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Calcium-activated Ionic Currents in Smooth Muscle Cells from Rabbit Superior Mesenteric Artery

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=ABSTRACT=

Intracellular free Ca^{2+} contributes to regulation of various events occurring in vascular smooth muscle cells. One of these events is modulating the membrane ion currents. Single smooth muscle cells were isolated from rabbit mesenteric artery. Three kinds of Ca^{2+}-activated currents were studied with the patch clamp method. Ca^{2+}-activated K^+ current with a large oscillation was recorded in the depolarized potential range. The single channel conductance of this current was about 250 pS. It was abolished by replacing intracellular K^+ with Cs^+. A Ca^{2+}-activated nonselective cation current was observed in both the depolarized and hyperpolarized potential ranges. And it was blocked by replacement of extracellular Na^+ with N-methylglucamine (NMG) or extracellular application of Cd^{2+}. Ca^{2+}-activated Cl^- current was revealed in the whole voltage range and was blocked by niflumic acid.

These results indicate that at least three kinds of Ca^{2+}-activated ionic currents exist in smooth muscle cells from rabbit superior mesenteric artery.

Key Words: Single smooth muscle cells, Ca^{2+}-activated currents, patch clamp, Single channel recording

INTRODUCTION

Intracellular Ca^{2+}, known to be an intracellular second messenger involved in many important biological mechanism, increases by influx through volatge-dependent channels (Tsien and Tsien, 1990; Yoshino et al, 1989) or surge from intracellular reservoirs (Yamazawa et al, 1992). Increase of Ca^{2+} activates some ion channels secondarily. Many investigators have reported ion channels activated by intracellular Ca^{2+},which are K^+ channel (Meech, 1978), nonselective cation channel (Colquhoun et al, 1981; Yellen, 1982) and Cl^- channel (Miledi and Parker, 1984; Evans and Marty, 1986). The existance and physiological properties of these Ca^{2+}-activated channels have been reported in many smooth muscles (Mirronneau and Savineau, 1980; Benham et al, 1985; Byrne and Large, 1987; Janssen and Sims, 1992). These three channels, however, were not recorded in every smooth muscle cell. And their physiological roles rarely have been reported except for the K^+ channel which was known to have a key role in the period of rapid depolarization (Mirronneau et al, 1981).

Caffeine penetrates the sarcolemma and acts on the sarcoplasmic reticulum to release Ca^{2+} into cytosol (Bianchi, 1962; Herz and Weber, 1965). The mechanism of action of caffeine was

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not known exactly because of its controversial effects on smooth muscle contraction. Some investigators have reported its contractile effects on rat uterine smooth muscle (Feinstein, 1966) others reported relaxing effects on guinea-pig taenia coli (Ito and Kuriyama, 1971). Although its action mechanism is not clear, however, it has been confirmed that this agent increases intracellular Ca^{2+} concentration.

In this study we isolated single smooth muscle cells and used the patch clamp technique (Hamill et al, 1981) to study which Ca^{2+}-activated currents might exist in single myocytes of superior mesenteric artery.

METHOD

Isolation of the single smooth muscle cells

We used New Zealand white rabbits of either sex, weighing 1.5~2kg. The rabbit were anesthesized with ether and the abdomen was opened to get the superior portion of the mesentery. Connective tissues, adipose tissues, the mesenteric vein and lymphatics were removed and the mesenteric artery was retained. The isolated mesenteric artery was divided into a few small pieces of about 1~3 mm^3. These procedure was performed in a physiological salt solution (containing in mM NaCl 140, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, NaH_2PO_4 0.25, HEPES 5, glucose 11, pH 7.4).

We performed the enzyme digestion procedure in Ca^{2+}- and Mg^{2+}-free physiological salt solution to obtain single myocytes from the mesenteric artery. The solution contained collagenase (Wako) 2mg/ml, bovine serum albumin 2mg/ml, trypsin inhibitor 1mg/ml and dithioerythritol 5 mM. Pieces of tissue mass were shaken in a test tube at 36°C for 45 minutes. After enzymatic digestion, gentle agitation with glass dropper were performed for 3 minutes. Isolated single cells were stored for 30 ~120 minutes at 4°C before each experiment.

Patch clamp recording experiment

Isolated single smooth muscle cells were dispersed in an experimental chamber (300 μl) on an inverted microscope (Olympus, IMT-II), and they were left for 20 minutes cells to become attached the bottom of the chamber. After 5 minutes, perfusion with the physiological salt solution we chose cells for study which were relaxed and had a sharp margin. Patch electrodes for whole cell mode recording were made with a vertical puller (model 700 C, DKI, U.S. A.) and had 3~5 M Ω tip resistance when filled with internal solution (containing in mM: CsCl 140, MgATP 5, $MgCl_2$ 5, NaH_2PO_4 1, EGTA 0.1, HEPES 5, pH 7.2). In the case of single channel recordings, intrapipette solution (containing in mM: KCl 140 $MgCl_2$ 1, MgATP 1, HEPES 10, EGTA 2, $CaCl_2$ 1.77, pH 7.4) and external perfusing solution (containing in mM: KCl 140, $MgCl_2$ 1, MgATP 1, HEPES 10, EGTA 2, $CaCl_2$ 0.87, pH 7.4) were changed, and electrodes with smaller tip diameter (5~10 M Ω) were used. Patch-clamp studies were performed with a standard amplifier (model AXOPATCH 1D, Axon, U.S.A.), and data were recorded to a personal computer (IBM) through a four-pole Bessel type filter at 500~1,000 Hz. Recoded data were analyzed with pClamp software (version 6.0, Axon, U.S.A.).

RESULTS

The recording of Ca^{2+}-activated K^+ current

We clamped membrane potential to −60 mV, and depolarized the potential with 7 episodes of step pulse increasing 20 mV per episode for 3 seconds (Fig. 1). From 0 mV large oscillatory Ca^{2+}-activated K^+ currents were observed. The oscillatory currents disappeared when intracellular Ca^{2+} concentrations were reduced by high EGTA (pCa over than 8, data not shown). Fig. 2 shows single channel recording data cor-

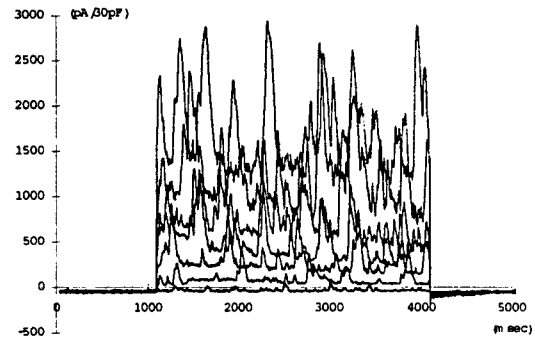

Fig. 1. An example of oscillatory outward currents elicited by 7 depolarized square pulses, −40 to 80 mV from a holding potential of −60 mV. External solution was a physiological salt solution and intrapipette solution contained 140 mV KCl and 0.1 mV EGTA.

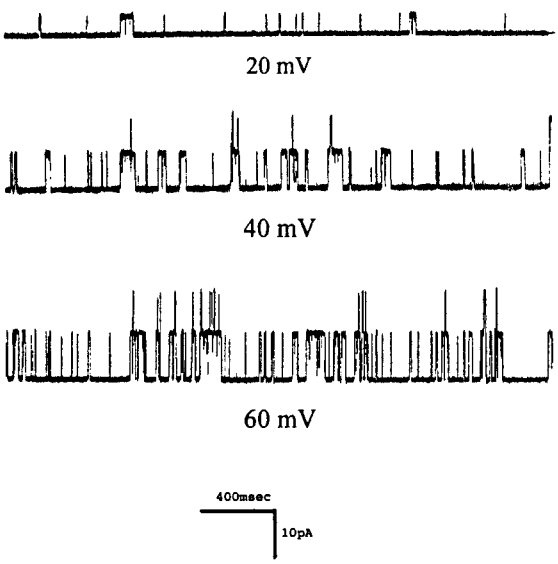

Fig. 2. Single channel recordings of Ca^{2+}-activated K^+ channel. Single channel conductance of this channel was about 250 pS.

responding to the previously mentioned large oscillatory currents in whole cell mode. The threshold for the oscillatory current was about −20∼−40 mV. These single channel

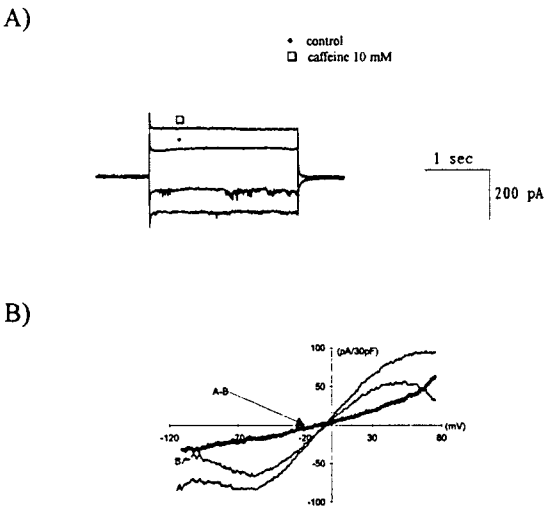

Fig. 3. Typical examples of caffeine-induced current. Uppeer traces are the curent records with depolarizing (80 mV) and hyperpolarizing (−140 mV) step pulses from a holding potential of −60 mV. Lower traces are its I-V relation obtained from slow ramp pulses.
(A: I-V relation in the presence of caffeine,
B: I-V relation in absence of caffeine,
A-B: difference between A and B)

currents showed sensitive dependency on intracellular Ca^{2+} concentration change. At increasing membrane potential depolarization, current amplitudes and opening probabilities were increased. Estimated from the I-V relation, the single channel conductance of this channel was about 250 pS.

Caffeine-induced current

By the replacement of intrapipette K^+ with Cs^+, the oscillatory K^+ currents disappeared. In this condition extracellular application of caffeine (10 mM) evoked outward currents in the depolarized membrane potential ranges, and inward currents in the hyperpolarized range (Fig. 3). These currents showed no time dependency. So we progressed to a further study with a ramp pulse (0.8 V/sec). The I-V relation

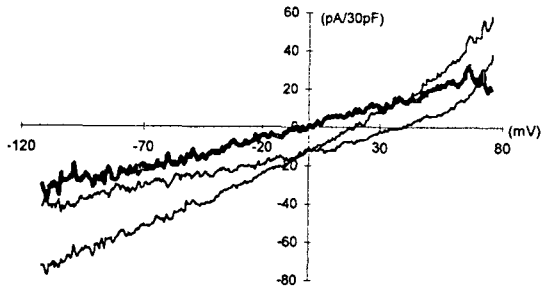

Fig. 4. I-V relation of caffeine-induced current with known blockers ($BaCl_2$ 2 mM, CsCl 2 mM, $NiCl_2$ 2 mM, 4-aminopyridine 1 mM, verapamil 2 μM and ouabain 10 μM).

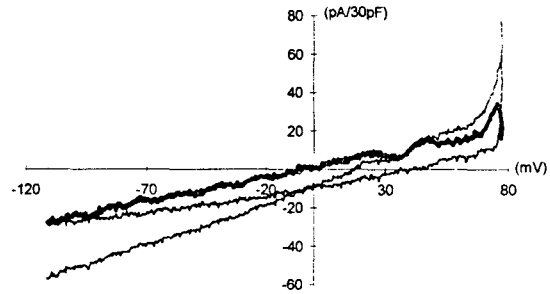

Fig. 6. I-IV relation of niflumic acid sensitive current which rellects chloride current. In this situation Cd^{2+} was used for the blocker of nonselective cationic current.

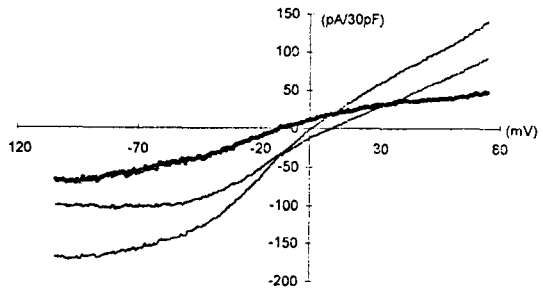

Fig. 5. I-V relation of Cd^{2+}-sensitive current which reflects Ca^{2+}-activated nonselective cationic current. In these conditions extracellular solution contains niflumic acid (20 μM) as a blocker of chloride current.

from −120 to 80 mV is shown in Fig. 3. The currents shown are almost linear throughout the voltage range.

Blocking of known currents: To eliminate currents except Ca^{2+}-activated nonselective current and Ca^{2+}-activated Cl^- current, we used specific blockers for each ion channel or transporter. The blockers and their concentrations were $BaCl_2$ 2 mM, CsCl 2 mM, $NiCl_2$ 2 mM, 4-aminopyridine 1 mM, verapamil 2 μM and ouabain 10 μM (Giles et al, 1985; Kimura et al, 1987; Osterrider et al, 1982). Application of these agents decreased the amplitude of caffeine-induced currents to about half of the control amplitudes (Fig. 4).

Isolation of Ca^{2+}-activated non-selective cation current: In these experimental conditions we used Cd^{2+} (2 mM) as a specific blocker of nonselective cation current (Inoue, 1991). We isolated Cd^{2+}-sensitive currents included in caffeine-induced currents by comparison between Cd^{2+}-absent and Cd^{2+}-present condition (Fig. 5). We used niflumic acid (20 μM; Pacaud et al, 1989) in extracellular solution to block Ca^{2+}-activated Cl^- current. Replacement of Na^+ with the nonpermeable cation N-methylglucamine (NMG) made no significant difference to the effects of Cd^{2+}. These currents represent Ca^{2+}-activated nonselective cation currents ranging from −50 to 50 pA in the voltage range of −100 to +60 mV.

Isolation of Ca^{2+}-activated Cl- current: We regarded the currents blocked by niflumic acid as a Ca^{2+}-activated Cl^- current (Fig. 6). Almost the same results have been obtained with use of N-phenylanthranilic acid instead of niflumic acid. Cd^{2+} was used to block the nonselective cation currents in these conditions. The amplitude of Cl^- current was almost same with that of nonselective current.

DISCUSSION

The physiological characteristics of smooth muscles are very complex and they have diversity according to their distribution between species or organs. The three types of Ca^{2+}-activated currents classified by Marty and his colleagues (1984), could not be recorded in every type of smooth muscle. Ca^{2+}-activated K^+ current was first described in chromaffin cells (Marty, 1981) and its activity has been confirmed in almost every type of smooth muscle cell. In the case of vascular smooth muscle cells, its existence and activity was first reported in guinea-pig mesenteric artery (Benham et al, 1986). Ca^{2+}-activated nonselective cation current and Cl^- current, however, have been diversely reported in various smooth muscle cells, in most of which it is controversial in its existence and properties. Pacaud and his colleagues (1989) reported Cl^- current of arterial smooth muscle cells from rat and Byrne and Large (1987) also reported Cl^- current from rat anococcygenous muscle. Sims (in canine stomach, 1992) and Loirand and his colleagues (in rat portal vein, 1991) have reported nonselctive cation currents.

From our experiment it could be suggested that 3 types of Ca^{2+}-activated current may exist in rabbit superior mesenteric arterial smooth muscle cell. Ca^{2+}-activated K^+ currents, recorded from our experiments, have similar physiological properties and single channel conductance (about 250 pS) to those reported in other smooth muscle cells (Benham et al, 1985), so their existence is indubitable. As for nonselective cation current and Cl^- current, however, there were difficuties in the study of their single channel properties and in isolating the current and small single channel conductance. We only obtained their macroscopic currents with the whole cell mode of the patch clamp method.

There are many organic and inorganic blockers which have blocking action on specific anion channel including the chloride channel (Soejima and Kokunbun, 1988). These agents have been used by many investigaters (Pacaud et al, 1989; Kokubun et al, 1991) but in the aspect of specificity reports show some controversial results. These controversies could also be applied to niflumic acid and Cd^{2+}, used in our experimental studies. For example Cd^{2+}, known by Inoue (1991) as a nonselective cation channel blocker, also affects other types of ion channel or transporters constituted with proteins (Klocker and Isenberg, 1985; Ohya et al, 1986; Aickin et al, 1987; Tokushige et al, 1984). It is difficult to conclude that Cd^{2+}-sensitive currents are nonselective cation currents, so we replaced extracellular Na^+ with the nonpermeable cation, N-methylglucamine(NMG), and obtained similar results from those of Cd^{2+}. As an anion channel blocker we also used N-phenylanthranilic acid (Ueda et al, 1990) and observed almost same results in comparison to data from niflumic acid. From the above observations we can suggest that there are nonselective cation currents and Cl^- currents in mesenteric arterial smooth muscle cells.

In the future it is necessary to study the physiological properties of Ca^{2+}-activated channels in terms of regulatory mechanisms and consequently their biological roles.

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