Two Ca\textsuperscript{2+}-dependent ATPases in Rat Liver Plasma Membrane

THE PREVIOUSLY PURIFIED (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase IS NOT A Ca\textsuperscript{2+}-PUMP BUT AN ECTO-ATPase

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We have shown that the rat liver plasma membrane has at least two (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPases. One of them has the properties of a plasma membrane Ca\textsuperscript{2+}-pump (Lin, S.-H. (1985) J. Biol. Chem. 260, 7850–7856); the other one, which we have purified (Lin, S.-H., and Fain, J. N. (1984) J. Biol. Chem. 259, 3016–3020) and characterized (Lin, S.-H. (1985) J. Biol. Chem. 260, 10976–10980) has no established function. In this study we present evidence that the purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase is a plasma membrane ecto-ATPase.

In hepatocytes in primary culture, we can detect Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activities by addition of ATP to the intact cells. The external localization of the active site of the ATPase was confirmed by the observation that the Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activities were the same for intact cells, saponin-treated cells, and cell homogenates. Less than 14% of total intracellular lactate dehydrogenase, a cytosolic enzyme, was released during a 30-min incubation of the hepatocytes with 2 mM ATP. This indicates that the hepatocytes maintained cytoplasmic membrane integrity during the 30-min incubation with ATP, and the Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activity measured in the intact cell preparation was due to cell surface ATPase activity.

The possibility that the ecto-Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase may be the same protein as the previously purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase was tested by comparing the properties of the ecto-ATPase with those of (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase. Both the ecto-ATPase and the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase have broad nucleotide-hydrolyzing activity, i.e. they both hydrolyze ATP, GTP, UTP, CTP, ADP, and GDP to a similar extent. The effect of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} on the ecto-ATPase activity is not additive indicating that both Ca\textsuperscript{2+} and Mg\textsuperscript{2+}-ATPase activities are part of the same enzyme. The ecto-ATPase activity, like the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase, is not sensitive to oligomycin, vanadate, N-ethylmaleimide and p-chloromercuribenzoate; and both the ecto-ATPase and purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase activities are insensitive to protease treatments. These properties indicate that the previously purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase is an ecto-ATPase and may function in regulating the effect of ATP and ADP on hepatocyte Ca\textsuperscript{2+} mobilization (Char-est, R., Blackmore, P. F., and Exton, J. H. (1985) J. Biol. Chem. 260, 15789–15794).

The cytosolic free calcium concentration of hepatocytes is in the range of 0.1 to 0.2 μM (Murphy et al., 1980). It is proposed that part of the Ca\textsuperscript{2+} gradient is maintained by a high affinity ATP-dependent Ca\textsuperscript{2+} transporter localized in the plasma membrane. The Ca\textsuperscript{2+} pumps of human erythrocyte membrane and rat heart sarcolemma have been characterized and purified (Niggligl et al., 1979; Caroni and Carafoli, 1981). In those tissues the plasma membrane Ca\textsuperscript{2+} pumps, like the muscle sarcoplasmic reticulum Ca\textsuperscript{2+} pump, possess ATPase activity which can be activated by Ca\textsuperscript{2+} in the presence of Mg\textsuperscript{2+}. However, no Ca\textsuperscript{2+}-stimulated ATPase activity could be found in rat liver plasma membrane under similar conditions. As a result, a high affinity Ca\textsuperscript{2+}-stimulated ATPase activity which was observed in the absence of exogenously added Mg\textsuperscript{2+} was thought to be the enzyme responsible for hepatocyte plasma membrane Ca\textsuperscript{2+} transport (Lotersztajn et al., 1981, 1984). We have purified the high affinity Ca\textsuperscript{2+}-stimulated ATPase (Lin and Fain, 1984). The properties of the high affinity (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase are different from those of the plasma membrane Ca\textsuperscript{2+} pump studied by reconstituting liver plasma membrane proteins into artificial liposomes (Lin, 1985a, 1985b). Further characterization of this ATPase demonstrated that this enzyme can be activated by either Ca\textsuperscript{2+} or Mg\textsuperscript{2+}; and it was also shown that this enzyme has broad nucleotide specificity, and its activity is not inhibited by inhibitors of known ion transporters (Lin, 1985b).

Since the high affinity (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase is not the liver plasma membrane Ca\textsuperscript{2+} pump, its physiological function remains unknown. Recently, Charest et al. (1985) reported that stimulation of isolated hepatocytes with ATP or ADP induced a rapid but transient increase of free cytosolic Ca\textsuperscript{2+} concentration indicating the existence of P2-purinergic receptor(s) in the hepatocyte plasma membranes. Further study also showed that the transient response was probably due to rapid hydrolysis of the ATP by a plasma membrane ecto-ATPase. As part of the effort to study the function of the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase, we tested the possibility that this enzyme is a plasma membrane ecto-enzyme with its nucleotide hydrolyzing site facing the outside of the cell. In this communication, we present evidence that Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activity can be detected by addition of ATP to the outside of intact hepatocytes. The properties of this enzyme indicate that it is the same enzyme that we previously purified (Lin and Fain, 1985).
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1984) and characterized (Lin, 1985b). Our finding that the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase hydrolyzes extracellular ATP and ADP suggests that it may play a role in terminating the effect of ATP and ADP on hepatocyte Ca\textsuperscript{2+} mobilization.

**EXPERIMENTAL PROCEDURES**

**Materials**—ATP (disodium salt), AMP-PNP,\textsuperscript{1} EGTA, ADP, AMP, GTP, GDP, ouabain, alginicmycin, and papain were obtained from Sigma. Trypsin and chymotrypsin were from CoopBerolmedical.

**Assay of ATPase Activities in Suspensions and Homogenates of Hepatocytes**—Hepatocytes were isolated from Sprague-Dawley rats (Charles River, CD strain) by collagenase digestion, as described by Seglen (1976). At the beginning of each experiment, the hepatocytes were washed twice with buffer A which contained 120 mM NaCl, 5 mM KCl, 20 mM Hepes/Tris (pH 7.4), and 2 mM EGTA. The hepatocytes were resuspended in buffer A and, half of them were homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at speed 5 for 2 s. Aliquots of the hepatocytes or the homogenates were added to tubes containing a small amount of water (for basal ATPase activity measurement) or Ca\textsuperscript{2+} solution to give a final concentration of 2 mM (for Ca\textsuperscript{2+}-ATPase activity), or Mg\textsuperscript{2+} solution to give a final concentration of 1 mM (for Mg\textsuperscript{2+}-ATPase activity). After incubation of cells or homogenates at 37 °C for 10 min, the assay was started by addition of ATP to a final concentration of 2 mM. The reaction was stopped by adding SDS to a final concentration of 2%. The ATPase activity was determined by measuring the inorganic phosphate released as described by Ames (1966), except that the time for color development was 20 min at 37 °C instead of 1 h at 37 °C.

**Cell Culture**—Primary hepatocyte cultures were prepared from 200-250 gm Sprague-Dawley rats (Holzman, Madison, WI) as described by Russell et al. (1984), except that after a 15-min attachment at 37 °C, the plating medium containing calf serum was replaced with 1.5 ml of Williams’ Medium E containing 150 nM insulin. The hepatocytes in collagen-coated dishes (3.75 × 10\textsuperscript{5} cells in 1.5 ml of Williams’ Medium E) were incubated at 37 °C for 24-48 h before use.

**Assay of ATPase Activities of Hepatocytes in Primary Culture and of Their Homogenates**—At the beginning of each experiment, the hepatocytes in culture dishes were rinsed twice (2 ml each time) with buffer A (for basal ATPase activity measurement), or buffer A plus 2 mM Ca\textsuperscript{2+} (for Ca\textsuperscript{2+}-ATPase activity measurement), or buffer A plus 1 mM Mg\textsuperscript{2+} (for Mg\textsuperscript{2+}-ATPase activity measurement). For the measurement of ATPase activity in intact cells, 2 ml of each solution was added, and ATPase assays were started by addition of ATP to each dish to a final concentration of 2 mM. After incubation of cells at room temperature with ATP for different periods of time as indicated, aliquots of the incubation medium (200 μl) were taken for inorganic phosphate determination at the beginning of the experiments.

The membrane-bound (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase activity was solubilized and purified from plasma membrane according to Lin and Fain (1984).

**RESULTS**

**Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase Activities of Intact and Disrupted Hepatocytes**—In the initial experiments, hepatocytes freshly prepared by liver perfusion and collagenase digestion were used. Although Ca\textsuperscript{2+}-stimulated and Mg\textsuperscript{2+}-stimulated ATPase activities could be detected by incubating intact hepatocytes with ATP, the lactate dehydrogenase activity of the cell supernatant was about 50% of that of the homogenate, indicating that freshly isolated hepatocytes were very leaky. In order to unambiguously demonstrate the side-ness of this plasma membrane enzyme, a hepatocyte preparation with minimum membrane leakage was required. Therefore, hepatocytes in primary culture were used in later experiments. Hepatocytes in primary culture were prepared by collagenase perfusion of rat livers. The method employed yielded a cell suspension containing greater than 95% hepatocytes, which were then plated on collagen-coated dishes. Under this condition, viable hepatocytes attach to the dish and the majority of damaged cells are washed off the dish at the beginning of the experiments.

Fig. 1A shows the time course of Ca\textsuperscript{2+}-stimulated and Mg\textsuperscript{2+}-stimulated ATPase activities of hepatocytes in primary culture. These activities were detected in the presence of 1.6 μM free Ca\textsuperscript{2+} or 25 μM free Mg\textsuperscript{2+}, respectively. Disruption of the hepatocytes by scraping the cells off the collagen-coated dishes followed by sonication for 1 min did not increase either the Ca\textsuperscript{2+}-ATPase or Mg\textsuperscript{2+}-ATPase activities (Fig. 1B). At the end of a 30-min incubation, aliquots of cell medium from the intact cells, and the disrupted cell preparations were taken for determination of lactate dehydrogenase activity. The lactate dehydrogenase activity of the cell medium from the intact cells was 14% of that of the disrupted cells (0.27 vs 1.92

\textsuperscript{1}The abbreviations used are: AMP-PNP, 5′-adenyl[β,γ-imido]diphosphate; CA, carboxyatractyside (oxelenepentilcetioleat); CaCl\textsubscript{2}, calcium chloride; D-2-hydroxyethyl) 1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.
ATPase activities of hepatocytes in primary culture and of disrupted hepatocytes. The ATPase activities are the same for both intact and sonically disrupted hepatocytes in primary culture and of disrupted hepatocytes. All the lactate dehydrogenase activities at the end of a 60-min incubation were 0.10, 1.49, and 0.80 units/10^6 cells for intact, saponin-treated, and homogenized cells, respectively. This indicates that, under the conditions used for ATPase assays, no further Ca^{2+}-ATPase and Mg^{2+}-ATPase can be detected by permeabilizing the cell.

**Nucleotide Specificity of Ecto-ATPase Activities**—In order to test the possibility that the ecto-Ca^{2+}-ATPase and Mg^{2+}-ATPase may be the same protein we previously purified (Lin and Fain, 1984) and characterized (Lin, 1985b), several properties of the previously characterized high affinity (Ca^{2+}-Mg^{2+})-nucleotidase were determined with the enzyme in intact hepatocytes. The nucleotide specificity of the ecto-ATPase activities is shown in Table I. Both Ca^{2+}- and Mg^{2+}-stimulated activities have broad substrate specificities. The relative nucleotide-hydrolyzing rates are about the same as that of the purified (Ca^{2+}-Mg^{2+})-ATPase (Lin, 1985b) except for AMP-PPNP hydrolysis. The hydrolysis of AMP-PNP in the presence of Ca^{2+} may be due to the presence of 5'-nucleotidase, which is known to be a hepatocyte ecto-enzyme, in the intact cell. The hydrolysis of AMP-PNP in the presence of Ca^{2+} may be due to the sequential hydrolysis of AMP-PNP by the Ca^{2+}-dependent adenine triphosphate pyrophosphohydrolase activity (Floyd and Torp-Pedersen, 1978) and the 5'-nucleotidase.

**Effect of Proteases on Ecto-ATPase and Purified ATPase Activities**—Since the ATP-hydrolyzing site of the ecto-ATPase is extracellular, it was interesting to see whether proteolysis would destroy the ATPase activity from the outside of the cells. As shown in Table II, the ecto-ATPase activity of hepatocytes in primary culture was not sensitive to the addition of 40 μg/ml saponin, which is known to permeabilize hepatocyte plasma membranes without disrupting mitochondria and endoplasmic reticulum (Joseph et al., 1984). As shown in Fig. 2, the Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in intact, saponin-treated, and homogenized cells are about the same. The lactate dehydrogenase activities at the end of a 60-min incubation were 0.10, 1.49, and 0.80 units/10^6 cells for intact, saponin-treated, and homogenized cells, respectively. This indicates that, under the conditions used for ATPase assays, no further Ca^{2+}-ATPase and Mg^{2+}-ATPase can be detected by permeabilizing the cell.

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**Effect of Proteases on Ecto-ATPase and Purified ATPase Activities**—Since the ATP-hydrolyzing site of the ecto-ATPase is extracellular, it was interesting to see whether proteolysis would destroy the ATPase activity from the outside of the cells. As shown in Table II, the ecto-ATPase activity of hepatocytes in primary culture was not sensitive to protease treatment.
p-Nitrophenyl phosphate was used for the reaction mixtures. The results represent the average of duplicate determinations.

No significant amount of Ca\(^{2+}\)-ATPase activity was lost with trypsin, chymotrypsin, or papain treatment. Although treatment of the purified enzyme with 50 \(\mu\text{g/ml}\) trypsin, chymotrypsin, or papain, respectively, at 37°C for 1 h, the membranes were then spun down with an Airfuge for 5 min, and the Ca\(^{2+}\)-ATPase activities were measured in both supernatant and pellet fractions. No significant amount of Ca\(^{2+}\)-ATPase activity was detected in the supernatant fraction (data not shown), suggesting that the ecto-ATPase enzyme was still membrane-bound after the protease treatment.

Effect of Inhibitors on Ecto-ATPase Activity—One of the distinct properties of the previously purified (Ca\(^{2+}\)-Mg\(^{2+}\))- ATPase was that its Ca\(^{2+}\)-stimulated ATPase activity was not sensitive to several known ATPase inhibitors (Lin, 1985b). The ecto-ATPase activity, like the previously purified (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase, was not inhibited by 0.5 mM vanadate, 5 mM ethylmaleimide, 100 \(\mu\text{M}\) p-chloromercuribenzoate, 1 mM ouabain, and 50 \(\mu\text{M}\) 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (not shown).

Effect of Ca\(^{2+}\) and Mg\(^{2+}\) Concentrations on ATPase Activity—The high affinity (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase of rat liver plasma membrane can be activated by either Ca\(^{2+}\) or Mg\(^{2+}\) (Lin, 1985b). Addition of both Ca\(^{2+}\) and Mg\(^{2+}\) to the ATPase assay medium gave the same ATPase activity as Mg\(^{2+}\) alone in both whole cell and disrupted cell preparations (Fig. 1, A and B). The nonadditive effect of Ca\(^{2+}\) and Mg\(^{2+}\) indicates that both activities are from the same enzyme.

As shown in Fig. 3A, in the absence of Mg\(^{2+}\) (the free Mg\(^{2+}\) concentration was less than 0.02 \(\mu\text{M}\)) the ecto-ATPase activity of intact hepatocytes is stimulated by Ca\(^{2+}\) in a concentration-dependent fashion. The Ca\(^{2+}\) concentration dependence curve is best fitted by one component which has the affinity for Ca\(^{2+}\) of 5.2 \(\mu\text{M}\). In the absence of added Ca\(^{2+}\), the ecto-ATPase
activity was stimulated by Mg\textsuperscript{2+}, with \( K_d \) for Mg\textsuperscript{2+} of around 5 \( \mu \)M free Mg\textsuperscript{2+} (Fig. 3B).

**DISCUSSION**

This paper reports the presence of an ecto-Ca\textsuperscript{2+}- and Mg\textsuperscript{2+}-stimulated ATPase in hepatocytes in primary culture. The external localization of the nucleotide-hydrolyzing site is supported by the observation that the Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activities are the same for both intact cells, saponin-treated cells, and homogenized cells. The properties of this ecto-ATPase suggest that the previously purified high affinity (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase of rat liver plasma membrane is the ecto-ATPase. First, the nucleotide specificity of the ecto-ATPase is the same as that of the purified enzyme; both of them are able to hydrolyze ATP, GTP, UTP, CTP, ADP, and GDP to a similar extent. Second, the ecto-ATPase activity can be activated by either Ca\textsuperscript{2+} or Mg\textsuperscript{2+}, and the effects of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} on this ecto-ATPase activity are not additive, indicating that both Ca\textsuperscript{2+} and Mg\textsuperscript{2+}-ATPase activities reside on the same enzyme. This is consistent with the property of the purified enzyme in which a nonadditive effect of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} on the enzymatic activity was observed. Third, the ecto-ATPase, like the purified enzyme, is not affected by oligo-nuclease, vanadate, N-ethylmaleimide, and p-chloromercuribenzoate. Furthermore, the activities of both the ecto-ATPase and purified ATPase are quite insensitive to protease treatments. Consistent with this conclusion is the recent finding by molecular cloning and sequencing of the gene for the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase that in this protein there is one hydrophobic segment which is localized near the C-terminal end of the protein and that there are many putative N-glycosylation sites in the rest of the protein. The molecular arrangement of this protein is similar to that of other membrane ectoenzymes which have been studied, i.e. alkaline phosphatase (Millan, 1986; Kam et al., 1985; Henthorn et al., 1986; Övitt et al., 1986; Berger et al., 1987) and \( \gamma \)-glutamyltransferrase (Laperche et al., 1986).

One approach to determine the sidedness of a membrane protein is to use specific antibodies. If the enzymatic reaction can be inhibited in the intact cell by an antibody specific for the enzyme, then one can conclude that the enzyme has its active site located on the outside of the cell. In order to use such an approach, an antibody against purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase was prepared by inoculating purified protein into the popliteal lymph nodes of a rabbit (Sigel et al., 1985; Henthorn et al., 1986). The antiserum reacts with the purified (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase by precipitating the enzyme complex was precipitated by protein A-Sepharose. Results from molecular cloning and sequencing of the gene for this ecto-ATPase shows that there are more than 15 potential asparagine glycosylation sites in the ecto-ATPase protein. The ecto-ATPase of liver plasma membranes was first observed as a high affinity (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase with a dissociation constant, \( K_d \), for Ca\textsuperscript{2+} in the range of 0.01 to 0.2 \( \mu \)M (Lotersztajn et al., 1981; Iwasa et al., 1982). The values of \( K_d \) of the detergent-solubilized and purified enzyme are 0.09 and 0.16 \( \mu \)M for Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, respectively (Lin, 1985b). In hepatocytes in primary culture, however, the \( K_d \) values for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} are 5.2 and 5.0 \( \mu \)M, respectively, which are higher than those of the purified enzyme. The factor(s) which gives such a difference between the purified enzyme and the enzyme in intact cells is not clear. It has been reported that there is an inhibitor (Lotersztajn and Pecker, 1982; Lotersztajn et al., 1985) and an activator (Lotersztajn et al., 1981) for the high affinity (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase of rat liver plasma membranes. Whether these two putative regulators are involved in the change of affinity for Ca\textsuperscript{2+} is unknown. Also it is possible that a GTP-binding protein may be involved in such a phenomenon. Consistent with this hypothesis is the recent finding by Lotersztajn et al. (1987) that the effect of glucagon on the (Ca\textsuperscript{2+}-Mg\textsuperscript{2+})-ATPase activity is mediated by a GTP-binding protein as revealed by the sensitivity of the effect to cholera toxin. Furthermore, the change of affinity for Ca\textsuperscript{2+} may be due to the difference between the oxidation-reduction status of the intracellular medium and the preparation medium. Finally, the local Ca\textsuperscript{2+} and Mg\textsuperscript{2+} concentrations for the enzyme may be quite different between the intact cells and the solubilized enzyme. Results from molecular cloning and sequencing of the gene for this ecto-ATPase showed that the C terminus of the postulated sequence for the ecto-ATPase contains a unique cAMP-dependent serine phosphorylation consensus sequence (Lys-Arg-X-X-Ser) (Krebs and Beavo, 1979). Whether phosphorylation or dephosphorylation of the ecto-ATPase is involved in changing the affinity of this enzyme for Ca\textsuperscript{2+} and Mg\textsuperscript{2+} is presently under investigation. As a result of the unexpected lower affinity for Ca\textsuperscript{2+} in intact cells, the concentration of Ca\textsuperscript{2+} used in the measurement of Ca\textsuperscript{2+}-ATPase activity, i.e. 1.5 \( \mu \)M free [Ca\textsuperscript{2+}], is lower than the \( K_d \) of the enzyme for Ca\textsuperscript{2+} in intact cells, while the concentration of Mg\textsuperscript{2+} used, i.e. 25 \( \mu \)M free [Mg\textsuperscript{2+}], is close to the concentration for maximal activation of Mg\textsuperscript{2+}-ATPase activity. This accounts for the observation that the Mg\textsuperscript{2+}-ATPase activity is higher than the Ca\textsuperscript{2+}-ATPase activity in several studies. In fact, in the presence of saturating amounts of Ca\textsuperscript{2+} (i.e. 100 \( \mu \)M free Ca\textsuperscript{2+}) or Mg\textsuperscript{2+} (i.e. 200 \( \mu \)M free Mg\textsuperscript{2+}), the Ca\textsuperscript{2+}-ATPase and Mg\textsuperscript{2+}-ATPase activities are the same (data not shown).

Most tissues contain ecto-ATPase activity which can be detected as a Ca\textsuperscript{2+}-stimulated ATPase activity. This ecto-ATPase activity on the plasma membrane is higher than Ca\textsuperscript{2+}-pump ATPase activity. In rat liver plasma membrane, the ATP-hydrolyzing activity of the ecto-ATPase is about 10 times that of the Ca\textsuperscript{2+}-pump ATPase (Lin, 1985a; Lin and

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Lin, S.-H. (1988) manuscript in preparation.
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Fain, 1984). And in rat corpus luteum, a 1000-fold difference between the transport rate and the rate of the Ca\(^{2+}\)-ATPase was observed (Minami and Pennistone, 1987). As a result, there is great confusion in deducing the significance of plasma membrane Ca\(^{2+}\)-stimulated ATPase activity. In the plasma membrane of several tissues, i.e. rat liver (Lin, 1985b), rat corpus luteum (Minami and Pennistone, 1987), rat kidney (Ghijsen et al., 1984) and intestinal basolateral membrane (Moy et al., 1986), and neutrophil plasma membranes (Ochs and Reed, 1984), two different Ca\(^{2+}\)-ATPase activities have been reported. In a recent report by Pavoine et al. (1987), it was claimed that the high affinity (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase of liver plasma membrane is a Ca\(^{2+}\) pump by reconstituting partially purified (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase into artificial liposomes and demonstrating a small amount of Ca\(^{2+}\) transport activity in such a preparation. The (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase preparation in that study, however, was not highly purified, since the specific activity of the enzyme was only 20-fold greater than that of plasma membrane (Lotersztajn et al., 1981). In our previous study, a 300-fold purification was obtained (Lin and Fain, 1984). As a result, it is possible that the Ca\(^{2+}\)-pumping activity observed in the partially purified preparation may be due to contamination of the preparation with the Ca\(^{2+}\)-pump protein. Furthermore, the nucleotide specificity and vanadate sensitivity of the transport system, two critical criteria which distinguish the Ca\(^{2+}\) pump from the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase, were not reported in that study. Therefore, the claim that the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase is a Ca\(^{2+}\) pump is dubious. The function of the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase which is not a calcium pump is not known.

Similar ecto-(Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activities have been found in several other tissues (for references see Lin, 1985b). Several observations (Charest et al., 1985; Dubyak and De Young, 1985) suggest that possible roles for the ecto-ATPase may be to terminate the effect of ATP on the cells or to participate in the ATP effect via its phosphatase activity. It was also interesting to find that the P\(_2\)-purinergic effect has broad nucleotide specificity (Dubyak and De Young, 1985; Buxton et al., 1986; Okajima et al., 1987) as does the nucleotide-hydrolyzing activity of the ecto-ATPase. These correlations between the properties of the P\(_2\)-purinergic effect and the ecto-ATPase activity raise the possibility that the ecto-ATPases may be the P\(_2\)-purinergic receptor. In the J774 mouse macrophage cell line, Steinberg and Silverstein (1987) showed that ecto-ATPase does not mediate the effects of ATP on these cells. In hepatocytes, this possibility is presently under investigation. In this report, we show that the major (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase in the plasma membrane is a membrane ecto-ATPase and suggest that its function may be in the regulation of P\(_2\)-purinergic receptor function. This result should be helpful in clarifying the confusion about the plasma membrane (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPases.

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