same manner.

Material

Oligomycin, ouabain, DCCD, cholate, desoxycholate, Triton and NEM were purchased from Sigma, St. Louis, MO. Bafilomycin and dog kidney brush border membranes were kindly supplied by Dr. Patrick Vinay, Hôpital Notre-Dame, Montreal. The monoclonal antibody E_{11} to the Mr 31000 subunit of bovine kidney vacuolar H^+ ATPase was a gift from Dr. Stephen Gluck, Washington University, St. Louis, MO) and the Schering 28080 was supplied by Schering Corporation, Bloomfield, NJ).

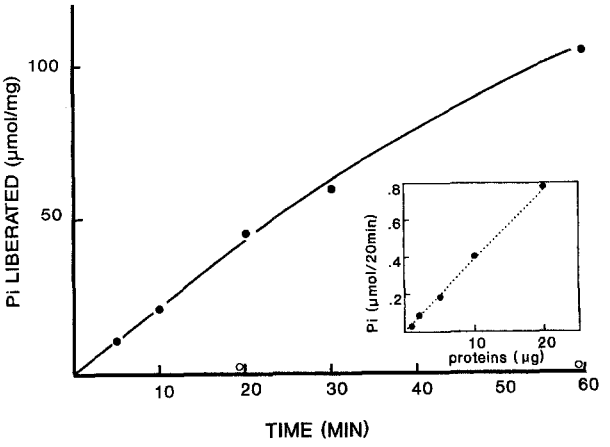

Fig. 1. (●) Time course of hydrolysis of 0.8 mM ATP at pH 7.5 by intact syncytial brush border membrane vesicles. (○): ATP hydrolysis in the absence of Mg^{2+}. Inset: variation of ATP hydrolysis with protein content.

Statistics

Results are expressed as means ± SEM. Statistical significance of the results obtained in two experimental conditions performed with the same placental tissue membranes were assessed by Student's paired *t* test.

Results

Effects of the Incubation Time and Membrane Protein on ATP Hydrolysis by Intact Vesicles

Figure 1 shows the time course of 0.8 mM ATP hydrolysis by intact brush border membrane vesicles, in the presence of 5 mM ouabain and 10 μM oligomycin, at pH 7.5. Hydrolysis increased linerly up to 20 min. When ATP hydrolysis was plotted against membrane proteins, a linear relationship was also observed (Fig. 1 inset). In the absence of Mg^{2+}, ATP hydrolysis was negligible.

Effects of ATP Concentration on ATP Hydrolysis

ATP hydrolysis increased with ATP concentrations according to a Michaelis-Menten relationship (Fig. 2). At ATP concentrations higher than 4 mM, a decline of ATP hydrolysis was observed, possibly because of an ADP accumulation (*not shown*). However, this phenomenon was present even when a regenerative system of ATP (6 mM phospho(enol)pyruvate and 15 U/ml pyruvate kinase) was added to the medium. The Eadie Hofstee plot analysis shows an apparent Km ATP of 0.18 ± 0.03 mM and a V max of 31 ± 1.7 μmol/mg/20 min.

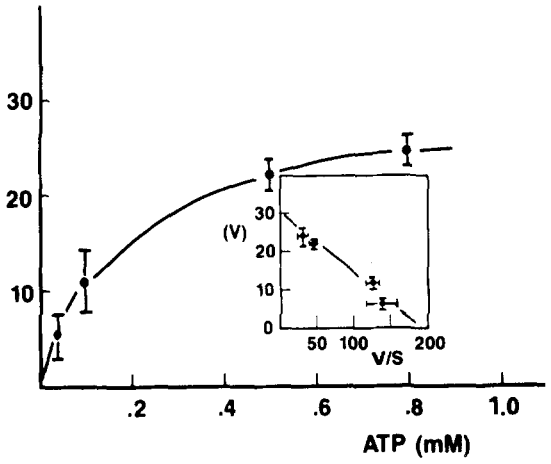

Fig. 2. Effect of ATP concentration on ATP hydrolysis at pH 7.5 by intact vesicles. Inset: Eadie-Hofstee representation of the same data. Apparent km: 0.18 ± 0.03 mM, Vmax 31 ± 1.66 μmol/mg/20 min. Data are the mean ± SEM of 5 experiments.

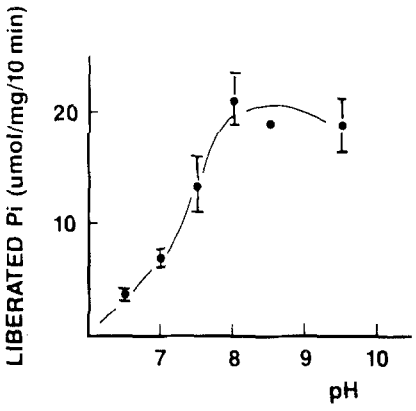

Fig. 3. Variations of 5 mM ATP hydrolysis by intact vesicles with pH. Intra- and extravesicular pH were the same. Data are the mean ± SEM of 4 experiments.

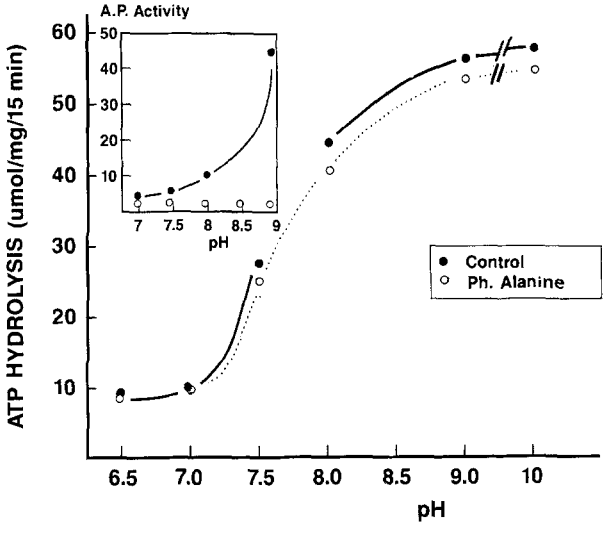

Fig. 4. Effect of alkaline phosphatase activity inhibition by 25 mM phenylalanine on 5 mM ATP hydrolysis by intact vesicles, at various pH. Inset: alkaline phosphatase activities in the same experiment. (●) Control experiment, (○) presence of phenylalanine in the incubation medium.

pH Dependency of ATPase Activities

Figure 3 represents the ATPase activity in the brush border membranes over the pH range of 6.5 to 9.5. ATP hydrolysis progressively increased to reach a maximal level at pH 8.0. In these experiments, pH were adjusted with 20 mM TRIS/HEPES; no bicarbonate was included in the medium. Addition of 10 to 60 mM Na bicarbonate to the medium while keeping the pH constant did not influence the ATPase activity (*not shown*).

Alkaline Phosphatase and ATP Hydrolysis

Because of the strong influence of alkaline pH on ATP hydrolysis, it was suspected that the high alkaline phosphatase content of the brush border membrane might be, at least partially, responsible for this ATPase activity. The strongest inhibitor of placental alkaline phosphatase is phenylalanine. Figure 4 represents the variation of ATP hydrolysis with pH, in the presence and absence of 25 mM phenylalanine. As expected, phenylalanine completely abolished the alkaline phosphatase activity (Fig. 4 inset), whereas no change in ATP hydrolysis was detected.

Pharmacological Characterization of ATPase Activity

Effect of Vanadate

As shown in Fig. 5, addition of 100 μM vanadate to the incubation medium slightly but significantly decreased the ATPase activity at pH 6.5 ($P < 0.05$). At higher pH, the observed difference, although within the same range, was not significant, probably due to the high degree of ATP hydrolysis and also to the larger standard deviations of data. Because of the slight sensitivity of this ATP hydrolysis to vanadate, suggesting a E_1 E_2 type of ATPase, we have also tested the effect of Schering 28080, an inhibitor of the K^+/H^+ ATPase. At concentrations from 10^{-8} to 10^{-5} M, Schering 28080 had no effect on ATP hydrolysis (Fig. 5, lower inset).

Effect of DCCD

In contrast, 100 μM DCCD produced a strong pH dependent inhibition of ouabain and oligomycin insensitive ATPase activity in the intact placental brush border

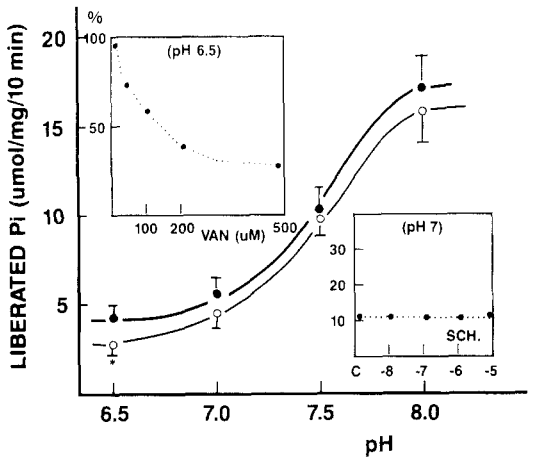

Fig. 5. Effect of 100 μM vanadate and Schering 28080 on 5 mM ATP hydrolysis by intact vesicles. (●) control experiments, (○) vanadate experiments. Data are the mean ± SEM of 4 experiments. Upper inset: dose-response curve of the effect of vanadate Lower inset: dose-response curve of the effect of Schering 28080. *: $P < 0.05$ compared to the control value.

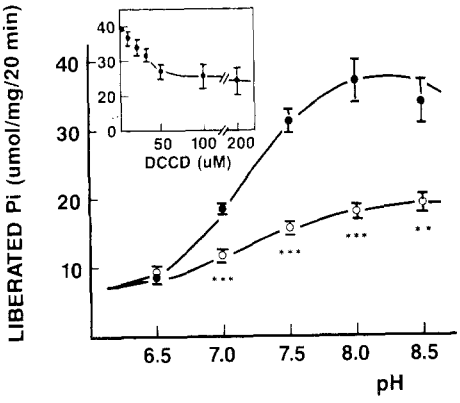

Fig. 6. Effect of 100 μM DCCD in the incubation medium on 0.8 mM ATP hydrolysis by intact vesicles. (●) control, (○) DCCD experiments. Inset: dose-response curve of the effect of DCCD. **: $P < 0.02$, ***: $P < 0.01$ compared to the control values (paired t test). Data are the mean values ± SEM of 4 experiments.

membrane vesicles. Whereas 100 μM DCCD had no effect on ATP hydrolysis at pH 6.5, it inhibited the ATPase activity by approximately 50% at pH 8.0 (Fig. 6). This action was dose-dependent with half-maximal inhibition occuring at 25 μM (or 2.5 μg/500 μl for 10 μg protein) (Fig. 6 Inset).

Effects of NEN and Bafilomycin in Intact Vesicles

DCCD is a rather a nonspecific ATPase inhibitor. To further characterize our brush border membrane ATPase, we tested the sensitivity of ATP hydrolysis to NEM and to Bafilomycin which inhibits preferentially the V type of H^+ ATPase. In these experiments, at pH 7.5 and above, 1 mM NEM decreased by less than 5% the ATPase activity of intact brush border membrane vesicles (Fig. 7), whereas Bafilomycin had no effect (*not shown*). The effects of NEM and DCCD were not additive (*not shown*).

Effect of NEM on ATPase Activity in Membranes Solubilized with DOC and Triton

In the experiments presented above, intact vesicles were incubated in media containing ATP with or without inhibitors. Since most of brush border membrane vesicles are right-side out, it is probable that the ATP binding site of the observed ATPase was located at the extracellular surface of the membrane. In order to detect additional activity at the cytosolic surface, vesicles were preincubated in media containing either DOC, or Triton. As shown in Fig. 8, a 30 min-preincubation with DOC at 20°C slightly decreased (by 24%) rather than increased

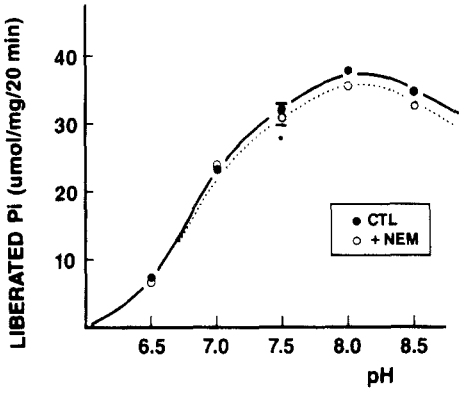

Fig. 7. Effect of 1 mM on ATPase activity in intact vesicles at various pH. *: $P < 0.05$ paired t test for the 3 experiments performed at pH 7.5.

the ATPase activity. This effect was dose-dependent with an ED_{50} value of approximately 0.07%. A similar decrease in ATP hydrolysis was observed with Triton (*not shown*). Addition of 1 mM NEM produced a slight decrease in ATP hydrolysis in opened as well as in intact vesicles (Fig. 8).

Effect of Bafilomycin on DOC-Treated Membranes

To further investigate the possibility of a H^+ ATPase activity, solubilized membranes were incubated in the presence and the absence of 10^{-7} M Bafilomycin. For comparison, brush border membranes from dog kidney, which are known to contain high H^+ ATPase activity (Noel et al., 1992), were treated in the same manner. Whereas Bafilomycin, like NEM, significantly decreased ATP hydrolysis in the kidney membranes, (56 ± 9%, n = 3), this inhibitor had no effect on the placental mem-

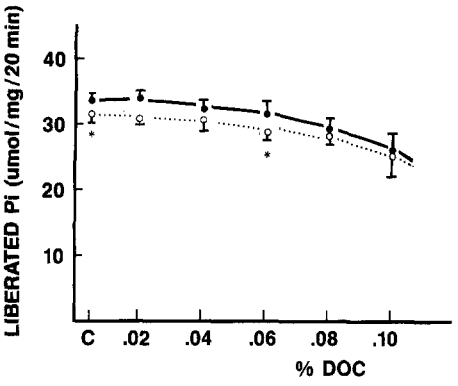

Fig. 8. Effect of NEM on the ATPase activity in membranes treated with DOC. The membranes were preincubated with increasing concentrations of DOC for 30 min at 20°C. Preincubation was terminated by a dilution of 1/150 in cold mannitol buffer. ATPase activity was measured at pH 7.5 in the absence (●) or the presence (○) of 1 mM NEM. Data are the mean ± SEM of 3 experiments. *: $P < 0.05$ compared to the control membranes.

branes (Fig. 9). Similarly, these inhibitors failed to affect ATP hydrolysis in membranes treated with Triton (*not shown*).

Effect of Cholate

Simon and Burckardt (1990) reported that treatment of brush border membranes from pig kidney with 1.2% cholate, reorientates NEM sensitive H^+ pumps from the inside to the outside of the vesicles. Addition of ATP to vesicles pretreated with cholate induced a H^+ uptake visualized with acridine orange. The inside-out oriented H^+ ATPase was sensitive to NEM, and was resistant to DCCD. Kulanthaivel et al. (1990) were successful in reproducing this technique with human placental brush border membranes. In an attempt to confirm these results and better characterize the small NEM sensitive ATPase observed in the DOC treated membranes, we incubated our brush border membranes with increasing concentrations of cholate, using the same technique as described by Simon and Burckardt. With increasing concentration of cholate, ATP hydrolysis progressively declined to reach 23% of the initial value with 1% cholate (Fig. 10). At this cholate concentration, alkaline phosphatase activity was 75% of the control value. In no experiment did a treatment with cholate increase the ATPase activity. As found with membranes solubilized in DOC, the addition of 1 mM NEM slightly decreased the ATP hydrolysis at all cholate concentrations tested. Treatment of the membranes with cholate did not influence the effect of DCCD either (*not shown*).

Immunocytochemical Experiments

Figure 11 shows immunocytochemical staining with the anti H^+ ATPase monoclonal antibody E_{11} on both normal human kidney and a fragment of placental villous tissue. Whereas the fluorescent antibody revealed the presence of H^+ ATPase in brush border microvilli and subvillar invaginations in proximal tubules and in the intercalated cells of cortical collecting ducts, no staining was observed in placental villosities.

Electron Microscopy Study

Under electron microscopy, both the human and rat placenta showed typical syncytiotrophoblast cells with numerous apical microvilli and subapical vesicles with and without coats. Careful observation of both apical and basal plasma membranes at high magnification failed to disclose the presence of the rows of 9 nm studs at regular intervals which are typical of the proton pump located within the plasma membrane.

Discussion

Our results show that the syncytiotrophoblast brush border membranes contain a high ouabain and oligomycin insensitive ATPase activity, which increases with alkaline pH. This ATPase is not alkaline phosphatase. It is strongly inhibited by DCCD and very slightly by NEM. The effects of DCCD and NEM are not influenced by solubilisation of the membranes in DOC or by treatment with cholate.

ATPases have been classified into three main classes: an E_1-E_2 type, an F_0-F_1 type and a vacuolar type (V type). The E_1-E_2 family of ATPases are phosphorylated, and dephosphorylation is necessary for the complete catalytic cycle, which is interrupted by the presence of vanadate. This ATPase group includes Na^+, K^+-ATPase, Ca^{2+}-ATPase and the gastric K^+-H^+ exchanger (Sachs, Faller & Rabon 1982). The F_0-F_1 family catalyzes electrogenic proton transport. These ATPases are not phosphorylated and therefore are resistant to vanadate. They bind DCCD and are located within the mitochondria. Following purification and reconstitution into liposomes, their activity increases as a function of H^+ concentration (Kagawa et al., 1979). They are inhibited by oligomycin or NaN_3. The V family of ATPases is also involved in proton transport. These ATPases are present in endosomes secretory granules, Golgi elements, endoplasmic reticulum, clathrin-coated vesicles (Yurko & Gluck, 1987) and with some distinct traits, in kidney microsomes (Gluck & Caldwell, 1987). They are vanadate and oligomycin resistant but they bind DCCD and are sensitive to NEM and Bafilomycin.

Finally, a bicarbonate-ATPase has also been described in rat renal mitochondria and brush border membranes (Kline-Saffran & Kinne, 1974, 1979). According to these authors, the bicarbonate ATPase of both origins

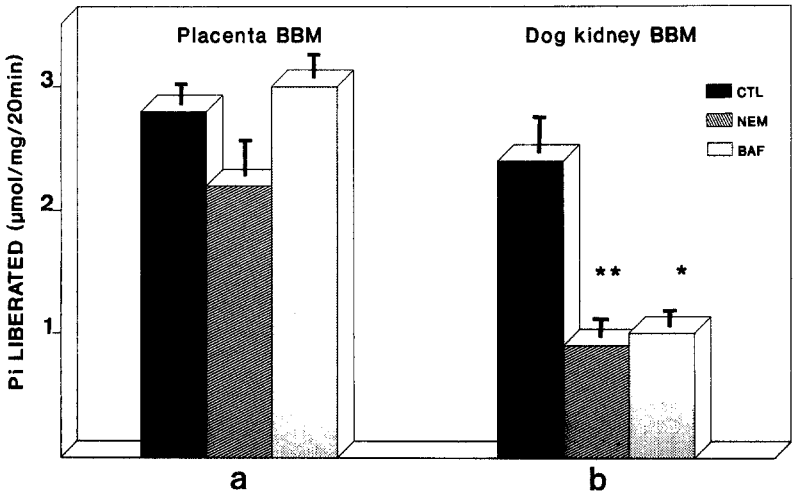

Fig. 9. Effect of 10^{-7} *M* Bafilomycin and 1 mM NEM on ATPase activity in DOC-treated brush border membranes from human placenta (*a*), and from dog renal proximal tubules (*b*). The medium contained (in mM): ATP, 25 imidazol histidine buffer pH 7,0, 100 NaCl, 5 KCl, 5 $MgCl_2$, 6 phospho(enol) pyruvate, 15 U/ml pyruvate kinase, 1 vanadate, 5 ouabain and 10 µM oligomycin.

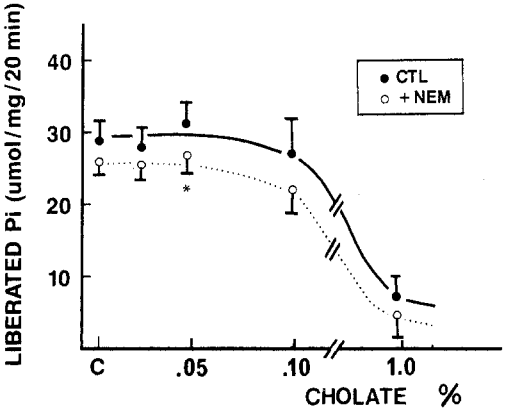

Fig. 10. Effect of 1 mM NEM on ATPase hydrolysis by membrane vesicles preincubated with various concentrations of cholate, on the 0.8 mM ATP hydrolysis. Membranes (3 mg protein/ml) were preincubated for 1 min at 35°C in a mannitol buffer (pH 7.5) containing cholate at the indicated concentration. Then the suspension was rapidly cooled at 4°C and dialyzed overnight in a 150 mM KCl, 20 mM TRIS HEPES pH 7.5. ATPase activity was measured the folowing day. Data are mean ± SEM of 3 experiments. * $P < 0.05$ compared to the control value.

is sensitive to oligomycin, but only the brush border membrane enzyme activity is inhibited by filipin, a cholesterol complexing antibiotic. A bicarbonate ATPase has also been reported in rabbit kidney (Ben Abdelkhalek, 1986) and in the kidney of a fresh water snail species (Bhouyain & Saleuddin, 1987). This enzyme is present in both mitochondrial and the microsomal fractions of the kidney.

The Nature of the Placental DCCD-Sensitive ATPase

In this study, the ATPase activity of syncytiotrophoblast brush border membranes was characterized in intact and solubilized vesicles, using specific inhibitors. The main conclusion is that this activity which is mostly sensitive to DCCD, does not belong to the E_1E_2 family since it is vanadate resistant. Its resistance to oligomycin and the low activity of succinate dehydrogenase in our brush border membranes precludes a mitochondrial origin. The DCCD sensitivity and oligomycin resistance suggest a H^+ ATPase although not of the V type since it binds ATP at the external surface of the right side out vesicles, as does an ecto-ATPase. Moreover, even after solubilization with DOC, the observed ATPase was not sensitive to Bafilomycin, the specific inhibition of V type H^+ ATPase, and no V type H^+ ATPase was detected by either electron microscopy or immunocytochemical examinations. Finally, it is unlikely that alkaline pH stimulates a H^+ ATPase.

The strong sensitivity to alkaline pH could reflect a bicarbonate activated ATPase; but our usual incubation media did not contain bicarbonate, and addition of Na bicarbonate while keeping the pH constant failed to affect the ATP hydrolysis.

Thus, supposedly, the only role this ecto-ATPase might play is to hydrolyze ATP in order to permit ADP transport. Since plasma does not contain ATP, it is possible that the high blood flow which characterizes the placental circulation results in some degree of hemolysis with ATP release from damaged red blood cells.

The Nature of the Vanadate and NEM Sensitive ATPase

The contribution of the vanadate and NEM sensitive ATPases to the total ATPase activity was very modest. The vanadate inhibitable ATPase was constant although significant at low pH only. This activity, observed in intact vesicles, might reflect contamination by basal plasma membranes. The presence of ouabain in the medium and

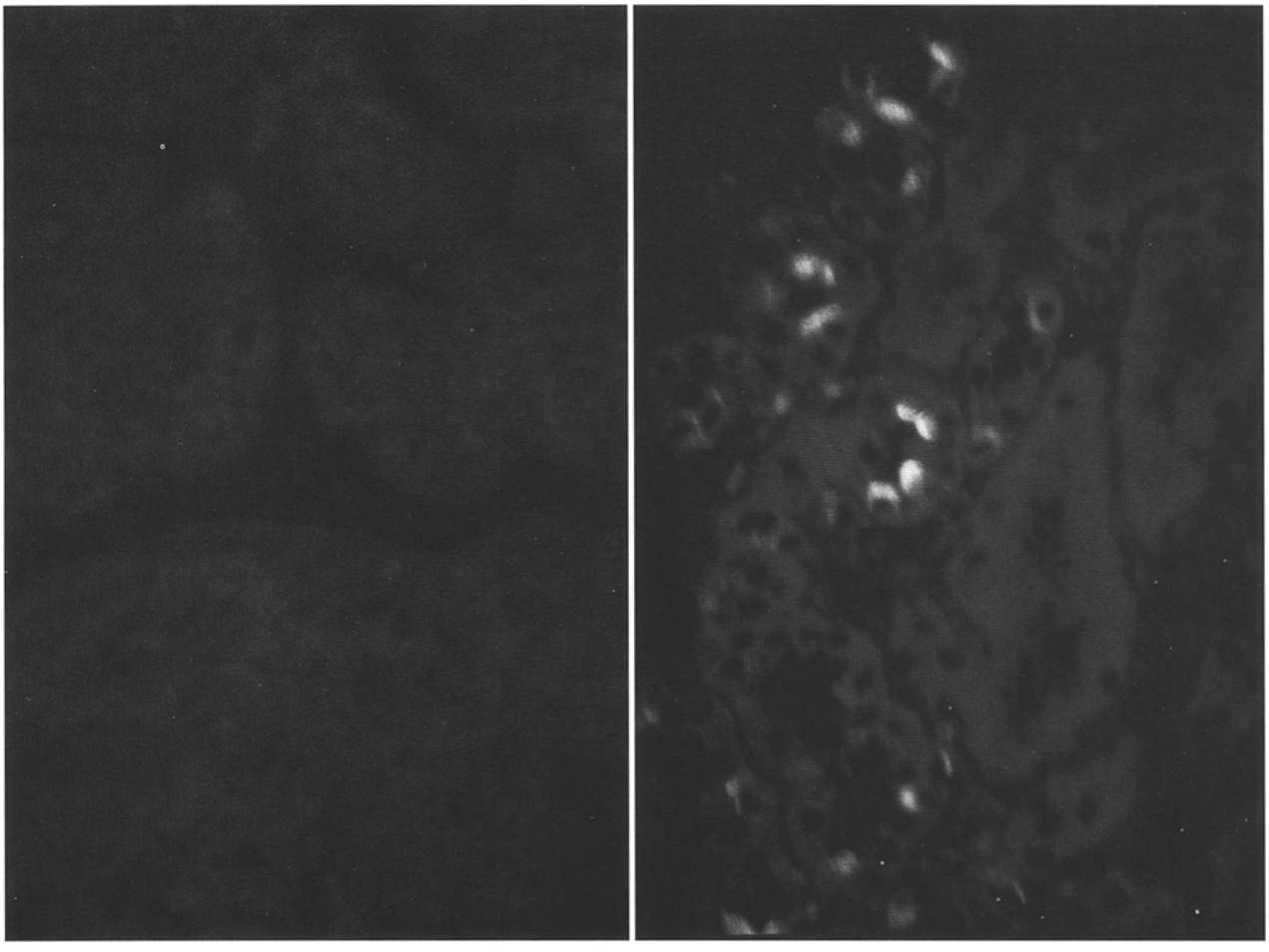

Fig. 11. Immunofluorescent staining of human midplacental tissue (left) and human kidney cortex (right) with anti H^+ ATPase antibody. Whereas staining of proximal tubule brush border and intercalated cells in cortical collecting duct is visible, no staining could be detected in placental tissue.

the lack of added calcium should prevent any Na^+/K^+ ATPase and Ca^{2+} ATPase activity. However, since no calcium binding substance such as EDTA was included in the medium, it is possible that traces of calcium were still bound to the membranes. Finally, the lack of sensitivity to Schering 28080 precludes the presence of H^+/K^+ ATPase.

A small NEM-sensitive ATPase activity was also present in intact membrane vesicles. Membrane solubilization did not increase this activity. As the DCCD sensitive ATPase, the NEM inhibitable activity was observed only in alkaline pH. Since the two ATPase activities (DCCD and NEM sensitive) were not additive, it is probable that they correspond to the same enzyme.

The Effect of Cholate

Our failure to preserve the ATPase activities in the membranes following treatment with 1% cholate is unexplained. Despite the short time of preincubation of membranes with cholate (1 min at 35°C) and an overnight dialysis at 4°C as described by Simon and Burckardt (1990), treatment with 1% cholate resulted in a more than 70% decrease in ATPase activity. Furthermore, at any concentration of cholate used, no increase in NEM-sensitive ATPase activity was detected, again indicating the absence of a H^+ pump in these membranes.

Conclusion

We have detected, in syncytiotrophoblast brush border membranes, four ATPases which bind ATP at the external surface of the membrane. The predominant ATPase strongly increases its activity with pH, is not influenced by bicarbonate but is inhibited by DCCD. We hypothesize that this ATPase is not a pump, but that it simply hydrolyzes ATP released by maternal red blood cells. A very low NEM-sensitive ATPase activity was also

present at alkaline pH in both intact and open vesicles. Since Bafilomycin did not influence ATP hydrolysis under any experimental conditions, and electron microscopy as well as immunocytochemical studies also failed to demonstrate a V type of H^+ ATPase activity, it is possible that both the NEM and the DCCD sensitive ATPases, whose actions are not additive, reflect the activity of the same enzyme. A small vanadate-sensitive ATPase activity was also detected in intact vesicles, which is believed to reflect a contamination by the basal plasma membrane Ca^{2+} pump. Finally, further investigations are warranted to characterize the nature of the DCCD resistant ATPase which, like the DCCD sensitive ATPase, binds ATP at the external surface of the membrane.

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