대한생리학회지 : 제23권 제 1 호

□ SPECIAL LECTURE □

Actions of Ca Antagonists on the Voltage Dependent Ca Channel in Mammalian Smooth Muscle Cell Membranes

Kenji Kitamura, Masahiro Okie and Hiroshi Kuriyama

Department of Pharmacology, Faculty of Medicine, Kyshu University, Fukuoka 812, Japan

INTRODUCTION

Intracellular free Ca concentrations play an essential role in regulation of the contractions relaxation cycle in mammalian smooth muscle cells, e.g. with concentrations of Ca above 0.1 - 0.3 μM, a contraction is generated and 1 - 5 μM Ca evokes the maximum amplitude of contraction in chemically skinned smooth muscle strips (Itoh et al, 1982, 1983). Increased Ca concentrations in the cytosol form the 4 Ca-calmodulin-myosin light chain kinase ternary complex and phosphorylates the 20 kD protein of myosin light chain thus leading to dephosphorylation of ATP by actin-dependent ATPase, the cycling of the cross bridge between actin and myosin is thus triggered (myosin-linked regulation of the contractile protein; Adelstein & Eisenberg, 1980; Hartshorne & Siemankowski, 1981; Driska et al, 1981; Aksoy et al, 1982; Kamm & Stull, 1985). When the concentration of Ca in the cytosol is decreased in the absence of influx, following an increase in the re-uptake of Ca into the sarcoplasmic reticulum or by increased extrusion of Ca through activations of the Ca pump or Na-Ca exchange diffusion, the phosphorylated myosin is dephosphorized by phosphatase and retrograde processes of the above steps occur. Relaxation of smooth muslce cells then occurs. However, even more complicated processes for the regulation of contraction-relaxation cycles have been postulated, namely, Ca not only plays an essential role in the Ca-calmodulin complex formation, it also seems to bind to leiotonin and this process may trigger production of the contraction, as processes similar to those occur in skeletal and cardiac muscles (actin-linked hypothesis; Ebashi 1984; Ebashi et al, 1977; Mikawa et al, 1977, 1978).

It was also reported that sustained contraction requires no further phosphorylation of the 20 kD protein of myosin, does not require the high cycling rate of cross bridge betweem actin and myosin, as estimated from the shortening velocity, and also does not require increases in the amount of free Ca (latch hypothesis; Murphy et al, 1983; Dillon et al, 1981; Chatterjee & Murphy, 1983; Chatterjee & Tejada, 1986; Rembold & Murphy, 1986).

As deduced from electrophysiological and mechanical responses, contraction of smooth muscle may occur with increases in the free Ca in the cytosol, such as increases in the voltage dependent or/with the receptor operated Ca influxes and the release of Ca from the sarcoplasmic reticulum plays a major role, albeit passively the inhibition of reuptake of Ca into the sarcoplasmic reticulum or inhibition of the Ca extrusion mechanism through the Ca pump or/ with the Na-Ca exchange diffusion may preserve the

Presented at the 40th annual meeting of the Korean Physiology Society, 28 October 1988.

Table. 1. Classification of voltage dependent Ca channel.

Feature of subtypes of voltage dependent Ca channels			
	T subtype	N subtype	L subytbe
Activating potential level	−70 mV	−10 mv	−10 mV
Inactivation potential level	−100∼−60 mV	−100∼−40 mV	−60∼−10 mV
Inactivation velocity	20∼50 ms	20∼50 ms	slow (>700 ms)
Unitary channel slope conductance	8∼10 ps	13 ps	25 ps
Ion selectivity	$I_{Ca} > I_{Ba}$	$I_{Ba} > I_{Ca} > I_{Ca}$	$I_{Ba} > I_{Ca}$
Inhibitors			
Cd^{2+} (kd)	+ (160 μM)	+++ (7 μM)	+++ (7 μM)
Ni (kd)	+++ (47 μM)	+ (280μM)	+ (280 μM)
dihydropyridine derivatives	−	−	+++
ω-conotoxin derivative	−	++	++ (nerve) − (smooth muscle)

L; long lasting type (I_{high}). T; transient outward current type (I_{low}). N; neither L nor T type

Table. 2. Ionic conductances of L- and T-subtypes measured from various smooth muscle cells

Unitary Ca-current conductance in smooth muscles					
Preparations	Conductance	Solution		Patch condition	Referecnes
		Outside	Inside		
Guinea-pig taenia coli	30pS	50mM $BaCl_2$	Tyrode	cell-attached	Yoshino & Yabu (1985)
	25pS 12pS	50mM $BaCl_2$	145mM KCl	cell-attached	Yoshino et al. (1988)
Guinea-pig aorta	12pS	110mM $BaCl_2$	Ringer	cell-attached	Caffirey et al. (1986)
Amphiuma stomach	12pS				
Rabbit ear artery	20pS 8pS	110mM$BaCl_2$	130mM CsCl	outside-out	Aaronson et al. (1986)
	25pS 8pS	80mM $BaCl_2$	120mM NaCl	outside-out	Benham et al. (1987)
Rabbit mesenteric artery	15pS 8pS	80mM $BaCl_2$	120mM NaCl	inside-out	Woley et al. (1986)
Rabbit intestine	20pS 25pS	50mM $BaCl_2$ 100mM $BaCl_2$	140mM KCl Krebs	cell-attached	Inoue et al. (1988)
Dog saphenous vein	18∼24pS 7∼9pS	90mM $BaCl_2$	130mM K aspartate	cell-attached	Yatani et al. (1987)

high concentrations of Ca in the cytosol (Bolton, 1979).

The voltage dependent Ca channel is composed of L-(I_{low}), T-(I_{high}), and N-subtypes in excitable cells, including nerve fibers and as deduced from electrical analysis, using voltage and patch clamp methods (Table 1). In smooth muscles, only two subtypes (L- and T-subtypes) are recognized (Table 2: Yoshino & Yabu, 1986; Yoshino et al, 1988; Cafsfrey et al, 1986; Aaronson et al, 1986; Benham et al, 1987; Worley et al, 1986; Yatani et al, 1987; Inoue et al, 1988). Furthermore, the distribution ratio of the L-/T-subtypes differes with the tissue. The voltage dependent Ca channel which is sensitive to Ca antagonists (dihydrophyridine derivatives such as nifedipine, nitrendipine, nicardipine, nimodipine, nisoldipine and etc., diltiazem, verapamil, gallopamil D-600 or flunarizine) is the L-subtype. This Ca antagonist sensitive protein (L-subtype) acts not only on the Ca channel but also as a Ca senser, as observed from the dihydrophyridine binding protein extracted from the transverse tubular structure and triad regions in skeletal muscles (Fig. 1).

This L-subtype is composed of α_1-(175 kD), α_2-(143 kD), β-(50-60 kD), γ-(30-33 kD) and s-subunits (24-27 kD; see Fig. 1; Borsotto et al, 1985; Curtis & Caterall, 1984; Flockerzi et al, 1986; Beent et al, 1986; Rios et al, 1987; Tanabe et al, 1987; Vandaele et al, 1987; Takahashi et al, 1987) senser (composed of 1873 amino acids and 212 kD proteins, Tanabe et al, 1987).

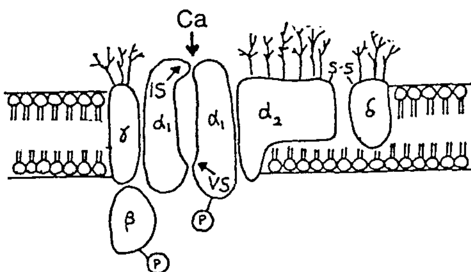

Fig. 1. Schematic arrangement of L-subtype of the voltage dependent Ca channel extracted from triad region of the skeletal muscle.

In this article, attention will be directed to the nature of the voltage dependent Ca channel in smooth muscle cell membranes, mainly on the Ca antagonists sensitive channel (L-subtype), and actions of Ca antagonists on this channel, determined using the voltage and patch clamp methods, will be discussed.

Effects of Ca antagonist on the action potential and contraction

In smooth muscle cells excited from various species and tissues, spontaneoulsy generated action potentials were recorded as burst discharges between silent intervals or with continuously generated action potentials. In some tissues, the action potential was only generated as a result of nerve stimulation, i.e. the action potential triggered on an excitatory junction potential or a slow depolarization. In the other tissues, neither direct muscle stimulation nor nerve stimulation produced the action potential (Kuriyama et al, 1982). In smooth muscle tissues, lack of the spike generation is due to either prevention of the generation of inward current by early generation of outward current (rabbit pulmonary and porcine coronary artery; Casteels et al, 1977; Ito et al, 1979) or lack of the channel required to generate the action potential (guinea-pig and rabbit iris and sphinctor pupil; Ito and Yoshidomi, 1985). Therefore, with few exceptions, in most of smooth muscle cell membranes the action potential is generated by the outward current pulse (depolarization), under condition of pretreatment with K channel blockers such as tetraethylammonium or 4-aminopyridine (Ito et al, 1980; Hara et al, 1982).

Activation of the voltage dependent Ca channels is induced by application of high concentrations of K. High K depolarizes the membrane, and the maximum slope of depolarization per tenfold change in K

concentrations, plotted on a logarithmic scale was 40-50 mV and this value was lower than the predicted K equilibrium potential (Ek) due to interferences of permeability and concentration gradient of other cations and anions (Kuriyama, 1970, 1981; Casteels, 1981; Brading, 1981).

When the spike potential was generated or the membrane was depolarized by electrical or high K stimulation, a contraction was generated. With application of Mn or Ca-free solution, the spike potential and contraction ceased but the K-induced depolarization remained. Therefore, the contraction evoked by these stimulations is thought to be generated by influxes of Ca due to activation of the voltage dependent Ca channel. The high K-induced contraction is composed of phasic and subsequently generated tonic components.

The amplitude of the tonic contraction, in comparison to that of the phasic contraction, differs with the tissue, e. g. the amplitude of tonic contraction was much the same as that of the phasic contraction in aorta, pulmonary artery, logitudinal muscle of ileum or fundus muscle of stomach, and the amplitude of tonic contraction was much smaller than the phasic contraction in circular pylorus muscle of the stomach, circular muscle of ileum or mesenteric artery (Kuriyama & Suzuki, 1975).

The phasic contraction is thought to be generated by the release of Ca from the sarcoplasmic reticulum due to activations of the Ca-induced Ca relaese mechanism triggered by influxes of Ca. The tonic contraction seems to be generated by influxes of Ca, as estimated using pharmacological approaches and these data were obtained from experimental animals.

The resting membrane potential of smooth muscle cells measured using the microelectrode method ranged between 45-75 mV, depending on the tissue. Applications of verapamil, diltiazem and flunarizine (over 1 μM) depolarized the membrane but the dihydrophyridine derivatives (nifedipine, nitrendipine, nisoldipine, nimodipine and nicardipine) did not. The above Ca antagonists blocked the spike potential evoked by depolarizing pulse or perivascular nerve stimulation (more detailed quantitative analysis will be given in the section of the voltage clamp method). Therefore, most of the spike component was due to activation of the Ca antagonist-sensitive voltage dependent Ca current.

The high K-induced contraction was also blocked by application of Ca antagonists, and the tonic component was more sensitive than the phasic one to Ca antagonists, e.g. the spike potential generated in the mesenteric artery was blocked by 10 mM nifedipine. The tonic component was more sensitive than the phasic component in the rabbit mesenteric artery (the 39 mM K-induced tonic contraction was blocked by 3 nM and the phasic contraction by 100 nM of nifedipine; Kanmura et al, 1983).

Nature of the voltage dependent inward current recorded using the voltage clamp procedures

Using the voltage clamp method, the voltage dependent Ca current (macrocurrent) can be recorded from freshly dispersed smooth muscle cells in various tissues from various species, i.e. when the depolarizing pulse (command pulse) was applied to collagenase dispersed smooth muslce cells of the rabbit intestine, at the holding potenital of −60 mV, a transient inward current and subsequently generated outward current was elicited. The outward currents were minimized by replacement of Cs-rich solution in the pipette and 20 mM tetraethylammonium in the bath.

The inward current was elicited at depolarization above −30 mV and reached the maximum value at +10 mV. The polarity of the inward current was reversed at about +60 mV. This inward current was dependent on intra- and extra-cellular Ca, and the increased extracellular Ca enhanced, and increased intracellular Ca accelerated inactivation of the Ca current (the Kd value for inhibition of the Ca current

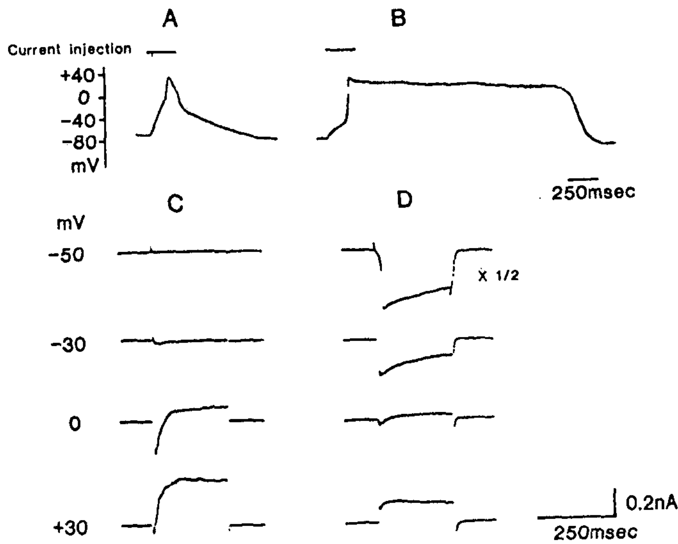

Fig. 2. Voltage dependent Ca current in Krebs solution and Na current in extremely low concentration of Ca in dispersed smooth muscle cell recorded from the guinea-pig portal vein using minoelectrode and whole cell voltage clamp methods (Ohya et al, 1986).

was 100 nM). This Ca current was blocked by Mn and Ca free solution, and the amplitude was enhanced by replacement of Ca with Ba. When extracellular Ca concentrations were reduced to below 1 μM, Na permeated the membrane and produced a long lasting action potential (plateau formation) and the large sustained inward current which reversed the polarity at a much lower membrane potential (about −20 mV) was blocked by Ca antagonists but not by tetrodotoxin. Therefore, with extremely low concentrations of Ca, Na permeates the Ca channel (Fig. 2). The nature of this voltage dependent Na current by activations of Ca channels recorded from the rabbit intestinal smooth muscle cell membrane was much the same as findings in case of the rabbit portal vein (Ohya et al, 1986, 1987).

Under perfusion with Krebs solution (pipette solution containing high Cs), the application of a command pulse to −10 mV from the holding potential of −80 mV in dispersed fresh smooth muscle of the rabbit pulmonary artery elicited an inward current comprising fast and slow components. In Ca-free solution containing 2.5 mM Mn and 134 mM Na, the major part of the inward current ceased, but the transient fast inward current remained. A reduction in extracellular Na reduced the amplitude of the fast inward current, and this current was blocked by application of tetrodotoxin, in the presence or absence of Ca (Km value of 8.7 nM).

The fast inward current was more sensitive to the potential shift, i.e. depolarization of the holding potential from −80 mV to −60 mV all but, inactivated the fast inward current. Ca antagonists, such as diltiazem and nicardipine, blocked the slow inward current, but these agents had no effect on the fast inward current. Chloramine-T (0.3 μM) blocked the fast but not the slow inward current. These results indicated that the fast inward current is generated by activations of the voltage dependent Na channel. Thus, the Na channel is present on the smooth muscle cell membrane of the pulmonary artery and activations of this channel produce the fast inward current (Okabe et al, 1988). Much the same results were also observed in case of muscle cells of the rabbit ileum (Okabe et al, personal communications). The physiological role of this Na current remains to be determined.

As described previously, biochemically purified Ca channel proteins from skeletal, cardiac and smooth muscles seem to have similarities, both chemically

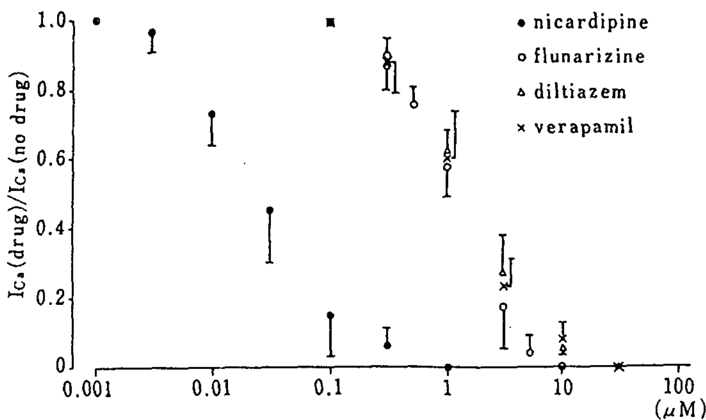

Fig. 3. Inibitory actions of Ca antagonists on the voltage dependent Ca current recorded from smooth muscle cells of the guinea-pig longitudinal layer (Terada et al, 1987).

and biophysically. However, when the effects of cyclic AMP were observed, the Ca current was enhanced in the cardiac muscles (α-and β-subunits of the Ca channel was phosphorylated cyclic AMP dependent protein kinase) but not in smooth muslces (ATP enhanced the Ca current instead of cyclic AMP) in the rabbit ileum (Ohya et al, 1987). Therefore, the nature of the Ca channel may not exactly the same between cardiac and smooth muscles.

Effects of Ca antagonists on the ionic current measured by the voltage clamp method in freshly dispersed smooth muscle cells

The voltage dependent Ca current: Following dispersion of smooth muscle cells from the rabbit small intestine, the voltage dependent Ca current (macro-current) was measured, using the voltage clamp method. To isolate the Ca current from the K current, high Cs was used as the pipette solution and thetraethylammonium was added to the bath. All Ca antagonists (nicardipine, verapamil, diltiazem and flunarizine) inhibited the amplitude of the Ca current, recorded at any given depolarizing pulses (command pulses). As shown in Fig. 3, the IC_{50} value on the maximum amplitude of the inward current (the holding potential of −60 mV and the command pulse of 0 mV) of nicardipine was 24 nM, and this value was roughly 50 times lower than values obtained with verapamil (IC_{50} value of 1.3 μM), diltiazem (1.4 μM) or flunarizine (1.4 μM). When the holding potential was kept at −80 mV, the concentration-response relationship observed under treatment with the above four Ca antagonists shifted to the right. When the depolarizing pulse was applied every 20 sec, the amplitude of the inward current remained unchanged. Nicardipine and flunarizine immediately, reduced the peak amplitude with or without repetitive stimulation but the inhibitory response was slow in case of diltiazem. In contrast, verapamil only inhibited the Ca current during stimulation. These slow inhibitions of the Ca current induced by the latter two agents depended on the frequency and number of stimulations. Nicardipine or flunarizine, but not diltiazem or verapamil shifted the voltage-dependent inactivation curve measured by applications of various depolarizing pulses (conditioning depolarization for 3 sec) to the left (Fig. 4). However, a long pre-pulse (10 sec) slightly shifted the above relationship to the left, in the presence of diltiazem or verapamil (Terada et al, 1987 a and b).

With regard to the smooth muscle cells of the rabbit portal vein and rabbit ileum, the inward Ca current was evident much the same extent. Since the Ca antagonist, especially gallopamil (D-600) and verapamil seem to act from inside of the cell membrane in cardiac muscle cells (Heschler et al, 1982),

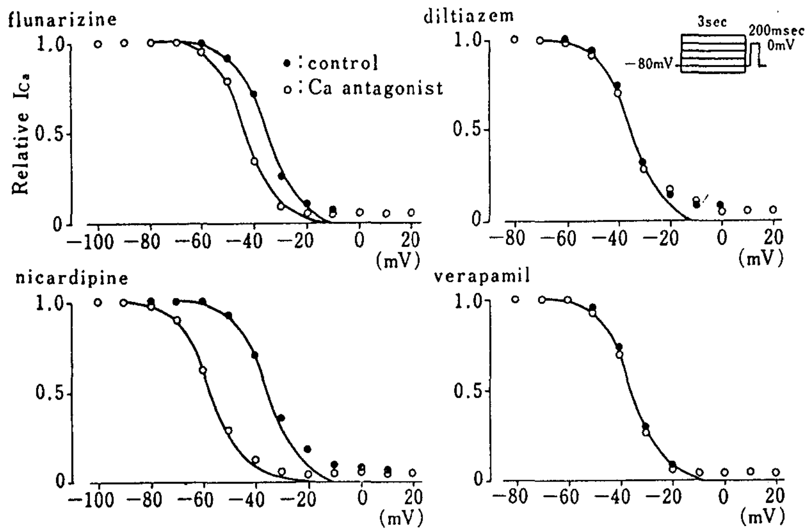

Fig. 4. Effects of various Ca antagonists on the inactivation of voltage dependent Ca currents. The experimental conditions was described in the text and parenthes (Terada et al, 1987).

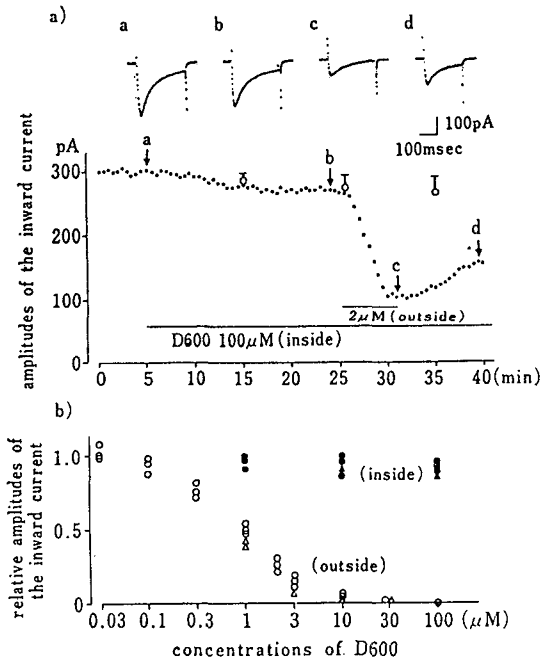

Fig. 5. Effects of intra- and extra-cellular applications of D600 (gallopamil) on the voltage dependent Ca current recorded from the dispersed smooth muscle cell of the guinea-pig ileum (Ohya et al, 1987).

the effects of intracellular perfusion of Ca antagonists on the Ca current were investigated. The intracellular perfusion of gallapamil did not modify the amplitude of the Ca inward current yet the extracellular bath application consistently inhibited the amplitude (Ohya et al, 1987). Therefore, here again we confirmed that the voltage dependent Ca channel in smooth muscle cell membrane may differ in nature that from cardiac muscle cells (Fig. 5).

Voltage dependent K current: The effects of Ca antagonists on the K current in the rabbit small intestine were examined, using the voltage clamp method. To eliminate influence of the Ca dependent K current, the voltage dependent K current was recorded in Ca-free solution containing 2.5 mM Mn. Nicardipine, verapamil, diltiazem and flunarizine inhibited the peak amplitude of the K current, in a concentration dependent manner. The IC_{50} value of the K outward current to nicardipine was 4.6 μM, to diltiazem was 30 μM, to verapamil was 14 μM and to flunarizine was 5.8 μM. As a consequence, the ratio of IC_{50} of K outward current/IC_{50} of Ca inward current was 190 for nicardipine, 21 for diltiazem, 11

for verapamil and 4 for flunarizine (Table 3).

Except for nicardipine, blocking concentrations of Ca antagonists on the action potential are not so different from the inhibitory concentrations of the K outward current. During application of a long depolarizing pulse (3 sec), amplitude of the voltage dependent K current decreased slowly with time. Diltiazem inhibited the K current with a slight prolongation of the decay time, while verapamil, nicardipine and flunarizine accelerated the inactivation. This inactivation curve observed below 0 mV was not modified by 10 μM diltiazem, 5 μM verapamil nor 3 μM nicardipine. This means that inhibition of the K current by Ca antagonists may also differ, i.e. diltiazem and other Ca antagonists behave in a different manner with regard to inactivation of the K current.

In smooth muscle cells of the small intestine (jejunum), the inhibitions of the Ca inward current by verapamil were markedly influenced by stimulus frequency of the command pulses, and did not depend on the time of exposure period to this drug. As shown in Fig. 6, these use- and frequency-dependent inhibitory actions were a typical feature in case of verapamil but such actions were weaker with diltiazem, flunarizine and nicardipine at the holding potenitial of -60 mV. When the holding potential was kept at -80 mV, even nicardipine showed a use-dependent inhibition of the Ca inward current. Therefore, Ca antagonists act at the open state of the Ca channel.

On the other hand, nicardipine and flunarizine showed a voltage-dependent inhibition of the Ca inward current, but no shift of the steady state inactivation was seen in cases of verapamil and diltiazem (the holding potential of -60 mV, the pre-conditioning depolarization 3 sec). In smooth

Table 3. Inhibitory actions of Ca antagonists on the voltage dependent Ca and K currents. The ratios of IC_{50} for the I_K/IC_{50} for the I_{Ca} were expressed

	$ID_{50}(I_K)$	$ID_{50}(I_{Ca})$	$ID_{50}(I_K)$ / $ID_{50}(I_{Ca})$
Nicardipine	4.6×10^{-6}M	2.4×10^{-8}M	190
Diltiazem	3.0×10^{-5}M	1.4×16^{-6}M	21
Verapamil	1.4×10^{-5}M	1.3×10^{-6}M	11
Flunarizine	5.8×10^{-6}M	1.4×10^{-6}M	4.1

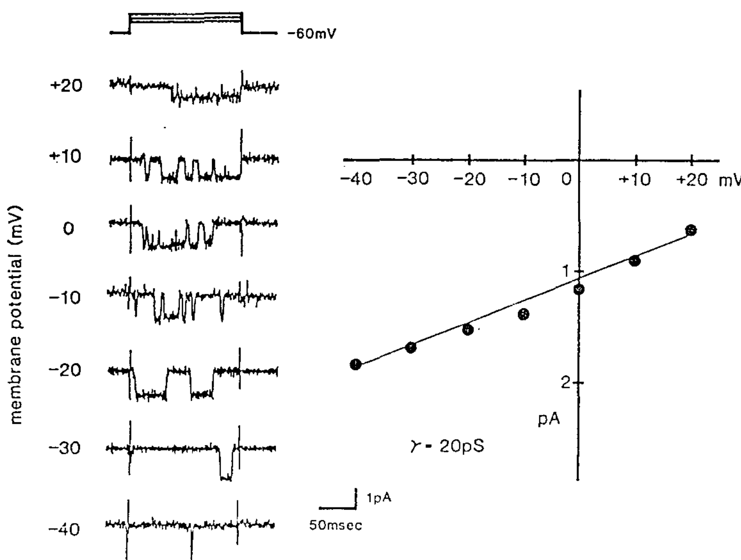

Fig. 6. Unitary Ba (Ca) current recorded from the smooth muscle cell of the guinea-pig ileum using the cell attached patch clamp method (Inoue, Y unpublished obervations).

muscle cells, application of 10 sec pre-conditioning pulse, the inactivation curve shifted slightly to the left by application of verapamil or diltiazem. Therefore, all the Ca antagonists we examined herein may induce an acceleration of the inactivation of the Ca inward current, but with different potencies. In cases of cardiac muscles, the voltage dependent inhibition of the Ca inward current has been reported (Ehara & Hoffmann, 1978; Sanguinetti & Kass, 1984; Uehara & Hume, 1985). Therefore, Ca antagonists more potently act at the inactive rather than at the resting state of the membrane (at −80 mV, the channel seems to be maintained at the resting state).

These results indicate that Ca antagonists ·block the Ca inward current with much the same mechanisms of inhibition, however, there are quantitaitive differences i.e. nicardipine more than other Ca antagonists acts more potently on the open state of the channel. Differences between the Ca channel in cardiac and smooth muscles here noted in cases of the action of gallopamil (D-600), i.e. this drug acts from the inside of the membrane in the cardiac muscle, but from the outside of the membrane in smooth muscles.

Effects of nifedipine on the unitary Ca current

To investigate in detail the action of nifedipine, as an example of dihydropyridine derivatives, the unitary Ca (Ba) current was recorded from dispersed smooth muslce cells of the rabbit ileum, using the cell attached patch clamp method (Fig. 6). With the cell attached configuration, only one type of unitary Ba current, a conductance of 25 pS in 100 mM Ba solution, was obtained. Presumably distributions of T-subtype of voltage dependent Ca channel may be rare (Inoue et al, 1988). However, the channel conductance depended on external concentrations of Ba with the dissociation constant of 19 mM and the maximum conductance of 42 pS (Inoue et al, 1988). There values were similar to those obtained from other smooth muscle cells (Benham et al, 1987). This means that the Ca channel in the smooth muscle cells of the rabbit ileum has one binding site for Ba (Ca) in the channel pore in the presence of high concentrations of Ba (Ca), and that the affinity for Ba ion is similar to events seen with cardiac muscles (Hess et al, 1984).

When the depolarization pulse (the holding potential of −60 mV) and the depolarization pulse of 0 mV, duration 150 msec with frequency of 0.5 Hz (high frequency stimulation) was applied, the unitary Ba current was observed with low open probability P value below 0.3), however, three-fourths of the depolarization pulses did not evoke the unitary Ba current (blank sweep). On the other hand, depolarization pulses, at a low frequency (0.033 Hz) reduced the blank sweep and increased the fraction of sweep, with a high open probability (P value above 0.5), i.e. changes in the mode of the probability tend to shift to mode 2 (high open probability), as observed in the case of the presence of a Ca agonist, Bay K 8644, in cardiac and smooth muscles (Hess et al, 1984; Inoue et al, 1987). Therefore, changes in the gating behavior of the Ca channels between modes may also depend on stimulus frequency of the membrane depolarization.

Using the whole cell voltage clamp method, the amplitude of the inward current was 1.0 nA, as evoked by the depolarization pulse of 0 mV from the holding a potential of −60 mV (100 mM in the bath). Using the cell attached patch clamp method, the amplitude of the unitary current measured at 0 mV was 1.25 pA. Therefore, about 800 channels simultaneously opened at the peak. If the open probability is assumed to be 0.12 (0.033 Hz), 7000 channels may be distributed on the membrane of a single cell (estimation of the surface area of the single cell from the rabbit ileal longitudinal layer being 5000 μM^2; Ohya et al, 1986). The density of the Ca channel of the surface membrane is estimated to be 1.4 μM^2. This number was larger than that obtained in the case of the dog saphenous vein (Caffrey et al, 1986; Yatani et

al, 1987).

Nifedipine reduced the open probability of the channel due to increase in the fraction of blank sweeps, to the greater extent than decrease in the time constant of the channel opening. Presumably, accelerations of transition from mode 2 to mode 0 (resting state of the channel) may occur directly in the presence of nifedipine, as estimated from the time constant of the channel opening obtained from only the current traces with open probability of below 0.3; reduction in the open probability of the Ca channel by nifedipine occurs within mode 2 and transition to mode 0, but may not occur through transition from mode 2 to mode 1. Nifedipine may act mainly on the inactivated state of the Ca channel and partly in the open state of the channel (Inoue et al, 1988), as described previously.

CONCLUSION

In this article, a brief introduction was made on the features of the voltage dependent Ca channel, in particular the Ca-antagonist sensitive channel (L-type), measured using the whole cell voltage clamp and patch clamp methods. Similarities and differences in the nature of voltage dependent Ca channel between cardiac and smooth muscle cell membranes were also given attention. In some species of smooth muscles, the T-type of the Ca channel is also distributed, but the role of this channel on physiological functions is yet to be determined. Regional differences in the nature of smooth muscles are remarkable and the channel property, not only the Ca channel but also other ion channels may also differ in visceral smooth muscle cells, including various regions in arterial and venal vascular smooth muscle tissues.

REFERENCES

Aronson PI, Benham CD, Bolton TB, Hess P, Lang RJ & Tsien RW (1986). Two types of single-channel and whole-cell calcium or barium currents in single smooth muscle cells of rabbit ear artery and the effect of noradrenaline. *J Physiol* 377, 36p

Adelstein RS & Eisenberg E (1980). Regulation and kinetics of the actin-myosin-ATP interaction. *Ann Rev Biochem* 49, 921-956

Aksoy MO, Murphy RA & Kamm KE (1982). Role of Ca^{2+} and myosin light chain phosphorylation in regulation of smooth muscle. *Am J Physiol* 242, C109-C116

Bean BP, Sturek M, Puga A & Hermsmeyer K (1986). Calcium channels in muscle cells isolated from rat mesenteric arteris: modulation by dihydropyridine drugs. *Circ Res* 59, 229-235

Benham CD, Hess P & Tsien RW (1987). Two types of calcium channels in single smooth muscle cells from rabbit ear artery studied with whole-cell and single-channel recordings. *Circ Res* 61 (suppl I), 110-116

Bolton TB (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol Rev* 59, 606-718

Borsotto M, Barhanin J, Fosset M & Lazdunski M (1985). The 1,4-dihydropyridine receptor associated with the skeletal muscle. *J Biol Chem* 260, 14255-14263

Brading AF (1981). Ionic distribution and mechanisms of transmembrane ion movements in smooth muscle. p65-92. In: Bülbring E, Brading AF, Jones AW & Tomita T, *Smooth Mucle.* Arnold, London.

Caffrey JM, Josephson IR & Brown AM (1986). calcium channels of amphibian stomach and mammalian aorta smooth muscle cells. *Biophys J* 49, 1237-1242

Casteels R (1981). Membrane potential in smooth muscle cells. In: Bülbring E, brading AF, Jones AW, Tomita T, *Smooth Muscle.* Arnold, London, p105-126.

Casteels R, Kitamura K, Kuriyama H & Suzuki H (1977). The membran properties of the smooth muscle cells of rabbit main pulmonary artery. *J Physiol* 217, 41-61

Chatterjee M & Murphy RA (1983). Calcium-dependent stress maintenance without myosin phosphorylaion in skinned smooth muscle. *Science* 221, 464-466

Chatterjee M & Tejada M (1986). Phorbol ester-induced contraction in chemically skinned vascular smooth muscle. *Am J Physiol* 251, C892-C903

Curtis BM & Caterall WA (1984). Purification of the

calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. *Biochem* 23, 2113-2118

Dillon PE, Aksoy MO, Driska SP & Murphy RA (1981). Myosin phosphorylation and crossbridge cycle in arterial smooth muscle. *Science* 211, 495-497

Driska PS, Aksoy MO & Murphy RA (1981). Myosin light chain phosphorylation associated with contraction in arterial smooth muscle. *Am J Physiol* 240, C222-C223

Ebashi S (1984). Actin-linked mechanism. In: Stephens NL, *Smooth Muscle Contraction.* (ed) Marcel Dekker, New York, p317-327.

Ebashi S, Mikawa T, Hirata M, Toyo-oka T & Nonomura Y (1977). Regulatory proteins of smooth muscle. In: Casteels R, Godfrained T D & Rüegg C *Excitation-Contraction Coupling in Smooth Muscle.* Elsevier, Amsterdam, p325-334.

Ehara T & Kaufmann R (1978). The voltage- and time-dependent effcts of (−)-verapamil on the slow inward current in isolated cat ventricular myocardium. *J Pharmacol Exp Ther* 207, 49-55

Flockerzi V, Oeken HJ, Hoffman F, Pelzer D, Cavalie A & Trautwein W (1986). Purifed dihydropyridine-binding site from skeletal muscle t-tubules is a functional calcium channel. *Nature* 232, 66-68

Hara Y, Kitamura K & Kuriyama H (1980). Actions of 4-aminopyridine on vascular tissues of the guinea-pig. *Br J Pharmacol* 68, 99-106

Hartshorne DJ & Siemankowski RF (1981). Regulation of smooth muscle actomyosin. *Ann Rev Physiol* 43, 519-630

Heschler J, Pelzer D, Trube G & Trautwein W (1982). Does the organic calcium channel blocker D600 act from inside or outside on the cardiac cell membrane? *Pflügers Arch* 393, 287-291

Hess P, Lansman JB, & Tsien RW (1984). Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca antagonists and antagonists. *Nature* 311, 538-544

Inoue Y, Kitamura K & Kuriyama H (1987). Some features of a single Ca channel obtained from rabbit ileal smooth muscle cell. *Folia Pharmacol* Japan 90, 90p

Inoue Y, Xiong ZL, Kitamura K & Kuriyama H (1988). Modulations produced by nifedipine of the unitary Ba-current of dispersed smooth muscle cells of the rabbit ileum. *Pflügers Arch* submitted

Ito Y, Kitamura K & Kuriyama H (1979). Effects of acetylcholine and catecholamines on the smooth muscle cells of porcine coronary artery. *J Physiol* 294, 595-611

Ito Y, Korenaga S & Tajima K (1980). Comparative study of the effects of 4-aminopyridine and tetraethylammonium on neuro-effector transmission in the guinea-pig vas deferns. *Br J Pharmacol* 69, 453-460

Itoh T, Kajiwara M, Kitamura K & Kuriyama H (1982). Roles of stored calcium on the mechanical response evoked in smooth muscle cells of porcine coronary artery. *J Physiol (Lond)* 322, 107-125

Itoh T, Kuriyama H & Ueno H (1983). Mechanisms of the nitroglycerine-induced vasodilation in vascular smooth muscles of the rabbit and pig. *J Physiol* 343, 233-252

Kamm KE & Stull JT (1985). The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. *Ann Rev Pharmacol* 25, 593-620

Kanmura Y, Itoh T, Suzuki H, Itoh Y & Kuriyama H (1983). Effects of nifedipine on smooth muscle cells of rabbit membrane artery. *J Pharmacol Exp Ther* 226, 238-348

Kuriyama H (1970). Effects of ions and drug on the electrical activity of smooth muscle In: Bulbring E, Brading AF, Jones AW, Tomita T (ed) *Smooth muscle.* Edward, Arnold, London p366-395

Kuriyama H (1981). Excitation-contraction coupling in various visceral smooth muscle. In: Bulbring E, Brandig AF, Jones AW, Tomita T (ed) *Smooth muscle.* Edward, Arnold, London. p171-198

Kuriyama H, Ito Y, Suzuki H, Kitamura K, Kajiwara M &Itoh T (1981). Chemical modulations of gated calcium current in vasculr smooth muscle membranes In: Ohnishi ST & Endo M (ed) *The Mechanisms of Gated Calcium Trasnport Across Biological Membranes.* Academic Press, p129-136.

Kuriyama H, Ito Y, Suzuki H, Kitamura K & Itoh T (1982). Factors modifying contraction-relaxation cycle in vascular smooth muscles. *Am J Physiol* 243, H641-H662

Kuriyama H & Suzuki H (1975). On the electrical and mechanical properties of the longitudiual and circular muscles of the guinea pig ileum. *INSERM* 50,

16-18

Lee KS & Tsien RW (1983). Mechanisms of calcium channel blockade by verapamil, D600, diltiazem and nitrendipine in single dialysed heart cells. *Nature* 302, 203-216

Mikawa T, Nonomura Y & Ebashi S (1977). Does phosphorylation of myosin light chain have direct relation to regulation in smooth muscle? *J Biochem* 82, 1789-1791

Mikawa T, Nonomura Y, Hirata S & Kikuchi S (1978). Involvement of an acidic protein in regulation of smooth muscle contaction by the tropomyosin-leiotonin system. *J Biochem* 1633-1636

Murphy RA, Aksoy MO, Dillon PF, Gerthoffer WT & Kamm KE (1983). The role of myosin light chain phosphorylation in regulation of cross-bridge cycle. *Fed Proc* 42, 51-56

Ohya Y, Kitamura K & Kuriyama H (1987). Cellular calcium regulates outward currents in rabbit intestinal smooth muscle cell. *Am J Physiol* 252, C401-C410

Ohya Y, Terada K, Kitamura K & Kuriyama H (1986). Membrane currents recorded from a fragment of rabbit intestinal smooth muscle cell. *Am J Physiol* 251, C335-C346

Ohya Y, Terada K, Kitamura K & Kuriyama H (1987). D600 blocks the Ca^{2+} channel from the outer surface of the membrane in smooth muscle membrane of rabbit intestine and portal vein. *Pflügers Arch* 408, 80-83

Ohya y, Kitamura K & Kuriyama H (1987). Modulation of ionic currents in smooth muscle cells of the rabbit intestine by intracellularly perfused ATP and cyclic AMP. *Pflügers Arch* 408, 465-673

Okabe K, Kitamura K & Kuriyama H (1988). The existence of a highly tetrodotoxin sensitive Na channel in freshly dispersed smooth muscle cells of rabbit main pulmonary atrery. *Pflügers Arch* 411, 423-428

Rembold CM & Murphy RA (1986). Myoplamic calcium, myosin phosphorylation, and regulation of the cross birdge cycle in swine arterial smooth muscle. *Circ Res* 58, 803-815

Rios E & Brum G (1987). Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* 325, 717-720

Sanguinette MC & Kass RS (1984). Voltage-dependent block of calcium channel current on the calf cardiac Purkinje fiber by dihydropyridine calcium channel antagonists. *Circ Res* 55, 336-348

Takahashi M, Seager MJ, Jones JF, Reber BFH & Catterall WA (1987). Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. *Proc Natl Acad Sci USA* 84, 5478-5482

Tanabe T, Takashima H, Mikami A, Flockerzi V, Takahashi H, Kanagawa K, Kojima M, Matsuo H, Hirose T & Numa S (1987). Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* 328, 313-318

Terada K, Kitamura K & Kuriyama H (1987). Different inhibitions of the voltage-dependent K^+ current by Ca^{2+} antagonists in the smooth muscle cell membrane of rabbit small intestine. *Pflügers Arch* 408, 558-564

Terada K, Kitamura K & Kuriyama H (1987). Blocking actions of Ca^{2+} antagonists on the Ca^{2+} channels in the smooth muscle cell membrane of rabbit small intestine. *Pflügers Arch* 408, 552-557

Uehara A & Hume JR (1985). Interactions of organic calcium channel antagonists with calcium channels in single frog atrial cells. *J Gen Physiol* 85, 621-647

Vandaele S, Fosset M, Galizzi JP & Lazdunski M (1987). Monoclonal antibodies that coimmunoprecipitate the 1,4-dihydropyridine and phenylalkylamine receptors and reveal Ca^{2+} channel structrue. *Biochmistry* 26, 5-9

Worley III JF, Deitmer JW & Nelson MT (1986). Single nisoldipine-sensitive calcium channels in smooth muscle cells insolated from rabbit mesenteric artery. *Proc Natl Acad Sci USA* 83, 5746-5750

Yatani A, Seidel CL, Allen J & Brown AM (1987). Whole-cell and single-channel calcium currents of isolated smooth muscle cells from saphenous vein *Cir Res* 60, 523-533

Yoshino M, Someya T, Nishio A & Yabu H (1988). Whole-cell and unitary Ca channel currents in mammalian intestinal smooth muscle cells: evidence for the existence of two types of Ca channels. *Pflügers Arch* 411, 229-231

Yoshino M & Yabu H (1985). Single Ca channel currents in mammalian smooth muscle cells. *Pflügers Arch* 404, 285-286

Yoshitomi T, Ito Y & Inomata H (1985). Adrenergic excitatory and cholinergic inhibitory innervations in the human iris dilator. *Exp Eye Res* 40, 453-459