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Role of Regucalcin as an Activator of Ca^{2+}-ATPase Activity in Rat Liver Microsomes

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Abstract The effect of Ca^{2+}-binding protein regucalcin on Ca^{2+}-ATPase activity in isolated rat liver microsomes was investigated. The presence of regucalcin (0.1–1.0 μM) in the enzyme reaction mixture led to a significant increase in Ca^{2+}-ATPase activity. Regucalcin significantly stimulated ATP-dependent $^{45}Ca^{2+}$ uptake by the microsomes. Thapsigargin (10^{-6} M), a specific inhibitor of microsomal Ca^{2+} pump enzyme (Ca^{2+}-ATPase), clearly inhibited regucalcin (0.5 μM)-increased microsomal Ca^{2+}-ATPase activity. Liver microsomal Ca^{2+}-ATPase activity was markedly decreased by N-ethylmaleimide (NEM; 2.5 mM), while the activity was clearly elevated by dithiothreitol (DTT; 2.5 mM), indicating that the sulfhydryl (SH) group of the enzyme is an active site. The effect of regucalcin (0.5 μM) in increasing Ca^{2+}-ATPase activity was completely inhibited by the presence of NEM (2.5 mM) or digitonin (10^{-2} %), a solubilizing reagent of membranous lipids. Moreover, the effect of regucalcin on enzyme activity was seen in the presence of Ca^{2+} ionophore (A23187; 10^{-7} M). The present study demonstrates that regucalcin can stimulate Ca^{2+} pump activity in rat liver microsomes, and that the protein may act the SH groups of microsomal Ca^{2+}-ATPase. J. Cell. Biochem. 74:663–669, 1999.

Key words: regucalcin; Ca^{2+} transport; Ca^{2+}-ATPase; liver microsome; rat liver

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. The Ca^{2+} effect in cells is modulated by calmodulin and other Ca^{2+}-binding proteins [Cheung, 1980; Bygrave and Benedetti, 1993; Heizmann and Hunziker, 1991]. Regucalcin, a novel Ca^{2+}-binding protein that does not contain the EF-hand motif as a Ca^{2+}-binding domain [Shimokawa and Yamaguchi, 1993], has been demonstrated to have an inhibitory effect on the activation of various enzymes by Ca^{2+} and calmodulin in liver and kidney cells [Yamaguchi and Tai, 1991; Yamaguchi and Mori, 1990; Kurota and Yamaguchi, 1997]. Regucalcin may play a regulatory role in cell function related to Ca^{2+} [Yamaguchi, 1992, 1998].

The rat regucalcin gene consists of seven exons and six introns, with several consensus regulatory elements upstream of the 5′-flanking region [Yamaguchi et al., 1996]. The gene is localized on the proximal end of rat chromosome Xq 11.1–12 [Shimokawa et al., 1995]. Expression of hepatic regucalcin mRNA is stimulated by Ca^{2+} signals, which are partly involved in the regulation of functional events in liver cells [Shimokawa and Yamaguchi, 1992]; the expression may be partly mediated through Ca^{2+}/calmodulin [Shimokawa and Yamaguchi, 1993]. Presumably, hepatic regucalcin mRNA expression is stimulated by Ca^{2+} signaling, and regucalcin plays a regulatory role in liver cell functions related to Ca^{2+}. Regucalcin may have a multifunctional role in cells.

Regucalcin has been shown to inhibit activation of the Ca^{2+}-dependent enzyme [Yamaguchi, 1992, 1998]. Moreover, regucalcin can stimulate Ca^{2+} pump activity in rat liver plasma membranes [Takahashi and Yamaguchi, 1994, 1997], suggesting that the protein plays a role in the regulation of intracellular Ca^{2+} homeostasis. The role of regucalcin in the control of Ca^{2+} concentration in liver cells, however, has not been fully clarified.

Therefore, the present study was undertaken to clarify the effect of regucalcin on Ca^{2+}-ATPase activity, which is related to ATP-dependent Ca^{2+} uptake by liver microsomes. We found

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regucalcin to have an activatory effect on hepatic microsomal Ca^{2+}-ATPase activity.

MATERIALS AND METHODS

Chemicals

Adenosine-5′-triphosphate (ATP), thapsigargin, ionophore A23187, N-ethylmaleimide, dithiothreitol, digitonin, dibutyryl cyclic adenosine-5′-monophosphate (DcAMP), and inositol 1,4,5-trisphosphate (IP_3) were purchased from Sigma Chemical Co. (St. Louis, MO). [^{45}Ca] calcium chloride (12.4 GBq/mg) was obtained from New England Nuclear (Boston, MA). Calcium chloride and all other chemicals were reagent grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Many reagents used were dissolved in distilled water then passed through an ion-exchange resin to remove metal ions.

Animals

Male Wistar rats, weighing 100–120 g, were used. They were obtained commercially from Japan SLC (Hamamatsu, Japan). The animals were fed commercial laboratory chow (solid) containing 57.5% carbohydrate, 1.1% Ca, and 1.1% P at a room temperature of 25°C, and were allowed distilled water freely.

Isolation of Regucalcin

Regucalcin is markedly expressed in rat liver cytosol [Shimokawa and Yamaguchi, 1993]. Regucalcin was isolated from rat liver cytosol. The livers were perfused with Tris-HCl buffer (pH 7.4), containing 100 mM Tris, 120 mM NaCl, 4 mM KCl, cooled to 4°C). The livers were removed, cut into small pieces, suspended 1:4 (wt/vol) in Tris-HCl buffer (pH 7.4), and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was spun at 5,500*g* in a refrigerated centrifuge for 10 min, and the supernatant was spun at 105,000*g* for 60 min. The resulting supernatant was heated at 60°C for 10 min and recentrifuged at 38,000*g* for 20 min. Regucalcin in the supernatant was purified to electrophoretic homogeneity by gel filtration on Sephadex G-75 and G-50, followed by ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose, as reported previously [Yamaguchi and Yamamoto, 1978].

Preparation of Liver Microsomes

Rats were killed by cardiac puncture, and the liver was perfused with ice-cold 250 mM sucrose solution, immediately cut into small pieces, suspended 1:9 in the homogenization medium containing 250 mM sucrose, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesuflonic acid (Hepes), 1.0 mM ethyleneglycol bis(2-amino-ethylether)-N,N,N′,N′,-tetraacetic acid (EGTA), and 1 mM dithiothreitol (DTT), pH 7.2, and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle [Moore and Kraus-Friedmann, 1983]. The homogenate was centrifuged at 1,000*g* for 10 min to remove nuclei, unbroken cells, and cell debris. The resultant supernatant was centrifuged at 7,700*g* for 20 min to remove the mitochondrial fraction. The postmitochondrial supernatant was then centrifuged at 110,000*g* for 60 min to sediment the microsomal fraction. The microsomal fraction was resuspended in 120 mM KCl, 10 mM Hepes, pH 6.8, to a final protein concentration of 10–15 mg/ml.

Assay of Ca^{2+}-ATPase

Mg^{2+}-ATPase activity was determined for 30 min at 25°C in a medium containing 42.7 mM Hepes-KOH buffer (pH 7.0), 0.1 M KCl, 5 mM $MgCl_2$, 5 mM NaN_3, 2 mM Tris-ethylene glycol bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA), 5 mM Tris-ATP, and the microsomes (100–150 µg as protein) in the absence or presence of A23187 (1 µM) [Heilmann et al., 1983]. The amount of inorganic phosphate released from ATP. E enzyme reaction was measured according to the method of Nakamura and Mori [1958]. (Ca^{2+} + Mg^{2+})-ATPase activity was measured in the same medium, but with Tris-EGTA replaced by 50 µM $CaCl_2$. Ca^{2+}-ATPase activity was calculated as the difference between (Ca^{2+} + Mg^{2+})-ATPase and Mg^{2+}-ATPase. Enzyme activity was expressed as nmol of inorganic phosphate released per min per mg protein. Protein concentration was determined by the method of Lowry et al. [1951].

ATP-Dependent $^{45}Ca^{2+}$ Uptake

$^{45}Ca^{2+}$ uptake was measured by the Millipore filtration technique [Moore and Kraus-Friedmann, 1983]. About 100–150 µg of protein/ml reaction of mixture was incubated for 1 min 37°C in 1 ml of medium containing 100 mM KCl, 20 mM Hepes, 1 mM NaN_3, 1 mM $MgCl_2$, 1 µM ruthenium red, and 100 µM $CaCl_2$ containing $^{45}Ca^{2+}$ (0.185 MBq), pH 6.8. At a designated time after the addition of 10 mM adenosine triphosphate (ATP), adjusted to pH 6.8 with

KOH, to initiate energy-dependent Ca^{2+} uptake, a 100-µl sample was filtered through a 0.22-µm pre-wetted Millipore filter. The precipitate was washed with 120 mM KCl/100 mM Hepes, pH 6.8, transferred to a scintillation vial and counted for radioactivity. $^{45}Ca^{2+}$ uptake is expressed as nmol of $^{45}Ca^{2+}$ accumulated per mg protein of the microsomes.

Statistical Analysis

Data were expressed as the mean ±SEM. Statistical differences were analyzed using Student's *t*-test. A *P*-value of 0.05 was considered to indicate a statistically significant difference.

RESULTS

Effect of Regucalcin on Liver Microsomal Ca^{2+}-ATPase Activity

The effect of calcium chloride addition on Ca^{2+}-ATPase activity in rat liver microsomes is examined in Figure 1. The addition of calcium chloride (5, 10, 25, 50, and 100 µM) in the enzyme reaction mixture led to a significant increase in Ca^{2+}-ATPase activity; the increase was saturated at 50 µM Ca^{2+}. In the presence of 50 µM $CaCl_2$, the addition of regucalcin (0.1, 0.25, 0.5, and 1.0 µM) produced a significant elevation in Ca^{2+}-ATPase activity. The effect of regucalcin reached to a maximum at the concentration of 0.5 µM (Fig. 2A). Meanwhile, hepatic microsomal Mg^{2+}-ATPase activity was not appreciably altered by the addition of regucalcin (0.1–1.0 µM) (Fig. 2B).

Thapsigargin is a specific inhibitor of the microsomal Ca^{2+} pump enzyme (Ca^{2+}-ATPase) [Thastrup et al., 1990]. Hepatic microsomal Ca^{2+}-ATPase activity was markedly decreased by the addition of thapsigargin (TP) in the enzyme reaction mixture (Fig. 3A). The inhibitory effect of TP was saturated at 10^{-6} M. The effect of regucalcin (0.5 µM) in increasing Ca^{2+}-ATPase activity was not significantly seen in the presence of TP (10^{-6} M) (Fig. 3B), indicating that regucalcin acts Ca^{2+} pump enzyme (Ca^{2+}-ATPase) in the microsomes.

The effect of regucalcin on ATP-dependent $^{45}Ca^{2+}$ uptake by liver microsomes is shown in Figure 4. The addition of ATP to the reaction mixture containing $^{45}Ca^{2+}$ led to microsomal $^{45}Ca^{2+}$ uptake (Fig. 4A). This uptake was significantly increased by the presence of regucalcin (0.5 µM) (Fig. 4A). A significant increase in microsomal $^{45}Ca^{2+}$ uptake by regucalcin was seen at 0.25 µM, and it was saturated at 1.0 µM (Fig. 4B). Thus, regucalcin had an activatory effect on liver microsomal Ca^{2+} pump.

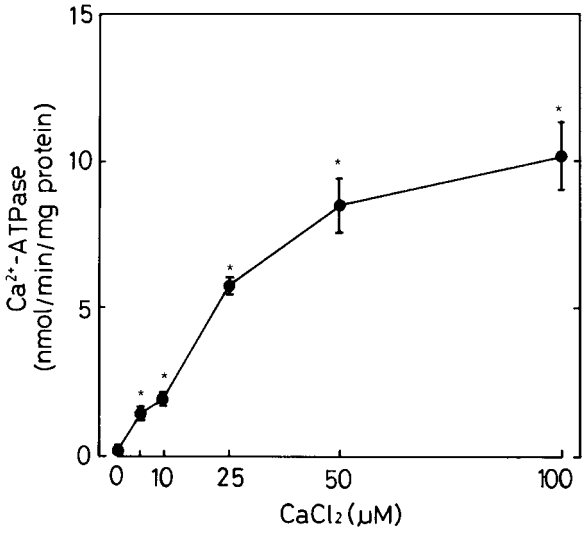

Fig. 1. Alteration in Ca^{2+}-ATPase activity with increasing concentrations of Ca^{2+} addition in rat liver microsomes. $CaCl_2$ was added to the enzyme reaction mixture, yielding concentrations of 5, 10, 25, 50, and 100 µM. Each value is the mean ±SEM of five experiments with separate rats. $*P < 0.01$, as compared with the control value without Ca^{2+} addition.

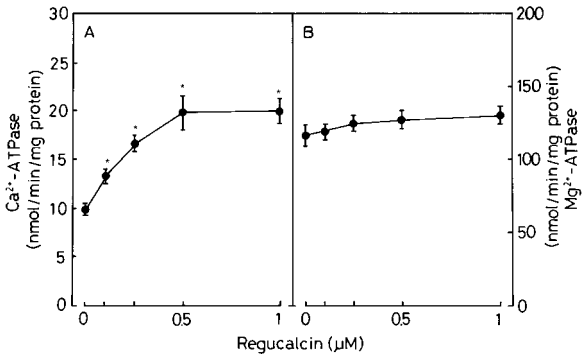

Fig. 2. Effect of regucalcin on Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in rat liver microsomes. Regucalcin was added to the enzyme reaction mixture, yielding concentrations of 0.1, 0.25, 0.5, and 1.0 µM in the presence **(A)** or absence **(B)** of 50 µM Ca^{2+}. Each value is the mean ±SEM of five experiments with separate rats. $*P < 0.01$, as compared with the control value without regucalcin addition.

Characterization of Regucalcin Action on Liver Microsomal Ca^{2+}-ATPase Activity

The effect of regucalcin on liver microsomal Ca^{2+}-ATPase activity in the presence of Ca^{2+} ionophore (A23187) is shown in Table I. The presence of A23187 (10^{-7} M) in the enzyme reaction mixture did not cause a significant

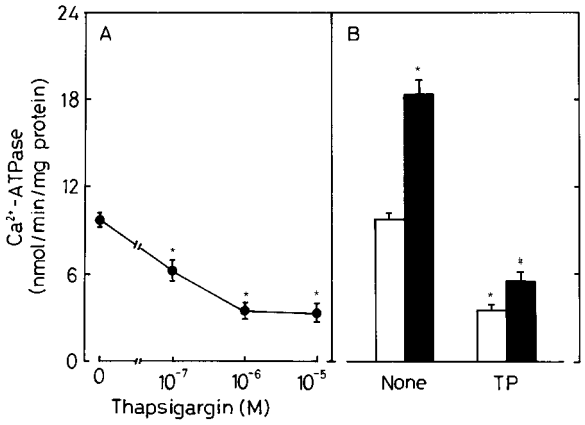

Fig. 3. Effect of thapsigarin, an inhibitor of microsomal Ca^{2+}-ATPase, on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. **A:** Thapsigargin (TP) was added to the enzyme reaction mixture, yielding concentrations of 10^{-7}–10^{-5} M. **B:** The enzyme reaction mixture contained either vehicle or regucalcin (0.5 μM) in the absence or presence of TP (10^{-5} M). Each value is the mean ±SEM of five experiments with separate rats. *$P < 0.01$, as compared with the control (none) value; #$P < 0.01$, as compared with the value of regucalcin alone. □, control; ■, regucalcin.

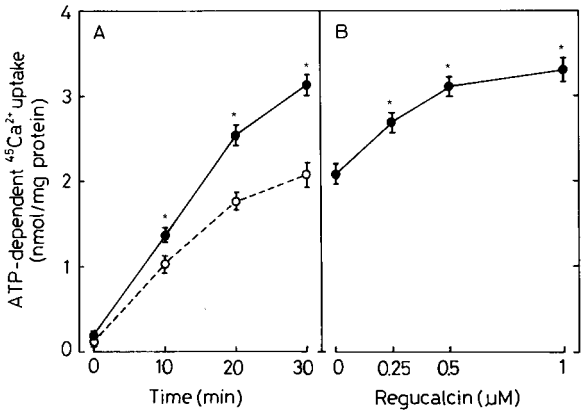

Fig. 4. Effect of regucalcin on ATP-dependent $^{45}Ca^{2+}$ uptake in rat liver microsomes. $^{45}Ca^{2+}$ uptake was measured as described in the experimental section. **A:** The microsomes were incubated for 10, 20, and 30 min after the addition of ATP in the absence or presence of regucalcin (0.5 μM). **B:** The microsomes were incubated for 30 min after the addition of ATP in the absence or presence of regucalcin (0.25, 0.5, and 1.0 μM). Each value is the mean ±SEM of five experiments with separate rats. ○, control; ●, regucalcin.

decrease in Ca^{2+}-ATPase activity, although (Ca^{2+} + Mg^{2+})-ATPase and Mg^{2+}-ATPase activity was reduced by the addition of A23187. The effect of RC (0.5 μM) in increasing Ca^{2+}-ATPase activity was also seen in the presence of A23187 (10^{-7} M). The effect of regucalcin was not dependent on Ca^{2+}.

TABLE I. Effect of Regucalcin on Liver Microsomal Ca^{2+}-ATPase Activity in the Presence of Ca^{2+} Ionophore[a]

Treatment	Enzyme activity (nmol/min/mg protein) (Ca^{2+} + Mg^{2+})-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase
Control	119.9 ± 1.9	115.1 ± 7.0	8.8 ± 0.8
A23187	101.7 ± 1.3*	94.3 ± 3.0*	7.4 ± 0.7
Regucalcin	130.2 ± 3.8*	113.0 ± 2.3*	18.8 ± 1.8*
A23187 + regucalcin	102.0 ± 4.1*	86.3 ± 7.2*	15.7 ± 2.0*

[a]The enzyme reaction mixture contained either vehicle or regucalin (0.5 μM) in the absence or presence of A23187 (10^{-7} M). Each value is the mean ± SEM of five experiments of separate rats.
*$P < 0.01$, as compared with the control value.

The effect of digitonin on regucalcin-increased Ca^{2+}-ATPase activity in hepatic microsomes is shown in Figure 5. Digitonin has a solubilization effect on membranous lipids [Murphy et al., 1980]. The presence of digitonin (10^{-3} and 10^{-2} %) in the enzyme reaction mixture did not have a significant effect on microsomal Ca^{2+}-ATPase activity. In the presence of digitonin, however, regucalcin (0.5 μM) could not increase Ca^{2+}-ATPase activity.

The effect of N-ethylmaleimide (NEM), a modifying reagent of sulfhydryl (SH) groups, on regucalcin-increased Ca^{2+}-ATPase activity in liver microsomes is shown in Figure 6. The presence of NEM (2.5 and 5.0 mM) in the enzyme reaction mixture caused a remarkable decrease in Ca^{2+}-ATPase activity (Fig. 6A). In the presence of NEM (2.5 mM), the effect of regucalcin (0.5 μM) in increasing Ca^{2+}-ATPase activity was not seen (Fig. 6B).

Liver microsomal Ca^{2+}-ATPase activity was markedly elevated by the presence of dithiothreitol (DTT), a protecting reagent of SH groups, in the enzyme reaction mixture (Fig. 7A). The effect of regucalcin (0.5 μM) or enzyme activity was not further enhanced in the presence of DTT (2.5 mM) (Fig. 7B).

Effect of Signaling Factors on Regucalcin-Increased Liver Microsomal Ca^{2+}-ATPase Activity

The effect of dibutyryl cAMP (DcAMP) on Ca^{2+}-ATPase activity in rat liver microsomes is shown in Figure 8. Microsomal Ca^{2+}-AMPase activity was significantly increased by the presence of DcAMP (10^{-6} to 10^{-4} M) in the enzyme

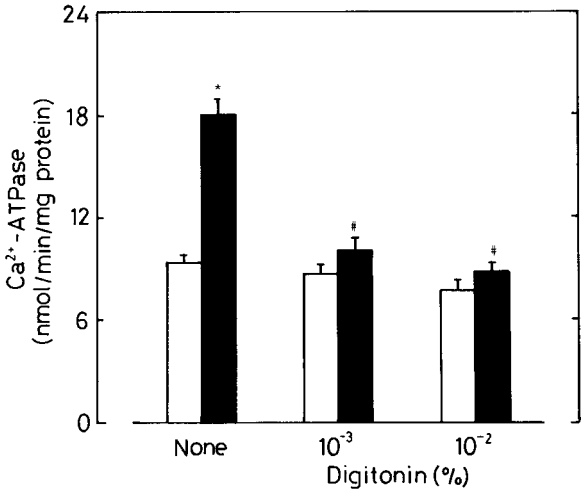

Fig. 5. Effect of digitonin on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. The enzyme reaction mixture contained either vehicle or digitonin (10^{-3} or 10^{-2} %) in the absence or presence of regucalcin (0.5 μM). Each value is the mean ±SEM of five experiments with separate rats. $*P <$ 0.01, as compared with the control (none) value; $\#P < 0.01$, as compared with the value of regucalcin alone. □, control; ■, regucalcin.

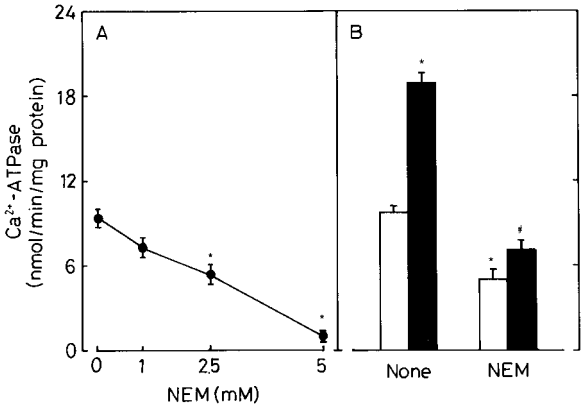

Fig. 6. Effect of N-ethylmaleimide (NEM) on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. **A:** NEM was added to the enzyme reaction mixture, yielding concentrations of 1.0, 2.5, and 5.0 mM. **B:** The enzyme reaction mixture contained either vehicle or NEM (2.5 mM) in the absence or presence of regucalcin (0.5 μM). Each value is the mean ±SEM of five experiments with separate rats. $*P <$ 0.01, as compared with the control (none) value; $\#P < 0.01$, as compared with the value of regucalcin alone. □, control; ■, regucalcin.

reaction mixture (Fig. 8A). This increase was not significantly enhanced by the presence of regucalcin (0.5 μM) (Fig. 8B).

The presence of inositol 1,4,5-trisphosphate (IP_3; 10^{-7} to 10^{-5} M) in the enzyme reaction mixture caused a significant increase in liver microsomal Ca^{2+}-ATPase activity (Fig. 9A). The effect of regucalcin (0.5 μM) in increasing Ca^{2+}-

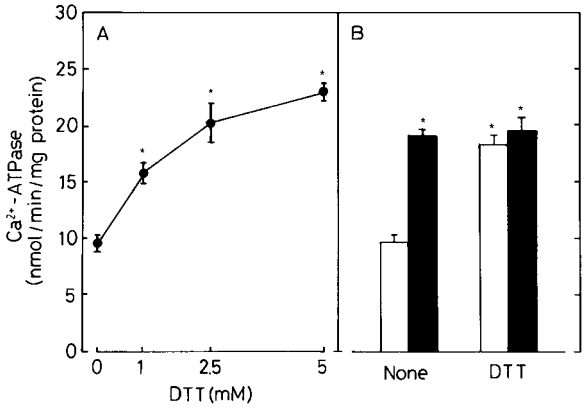

Fig. 7. Effect of dithiothreitol (DTT) on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. **A:** DTT was added to the enzyme reaction mixture, yielding concentrations of 1.0, 2.5, and 5.0 mM. **B:** The enzyme reaction mixture contained either vehicle or DTT (2.5 mM) in the absence or presence of regucalcin (0.5 μM). Each value is the mean ±SEM of five experiments with separate rats. $*P <$ 0.01, as compared with the control (none) value. □, control; ■, regucalcin.

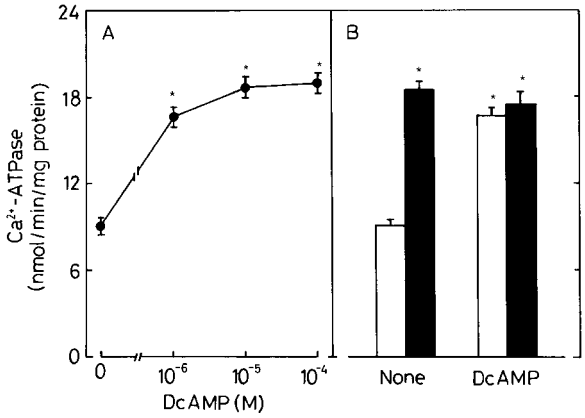

Fig. 8. Effect of dibutyryl cyclic adenosine monophosphate (DcAMP) on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. **A:** DcAMP was added to the enzyme reaction mixture, yielding concentrations of 10^{-6}–10^{-4} M. **B:** The enzyme reaction mixture contained either vehicle or DcAMP (10^{-6} M) in the absence or presence of regucalcin (0.5 μM). Each value is the mean ±SEM of five experiments with separate rats. $*P <$ 0.01, as compared with the control (none) value. □, control; ■, regucalcin.

ATPase activity was not significantly altered by the presence of IP_3 (10^{-6} M) (Fig. 9B).

DISCUSSION

Regucalcin has been shown to activate Ca^{2+} pump enzyme (Ca^{2+}-ATPase) in isolated rat liver plasma membranes [Takahashi and Yamaguchi, 1994, 1997], suggesting that the protein plays a role in the regulation of intracellular Ca^{2+} homeostasis. Furthermore, the pres-

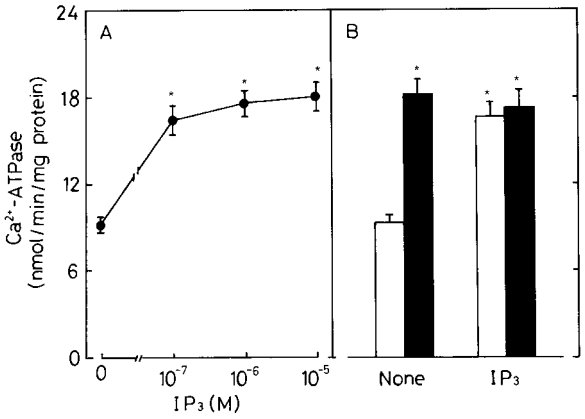

Fig. 9. Effect of inositol 1,4,5-trisphosphate (IP_3) on regucalcin-increased Ca^{2+}-ATPase activity in rat liver microsomes. **A:** IP_3 was added to the enzyme reaction mixture, yielding concentrations of 10^{-7}–10^{-5} M. **B:** The enzyme reaction mixture contained either vehicle or IP_3 (10^{-7} M) in the absence or presence of regucalcin (0.5 μM). Each value is the mean ±SEM of five experiments with separate rats. *$P < 0.01$, as compared with the control (none) value. □, control; ■, regucalcin.

ent study was undertaken to clarify the effect of regucalcin on Ca^{2+} pump activity in isolated rat liver microsomes. Hepatic microsomal Ca^{2+} sequestration is involved in Ca^{2+}-ATPase [Heilmann et al., 1983; Kraus-Friedmann, 1990]. Regucalcin has been found to increase Ca^{2+}-ATPase activity and ATP-dependent Ca^{2+} uptake in hepatic microsomes.

The effect of regucalcin in increasing liver microsomal Ca^{2+}-ATPase activity was not seen in the presence of digitonin, a solubilization reagent of membranous lipids [Murphy et al., 1980], in the enzyme reaction mixture. This result suggests that regucalcin acts on Ca^{2+}-ATPase by its binding on the microsomal membranous lipids. Moreover, regucalcin-increased microsomal Ca^{2+}-ATPase activity was completely inhibited by the presence of NEM, a modifying reagent of SH groups, while the effect of regucalcin was not further enhanced by the addition of DTT, a protecting reagent of SH groups. It has been reported that liver microsomal Ca^{2+} sequestration is critically dependent on the SH groups of protein, and that modification of protein thiols may be an important mechanism for the inhibition of microsomal Ca^{2+} sequestration by a variety of toxic agents [Thor et al., 1985]. On the basis of our results, it is assumed that regucalcin acts on the SH groups, which may be an active site of Ca^{2+}-ATPase in hepatic microsomes.

Ca^{2+} ionophore (A23187) can stimulate release of Ca^{2+} from liver microsomes [Kraus-Friedmann, 1990]. Whether the effect of regucalcin on liver microsomal Ca^{2+}-ATPase activity is involved in the microsomal Ca^{2+} was examined. Regucalcin was able to increase hepatic microsomal Ca^{2+}-ATPase activity in the presence of Ca^{2+} ionophore (A23187), suggesting that regucalcin activates the enzyme independent on Ca^{2+}, which is related to the microsomal Ca^{2+} transport. Presumably, regucalcin binds to the microsomal membranes and directly activates Ca^{2+}-ATPase by acting on the SH groups.

Cyclic adenosine monophosphate (cAMP) and inositol 1,4,5-trisphosphate (IP_3) are intracellular signaling factors [Joseph and Williamson, 1989; Rasmussen, 1970]. It is known that cAMP and IP_3 can stimulate Ca^{2+} release from liver microsomes [Staddon and Hansford, 1989; Kraus-Friedmann, 1990; Joseph and Williamson, 1989]. Whether the effect of regucalcin on Ca^{2+}-ATPase activity in liver microsomes is involved in the action of cAMP or IP_3 was examined. The presence of dibutyryl cAMP (DcAMP) or IP_3 in the enzyme reaction mixture caused a significant increase in liver microsomal Ca^{2+}-ATPase activity. These results suggest the possibility that cAMP or IP_3 can stimulate ATP-dependent Ca^{2+} uptake. Meanwhile, regucalcin-increased liver microsomal Ca^{2+}-ATPase activity was not significantly enhanced by the presence of DcAMP or IP_3. Regucalcin largely exists in the cytoplasm of liver cells [Yamaguchi and Isogai, 1993]. Liver cytoplasm regucalcin concentration is estimated at about 5 μM. Intracellular cAMP and IP_3, which are generated by hormonal stimulation, may not have physiological significance for Ca^{2+} sequestration system in liver cells in which regucalcin is largely present.

An activator of liver microsomal Ca^{2+}-ATPase is not fully known [Kraus-Friedmann, 1990]. Yet the endoplasmic reticulum contains calmodulin and the Ca^{2+} sequestration process does respond to calmodulin [Kraus-Friedmann et al., 1988; Fomulshi and Carafoli, 1984]. It is also possible that the role of calmodulin in the endoplasmic reticulum is more related to Ca^{2+} release than to uptake, as has been suggested in the sarcoplasmic reticulum [Smith et al., 1989]. Presumably, regucalcin is unique as an activator of Ca^{2+}-ATPase in rat liver microsomes.

Regucalcin has been demonstrated to stimulate Ca^{2+} pump activity in rat liver plasma membranes [Takahashi and Yamaguchi, 1994,

1997]. In addition, the present study finds that regucalcin has an activatory effect on ATP-dependent Ca^{2+} uptake in the endoplasmic reticulum of liver cells. Thus, regucalcin may play an important role in the regulation of Ca^{2+} concentration in the cytoplasm of liver cells.

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