Review article

Properties of the Inward Ionic Currents and Their Regulating Agents in Smooth Muscle Cells

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Introduction

After Hamil et al. (1981) introduced the patch clamp procedure for the investigation of ionic channels, studies on the biophysical features of smooth muscle membranes have progressed remarkably. From results with the patch and voltage clamp procedures, more than two voltage-dependent Ca channels, and several Ca-dependent and Ca-independent K channels have been classified from their physiological and pharmacological properties. With the whole cell patch clamp technique, a Na current was also isolated from vascular smooth muscles. In this article, we want to review recent advances in biophysical investigation of the ionic current of smooth muscle membranes. We will focus on the inward currents, following through the voltage-dependent and receptor-operated Ca permeable channels and voltage-dependent Na channel in relation to some drug actions.

Features of the voltage-dependent calcium-inward current

Using the patch clamp method (inside-out, outside-out or cell attached configuration) or the whole cell voltage clamp method, inward currents carried by Ca, Na and Cl ions have been recorded from smooth muscle cells. The Ca (Ba)-inward current which can produce the spontaneous or evoked action potentials is commonly observed in many smooth muscle tissues, and the voltage dependent Ca-inward current can be recorded from electrically quiescent (no response of the cell membrane to either electrical and drug stimulation) smooth muscle cells in various ionic environments. The Na current was obtained from some smooth muscle cells using the whole cell voltage clamp procedure, but this voltage-dependent Na-current is revealed by neither the classical microelectrode nor single channel current recording procedures.

Various features of the voltage-dependent Ca channel obtained from many different smooth muscle tissues with the whole cell voltage clamp and patch clamp methods are summarized in Table 1 (*A* and *B*). The activation and

Table 1A. Unitary Ca-current conductance in smooth muscles

Preparations	Conductance	Solution		Patch Configuration	References
		outside	inside		
Guinea-pig taenia coli	30pS	50mmol/l $BaCl_2$	Tyrode	cell-attached	Yoshino and Yabu (1985)
	25pS	50mmol/l Ba	145mmol/l KCl	cell-attached	Yoshino et al. (1988)
	12pS				
Guinea-pig aorta	12pS	110mmol/l $BaCl_2$	Ringer	cell-attached	Cafferey et al. (1986)
Amphiuma stomach	12pS				
Rabbit ear artery	20pS	110mmol/l $BaCl_2$	130mmol/l CsCl	outside-out	Aaronson et al. (1986)
	8pS				
	25pS	80mmol/l $BaCl_2$	120mmol/l NaCl	outside-out	Benham et al. (1987b)
	8pS				
Rabbit mesenteric artery	15pS	80mmol/l $BaCl_2$	120mmol/l NaCl	inside-out	Worley et al. (1986)
	8pS				
Rabbit intestine	20pS	50mmol/l $BaCl_2$	140mmol/l KCl	cell-attached	Inoue et al. (1989)
	25pS	100mmol/l $BaCl_2$	Krebs		
			130 mmol/l	cell-attached	Yatani et al. (1987)
Dog saphenous vein	18 24pS	90mmol/l $BaCl_2$	Kaspartate		
	7 9 pS				

Table 1B. Macroscopic Ca-current measured in smooth muscles

Preparation		Activating potential	Maximum activating potential (max current)	50 % activating potential (slope factor)	Inactivating potential	50 % inactivating potential (slope factor)	Solution	Temperature	References
Rabbit	ileum	−30 mV	+10 mV (0.3 nA)	−10 mV*	−60 mV	−30 mV (7 mV)	2.8 mmol l^{-1} $CaCl_2$	room temp.	Ohya et al. (1986)
	pulmonary artery	−40 mV	0 mV (100 pA)	−20 mV*	−70 mV	−40 mV (8.6 mV)	2.5 mmol l^{-1} $CaCl_2$	room temp.	Okabe et al. (1987b)
	mesenteric artery	−20 mV	+20 mV (0.2 nA)	0 mV*	−30 mV	0 mV (8.5 mV)	100 mmol l^{-1} $BaCl_2$	room temp.	Terada et al. (1987)
	portal vein	−30 mV	0 mV (0.4 nA)	−17 mV*	−50 mV	−38 mV (7.2 mV)	2.5 mmol l^{-1} $CaCl_2$	room temp.	Ohya et al. (1988b)

Guinea-pig	urinary bladder	−30 mV	−10 mV (0.95 nA)	−14 mV (−6 mV)	−60 mV	−43 mV (6 mV)	3.6 mmol.l^{-1} $CaCl_2$	35 °C	Klöckner and Isenberg (1985a)
	taenia coli	−30 mV	+20 mV (0.55 nA)	−5 mV*			2.5 mmol.l^{-1} $CaCl_2$	22—24 °C	Gamkevich et al. (1986)
	ileum	−40 mV	−10 mV (0.3 nA)	−27 mV (−8 mV)	−80 mV	−55 mV (8 mV)	1.5 mmol.l^{-1} $CaCl_2$ 1.5 mmol.l^{-1} $BaCl_2$	20—22 °C	Droogmans and Callewaert (1986)
Rat	vas deferens	−30 mV	0 mV (0.3 nA)	−15 mV*			1.8 mmol.l^{-1} $CaCl_2$	35 °C	Nakazawa and Matsuki (1987)
	aorta (cultured)	−30 mV	+20 mV (30 pA)	+5 mV*	−80 mV	−37 mV (22 mV)	20 mmol.l^{-1} $CaCl_2$	room temp.	Toro and Stefani (1987)
	mesentlric artery (cultured)	−30 mV 0 mV	+10 mV (5 pA) −10 mV +40 mV (25 pA)	−10 mV +20 V	−50 mV −20 mV	−41 mV (5.1 mV) −5 mV	115 mmol.l^{-1} $BaCl_2$	25 °C	Bean et al. (1986)
	azygos vein (cultured)	−40 mV −10 mV	−10 mV (1 nA) +50 mV (0.4 nA)	−30 mV* +20 mV*	−60 mV −20 mV	−45 mV −5 mV	10 mmol.l^{-1} $CaCl_2$		Sturek and Hermsmeyer (1986)
Toad	stomach	−30 mV	+10 mV (0.9 nA)	−5 mV*	−90 mV	−65 mV (9.6 mV)	50 mmol.l^{-1} $CaCl_2$	room temp.	Walsh and Singer (1987)
		−40 mV	0 mV (30 pA)	−20 mV*	−70 mV	−53 mV (5.3 mV)			
Dog	saphenous vein	−10 mV	+30 mV (100 pA)	+10 mV*	−30 mV	−18 mV (5.2 mV)	20 mmol.l^{-1} $CaCl_2$	room temp.	Yatani et al. (1987)

* estimated from I—V curve

inactivation potential levels for the Ca-current and the potential which produces the maximum Ca current are roughly the same in all smooth muscle cells studied. However, in some of the vascular smooth muscle cells, it is difficult to record the Ca-inward current in physiological salt solution containing 2.5 mmol . l^{-1} Ca but it is possible to record a Ba-inward current. Alternatively the Ca-inward current can be recorded with increased concentrations (above 10 mmol . l^{-1}) of Ca in the bath (Bean et al. 1986; Toro and Stefani 1987; Terada et al. 1987c.).

Many investigators have examined the binding affinity of [^{3}H]-dihydropyridines which have been reported to bind specifically to L subtype of the voltage-dependent Ca channel (see later) for various tissues, and reported that the K_d value is 0.1—0.2 nmol . l^{-1} in brain, heart and gastrointestinal smooth muscle membrane, whereas in skeletal muscles it is 1—2 nmol . l^{-1} (Bolger et al. 1983; Ehlert et al. 1982; Fosset et al. 1983). Sumimoto et al. (1988) reported that in dispersed smooth muscle cells of the porcine coronary artery, the K_d values for [^{3}H]-dihydropyridines show relatively high values of 1—2 nmol . l^{-1}. However, the affinity increased to 0.5—1.3 nmol . l^{-1}, when the microsomal fraction was used. This K_d value is almost the same as those observed for cardiac muscle and gastrointestinal smooth muscle cells in EGTA-treated microsomal fraction. Therefore, the affinity of the dihydropyridine derivatives to the binding sites my be changed by different environments. Sumimoto et al. (1988) noted in the porcine coronary artery that the value of B_{max} for the specific [^{3}H]-nifedipine binding is 60 fmol/10^6 cells which is equivalent to 36,000 binding sites per cell, and by assuming that the surface area of the smooth muscle cell is 5000 μm^2 (Gabella 1981), the density of the nifedipine binding site in the porcine coronary artery is calculated to be 7 sites per μm^2. This value agrees well with that observed for the guinea pig ileal smooth muscle cells (Bolger et al. 1983). On the other hand, the density of the binding sites for 1,4-dihydropyridine derivatives to the tubular structure in skeletal muscles is reported to be 65 pmol/mg proteins, and those in cardiac and vascular smooth muscle are calculated to be 1—2 pmol/mg protein and 0.2 pmol/mg protein, respectively (Triggle and Janis 1987). Unfortunately, the density of Ca channels estimated from the binding site for 1,4-dihydropyridine derivatives and from the Ca current measured by the patch or whole cell clamp procedures are not completely comparable (see later).

Activation and inactivation of the Ca-inward current

The amplitude of the Ca-inward current increases as the Ca concentration increases, and the amplitude of the Ca-current is not affected by $[Na]_o$ or addition of Ba up to 1 mmol/l. Thus, the Ca channel is highly selective to Ca rather than other permeable ions. The amplitude of the inward current is

markedly enhanced by replacing Ca with Ba in the bath (Ohya et al. 1986; Ganitkevich et al. 1986). Although $[Na]_o$ does not affect the Ca inward current, when $[Ca]_o$ is reduced to below 0.1 mmol/l, the duration of the action potential evoked by a depolarization pulse is prolonged (plateau formation). This prolongation of the action potential is due to generation of a Na- inward current passing through the Ca channels, because it is not blocked by tetrodotoxin (TTX) but is blocked by Mn or Ca channel blockers, such as nifedipine or diltiazem. When concentrations of Ca are increased to 0.1 μmol/l in the bath or a few mmol/l of Ca is added in the Ba solution, both the Na- and Ba-currents are markedly inhibited (Ohya et al. 1986; Jmari et al. 1987).

The resting membrane potential in many mammalian smooth muscle cells ranges between -50 mV and -70 mV (Creed 1979), while the voltage dependent inactivation of the Ca current occurs at a more negative membrane potential than the resting potential (Table 1*B*). For example, in the rabbit pulmonary artery, the membrane potential is about -50 mV, but the inactivation occurs at -70 mV. The membrane potential at which the Ca current was inhibited to half ($V_{0.5}$) is estimated to be -40 mV. This means that at the resting membrane potential, a quarter of the available Ca channels has already been inactivated. Similarly in frog stomach muscles, the membrane potential produced half inactivation ($V_{0.5}$) of the inward current was -65 mV (Walsh and Singer 1987). Thus, the inactivation process occurs beyond the level to evoke the Ca-inward current. When the inactivation curve is plotted 100 ms prepulse (conditioning pulse), a U shaped curve is obtained, and when various intracellular concentrations of free Ca, $[Ca]_i$, are perfused instead of given prepulses, the inactivation is enhanced in proportion to the increase in the $[Ca]_i$. These results indicate that the Ca-inward current itself regulates the Ca-inward current, and the inactivation of the Ca-inward current occurs in a manner dependent on voltage and intracellular Ca (Ohya et al. 1986; Jmari et al. 1986; Ganitkevich et al. 1986).

When decay of the Ca-inward current is compared with the Ba- or Na-inward currents through the Ca channel, inactivation occurs much faster for the Ca-inward current than the Ba-inward current. On the other hand, administration of EGTA into the pipette produces a slow decay of the Ca-inward current. When the inactivation process is estimated from the peak amplitude of the inward current, the appearance of inactivation has a causal relation with the amount of free Ca in the cytosol (Ohya et al. 1988a), i.e. when various concentrations of Ca are perfused into the cell by the intracellular perfusion technique, the inhibition of the amplitude is accelerated in proportion to the intracellular concentration of Ca. When $[Ca]_i$ is increased to 100 nmol/l, the peak amplitude of the Ca-inward current is reduced to half, and with perfusion of 1 μmol/l Ca it ceases completely. The Hill coefficient (n_H) for the inhibition

of $[Ca]_i$ for the Ca inward current is 1 and the K_d is 100 nmol/l. However, intracellular perfusion with Ba does not accelerate the inactivation of the Ca-inward current (Ohya et al. 1988a). Therefore, inactivation of the Ca-inward current plays an important role, recurrently, in regulation of the $[Ca]_i$, and this mechanism may prevent an excessive increase of the $[Ca]_i$ in the cytosol. It is well known that increases in the $[Ca]_i$ above 100 nmol/l evoke contraction and 1 μmol/l Ca evokes a submaximum contraction in various vascular beds (Kuriyama et al. 1982). Thus, a negative- feedback regulatory mechanism may occur on the Ca influx through the voltage- and Ca-dependent processes.

Classification of the Ca-inward current from measurements of the unitary and macroscopic currents

Carbone and Lux (1984) classified the Ca current obtained from the chick dorsal root ganglion cell into two subtypes; transient (T-type; 8-10 pS) and long-lasting (L-type; 25 pS) types. Furthermore, Nowycky et al. (1985) found an additional Ca current, neither T- nor L-subtype (N-type; 13 pS) in the same cell (Table 2). From the features of the macroscopic current, two types of Ca channel have also been elucidated in many excitable cells (channel I and II, Hagiwara et al. 1975; inactivation type and non-inactivation types, Llinas and Yarom 1981). Classification and characterization of the Ca-unitary current recorded from cardiac muscle have been investigated in detail (Reuter et al. 1982; Cavalie et al. 1983, 1986; Nilius 1985; Hess et al. 1986; Lansman et al. 1986). In smooth muscle cells, the Ca channel is also classified into two subtypes (Bean et al. 1986; Sturek and Hermsmeyer 1986; Friedman et al. 1986; Loirand et al. 1986; Worley et al. 1986; Benham et al. 1987b; Yatani et al. 1987; Jmari et al. 1987; Yoshino et al. 1988). On the other hand, one type of Ca unitary current is recorded from rat aorta and rabbit ileal smooth muscle cells (Caffrey et al. 1986; Inoue et al. 1989).

From characteristics of the Ca channel in many excitable cells, the Ca currents are classified by various criteria, i.e., slope conductance of the Ca-unitary current, the threshold required to activate the Ca channel, the time constants of the inactivation process, the permeability of the channel to Ca compared with Ba, and the sensitivities to 1,4-dihydropyridine derivatives and to divalent cations such as Ni or Cd. However, analysis of subtypes from the results obtained by the whole cell clamp method (macroscopic-current recording) requires some caution, for instance, the decay of the Ca current (inactivation) occurs in a voltage and Ca dependent manner, and the Ba current shows only voltage dependent inactivation (Table 2). In smooth muscle cells of the rabbit mesenteric artery, the voltage- dependent Ca channel is classified into two subtypes from the slope conductance, both channel subtypes are inhibited by

Table 2. Features of voltage dependent Ca channel subtypes

	T subtype	N subtype	L subtype
Activating potential level	-70 mV	-10 mV	-10 mV
Inactivating potential level	$-100 \sim -60$ mV	$-100 \sim -40$ mV	$-60 \sim -10$ mV
Inactivating potential level	$20 \sim 50$ ms	$20 \sim 50$ ms	slow (> 700 ms)
Unitary channel slope conductance	$8 \sim 10$ pS	13 pS	25 pS
Ion selectivity	$I_{Ca} > I_{Ba}$	$I_{Ba} > I_{Ca}$	$I_{Ba} > I_{Ca}$
Inhibitors			
Cd^{2+} (Kd)	$+$ ($160\,\mu$mol.l^{-1})	$+++$ ($7\,\mu$mol.l^{-1})	$+++$ ($7\,\mu$mol.l^{-1})
Ni(Kd)	$+++$ ($47\,\mu$mol.l^{-1})	$+$ ($280\,\mu$mol.l^{-1})	$+$ ($280\,\mu$mol.l^{-1})
dihydropyridine derivatives	$-$	$-$	$+++$
ω — conotoxin derivative	$-$	$++$	$++$(nerve)
			$-$(smooth muscle)

low concentrations of nisoldipine, a 1,4-dihydropyrine derivative (Worley et al. 1986).

Effects of Ca antagonists on the Ca inward current

As stated previously, the B_{max} value of the [^{3}H]-nitrendipine binding in smooth muscles is much lower than that in tubular structure of skeletal muscles (Triggle and Janis 1987). Thus, biochemical research to determine the amino acid sequence of the Ca channel (L subtype) has usually been carried out using the transverse tubules of skeletal muscles, and was found to be composed of the α_1 — (175 kD), α_2 — (143 kD), β — (54 kD), γ — (30 kD) and δ — (24—27 kD) subunits. The 1,4-dihydropyridine binding protein, forming the Ca permeable path, is thought to be the α_1-subunit and the path is composed of hydrophobic amino acid sequences. There are two phosphorylation sites in the channel protein; one is located at a site facing the cytosol of the α_1-subunit and the other site is in the β-subunit (Glossman and Ferry 1983; Curtis and Caterall 1984, 1986; Borsotto et al. 1985; Galizzi et al. 1986; Takahashi et al. 1987; Tanabe et al. 1987).

Mechanisms of action of Ca entry blocker (Ca channel blocker, slow channel blocker) on the voltage-dependent Ca channel have already been reviewed by many investigators (Fleckenstein 1983; Godfraind 1985). In this article, we focus attention on the actions of Ca channel blockers on the Ca channels elucidated as unitary- and macroscopic-currents.

Ca channel blockers are classified into 1,4-dihydropyridine derivatives

(nifedipine, nicardipine, nitrendipine, nisoldipine, nimodipine, felodipine, and others), phenylalkylamine derivatives (verapamil, gallopamil, tiapamil), benzothiazepine derivative (diltiazem), diphenylpiperazine derivatives (cinnarizine, flunarizine) and others, such as bepridil or prenylamine. Since highly selective Ca channel blockers are only few, we define Ca channel blockers as organic compounds which rather selectively block the entry of Ca through the voltage-dependent Ca channel. To explain the action of Ca channel blockers, the modulated receptor hypothesis is adopted (Hille 1977), i.e. the state of the channel is classified into resting, open and inactivated states. In cardiac muscles 1,4-dihydropyridine derivatives act on the resting as well as inactivated states (Sanguinetti and Kass 1984; Uehara and Hume 1985). On the other hand, phenylalkylamine derivatives and benzothiazepine derivatives act on the open state as well as inactivated state, because the inhibition is predominantly observed with repetitive firing of cardiac muscle cells (Uehara and Hume 1985; McDonald et al. 1984b).

Using the patch clamp procedures, Hess et al. (1984) classified the state of Ca channels by its channel kinetics into mode 0: — no channel open, mode 1: — channel opening with short open time and mode 2: — channel opening with long open time. The state of the Ca channel changes very slowly between these modes. Nitrendipine in low concentrations, enhances the channel opening by prolongating the open time of the channel (mode 1 to mode 2) in the same manner as observed on application of Bay K 8644, a 1,4-dihydropyridine derivative Ca channel agonist (Lee and Tsien 1983; Trautwein and Pelzer 1983; McDonald et al. 1984 a and b; Ochi et al. 1984; Kokubun and Reuter 1984; Hess et al. 1984; Hume 1984; Trautwein and Pelzer 1985), but high concentrations of nitrendipine reduce the open probability. As a consequence, sweeps with no channel opening are increased (mode 1 to mode 0; Hess et al. 1984).

The effects of various Ca channel blockers were investigated on smooth muscle cells of the rabbit small intestine. 1,4-dihydropyridine derivatives such as nifedipine or nicardipine inhibit the peak Ca-inward current, in a concentration dependent manner, at the holding potential of $-60\,\text{mV}$ (ID_{50} value of $24\,\text{nmol}\,.\,l^{-1}$) (Terada et al. 1987a). CV-4093 or KW-3049, newly synthesized long acting dihydropyridine derivatives applied to rabbit arterial muscle cells produce an inhibitory action on the Ca-inward current similar to that observed on application of nicardipine. However, the inhibitory action of both drugs lasted much longer than that of nicardipine (Terada et al. 1987c; Okabe et al. 1987b). The inhibition induced by either agent occurs to a greater extent on the Ca inward current evoked by higher than lower depolarization pulses. Both CV-4093 and KW-3049, as described previously for nitrendipine in cardiac muscles, show a dual action on the Ca-inward current (Kokubun and Reuter 1984; Hess et al. 1984; Affolter and Coronado 1985; Brown et al. 1986; Bean

et al. 1986). 1,4-dihydropyrine derivatives shifted the voltage-dependent inactivation curve to more negative membrane potential levels, and tonic inhibition of the Ca-inward current was observed within a few minutes after application of the drugs. CV-4093 and KW-3049 showed a slow inhibition of the Ca-inward current, but the inhibition occurred without any relation to the number or frequency of stimulation, i.e. the inhibitory action was not "use dependent". These observations indicate that 1,4-dihydropyridine derivatives act mainly on the Ca channel in the inactivated state (Terada et al. 1987c; Okabe et al. 1987b). However, these agents also accelerate the decay of the Ba inward current, suggesting that they also act as open channel blockers.

In contrast with 1,4-dihydropyridine derivatives, verapamil inhibits the Ca channel in a use dependent manner, in the longitudinal smooth muscle cells of the guinea-pig ileum ($ID_{50} = 1.3\,\mu\text{mol}\,.\,l^{-1}$), i.e. the inhibition of the Ca current depends on the number or frequency of stimulation at the holding potential of either -60 mV or -80 mV. Verapamil also shifts the steady state inactivation curve to the left on application of a conditioning pulse of 10 s but not on application of short pulses (3 s). These results suggest that verapamil acts mainly on the Ca channel in the open state (Terada et al. 1987a). In cardiac muscle cells, gallopamil and verapamil were reported to act from inside the cell (Heschler et al. 1982), but Ohya et al. (1987c) noted that both agents act on extracellular application but no effect occurred with intracellular perfusion.

Diltiazem also inhibits the Ca-inward current ($ID_{50} = 1.4\,\mu\text{mol}\,.\,l^{-1}$) with a weaker potency in comparison to that of the 1,4-dihydropyridine derivatives. This agent inhibited the channel partly in a use dependent manner and partly by binding at the inactivated state (Terada et al. 1987 a). Diltiazem inhibits the Ca channel more potently on addition to the bath rather than intracellular perfusion (Ohya et al. 1987c). From receptor binding experiments, using [^{3}H]-nitrendipine on the fragmented membrane of smooth muscle cells, diltiazem shows a positive allosteric action, but verapamil shows a negative allosteric action on the 1,4-dihydropyridine binding site. On the other hand, both diltiazem and verapamil additively inhibit the action of 1,4-dihydropyridine as estimated from the amplitude of the Ca-current recorded from dispersed smooth muscle cells. The effects of diltiazem and verapamil on the 1,4-dihydropyridine-induced inhibition of the depolarization-induced contraction occur non-competitively (Kanmura et al. 1983).

Flunarizine inhibits the Ca spike (Van Neuten et al. 1978; Nakayama and Kasuya 1980; Godfraind and Dieu 1981) by blocking the Ca inward current. The inhibition of the Ca inward current occurs in concentration dependent (ID_{50} was 1.4 μmol/l), voltage and use dependent manners. Furthermore, intracelullar application of flunarizine does not modify the Ca inward current (Terada et al. 1987d).

Ca channel blockers (nicardipine, diltiazem, verapamil and flunarizine) also act on the K-outward current of the rabbit small intestine as measured using the whole cell voltage clamp method (Terada et al. 1987b). To eliminate the influence of the Ca dependent K-current, the voltage dependent K-current was recorded in 2.5 mmol.l^{-1} Mn containing solution (Ca-free). These agents inhibit this voltage dependent K-current in a concentration dependent manner (the ID_{50} values for nicardipine, diltiazem, verapamil and flunarizine are, 4.5 μmol.l^{-1}, 30 μmol.l^{-1}, 14 μmol.l^{-1} and 5.8 μmol.l^{-1}, respectively). The voltage dependent inactivation of the K-current observed below 0 mV, is not modified by 10 μmol.l^{-1} diltiazem, 5 μmol.l^{-1} verapamil or 30 μmol.l^{-1} nicardipine. Nicardipine, verapamil and flunarizine accelerate the decay of the outward current while diltiazem does not. Therefore, the mode of inhibition of the voltage dependent K-current induced by nicardipine or verapamil differs from that by diltiazem. The Ca dependent K-current is also inhibited by high concentrations of nicardipine (Ohya, unpublished observation). The selectivity of some Ca channel blockers for both the Ca and K channels observed on smooth muscle cells is low in comparison to that seen from frog atrial cells (Hume 1984). In cardiac muscle cells, the ratios of the ID_{50} values (K-current/Ca-current) are 2000 for nisoldipine 1000 for gallopamil and 76 for diltiazem, whereas in ileal smooth muscle cells, they are 190 for nicardipine, 11 for verapamil, 21 for diltiazem and 4 for flunarizine (Terada et al. 1987, a,b,d). In helix neurons, Nishi et al. (1983) reported that the selectivities of Ca channel blockers (nifedipine, verapamil and diltiazem) for the Ca current against the time dependent K current are in the range of 1—17. High concentrations of gallopamil or nitrendipine also block the Na channel in the human or neonatal rat cardiac myocytes (Bustamante 1985; Yatani and Brown 1985). These differences in selectivity are mainly caused by differences in the potency of inhibition of the Ca current, and not the K-current. Therefore, the inhibition induced by Ca antagonist on the K- and Na-currents seems to be a nonselective blocking action at high concentrations of Ca channel blockers. Thus, we should be aware of an inhibitory action on the K and Na channels by Ca channel blockers on smooth muscle cells, because diltiazem, verapamil and flunarizine require relatively high doses in comparison to 1,4-dihydropyridine derivatives in clinical uses.

Factors and drugs modifying the Ca channel other than Ca channel blockers

Cyclic nucleotides:

Adrenaline and isoprenaline accelerate the activation of the L-subtype of the voltage dependent Ca channel in cardiac muscles (Heschler et al. 1986). As described previously, the α_1-subunit and β-subunit of the 1,4-dihydropyridine

derivative binding protein contain the phosphorylation site. The accelerating action of catecholamines is thought to be due to phosphorylation of channel protein by cyclic AMP synthesized through activation of adenylate cyclase (catalytic subunit) with protein kinase A (A-kinase), and also activation of the related GTP-binding protein (G_s- or N_o-protein). In contrast, extracellular application of dibutyryl cyclic AMP and intracellular perfusion of cyclic AMP to the smooth muscle cells of the guinea-pig ileum do not modify the voltage dependent Ca channel (Ohya et al. 1987b), and no action of dibutyryl cyclic AMP was reported in the guinea-pig urinary bladder (Klöckner and Isenberg 1985a). In the rabbit ear artery, Aaronson et al. (1986) reported that noradrenaline (NAd) accelerates the L- and T-subtypes of the Ca inward current, while Droogmans et al. (1987) noted that this agent inhibits the Ca inward current and this inhibition has no causal relation with cyclic GMP and cyclic AMP. However, ineffectiveness of cyclic nucleotides does not indicate that the phosphorylation process is not required to activate the Ca channel, because the presence of ATP but not AMP-PNP is absolutely necessary to generate the Ca-current. ATP enhances the amplitude of Ca-currents in a concentration dependent manner and prevents the run down phenomenon of the amplitude of Ca-current (Ohya et al. 1987b).

Acetylcholine (ACh) inhibits the voltage dependent Ca-current in frog ventricular muscles through synthesis of cyclic GMP (Fischmeister and Hartzell 1987). While in smooth muscle cells, cyclic GMP had no effect on the Ca-inward current (Ohya, unpublished observations). In vascular smooth muscle cells, ACh increases the amount of cyclic GMP mainly through release of endothelium-derived relaxing factor (EDRF; Furchgott et al. 1981; Furchgott 1983). This cyclic GMP synthesized by EDRF in the cytosol of smooth muscle cells is due to activation of soluble guanylate cyclase (Rapoport and Murad 1983; Ignarro and Dadwitz 1985; Murad 1986).

Biological poisons

Maitotoxin: Maitotoxin is isolated from the dinoflagellate, *Gambierdiscus toxicus*, and possesses an excitatory action on the contraction evoked in the guinea-pig intestine, and this excitatory action is inhibited by nitrendipine but not by tetrodotoxin. Therefore, this poison may activate the voltage dependent Ca channel (Ohizumi et al. 1983). Maitotoxin also accelerates the ^{45}Ca influx in cultured chicken cardiac muscle cells which is inhibited by nitrendipine.

Gonioporatoxin: In the transverse tubules of the rabbit skeletal muscles, gonioporatoxin inhibits the binding of a 1,4-dihydropyridine derivative (PN 200—110) to the channel protein (Ohizumi et al. 1983; Kobayashi et al. 1986, 1987). Presumably, this agent activates the L-subtype of the voltage dependent Ca channel. However, there is little information on the actions of gonioporatoxin.

ω-conotoxin: ω-conotoxin is purified from poisons of *Conus geographus*, and this toxin possesses the property to inhibit the L- and N-subtypes but not the T-subtype (but using the patch clamp method, this agent transiently inhibits the T-subtype) in chicken dorsal root ganglion cells. This agent has no effect on the Na- and K-currents, or on synthesis and actions of the second messenger (Kerr and Yoshikami 1984; McClesky et al. 1987). However, this toxin does not bind to the L-subtype of Ca channels at the sarcolemma in cardiac and smooth muscle cells or to fragments of the transverse tubular system of skeletal muscle cells, but it does bind to nerve cell membranes (Cruz et al. 1987). It is also reported that this binding site differs from that of the 1,4-dihydropyridine derivative (Cruz and Olivera 1986). Therefore, the binding of ω-conotoxin to the voltage dependent Ca channel may possess tissue specificity. This also indicates that the property of the L-subtype observed in nerve and smooth muscle cells may differ.

Other agents: In peripheral nerve fibres, secretory glands and also in the dorsal root ganglion cells, neuropeptides such as leucine-enkephalin and somatostatin inhibit the type II (N-subtype) Ca inward current, in a concentration dependent manner. The receptor for enkephalin is thought to be the δ-opiate receptor (Tsunoo et al. 1986; McDonald and Werz 1986). Since dynorphin A inhibits but enkephalin fails to inhibit the Ca-current recorded from the dorsal root ganglion, the opiate receptor in the Ca channels seems to be the κ-but neither μ nor δ-opiate receptor (McDonald and Werz 1986). The action of enkephalin requires the presence of GTP-binding protein (Hescheler et al. 1987). Chlorpromazine and benzodiazepine derivatives (clonazepam, diazepam, nitrazepam) also inhibit the Ca inward current and accelerate the inactivation process in the squid giant axon. In smooth muscle tissues, such actions of chlorpromazine have been postulated from the results obtained by mechanical recording methods. These drugs act mainly on the type I (T-) subtype of the Ca-current but also a little on type II (N-) subtype (McCleskey et al. 1986). Phenytoin (diphenylhydantoin), an anti-epileptic agent, inhibits activation of the type I (T-subtype) of the voltage dependent Ca channel of the squid giant axon (M. Yoshii, personal communication). Systematic investigations of various drugs which act on the central nervous system have not been carried out on the Ca-inward current in smooth muscle cells.

The receptor activated calcium inward current

Activation of a receptor by its own agonist may directly modify the Ca channel and increase the Ca influx. Until now, the activation of the Ca influx recorded as a unitary current by direct stimulation of the receptor has only been demonstrated in the rabbit ear artery on application of ATP (activation of the purinergic receptor; P_2-subtype) using the patch clamp method (Benham and Tsien 1987) (Table 3).

Table 3. Receptor operated ion channel

Substance P receptor toad stomach	inhibition of K channel (M-current)	Sims et al. (1986)
Muscarinic receptor toad stomach	inhibition of K channel (M-current)	Sims et al. (1986)
rabbit jejunum	voltage dependent noselective cation channel	Benham et al. (1985a)
guinea-pig ileum	voltage dependent nonselective cation channel (mainly Na channel)	Inoue et al. (1987)
Purinergic receptor rabbit ear artery	voltage dependent Ca Na channel	Benham and Tsien (1987)
Adrenergic receptor rat anococcygeus muscle	voltage dependent Cl channel	Byrne and Large (1987a and b)

Acetylcholine: In the toad stomach muscles, ACh does not modify the Na and Cl permeabilities but consistently increases the voltage dependent Ca-inward current elicited by the depolarization, and inhibits the K-current (M current) (Sims et al. 1985; Clapp et al. 1987). In the longitudinal smooth muscle cells of the rabbit jejunum, Benham et al. (1985a) reported that the ACh-induced depolarization is due to activation of nonselective permeation for Na and K ions as estimated from the reversal potential value for ACh in various ionic solutions. Inoue et al. (1987) also reported on the guinea-pig small intestine that ACh opens a non-selective cation channel with a single channel conductance of 20—25 pS. The inward current can be evoked in the absence of Ca or Cl but the current is reduced in Na-deficient solution. This inward current is, therefore, mainly due to activation of the Na channel and contributions by K and Cl for regulating the inward current may be small in physiological ionic conditions.
Catecholamines: Noradrenaline (NAd), as an activator of α-adrenoceptor, depolarizes the membrane in the guinea-pig pulmonary artery and this is associated with an increase in the ionic conductance of the membrane due to an increase in the inward current (Byrne and Large 1987a), although in whole tissue preparations, the depolarization of the membrane by NAd is associated with a decrease in the membrane conductance. In the rat anococcygeous muscle, NAd activated the Cl channel (Byrne and Large 1987b). On the other hand, in the rabbit ear artery no such inward current is recorded in response to NAd in the resting state (-50 mV), but NAd inhibits the voltage dependent Ca-current evoked by various levels of the command pulse (Droogmans et al. 1987). They

noted that the effects of NAd are not modified by addition of cyclic AMP or cyclic GMP to the pipette solution. In contrast with the above observations, Aaronson et al. (1986) presented a short note on the rabbit ear artery stating that NAd enhances both the slow and fast inward currents (T- and L-subtypes) evoked by the depolarization. Furthermore, Pacaud et al. (1987) reported that NAd stimulates the fast Ca current but inhibits the slow Ca current due to the release of Ca from the sarcoplasmic reticulum. Further detailed experiments are required to clarify the action of NAd on the membrane current to determine whether the response is generated by the receptor mediated response or through synthesis of second messengers. Concerning the action of the second messenger, intracellular perfusion of cyclic AMP did not modify the membrane currents and the resting membrane potential in smooth muscle cells (urinary bladder, Klöckner and Isenberg 1985a; ear artery, Droogmans et al. 1987; small intestine, Ohya et al. 1987b). Furthermore, $InsP_3$ has no effect on the Ca-current observed by intracellular perfusion technique (Ohya et al. 1988b).

Adenosine triphosphate (ATP): Responses of smooth muscle cells to ATP differ in different tissues, e.g. ATP hyperpolarizes intestinal smooth muscle cells due to activation of the apamine-sensitive Ca-dependent K channel (Yamanaka et al. 1985). On the other hand, in smooth muscle cells of some vascular tissues, ATP depolarizes the membrane (Benham et al. 1987a). In the rat vas deferens, Nakazawa and Matsuki (1987) reported that ATP produces a transient inward current. Using the cell attached patch clamp procedure, a unitary inward current can be recorded and the reversal potential is estimated to be about 0 mV. The slope conductance as estimated from the current-voltage relationship is calculated to be 20 pS. Thus, the ion selectivity of this channel is poor. In the rabbit ear artery, Benham and Tsien (1987) reported that ATP evokes a transient inward current with a reversal potential close to 0 mV and this inward current does not depend on the membrane potential. Substitution of Cl, K (with Na) or Ca (with Ba) does not affect the response induced by ATP and the inward current was still observed in 110 mmol.l^{-1} Ca, 110 mmol.l^{-1} Ba or 110 mmol.l^{-1} Mg solution, thus suggesting that the ATP-activating channel is cation selective and allows permeation of monovalent, and possibly divalent cations. The conductance of the single channel activated by ATP is reported to be about 5 pS with 110 mmol.l^{-1} Ca or Ba in the bath. The selectivity for Ca relative to Na, examined is about 3 : 1. Since the ATP-activated Ca-current is recorded in low cytosolic Ca concentration (10 nmol.l^{-1}), and $ATP_\gamma S$ or α,β-methylene ATP has no effect on this current, it is possible that the contribution of the signal transductor synthesized by ATP around the membrane cannot be ruled out. On the other hand, intracellular perfusion of ATP prevents a "run down" phenomenon of the Ca-inward current as described previously, and

enhances the Ca-inward current possibly due to phosphorylation of the Ca channel (Ohya et al. 1987b).

Features of the sodium-inward current

In the presence of very low concentrations of [Ca_o] (0.1 μmol.l^{-1}), a Na-current is generated through the Ca channel in smooth muscle cells similar to that observed in the cardiac muscle cells (Klöckner and Isenberg 1985a; Ohya et al. 1986; Loirand et al. 1986; Benham et al. 1986; Walsh and Singer 1987). In several smooth muscle cells, a Na-current which is resistant to inorganic (Ni, Cd or others) and organic Ca channel blockers is found.Amédée et al. (1986) reported the presence of a low affinity binding site for labelled TTX in the pregnant rat myometrium. Sturek and Hermsmeyer (1986) reported the presence of a Na channel in primary cultured smooth muscle cells obtained from azygos vein of neonatal rat, which is thought to be related to the pacemaker activity in this smooth muscle cells.

In general, the Na channel in smooth muscle cells is thought to be the TTX resistant type (venous smooth muscle cells of the neonatal rat, K_d value of 30 μmol.l^{-1}, Sturek and Hermsmeyer 1986; rat myometrium, K_d value of 2 μmol.l^{-1} Amédée 1986). However, in the rabbit pulmonary artery, Okabe et al. (1988a) recorded the presence of a voltage dependent Na channel which is sensitive to TTX using the whole cell patch clamp method. At a holding potential of -80 mV, application of a command pulse of -10 mV produces a fast (rapidly inactivated; peak amplitude within 1 ms) and slow inward current (slowly inactivated; peak amplitude within several ms). On superfusion with Ca-free solution, the slow inward current ceases but a part of the inward current, the fast inward current, remains the same. At a holding potential of -60 mV, this fast inward current is almost inactivated, i.e. the inactivation of the fast inward current occurs at a lower membrane potential than that of the slow inward current. The fast inward current reduces the amplitude in inverse proportion to the concentration of Na, and is blocked by low concentrations of TTX (above 1 nmol.l^{-1}; the dissociation constant, $K_d = 8.7$ nmol.l^{-1}). This TTX-sensitivity of the channel is similar to that reported in nerve fibres rather than cardiac muscle cells (Chiu et al. 1984; Benoit et al. 1985; Benndorf et al. 1985; Pidoplichko 1986) (Table 4*A*). Nicardipine, a 1,4-dihydropyridine derivative, has no effect on the fast current but blocks the slow current. The fast inward current is, therefore, generated by the permeation of Na ion through the Na channel. Furthermore, chloramine-T, a modulator of inactivation of the fast Na channels of nerve fibres (Wong 1984; Schmidtmayer 1985) suppresses the inactivation of the Na inward current and inhibits the Ca-inward current in the smooth muscle cells of the rabbit main pulmonary artery (Okabe et al. 1988a).

Table 4A. Classification of the Na channel in smooth muscle

Channel type	Preparation	References
TTX sensitive Na channel (IC_{50} = 8nmol . l^{-1})	rabbit pulmonary artery rat portal vein	Okabe et al. (1988) Okabe et al. (1989)
TTX resistant Na channel IC_{50} = 30μmol . l^{-1} = 2μmol . l^{-1}	rat neonatal cell from azygos vein (cultured) rat myometrium	Sturek and Hermsmeyer (1986) Amédée et al. (1986)

Table 4B. Anion channel in smooth muscle

channel	conductance	solution		preparation	reference
		inside	outside		
Cl channel (Ca sensitive)	340pS	150 mmol . l^{-1} TEA Cl	150 mmol . l^{-1} TEA Cl	A7r5 cell line (rat aorta)	Soejima and Kokubun (1988)

The presence of such highly TTX sensitive Na-currents was also reported in the rat portal vein (Okabe et al. 1988b). However, the membrane potentials of most smooth muscles are relatively low (−70 to −45 mV), and a part of the Na-current may already be inactivated. Furthermore, in the case of the rabbit pulmonary artery, a spontaneous action potential cannot be recorded. The role of the Na channel in smooth muscle cells should be clarified.

Nature of the chloride-inward current

In smooth muscles, Cl ions play an important role in maintaining the resting membrane potential and also in the membrane responses mediated by agonists. In the rat anococcygeous muscle, NAd increased the Cl conductance and the reversal potential level for NAd could be explained by the Cl equilibrium potential (Bryne and Large 1987b). Using the patch clamp method, Soejima and Kokubun (1988) revealed the presence of a voltage dependent Cl channel in cultured arterial smooth muscle cells (Table 4*B*). However, further experiments are required to clarify features of the Cl channel in smooth muscles.

Conclusion

In this mini-review, we have introduced recent advances on investigations of the inward current recorded from smooth muscle cells obtained with the patch and whole cell clamp methods. At least two different voltage dependent Ca channels

are classified from the value of the unitary current conductances, sensitivity to Ca channel blocker and also the biophysical characteristics. It is worth to note that even in the same L-subtypes the unitary conductance of the Ca channels in visceral smooth muscle differ from those in arterial muscle cells. Features of these inward currents have similarities to and differences from those observed in cardiac and nerve cells. In comparison to cardiac muscle cell membranes, the voltage dependent Ca channel (L-subtype) in smooth muscle cell membranes is distinguished by the action of cyclic AMP and also by the site of action of Ca channel blockers. It is plausible to postulate that the presence of two different Ca channels (L- and T- subtypes) is not a prerequisite to preserve the physiological function of smooth muscles, because action potentials evoked in smooth muscles cease on application of low concentrations of 1,4-dihydropyridine derivatives. As concerns a receptor activated ionic current, for example the puringergic receptor, the channel is voltage independent and is nonselective to cations. A Na-inward current is also recorded from smooth muscle cells and the channels responsible for the Na-current are classified into TTX sensitive (high affinity) and resistant (low affinity) channels. However, the role of the Na-current observed in vascular smooth muscles on the physiological function is not yet clarified.

Features of the outward currents recorded from smooth muscles are not discussed in this review. Several Ca-dependent and Ca-independent K channels have been recorded using the patch and whole cell clamp procedures (Benham et al. 1983, 1985b, 1986; Berger et al. 1984; Singer and Walsh 1984; Inoue et al. 1985, 1986; Klöckner and Isenberg 1985b; McCann and Welsh 1986, 1987; Walsh and Singer 1987; Ohya et al. 1987a; Okabe et al. 1987a). We may review on K-outward current in the near future.

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