Journal of Cellular Biochemistry 78:541–549 (2000)

Role of Endogenous Regucalcin in the Regulation of Ca^{2+}-ATPase Activity in Rat Liver Nuclei

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Abstract The role of endogenous regucalcin in the regulation of Ca^{2+}-ATPase, a Ca^{2+} sequestrating enzyme, in rat liver nuclei was investigated. Nuclear Ca^{2+}-ATPase activity was significantly reduced by the addition of regucalcin (0.1–0.5 μM) into the enzyme reaction mixture. The presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml) caused a significant elevation of Ca^{2+}-ATPase activity; this effect was completely abolished by the addition of regucalcin (0.1 μM). The effect of anti-regucalcin antibody (50 ng/ml) in increasing Ca^{2+}-ATPase activity was completely prevented by the presence of thapsigargin (10^{-6} M), an inhibitor of Ca^{2+} sequestrating enzyme, *N*-ethylmaleimide (1 mM), a modifying reagent of thiol groups, or vanadate (10^{-5} M), an inhibitor of phosphorylation of the enzyme by ATP, which revealed an inhibitory effect on nuclear Ca^{2+}-ATPase activity. Meanwhile, the effect of anti-regucalcin antibody (50 ng/ml) was significantly enhanced by the addition of calmodulin (5 μg/ml), which could increase nuclear Ca^{2+}-ATPase activity. In addition, the effect of antibody (50 ng/ml) was significantly reduced by the presence of trifluoperazine (20 μM), an antagonist of calmodulin. These results suggest that the endogenous regucalcin in liver nuclei has a suppressive effect on nuclear Ca^{2+}-ATPase activity, and that regucalcin can inhibit an activating effect of calmodulin on the enzyme. J. Cell. Biochem. 78:541–549, 2000.

Key words: regucalcin; Ca^{2+}-ATPase; calmodulin; calcium; rat liver nuclei

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. The Ca^{2+} effect in cells is amplified by calmodulin and protein kinase C, which is related to a signal transduction caused by hormonal stimulation [Cheung, 1980; Nishizuka, 1986; Heizmann and Hunziker, 1991; Kraus-Friedman and Feng, 1996]. A novel Ca^{2+}-binding protein, regucalcin, has been shown to regulate the Ca^{2+}-dependent signaling system; the protein inhibits Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C [Mori and Yamaguchi, 1990; Yamaguchi and Mori, 1990; Yamaguchi and Katsumata, 1999].

Regucalcin is mainly expressed in the liver [Shimokawa and Yamaguchi, 1992, 1993; Yamaguchi and Isogai, 1993]. The expression of hepatic regucalcin mRNA is partly mediated through Ca^{2+} signaling factors [Murata and Yamaguchi, 1999]. Regucalcin mRNA has been demonstrated to be enhanced in regenerating rat liver, which induces a proliferation of liver cells after a partial hepatectomy [Yamaguchi and Kanayama, 1995]. Regucalcin has an inhibitory effect on DNA and RNA syntheses in the nuclei isolated from regenerating rat liver, suggesting that the protein plays a role in the regulation of nuclear function in proliferative liver cells [Yamaguchi and Kanayama, 1996; Yamaguchi and Ueoka, 1997]. The action of regucalcin on liver nuclear function, however, remains to be elucidated.

There has been growing evidence that Ca^{2+} plays a role in liver nuclear functions [Jones et al., 1989; Allbritton et al., 1994; Csermely et al., 1995]. Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C have been shown to exist in liver nuclei [Backs and Carafoli, 1987; Block et al., 1992]. The existence of an adenosine 5′-triphosphatase (ATP)-stimulated Ca^{2+} sequestration system in rat liver nuclei that generates a net increase in nuclear matrix-free Ca^{2+} concentration has been reported [Nicotera et al., 1989; Lanini et al.,

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Received 1 February 2000; Accepted 24 February 2000

1992; Yamaguchi, 1992; Yamaguchi and Oishi, 1993]. The role of regucalcin in the regulation of Ca^{2+}-ATPase activity in liver nuclei, however, has not been clarified so far. This may be important as a mechanism of regucalcin action in the regulation of liver nuclear functions that are related to Ca^{2+}.

The present study was undertaken to determine the effect of endogenous regucalcin on Ca^{2+}-ATPase activity in rat liver nuclei using anti-regucalcin monoclonal antibody, because regucalcin is present in the liver nuclei [Omura and Yamaguchi, 1999]. We found that endogenous regucalcin had an inhibitory effect on Ca^{2+}-ATPase activity in rat liver nuclei.

MATERIALS AND METHODS

Chemicals

ATP, thapsigargin, *N*-ethylmaleimide (NEM), dithiothreitol (DTT), trifluoperazine (TFP), and calmodulin (52,000 U/mg protein from bovine brain) were obtained from Sigma Chemical Co. (St. Louis, MO). Calcium chloride, vanadate (neutralized with KOH), and other reagents were purchased from Wako Pure Chemical Co. (Osaka, Japan). The reagents were dissolved in distilled water and then passed through ion-exchange resin to remove metal ions.

Animals

Male Wistar rats, weighing 100–120 g, were used. They were obtained commercially from Japan SLC, Inc. (Hamamatsu, Japan). Animals were fed commercial laboratory chow (solid) containing 57.5% carbohydrate, 1.1% calcium, and 1.1% phosphorus at a room temperature of 25°C, and were allowed distilled water freely. After 1 week on the diet, animals were killed by bleeding.

Isolation of Regucalcin

Regucalcin was isolated from rat liver. Livers were perfused with Tris-HCl buffer (pH 7.4, containing 100 mM Tris, 120 mM NaCl, 4 mM KCl, cooled to 4°C). Livers were removed, cut into small pieces, suspended 1:4 in Tris-HCl buffer (pH 7.4), and 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-cellulose, as reported previously [Yamaguchi and Yamamoto, 1978].

Anti-Regucalcin Antibody

Anti-regucalcin antibody was a monoclonal antibody raised against regucalcin prepared using standard methods [Omura and Yamaguchi, 1998]. Mice (BALB/C, Japan SLC) were subcutaneously injected with 0.1 mg per animal of antigen (rat liver regucalcin) emulsified with Freund's complete adjuvant, and 19 days later antigen (0.25 mg/animal) was intraperitoneally injected with Freund's incomplete adjuvant. Animals were killed by bleeding 3 days after the last injection. Spleen cells were prepared from immunized mouse and fused into myeloma cells. Anti-regucalcin monoclonal antibody–producing cells (hybridoma cells) were obtained by screening. The IgG from hybridoma cells was isolated through protein A-agarose column (Sigma).

Isolation of Nuclei

Liver nuclei were isolated by the procedure of Jones et al. [1989] with a minor modification. Rats were killed by cardiac puncture, and the livers were perfused with ~10 ml of ice-cold TKM solution (50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM $MgCl_2$) to remove blood. Livers were then removed, cut into small pieces, and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle in 40 ml of the same solution containing 0.25 M sucrose and 1.0 mM ethyleneglycol-bis-(aminoethylether) N,N,N′,N′-tetraacetic acid (EGTA). The homogenate was filtered through three layers of cheesecloth. The nuclei were pelleted by centrifugation at 700*g* for 10 min. The pellets were homogenized (five strokes) in 40 ml of the same solution and centrifuged again at 700 g for 10 min. The pellet was resuspended in 24 ml of the same solution by homogenization (five strokes), and 6 ml was added to each of four tubes containing 12 ml of TKM included 2.3 M sucrose. The tubes were gently mixed, and a 6-ml cushion (TKM containing 2.3 M sucrose) was carefully layered on the bottom of each tube. The tubes were centrifuged at 37,000*g* for 30 min. The upper layer and the sucrose cushion were removed with an aspirator. The resulting pellet of highly purified nuclei was resuspended in the incubation medium (125 mM KCl, 2 mM potas-

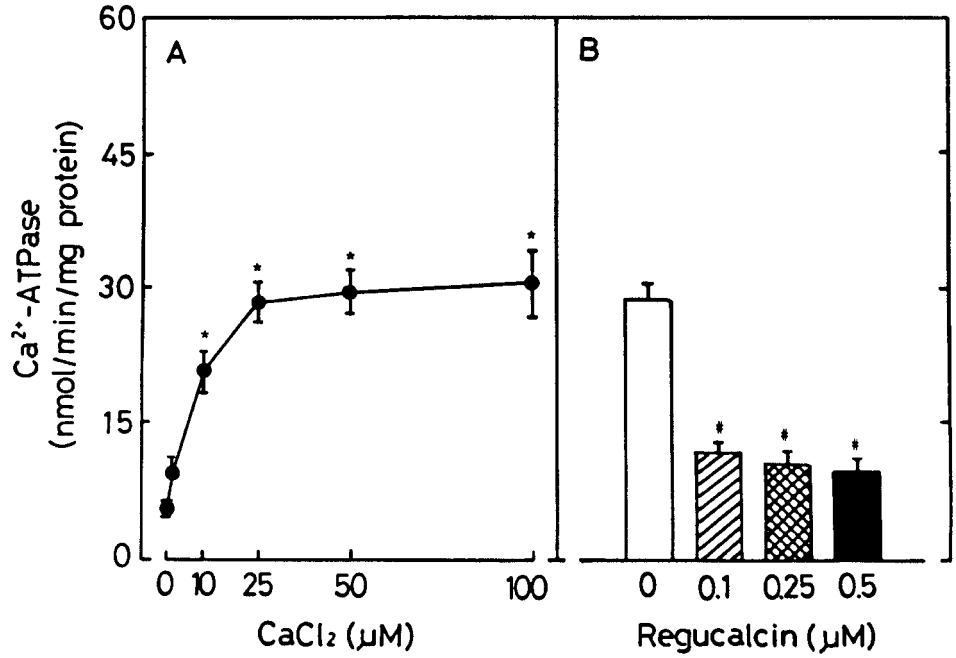

Fig. 1. Effect of calcium chloride ($CaCl_2$) on Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme activity was measured for 10 min in the reaction mixture containing $CaCl_2$ (1, 10, 25, 50, and 100 μM) in the presence of the nuclei as described in the Materials and Methods section. **B:** The enzyme reaction mixture contained either vehicle or regucalcin (0.1, 0.25, and 0.5 μM) in the presence of $CaCl_2$ (25 μM). Each value represents the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P < 0.01$, compared with the value without $CaCl_2$ addition. $\#P < 0.01$, compared with the control value with $CaCl_2$ (25 μM) addition.

sium phosphate, 25 mM HEPES, 4 mM $MgCl_2$, pH 7.0) by hand homogenization. Assay of marker enzymes, as reported previously [Yamaguchi and Oishi, 1993], showed that there was <5% contamination by microsomes, plasma membranes, or mitochondria.

DNA content in the nuclei was determined by using the diphenylamine reaction [Burton, 1956]. Protein concentration was measured as described by Lowry et al. [1951] using bovine serum albumin as the standard.

Ca^{2+}-ATPase Assay

Basal, Mg^{2+}, and Ca^{2+} + Mg^{2+}-ATPase activities were determined by modification of the method of Zhang et al. [1990]. The incubation medium (2.5 ml) contained 100 mM KCl, 20 mM HEPES, and nuclear protein (0.5–0.8 mg/ml; 0.15–0.25 mg DNA/ml). In the Mg^{2+}-, and Ca^{2+} + Mg^{2+}-ATPase assay, either 5 mM $MgCl_2$, 25 μM $CaCl_2$, or both were added to the incubation mediums, respectively [Yamaguchi and Oishi, 1993]. The reaction was started by adding 2 mM ATP. After incubation at 37°C for 10 min, the ice-cold trichloroacetic acid was added to a final concentration of 7% (wt/vol) to stop the reaction. The protein-free supernatant was obtained after centrifugation at 5,000*g* for 5 min and was assayed for inorganic phosphate concentration [Nakamura and Mori, 1958]. Enzyme activity was expressed as nanomoles of inorganic phosphate released per minute per milligram of protein. Ca^{2+}-ATPase activity was calculated by subtracting Mg^{2+}-ATPase activity from (Ca^{2+}–Mg^{2+})-ATPase activity.

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 statistically significant differences.

RESULTS

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

The effect of calcium chloride addition on Ca^{2+}-ATPase activity in rat liver nuclei was examined (Fig. 1A). The addition of calcium

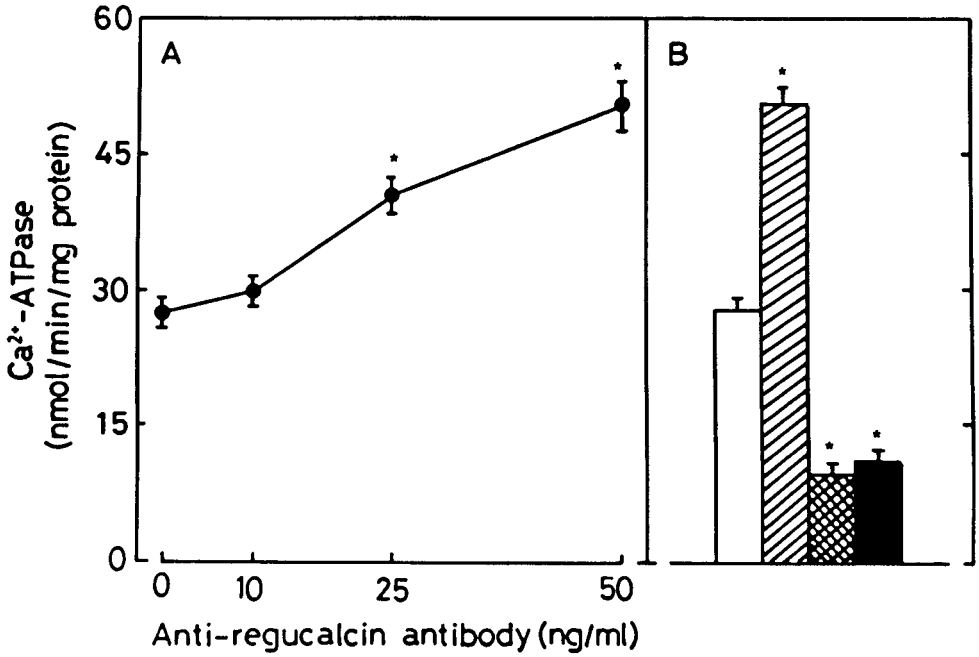

Fig. 2. Effect of anti-regucalcin monoclonal antibody on Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme reaction mixture contained either vehicle or anti-regucalcin monoclonal antibody (10, 25, and 50 ng/ml) in the presence of $CaCl_2$ (25 μM). **B:** The reaction mixture contained either vehicle, the antibody (50 ng/ml), regucalcin (0.1 μM), the antibody (50 ng/ml) plus regucalcin (0.1 μM) in the presence of $CaCl_2$ (25 μM). Each value represents the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P < 0.01$, compared with the control value without the antibody addition. White bar, control; hatched bar, antibody; cross-hatched bar, regucalcin; black bar, antibody plus regucalcin.

(10–100 μM) into the enzyme reaction mixture caused a remarkable elevation of Ca^{2+}-ATPase activity in liver nuclei; the increase reached to a maximum level at 25 μM calcium addition. Ca^{2+}-ATPase activity was calculated by subtracting Mg^{2+}-ATPase activity and (Ca^{2+}–Mg^{2+})-ATPase activity; Mg^{2+}-ATPase activity and (Ca^{2+}–Mg^{2+})-ATPase activities in the presence of 25 μM Ca^{2+} were 354.6 ± 7.8 and 382.2 ± 4.5 (nmol/min/mg protein), respectively. Meanwhile, liver nuclear Ca^{2+}-ATPase activity was significantly reduced by the addition of regucalcin (0.1, 0.25, and 0.5 μM) (Fig. 1B). The effect of regucalcin was saturated at 0.1 μM. However, regucalcin (0.1 μM) had no effect on Mg^{2+}-ATPase activity (data not shown).

The effect of anti-regucalcin monoclonal antibody on Ca^{2+}-ATPase activity in rat liver nuclei is shown in Figure 2. The presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml) in the enzyme reaction mixture containing liver nuclei caused a significant increase in Ca^{2+}-ATPase activity (Fig. 2A). The effect of the antibody (50 ng/ml) in elevating Ca^{2+}-ATPase activity was completely abolished by the addition of regucalcin (0.1 μM) (Fig. 2B). Anti-regucalcin monoclonal antibody (50 ng/ml) did not have an effect on liver nuclear Mg^{2+}-ATPase activity (data not shown). The result indicates that the endogenous regucalcin has a suppressive effect on Ca^{2+}-ATPase activity in liver nuclei. Regucalcin has been shown to be localized in rat liver nuclei [Omura and Yamaguchi, 1999].

Characterization of Anti-Regucalcin Antibody Effect on Nuclear Ca^{2+}-ATPase Activity

The effect of thapsigargin, an inhibitor of microsomal Ca^{2+}-ATPase (Ca^{2+} pump enzyme) activity [Lanini et al., 1992], on the anti-regucalcin monoclonal antibody (50 ng/ml)-raised Ca^{2+}-ATPase activity in the liver nuclei is shown in Figure 3. The presence of thapsigargin (10^{-6} or 10^{-5} M) in the enzyme reaction mixture caused a significant decrease in Ca^{2+}-ATPase activity (Fig. 3A). The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in elevating Ca^{2+}-ATPase activity in liver nuclei was completely blocked by the presence of

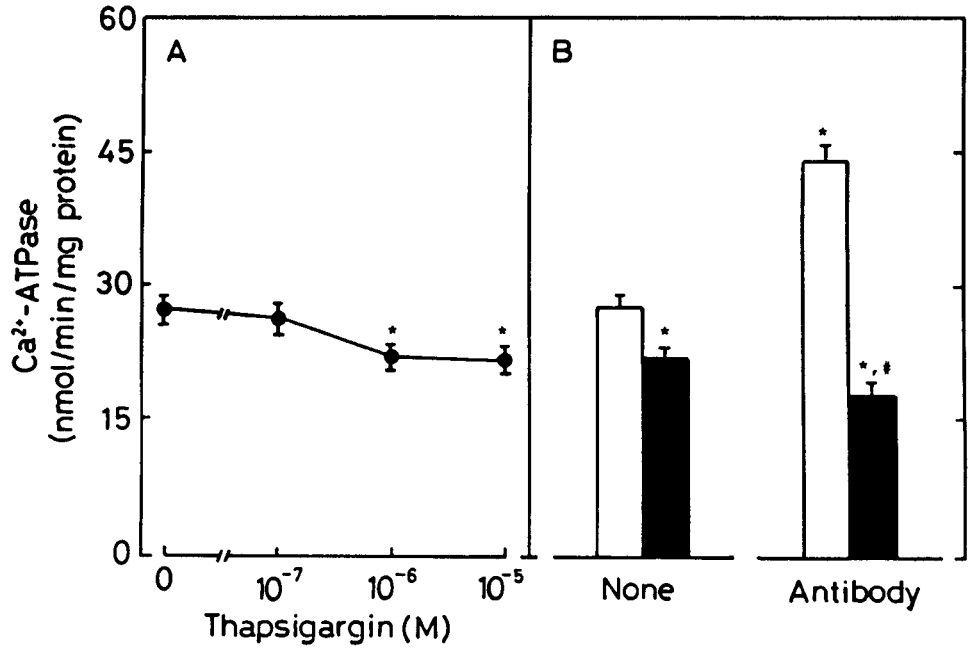

Fig. 3. Effect of thapsigargin on the anti-regucalcin monoclonal antibody-increased Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme reaction mixture contained either vehicle or thapsigargin (10^{-7}–10^{-5} M) in the presence of $CaCl_2$ (25 μM). **B:** The reaction mixture contained either vehicle or thapsigargin (10^{-5} M) in the presence of antibody (50 ng/ml) with $CaCl_2$ (25 μM) addition. Each value is the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P < 0.01$, compared with the value without thapsigargin addition. $\#P < 0.01$, compared with the value for antibody alone. White bars, control; black bars, thapsigargin.

thapsigargin (10^{-6} M) (Fig. 3B). The result indicates that the endogenous regucalcin in liver nuclei has a suppressive effect on thapsigargin-sensitive Ca^{2+}-ATPase activity, which may be involved in Ca^{2+} sequestration in the nuclei [Yamaguchi and Oishi, 1993].

The effect of thiol (SH) group-acting reagents on Ca^{2+}-ATPase activity in rat liver nuclei is shown in Figure 4. Liver nuclear Ca^{2+}-ATPase activity was significantly raised by the addition of DTT (1, 2.5, and 5 mM), a protecting reagent of SH groups (Fig. 4A). The enzyme activity was significantly decreased by NEM (1, 2.5, and 5 mM), a modifying reagent of SH groups (Fig. 4B). The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in increasing Ca^{2+}-ATPase activity was completely prevented by the presence of NEM (1 mM) (Fig. 4C), indicating that the endogenous regucalcin acts on the SH groups of the enzyme.

The effect of vanadate, an inhibitor of phosphorylation of Ca^{2+}-ATPase by ATP [Chen and Junger, 1983], on Ca^{2+}-ATPase activity in rat liver nuclei is shown in Figure 5. Liver nuclear Ca^{2+}-ATPase activity was significantly decreased by vanadate (10^{-5} or 10^{-4} M) (Fig. 5A). In the presence of vanadate (10^{-5} M), Ca^{2+}-ATPase activity was not raised by anti-regucalcin monoclonal antibody (50 ng/ml) (Fig. 5B).

Effect of Anti-Regucalcin Antibody on Nuclear Ca^{2+}-ATPase Activity in the Presence of Calmodulin

The effect of calmodulin addition on Ca^{2+}-ATPase activity in rat liver nuclei is shown in Figure 6. Liver nuclear Ca^{2+}-ATPase activity was significantly raised by the addition of calmodulin (1, 2.5, and 5 μg/ml) into the enzyme reaction mixture (Fig. 6A). The effect of calmodulin (5 μg/ml) was significantly enhanced in the presence of anti-regucalcin monoclonal antibody (50 ng/ml) (Fig. 6B). The presence of trifluoperazine (TFP; 50 or 100 μM), an antagonist of calmodulin [Vincenzi, 1982], in the enzyme reaction mixture caused a remarkable reduction of nuclear Ca^{2+}-ATPase activity (Fig. 7A). The effect of anti-regucalcin monoclonal antibody (50 ng/ml) in elevating Ca^{2+}-ATPase activity was significantly prevented by the presence of TFP (20 μM).

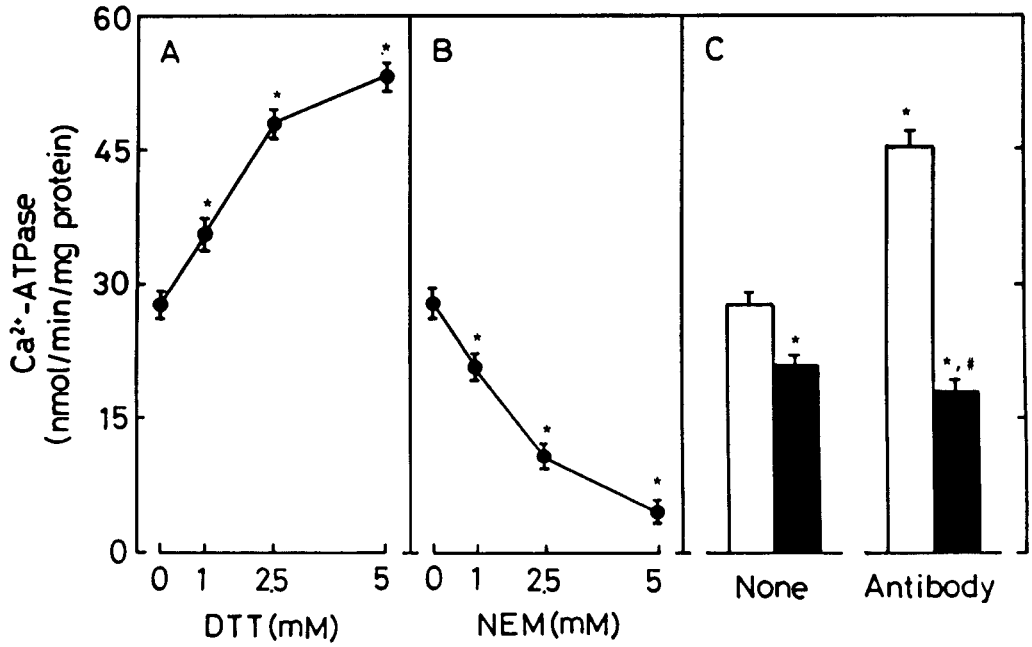

Fig. 4. Effect of dithiothreitol (DTT) or *N*-ethymaleimide (NEM) on Ca^{2+}-ATPase activity in rat liver nuclei. The enzyme reaction mixture contained either vehicle, DTT (1, 2.5, and 5 mM) (**A**), NEM (1, 2.5, and 5 mM) (**B**) or NEM (1 mM) in the absence or presence of anti-regucalcin monoclonal antibody (50 ng/ml) (**C**) with $CaCl_2$ (25 μM) addition. Each value is the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P <$ 0.01, compared with the control value without DTT or NEM addition. $\#P < 0.01$, compared with the value for antibody alone. White bars, control; black bars, NEM (1 mM).

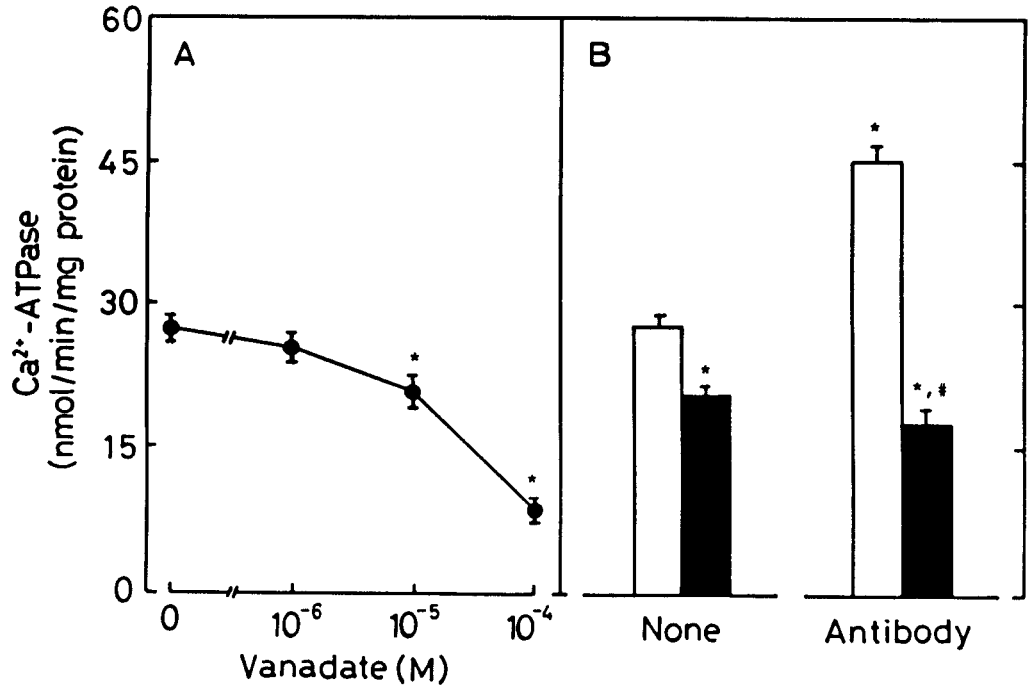

Fig. 5. Effect of vanadate on the anti-regucalcin monoclonal antibody-increased Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme reaction mixture contained either vehicle or vanadate (10^{-6}–10^{-4} M) in the presence of $CaCl_2$ (25 μM). **B:** The reaction mixture contained either vehicle, vanadate (10^{-5} M), anti-regucalcin monoclonal antibody (50 ng/ml), or vanadate (10^{-5} M) plus the antibody (50 ng/ml) with $CaCl_2$ (25 μM) addition. Each value is the the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P <$ 0.01, compared with the control value without vanadate addition. $\#P < 0.01$, compared with the value for antibody alone. White bars, control; black bars, vanadate (10^{-5} M).

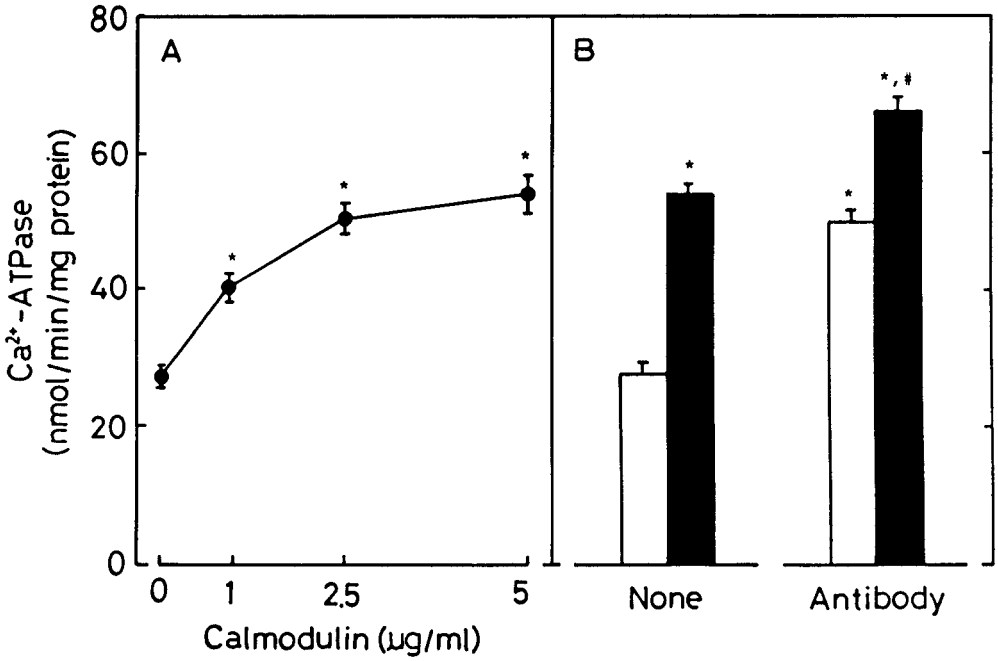

Fig. 6. Effect of calmodulin on the anti-regucalcin monoclonal antibody-increased Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme reaction mixture contained either vehicle or calmodulin (1, 2.5, and 5 μg/ml) in the presence of $CaCl_2$ (25 μM). **B:** The reaction mixture contained either vehicle, calmodulin (5 μg/ml), anti-regucalcin monoclonal antibody (50 ng/ml), or calmodulin (5 μg/ml) plus the antibody (50 ng/ml) in the presence of $CaCl_2$ (25 μM). Each value is the the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P < 0.01$, compared with the control value without calmodulin. $\#P < 0.01$, compared with the value for antibody alone. White bars, control; black bars, calmodulin.

DISCUSSION

Recently, it has become apparent that Ca^{2+} is involved in the control of key nuclear events [Csermely et al., 1995; Malviya and Rogue, 1998]. Free Ca^{2+} in the nuclei is regulated independently from free Ca^{2+} in the surrounding cytoplasm. Ca^{2+} transporting systems, allowing for an autonomous regulation of Ca^{2+} levels, have been identified on the nuclear envelope; a sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) corresponding to the SERCA26 isoform, which is driver as an ATP-dependent calcium uptake mechanism, has been located on the outer nuclear membrane in rat liver nuclei [Lanini et al., 1992]. The autonomous regulation of nuclear free Ca^{2+} has been shown to subserve specific functions. In particular, nuclear Ca^{2+} is involved in the regulation of gene expression [Malviya and Rogue, 1998; Hardingham et al., 1997].

The regulatory mechanism for Ca^{2+}-ATPase in liver nuclei has not been fully clarified. cAMP-dependent protein kinase has been shown to phosphorylate and activate Ca^{2+}-ATPase in rat liver nuclei [Rogue et al., 1998]. Previously, we reported the existence of an ATP-dependent Ca^{2+} transport system in rat liver nuclei with a Ca^{2+} electrode technique [Yamaguchi, 1992; Yamaguchi and Oishi, 1993; Oishi and Yamaguchi, 1994]. Phorbol 12-myristate 13-acetate has been shown to activate Ca^{2+}-ATPase in rat liver nuclei [Oishi and Yamaguchi, 1994].

A novel Ca^{2+}-binding protein, regucalcin, could inhibit Ca^{2+}-ATPase activity in rat liver nuclei. Moreover, the presence of anti-regucalcin monoclonal antibody caused a significant elevation of Ca^{2+}-ATPase activity in the nuclei, and this increase was completely abolished by the addition of exogenous regucalcin. These results indicate that Ca^{2+}-ATPase activity is suppressed by the endogenous regucalcin in liver nuclei. Regucalcin has been demonstrated to localize in rat liver nuclei [Omura and Yamaguchi, 1999]. The present finding suggests that regucalcin plays a role in the control of Ca^{2+}-ATPase activity in liver nuclei. Presumably, regucalcin inhibits an ATP-dependent Ca^{2+} uptake in liver nuclei. This remains to be elucidated.

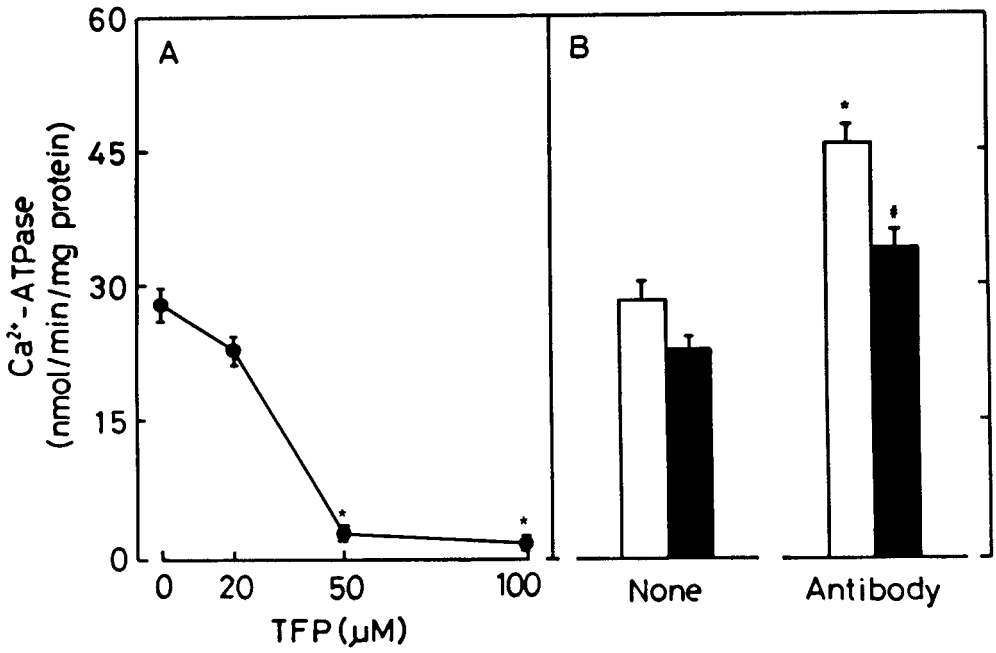

Fig. 7. Effect of trifluoperazine (TFP) on the anti-regucalcin monoclonal antibody-increased Ca^{2+}-ATPase activity in rat liver nuclei. **A:** The enzyme reaction mixture contained either vehicle or TFP (20, 50, and 100 μM) in the presence of $CaCl_2$ (25 mM). **B:** The reaction mixture contained either vehicle, TFP (20 μM), anti-regucalcin monoclonal antibody (50 ng/ml), or TFP (20 μM) plus the antibody (50 ng/ml) with $CaCl_2$ (25 μM) addition. Each value is the mean ± SEM of five separate experiments using different preparations of liver nuclei. $*P < 0.01$, compared with the control value without TFP. $\#P < 0.01$, compared with the value for antibody alone. White bars, control; black bars, TFP.

The characterization of endogenous regucalcin action in the regulation of liver nuclear Ca^{2+}-ATPase activity was examined. The effect of anti-regucalcin monoclonal antibody in elevating nuclear Ca^{2+}-ATPase activity was completely blocked by the presence of thapsigargin, NEM, or vanadate in the enzyme reaction mixture. Endogenous regucalcin in the nuclei may act on a thapsigargin-sensitive Ca^{2+}-ATPase, which is a sarcoendoplasmic reticulum Ca^{2+}-ATPase on the nuclear envelope. Regucalcin may act on the SH groups of the enzyme, and it may inhibit the phosphorylation of the enzyme by ATP.

The existence of calmodulin and Ca^{2+}/calmodulin-dependent protein kinase has been reported [Backs and Carafoli, 1987]. Liver nuclear Ca^{2+}-ATPase activity was significantly raised by the addition of calmodulin into the enzyme reaction mixture. The presence of TFP, an antagonist of calmodulin [Vincenzi, 1982], caused an appreciable reduction of nuclear Ca^{2+}-ATPase activity. These results suggest that calmodulin, which is present in the nuclei, activates Ca^{2+}-ATPase. The effect of anti-regucalcin monoclonal antibody in elevating nuclear Ca^{2+}-ATPase activity was significantly enhanced by the addition of calmodulin, and the antibody's effect was prevented by TFP. Regucalcin has been shown to bind to calmodulin [Omura and Yamaguchi, 1998]. Presumably, regucalcin can antagonize in the regulation of Ca^{2+}-ATPase activity in rat liver nuclei.

Nuclear Ca^{2+} may be involved in the regulation of gene expression [Malviya and Rogue, 1998]. A Ca^{2+}-binding protein, regucalcin, has been shown to inhibit Ca^{2+}-dependent protein kinase and protein phosphatase activities in rat liver nuclei [Katsumata and Yamaguchi, 1998; Omura and Yamaguchi, 1999], and regucalcin could inhibit DNA and RNA syntheses in the nuclei with a proliferative cells of regenerating rat liver [Yamaguchi and Kanayama, 1996; Yamaguchi and Ueoka, 1997]. The present finding, i.e., that regucalcin can inhibit Ca^{2+}-ATPase activity in liver nuclei, may further support the view that regucalcin plays an inhibitory role in the regulation of nuclear functions.

In conclusion, it has been demonstrated that endogenous regucalcin has an inhibitory effect on Ca^{2+}-ATPase activity in rat liver nuclei.

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