Simultaneous Activation of Ca^{2+}-Dependent K^{+} and Cl^{-} Currents by Various Forms of Stimulation in the Membrane of Smooth Muscle Cells from the Rabbit Basilar Artery

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Abstract

In smooth muscle cells isolated from cerebral blood vessels, histamine activates Cl^{-} channels through an elevation of intracellular Ca^{2+}. We investigated whether Cl^{-} currents were also evoked by adenosine triphosphate (ATP) or caffeine in isolated smooth muscle cells from the rabbit basilar artery using the perforated patch-clamp technique. Bath application of 10 μM ATP or 1 mM caffeine (holding potential -60 mV) activated transient inward currents. With prolonged bath application of 10 μM ATP or 1 mM caffeine, oscillatory inward current were sporadically generated. At a holding potential of -40 mV, transient Cl^{-} currents were induced by 10 μM histamine, 10 μM ATP, and 1 mM caffeine, following activation of a K^{+} current. At -10 or -20 mV, histamine predominantly activated the K^{+} current. A repetitively activated outward current was induced by membrane depolarization. These results suggest that oscillations in intracellular Ca^{2+} induced by histamine, ATP, and caffeine caused Cl^{-}--current activation at the resting membrane potential. This Cl^{-} current may depolarize the membrane and, thus activate voltage-dependent currents, including a Ca^{2+}-dependent K^{+} current. Both the Ca^{2+}-dependent K^{+} and Cl^{-} currents induced by various stimuli may contribute to the modulation of Ca^{2+} influx by reinforcing membrane depolarization.

Key Words: ionic current, ATP, caffeine, smooth muscle, cerebral artery, patch clamp

Introduction

In a previous report, we have demonstrated that in vascular smooth muscle cells of rabbit basilar artery, histamine acts on the H_{1} receptor, increases intracellular Ca^{2+} and opens Ca^{2+}-dependent Cl^{-} channels (Kamouchi et al., 1997). Generation of these Ca^{2+}-dependent Cl^{-} currents was periodic and observed throughout the time for which histamine was present. As
the activity of the Ca²⁺-dependent Cl⁻ current depends upon the concentration of intracellular Ca²⁺ ([Ca²⁺]ᵢ), such a periodic generation of the Cl⁻ current might indicate a periodic change in [Ca²⁺], (Désilets et al., 1989; Janssen and Sims, 1993; Wang et al., 1993; Hogg et al., 1993). Indeed, [Ca²⁺], oscillations have been demonstrated in many types of excitable and nonexcitable cells (Jacob et al., 1988; Berridge, 1993; Berridge et al., 1989; Tsien et al., 1990; Berridge, 1990; Meyer et al., 1991; Harootunian et al., 1991; Iino et al., 1994).

Spontaneous transient outward currents (STOCs) have been shown to be due to the periodic activation of a Ca²⁺-dependent K⁺ current in studies using the patch-clamp technique in smooth muscle cells. Moreover, there is an evidence indicating a causal relation between STOCs and Ca²⁺ release from the intracellular store sites (Benham et al., 1986; Ohya et al., 1987; Komori et al., 1991; Kitamura et al., 1992). However, the frequency with which STOCs were generated coincided neither with the oscillations in [Ca²⁺], measured by the fluorescence dye, fura-2, nor with contractions (Itoh et al., 1983; Iino et al., 1993).

Various vasoactive neurotransmitters play important roles in the regulation of cerebral circulation. Histamine and ATP are candidates of neurotransmitters in the cerebral circulation (Karashima et al., 1981; Fujiwara et al., 1982; Suzuki et al., 1982). These agonists, and caffeine, increase [Ca²⁺]ᵢ by inducing the release of Ca²⁺ from the sarcoplasmic reticulum (SR) via activation of inositol 1,4,5-trisphosphate (InsP₃)-, or ryanodine-sensitive Ca²⁺ release channels (Itoh et al., 1992; Berridge, 1993; Hirose et al., 1994).

As an increase in Cl⁻ conductance would depolarize the membrane, activation of Cl⁻ channels could play an important role in the cell excitation induced by neurotransmitters in cerebral arteries. In the present experiments, we investigated whether agonist, or other forms of stimulation thought to modulate Ca²⁺ release from the SR, could open Ca²⁺-dependent Cl⁻ channels in isolated smooth muscle cells of the rabbit basilar artery. We also looked for evidence for existence of Ca²⁺-dependent K⁺ channels, since the presence of both channels together would provide for a dual control of membrane potential (K⁺ currents causing hyperpolarization).

**Methods**

**Cell isolation**

Male albino rabbits (Nippon White, 2.0-2.2 Kg) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.) and exsanguinated. The whole brain was removed and placed in physiological salt solution (PSS) containing 0.5 mM Ca²⁺ and 1.5 mM Mg²⁺. The proximal portion of the basilar artery was dissected free and incubated for 20 min at 35°C in nominally Ca²⁺-free PSS with collagenase (2.0 mg/ml, Wako Pure Chem., Osaka, Japan), papain (1.0 mg/ml, Sigma Chem., St Louis, MO, USA), bovine serum albumin (BSA; fraction V, essentially fatty acid free; 1.0 mg/ml, Sigma Chem.) and dithiothreitol (1 mM, Sigma Chem.). The treated tissue was cut into small pieces in nominally Ca²⁺-free PSS and then gently agitated using a blunt-tipped pipette to disperse the single cells. The isolated cells were stored in Ca²⁺-free PSS containing BSA (1.0 mg/ml).
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Solutions

The ionic composition of PSS (bathing solution) was (in mM): NaCl 134, KCl 6.2, CaCl₂ 1.8, MgCl₂ 1.0, glucose 12.1, and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) 10. The composition of the high K⁺ solution (pipette solution) was (in mM): KCl 140, MgCl₂ 2.0, ethylene glycol-bis (β-aminoethyl ether)-N, N’, N’’-tetraacetic acid (EGTA) 0.1, and HEPES 5. To make Ca²⁺-free PSS, Ca²⁺ was replaced in the above PSS by an equimolar concentration of Mg²⁺. The pH of the solutions was adjusted with tris (hydroxy-methyl) aminomethane to 7.35.

Drugs

Drugs used in the present experiments were histamine, ATP, caffeine, sodium nitroprusside and nystatin (all from Sigma Chem.). These drugs, except nystatin, were dissolved in deionized water to make a stock solution, which was diluted in PSS before use. Nystatin was dissolved in dimethylsulfoxide (50 mg/ml; stored in a light-protected bottle) and diluted in PSS with sonication (Branson 2200; Yamato, Tokyo) just before use.

All drugs were added in PSS by superfusion through the bath at a flow rate of 2 ml/min. Data are expressed as mean±SD, except the amplitude and interval of spontaneously transient inward currents which are shown as mean±SEM.

Current recording

Whole-cell currents were recorded at room temperature (20-25°C) using nystatin-perforated patch-clamp recording (originally described by Horn et al., 1988) by way of a patch clamp amplifier (Axopatch-1D, Axon Instrum., Burlingame, CA, USA). Patch electrodes (2-4 MΩ) were prepared using an electrode puller (PP-83, Narishige Sci. Instrum. Lab., Tokyo), and the tip was polished with a heat polisher (MF-83, Narishige Sci. Instrum. Lab.). To obtain the perforated patch, the tip of a pipette was first dipped in the internal high K⁺ solution, and then a solution containing nystatin (100-200 μg/ml) was backfilled into the pipette. When a giga-ohm seal had been maintained for several min, chemical perforation was deemed to have been successfully achieved. Ramp voltage pulses were applied by means of a personal computer (Deskpro/25, Compaq, USA) using “PCLAMP 5.5.” software (Axon Instrum.). Data were stored using a videotape recorder (NV-FS1, National, Tokyo) after digitizing through a pulse code modulator (PCM-501ES, Sony, Tokyo). Traces were obtained later on a laser printer (L-880S, Kyocera, Kyoto, Japan; resolution 300 dpi) by passing the recorded signals through an 8-pole Bessel filter (VT filter 3334; NF Elec. Instrum., Yokohama) at an fc of 300 Hz.

Results

Transient inward currents evoked by histamine, ATP, and caffeine

When the membrane of isolated smooth muscle cells from the rabbit basilar artery was held at −60 mV in PSS, histamine (10 μM) produced a transient inward current (Fig. 1A). This current is known to be inhibited by niflumic acid (a Cl⁻ channel blocker), to be modified by a
reduction in the Cl⁻ concentration in the bath, and has been determined to be a Ca²⁺-activated Cl⁻ current (Kamouchi et al., 1997). A similar transient inward current was evoked by superfusion with either ATP or caffeine. As shown in Fig. 1B and 1C, external application of ATP (5 μM) or caffeine (1 mM) evoked an inward current at a holding potential of -60 mV. The mean values for the peak amplitude of the inward currents evoked by 5 μM ATP and 1 mM caffeine were 16.3 ± 7.1 pA (n = 7) and 32.9 ± 8.7 pA (n = 7), respectively. These mean currents were both smaller than the mean of those evoked by 10 μM histamine (79.2 ± 55.8 pA, n = 45; Kamouchi et al., 1997). However, the time-to-peak for the inward current (Tpeak), the time taken for the inward current to decay by 90% (DIC90), and the reversal potential of the current (Erev) when the current was evoked by ATP were not different from the corresponding values reported for histamine (Table 1). On the other hand, the values obtained for the caffeine-induced current were slightly larger (Tpeak and DIC90) or more negative (Erev) than those for the histamine-, and ATP-induced inward currents (Table 1).

Oscillatory inward currents induced by histamine, ATP, and caffeine

Prolonged application of ATP (10 μM) at a holding potential of -60 mV produced oscillatory inward currents (sporadically transient inward currents; STICs) in some cells (4 out of 12 cells) after the disappearance of the transient inward current that was evoked initially (Fig.
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Table 1 Characteristics of transient inward currents induced by histamine, ATP, and caffeine

|                  | histamine 10 μM | ATP 10 μM    | caffeine 1 mM |
|------------------|-----------------|--------------|---------------|
| T<sub>p<sub>eq</sub></sub>(s) | 0.42 ± 0.31     | 0.30 ± 0.14  | 2.26 ± 0.38*  |
| DIC<sub>90</sub>(s)    | 1.86 ± 0.97     | 1.04 ± 0.44  | 6.40 ± 1.03*  |
| amplitude (pA)      | 79.2 ± 55.8     | 16.3 ± 7.1*  | 32.9 ± 8.7*   |
| n                 | 45              | 7            | 7             |
| E<sub>rev</sub> (mV)  | −6.3 ± 4.4      | −4.5 ± 7.1   | −16.5 ± 7.3*  |

Inward currents were evoked by histamine, ATP, or caffeine at a holding potential of −60 mV. Values are the time mean ± SD. n indicates number of currents. Rise time (T<sub>p<sub>eq</sub></sub>) is expressed as the time from onset to peak for each current. Ninety percent decay time (DIC<sub>90</sub>) is the time taken by the inward current to decay from peak to 10% peak amplitude. Reversal potential (E<sub>rev</sub>) was evaluated by application of ramp depolarizing pulse (−120 to +60 mV, duration 500 ms). *indicates significant difference from the corresponding value for histamine (p<0.05). All of the histamine data is taken from our previous paper (Kamouchi et al., 1996).

Fig. 2. Spontaneous inward current oscillations induced by 10 μM ATP (A), 1 mM caffeine (B & C), and 10 μM histamine (D). Drugs were present in the bath for the time indicated by the bars. The membrane was held at −60 mV (A), −40 mV (B), or −20 mV (C & D). Note the different time calibrations.

In the majority of the cells (8 out of 12 cells), ATP did not evoke STICs. The STICs showed a relatively constant amplitude and interval at a given holding potential; their mean amplitude and interval being 23.0 ± 5.4 pA and 11.2 ± 2.0 sec, respectively (n = 4, Table 2). When caffeine (1 mM), rather than ATP, was superfused in the bath, STICs were elicited in 3 out of 14 cells at holding potentials of −60 or −40 mV (Fig. 2B). As was the case for the initial
Table 2. Comparison of the amplitudes of and intervals between the oscillatory inward currents in responses to histamine, ATP, and caffeine

|                | histamine 10 μM | ATP 10 μM | caffeine 1 mM |
|----------------|-----------------|-----------|---------------|
| amplitude (pA) | 45.0±6.6        | 23.0±5.4  | 27.3±5.8      |
| interval (sec)  | 20.3±1.7        | 11.2±2.0  | 27.3±5.8      |
| n              | 8               | 4         | 3             |

Oscillatory inward currents were evoked by histamine, ATP, or caffeine at −60 mV. Values are mean±SEM. n indicates number of cells. The interval was calculated as the mean for n different cells of the mean time between oscillatory inward currents in each cell.

transient inward current, STICs induced by caffeine tended to show a longer T_{peak} and a longer DIC_{90} than the STICs evoked by histamine or ATP (data not shown). The interval between the caffeine-induced oscillatory currents was 27.3±5.8 sec (n=3, Table 2). The caffeine-induced STICs decreased gradually in amplitude and disappeared after about several min (Fig. 2B). When the holding potential was elevated to −20 mV, 1 mM caffeine predominantly activated spontaneous transient outward currents (STOCs) as well as some small amplitude STICs (Fig. 2C). At the same holding potential (−20 mV), histamine produced discreet STICs as well as STOCs (Fig. 2D).

Fig. 3 shows, on an expanded time-scale, typical examples of the membrane responses induced by 10 μM ATP and 10 μM histamine at a holding potential of −40 mV. Histamine and ATP produced both transient inward and outward currents more or less simultaneously, though the transient outward current was always the first to appear. In most responses, STOCs and STICs were intermingled (Figs. 2B–D, 3B), but as shown in Fig. 3A, STOCs were sometimes evoked without associated STICs.

**Spontaneously transient outward current (STOCs)**

Although STOCs were hardly recorded at all at a holding potential of −60 mV, they were recorded frequently at more positive potentials. When the holding potential was elevated to
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Fig. 4. Periodic appearance of spontaneous transient outward currents. A: Spontaneous transient outward currents recorded at -10 mV before (a) and during (b) application of 2 μM sodium nitroprusside. B: Burst-like activation of transient outward currents induced by application of 10 μM histamine at a holding potential of -10 mV. Drugs were present in the bath for the time indicated by the bars.

-20 mV, or to even more positive potentials, STOCs were predominant with the nystatin-perforated patch configuration. At a holding potential of -10 mV, the majority of cells (22 out of 25) continuously generated STOCs; however, some cells (3 out of 25) periodically generated clusters of STOCs (Fig. 4A). Such clusters were completely blocked by the addition of either 1 μM nicardipine, 100 nM charybdotoxin (data not shown) or 2 μM sodium nitroprusside (Fig. 4Ab). When the membrane potential was kept at -10 mV, histamine (10 μM) no longer produced an inward current, but only transient outward currents (Fig. 4B). Like the periodically generated STOCs, these histamine-induced outward currents did not fuse with each other completely.

Discussion

Histamine and ATP, putative excitatory neurotransmitters in the cerebro-vascular system, each depolarizes the membrane and produce contraction in the smooth muscle cells of cerebral arteries (Fujiwara et al., 1982; Karashima et al., 1981; Suzuki et al., 1982; Ogata et al., 1996). The ionic mechanisms underlying the membrane depolarization and contraction induced by these agonists have been documented: they involve the activation of non-selective cation channels and the release of Ca$^{2+}$ from the SR (Xiong et al., 1991; Wang et al., 1993; Itoh et al., 1992; Hirose et al., 1994). Cl$^{-}$ currents have been also reported to be activated by histamine, and by norepinephrine, in ear and pulmonary arteries (Amédee et al., 1990a; 1990b; Wang et al., 1993). In our previous paper (Kamouchi et al., 1996), we demonstrated that histamine induced a transient inward current at a holding potential close to the resting membrane potential of the rabbit basilar artery (-50 mV; Fujiwara et al., 1982; Ogata et al., 1996). In that paper, we suggested that this might be due to the opening of a Ca$^{2+}$-dependent Cl$^{-}$ channel, rather than to activation of a non-selective cation channel as also observed in the rabbit pulmonary artery (Wang et al., 1993). Possibly, activation of a Ca$^{2+}$-dependent Cl$^{-}$
channel by histamine might be a secondary effect, caused by an elevation of [Ca\textsuperscript{2+}]\textsubscript{i} via activation of the H\textsubscript{1} receptor. Indeed, in the present experiments, we were able to obtain similar responses by applying ATP or caffeine, and the electrical features of the ATP-induced inward current (such as T\textsubscript{peak}, DIC\textsubscript{90}, and E\textsubscript{rev}) were well in accord with those previously reported for histamine. These findings suggest that activation of Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} currents by such agonists in the rabbit basilar artery may involve a common mechanism; that is, release of Ca\textsuperscript{2+} from the SR (as observed in other smooth muscle cells: Ohta \textit{et al.}, 1993; Ito \textit{et al.}, 1993; Hogg \textit{et al.}, 1994).

Caffeine also induced a transient inward current at a holding potential of −60 mV, but with a slightly more negative E\textsubscript{rev} and longer T\textsubscript{peak} and DIC\textsubscript{90} than those obtained with histamine or ATP. The reasons for these differences in the electrical features of the inward currents induced by caffeine and agonists are unknown. However, the more negative E\textsubscript{rev} may suggest that caffeine activates a Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current as well as, or more strongly than, the Cl\textsuperscript{-} current activated by the agonists. In the rabbit pulmonary artery, Wang \textit{et al.} (1993) also reported that caffeine elicited a larger outward current component than inward component. As caffeine is known to open the ryanodine-sensitive Ca\textsuperscript{2+} release (RSCR) channels in the SR, it may be that Ca\textsuperscript{2+} released from RSCR channels and InsP\textsubscript{3}-sensitive Ca\textsuperscript{2+} release (IIICR) channels activates the same Ca\textsuperscript{2+}-dependent K\textsuperscript{+} and Cl\textsuperscript{-} channels, but in a quantitatively different fashion.

The voltage-dependent difference in the amplitude of the Ca\textsuperscript{2+}-dependent K\textsuperscript{+} and Cl\textsuperscript{-} currents could be due to the effect of their differing equilibrium potentials on the voltage-dependent component of their activation. On the other hand, the time-dependent differences between the K\textsuperscript{+} and Cl\textsuperscript{-} currents might be due, at least in part, to the difference in their Ca\textsuperscript{2+} sensitivities, as Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel activation is known to require a much larger [Ca\textsuperscript{2+}], than that required for activation of Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} channels (Inoue \textit{et al.}, 1985; Markwardt \textit{et al.}, 1992; Klöckner, 1993). In addition to this, the cytosolic conditions, such as the concentrations of ATP and other nucleotides and the activity of various kinases and other modulators, would also greatly affect their time course (Komori \textit{et al.}, 1989; Kitamura \textit{et al.}, 1992; Lee \textit{et al.}, 1994). It has been shown that, in the portal vein and pulmonary artery, STOCs are transiently activated and then suppressed by the application of agonists, and that this suppression can be mimicked by PKC activators and prevented by PKC inhibitors (Kitamura \textit{et al.}, 1992; Wang \textit{et al.}, 1993).

Only a few papers have been published concerning the periodic generation of STICs or STOCs in smooth muscle cells (canine and guinea-pig trachea, Janssen \textit{et al.}, 1992; guinea-pig ileum, Komori \textit{et al.}, 1992; rabbit pulmonary artery, Wang \textit{et al.}, 1993; Lee \textit{et al.}, 1994). The present experiments and our previous work (Kamouchi \textit{et al.}, 1996) provide direct evidence of the simultaneous, periodic generation of STICs and STOCs, suggesting the presence of a common Ca\textsuperscript{2+} source for their activation.

The periodic occurrence of STICs was rare in the present study (4 out of 12 cells for ATP and 3 out of 14 cells for caffeine). Appropriate conditions for the regulation of [Ca\textsuperscript{2+}], such as a low concentration of EGTA, or other Ca\textsuperscript{2+} chelating agent, in the pipette, have been found to be needed for the generation of STICs in a conventional whole-cell recording situation.
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(Wang et al., 1993). As nystatin-perforated whole-cell voltage-clamp recording preserved agonist-induced inward currents and STICs for much longer than conventional whole-cell voltage-clamp recording, unknown endogenous modulators may also regulate the generation of these currents (Amédée et al., 1990a). This means that differences between cells and/or procedures for cell isolation leading to differences in intracellular Ca\textsuperscript{2+} buffering conditions, may lead to greater or lesser degrees of impairment of the mechanisms that regulate Ca\textsuperscript{2+} oscillations. Confocal images of Ca\textsuperscript{2+} have provided evidence that noradrenaline produces an increase in [Ca\textsuperscript{2+}], in certain cells, but not in all cells, in the rat tail artery (Iino et al., 1994). Further studies would be required to elucidate the mechanism(s) underlying differences in the generation of STICs in different cerebral arterial cells.

It is of interest that, in the present experiments in rabbit basilar artery, each STOC appeared as a spike-like response with a very short duration and high frequency of appearance (by comparison with those of the STICs seen in the same cells; see Figs. 2-4). Similar STOCs have been commonly reported in various smooth muscle cells in studies using conventional whole-cell voltage-clamp recording (Benham et al., 1986; Ohya et al., 1987; Kitamura et al., 1992). It has been suggested that spontaneous Ca\textsuperscript{2+} leakage from the Ca\textsuperscript{2+}-overloaded SR might be the mechanism underlying the generation of STOCs (Kass et al., 1982; Ohya et al., 1987). However, while STOCs appeared with high frequency, but with various amplitudes, in vascular and visceral smooth muscle cells, their frequency did not coincide with that of Ca\textsuperscript{2+} release, as monitored using Ca\textsuperscript{2+}-sensitive dye or contractions (Itoh et al., 1983; Benham et al., 1986; Ohya et al., 1987; Kitamura et al., 1992; Iino et al., 1994). The periodic appearance of clusters of STOCs might seem to favor the idea of a periodic discharge of Ca\textsuperscript{2+} from SR and, if this is the case, other regulatory mechanisms might be involved in the generation of clusters of STOCs. In the present experiments, the periodic generation of clusters of STOCs has been clearly demonstrated on membrane depolarization alone, as well as on caffeine stimulation, suggesting that the CICR mechanism, through L-type Ca\textsuperscript{2+} channels, is also involved in the periodic release of Ca\textsuperscript{2+} from the SR.

The present study is the first to show that ATP and caffeine can activate transient and oscillatory inward currents in smooth muscle cells from cerebral blood vessels at or near their resting membrane potential, when intracellular conditions have been preserved by the use of quasi-physiological conditions. It has been reported that extracellular Ca\textsuperscript{2+} makes an important contribution to the regulation of [Ca\textsuperscript{2+}], in cerebral arteries, as agonist-induced contraction is more strongly inhibited by Ca\textsuperscript{2+} channel blockers in such vessels than in other systemic arteries (Bevan, 1983; Peroutka et al., 1984). Agonist-induced oscillations or spontaneous oscillations in [Ca\textsuperscript{2+}], at the resting membrane potential may lead to the depolarization of the membrane of cerebral arterial cells as a result of the activation of Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} channels, as well as of non-selective cation channels, and subsequently trigger the voltage-dependent Ca\textsuperscript{2+} channels to produce an action potential. If this is so, Ca\textsuperscript{2+}-dependent Cl\textsuperscript{-} channels would have a key role in the regulation of the membrane potential in cerebral arteries. Excess [Ca\textsuperscript{2+}] would also activate Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels to fix the membrane at a certain voltage and prevent Ca\textsuperscript{2+} influx. Thus, in this scheme, both Ca\textsuperscript{2+}-dependent currents would have important physiological roles in cell excitation in the cerebral circulation during
agonist and other forms of stimulation.

Finally, it has been demonstrated that spontaneous vasomotion occurs in the rat basilar artery in vivo (Fujii et al., 1990). The mechanism underlying this vasomotion is still unclear, but the results obtained in this study may provide a clue to its identity. Further experiments need to be carried out to elucidate the possible role under pathophysiological conditions of oscillations in intracellular Ca\(^{2+}\), which would modulate the membrane potential via Ca\(^{2+}\)-dependent Cl\(^{-}\) and K\(^{+}\) channels.

Acknowledgment

The authors are grateful to Dr. R.J. Timms for the language editing. This study was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan (to K.K.).

References

Amédée, T., Benham, C.D., Bolton, T.B., Byrne, N.G. and Large, W.A. (1990a). Potassium, chloride and non-selective cation conductances opened by noradrenaline in rabbit ear artery cells. J. Physiol. 423 : 551-568.

Amédée, T., Large, W.A. and Wang, Q. (1990b). Characteristics of chloride currents activated by noradrenaline in rabbit ear artery cells. J. Physiol. 428 : 501-516.

Benham, C.D. and Bolton, T.B. (1986). Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. J. Physiol. 381 : 385-406.

Berridge, M.J. (1990). Calcium oscillations. J. Biol. Chem. 265 : 9583-9586.

Berridge, M.J. (1993). Inositol trisphosphate and calcium signalling. Nature 361 : 315-325.

Berridge, M.J., Irvine, R.F. (1989). Inositol phosphates and cell signalling. Nature 341 : 197-205.

Bevan, J.A. (1983). Diltiazem selectively inhibits cerebrovascular extrinsic but not intrinsic myogenic tone. A review. Circ. Res. 52 (Suppl. 1) : 104-109.

Désilets, M., Driska, S.P. and Baumgarten, C.M. (1989). Current fluctuations and oscillations in smooth muscle cells from hog carotid artery. Role of the sarcoplasmic reticulum. Circ. Res. 65 : 708-722.

Fujii, K., Heistad, D.D. and Faraci, F.M. (1990). Ionic mechanisms in spontaneous vasomotion of the rat basilar artery in vivo. J. Physiol. 430 : 389-398.

Fujiwara, S., Itoh, T. and Suzuki, H. (1982). Membrane properties and excitatory neuromuscular transmission in the smooth muscle of dog cerebral arteries. Br. J. Pharmacol. 77 : 197-208.

Harootunian, A.T., Kao, J.P., Paranjpe, S. and Tsien, R.Y. (1991). Generation of calcium oscillations in fibroblasts by positive feedback between calcium and IP3. Science 251 : 75-78.

Hogg, R.C., Wang, Q., and Large, W.A. (1993). Time course of spontaneous calcium-activated chloride currents in smooth muscle cells from the rabbit portal vein. J. Physiol. 464 : 15-31.

Hogg, R.C., Wang, Q., and Large, W.A. (1994). Action of niflumic acid on evoked and spontaneous calcium-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. Br. J. Pharmacol. 112 : 977-984.

Iino, M., Kasai, H., Yamazawa, T. (1994). Visualization of neural control of intracellular Ca\(^{2+}\) concentration in single vascular smooth muscle cells in situ. EMBO. J. 13 : 5026-5031

Inoue, R., Kitamura, K., Kuriyama, H. (1985). Two Ca-dependent K-channels classified by application of tetraethylammonium distribute to smooth muscle membranes of the rabbit portal
Membrane currents evoked by Ca release

Ito, S., Ohta, T., Nakazato, K. (1993). Inward current activated by carbachol in rat intestinal smooth muscle cells. *J. Physiol.* **470**: 395–409.

Itoh, T., Kajikuri, J., Kuriyama, H. (1992). Characteristic features of noradrenaline-induced Ca\(^{2+}\) mobilization and tension in arterial smooth muscle of the rabbit. *J. Physiol.* **457**: 297–314.

Itoh, T., Kuriyama, H., Suzuki, H. (1983). Differences and similarities in the noradrenaline- and caffeine-induced mechanical responses in the rabbit mesenteric artery. *J. Physiol.* **337**: 609–629.

Jacob, R., Merritt, J.E., Hallam, T.J., Rink, T.J. (1988). Repetitive spikes in cytoplasmic calcium evoked by histamine in human endothelial cells. *Nature* **335**: 40–45.

Janssen, L.J. and Sims, S.M. (1993). Histamine activates Cl\(^-\) and K\(^+\) currents in guinea-pig tracheal myocytes: convergence with muscarinic signalling pathway. *J. Physiol.* **456**: 661–677.

Kamouchi, M., Ogata, R., Fujishima, M., Ito, Y. and Kitamura, K. (1996). Membrane currents evoked by histamine in rabbit basilar artery. *Am. J. Physiol.* **272**: H638–H647.

Kamouchi, M., Ogata, R., Fujishima, M., Ito, Y. and Kitamura, K. (1996). Membrane currents evoked by histamine in rabbit basilar artery. *Am. J. Physiol.* **272**: H638–H647.

Karashima, T. and Kuriyama, H. (1981). Electrical properties of smooth muscle cell membrane and neuromuscular transmission in the guinea-pig basilar artery. *J. Physiol.* **272**: H638–H647.

Klockner, U. (1993). Intracellular calcium ions activate a low-conductance chloride channel in smooth muscle cell isolated from human mesenteric artery. *Pflügers Arch.* **424**: 231–237.

Komori, S. and Bolton, T.B. (1989). Actions of guanine nucleotides and cyclic nucleotides on calcium stores in single patch-clamped smooth muscle cells from rabbit portal vein. *Br. J. Pharmacol.* **97**: 973–982.

Komori, S. and Bolton, T.B. (1991). Calcium release induced by inositol 1,4,5-trisphosphate in single rabbit intestinal smooth muscle cells. *J. Physiol.* **433**: 495–517.

Komori, S., Kawai, M., Takewaki, T. and Ohashi, H. (1992). GTP-binding protein involvement in membrane currents evoked by carbachol and histamine in guinea-pig ileal muscle. *J. Physiol.* **450**: 105–126.

Lamb, F.S. and Shibata, E.F. (1994). Calcium-activated chloride current in rabbit coronary artery myocytes. *Circ. Res.* **75**: 742–750.

Lee, S.H. and Earm, Y.E. (1994). Caffeine induces periodic oscillations of Ca\(^{2+}\)-activated K\(^+\) current in pulmonary arterial smooth muscle cells. *Pflügers Arch.* **426**: 189–198.

Markwardt, F. and Isenberg, G. (1992). Gating of maxi-K\(^+\) channels studied by Ca\(^{2+}\) concentration jumps in excised inside-out multi-channel patches (myocytes from guinea pig urinary bladder). *J. Gen. Physiol.* **99**: 841–862.

Meyer, T. and Stryer, L. (1991). Calcium spiking. *Ann. Rev. Biophys. Biophys. Chem.* **20**: 153–174.

Ogata, R., Inoue, Y., Nakano, H., Ito, Y., Kitamura, K. (1996). Oestradiol-induced relaxation of rabbit basilar artery by inhibition of voltage-dependent Ca channels through GTP-binding protein. *Br. J. Pharmacol.* **117**: 351–359.

Ohta, T., Ito, S. and Nakazato, Y. (1993). Chloride currents activated by caffeine in rat intestinal smooth muscle cells. *J. Physiol.* **465**: 149–162.

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

Peroutka, S.J., Banghart, S.B. and Allen, G.S. (1984). Relative potency and selectivity of calcium antagonists used in the treatment of migraine. *Headache.* **24**: 55–58.

Suzuki, H. and Fujiwara, S. (1982). Neurogenic electrical responses of single smooth muscle cells of
the dog middle cerebral artery. *Circ. Res.* 51: 751-759.

Tsien, R.W. and Tsien, R.Y. (1990). Calcium channels, stores, and oscillation. *Annu. Rev. Cell. Biol.* 6: 715-760.

Wang, Q., Large, W.A. (1993). Action of histamine on single smooth muscle cells dispersed from the rabbit pulmonary artery. *J. Physiol.* 468: 125-139.

Xiong, Z., Kitamura, K., Kuriyama, H. (1991). ATP activates cationic currents and modulates the calcium current through GTP-binding protein in rabbit portal vein. *J. Physiol.* 440: 143-165

(Received September 16, 1998 : Accepted October 2nd, 1998)