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REGULATORY EFFECTS OF ZINC AND COPPER ON THE CALCIUM TRANSPORT SYSTEM IN RAT LIVER NUCLEI

RELATION TO SH GROUPS IN THE RELEASING MECHANISM

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Abstract—In isolated hepatic nuclei, the heavy metals Zn^{2+} and Cu^{2+} (10 μM) inhibited Ca^{2+} uptake and caused a prompt release of Ca^{2+} from preloaded nuclei in a concentration-dependent manner, with Zn^{2+} being more effective than Cu^{2+}. The sulfhydryl group reducing agent dithiothreitol (DTT) protected the nuclei from the effects of heavy metals; DDT (1 mM) almost completely blocked Zn^{2+}- or Cu^{2+}-induced Ca^{2+} release and inhibition of Ca^{2+} uptake. The sulfhydryl modifying reagent *N*-ethylmaleimide (NEM; 0.2 mM) also caused Ca^{2+} release, but it did not have an appreciable effect on Ca^{2+} uptake. Furthermore, in the presence of NEM heavy metals did not evoke Ca^{2+} release. The present study demonstrates that Zn^{2+} and Cu^{2+} have a stimulatory effect on Ca^{2+} release from isolated rat liver nuclei, and that the SH group may play an important role in the Ca^{2+}-releasing mechanism in liver nuclei.

Ca^{2+} plays an important role in the regulation of many cell functions [1], and its role in liver metabolism has been demonstrated [2, 3]. Liver metabolism is regulated by Ca^{2+} which is increased in the cytosol of liver cells by hormonal stimulation. In recent years, there has also been growing evidence that Ca^{2+} plays a role in liver nuclear function [4–9]. Calmodulin, a Ca^{2+}-binding protein which can amplify the effect of Ca^{2+} [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]. It has also been reported that isolated rat liver nuclei contain a DNA endonuclease activity dependent upon Ca^{2+} in the submicromolar range, and that Ca^{2+} causes extensive DNA hydrolysis [11]. Presumably, Ca^{2+} and calmodulin regulate liver nuclear function. On the other hand, it has been demonstrated recently that regucalcin, a novel Ca^{2+}-binding protein which reverses the effect of Ca^{2+} effect on many enzymes in liver cells [12, 13], can inhibit Ca^{2+}-activated DNA fragmentation in isolated rat liver nuclei [14], and that the protein evokes Ca^{2+} release from the nuclei [15]. Thus, the nuclear Ca^{2+} transport system may be involved in the regulation of liver nuclear function. However, little is known about the regulatory mechanisms by which Ca^{2+} is released from liver nuclei.

Heavy metals which interact with SH groups have been reported to evoke Ca^{2+} release from sarcoplasmic reticulum and hepatic microsomal vesicles [16, 17]. It was suggested that the heavy metal-induced Ca^{2+} release is due to their interaction with and modification of essential protein sulfhydryl groups [18, 19]. Heavy metals serve as useful tools for probing the mechanism of Ca^{2+} release. The elucidation of the mechanism by which Ca^{2+} is released from intracellular sources is important not only in the sarcoplasmic reticulum and microsomes, but also in the nuclei. The present studies were undertaken to evaluate the possible role of SH groups in the hepatic nuclear Ca^{2+} transport system. It was found that the heavy metals Zn^{2+} and Cu^{2+} evoked Ca^{2+} release from rat liver nuclei, suggesting a possible role of the SH groups in the releasing mechanism.

MATERIALS AND METHODS

Chemicals. ATP, dithiothreitol (DTT)† and *N*-ethylmaleimide (NEM) were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). $CaCl_2 \cdot 2H_2O$, $ZnSO_4$, $CuSO_4$ and other reagents were purchased from the 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°, and were allowed distilled water freely. After 1 week on the diet animals were killed by bleeding.

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,

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† Abbreviations: DTT, dithiothreitol; NEM, *N*-ethylmaleimide; EGTA, [ethylene bis(oxyethylenenitrilo)]-tetraacetic acid; and Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

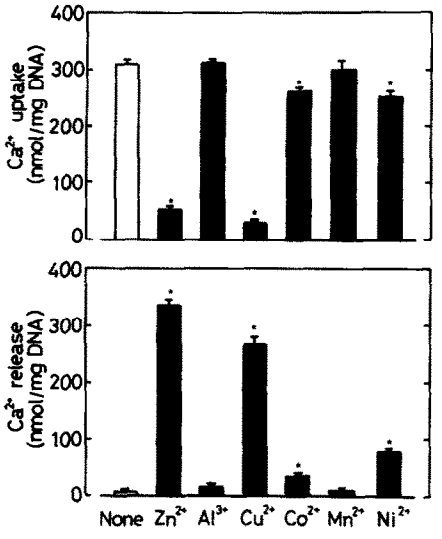

Fig. 1. Effects of heavy metals on Ca^{2+} uptake and release in rat liver nuclei. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode. The reaction system contained 5 mL of Ca^{2+} transport medium which consisted of 100 mM KCl, 20 mM Hepes, pH 6.8, 5 mM $MgCl_2$, 40 μM $CaCl_2$, and 2.0 mM ATP. The heavy metal compounds were $ZnSO_4$, $Al_2(SO_4)_3$, $CuSO_4$, $CoSO_4$, $MnSO_4$, and $NiSO_4$. All of the heavy metal solutions were adjusted to pH 7. The pH of the reaction system was not changed when 100 μM (final concentration) heavy metal was added. The total Ca^{2+} uptake and Ca^{2+} release in 10 min were determined by the difference between Ca^{2+} concentrations before and after the addition of A23187 (final concentration 5 μM). Data are means ± SEM of five separate experiments with different nuclear preparations. Key: (*) $P < 0.01$ as compared to the control.

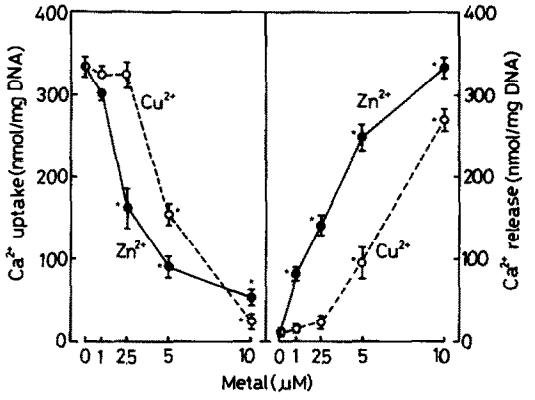

Fig. 2. Concentration–response curves of heavy metal-induced Ca^{2+} release and inhibition of Ca^{2+} uptake in rat liver nuclei. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode as described in the legend of Fig. 1. The total Ca^{2+} uptake and Ca^{2+} release in 10 min were determined by the difference between Ca^{2+} concentration before and after the addition of A23187. Data are means ± SEM of five separate experiments with different nuclear preparations. Key: (*) $P < 0.01$ as compared to the control.

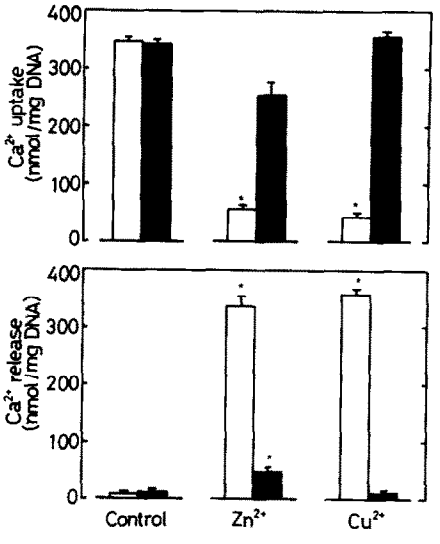

Fig. 3. Effect of dithiothreitol (DTT) on heavy metal-induced Ca^{2+} release and inhibition of Ca^{2+} uptake in rat liver nuclei. DTT (final concentration 1 mM) was added at approximately 1 min before the administration of 10 μM Zn^{2+} or Cu^{2+}. Ca^{2+} uptake and release in 10 min were determined with a Ca^{2+} electrode as described in the legend of Fig. 1. Data are means ± SEM of five separate experiments using different nuclear preparations. Key: (*) $P < 0.01$ as compared to the control without the addition of metal; (□) without DTT; and (■) with DTT.

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 [ethylene bis(oxyethylenenitrilo)]-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 in 2.3 M sucrose solution. The tubes were mixed gently, 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 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (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 5% contamination by microsomes, plasma membranes,

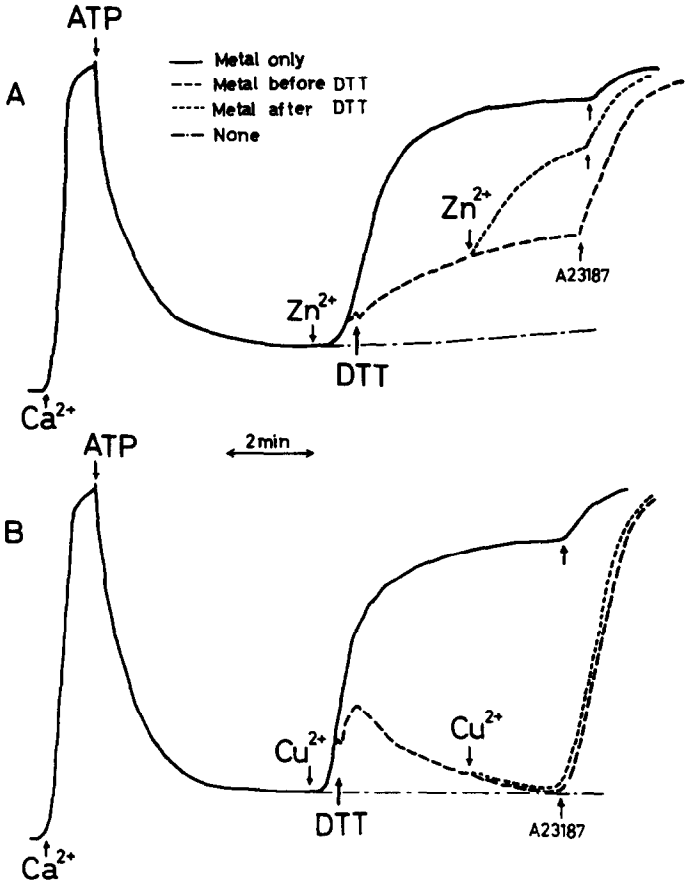

Fig. 4. Influence of the time of addition of DTT on its protective effect against heavy metals. Ca^{2+} release was determined with a Ca^{2+} electrode as described in the legend of Fig. 1. DTT (final concentration 1 mM) was added at approximately 1.0 min after the addition of the heavy metals (final concentration 10 μM).

or mitochondria. DNA content in the nuclei was determined with the diphenylamine reaction [20].

Ca^{2+} *transport assay*. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode [19]. A reaction mixture (5.0 mL) composed of 100 mM KCl, 20 mM Hepes, 5 mM $MgCl_2$ and the other desired reagents was used with the electrode (Orion, model EA 940, Cambridge, MA, U.S.A.). Nuclear DNA was approximately 70–90 μg/mL of the 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°. 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. Ca^{2+} release was monitored, as Ca^{2+} uptake was completely saturated within 10 min: the nuclei were incubated in the presence of 40 μM Ca^{2+} and 2.0 mM ATP for 10 min at 37° in the Ca^{2+} uptake assay buffer, and then metals and/or other reagents were added to the incubation mixture. Ca^{2+} uptake and release are expressed as nanomoles of total Ca^{2+} per milligram of nucleic DNA.

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.

RESULTS

The effects of the heavy metals Zn^{2+}, Al^{3+}, Cu^{2+}, Co^{2+}, Mn^{2+}, and Ni^{2+} on Ca^{2+} uptake and release in isolated rat liver nuclei are presented in Fig. 1. Inclusion of Zn^{2+} and Cu^{2+} in the incubation medium at a 10 μM concentration markedly inhibited ATP-stimulated Ca^{2+} uptake, whereas the effects of the other metals were slight. On the other hand, when the same concentration of metals was added to vesicles previously loaded with Ca^{2+}, a remarkable release of Ca^{2+} was observed with Zn^{2+} and Cu^{2+}. The effects of the other metals were slight, although Co^{2+} and Ni^{2+} had a significant effect on Ca^{2+} release. Thus, of the various metals tested, only

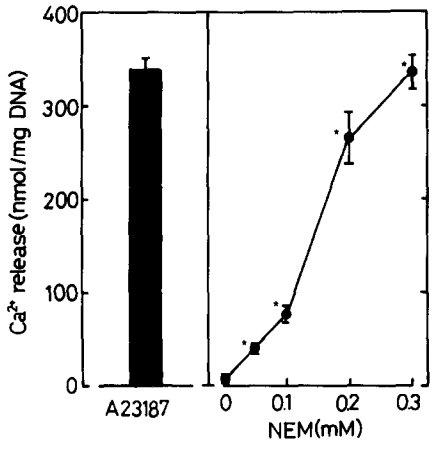

Fig. 5. Effect of *N*-ethylmaleimide (NEM) on Ca^{2+} release in rat liver nuclei. NEM was added at approximately 10 min after the addition of 2 mM ATP (final concentration). The total Ca^{2+} uptake in 10 min was determined by the difference between Ca^{2+} concentrations before and after the addition of 5 μM A23187. Ca^{2+} release was determined with a Ca^{2+} electrode as described in the legend of Fig. 1. Data are means ± SEM of five separate experiments using different nuclear preparations. Key: (*) $P < 0.01$ as compared to the control without NEM addition.

Zn^{2+} and Cu^{2+} had a potent effect on Ca^{2+} transport in rat liver nuclei.

The concentration dependence of these processes on Zn^{2+} and Cu^{2+} is shown in Fig. 2. Both Ca^{2+} uptake and release in rat liver nuclei were clearly affected by the heavy metals at a 5 μM concentration. At a 2.5 μM concentration an almost 50% inhibition of Ca^{2+} uptake was evident with Zn^{2+} but not with Cu^{2+}. With 1 μM Zn^{2+}, Ca^{2+} release was increased significantly, although the metal had no effect on Ca^{2+} uptake at this concentration. To test the possibility that the effects of the metals were due to their interaction with critical SH groups, these effects were examined with the sulfhydryl group protecting agent DTT (Fig. 3). The presence of 1 mM DTT in the incubation mixture did not affect Ca^{2+} uptake and release in the liver nuclei. Inclusion of 1 mM DTT clearly prevented Zn^{2+} (10 μM)-induced inhibition of Ca^{2+} uptake by the liver nuclei. The same effect of DTT was seen in respect to Ca^{2+} release from the liver nuclei.

The effect of DTT on Ca^{2+} release from the liver nuclei showed a different pattern according to the time of the addition of the metals into the incubation mixture (Fig. 4). When Zn^{2+} or Cu^{2+} was added to nuclei previously loaded with Ca^{2+}, a release of Ca^{2+} was evoked. DTT (1 mM) was included during 1.0 min after the addition of the heavy metals. The Cu^{2+} (10 μM)-induced Ca^{2+} release was prevented completely, while the Ca^{2+}-releasing effect of Zn^{2+} was prevented partially. Re-addition of Zn^{2+} (10 μM) evoked Ca^{2+} release, whereas the re-addition of Cu^{2+} (10 μM) did not.

The effect of NEM, a SH group reagent, on Ca^{2+} transport in rat liver nuclei is shown in Fig. 5. When NEM was added to Ca^{2+}-loaded nuclei, the Ca^{2+} release was increased linearly with the increase in NEM concentration (0.05 to 0.3 mM). An appreciable effect of NEM on Ca^{2+} release was observed at 0.05 mM. With a 0.3 mM concentration, the effect of NEM was almost complete, as compared with the effect of A23187 (5 μM) on Ca^{2+} release.

When NEM (0.2 mM) was included in the incubation mixture before the addition of ATP, Ca^{2+} uptake following ATP addition was seen clearly, indicating that NEM did not have an effect on Ca^{2+} uptake by the liver nuclei (Fig. 6A). Beginning 4.0 min after the addition of ATP, Ca^{2+} release was evoked from nuclei previously loaded with metal. This suggested that there was a relationship between the SH group and Ca^{2+} release. When NEM (0.2 mM) was included in Ca^{2+}-loaded nuclei, the reagent evoked Ca^{2+} release from the nuclei. The NEM-induced Ca^{2+} release was not enhanced by the addition of Zn^{2+} (10 μM) (Fig. 6B).

DISCUSSION

The importance of the SH group for Ca^{2+} sequestering in the sarcoplasmic reticulum and hepatic microsomes has generally been recognized (see Introduction). A previous investigation showed that Ca^{2+} uptake and release in isolated rat liver nuclei are not altered by ryanodine, thapsigargin and 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone [15], indicating that the nuclear fraction does not contain sarcoplasmic reticulum or hepatic microsomes. The data presented show that Zn^{2+} and Cu^{2+}, which are important as essential trace metals, have a marked effect on Ca^{2+} sequestering systems in rat liver nuclei, causing inhibition of Ca^{2+} uptake and evoking a fast release of previously accumulated Ca^{2+}. Also, Cd^{2+} and Hg^{2+}, which can interact with the SH group, caused a significant inhibition of Ca^{2+} uptake and a corresponding elevation of Ca^{2+} release in isolated rat liver nuclei (data not shown in Fig. 1). It is interesting, however, that Zn^{2+} and Cu^{2+} are distributed endogenously in hepatic cytosol as essential metals, whereas Cd^{2+} and Hg^{2+} are toxic metals. Among the SH group reagents that were used in the present study, Zn^{2+} had a more pronounced effect on Ca^{2+} release than Cu^{2+} and the SH group reagent NEM. This difference may be due to the higher affinities of Zn^{2+} and Cu^{2+} to specific SH groups involved in this process. It was shown previously that Cu^{2+} but not Zn^{2+} can oxidize the SH group [21]. However, Zn^{2+} has the ability to bind SH groups. The stoichiometric ratio of Zn^{2+} binding to glutathione (GSH) is 1 Zn/GSH [21]. Thus, the quantitative differences found among the metals can be explained by their chemical characteristics. Meanwhile, Zn^{2+} and Cu^{2+} tend to be oxidized and reduced a great deal, and generate oxygen radicals when they are added without being chelated. This does not exclude the possibility that the effects of Zn^{2+} and Cu^{2+} on liver nuclear Ca^{2+} transports may be due in part to nonspecific oxidation reduction reactions of the metals.

It is well documented that DTT contains SH groups and has the ability to maintain protein thiol groups in the reduced state [21, 22]. DTT is a

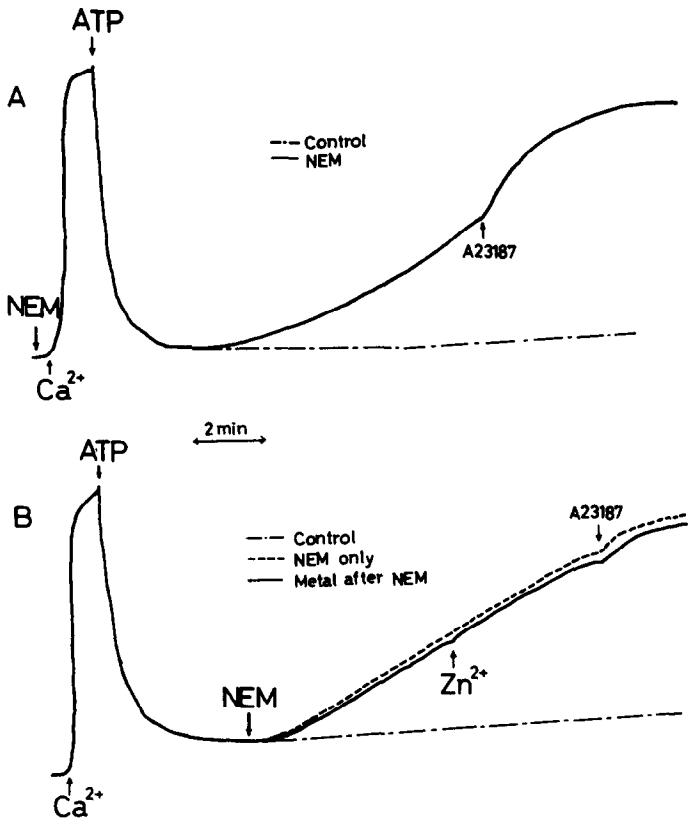

Fig. 6. Influence of the time of addition of NEM on Ca^{2+} release in rat liver nuclei. Ca^{2+} release was determined with a Ca^{2+} electrode as described in the legend of Fig. 1. NEM (final concentration 0.2 mM) was added at approximately 1.0 min before the addition of 2 mM ATP (final concentration) (A) or 4.0 min after the addition of ATP (B). Zn^{2+} (final concentration 10 μM) was added at approximately 6.0 min after the addition of NEM (B).

permeable SH group protection reagent [23]. It was found that DTT protects the liver nuclei from the effects of Zn^{2+} and Cu^{2+} on Ca^{2+} uptake and Ca^{2+} release. This could mean that the affected SH groups are located on the intraluminal and/or external side of the nuclei. DTT completely prevented the Cu^{2+}-induced Ca^{2+} release, while it had a partial effect on the action of Zn^{2+}. The different degree of protection provided by the sulfhydryl reagent may be due to the differences in affinity for heavy metal ions.

The sulfhydryl modifying reagent NEM evoked a release of previously accumulated Ca^{2+} in the liver nuclei, while it did not inhibit Ca^{2+} uptake. This may imply that the Ca^{2+}-releasing system is affected by the SH reagent NEM with higher affinity than Ca^{2+} uptake in the liver nuclei. Addition of Zn^{2+} did not increase the rate of release above the observed rate of Ca^{2+} release with NEM. This was also seen in the case of Cu^{2+} addition (data not shown). The effective sites of heavy metals evoking Ca^{2+} release may be involved in the SH groups.

It is clear that the fast rate of Ca^{2+} release cannot be attributed entirely to the inhibition of Ca^{2+} uptake. NEM increased Ca^{2+} release from the liver nuclei, while it did not have an appreciable effect on Ca^{2+} uptake. Also, Zn^{2+}- and Cu^{2+}-induced increases in the nuclear Ca^{2+} release may be due in part to the inhibitory effect of Ca^{2+} uptake by the heavy metals. However, Zn^{2+} and Cu^{2+} at a comparatively low concentration had a potent effect on Ca^{2+} release rather than Ca^{2+} uptake in the liver nuclei. Thus, the inhibition of Ca^{2+} uptake is not sufficient to explain the observed effect of the SH group modifying reagents on Ca^{2+} release from the liver nuclei. The present data are suggestive of the opening of a channel in the liver nuclei as was proposed in the case of the sarcoplasmic reticulum [16].

It is uncertain whether the Ca^{2+} channel is located on liver nuclei. An interesting question in respect to the heavy metal-activated Ca^{2+} channel is whether it is part of the Ca^{2+}-ATPase or comprises a separate protein. It is suggested that the heavy metal-responsive Ca^{2+} channel(s) resides in a protein which is separate from the Ca^{2+}-ATPase in sarcoplasmic reticulum vesicles [24, 25]. On the other hand, Chiesi suggested that the rapid Ca^{2+} efflux observed upon the addition of cupric phenanthroline to sarcoplasmic reticulum vesicles is due to Ca^{2+} channels that are

formed by chemical modification of the Ca^{2+}-ATPase itself [26]. No structure homologous to the sarcoplasmic reticulum triad has been identified in the liver thus far, nor is the endoplasmic reticulum connected to the plasma membrane. There are no reports about the nature of Ca^{2+} channels located in the liver nuclei fraction. The question of whether heavy metals (Zn^{2+} and Cu^{2+}) open a channel which is the Ca^{2+}-ATPase itself or a separate protein(s) remains to be resolved.

The present study suggests the possibility of the presence of SH group gated Ca^{2+} channels in the liver nuclei. The physiological relevance of this finding to the regulation of liver nuclear Ca^{2+} distribution and the nuclear function remains to be elucidated. It is likely, however, that these channels are involved in the regulation of Ca^{2+}-activated DNA fragmentation [14] and Ca^{2+} release in the nuclei by the hepatic Ca^{2+}-binding protein regucalcin [15] observed previously. Further investigation is in progress to clarify the Ca^{2+}-transporting mechanism in liver nuclei.

REFERENCES

1. Rasmussen J, Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* **170**: 404–412, 1970.
2. Williamson JR, Cooper RK and Hoek JB, Role of calcium in the hormonal regulation of liver metabolism. *Biochim Biophys Acta* **639**: 243–295, 1981.
3. Reihart PH, Taylor WM and 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 and 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 and 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 and MacManus JP, Calmodulin stimulates DNA synthesis by rat liver cells. *Biochem Biophys Res Commun* **95**: 745–749, 1980.
7. Cruise J, Houck KA and Michalopoulos GK, Induction of DNA synthesis in cultured rat hepatocytes through stimulation of α_1-adrenoreceptor by norepinephrine. *Nature* **227**: 749–751, 1985.
8. Pujol MJ, Soriano M, Aliqué R, Carafolli E and 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 and 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 pivotal role in cellular regulation. *Science* **207**: 19–27, 1980.
11. Jones DP, McConkey DJ, Nicotera P and Orrenius S, Calcium-activated DNA fragmentation in rat liver nuclei. *J Biol Chem* **264**: 6398–6403, 1989.
12. Yamaguchi M and 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.
13. Yamaguchi M and 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.
14. Yamaguchi M and 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.
15. Yamaguchi M, Effect of calcium-binding protein regucalcin on Ca^{2+} transport system in rat liver nuclei: Stimulation of Ca^{2+} release. *Mol Cell Biochem* **113**: 63–70, 1992.
16. Bindoli A and Fleischer S, Induced calcium release in skeletal muscle sarcoplasmic reticulum by sulfhydryl reagents and chlorpromazine. *Arch Biochem Biophys* **221**: 458–466, 1983.
17. Kraus-Friedmann N, Hormonal regulation of hepatic gluconeogenesis. *Physiol Rev* **64**: 170–259, 1984.
18. Abramson JJ, Trim JL, Weden L and Salama G, Heavy metals induce rapid calcium release from sarcoplasmic reticulum vesicles isolated from skeletal muscle. *Proc Natl Acad Sci USA* **80**: 1526–1530, 1983.
19. Zhang GH, Yamaguchi M, Kimura S, Higham S and 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.
20. 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.
21. Christie NT and Costa M, *In vitro* assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: Interactions with membranes, glutathione, metallothionein, and DNA. *Biol Trace Elem Res* **6**: 139–158, 1984.
22. Bellomo G, Jewell SA, Thor H and Orrenius S, Regulation of intracellular calcium compartmentation: Studies with isolated hepatocytes and *t*-butyl hydroperoxide. *Proc Natl Acad Sci USA* **79**: 6842–6846, 1982.
23. Graf P and Sies H, Hepatic uptake of cadmium and its biliary release as affected by dithioerythritol and glutathione. *Biochem Pharmacol* **33**: 639–643, 1984.
24. Salama G and Abramson J, Silver ions trigger Ca^{2+} release by acting at the apparent physiological release site in sarcoplasmic reticulum. *J Biol Chem* **259**: 13363–13369, 1984.
25. Palade P, Drug-induced Ca^{2+} release from isolated sarcoplasmic reticulum. II. Releases involving a Ca^{2+}-induced Ca^{2+} release channel. *J Biol Chem* **262**: 6142–6148, 1987.
26. Chiesi M, Cross-linking agents induce rapid calcium release from skeletal muscle sarcoplasmic reticulum. *Biochemstry* **23**: 3899–3907, 1984.