Journal of Cellular Biochemistry 71:569–576 (1998)

Inhibitory Effect of Calcium-Binding Protein Regucalcin on Protein Kinase Activity in the Nuclei of Regenerating Rat Liver

Toru Katsumata and Masayoshi Yamaguchi*

Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, Shizuoka City 422-8526, Japan

Abstract The effect of Ca^{2+}-binding protein regucalcin on protein kinase activity in the nuclei of normal and regenerating rat livers was investigated. Protein kinase activity in the nuclei isolated from normal rat liver was significantly increased by addition of Ca^{2+} (500 μM) and calmodulin (10 μg/ml) in the enzyme reaction mixture. Nuclear protein kinase activity was significantly decreased in the presence of EGTA (1.0 mM), trifluoperazine (TFP; 20 μM), dibucaine (10^{-4} M), or staurosporine (10^{-7} M), indicating that Ca^{2+}-dependent protein kinases are present in the nuclei. Protein kinase activity was significantly elevated in the liver nuclei obtained at 6 to 48 h after a partial hepatectomy. Hepatectomy-increased nuclear protein kinase activity was significantly decreased in the presence of EGTA (1.0 mM), TFP (20 μM), or staurosporine (10^{-7} M) in the enzyme reaction mixture. The presence of regucalcin (0.1–0.5 μM) caused a significant decrease in protein kinase activity in the nuclei obtained from normal and regenerating rat livers. Meanwhile, the nuclear protein kinase activity from normal and regenerating livers was significantly elevated in the presence of anti-regucalcin monoclonal antibody (50–200 ng/ml). The present study suggests that regucalcin plays a role in the regulation of protein kinase activity in the nuclei of proliferative liver cells. J. Cell. Biochem. 71:569–576, 1998.

Key words: regucalcin; calmodulin; protein kinase; calcium-binding protein; liver nuclei; regenerating rat liver

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 due to hormonal stimulation [Cheung, 1980; Nishizuka, 1986; Heizmann and Hunziker, 1991; Kraus-Friedman and Feng, 1996]. A novel Ca^{2+}-binding protein, regucalcin, has been demonstrated 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]. Regucalcin may play a role as a regulatory protein in the Ca^{2+}-signaling process.

The regucalcin gene is localized on rat chromosome Xq11. 1-12 proximal end, and regucalcin messenger ribonucleic acid (mRNA) is mainly expressed in liver [Shimokawa and Yamaguchi, 1993b; Shimokawa et al., 1995; Murata and Yamaguchi, 1998]. The expression of hepatic regucalcin mRNA is clearly stimulated by the administration of calcium chloride to rats; the expression may be mediated through Ca^{2+}/calmodulin [Shimokawa and Yamaguchi, 1993a; Murata and Yamaguchi, 1998]. Moreover, 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].

There has been growing evidence that Ca^{2+} plays a role in liver nuclear function [Jones et al., 1989; Backs et al., 1990; Allbritton et al., 1994]. The existence of an ATP-stimulated Ca^{2+} seqestration system in rat liver nuclei that generates a net increase in nuclear matrix free Ca^{2+} concentration has been reported [Nicotera et al., 1989; Yamaguchi and Oishi, 1993]. Calmodulin exists in rat liver nuclei [Backs and

*Correspondence to: Masayoshi Yamaguchi, Ph.D., Laboratory of Endocrinology and Molecular Metabolism, Graduate School of Nutritional Sciences, University of Shizuoka, 52-1 Yada, Shizuoka City 422-8526, Japan.

Received 8 June 1998; Accepted 10 July 1998

Carafoli, 1987], and the protein stimulates deoxyribonucleic acid (DNA) synthesis by liver cells [Backs et al., 1990]. 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, has not been fully clarified.

It has been reported that Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C exist in liver nuclei [Backs and Carafoli, 1987; Block et al., 1992]. The present study, therefore, was undertaken to clarify the effect of regucalcin on Ca^{2+}-dependent protein kinase activity in the nuclei isolated from normal and regenerating rat livers. It was found that regucalcin can inhibit nuclear protein kinase activity in regenerating rat liver.

MATERIALS AND METHODS

Chemicals

Calmodulin [52,000 units (U)/mg protein from bovine brain], calcineurin (from bovine brain), adenosine triphosphate (ATP), etylene glycol bis(2-aminothylether)N,N,N′,N′-tetraacetic acid (EGTA, pH 7.0), trifluoperazine, dibucaine, staurosporine, and nonimmune IgG were obtained from Sigma Chemical Co. (St. Louis, MO). [γ-^{32}P]ATP (111 TBq/mmol) was purchased from New England Nuclear (Boston, MA). NHS-LC-Biotin was obtained from Pierce (Rockford, IL). Streptavidin-peroxidase conjugate was obtained from Tago, Inc. (Burlingame, CA). Calcium chloride and all other chemicals were reagent grade from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All reagents used were dissolved in distilled water then passed through an ion-exchange resin to remove metal ions.

Animals and Hepatectomy

Male Wistar rats, weighing 100–120 g, were used. They were obtained commercially from the Japan SLC Inc. (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. Partial hepatectomy was carried out by excision of two-thirds of the liver (the medium and left lateral lobes), as described by Higgins and Anderson [1931]. Rats were anesthetized with ether before surgery. In sham-operated rats, an incision was made, and the liver was manipulated, but not ligated. Surgery was routinely performed at 10:00–12:00.

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 approximately 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 including 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 potassium phosphate, 25 mM Hepes, 4 mM $MgCl_2$, pH 7.0) by hand homogenization. Assay of marker enzymes, as reported previously [Yamaguchi and Kanayama, 1996], showed that there was less than 5% contamination by microsomes, plasma membranes, or mitochondria.

DNA content in the nuclei was determined using the diphenylamine reaction [Burton, 1956]. Nuclear protein concentration was measured by the method of Lowry et al. using bovine albumin as standard [1951]. The amount of calcium in the nuclei was determined by atomic absorption spectrophotometry after digestion with nitric acid [Yamaguchi et al., 1975]. Calcium content was expressed microgram per gram of nuclear protein.

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 (weight:volume) in Tris-HCl buffer (pH 7.4); 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].

Anti-Regucalcin Antibody

Anti-regucalcin antibody was a monoclonal antibody raised against regucalcin prepared using standard methods [Omura and Yamaguchi, 1989]. 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 a protein A-agarose column (Sigma).

Assay of Protein Kinase Activity

Ca^{2+}/calmodulin-dependent protein kinase activity was measured at 30°C in an incubation volume of 500 µl as described by Connelly et al. [1987]. Phosphorylation of the substrate was performed in a reaction mixture containing liver nuclei (500–700 µg protein/ml; 400–700 µg DNA/ml), 50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 0.2 mM EGTA, 20 µM ATP, 5.55 TBq [γ-^{32}p] ATP in the presence or absence of 0.5 mM $CaCl_2$ and calmodulin (10 µg/ml). In separate experiments, the enzyme reaction mixture contained regucalcin (0.1–0.5 µM) and other reagents without Ca^{2+} and calmodulin addition. The phosphorylation reaction was terminated by the addition of 2 ml of ice-cold 5% trichloroacetic acid containing 10 mM H_3PO_4. The radioactivity retained on GF/B glass fiber filters after filtration was determined by counting the dried filters in 2 ml of scintillation fluid. Ca^{2+}/calmodulin-dependent protein kinase activity was determined after substracting the incorporation in the absence of Ca^{2+} and calmodulin. The enzyme activity was expressed as the radioactivity (dpm) of [^{32}P]phosphate phosphorylated per milligram of the nuclear protein during incubation (10 min).

Statistical Analysis

Data were expressed as the mean ± SEM. Statistical differences were analyzed using Student's *t*-test. A *P* value of less than .05 was considered to indicate a statistically significant difference.

RESULTS

Ca^{2+}-Dependent Protein Kinase Activity in Rat Liver Nuclei

The phosphorylation of the nuclear protein using isolated rat liver nuclei was examined. The phosphorylation of nuclear protein rapidly occurred by incubation at an earlier time (2.5 min) (Fig. 1). Phosphorylation was not increased by further increasing incubation times (2.5–30 min). The result indicates that protein kinase activity exists in liver nuclei isolated from normal rats. This activity was further increased by the addition of calcium chloride (Ca^{2+}; 500 µM) plus calmodulin (10 µg/ml) to the reaction mixture (Fig. 1). This demonstrates that Ca^{2+}/calmodulin-dependent protein kinase is present in the liver nuclei.

The effect of EGTA or trifluoperazine (TFP) on protein kinase activity in the liver nuclei isolated from normal rats is shown in Figure 2. Nuclear protein kinase activity was significantly decreased in the presence of EGTA (1.0 mM), a chelator of Ca^{2+}, or TFP (20 µM), an antagonist of Ca^{2+}/calmodulin action, at 5 and 10 min of the enzyme reaction. Endogenous Ca^{2+}/calmodulin in the liver nuclei seemed to be involved in the phosphorylation. Calcium content in isolated rat liver nuclei was 351 ± 18 (µg/g protein; mean ± SEM of five rats). Liver

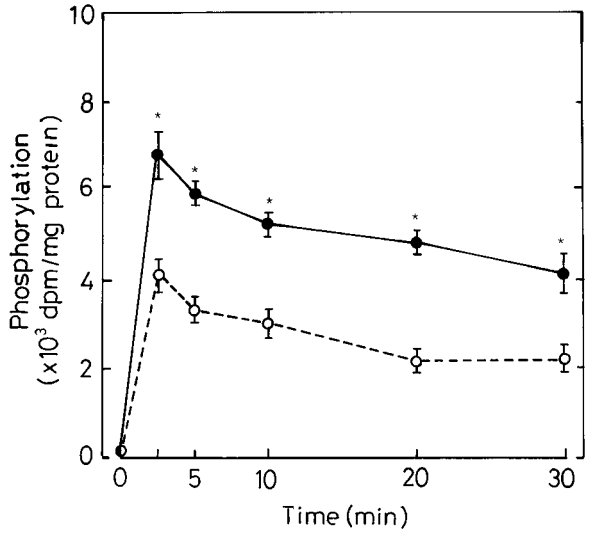

Fig. 1. Alteration in protein kinase activity in the nuclei isolated from normal rat liver without or with Ca^{2+}/calmodulin addition. The enzyme reaction mixture was incubated for 2.5–30 min in the absence or presence of $CaCl_2$ (500 μM) and calmodulin (10 μg/ml). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the value without Ca^{2+}/calmodulin addition. Open circles: Control; solid circles: Ca^{2+}/calmodulin addition.

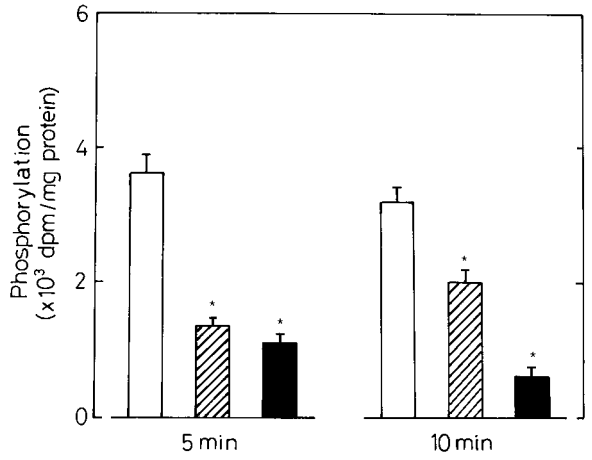

Fig. 2. Effect of Ca^{2+}/calmodulin antagonist on protein kinase activity in the nuclei isolated from normal rat liver. The enzyme reaction mixture was incubated for 5 or 10 min in the absence or presence of EGTA (1 mM) or trifluoperazine (TFP; 20 μM). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value without EGTA or TFP addition. Open bars: control, hatched bars: EGTA, solid bars: TFP.

nuclei contained a considerable amount of calcium.

The effect of staurosporine or dibucaine on protein kinase activity in isolated rat liver nuclei is shown in Figure 3. The enzyme reaction

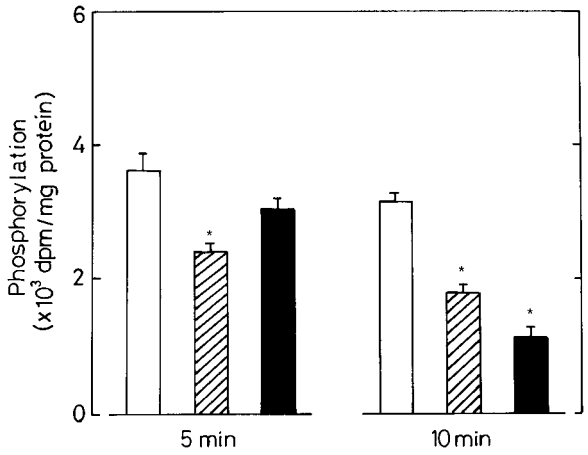

Fig. 3. Effect of staurosporine or dibucaine on protein kinase activity in the nuclei isolated from normal rat liver. The enzyme reaction mixture was incubated for 5 or 10 min in the absence or presence of staurosporine (10^{-7} M) or dibucaine (10^{-4} M). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value without staurosporine or dibucaine. Open bars: control; hatched bars: staurosporine; solid bars: dibucaine.

mixture was incubated for 5 and 10 min in the presence of staurosporine (10^{-7} M), an inhibitor of protein kinase C, or dibucaine (10^{-4} M), an inhibitor of Ca^{2+}/calmodulin-dependent protein kinase. Nuclear protein kinase activity was significantly decreased in the presence of staurosporine or dibucaine after a 10-min incubation.

Alteration in Protein Kinase Activity in the Nuclei Isolated From Regenerating Rat Liver

The alteration in protein kinase activity in the nuclei isolated from regenerating rat liver is shown in Figure 4. Partial hepatectomy was carried out by excision of two-thirds of the liver. Also, rats were sham-operated. Nuclear protein kinase activity was significantly enhanced in regenerating liver at 6 h after a partial hepatectomy. This increase was also seen at 24 and 48 h after a partial hepatectomy. At 72 h after a partial hepatectomy, however, the nuclear protein kinase activity was restored to the control levels of sham-operated rats. Meanwhile, protein kinase activity in the liver nuclei isolated from sham-operated rats was not significantly altered by the operation.

The effect of EGTA, TFP, and staurosporine on protein kinase activity in the nuclei isolated from regenerating rat liver is shown in Figure 5. Rats were sacrificed by bleeding at 24 and 48 h after a partial hepatectomy. Protein kinase

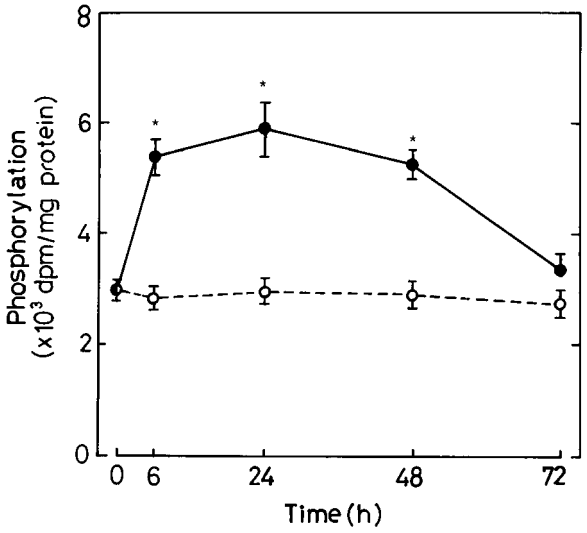

Fig. 4. Alteration in protein kinase activity in the nuclei isolated from regenerating rat liver. Rats were partially hepatectomized, and 6, 24, 48, and 72 h later the animals were sacrificed by bleeding. The enzyme reaction mixture was incubated for 10 min without Ca^{2+}/calmodulin addition. Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value obtained from sham-operated rats. Open circles: sham-operated (control) rats; solid circles: partial hepatectomized rats.

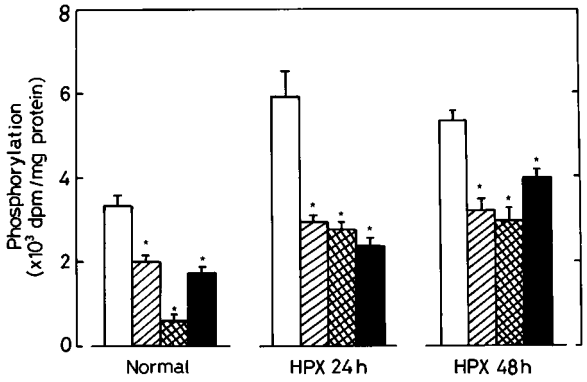

Fig. 5. Effect of EGTA, trifluoperazine, and staurosporine on protein kinase activity in the nuclei isolated from regenerating rat liver. Rats were partially hepatectomized, and 24 or 48 h later the animals were sacrificed by bleeding. The enzyme reaction mixture was incubated for 10 min without or with EGTA (1 mM), trifuoperazine (TFP; 20 μM), or staurosporine (10^{-7} M). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value without inhibitor addition. Open bars: control; hatched bars: EGTA; cross-hatched bars: TFP; solid bars: staurosporine.

activity in the nuclei obtained at 24 or 48 h after a partial hepatectomy was significantly decreased in the presence of EGTA (1.0 mM), TFP (20 μM), or staurosporine (10^{-7} M) in the enzyme reaction mixture. Also, these inhibitors

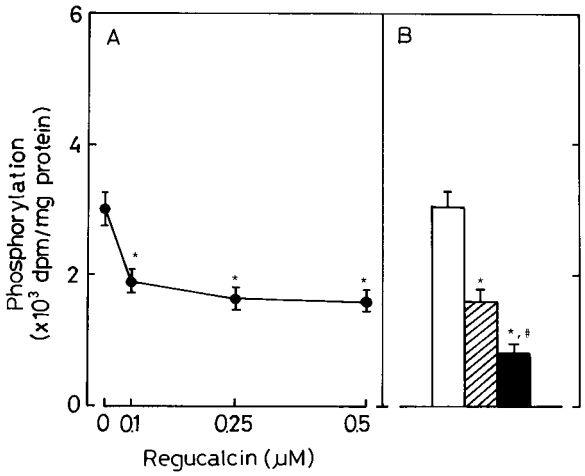

Fig. 6. Effect of Ca^{2+}-binding protein regucalcin on protein kinase activity in the nuclei isolated from normal rat liver. The enzyme reaction mixture was incubated for 10 min. **A:** The reaction mixture contained either vehicle or regucalcin (RC; 0.1, 0.25, and 0.5 μM). **B:** The reaction mixture contained either vehicle (control), EGTA (1 mM), or EGTA (1 mM) plus RC (0.25 μM). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value without EGTA or RC addition. #$P < .01$ as compared with the value of EGTA addition. Open bars: control; hatched bars: EGTA; solid bars: EGTA plus RC.

had an effect on protein kinase activity in the nuclei isolated from normal rat liver.

Effect of Regucalcin on Protein Kinase Activity in Isolated Rat Liver Nuclei

The effect of regucalcin, a Ca^{2+}-binding protein, on protein kinase activity in the nuclei isolated from normal rat liver is shown in Figure 6. The presence of regucalcin (0.1, 0.25, and 0.5 μM) in the enzyme reaction mixture caused a significant decrease in nuclear protein kinase activity. The inhibitory effect of regucalcin was saturated at the concentration of 0.25 and 0.5 μM (Fig. 6A). In the presence of EGTA (1.0 mM), regucalcin (0.25 μM) had a significant effect on nuclear protein kinase activity (Fig. 6B).

The effect of regucalcin on protein kinase activity in the nuclei isolated from regenerating rat liver is shown in Figure 7. Rats were sacrificed by bleeding at 24 h after a partial hepatectomy. Nuclear protein kinase activity was significantly decreased in the presence of regucalcin (0.1 μM) in the enzyme reaction mixture (Fig. 6A). The inhibition was saturated at the concentration of 0.25 μM regucalcin. This effect of regucalcin (0.25 μM) on the enzyme

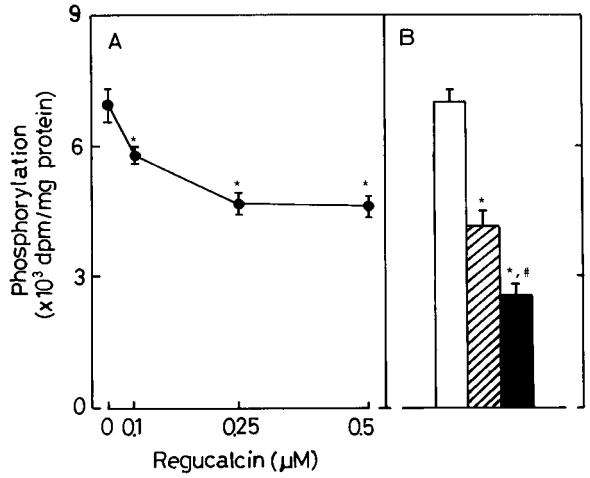

Fig. 7. Effect of Ca^{2+}-binding protein regucalcin on protein kinase activity in the nuclei isolated from regenerating rat liver. Rats were partially hepatectomized, and 24 h later the animals were sacrificed by bleeding. The enzyme reaction mixture was incubated for 10 min. **A:** The reaction mixture contained either vehicle or regucalcin (0.1, 0.25, and 0.5 μM). **B:** The reaction mixture contained either vehicle (control), EGTA (1 mM), or EGTA (1 mM) plus regucalcin (0.25 μM). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value without EGTA or regucalcin addition. #$P < .01$ as compared with the value of EGTA addition. Open bars: control; hatched bars: EGTA; solid bars: EGTA plus regucalcin.

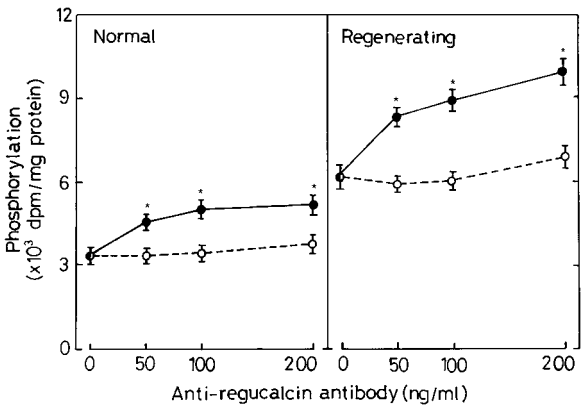

Fig. 8. Effect of anti-regucalcin antibody on protein kinase activity in the nuclei isolated from normal rat liver or regenerating rat liver. Rats were sham-operated or partially hepatectomized, and 24 h later the animals were sacrificed by bleeding. The enzyme reaction mixture was incubated for 10 min in the presence of either vehicle, nonimmune IgG (50–200 ng/ml), or anti-regucalcin antibody (50–200 ng/ml). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value with nonimmune IgG addition. Open circles: control (nonimmune IgG); solid circles: anti-regucalcin antibody.

activity was also seen in the presence of EGTA (1.0 mM) (Fig. 7B).

The effect of anti-regucalcin monoclonal antibody on protein kinase activity in isolated rat liver nuclei is shown in Figure 8. The presence of anti-regucalcin monoclonal antibody (50–200 ng/ml) in the enzyme reaction mixture caused a significant increase in the nuclei isolated from normal rat liver, whereas the enzyme activity was not significantly altered by the addition of nonimmune IgG (50, 100, and 200 ng/ml). Meanwhile, protein kinase activity in the liver nuclei obtained at 24 h after a partial hepatectomy was significantly elevated in the presence of anti-regucalcin monoclonal antibody (50–200 ng/ml); such effect was not seen in the presence of nonimmuned IgG (50–200 ng/ml). The net increase in the enzyme activity caused by anti-regucalcin antibody was about twofold in the nuclei of regenerating liver as compared with that of normal rat liver nuclei.

The effect of anti-regucalcin monoclonal antibody (200 ng/ml) to enhance the nuclear protein kinase activity in normal rat liver and regenerating rat liver was completely abolished by the addition of regucalcin (0.25 μM) in the enzyme reaction mixture (Fig. 9).

DISCUSSION

The present study demonstrates that phosphorylation in nuclei isolated from normal rat liver is stimulated by the addition of Ca^{2+}/calmodulin, indicating the existence of Ca^{2+}/calmodulin-dependent protein kinase in the nuclei. Moreover, phosphorylation in liver nuclei was decreased by the addition of EGTA, trifluoperazine (TFP), dibucaine, or staurosporine in the reaction mixture. TFP and dibucaine are an antagonist of calmodulin [Vincenzi, 1982], and staurosporine is an inhibitor of protein kinase C [Tamaoki et al., 1986]. Thus, protein kinases, which are related to Ca^{2+} signaling, are present in the nuclei isolated from rat liver.

Protein kinase activity was enhanced in the nuclei of regenerating rat liver after a partial hepatectomy, indicating that nuclear phosphorylation is stimulated in proliferative liver cells. This enhancement was clearly inhibited in the presence of EGTA, TFP, or staurosporine in the reaction mixture. Presumably, protein kinases, which are involved in Ca^{2+} signaling, participate in the enhancement of nuclear phosphorylation in regenerating liver.

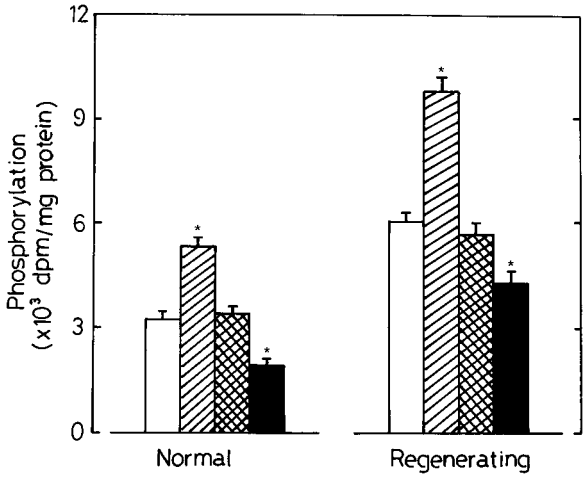

Fig. 9. Effect of regucalcin addition on anti-regucalcin antibody-increased protein kinase activity in the nuclei isolated from normal rat liver or regenerating rat liver. Rats were sham-operated or partially hepatectomized, and 24 h later the animals were sacrificed by bleeding. The enzyme reaction mixture was incubated for 10 min in the presence of either vehicle, anti-regucalcin antibody (ARA; 200 ng/ml), ARA (200 ng/ml) plus regucalcin (0.25 μM) or regucalcin (0.25 μM). Each value is the mean ± SEM of five rats with different experiments. *$P < .01$ as compared with the control value. Open bars: control; hatched bars: anti-regucalcin antibody; cross-hatched bars: anti-regucalcin antibody plus regucalcin; solid bars: regucalcin.

The expression of Ca^{2+}-binding protein regucalcin mRNA in the liver has been demonstrated to be stimulated in regenerating rat liver after a partial hepatectomy [Yamaguchi and Kanayama, 1995]. Regucalcin can inhibit DNA and RNA syntheses in the nuclei isolated from regenerating rat liver [Yamaguchi and Kanayama, 1996; Yamaguchi and Ueoka, 1997]. These observations suggest that regucalcin plays a role in the regulation of the proliferation of liver cells after a partial hepatectomy. The regulatory mechanism of regucalcin in the liver nuclei, however, is not fully clarified. The present finding that regucalcin can inhibit protein kinase activity in the nuclei of regenerating rat liver may provide a possible mechanism in the regulation of proliferation of liver cells.

The effect of regucalcin to inhibit protein kinase activity in the nuclei isolated from normal and regenerating rat livers was seen in the presence of a physiological concentration of regucalcin [Yamaguchi and Isogai, 1993]. The regucalcin effect was also revealed in the presence of EGTA, a chelator of Ca^{2+}, although the chelator caused a significant decrease in the nuclear protein kinase activity. Regucalcin may have an inhibitory effect on Ca^{2+} signaling–dependent protein kinase including Ca^{2+}/calmodulin-dependent protein kinase and protein kinase C in the nuclei isolated from normal and regenerating rat livers. In addition, regucalcin may be able to inhibit other protein kinases besides Ca^{2+} signaling–dependent protein kinases in liver nuclei.

The phosphorylation in the nuclei isolated from normal and regenerating rat livers was significantly stimulated in the presence of anti-regucalcin antibody in the reaction mixture. Such stimulation was completely abolished by the addition of exogenous regucalcin. These results suggest that endogenous regucalcin in the nuclei is involved in the regulation of protein kinase activity. The net increase in protein kinase activity caused by the addition of anti-regucalcin antibody was about twofold in the nuclei isolated from regenerating rat liver as compared with that of normal rat liver. This increase may result from an elevation of endogenous regucalcin in regenerating liver nuclei, if hepatic regucalcin mRNA expression is raised after a partial hepatectomy [Yamaguchi and Yamanaka, 1995]. At present, whether regucalcin in liver cytoplasm is transported into the nuclear matrix is unknown. It appears, however, that regucalcin may translocate to the nuclei in regenerating liver. This remains to be elucidated.

There has been growing evidence that Ca^{2+} plays a role in the regulation of liver nuclear function [Block et al., 1992; Csermely et al., 1995]. This regulatory mechanism has not been fully resolved. Ca^{2+}-binding protein regucalcin has an inhibitory effect on DNA and RNA syntheses in liver nuclei [Yamaguchi and Kanayama, 1996; Yamaguchi and Ueoka, 1997], and it can also inhibit hepatic nuclear protein kinase activity. Presumably, regucalcin plays a role as a regulatory protein in liver nuclear function.

In conclusion, it has been demonstrated that regucalcin can inhibit protein kinase activity in the nuclei of normal and regenerating rat livers.

REFERENCES

Allbritton NL, Oancea E, Kuhn MA, Meyer T (1994): Source of nuclear calcium signals. Proc Natl Acad Sci USA 91: 12458–12462.

Backs O, Carafoli E (1987): Calmodulin and calmodulin-binding proteins in liver cell nuclei. J Biol Chem 262: 10786–10790.

Backs O, Lanini L, Serratosa J, Coll MJ, Bastors R, Aline R, Ruis E, Carafoli E (1990): Calmodulin-binding proteins in the nuclei of quiescent and proliferatively activated rat liver cells. J Biol Chem 265:18595–18600.

Block C, Freyermuth S, Beyersmann D, Malviya AN (1992): Role of cadmium in activating nuclear protein kinase C and the enzyme binding to nuclear protein. J Biol Chem 267:19824–19828.

Burton A (1956): Study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323.

Cheung WY (1980): Calmodulin plays a pivotal role in cellular regulation. Science 202:19–27.

Connelly PA, Sisk RB, Schulman H, Garrison JC (1987): Evidence for the activation of the multifunction Ca^{2+}/calmodulin-dependent protein kinase in response to hormones that increase infracellular Ca^{2+}. J Biol Chem 262:10154–10163.

Csermely P, Schnaider T, Szanto I (1995): Signalling and transport through the nuclear membrane. Biochim Biophys Acta 1241:425–452.

Heizman CW, Hunziker W (1991): Intracellular calcium-binding proteins: more sites than in sights. Trends Biochem Sci 16:98–103.

Higgins GH, Anderson RM (1931): Experimental pathology of the liver. Restoration of the liver of the white rat following partial surgical removal. Arch Pathol 12:186–202.

Jones DP, McConkey DJ, Nicotera P, Orrenius S (1989): Calcium activated DNA fragmentation in rat liver nuclei. J Biol Chem 264:6398–6403.

Kraus-Friedman N, Feng L (1996): The role of intracellular Ca^{2+} in the regulation of gluconeogenesis. Metabolism 42:389–403.

Lowry Oh, Rosebrough MJ, Farr Al, Randall FJ (1951): Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–273.

Mori S, Yamaguchi M (1990): Hepatic calcium-binding protein regucalcin decreases Ca^{2+}/calmodulin-dependent protein kinase activity in rat liver cytosol. Chem Pharm Bull 38:2216–2218.

Murata T, Yamaguchi M (1998): Ca^{2+} administration stimulates the binding of AP-1 factor to the 5′-flanking region of the rat gene for the Ca^{2+}-binding protein regucalcin. Biochem J 329:157–163.

Nicotera P, McConkey DJ, Jones DP, Orrenius S (1989): ATP stimulates Ca^{2+} uptake and increases the free Ca^{2+} concentration in isolated rat liver nuclei. Proc Natl Acad Sci USA 86:453–457.

Nishizuka WY (1986): Studies and perspectives of protein kinases C. Science 233:305–312.

Omura M, Yamaguchi M (1998): Effect of anti-regucalcin antibody on neutral phosphatase activity in rat liver cytosol: involvement of endogenous regucalcin. Mol Cell Biochem (in press).

Shimokawa N, Yamaguchi M (1993a): Expression of hepatic calcium-binding protein regucalcin mRNA is mediated through Ca^{2+}/calmodulin in rat liver. FEBS Lett 316:79–84.

Shimokawa N, Yamaguchi M (1993b): Molecular cloning and sequencing of the cDNA coding for a calcium-binding protein regucalcin from rat liver. FEBS Lett 327:251–255.

Shimokawa N, Matsuda Y, Yamaguchi M (1995): Genomic cloning and chromosomal assignment of rat regucalcin gene. Mol Cell Biochem 151:157–163.

Tamaoki T, Nomoto H, Takahashi I, Kato Y, Morimoto M, Tomita E (1986): Staurosporin, a potent inhibitor of phospholipid/Ca^{2+}-dependent protein kinase. Biochem Biophys Res Commun 135:397–402.

Vincenzi FF (1982): Pharmacology of calmodulin antagonism. In Godfraind T, Albertini A, Paoletti R (eds): "Calcium Modulators."Amsterdam: Elsevier Biomedical Press, pp 67–80.

Yamaguchi M, Isogai M (1993): Tissue concentration of calcium-binding protein regucalcin in rats by enzyme-linked immunoadsorbent assay. Mol Cell Biochem 122: 65–68.

Yamaguchi M, Kanayama Y (1995): Enhanced expression of calcium-binding protein regucalcin mRNA in regenerating rat liver. J Cell Biochem 57:185–190.

Yamaguchi M, Kanayama Y (1996): Calcium-binding protein regucalcin inhibits deoxyribonucleic acid synthesis in the nuclei of regenerating rat liver. Mol Cell Biochem 162:121–126.

Yamaguchi M, Mori S (1990): Inhibitory effect of calcium-binding protein regucalcin on protein kinase C activity in rat liver cytosol. Biochem Med Metab Biol 43:140–146.

Yamaguchi M, Oishi K (1993): Characterization of Ca^{2+}-stimulated adenosine 5′-triphosphatase and Ca^{2+} sequestering in rat liver nuclei. Mol Cell Biochem 125:43–49.

Yamaguchi M, Ueoka S (1997): Inhibitory effect of calcium-binding protein regucalcin on ribonucleic acid synthesis in isolated rat liver nuclei. Mol Cell Biochem 173:169–175.

Yamaguchi M, Yamamoto T (1978): Purification of calcium binding substance from soluble fraction of normal rat liver. Chem Pharm Bull 26:1915–1918.

Yamaguchi M, Takei Y, Yamamoto T (1975): Effect of thyrocalcitoninin liver of intact and thyroparathyroidectomized rats. Endocrinology 96:1004–1008.