Biochemical Pharmacology, Vol. 45, No. 7, pp. 1471–1475, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

REGULATORY EFFECT OF ARACHIDONIC ACID ON THE CALCIUM TRANSPORT SYSTEM IN RAT LIVER NUCLEI

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(*Received* 21 *October* 1992; *accepted* 11 *December* 1992)

Abstract—The effect of arachidonic acid (AA) 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 ATP (0.5–2.0 mM), while uptake was negligible in the presence of 2.0 mM ADP or AMP. AA (10–100 μM) caused a marked inhibition of Ca^{2+} uptake following the addition of 2.0 mM ATP. Also, Ca^{2+}, which accumulated in the nuclei during 6 min after ATP addition, was clearly released by the addition of AA (10–100 μM). The alterations were concentration dependent. The nuclear Ca^{2+} uptake and release were not altered significantly by the presence of prostaglandin E_2 (10 and 20 μM), prostaglandin H_2 (1 and 4 μM), thromboxane B_2 (1 and 4 μM), leukotriene A_4 (1 and 4 μM), Ins(1,4,5)P_3 (1 and 10 μM) or dibutyryl cAMP (10 and 50 μM). Only, 5-hydroxy-eicosatetraenoic acid (5-HETE) at 4 μM caused a significant inhibition of nuclear Ca^{2+} uptake and an appreciable increase in Ca^{2+} release; the 1 μM concentration had no effect. These results indicate that AA, one of the prostanoids, has a unique effect on Ca^{2+} uptake and release in rat liver nuclei. The finding suggests that AA has a regulatory effect on the Ca^{2+} transport system in liver nuclei.

It is well known that Ca^{2+} plays an important role in the regulation of many cell functions, and the role of Ca^{2+} in liver metabolism has been demonstrated [1, 2]. Liver metabolism is regulated by an increase of Ca^{2+} in the cytosol of liver cells due to hormonal stimulation. There is growing evidence that Ca^{2+} plays a role in liver nuclear function [3–8]. Calmodulin, a calcium-binding protein which can amplify the effect of Ca^{2+} exists in rat liver nuclei [5, 8]. The existence of an ATP-stimulated Ca^{2+} sequestration system in rat liver nuclei which generates a net increase in nuclear matrix free Ca^{2+} has been reported [6]. Also, isolated rat liver nuclei contain an enzyme named a DNA endonuclease with activity dependent upon Ca^{2+} in the submicromolar range, and Ca^{2+} results in extensive DNA hydrolysis [7, 9]. The role of Ca^{2+} in liver nuclei function, however, needs further investigation.

More recently, it has been demonstrated using a Ca^{2+} electrode technique, that an ATP-dependent Ca^{2+} uptake system exists in isolated rat liver nuclei [10], and that Ca^{2+} is released from the Ca^{2+}-loaded nuclei [10, 11]. The nuclear Ca^{2+} transport system in the liver may play an important role in the regulation of Ca^{2+} homeostasis in the nuclei. The regulatory factor in the liver nuclear Ca^{2+} transport system, however, is poorly understood. It has been reported that arachidonic acid (AA)† can induce Ca^{2+} mobilization from rat liver microsomes [12]. Therefore, the present investigation was undertaken to clarify the effect of AA, which is generated by hormonal stimulation, on the Ca^{2+} transport system in isolated rat liver nuclei. It was found that AA uniquely inhibits Ca^{2+} uptake and stimulates Ca^{2+} release in the liver nuclei.

MATERIALS AND METHODS

Chemicals. ATP, AA, prostaglandin E_2 (PGE_2), prostaglandin H_2 (PGH_2), thromboxane B_2 (TXB_2), 5-hydroxy-eicosatetraenoic acid (5-HETE), leukotriene A_4 methyl ester (LTA_4), D-*myo*-inositol-1,4,5-triphosphate [Ins(1,4,5)P_3], and dibutyryl adenosine-3′,5′-cyclic monophosphate (dibutyryl cAMP) were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). $CaCl_2 \cdot 2H_2O$ and other reagents were purchased from the Wako Pure Chemical Co. (Osaka, Japan). The reagents were dissolved in distilled water which passed through ion-exchange resin to remove metal ions. AA and its related compounds were dissolved in ethanol and hexane.

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.* [7] with a minor modification. Rats were killed by cardiac puncture, and the livers were perfused with approximately

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† Abbreviations: AA, arachidonic acid; PGE_2, prostaglandin E_2; PGH_2, prostaglandin H_2; TXB_2, thromboxane B_2; 5-HETE, 5-hydroxy-eicosatetraenoic acid; LTA_4, leukotriene A_4 methyl ester; DTT, dithiothreitol; Ins(1,4,5)P_3, D-*myo*-inositol-1,4,5-triphosphate; dibutyryl cAMP, dibutyryl adenosine-3′,5′-cyclic monophosphate; and EGTA, ethyleneglycol-bis-(aminoethylether)*N*,*N*′-tetraacetic acid.

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*′-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 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 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 (glucose-6-phosphatase, 5′-nucleotidase, succinate dehydrogenase) 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 [13].

Ca^{2+} *transport assay.* Ca^{2+} uptake and release were determined with a Ca^{2+} electrode [10, 14]. A reaction mixture (5.0 mL) composed of 100 mM KCl, 20 mM Hepes, pH 6.8, 5.0 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 (neutralized with KOH) 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 by the addition of 2.0 mM ATP. Various agents were added to the incubation medium 6 min after the addition of 2.0 mM ATP and then Ca^{2+} release was measured for 10 min. Ca^{2+} uptake and release are expressed as nanomoles of total Ca^{2+} per milligram 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.

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. The uptake was saturated 6 min after ATP addition.

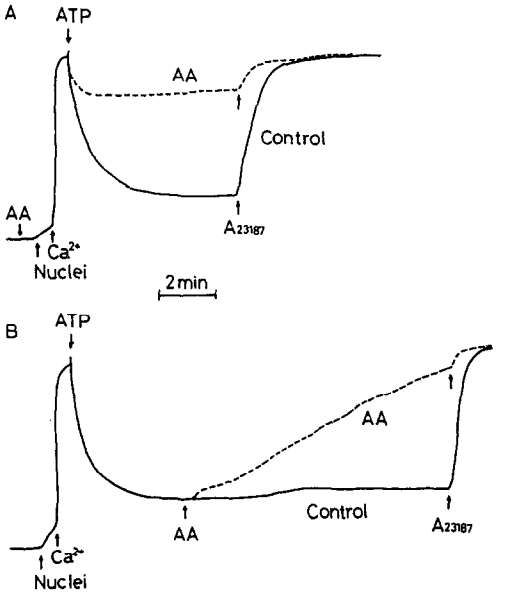

Fig. 1. Influence of the time of addition of arachidonic acid (AA) on Ca^{2+} uptake and release in rat liver nuclei. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode. The reaction system contained 5 mL of Ca^{2+} transport medium which consisted of 100 mM KCl, 20 mM Hepes, pH 6.8, 5 mM $MgCl_2$, 40 μM $CaCl_2$, and 2.0 mM ATP. AA (final concentration 100 μM) was added at about 1.0 min before the addition of 2.0 mM ATP (A) or at 6 min after ATP addition (B). The pH of the reaction system was not changed when AA was added.

Ca^{2+} sequestration was dependent on the concentration of ATP; maximum uptake was reached with 1.0 mM ATP. ATP-independent Ca^{2+} accumulation was not directly related to the level of free Ca^{2+} in the incubation medium and was negligible.

The time-dependent effect of arachidonic acid (AA) on Ca^{2+} uptake and release in the nuclei isolated from rat liver is shown in Fig. 1. When AA (100 μM) was included in the incubation mixture before ATP addition, an inhibition of Ca^{2+} uptake following ATP addition was seen at an early time point (Fig. 1A). Meanwhile, when AA was added 6 min after ATP addition, Ca^{2+} release increased progressively (Fig. 1B). Thus, AA had an inhibitory effect on Ca^{2+} uptake by the nuclei, and it also had a stimulatory effect on Ca^{2+} release from the nuclei.

The effect of increasing concentrations of AA on Ca^{2+} uptake and release in the nuclei isolated from rat liver is shown in Fig. 2. The presence of AA (10–100 μM) in the incubation medium caused a significant inhibition of nuclear Ca^{2+} uptake after the addition of 2.0 mM ATP. When AA was added at the time point (6 min) at which Ca^{2+} uptake was saturated by the presence of 2.0 mM ATP in the incubation medium, Ca^{2+} release increased linearly with the increase in AA concentration (10–100 μM). An appreciable effect of AA on Ca^{2+} release was observed at a 10 μM concentration. At 100 μM AA, Ca^{2+} uptake was decreased to about 40% of the

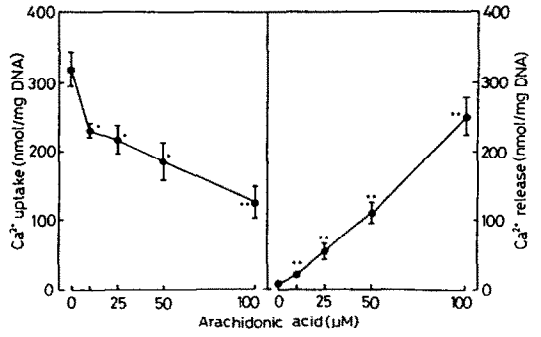

Fig. 2. Effect of arachidonic acid (AA) on Ca^{2+} uptake and release in rat liver nuclei. Ca^{2+} uptake and release were determined with a Ca^{2+} electrode as described in the legend to Fig. 1. The total Ca^{2+} uptake and release were determined by the difference between Ca^{2+} concentrations before and after A23187 addition (final concentration 5 μM). Ca^{2+} uptake in the presence of AA in the incubation mixture was measured at 6 min after ATP addition. Ca^{2+} release was observed for 10 min following AA addition at 6 min after ATP addition. Data are the means ± SEM of five separate experiments with different nuclear preparations. Key: (*) $P < 0.05$, and (**) $P < 0.01$ as compared with the control (none) values.

Table 1. Effects of leukotriene A_4 (LTA_4) and 5-hydroxy-eicosatetraenoic acid (5-HETE) on Ca^{2+} uptake and release in isolated rat liver nuclei

Treatment	Ca^{2+} uptake (nmol/mg DNA)	Ca^{2+} release (nmol/mg DNA)
Control	327.7 ± 15.3	12.0 ± 4.0
LTA_4, 1 μM	315.2 ± 41.9	12.3 ± 3.8
LTA_4, 4 μM	279.8 ± 8.2	12.3 ± 4.1
5-HETE, 1 μM	303.2 ± 2.6	15.7 ± 3.1
5-HETE, 4 μM	250.5 ± 12.0*	42.8 ± 9.5*

Ca^{2+} uptake and release were determined with a Ca^{2+} electrode. The Ca^{2+} uptake took 6 min after addition of 2.0 mM ATP in the presence of various agents. These agents were added to the incubation medium at 6 min after 2.0 mM ATP addition, and then the Ca^{2+} release was measured for 10 min. Data are the means ± SEM of four separate experiments using different preparations of liver nuclei.

* $P < 0.01$, as compared with the control value.

control value, and the reagent released about 80% of Ca^{2+} accumulated into the nuclei after ATP addition.

The effects of prostaglandins and TXB_2 on the Ca^{2+} transport system in rat liver nuclei were examined. The presence of PGE_2 (10 and 20 μM), PGH_2 (1 and 4 μM) or TXB_2 (1 and 4 μM) in the incubation mixture before ATP addition had no effect on nuclear Ca^{2+} uptake following ATP addition (data not shown). When these reagents were added to Ca^{2+}-loaded nuclei, Ca^{2+} release was not seen (data not shown). These results indicate that PGE_2, PGH_2 and TXB_2 did not have an appreciable effect on the Ca^{2+} transport system in isolated rat liver nuclei.

The effects of leukotriene A_4 (LTA_4) and 5-hydroxy-eicosatetraenoic acid (5-HETE) on the Ca^{2+} transport system in rat liver nuclei are shown in Table 1. LTA_4 (1 and 4 μM) did not have an appreciable effect on Ca^{2+} uptake and release in liver nuclei. On the other hand, 5-HETE at 4 μM caused a significant inhibition of nuclear Ca^{2+} uptake and it induced an appreciable increase in Ca^{2+} release from the Ca^{2+}-loaded nuclei. Such effects of 5-HETE were not seen at the 1 μM concentration.

The interaction of PGE_2 or dithiothreitol (DTT) on the AA-induced alteration of the Ca^{2+} transport system in rat liver nuclei is shown in Table 2. The presence of PGE_2 (10 μM) or DTT (100 μM) in the incubation mixture before ATP addition did not have an appreciable effect on the AA (25 and 100 μM)-induced inhibition of nuclear Ca^{2+} uptake. The stimulatory effect of AA (25 and 100 μM) on Ca^{2+} release from the Ca^{2+}-loaded nuclei was not altered by the addition of PGE_2 (10 μM) or DTT (100 μM).

The effects of Ins(1,4,5)P_3 and dibutyryl cAMP on the Ca^{2+} transport system in rat liver nuclei also were examined with a Ca^{2+} electrode. Ca^{2+} uptake took 6 min after the addition of 2.0 mM ATP in the presence of these agents. The agents were added to the incubation medium at 6 min after 2.0 mM ATP addition, and then Ca^{2+} release was measured for 10 min. The presence of Ins(1,4,5)P_3 (1–10 μM) or dibutyryl cAMP (10 and 50 μM) in the incubation mixture before ATP addition had no effect on Ca^{2} uptake by liver nuclei (data not shown). Also, the addition of Ins(1,4,5)P_3 (1–10 μM) or dibutyryl cAMP (10 and 50 μM) did not stimulate Ca^{2+} release from the Ca^{2+}-loaded nuclei (data not shown). Thus, the factors, which are generated by hormonal stimulation, did not have an appreciable effect on the Ca^{2+} transport system in rat liver nuclei. AA had a unique effect on liver nuclear Ca^{2+} transport.

DISCUSSION

It has been reported recently, that an ATP-stimulated Ca^{2+} sequestration system exists in rat liver nuclei [6, 15]. This system may play an important role in the regulation of intranuclear Ca^{2+}-dependent processes [3–9]. With a Ca^{2+} electrode technique, the more recent investigations clearly demonstrate that the ATP-dependent Ca^{2+} uptake and the Ca^{2+} release system from Ca^{2+}-loaded nuclei exist in the nuclei isolated from rat liver [10, 11]. Furthermore, the present study was undertaken to clarify the effect of regulatory factors on the Ca^{2+} transport system in rat liver nuclei. It was found that AA can inhibit nuclear Ca^{2+} uptake and stimulate Ca^{2+} release from Ca^{2+}-loaded nuclei in rat liver. This finding suggests that AA plays a role in the regulation of intracellular Ca^{2+} homeostasis in rat liver.

The effects of other metabolites related to AA metabolism on the Ca^{2+} transport system in rat liver nuclei were examined. PGE_2 (10 and 20 μM), PGH_2 (1 and 4 μM), TXB_2 (1 and 4 μM) and LTA_4 (1 and 4 μM) did not have an appreciable effect on Ca^{2+} uptake and release in isolated rat liver nuclei. 5-HETE at 4 μM caused a significant inhibition of nuclear Ca^{2+} uptake and stimulation of Ca^{2+} release

Table 2. Interaction of prostaglandin E_2 (PGE_2) or dithiothreitol (DTT) on arachidonic acid (AA)-induced alteration of Ca^{2+} uptake and release in isolated rat liver nuclei

Treatment	Ca^{2+} uptake (nmol/mg DNA)	Ca^{2+} release (nmol/mg DNA)
Control	323.7 ± 19.7	11.5 ± 1.4
AA (25 μM)	215.2 ± 11.5*	59.0 ± 6.8*
AA (100 μM)	94.8 ± 6.4*	167.8 ± 26.9*
AA (25 μM) + PGE_2 (10 μM)	222.2 ± 27.8*	73.4 ± 12.1*
AA (100 μM) + PGE_2 (10 μM)	98.6 ± 20.9*	215.5 ± 10.4*
PGE_2 (10 μM)	358.7 ± 19.5	11.3 ± 4.4
AA (25 μM) + DTT (100 μM)	180.4 ± 6.8*	67.6 ± 9.5*
AA (100 μM) + DTT (100 μM)	80.2 ± 8.5*	134.5 ± 12.5*
DTT (100 μM)	327.0 ± 32.4	11.5 ± 1.9

Ca^{2+} uptake and release were determined with a Ca^{2+} electrode. The Ca^{2+} uptake took 6 min after the addition of 2.0 mM ATP in the presence of various agents. These agents were added simultaneously to the incubation medium at 6 min after 2.0 mM ATP addition, and then Ca^{2+} release was measured for 10 min. Data are the means ± SEM of five separate experiments of liver nuclei.

* $P < 0.01$, as compared with the control.

from the Ca^{2+}-loaded nuclei, although the 1 μM concentration had no effect on the Ca^{2+} transport system in the nuclei. There may be a higher concentration of AA in comparison with that of other metabolites involved in AA metabolism in liver cells. Of various prostanoids, AA may uniquely regulate the Ca^{2+} transport system in liver nuclei.

The AA-induced inhibition of Ca^{2+} uptake and stimulation of Ca^{2+} release in rat liver nuclei were not altered appreciably by the presence of PGE_2 with a chemical structure similar to that of AA. This suggests that AA has a specific effect on the membranes of liver nuclei. Meanwhile, DTT did not influence nuclear Ca^{2+} transport in the presence of AA. It is known that DTT can protect both inhibition of Ca^{2+} uptake and stimulation of Ca^{2+} release induced by the SH group-modifying reagents [11, 14]. Presumably, the effect of AA on nuclear Ca^{2+} transport does not involve the SH groups of the membranous proteins in liver nuclei, although the binding sites of AA may be on nuclear membranes. The mechanism by which AA acts on Ca^{2+} uptake and release in liver nuclei, however, remains to be elucidated.

The second messengers, Ins(1,4,5)P_3 and cAMP, which are generated by hormonal stimulation did not have an appreciable effect on Ca^{2+} uptake and release in isolated rat liver nuclei. Ins(1,4,5)P_3 can stimulate Ca^{2+} release from rat liver microsomes [16, 17]. More recently, it was reported that Ins(1,4,5)P_3 can cause Ca^{2+} release from isolated rat liver nuclei [18], although the effect is temporal and weak. In the present study, addition of Ins(1,4,5)P_3 (1 and 10 μM as final concentrations) had no effect on nuclear Ca^{2+} uptake and release. We do not have the explanation of this discrepancy of Ins(1,4,5)P_3 effect on nuclear Ca^{2+} release. However, in the experiment of Nicotera *et al.* [18], Ins(1,4,5)P_3 (5 μM) was added in the incubation mixture at a time point when the nuclear Ca^{2+} uptake was not completely saturated. Further investigation may be needed to clarify the effect of Ins(1,4,5)P_3 on Ca^{2+} release from liver nuclei. Moreover, dibutyryl cAMP (10 and 50 μM) did not cause a significant alteration of nuclear Ca^{2+} uptake and release. The data presented demonstrate that Ins(1,4,5)P_3 and cAMP do not have an effect on the Ca^{2+} transport system in isolated rat liver nuclei.

The activation of phospholipase A_2 induced by hormonal stimulation generates AA from the phospholipids of hepatic plasma membrane. Since AA stimulates Ca^{2+} release from rat liver microsomes [12] and it induces both nuclear Ca^{2+} uptake inhibition and Ca^{2+} release stimulation, AA may have a potent effect on the cytosolic Ca^{2+} level in liver cells. In addition, the nuclear effect of AA may contribute to the elevation of the cytosolic Ca^{2+} concentration in liver cells following hormonal stimulation. Presumably, AA is important as a regulatory factor of intracellular Ca^{2+} metabolism in liver cells.

In conclusion, the present investigation clearly demonstrates that AA has an inhibitory effect on Ca^{2+} uptake and a stimulatory effect on Ca^{2+} release in rat liver nuclei. AA may play a role in the regulation of Ca^{2+} transport in liver nuclei.

Acknowledgement—This work was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 04671362) from the Ministry of Education, Japan.

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