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# Effects of $Cd^{2+}$ on the Contractility in the Antral Circular Muscle of Guinea-pig Stomach

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=ABSTRACT=

**The effects of $Cd^{2+}$ on spontaneous contraction, and the contractures induced by 0mM $Na^+$, 60mM $K^+$ and $10^{-6}$ M acetylcholine, 1mM caffeine were studied in order to elucidate diverse actions of $Cd^{2+}$ on the $Ca^{2+}$ mobilization related with contractility in the antral circular muscle of guinea-pig stomach. $Cd^{2+}$ inhibited the spontaneous contraction in a does - dependent manner ($10^{-6}$ M - $10^{-4}$ M). $Cd^{2+}$ ($3\times10^{-5}$M) suppressed 60 mM $K^+$-induced contracture composed of a phasic and a tonic response and the increased tonic response by the increased external $Ca^{2+}$ concentration. $Cd^{2+}$ also suppressed acetylcholine - induced contracture composed of repetitive phasic and a tonic component and the increased tonic response by the increased external $Ca^{2+}$ concentration. Caffeine in the concentration of 1mM evoked contracture but $Cd^{2+}$ suppressed the contracture. $Cd^{2+}$ suppressed the amplitude of the $Na^+$-free contracture dose-dependently and the amplitude of $Na^+$-free contracture almost decreased to 20% of control amplitude in the concentration of $10^{-4}$ M $Cd^{2+}$.**

**From the above results, it is suggested that $Cd^{2+}$ may inhibit not only $Ca^{2+}$ influx via voltage-sensitive, receptor-operated $Ca^{2+}$ channel and Na/Ca exchange but also intracellular $Ca^{2+}$ release from the sarcoplasmic reticulum in the antral circular muscle of guinea-pig stomach.**

**Key Words:** $Cd^{2+}$, Contractility, Antral Circular Muscle, Guinea-pig stomach.

## INTRODUCTION

Most of the $Ca^{2+}$-regulated processes appear to be controlled by intracellular $Ca^{2+}$ concentration at the range of $10^{-7}$-$10^{-5}$ M (Constantin,1977). Indeed, direct measurements in a variety of cell types, with $Ca^{2+}$-selective microelectrodes, indicate that intracellular $Ca^{2+}$ concentration is normally in the range of about $5\times10^{-8}$-$10^{-7}$ M in most resting cells (Blinks et al., 1982 ; Campbell, 1983 ; Tsien and Rink, 1983). Thus, even small changes in intracellular $Ca^{2+}$ concentrations can be associated with a large signal (Blaustein, 1985). Furthermore, most cells possess several coordinate systems to regulate intracellular $Ca^{2+}$ concentrations both at rest and during activity.

Several different systems may contribute to the regulation of intracellular $Ca^{2+}$ concentration (Sheu & Blaustein, 1986). These include the followings : (a) voltage-gated and chemical-receptor-operated $Ca^{2+}$ channels that mediate $Ca^{2+}$ entry from the extracellular fluid ; (b) the endoplasmic reticulum(sarcoplasmic reticulum in muscle), which sequesters $Ca^{2+}$ by an ATP-driven transport mechanism and can then release $Ca^{2+}$ back into the cytosol during cell activation ; (c) mitochondria, which can also sequester $Ca^{2+}$ by an ATP-dependent transport mechanism and can release $Ca^{2+}$ by a $Na^+$-dependent mechanism ; (d) cytoplasmic $Ca^{2+}$-binding proteins, such as calmodulin and parvalbumin, that can buffer $Ca^{2+}$ ; (e) an ATP-

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driven $Ca^{2+}$ pump that extrudes $Ca^{2+}$ across the plasma membrane; (f) a $Na^+/Ca^{2+}$ exchange system that can move $Ca^{2+}$ either into or out of the cytosol, across the plasma membrane, depending on the prevailing $Na^+$ electrochemical gradient.

Cd ions have been found to be a selective blocker of the slow inward calcium currentrent in various tissues (Kostyuk et al., 1977 ; Lee & Tsien, 1982 ; Giles et al., 1983 ; Josephson et al., 1984 ; Gautier et al., 1987). But the blocking mechanism of Cd ion on smooth muscles is yet poorly understood. The effect of Cd ions on smooth muscle contractions may involve not only voltage-sensitive $Ca^{2+}$-channel blockage but also other $Ca^{2+}$-regulatory processes. The present study was carried out to study the effect of Cd ion on spontaneous contraction and the contractures induced by $Na^+$, $K^+$, and acetylcholine, caffeine in the antral circular muscle of guinea-pig stomach.

## METHOD

### Preparation of antral circular muscle

Guinea-pigs of either sex weighing about 300g were used. The animals were killed by a blow on the hind neck and exsanguinated by cutting both the carotid arteries. The stomach was extracted quickly and transferred into a chamber containing oxygenated Tyrode solution. The antral part was isolated by removing the other parts with a scissors. The antral part was cut in the longitudinal direction along the lesser curvature.

The content within stomach and the mucosal layer were removed from the muscle layers in phosphate-buffered Tyrode solution aerated with oxygen in the room temperature. The strips of the antral circular muscle were prepared with the size, 5 mm in length and 1 mm in width. The loop was made with the fine cotton thread for connecting with the hook of the force transducer in the one end of the muscle strip. After the muscle strips were rested for 1 hour in the preparation chamber, then the muscle strip was transferred into the experimental chamber.

### Solutions

Preparation solution: phosphate-buffered Tyrode solution contains NaCl 147 mM, KCl 4 mM, $MgCl_2 \cdot 6H_2O$ 1.05 mM, $CaCl_2 \cdot 2H_2O$ 2mM, $NaH_2PO_4 \cdot 2H_2O$ 0.42mM, $Na_2HPO_4 \cdot 12H_2O$ 1.81 mM, glucose 5.5 mM, pH 7.4.

Working solution: tris-buffered normal tyrode solution contains NaCl 147 mM, KCl 4 mM, $CaCl_2 \cdot 2H_2O$ 2 mM, $MgCl_2 \cdot 6H_2O$ 1.05 mM, tris-HCl 5 mM, glucose 5.5 mM, pH 7.4.

Above solutions were equilibrated with 100% $O_2$. $Na^+$-free solutions were made by replacing NaCl isosmotically with tris-Cl. The change in the $Na^+$ concentration was made by replacing NaCl isosmotically with tris-Cl.

### Experimental apparatus and protocol

Experimental chamber was made of perspex (derivative of leucite) and horizontal type. The input and output of the flow was done with hydrostatic pressure. Experimental temperature was maintained at about 35°C with constant temperature circulator(Haake).

The muscle strips were allowed to relax at the horizontal chamber for 1 hour in tris-buffered Tyrode solution at 35°C. equilibrated with 100% $O_2$. Then isometric contractions were recorded by using a force transducer (Harvard & Grass FT-$O_3$) and a recorder (Harvard & Device). When spontaneous activity reached the steady-state, length-tension curves were obtained and all experiments were performed at optimal length.

## RESULTS

The antral circular muscle of the guinea-pig stomach showed spontaneous contractions (Fig.1). The amplitudes of the contractions showed little change in the concentration of $10^{-6}$ M Cd ion. But the amplitudes of the contractions decreased above the concentration of $10^{-6}$ M Cd ion in a dose-dependent manner and were

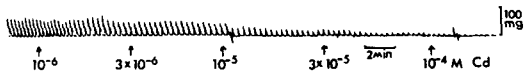


*Fig. 1. Effect of $Cd^{2+}$ on the spontaneous contractions in the antral circular muscle of guinea-pig stomach. The amplitudes of the spontaneous contractions decreased in a does-dependent manner in the range of $10^{-6}$ M - $10^{-4}$ M $Cd^{2+}$ and the contractions were almost suppressed in the concentration of $3 \times 10^{-5}$M $Cd^{2+}$. The frequencies of the spontaneous contractions decreased slightly in lower concentration of $Cd^{2+}$ but the degree of the frequency decrement had little changed even in higher concentration.*

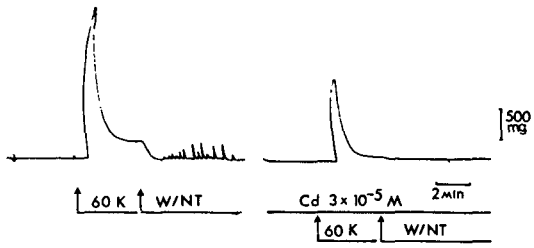


*Fig. 2. Effect of $Cd^{2+}$ on the 60mM $K^+$-induced contracture in the antral circular muscle of guinea-pig stomach. When 60mM $K^+$-Tyrode solution was applied to the antral circular muscle, a contracture composed of a pha sic and a tonic response was induced (left side panel). The contracture was suppressed by the pretreatment of $3 \times 10^{-5}$ M $Cd^{2+}$ for 10 minutes (right side panel).*

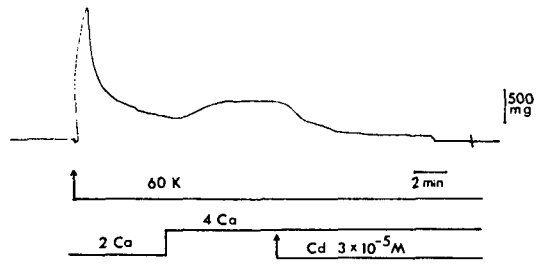


*Fig. 3. Effect of $Cd^{2+}$ on the increased tonic response by external $Ca^{2+}$ in the 60mM K-induced contracture. The initial, rapidly developed tension(phasic) gradually declined to a level which was sustained(tonic). The developed tonic response increased as the concentration of external $Ca^{2+}$ increased from 2mM to 4mM but the increased tension was suppressed when $3 \times 10^{-5}$ M $Cd^{2+}$ was applied.*

almost suppressed in the concentration of $3 \times 10^{-5}$ M Cd ion. The frequencies of the spontaneous contractions decreased slightly in lower concentration of Cd ion but the degree of the frequency decrement little changed even in higher concentration of Cd ion. The effect of $Cd^{2+}$ on 60 mM $K^+$-induced contracture was investigated in order to elucidate an effect of $Cd^{2+}$ on the $Ca^{2+}$ influx via voltage-sensitive $Ca^{2+}$-channel. 60 mM $K^+$-Tyrode solution was made by substituting Na ion with equimolar K ion. When 60 mM $K^+$-Tyrode solution was applied to the antral circular muscle of guinea-pig stomach, a contracture was induced. The contracture was composed of a phasic and a tonic response (Fig. 2 left side panel). After the antral circular muscle was treated with $3 \times 10^{-5}$ M Cd ion for 10 minutes, the muscle was exposed to 60 mM $K^+$-Tyrode solution. Then the phasic and the tonic response of the contracture were both suppressed (Fig. 2 right side panel).

Next experiment was carried out to confirm the probability that $Cd^{2+}$ may have an antagonizing effect on the $Ca^{2+}$ influx via voltage-sensitive $Ca^{2+}$ channel. In the contracture induced by 60 mM K-Tyrode solution, the initial, rapidly developed tension(phasic) gradually declined to a level which was sustained(tonic), the developed tonic response increased as the concentration of external Ca ion increased from 2mM to 4mM but the increased tension was suppressed when $3 \times 10^{-5}$ M Cd ion was applied (Fig. 3). It is suggested that Cd ion may inhibit $Ca^{2+}$ influx through voltage - sensitive $Ca^{2+}$ channel (Fig. 2,3).

Next, effect of Cd ion on the acetylcholine-

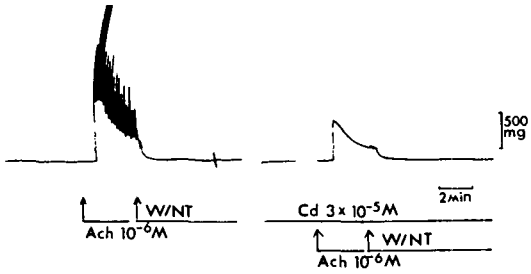


*Fig. 4. Effect of $Cd^{2+}$ on the acetylcholine-induced contracture in the antral circular muscle of guinea-pig stomach. When the antral circular muscle was exposed to $10^{-6}$ M acetylcholine - containing Tyrode solution, a contracture composed of repetitive phasic and a tonic response was induced (left side panel). The contracture was suppressed by the pretreatment of $3 \times 10^{-5}$ M $Cd^{2+}$ for 10 minutes (right side panel).*

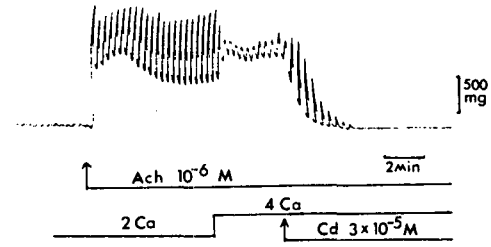


*Fig. 5. Effect of $Cd^{2+}$ on the increased tonic response by external $Ca^{2+}$ in the acetylcholine - induced contracture. The initial, developed tension reached maximum gradually with repetitive phasic contractions and declined to a plateau level. The developed tonic response increased when external $Ca^{2+}$ increased from 2mM to 4mM but the increased tension was suppressed by the application of $3 \times 10^{-5}$ M $Cd^{2+}$.*

induced contracture was observed in order to elucidate an effect of $Cd^{2+}$ on the $Ca^{2+}$ influx via acetylcholine-receptor operated $Ca^{2+}$ channel. When the antral circular muscle was exposed to $10^{-6}$ M acetylcholine-containing normal Tyrode solution, a contracture was induced. The contracture was composed of repetitive phasic and a tonic response (Fig. 4 left side panel). After the antral circular muscle was treated with $3 \times 10^{-5}$ M Cd ion for 10 minutes, the muscle was exposed to $10^{-6}$ M acetylcholine-containing Tyrode solution. Then the repetitive phasic contractions disappeared and the tonic response was suppressed (Fig.4 right side panel).

Next experiment was carried out to confirm the probability that $Cd^{2+}$ may have an antagonizing effect on the $Ca^{2+}$ influx via acetylcholine-receptor operated $Ca^{2+}$ channel.

In the contracture induced by $10^{-6}$ M acetylcholine, the developed tension increased gradually to maximum with repetitive phasic contractions and declined to a plateau level, the developed tonic contracture increased when the concentration of external Ca ion increased from 2mM to 4mM and the increased tension was suppressed by the application of $3 \times 10^{-5}$ M Cd

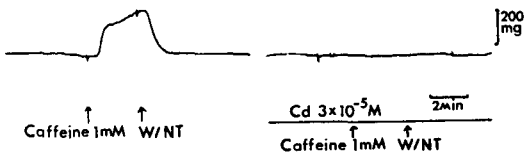


*Fig. 6. Effect of $Cd^{2+}$ on the caffeine - induced contracture in the antral circular muscle of guinea-pig stomach. When 1mM caffeine - containing normal Tyrode solution was applied to the antral circular muscle, a contracture was developed (left side panel). The contracture was suppressed by the pretreatment of $3 \times 10^{-5}$ M $Cd^{2+}$ for 10 minutes (right side panel).*

ion (Fig. 5). Thus Cd ion may inhibit $Ca^{2+}$ influx via acetylcholine - receptor operated $Ca^{2+}$ channel.

Effect of Cd ion on the caffeine-induced contracture was observed in order to an effect of $Cd^{2+}$ on the $Ca^{2+}$ release from sarcoplasmic reticulum. Caffeine is known to be a stimulator of Ca ion release from sarcoplasmic reticulum in various tissues. When 1mM caffeine - con-

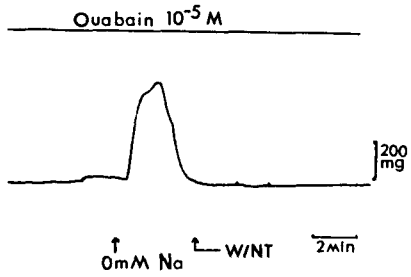


*Fig. 7. Induction of contracture by $Na^+$-free Tyrode solution in the antral circular muscle of guinea-pig stomach. The antral circular muscle was treated with $10^{-5}$ M ouabain for more than 30 minutes in order to increase intracellular $Na^+$ concentration. Then $Na^+$-free Tyrode solution was applied, a contracture was developed and relaxed spontaneously.*

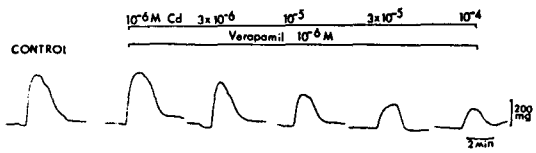


*Fig. 8. Effect of $Cd^{2+}$ on the $Na^+$-free contracture in the antral circular muscle of guinea-pig stomach. In order to exclude the possible effect of $Cd^{2+}$ through $Ca^{2+}$ channel on $Na^+$-free contracture, $10^{-6}$ M verapamil was treated for 10 minutes before $Cd^{2+}$ was applied to the muscle. $Cd^{2+}$ in the concentration of $10^{-6}$ M had little effect on the contracture but from $3\times10^{-6}$ M suppressed the amplitude of the contracture in a dose-dependent manner.*

taining normal Tyrode solution was applied to the antral circular muscle, a contracture was developed (Fig. 6 left side panel). After the antral circular muscle was treated with $3\times10^{-5}$ M Cd ion for 10 minutes, 1mM caffeine was applied to the muscle. Then the caffeine-induced contracture was suppressed (Fig. 6 right side panel).

After the antral circular muscle was treated with $10^{-5}$ M ouabain for more than 30 minutes in order to increase intracellular sodium ion

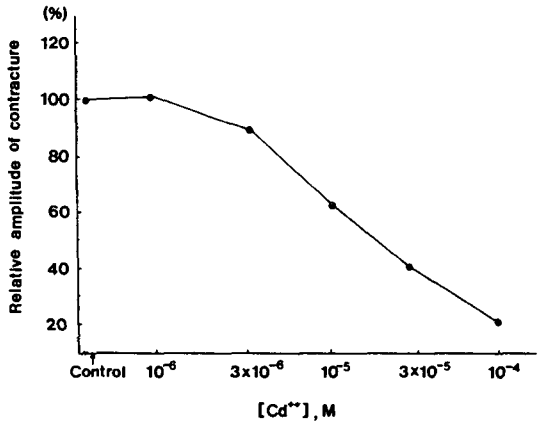


*Fig. 9. Dose-response curve between external $Cd^{2+}$ concentrations and the amplitudes of $Na^+$-free contractures. The amplitudes of $Na^+$-free contractures at various external $Cd^{2+}$ concentrations were expressed as a percentage of the control amplitude. $Cd^{2+}$ in high concentration decreased the amplitude of the contracture prominently and the amplitude of the contracture almost decreased to 20% of control amplitude in the concentration of $10^{-4}$ M $Cd^{2+}$.*

concentration, $Na^+$-free Tyrode solution ($Na^+$ was replaced by equimolar tris) was applied. Then a contracture was developed and relaxed spontaneously. The amplitude of $Na^+$-free contracture was considered as the index of $Ca^{2+}$ influx via Na/Ca exchange mechanism (Fig. 7 Kim et al., 1992).

In order to exclude the possible effect of Cd ion through Ca-channel on $Na^+$-free contracture, $10^{-6}$ M verapamil was treated for 10 minutes before Cd ion was applied to the antral circular muscle. Cd ion in the concentration of $10^{-6}$ M had little effect on the amplitude of $Na^+$-free contracture but from the concentration of $3\times10^{-6}$M suppressed the contractures in a does-dependent manner (Fig. 8).

Relationship between Cd ion concentrations and the amplitudes of $Na^+$-free contractures was plotted with does-response curve (Fig. 9). The amplitudes of $Na^+$-free contractures at various external Cd ion concentrations were expressed as a percentage of the amplitude of the control.

Cd ion in high concentration inhibited $Na^+$-free contracture prominently and the amplitude of $Na^+$-free contracture almost decreased to 20% of control amplitude in the concentration of $10^{-4}$ M Cd ion.

## DISSCUSSION

Unitary smooth muscles have spontaneous myogenic activities. These activities are generated by pacemaker potentials or slow depolarization (slow wave) of the membrane. It is well known that the influx of extracellular $Ca^{2+}$ is essential for generation of the slow depolarization of the membrane which is related to the spontaneous contractions (Tomita, 1981). From the present result that Cd ion caused a dose-dependent inhibition on the spontaeous contractions (Fig. 1), it is suggested that Cd ion may inhibit the influx of extracellular $Ca^{2+}$ dose-dependently and in this way disturb the contractions which, physiologically are regulated by $Ca^{2+}$.

When excess $K^+$ concentration is applied to smooth muscle tissues, the $K^+$-induced contracture is composed of a phasic and a tonic response. The initial, rapidly developed tension (phasic) gradually declines to a level which is sustained (tonic). Urakawa and Holland (1964) proposed that for the phasic contraction, sufficient $Ca^{2+}$ is released from a intracellular site to initiate contraction, whereas for the tonic contraction an adequate amount of extracellular $Ca^{2+}$ penetrates the plasma membrane to initiate contraction. On the other hand, Imai and Takeda(1967) postulated that the tonic response of the contracture was due to release of bound calcium from the smooth muscle. In the sarcoplasmic reticulum of skeletal muscle, there are at least two different mechanisms for inducing a release of stored calcium, i.e. $Ca^+$-release induced by the membrane depolarization and calcium itself. In the smooth muscle, the membrane depolarization by excess extracellular $K^+$ concentration also induce intracellular $Ca^{2+}$ release from sarcoplasmic reticulum and increases the influx of extracellular $Ca^{2+}$. However, the extracellular $Ca^{2+}$ concentration determines the amplitude of the $K^+$-induced contracture (Kuriyama, 1977). The present result (Fig. 2,3) concerning the inhibitory effect of Cd ion on the $K^+$-induced contracture give rise to the existence of an antagonism between $Cd^{2+}$ and $Ca^{2+}$ about voltage-sensitive Ca-channel. Evidence for blockade at a $Ca^{2+}$ - channel comes from the observation that the inhibitory action of $Cd^{2+}$ on the increased $Ca^{2+}$ influx by the increased external $Ca^{2+}$ could be induced by external $Cd^{2+}$.

Divalent cations such as $Cd^{2+}$, $Mn^{2+}$, $Ni^{2+}$ mainly owing to their molecular similarity to $Ca^{2+}$, induce a variety of cellular consequences. For example such divalent cations have been regarded as voltage-sensitive $Ca^{2+}$ channel blockers(Kl ckner and Isenberg, 1985 ; Ohya et al., 1986) and they have been used to separate receptor-operated $Ca^{2+}$ influx from $Ca^{2+}$ influx via voltage - sensitive $Ca^{2+}$ channel (Bolton, 1979 ; Brading & Sneddon, 1980). It has not been resolved whether acetylcholine act only the surface membrane or also on the intracelluar $Ca^{2+}$-storage site in inducing a contracture of the gastric smooth muscle. The tonic response of acetylcholine-induced contracture increased when external $Ca^{2+}$ concentration increased (Fig. 5). This increased portion of the tonic response will be resulted from $Ca^{2+}$ influx due to increased external $Ca^{2+}$ concentration. The increased portion of the tonic response was suppressed by $Cd^{2+}$. So it was believed the possibility that $Cd^{2+}$ may inhibit $Ca^{2+}$ influx via acetylcholine-receptor operated $Ca^{2+}$ channel, thereby suppressing acetylcholine-induced contracture.

Caffeine induces $Ca^{2+}$ release from the sarcoplasmic reticulum in skeletal muscle fibers. Caffeine has been also shown to contract various smooth muscles by mobilizing an intracellular $Ca^{2+}$ release from the sarcoplasmic reticulum (Weber & Hertz, 1968 ; Casteels & Raeymaekers, 1979 ; Haeusler et al., 1981 ; Itoh et al., 1982 ; 1983 ; Leijten & Van Breemen, 1984). Cd ion may have an inhibitory effect on the intracellular $Ca^{2+}$ release from the sarcoplasmic reticulum from the point of the pre-

sent result that Cd ion suppressed the caffeine - induced contracture (Fig. 6).

The antagonistic action of extracellular Ca and Na ions on cardiac contractile strength described for frog heart (L ttgau & Niedergeke, 1958) had been observed. Reuter & Seitz (1968) found evidence for a membrane carrier system in the heart involving $Na^+$ and $Ca^{2+}$ and demonstrated that $Ca^{2+}$ efflux rate was sensitive to the concentration gradient for $Na^+$ across the sarcolemma. Next Baker et al.(1969) demonstrated, in the internally perfused squid axon, that in addition to inward $Na^+$ movement, exchange also occurred in the opposite direction -i.e. elevation of internal $Na^+$ increased $Ca^{2+}$ influx. Removal of external $Na^+$ induces a contracture in the heart which is attributable to an influx of extracellular Ca ion through the Na/Ca exchange mechanism (chapman,1979). In the gastric smooth muscle $Na^+$-free contracture was also observed as an evidence of $Ca^+$ influx via Na/Ca exchange mechanism (Kim et al., 1992). Therefore it is fair to assume that the amplitude of the $Na^+$-free contracture may be an index of the amount of $Ca^+$ influx via Na/Ca exchange mechanism. There is no specific inhibitor of Na/Ca exchange at present. But $Ca^{2+}$ channel blockers were known to inhibit Na/Ca exchange non-specifically. The ability of inorganic $Ca^{2+}$ channel blockers to interfere with the $Na^+$-free contracture was first noted for Mn ion (Chapman and Ochi, 1971). Coraboeuf et al (1981) noted that $Mn^{2+}$ in the concentration of 4mM did not alter the $Na^+$-free contracture but in 20mM depressed the contracture in the dog purkinje fibers.

In the present result (Fig. 8,9), $Cd^{2+}$ in the concentration of $10^{-6}$ M had little effect on the amplitude of the $Na^+$-free contracture but in higher concentration than $10^{-6}$ M suppressed the contracture dose - dependently and the contracture almost decreased to 20% of control amplitude in the concentration of $10^{-4}$ M $Cd^{2+}$. Transarcolemmal transport of the Na and Ca ions via an exchanger or carrier can be considered in the Na/Ca exchange (Langer, 1982). The reason to dose-dependent inhibition of $Cd^{2+}$ on the $Na^+$-free contracture can be explained by the assumption that increments in extracellular $Cd^{2+}$ concentrations decrease extracellular $Ca^{2+}$ binding to the Na/Ca exchange carrier through competitive binding of $Cd^{2+}$ to the carrier against $Ca^{2+}$, and so, the amount of $Ca^{2+}$ influx via Na/Ca exchange carrier will be reduced.

From the above results, it is suggested that $Cd^{2+}$ may inhibit not only $Ca^{2+}$ influx via voltage - sensitive, receptor - operated $Ca^{2+}$ channel and Na/Ca exchange but also intracellular $Ca^{2+}$ release from the sarcoplasmic reticulum in the antral circular muscle of guinea-pig stomach.

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