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Influences of Divalent Cations and Membrane Phosphorylation Inhibitors on Na^+-Ca^{++} Exchange in Synaptosomes

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ABSTRACT

Verapamil, tetrodotoxin and tetraethylammonium chloride in the stated amount did not affect the Na^+ induced Ca^{++} release. Cd^{++} and Zn^{++} significantly inhibited the Na^+ induced Ca^{++} release. Mn^{++} also inhibited Na^+-Ca^{++} exchange. Cd^{++} inhibited Na^+-Ca^{++} exchange noncompetitively with an apparent inhibition constant (Ki) of 100 μM. Cd^{++} caused loss of sulfhydryl group, whereas Zn^{++} did not show any significant effect. Cd^{++} and Zn^{++} effectively inhibited Na^+-Ca^{++} ATPase and slightly inhibited Ca^{++}-Mg^{++} ATPase. Carbonyl cyanide chlorophenylhydrazone, 2, 4-dinitrophenol and sodium arsenate stimulated the Na^+ induced Ca^{++} release. Dibucaine and oligomycin slightly inhibited it.

The results suggest that the Na^+-Ca^{++} exchange on the synaptosomal plasma membrane may be not accomplished by ion channels. The Na^+-Ca^{++} exchange is sensitively inhibited by Cd^{++} and this transport process appears to be partially regulated by sulfhydryl groups of the synaptosomal plasma membrane.

It is also postulated that Na^+-Ca^{++} exchange is suppressed during the phosphorylation reaction of protein component on the neuronal membrane.

Key Words: Divalent cations, Membrane phosphorylation inhibitors, Na^+-Ca^{++} exchange at synaptosomes

INTRODUCTION

A rise in the concentration of internal ionized calcium may be requisite for the release of neurotransmitters at nerve terminals (Krnjević, 1974). The release process is normally triggered by Ca^{++} influx through Ca^{++} channels that open in response to depolarization of the plasma membrane (Nachshen and Blaustein, 1980; Cho *et al.*, 1988). The rapid termination of neurotransmitter release, with a time constant of < 1 ms, is probably ascribed to the reduction of internal calcium concentration and not to inactivation of the release process itself (Katz and Miledi, 1968; Llinas *et al.*, 1976).

In order to remain in steady state calcium balance, Ca^{++} which enters the terminals during activity must be subsequently extruded. Extrusion of Ca^{++} against its concentration gradient is partially accomplished by a Na^+-Ca^{++} exchange system that utilizes energy derived from the sodium electrochemical gradient to exchange internal Ca^{++} for external Na^+ and by a Ca^{++}-Mg^{++} ATPase which utilizes ATPase at the plasma membrane (Goddard and Robinson, 1976). In addition, the intracellular calcium concentration can be lowered by the action of intracellular calcium storage sites, such as mitochondria (Reichardt and Kelly, 1983).

In nerve terminals, it is uncertain which system plays a major role in $[Ca^{++}]i$ regulation and the relative roles of Na^+-Ca^{++} ATPase and Ca^{++}-Mg^{++} ATPase in the extrusion of intracellular calcium is unknown. However, some reports have suggested that role of Na^+-Ca^{++} exchange is greater than that of mitochondria (Sanchez-

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Armass and Blaustein, 1987). On the other hand, it is well established that mitochondria and Ca^{++}-Mg^{++} ATPase play a critical role in the regulation of $[Ca^{++}]i$ in heart muscle (Carafoli and Crompton, 1978) and red blood cell (Lee and Shin, 1969).

The Na^+-Ca^{++} exchange appears to be implicated in the process of neurotransmitter release at nerve ending in the squid giant synapse and frog neuromuscular junction (Atwood *et al.*, 1983; Misler and Hurlbut, 1983). It is suggested that the Na^+ electrochemical gradient influences Ca^{++} dependent transmitter release. The Na^+-Ca^{++} exchange at the neuronal membrane is mediated by carrier, which are known to have a stoichiometry of either 3 Na^+/Ca^{++} or even 4 Na^+/Ca^{++} (Mullins, 1981). However, character or property of carrier itself is still not clarified. In addition, factors which affect the carrier protein are somewhat unknown.

Thus, in the present study, effects of specific ion channel blockers, divalent cations, uncouplers, calmodulin inhibitor and Na^+-K^+ ATPase inhibitor on Na^+-Ca^{++} exchange at rat cerebral synaptosomes were investigated. Influences of divalent cations on synaptosomal Na^+-Ca^{++} ATPase activities and sulfhydryl group were also examined.

MATERIALS AND METHODS

Chemicals

Diltiazem, verapamil, tetrodotoxin, tetraethylammonium chloride, calcium chloride, $ZnCl_2$, 2, 4-dinitrophenol, carbonyl cyanide m-chlorophenylhydrazone (CCCP), sodium arsenate, dibucaine, oligomycin, ouabain and arsenazo III were purchased from Sigma Chemical Co. KCl was obtained from E. Merck Co.; $CaCl_2$ and NaCl from Kanto Chemical Co., Inc.; Maleic acid from Junsei Chemical Co., Ltd.; ^{45}Ca from DuPont., NEN products, Boston Mass. All chemicals were of analytic reagent grade.

1. Preparation of cerebral synaptosomes

Synaptosomal particles were prepared from rat cerebrum according to the method of Hajős (1975). Male Sprague-Dawley rats weighing about 150 ~200 g were used. The animals were killed by decapitation. After removing blood clot, the brains were placed in 9 volume of cold 0.3 M sucrose and homogenized with a teflon glass homogenizer. The pellet obtained after centrifugation at 1,500 g for 10 min was washed by resuspension in 0.3 M sucrose of approximately the original volume. Suspension was centrifuged at 1,500 g for 10 min. The supernatants were recentrifuged at 9,000 g for 20 min. The pellet was dispersed in 5 ml of 0.3 M sucrose. The suspension was layered over 20 ml of 0.8 M sucrose and centrifuged at 9,000 g for 25 min.

A synaptosomal fraction was obtained at 0.8 M sucrose layer. The synaptosomal fraction was diluted with 6 volume of diluting solution (contains 145 mM NaCl, 5 mM KCl, 10 mM dextrose, 1.3 mM $MgCl_2$, 20 mM Tris-maleate, pH 7.4) and centrifuged at 15,000 g for 20 min.

The pellet was resuspended in diluting solution for Ca^{++} transport experiments. Protein concentration was determined by the method of Lowry *et al.* (1951).

2. Measurement of Na^+-Ca^{++} exchange in synaptosomes

A) Arsenazo III method: The Na^+ induced Ca^{++} release from synaptosomes were measured through the absorbance change of calcium sensitive dye, arsenazo III, at 675-685 nm with a dual wave-length-split beam spectrophotometer (Aminco Chance dual wavelength-split beam recording spectrophotometer) (Ahmed and Connor, 1979; Åkerman and Heinonen, 1983).

Measurement of Na^+-Ca^{++} exchange in synaptosomes, were done in the reaction mixture containing 2 mg protein/ml synaptosomal particles, 130 mM KCl, 1.3 mM $MgCl_2$, 10 mM dextrose, 20 mM Tris-maleate, pH 7.4, 100 μm arsenazo III and other compound. After 5 min preincubation at 30℃, 10 μl of 9 mM $CaCl_2$ (final 30 μM) was added to reaction medium, and thereby Ca^{++} uptake by synaptosomes was produced. After the maximum Ca^{++} uptake by synaptosomes, calcium release was initiated by addition of 20 mM NaCl and amount of Ca^{++} released was spectrophotometrically measured, and was expressed in nmol Ca^{++} per mg protein.

B) $^{45}Ca^{++}$ method: The Na^+ induced Ca^{++} release from preloaded synaptosomes was measured, with the millipore filter method employing $^{45}Ca^{++}$ (Lee and Choi, 1966). The reaction medium was the same as described in arsenazo III method and contained $^{45}Ca^{++}$ (0.4 μCi/ml) instead of arsenazo III.

The reaction was carried out at 25℃ and at stated times, 0.3 ml sample were taken and filtered

with 0.45 μM milipore filter under negative pressure. 0.1 ml each of filtered samples were placed in vials containing 10 ml of scintillation cocktail and then radioactivities were measured in a Beckman LS 100 C liquid scintillation counter. The amount of released Ca^{++} was measured and expressed in nmol of Ca^{++} per mg protein.

3. Measurement of ATPase activity

Synaptosomal particles (0.1 mg/ml) were incubated in the reaction medium containing 50 mM imidazole-HCl, pH 7.4; 120 mM NaCl, 2 mM $CaCl_2$, 0.1 mM ouabain for Na^+-Ca^{++} ATPase; 5 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.1 mM ouabain for Ca^{++}-Mg^{++} ATPase.

After 10 min preincubation at 37℃, the reaction was initiated by addition of 0.1 ml of 20 mM Tris-ATP (final 2 mM) and the total volume was 1.0 ml. After 10 min incubation at 37℃, the reaction was stopped with 1.0 ml of 15% cold trichloroacetic acid and the mixture was centrifuged at 1,000 g for 10 min. One ml of the supernatant was used for determination of Pi according to the method of Horwitt (1952). ATPase activities were expressed in μmol Pi liberated per mg protein per hour.

4. Measurement of sulfhydryl groups in synaptosomes

The sulfhydryl (SH) groups was estimated by measuring sulfhydryl-DTNB complex. Measurement of SH groups was done in 1.0 ml reaction medium containing 120 mM NaCl, 50 mM imidazole-HCl, pH 7.4, 2 mM $CaCl_2$, 1 mg/ml protein. The reaction was started by addition of synaptosome.

The reaction was incubated at 37℃ for 20 min and was stopped by addition of 2.0 ml, 0.5% SDS in 100 mM Tris-HCl, pH 8.0. The reaction medium was added by 20 μl of 10 mM DTNB in water bath at 37℃ for 10 min. The SH groups was measured at 412 nm and expressed in nmol/mg protein, using the molar extinction coefficient for p-nitrothiophenol of 1.36×10^4/M/cm (Ellman, 1959).

RESULTS

Effects of specific ion channel blocker on the Na^+-Ca^{++} exchange

Synaptosomes have the membrane potential and can accumulate calcium when triggered by depolarizing agents, such as K^+ and veratridine (Blaustein, 1975). In the present study, Ca^{++} loaded at synaptosomes was made by addition of K^+. Ca^{++} efflux from the Ca^{++} loaded synaptosomes was produced by addition of Na^+. As shown in Fig. 1, extracellular Na^+ induced Ca^{++} release from the Ca^{++} loaded synaptosomes in a dose dependent manner. At 20 mM Na^+, the amount of Ca^{++} released was 1.081 nmol/5 min/mg protein.

To explore the character of carrier-mediated exchange of Na^+ for Ca^{++}, effects of specific ion channel blockers on the Na^+-Ca^{++} exchange were investigated. It postulates that Na^+-Ca^{++} exchange is not accomplished through ion channels, because Ca^{++} channel blockers, diltiazem and verapamil, Na^+ channel blockers, tetrodotoxin and K^+ channel blocker, tetraethylammonium chloride in the

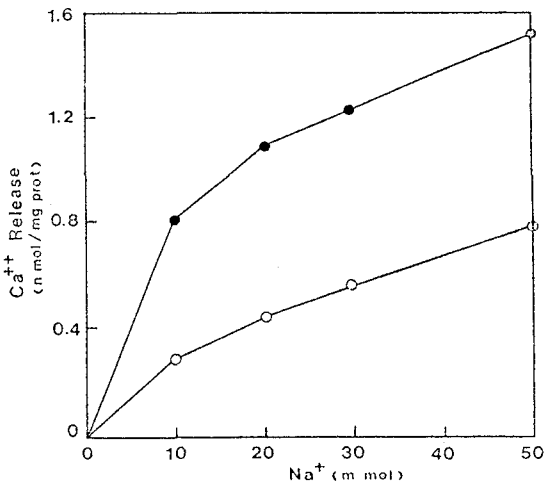

Fig. 1. Na^+ induced Ca^{++} release from preloaded synaptosomes. Na^+ dependent Ca^{++} release from the preloaded synaptosomes was measured through the absorbance changes of calcium sensitive dye, arsenazo III, at 675~685 nm. The reaction medium contained 2 mg/ml of synaptosomes, 130 mM KCl, 1.3 mM $MgCl_2$, 10 mM dextrose, 20 mM Tris-maleate pH 7.4, 30 μM $CaCl_2$ and 100 μM arsenazo III. Ca^{++} release from the preloaded synaptosomes was produced by addition of various concentration of Na^+. Each point represents an average of 5~7 experiments. O, Ca^{++} release during 1 min; ●, Ca^{++} release during 5 min.

Table 1. Effects of specific ion channel blockers on the Na^+ dependent Ca^{++} release

Compounds		Na^+ dependent Ca^{++} release (nmol/mg prot)	
		During 1 min	During 5 min
None		0.44±0.02	1.08±0.03
Diltiazem	100 μM	0.43±0.00	1.00±0.02
Verapamil	100 μM	0.43±0.01	0.98±0.02
TTX	10 μM	0.43±0.02	1.01±0.01
TEA	100 μM	0.41±0.01	1.05±0.03

The values were estimated from experiments as in Fig. 1. The reaction medium contained 2 mg/ml synaptosome, 130 mM KCl, 1.3 mM $MgCl_2$, 10 mM dextrose, 20 mM Tris-maleate, pH 7.4, 30 μM $CaCl_2$ and 100 μM arsenazo III with various agents.
Each value represents mean±S.E. of 4 experiments. The agent were present during preincubation period.

amount used all did not affect the Na^+ induced Ca^{++} release (Table 1).

Inhibition of the Na^+-Ca^{++} exchange by Cd^{++}, Mn^{++} and Zn^{++}

Divalent cations are known to block Ca^{++} channels in various systems (Nachshen, 1984) and among them, Mg^{++}, Mn^{++} and Zn^{++} also inhibit the Na^+-Ca^{++} exchange (Coutinho *et al.*, 1984). Cd^{++} has been shown to block tetrodotoxin (TTX)-resistant Na^+ fluxes in cardiac cells and skeletal muscle cells (Frelin *et al.*, 1986).

The result represented in Table 2 showed that Cd^{++} and Zn^{++} significantly inhibited the Na^+-Ca^{++} exchange, and at 0.1 mM, they inhibited Na^+ induced Ca^{++} release as much as 33~49% after 5 min incubation. Mn^{++} also inhibited the Na^+-Ca^{++} exchange. On the other hand, in the Na^+ free media, these metal ions did not cause the Ca^{++} release from Ca^{++} loaded synaptosomes (data not shown).

The inhibition constant for Cd^{++} in the Na^+ induced Ca^{++} release was estimated by a Dixon plot of the data, which were obtained in the presence of 10 mM, 20 mM and 50 mM Na^+ respectively. Fig. 2 showed that Cd^{++} inhibited the Na^+-Ca^{++} exchange noncompetitively with an apparent inhibition contant (Ki) of 100 μM.

Table 2. Inhibition of the Na^+ dependent Ca^{++} release by Cd^{++}, Mn^{++} and Zn^{++}

Compounds		Na^+ dependent Ca^{++} release (nmol/mg prot)			
		During 1 min	△%	During 5 min	△%
None		0.44±0.02		1.08±0.03	
Cd^{++}	100 μM	0.13±0.03	−70	0.55±0.05	−49
	50 μM	0.25±0.03	−43	0.59±0.06	−45
	10 μM	0.44±0.01	0	0.97±0.03	−10
	1 μM	0.44±0.01	0	1.08±0.03	0
Mn^{++}	100 μM	0.31±0.03	−30	0.72±0.06	−33
	50 μM	0.36±0.02	−18	0.84±0.06	−22
	10 μM	0.45±0.02	+ 2	1.06±0.01	− 2
Zn^{++}	50 μM	0.13±0.01	−70	0.29±0.03	−73
	25 μM	0.24±0.01	−45	0.78±0.02	−28

The values were estimated from experiments as in Fig. 1. The reaction medium were the same as described in Table 1. Each value represents mean±S.E. of 4 experiments. The divalent cations were present during preincubation period.

Effects of Cd^{++} and Zn^{++} on the synaptosomal sulfhydryl groups

Since it is well known that tissue sulfhydryl groups play an important role in cellular function and integrity of macromolecules (Torchinskii, 1974), it is suggested that the postulation that oxidoreduction of sulfhydryl group may affect the biological macromolecules including Na^+-Ca^{++} exchanger. Thus, the possibility that divalent cation induced the oxidation sulfhydryl group was examined. As shown in Table 3, Cd^{++} caused loss of sulfhydryl group. On the other hand, Zn^{++} id not show any significant effect.

Effects of Cd^{++} and Zn^{++} on Na^+-Ca^{++} ATPase and Ca^{++}-Mg^{++} ATPase activities

In order to study further the mechanism by which Cd^{++} and Zn^{++} inhibited the Na^+ induced Ca^{++} release, influence of the divalent cations on Na^+-Ca^{++} ATPase and Ca^{++}-Mg^{++} ATPase activity was investigated. As can be seen in Table 4 and Table 5, Cd^{++} and Zn^{++} effectively inhibited Na^+-Ca^{++} ATPase and slightly inhibited Ca^{++}-Mg^{++} ATPase.

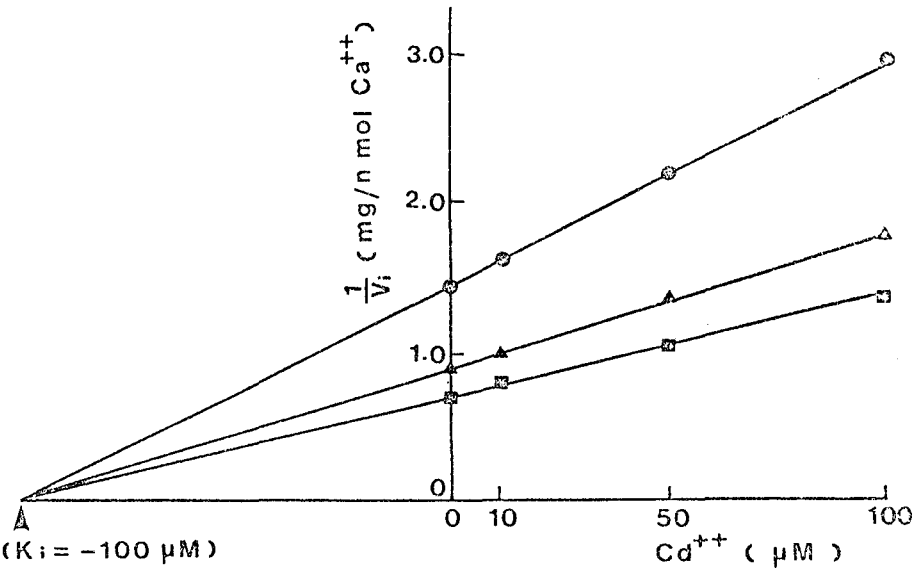

Fig. 2. Na^+ and Cd^{++} interaction on Na^+-Ca^{++} exchange. Dixon plot analysis for the inhibiting action of Cd^{++} on the Na^+ induced Ca^{++} release from the preloaded synaptosomes. The reaction medium were the same as described in Fig. 1. and contained 0.4 μCi $^{45}Ca^{++}$ instead of arsenazo III. Ca^{++} release was measured with the millipore filter method. Each point represents mean of 4 experiments. Ki is an apparent inhibition constant. Vi is an amount of Ca^{++} released during 5 min incubation. 10 mM (●), 20 mM (▲) and 50 mM (■) of Na^+.

Table 3. Oxidative action of Cd^{++} and Zn^{++} on sulfhydryl groups of synaptosomes

Compounds		Sulfhydryl content (nmol/mg prot)	△%
None		42.53±0.26	
Cd^{++}	100 μM	33.60±0.31	−21
	10 μM	37.00±0.11	−13
Zn^{++}	100 μM	40.30±0.31	− 5
	50 μM	40.07±0.15	− 6

The reaction medium contained 120 mM NaCl, 50 mM imidazole-HCl pH 7.4, 2 mM $CaCl_2$, 1 mg/ml synaptosome and divalent cations, and reaction was performed for 20 min at 37℃. Sulfhydryl content of intact synaptosome is represented as 100%. Each value represents mean±S.E. of 5 experiments.

Table 4. Inactivation of Na^+-Ca^{++} ATPase by Cd^{++} and Zn^{++}

Compounds		Activity μmol Pi/mg prot/hr	△%
None		6.50±0.30	
Cd^{++}	100 μM	4.08±0.31	−37
	10 μM	4.26±0.23	−34
Zn^{++}	50 μM	3.58±0.31	−45
	25 μM	3.84±0.37	−41

The reaction medium contained 120 mM NaCl, 2 mM $CaCl_2$, 1 mM ouabain and divalent ions. Each value represents mean±S.E. of 5 experiments.

Stimulation of the Na^+-Ca^{++} exchange by inhibitors of membrane phosphorylation and ouabain

To examine the possibility that phosphorylation of membrane protein may be involved in the Na^+-Ca^{++} exchanging process, effects of phosphorylation inhibitors on the Na^+ induced Ca^{++} release were observed.

Table 6 showed that carbonyl cyanide chlorophenylhydrazone, 2, 4-dinitrophenol and sodium arsenate stimulated the Na^+ induced Ca^{++} release.

Dibucaine, a calmodulin inhibitor and oligomycin, a inhibitor of ATP hydrolysis slightly inhibited the Na^+ induced Ca^{++} release, whereas

Table 5. Effects of Cd^{++} and Zn^{++} on Ca^{++}-Mg^{++} ATPase activity

Compounds		Activity	
		μmol Pi/mg prot/hr	△%
None		3.52±0.10	
Cd^{++}	100 μM	2.77±0.12	−21
	10 μM	3.35±0.05	− 5
Zn^{++}	50 μM	3.22±0.08	− 9
	25 μM	3.35±0.10	− 5

The reaction medium contained 5 mM $MgCl_2$ 2 mM $CaCl_2$, 1 mM ouabain and divalent cations (Cd^{++} and Zn^{++}). Each value represents mean±S.E. of 5 experiments.

Table 6. Stimulation of the Na^+ dependent Ca^{++} release by carbonyl cyanide chlorophenylhydrazone (CCCP), 2, 4-dinitrophenol and sodium arsenate

Compounds	Na^+ dependent Ca^{++} release (nmol/mg prot)			
	During 1 min	△%	During 5 min	△%
None	0.44±0.22		1.08±0.03	
CCCP				
100 μM	1.21±0.03	+175	2.17±0.02	+101
10 μM	0.69±0.02	+ 57	1.40±0.02	+ 30
Sodium arsenate				
100 μM	0.59±0.03	+34	1.29±0.03	+19
10 μM	0.54±0.08	+23	1.22±0.03	+13
2,4-dinitrophenol				
100 μM	0.59±0.02	+34	1.24±0.04	+15
10 μM	0.56±0.02	+27	1.14±0.05	+ 6

The values were estimated from experiments as in Fig. 1. The reaction medium were the same as described in Table 1. Each value represents mean±S.E. of 4 experiments. Agents were present during preincubation period.

ouabain slightly stimulated it (Table 7).

DISCUSSION

Synaptosomes which retain considerable degree of functional integrity have the membrane potential, and can accumulate Ca^{++} and release neurotransmitters when triggered by depolarizing agents, such as K^+ and veratridine (Blaustein, 1975). The K^+ depolarization has been the more common method to alter membrane potential with concomitant entry of Ca^{++} (Åkerman and Nicholls, 1981). As in the case of the other neuronal tissues including squid axons, the efflux of Ca^{++} from preloaded synaptosomes also requires the presence of Na^+ in bathing medium (Blaustein and Oborn, 1975).

Table 7. Effects of dibucaine, oligomycin and ouabain on the Na^+ dependent Ca^{++} release

Compounds	Na^+ dependent Ca^{++} release (nmol/mg prot)			
	During 1 min	△%	During 5 min	△%
None	0.44±0.02		1.08±0.03	
Dibucaine				
100 μM	0.37±0.03	−16	0.94±0.05	−13
10 μM	0.43±0.02	− 2	0.96±0.02	−11
Oligomycin				
100 μM	0.38±0.02	−14	0.97±0.02	−10
10 μM	0.40±0.02	− 9	1.05±0.00	− 3
Ouabain				
100 μM	0.52±0.01	+18	1.35±0.01	+25
10 μM	0.41±0.04	− 7	1.19±0.03	+10

The values were estimated from experiments as in Fig. 1. The reaction medium were the same as described in Table 1. Each value represents mean±S.E. of 4 experiments. Agents were present during preincubation period.

This finding was investigated in the present study (Fig. 1), and in the presence of 20 mM of Na^+ about 35% of K^+ depolarization induced Ca^{++} uptake was released (data not shown). Without extracellular Na^+, spontaneous Ca^{++} release from preloaded synaptosomes was not detectable.

Neuronal membranes appear to possess both the Na^+-Ca^{++} exchange and the ATP-dependent Ca^{++} transport mechanisms (Goddard and Robinson, 1976). Some experiments have indicated that the Na^+-Ca^{++} exchange mechanism plays a major role in the regulation of elevated intracellular calcium and mitochondria have a relatively minor role (Sanchez-Armass and Blaustein, 1987). On the other hand, the relative roles of Na^+-Ca^{++} exchange and Ca^{++}-Mg^{++} ATPase in the extrusion of intracellular calcium are still unknown. The Na^+-Ca^{++} exchange is known as a carrier mediated process and utilizes energy from the Na^+ electrochemical gradient, in which the Ca^{++} efflux may involve an exchange of three Na^+ for one Ca^{++} (Blaustein and Nelson, 1982).

It is suggested that Na^+-Ca^{++} exchange plays an important role in the extrusion of the Ca^{++} that enters during activity. The Na^+-Ca^{++} exchange can be inhibited by La^{+++}, Mn^{++} and Zn^{++} (Blaustein and Ector, 1976), and these ions are also known to block the Ca^{++} channel. However, properties of Na^+-Ca^{++} exchanger and influences of certain factors on this transport system are still not clarified. Thus, effects of specific ion channel blockers, uncouplers of oxidative phosphorylation and divalent cations on the Na^+-Ca^{++} exchange were investigated in this study.

The Na^+ dependent Ca^{++} release from preloaded synaptosomes was not affected by verapamil, tetrodotoxin and tetraethylammonium chloride. Accordingly, Table 1 suggests that Na^+-Ca^{++} exchange may not be mediated through voltage sensitive Ca^{++}, Na^+ and K^+ channel.

On the other hand, divalent cations, such as Cd^{++}, Mn^{++} and Zn^{++} which are known to block Ca^{++} channels, effectively inhibited the Na^+ induced Ca^{++} release.

In the Na^+ free medium, these cations did not cause Ca^{++} release from preloaded synaptosomes (data not shown). Inhibitory effect of Mn^{++} and Zn^{++} on Na^+-Ca^{++} eschange is in agreement with previous studies in synaptosomes from sheep brain cortex (Coutinho *et al.*, 1984). If Na^+-Ca^{++} exchange is accomplished through ion channels, particularly Ca^{++} channels, effect of organic Ca^{++} channel blockers such as verapamil and that of divalent cation on Na^+-Ca^{++} exchange would be same, but their influences as observed in this study were different. Thus, in order to explain the discrepancy between the actions of organic Ca^{++} channel blockers and divalent cations on Na^+-Ca^{++} exhange, effect of Cd^{++} on the Na^+ binding site on the synaptosomes was examined, because the Na^+-Ca^{++} exhange is regarded as a carrier mediated transport and Cd^{++} appears to inhibit the Ca^{++} uptake by bovine aortic microsomes (Sumida *et al.*, 1986).

As can be seen in Fig. 2, Cd^{++} noncompetitively inhibited the Na^+ induced Ca^{++} release with an apparent inhibition constant (Ki) of 100 μM. Accordingly, it is suggested that Cd^{++} binds to a site other than Na^+ binding site and then indirectly inhibits Na^+ binding to synaptosomes. This finding further suggests that Na^+-Ca^{++} exchange is probably not associated with ion channels. Since it is reported that inhibitory action of Cd^{++} and Hg^{++} on microsomal Ca^{++} uptake is effectively abolished by thiol reagents, dithiothreitol and cysteine (Sumida *et al.*, 1986), the Cd^{++} induced oxidation of sulfhydryl groups was investigated. The result represented in Table 3 indicates that inhibition of the Na^+ induced Ca^{++} release by Cd^{++} may be partially attributed to oxidation of the reduced form of sulfhydryl groups of synaptosomal protein.

The inhibitory action mechanism of Cd^{++} on the Na^+ induced Ca^{++} release was further elucidate with respect to its effect on the Na^+-Ca^{++} ATPase and Ca^{++}-Mg^{++} ATPase activities. Cd^{++} and Zn^{++} may inhibit the Na^+ induced Ca^{++} release by their inhibitory action on Na^+-Ca^{++} ATPase. And since Ca^{++} transport is known to be attained bidirectionally at the plasma membrane, the inhibitory action of Cd^{++} on Ca^{++} "pumping out" ATPase may also explain its action on Ca^{++} efflux.

The increased permeability for Ca^{++} by glutamate and K^+ may be mediated through their actions on membrane phosphorylation (De Lorenzo and Freedman, 1978; Watkins and Evans, 1981; Dunkley *et al.*, 1986). It is observed that glutamate-and K^+-induced Ca^{++} influx at synaptosomes was inhibited by 2, 4-dinitrophenol (Lee *et al.*, 1988). However, the Na^+ dependent Ca^{++} release was stimulated by carbonyl cyanide chlorophenyl hydrazone, 2, 4-dinitrophenol and sodium arsenate, as shown in Table 6. The stimulatory effect of membrane phosphorylation inhibitors on Na^+-Ca^{++} exchange is in agreement with previous report (Gill *et al.*, 1981).

On the other hand, in Na^+ free medium, phosphorylation inhibitors did not cause Ca^{++} release from the preloaded synaptosomes (data not shown). Thus, the results suggest that Na^+-Ca^{++} exchange may be partially inhibited during the phosphorylation reaction of the plasma membrane protein. Accordingly, if the phosphorylation reaction is inhibited under a certain condition, Na^+ dependent Ca^{++} release will be promoted.

Table 7 suggests that the Na^+ dependent Ca^{++} release can be accelerated, when Na^+-K^+ ATPase is inhibited and this process may be energy dependent.

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═국문초록═

이가 양이온과 세포막 인산화 반응의 억제제가 Synaptosome에서의 소듐-칼슘 교환이동에 미치는 영향

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소듐에 의한 칼슘의 유리는 verapamil, TTX, TEA의 영향을 받지 않았다. 100 μM Cd^{++}과 Zn^{++}은 소듐에 의한 칼슘 유출을 유의하게 억제하였다. Cd^{++}은 Ki 100 μM로써 비상경적으로 소듐-칼슘 교환이동을 억제하였다. Cd^{++}은 SH기의 산화를 초래하였으나, Zn^{++}은 거의 영향을 나타내지 않았다. Cd^{++}과 Zn^{++}은 Na^{+}-Ca^{++} ATPase를 효과적으로 억제하였으나 Ca^{++}-Mg^{++} ATPase를 약간 억제시켰다. Carbonyl cyanide chlorophenylhydrazone, 2,4-dinitrophenol과 sodium arsenate는 소듐에 의한 칼슘 유리를 촉진하였다. Dibucaine과 oligomycin은 소듐에 의한 칼슘의 유리를 약간 억제하였으나, 이에 반하여 ouabain은 약간 촉진하였다.

이상의 실험 결과로부터 신경 세포막에서의 소듐-칼슘 교환은 이온 통로를 통하여 이루어지지 않을 것으로 시사되었다. 소듐-칼슘 교환이동은 Cd^{++}에 민감하게 억제되고 이 이동기전에 synaptosome 막의 SH기가 관여할 것으로 사료되었다. 또한 소듐-칼슘 교환은 세포막 단백질 성분의 인산화 반응 동안에 억압될 것으로 추정되었다.