Molecular and Cellular Biochemistry **113**: 63–70, 1992.
© 1992 *Kluwer Academic Publishers. Printed in the Netherlands.*

Effect of calcium-binding protein regucalcin on Ca^{2+} transport system in rat liver nuclei: stimulation of Ca^{2+} release

Masayoshi Yamaguchi
Laboratory of Metabolism and Endocrinology, Graduate School of Nutritional Sciences, University of Shizuoka, 395 Yada, Shizuoka City 422, Japan

Received 30 September 1991; accepted 13 February 1992

Abstract

The effect of regucalcin, a calcium-binding protein isolated from rat liver cytosol, on Ca^{2+} transport in rat liver nuclei was investigated. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode. Ca^{2+} uptake increased dependent on adenosine triphosphate (ATP; 0.5–2.0 mM), while the uptake was negligible in the presence of 2 mM ADP or AMP. Regucalcin (0.5–2.0 μM) had no effect on Ca^{2+} uptake following addition of 2.0 mM ATP. Meanwhile, Ca^{2+}, which accumulated in the nuclei during 10 min after ATP addition, was significantly released by the addition of regucalcin. This release was dose-dependent (0.1–2.0 μM). Vanadate (100 μM) and guanosine triphosphate (100 μM) did not cause a significant release of Ca^{2+} from the nuclei. Trifluoroperazine (TFP; 50 μM), an antagonist of calmodulin, significantly increased Ca^{2+} release from the nuclei. The presence of regucalcin (0.5 μM) further enhanced the TFP effect. These results indicate that regucalcin stimulates Ca^{2+} release from liver nuclei, and that the effect is not influenced by calmodulin antagonist. The finding suggests that regucalcin can regulate the Ca^{2+} transport system in rat liver nuclei. (Mol Cell Biochem **113:** 63–70, 1992)

Key words: regucalcin, calcium-binding protein, calcium transport, rat liver nuclei

Introduction

It is well known that Ca^{2+} plays an important role in the regulation of many cell functions [1]. The role of Ca^{2+} in liver metabolism has been demonstrated in recent investigations [2, 3]. Liver metabolism is regulated by the increase of Ca^{2+} in the cytosol of liver cells due to hormonal stimulation. There are growing evidences that Ca^{2+} plays a role in liver nuclear function [4–9]. Calmodulin, a calcium-binding protein which can amplify Ca^{2+} effect [10], exists in rat liver nuclei [4]. The existence of an ATP-stimulated Ca^{2+} sequestration system in rat liver nuclei that requires calmodulin and generates a net increase in nuclear matrix free Ca^{2+} concentration has been reported [5]. Calmodulin stimulates DNA synthesis by liver cells [6]. Recently, it has been reported that isolated rat liver nuclei contain a DNA endonuclease activity dependent upon Ca^{2+} in the submicromolar range, and that Ca^{2+} results in ex-

Address for offprints: M. Yamaguchi, Laboratory of Metabolism and Endocrinology, Graduate School of Nutritional Sciences, University of Shizuoka, 395 Yada, Shizuoka City 422, Japan

tensive DNA hydrolysis [11]. Thus, Ca^{2+} and calmodulin may regulate liver nuclear function.

On the other hand, a novel calcium-binding protein (regucalcin), which differs from calmodulin [10] and other calcium-binding proteins (caligulin [12], calregulin [13] and calreticulin [14]) is distributed in rat liver cytosol [15, 16]. Regucalcin may play a cell physiological role different from those of other calcium-binding proteins in liver cell functions; regucalcin can reverse the effect of Ca^{2+} of many enzymes in liver cells [17–19]. Regucalcin may play a role as a regulatory protein for Ca^{2+} effects in liver cells. More recently, it has been found that regucalcin can inhibit the Ca^{2+}-activated DNA fragmentation due to binding the metal, suggesting a role in the regulation of liver nuclear functions [20]. Furthermore, the present investigation was undertaken to clarify whether regucalcin has an effect on Ca^{2+} transport system in isolated rat liver nuclei. It was found that regucalcin can stimulate release of Ca^{2+} accumulated in the nuclei.

Materials and methods

Chemicals

Adenosine 5′-triphosphate (ATP), guanosine 5′-triphosphate (GTP), ethyleneglycol-bis-(aminoethylether)N,N′-tetraacetic acid (EGTA), and trifluoroperazine were obtained from Sigma Chemical Co. (St. Louis, MO, USA). $CaCl_2 \cdot 2H_2O$ 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.

Isolation of regucalcin

Male Wistar rats, weighing 100–120 g, were used. They were obtained commercially from 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. After one week on this diet animals were killed by bleeding. 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 in Tris-HCl buffer (pH 7.4) and the homogenate was spun at 5500 × g in a refrigerated centrifuge for 10 min and the supernatant was spun at 105000 × g for 60 min. The resulting supernatant was heated at 60° C for 10 min and recentrifuged at 38000 × 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 [15]. Protein concentration was determined by the method of Lowry et al. [21] using albumin as a standard.

Isolation of nuclei

Liver nuclei were isolated by the procedure of Jones et al. [11] with a minor modification. Rats were killed by cardiac puncture, and the liver was 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 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 were added to each of four tubes containing 12 ml of TKM containing 2.3 M sucrose solution. 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 37000 × 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 (glucose-6-phosphatase, 5′-nucleotidase, succinate dehydrogenase) showed that there was less than about 5% contamination by microsomes, plasma membranes, or mitochondria. DNA content in the nuclei was determined with the diphenylamine reaction [22].

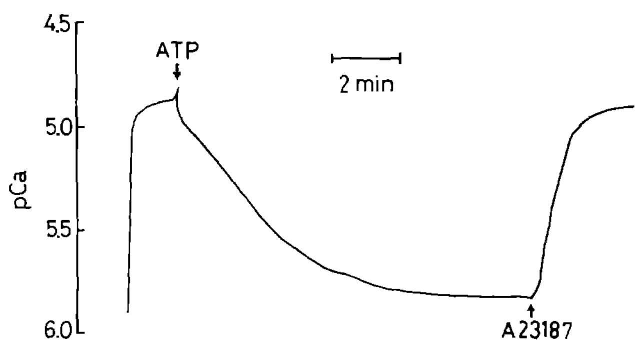

Fig. 1. ATP-stimulated Ca^{2+} uptake in isolated rat liver nuclei. Ca^{2+} uptake was determined with a Ca^{2+} electrode. The reaction system contained 5.0 ml of Ca^{2+} transport medium which contained 100 mM KCl, 20 mM Hepes, pH 6.8, 5.0 mM $MgCl_2$, 40 μM $CaCl_2$, 2.0 mM ATP, and liver nuclei (70–90 μg DNA/ml).

Ca^{2+} transport assay

Ca^{2+} uptake and release were determined with a Ca^{2+} electrode [23]. With the electrode (Orion, Model EA 940, Cambridge, MA, USA), a reaction mixture (5.0 ml) composed of 100 mM KCl, 20 mM Hepes, 5 mM $MgCl_2$ and the desired other reagents was used. Nuclear DNA was approximately 70–90 μg/ml of reaction mixture. Ca^{2+} concentration was adjusted to the desired Ca^{2+} level (about 40 μM) with 10 mM $CaCl_2$, and uptake was initiated by the addition of 0.1 M ATP to a final concentration of 2.0 mM at 37° C. The Ca^{2+} electrode was calibrated using Ca^{2+}-EGTA buffers of known ionized Ca^{2+} concentrations, which were prepared and standardized using a Ca^{2+} standard solution purchased from Orion Associates Inc. In the Ca^{2+} release assay, the nuclei were incubated in the presence of 40 μM Ca^{2+} and 2.0 mM ATP for 10 min at 37° C in the Ca^{2+} uptake assay buffer, and then regucalcin and/or other reagents was added to the incubation mixture. Ca^{2+} uptake and release are expressed as nmoles of total Ca^{2+} per mg DNA of the nuclei.

Statistical methods

The significance of differences between values was estimated by using Student's *t*-test; p values of less than 0.05 were considered to indicate statistically significant differences.

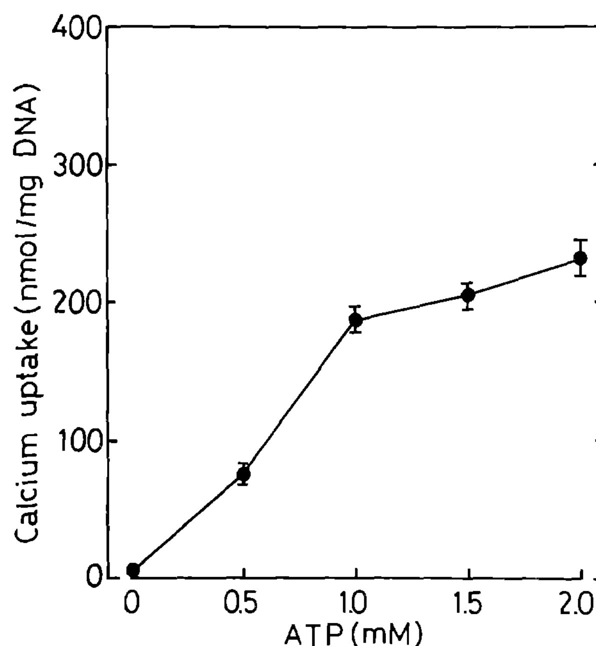

Fig. 2. Effect of increasing concentrations of ATP on Ca^{2+} uptake in isolated rat liver nuclei. Ca^{2+} uptake was determined with a Ca^{2+} electrode as described in the legend to Fig. 1. The total Ca^{2+} uptake in 10 min after ATP addition was determined by the differences between Ca^{2+} concentration before and after A23187 addition. The data presented are means ± SEM of five separate experiments with different nuclear preparations.

Results

When Ca^{2+} uptake by rat liver nuclei was determined with a Ca^{2+} electrode, addition of 2.0 mM ATP to the nuclei incubated in a medium containing 40 μM Ca^{2+} resulted in rapid nuclear Ca^{2+} uptake (Fig. 1). The uptake was saturated during 10 min after ATP addition. Ca^{2+} sequestration was dependent on the concentration of ATP; the uptake reached to maximum with 1.0 mM ATP (Fig. 2). This observation coincided with the results of filtration methods reported by Nicotera et al. [5]. ATP-independent Ca^{2+} accumulation was not directly related to the level of free Ca^{2+} in the incubation medium and was negligible. Maintenance of the nuclear Ca^{2+} gradient depends on the presence of ATP, since deficiency of ATP from the incubation medium resulted in release of Ca^{2+} from loaded nuclei, whereas readdition of ATP resulted in rapid reuptake of Ca^{2+} (data not shown). Also, Ca^{2+} sequestration was dependent on the Ca^{2+} concentration in the medium containing ATP (Fig. 3).

The effect of various agents on Ca^{2+} uptake by rat

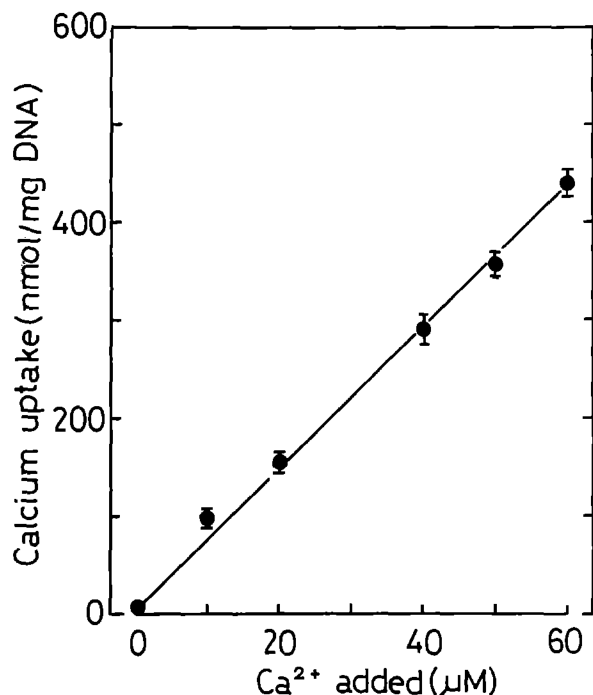

Fig. 3. Ca^{2+} uptake with increasing concentrations of Ca^{2+} in isolated rat liver nuclei. Ca^{2+} uptake was determined with a Ca^{2+} electrode as described in the legend to Fig. 1 with varying medium Ca^{2+} concentration. The total Ca^{2+} uptake in 10 min after 2.0 mM ATP addition was determined by the difference between Ca^{2+} concentration before and after A23187 addition. The data presented are means ± SEM of five separate experiments with different nuclear preparations.

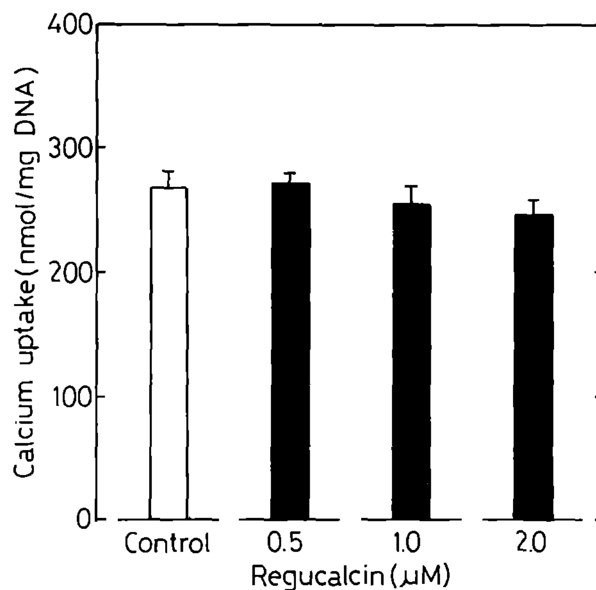

Fig. 4. Effect of regucalcin on Ca^{2+} uptake in isolated rat liver nuclei. Ca^{2+} uptake was determined with a Ca^{2+} electrode as described in the legend to Fig. 1 in the presence of regucalcin (0.5–2.0 µM). The total Ca^{2+} uptake in 10 min after ATP addition was determined by the difference between Ca^{2+} concentration before and after A23187 addition. The data presented are means ± SEM of five separate experiments with different nuclear preparations.

liver nuclei is shown in Table 1. Comparison of the effectiveness of ATP, ADP, and AMP to support nuclear Ca^{2+} accumulation indicated a preferential dependency on ATP. Vanadate, an inhibitor of ATP-dependent ion pumps [5, 23], partially inhibited nuclear Ca^{2+} uptake. Also, the effects of inhibitors of mitochondrial (sodium azid) [24] and microsomal [2,5-di(tert-butyl)-1,4-benzohydroquinone] [25] Ca^{2+} sequestration on nuclear Ca^{2+} uptake were determined. The agents did not have an appreciable effect on nuclear Ca^{2+} uptake. In contrast, incubation of isolated nuclei with trifluoroperazine, an inhibitor of calmodulin [18], resulted in marked inhibition of Ca^{2+} uptake. Since calmodulin is not involved in the activation of the hepatic plasma membrane Ca^{2+} pump [26], this result exclude the possibility that the observed Ca^{2+} uptake was due to

Table 1. Effect of various agents on Ca^{2+} uptake in isolated rat liver nuclei.

Treatment	Ca^{2+} uptake (nmol/mg DNA)	% of control
Control (+2.0 mM ATP)	294.0 ± 11.5	100
ADP (2.0 mM)	37.8 ± 4.2*	12.9
AMP (2.0 mM)	24.0 ± 2.9*	8.2
Vanadate (100 µM)	221.5 ± 13.1*	75.3
Sodium azide (25 µM)	288.6 ± 15.2	98.2
2,5-Di(tert-butyl)-1,4-benzohydroquinone (10 µM)	289.1 ± 19.7	98.3
Trifluoroperazine (50 µM)	193.4 ± 8.7*	65.8
Trifluoroperazine (100 µM)	61.9 ± 7.2*	21.1

Ca^{2+} uptake was determined with a Ca^{2+} electrode. The Ca^{2+} uptake took 10 min after addition of 2.0 mM ATP in the presence of various agents. ADP and AMP used were exchanged for ATP. The data presented are the means ± SEM of five separate experiments using different preparations of liver nuclei. * $P < 0.01$, as compared with the value of control.

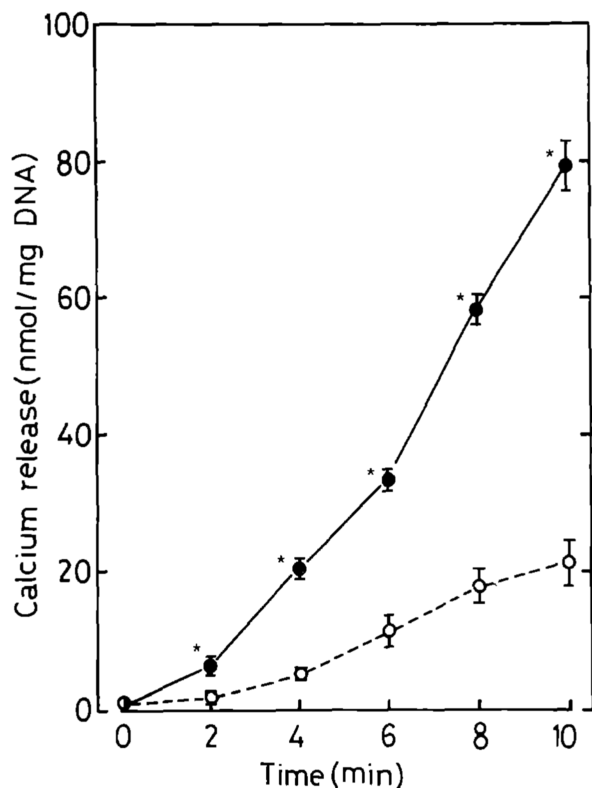

Fig. 5. Effect of regucalcin on Ca^{2+} release from isolated rat liver nuclei. Ca^{2+} release was determined with a Ca^{2+} electrode. The reaction mixture was incubated for 10 min in medium containing 100 mM KCl, 20 mM Hepes, pH 6.8, 5.0 mM $MgCl_2$, 40 μM $CaCl_2$, 2.0 mM ATP, and liver nuclei (70–90 μg DNA/ml), and then regucalcin (0.5 μM) was added into the incubation medium. The total Ca^{2+} release at time point indicated after regucalcin addition was determined by the difference between Ca^{2+} concentration before and after A23187 addition. The data presented are means ± SEM of five separate experiments with different nuclear preparations. * $P < 0.01$, as compared with the value of control at each time point. ○; control, ●; regucalcin.

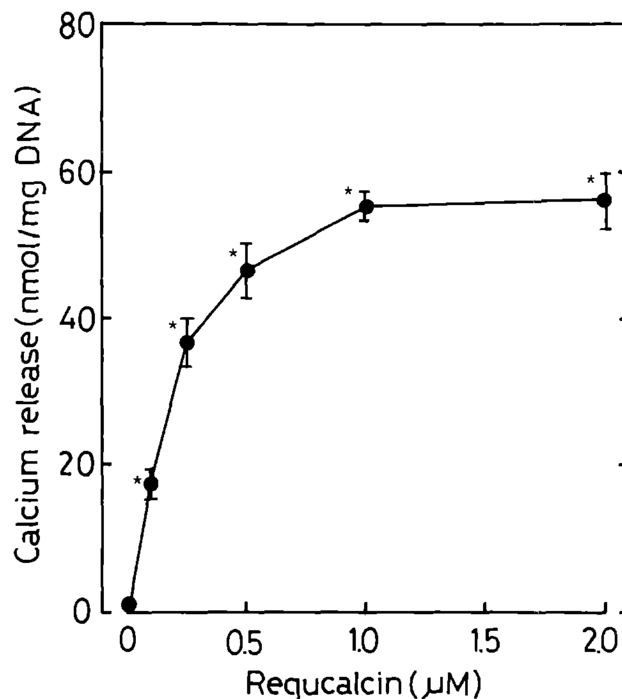

Fig. 6. Effect of increasing concentrations of regucalcin on Ca^{2+} release from isolated rat liver nuclei. Ca^{2+} release was determined with a Ca^{2+} electrode as described in the legend to Fig. 4. The total Ca^{2+} release in 10 min after regucalcin (0.1, 0.25, 0.5, 1.0, and 2.0 μM) addition was determined by the difference between Ca^{2+} concentrations before and after A23187 addition. The data presented are means ± SEM of five separate experiments with different nuclear preparations. * $P < 0.01$, as compared with the value of control without regucalcin.

contamination with inverted plasma membrane vesicles.

The effect of regucalcin on Ca^{2+} sequestration in rat liver nuclei is shown in Fig. 4. The presence of regucalcin (0.5–2.0 μM) in incubation medium did not cause a significant alteration of nuclear Ca^{2+} uptake after addition of 2.0 mM ATP, although it has been shown that the regucalcin concentration used can cause an inhibition of Ca^{2+} uptake by rat liver microsomes [27].

To clarify whether regucalcin has an effect on Ca^{2+} release from rat liver nuclei, the protein was added at time point (10 min) which the Ca^{2+} uptake was saturated by the presence of 2.0 mM ATP in incubation medium. In the absence of regucalcin, Ca^{2+} release from the nuclei was gradually increased with increasing incubation time (Fig. 5). This increase of Ca^{2+} release was blocked by readdition of 2.0 mM ATP (data not shown). However, addition of regucalcin (0.5 μM) resulted in remarkable increase of Ca^{2+} release from the nuclei. The Ca^{2+} release was depended on the regucalcin concentration, being half-maximal at about 0.2 μM regucalcin (Fig. 6).

The effect of various agents on Ca^{2+} release from rat liver nuclei is shown in Table 2. The nuclei were incubated for 10 min after 2.0 mM ATP addition into incubation medium. After saturation of the Ca^{2+} sequestration, agents were added into the incubation medium. The Ca^{2+} release was not significantly stimulated by addition of 100 μM vanadate and 100 μM GTP. Meanwhile, addition of 50 μM trifluoroperazine resulted in remarkable increase of Ca^{2+} release from the nuclei. Moreover, the presence of regucalcin (0.5 μM) further enhanced the trifluoroperazine (50 μM)-induced stimulation of Ca^{2+} release from the nuclei. The trifluoroperazine effect was not blocked by addition of heparin (20 μg/ml) or dithiothreitol (100 μM) (data not shown).

However, the regucalcin-stimulated Ca^{2+} release was inhibited by the presence of heparin (20 μg/ml) or dithiothreitol (100 μM).

Discussion

More recently, Nicotera et al. has reported the existence of an ATP-stimulated Ca^{2+} sequestration system in rat liver nuclei that requires calmodulin and generates a net increase in nuclear matrix free Ca^{2+} concentration [5]. This system may play an important role in the regulation of intranuclear Ca^{2+}-dependent processes [4–9]. With a Ca^{2+} electrode technique, the present study clearly demonstrates that the ATP-dependent Ca^{2+} uptake system exists in the nuclei isolated from rat liver. The nuclear Ca^{2+} uptake was not influenced by the presence of inhibitors for Ca^{2+} sequestration in the mitochondria, microsomes and plasma membranes of liver cells, indicating that the uptake is not contributed by other organelles. Trifluoperazine, an antagonist of calmodulin, resulted in remarkable inhibition of ATP-stimulated Ca^{2+} sequestration by rat liver nuclei. This observation further supported the previous finding that nuclear Ca^{2+} sequestration may be mediated by a calmodulin-dependent Ca^{2+} pump [5]. Thus, the existence of ATP-dependent Ca^{2+} uptake system in liver nuclei was confirmed by the technique with the Ca^{2+} electrode.

Whether regucalcin, a calcium-binding protein isolated from rat liver cytosol [15, 16], can act on ATP-dependent Ca^{2+} transport systems in rat liver nuclei was examined with the Ca^{2+} electrode. The presence of regucalcin (0.5–2.0 μM) did not have an effect on the nuclear Ca^{2+} uptake. Meanwhile, the gradual Ca^{2+} release from liver nuclei generated when the Ca^{2+} sequestration was saturated during 10 min after ATP addition. The Ca^{2+} release was blocked by readdition of ATP into incubation medium. The deficiency of ATP may partly induce Ca^{2+} release from liver nuclei. Trifluoroperazine, which inhibited ATP-stimulated Ca^{2+} sequestration in the nuclei, resulted in Ca^{2+} release from the nuclei. The present finding suggests that nuclear Ca^{2+} sequestration is mediated by a calmodulin-dependent Ca^{2+} pump and this inhibition induces Ca^{2+} release from nuclei. On the other hand, when nuclear Ca^{2+} sequestration was saturated, addition of regucalcin (0.1–2.0 μM) resulted in remarkable increase of Ca^{2+} release from the nuclei. Regucalcin could significantly enhanced the trifluoroperazine-induced increase in Ca^{2+} release from the nuclei. Thus, regucalcin had a stimulatory effect on nuclear Ca^{2+} release, whereas the Ca^{2+} uptake was not influenced by the protein. Presumably, the regucalcin effect differs from that of calmodulin.

At present, the characteristics of the ATP-dependent Ca^{2+} uptake and the release systems are not clarified fully. The present observation, that liver nuclei generates a spontaneous release of Ca^{2+}, suggested the existence of Ca^{2+}-releasing channels on liver nuclei. Addition of GTP (100 μM), which can stimulate liver microsomal Ca^{2+} release [28], did not cause Ca^{2+} release from liver nuclei. The stimulatory effect of regucalcin on nuclear Ca^{2+} release was inhibited by coexistence of heparin, which can block liver microsomal Ca^{2+} release stimulated by inositol-1,4,5-triphosphate [29]. Also, the Ca^{2+}-releasing effect of regucalcin was not seen in the presence of dithiothreitol, a SH group-protecting agent

Table 2. Effect of various agents on Ca^{2+} release from isolated rat liver nuclei.

Treatment	Ca^{2+} release (nmol/mg DNA)	% of control
Control	23.9 ± 5.2	100
Regucalcin (0.5 μM)	46.5 ± 3.9*	194.6
Vanadate (100 μM)	29.3 ± 2.4	122.6
GTP (100 μM)	19.5 ± 3.5	81.6
Trifluoroperazine (50 μM)	77.8 ± 7.1*	325.5
Trifluoroperazine (50 μM) + regucalcin (0.5 μM)	131.5 ± 18.0*,**	550.2
Heparin (20 μg/ml)	20.2 ± 4.0	84.5
Heparin (20 μg/ml) + regucalcin (0.5 μM)	25.3 ± 2.9	105.9
Dithiothreitol (100 μM)	15.5 ± 3.8	64.9
Dithiothreitol (100 μM) + regucalcin (0.5 μM)	24.4 ± 2.7	102.1

Ca^{2+} release was determined with a Ca^{2+} electrode. Various agents were simultaneously added into incubation medium at 10 min after 2.0 mM ATP addition, and then the Ca^{2+} release was measured for 10 min. The data presented are the means ± SEM of five separate experiments using different preparations of liver nuclei. * $P < 0.01$, as compared with the value of control. ** $P < 0.01$, as compared with the value of trifluoroperazine alone.

[23]. The regucalcin sensitive Ca^{2+} channels for the releasing may be involved in inositol 1,4,5-triphosphate and SH group sensitivities in nuclear membranes. This, however, remains to be elucidated.

It has been reported that regucalcin can inhibit Ca^{2+}-activated DNA fragmentation in rat liver nuclei, suggesting that this effect is based on Ca^{2+} binding of regucalcin [20]. The Ca^{2+} binding constant of regucalcin was found to be $4.19 \times 10^5\ M^{-1}$ by equilibrium dialysis, and there appears to be 6–7 high affinity binding sites for Ca^{2+} per molecule of protein [16]. However, since regucalcin can stimulate Ca^{2+} release from liver nuclei, the inhibitory effect of regucalcin on the Ca^{2+}-activated DNA fragmentation in liver nuclei may be partly contributed by the regucalcin-stimulated Ca^{2+} release from the nuclei. Presumably, regucalcin plays a regulatory role in the regulation of liver nuclear function involved in Ca^{2+}.

In conclusion, the present study demonstrates that the ATP-stimulated Ca^{2+} sequestration and the Ca^{2+} release systems exist in rat liver nuclei estimating with a Ca^{2+} electrode technique. Regucalcin could stimulate Ca^{2+} release from the nuclei. The finding suggests that regucalcin partly regulate liver nuclear function through the Ca^{2+} releasing effect.

References

1. Rasmussen J: Cell communication, calcium ion, and cyclic adenosine monophosphate. Science 170: 404–412, 1970
2. Williamson JR, Cooper RK, Hoek JB: Role of calcium in the hormonal regulation of liver metabolism. Biochim Biophys Acta 639: 243–295, 1981
3. Reinhart PH, Taylor WM, Bygrave FL: The role of calcium ions in the mechanisms of action of α-adrenergic agonists in rat liver. Biochem J 223: 1–13, 1984
4. Bachs O, Carafolli E: Calmodulin and calmodulin-binding proteins in liver cell nuclei. J Biol Chem 262: 10786–10790, 1987
5. Nicotera P, McConkey DJ, Jones DP, Orrenius S: 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, 1989
6. Boyton AL, Whitfield JF, MacManus JP: Calmodulin stimulates DNA synthesis by rat liver cells. Biochim Biophys Res Commun 95: 745–749, 1980
7. Cruise J, Houck KA, Michalopoulos GK: Induction of DNA synthesis in cultured rat hepatocytes through stimulation of α-adrenoreceptor by norepinephrine. Nature 227: 749–751, 1985
8. Pujol MJ, Soriano M, Alique R, Carafolli E, Bachs O: Effect of α-adrenergic blockers on calmodulin association with the nuclear matrix of rat liver cells during proliferative activation. J Biol Chem 264: 18863–18865, 1989
9. Bachs O, Lanini L, Serratosa J, Coll MJ, Bastos R, Aligue R, Rius E, Carafolli E: Calmodulin-binding proteins in the nuclei of quiescent and proliferatively activated rat liver cells. J Biol Chem 265: 18595–18600, 1990
10. Cheung WY: Calmodulin plays a pirotal role in cellular regulation. Science 202: 19–27, 1980
11. Jones DP, McConkey DJ, Nicotera P, Orrenius S: Calcium-activated DNA fragmentation in rat liver nuclei. J Biol Chem 264: 6398–6403, 1989
12. Waisman DM, Muranyi J, Ahmed M: Identification of a novel calcium binding protein from bovine brain. FEBS Lett 164: 80–84, 1983
13. Waisman DM, Salimath BP, Anderson MJ: Isolation and characterization of CAB-63, a novel calcium-binding protein. J Biol Chem 260: 1652–1660, 1985
14. Fliegel L, Burns K, MacLennan DH, Reithmeier RAF, Michalak M: Molecular cloning of the high affinity calcium-binding protein (calreticulin) of skeletal muscle sarcoplasmic reticulum. J Biol Chem 264: 21522–21528, 1989
15. Yamaguchi M, Yamamoto T: Purification of calcium binding substance from soluble fraction of normal rat liver. Chem Pharm Bull 26: 1915–1918, 1978
16. Yamaguchi M, Sugii K: Properties of calcium-binding protein isolated from the soluble fraction of normal rat liver. Chem Pharm Bull 29: 567–570, 1981
17. Yamaguchi M, Mori S: Inhibitory effect of calcium-binding protein regucalcin on protein kinase C activity in rat liver cytosol. Biochem Med Metab Biol 43: 140–146, 1990
18. Mori S, Yamaguchi M: Hepatic calcium-binding protein regucalcin decreases Ca^{2+}/calmodulin-dependent protein kinase activity in rat liver cytosol. Chem Pharm Bull 38: 2216–2218, 1990
19. Yamaguchi M, Tai H: Inhibitory effect of calcium-binding protein regucalcin on Ca^{2+}/calmodulin-dependent cyclic nucleotide phosphodiesterase activity in rat liver cytosol. Mol Cell Biochem 106: 25–30, 1991
20. Yamaguchi M, Sakurai T: Inhibitory effect of calcium-binding protein regucalcin on Ca^{2+}-activated DNA fragmentation in rat liver nuclei. FEBS Lett 279: 281–284, 1991
21. Lowry OH, Rosebrough NJ, Farr AL, Randall FJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–273, 1951
22. Burton K: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62: 315–323, 1956
23. Zhang GH, Yamaguchi M, Kimura S, Higham S, Kraus-Friedmann N: Effects of heavy metal on rat liver microsomal Ca^{2+}-ATPase and Ca^{2+} sequestering. Relation to SH groups. J Biol Chem 265: 2184–2189, 1990
24. Nicholls D, Åkerman K: Mitochondrial calcium transport. Biochim Biophys Acta 683: 57–88, 1982
25. Llopis J, Chow SB, Kass GEN, Gahm A, Orrenius S: Comparison between the effects of the microsomal Ca^{2+}-translocase inhibitors thapsigargin and 2,5-di-(t-butyl)-1,4-benzohydroquinone on cellular calcium fluxes. Biochem J 277: 553–556, 1991
26. Lotersztajn S, Hanoune J, Pecker F: A high affinity calcium-stimulated magnesium-dependent ATPase in rat liver plasma membranes. Dependence on an endogenous protein activator distinct from calmodulin. J Biol Chem 256: 11209–11215, 1981
27. Yamaguchi M, Mori S: Effect of the calcium-binding protein regucalcin on the Ca^{2+} transport system in rat liver microsomes:

The protein stimulates Ca^{2+} release. Chem Pharm Bull 37: 3037–3041, 1989

28. Dawson AP: GTP enhances inositol triphosphate-stimulated Ca^{2+} release from rat liver microsomes. FEBS Lett 185: 147–150, 1985
29. Cullen PJ, Comerford JG, Dawson AP: Heparin inhibits the inositol 1,4,5-triphosphate-induced Ca^{2+} release from rat liver microsomes. FEBS Lett 228: 57–59, 1988