Role of membrane potential in vasomotion of isolated pressurized rat arteries

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Abstract

Vasomotion, the phenomenon of vessel diameter oscillation, regulates blood flow and resistance. The main parameters implicated in vasomotion are particularly the membrane potential and the cytosolic free calcium in smooth muscle cells. In this study, these parameters were measured in rat perfused-pressurized mesenteric artery segments. The application of norepinephrine (NE) caused rhythmic diameter contractions and membrane potential oscillations (amplitude; 5.3 ± 0.3 mV, frequency; 0.09 ± 0.01 Hz). Verapamil (1 μM) abolished this vasomotion. During vasomotion, 10^{-5} M ouabain (Na^+–K^+ ATPase inhibitor) decreased the amplitude of the electrical oscillations but not their frequency (amplitude; 3.7 ± 0.3 mV, frequency; 0.08 ± 0.002 Hz). Although a high concentration of ouabain (10^{-3} M) (which exhibits non-specific effects) abolished both electrical membrane potential oscillations and vasomotion, we conclude that the Na+–K+ ATPase could not be implicated in the generation of the membrane potential oscillations. We conclude that in rat perfused-pressurized mesenteric artery, the slow wave membrane type of potential oscillation by rhythmically gating voltage-dependent calcium channels, is responsible for the oscillation of intracellular calcium and thus vasomotion.
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Introduction

Vasomotion has been known as spontaneous variations in the muscular tone in small, resistance-type arteries and arterioles. It was suggested that this phenomenon of diameter oscillations regulates blood

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flow and resistance. However, the mechanism behind rhythmic contractions and relaxations of small arteries has not yet been fully understood despite numerous publications in that field. One of the experimental models for the rhythmic vascular movement was isolated *in vitro* segment of mesenteric artery of the rats [1–4]. In these experiments, inhibition of calcium-induced calcium release by ryanodine abolishes vasomotion, whereas stimulation of intracellular calcium pool release by caffeine increases the frequency of the movement [4]. In the same way, an agonist that increases cytosolic free calcium concentration also increases the frequency [4]. On the contrary, the inhibition of the $Na^+–K^+$ ATPase or voltage-dependent calcium channels abolishes vasomotion [3].

These studies concluded that the rhythmicity of the mesenteric artery results from an interplay between membrane activation and intracellular calcium stores [4]. Spontaneous intermittent release of calcium from the sarcoplasmic reticulum, which is initially unsynchronized between the vascular smooth muscle cells, becomes synchronized to initiate vasomotion [5]. Indeed calcium sparks and puffs have been observed in single cells of intact, pressurized rat mesenteric small arteries during activation with phenylephrine [6]. In this last article the electrophysiological measurements were not done on the perfused vessel, but on a vessel mounted in a myograph.

Reactivity of isolated arteries depends on the in vitro used methods [7]. Therefore, we standardized measurement protocols. By that way, the membrane potential of smooth muscle cells was determined while monitoring the diameter of the artery. Separately but on the same model, we observed the cytosolic free calcium of the smooth muscle cell together with the vessel diameter. The goal of this study was to measure these parameters during norepinephrine-induced vasomotion of the rat mesenteric artery in the pressurized and perfused condition.

Method

Materials

Male Sprague–Dawley rats aged 6 to 10 weeks, and weighing 200–350 gr. were obtained at the animal house of the University and treated in agreement with the Care of Animals (edited by "l'Académie Suisse des Sciences Médicales" and "la Société Hélvétique des Sciences Naturelles"). The rats were anesthetized with chloralose–urethane [1 g/kg intraperitoneally (i.p)] (Sigma, St. Louis, MO, U.S.A). A first or second order branch of the mesenteric artery (MA) was taken from the mesenteric arcade and placed in Krebs–Ringer bicarbonate solution (room temperature) [(in mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, and glucose 11.1, aerated with 95% O_2/5% CO_2 (control solution)]. The blood vessel was cleaned of surrounding tissues and cut into a segments 10–12 mm long. The tissue was mounted on the micro-organ cannulation chamber (0.5 ml capacity).

Cannulation

The artery was cannulated at both ends, using stainless steel needles, to which they were secured by 8-0 nylon filaments. One cannula was connected to an infusion syringe pump that contained the intraluminal fluid (control solution) and continuously infused at a rate of 50 μl/min. The other was connected to a pressure transducer (Gould P-23, U.K) to continuously record the intraluminal pressure by PowerLab data recording system (AD Instrument, U.S.A). After cannulation, the pressure was set to

50 mmHg, and the vessel was stretched until any buckling disappeared. Only vessels without leaks were used. The micro-organ cannulation chamber was continuously refreshed by superfusion fluid (control solution; 37 °C) at a rate of 1.5 ml/min. The arteries were superfused and infused with control solution for 60 min before the recording of responses was started. Norepinephrine (NE: 10^{-6} M to 2×10^{-6} M) was added to the superfusion solution to induce vasomotion. When vasomotion was present, the magnitude of membrane potential and external diameter change responses were recorded. To monitor the external diameter, a video-dimension analyzer (V94, Living systems, U.S.A) with videomicroscopy was mounted on the microscope. The diameter change in the middle of the vessel segment was continuously recorded on the PowerLab system.

Membrane potential measurement

The membrane potential of arterial vascular smooth muscle cells (VSMC) was measured with glass microelectrodes (WPI, Florida, U.S.A) filled with 3 M KCl solution (tip resistance, $40–80$ MΩ). The electrical signal was amplified (Intra 767 amplifier, WPI, U.S.A) from recordings obtained from the adventitial side of the vessel. The membrane potential was monitored continuously using an oscilloscope (Type1425, Gould, U.K) and data was recorded using the PowerLab system. The criteria for accepting a record were a sharp penetration and rise to 0 mV when the electrode was withdrawn from the recorded cell.

Intracellular calcium concentration measurement

Fifty micrograms of Fura-2 AM® were dissolved in 50 µl dimethyl sulfoxide (DMSO) containing 2% pluronic solution and suspended in 5 ml modified Krebs–Ringer bicarbonate solution [(in mM): NaCl 145, KCl 5, $CaCl_2$ 1, $MgSO_4$ 0.5, Na_2HPO_4 1, Hepes 20 and Tris Base 23] (loading solution). For the measurement of VSMC intracellular calcium concentration, the tissue was superfused with the loading solution for 1.5 hours at room temperature followed by a washout period of 30 minutes at 37 °C. By that way, the VSMCs were selectively loaded as only outlines of cells perpendicular to the vessel axis, and no endothelial cells which are parallel to the vessel axis, could be seen on the fluorescence images [8]. Excitation was achieved by fluorescence microscopy using a 100 W Xenon light source and a filter wheel rotating at about 4 Hz and containing 340- and 380-nm interference filters. Emitted fluorescence was collected using a CCD camera (Hamamatsu Orcca 4742-95) and used to monitor vessel diameter and changes in intracellular Ca^{2+} levels. Fluorescence intensity data was subsequently analyzed using Openlab software (Improvision, UK) for calculation of the ratioed fluorescence intensities resulting from excitation at 340 and 380 nm. The dye concentration used in this study was 10 µM. Since, the diameter responses in the presence and the absence of the dye don't show any statistically significant differences, we can conclude that the possible calcium buffering of the dye was negligible in this case. The fluorescence signal was not calibrated. Therefore, only variation amplitudes and dynamics were considered in this study.

Drugs

The drugs used were norepinephrine (NE), acetylcholine (ACh), ouabain, verapamil (all from Sigma, St. Louis, MO, U.S.A) and Fura-2 AM® (Molecular Probes, Leiden, Netherlands). Ouabain was dissolved in control solution directly. The other drugs were prepared with distilled water.

Analysis of data

Data are expressed as means $\pm$ SEM. Statistical significance was tested using Student's t-test on paired data; $P < 0.05$ was regarded as significant; n represents the number of animals examined.

Results

Smooth muscle cell membrane potential

The resting VSMC membrane potential of cannulated arteries was -52.2 ± 2.2 mV (n = 9; mean $\pm$ SE) (Fig. 1). The mean resting external diameter of the perfused vessels was 473 ± 30 μm (n = 6; mean $\pm$ SEM) (Fig. 1). Stimulation with NE caused vasoconstriction and vasomotion. Vasomotion was absent without stimulation with NE. The application of 10^{-6} M NE decreased the vessel diameter of the
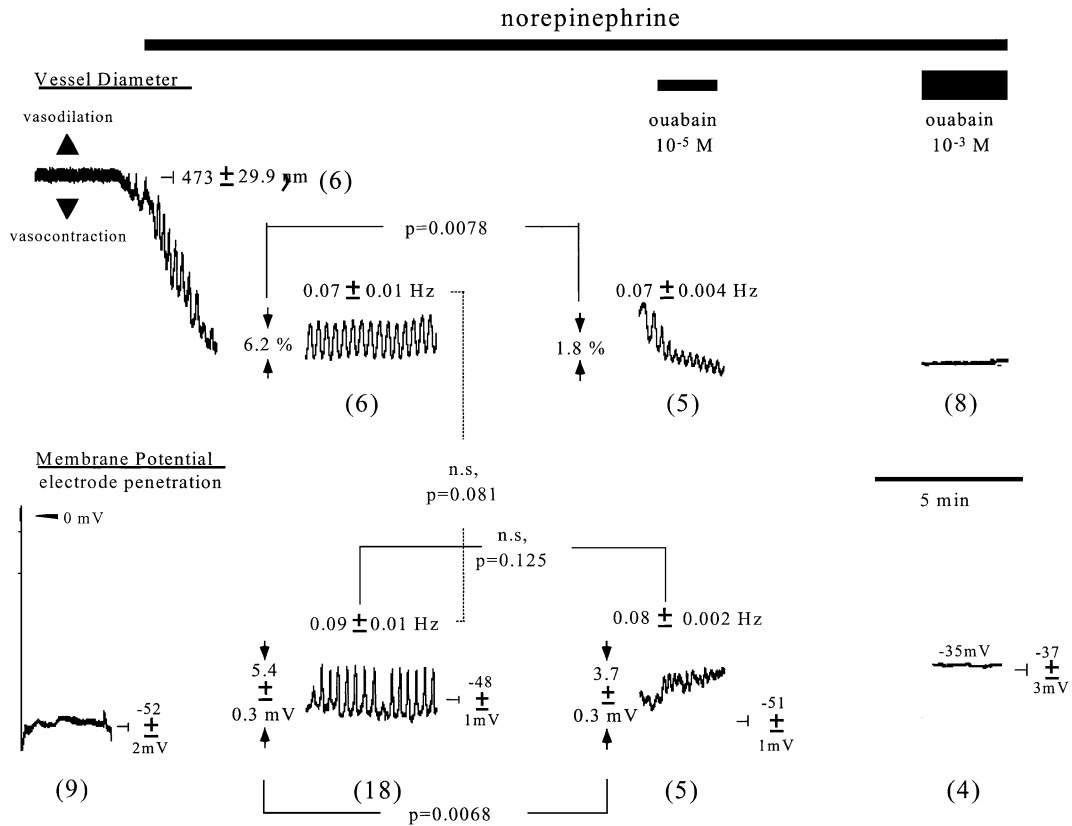

Fig. 1. Summarizing table and representative recordings of vessel diameter and membrane potential changes in response to superfusion of noradrenaline before and during application of ouabain (10^{-5} M and 10^{-3} M). Both diameter and membrane potential changes were recorded simultaneously from the same tissue. Data are shown as mean $\pm$ SEM. Number in parentheses indicates number of experiments. Arrows show the amplitude of oscillations (% in vessel diameter study and mV in membrane potential study).

perfused vessel by 38.1% (100% refer to the resting diameter). Subsequently, this vasoconstriction was accompanied by a rhythmic oscillation of the vessel diameter with a frequency of 0.07 ± 0.01 Hz and an amplitude of $6.20 \pm 0.01\%$ (n = 6) of the resting diameter (Fig. 1). The membrane potential of VSMC during spontaneous rhythmic contractions was also recorded. In this experiment, NE depolarized membrane potentials to -46.2 ± 1.7 mV (n = 18; mean $\pm$ SE) (Fig. 1). The presence of NE also induced oscillations in membrane potential (Fig 1). The frequency of membrane potential oscillations was 0.09 ± 0.01 Hz with an amplitude of 5.4 ± 0.3 mV (n = 18; mean $\pm$ SE). The frequency of membrane potential oscillations was not significantly different from that of the diameter oscillations (p = 0.081) (Fig. 1). This oscillation is 6 times out of 18 times synchronous with vasomotion.

This asynchronism can be explained by the observation that the entire vessel segment did not always contract synchronously over its entire length and the recording electrode was not positioned where the diameter was measured. To confirm that this membrane potential oscillation was not a movement artifact but originates from the VMSC conductivity changes, acetylcholine (ACh, 10^{-4} M) was applied. It is an endothelium-dependent vasodilator which hyperpolarizes the smooth muscle cells in an endothelium-dependent manner [9]. ACh induced membrane hyperpolarization during slow wave membrane potential change and vasodilation of the cannulated vessel simultaneously (Figs. 2 and 3B).

$Na^+ - K^+$ ATPase

Ouabain (10^{-3} and 10^{-5} M) was applied to the vessel only when successful cell impalement was obtained. The effect of ouabain was observed during the establishment of its action. In the presence of 10^{-5} M ouabain, an inhibitor of the $Na^+ - K^+$ ATPase, the mean membrane potential was -51 ± 1 mV (n = 5; mean $\pm$ SE). This value was not significantly different from the mean resting membrane potential. During vasomotion evoked by NE, 10^{-5} M ouabain decreased the amplitude of slow wave oscillations to 3.7 ± 0.3 mV (n = 5; mean $\pm$ SE). There was a significant difference in amplitude compared with the absence of ouabain (p < 0.01). On the other hand, there was no significant difference in frequency of slow wave oscillations between the presence (0.080 ± 0.002 Hz) and the absence of ouabain (p = 0.125) (Fig. 1). In the presence of NE, ouabain (10^{-5} M) caused a weak constriction, the diameter of the artery was then 326 ± 9.7 µm (n = 5; mean $\pm$ SE).
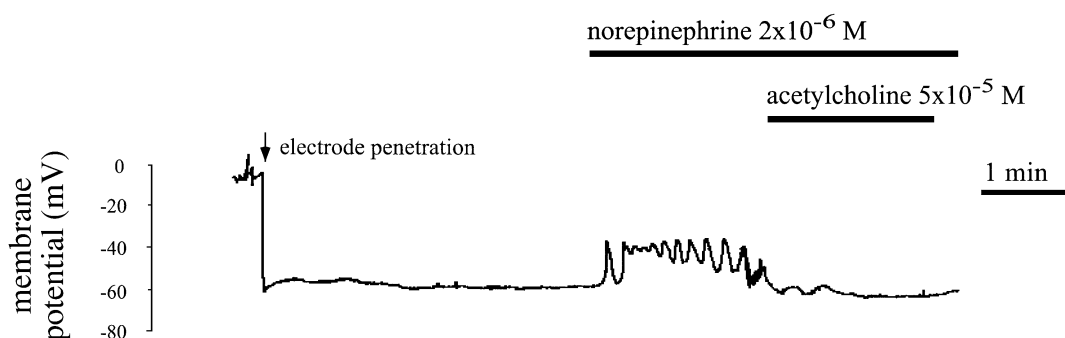

Fig. 2. Measurements of smooth muscle cell membrane potential during vasomotion evoked by NE. Acetylcholine (10^{-4} M) abolished membrane potential slow wave and membrane hyperpolarization simultaneously.
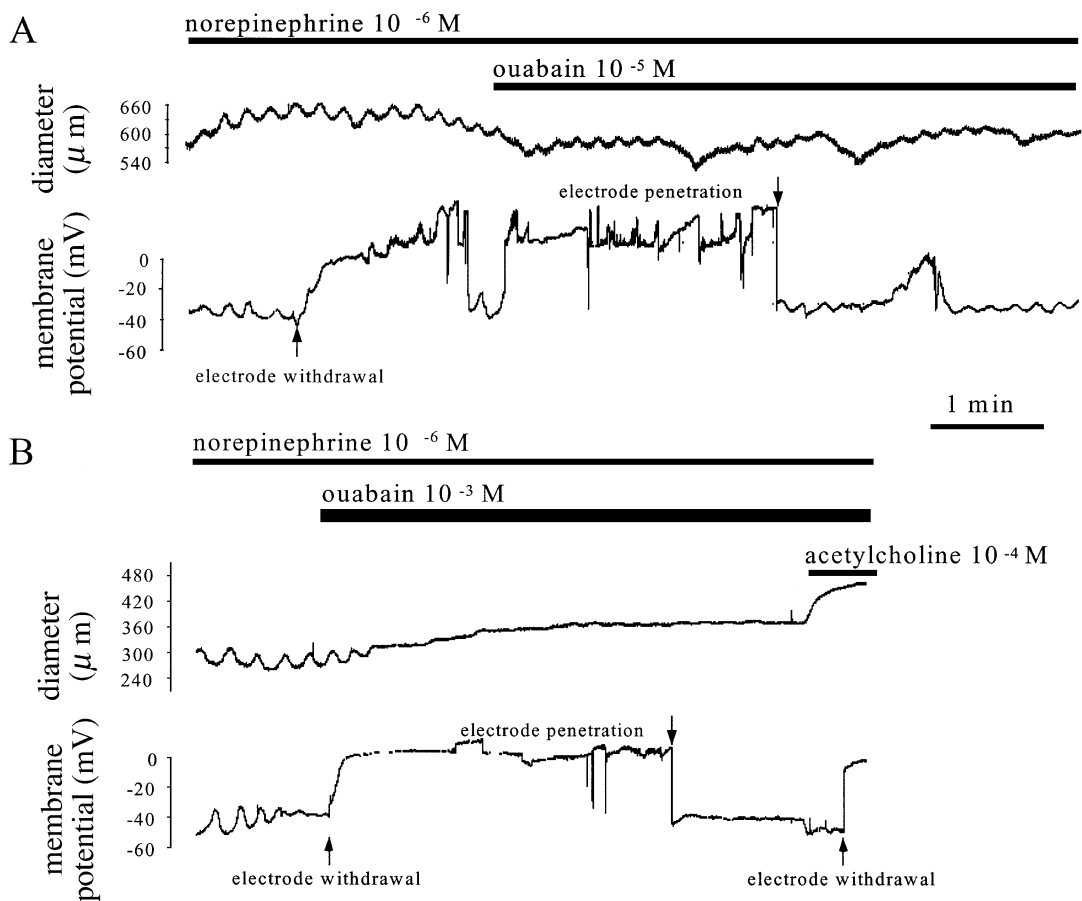
A

norepinephrine 10⁻⁶ M

Fig. 3. Effect of 10^{-5} M (A) and 10^{-3} M (B) ouabain on vessel diameter change and smooth muscle cell electrical slow wave oscillations elicited by 1 μM noradrenaline. In the presence of ouabain, acetylcholine (10^{-4} M) stimulated vasodilation and membrane hyperpolarization simultaneously (B).

A high concentration of ouabain (10^{-3} M) did not contract further the vessels (324 ± 19 μm, n = 8; mean $\pm$ SE). However, it abolished both vasomotion (n = 8) and electrical slow wave oscillations (n = 4, mean membrane potential was -36.6 ± 2.5 mV) (Fig. 1 and Fig. 3A and B, typical trace). To confirm that this constant membrane potential was associated with a functional cell, ACh (10^{-4} M) was used. ACh produced membrane hyperpolarization of the recorded cell and vasodilation of the cannulated vessel in the presence of 1 mM ouabain with 1 μM NE (Fig. 3B).

Smooth muscle cell cytosolic free calcium

Application of NE (0.5 μM) induced rhythmic contractions and relaxations in pressurized mesenteric artery (Fig. 4A). Simultaneously, NE caused smooth muscle cell cytosolic free calcium oscillations. Cytosolic free calcium in VSMC oscillated in parallel with vasomotion. Intraluminal application of ACh
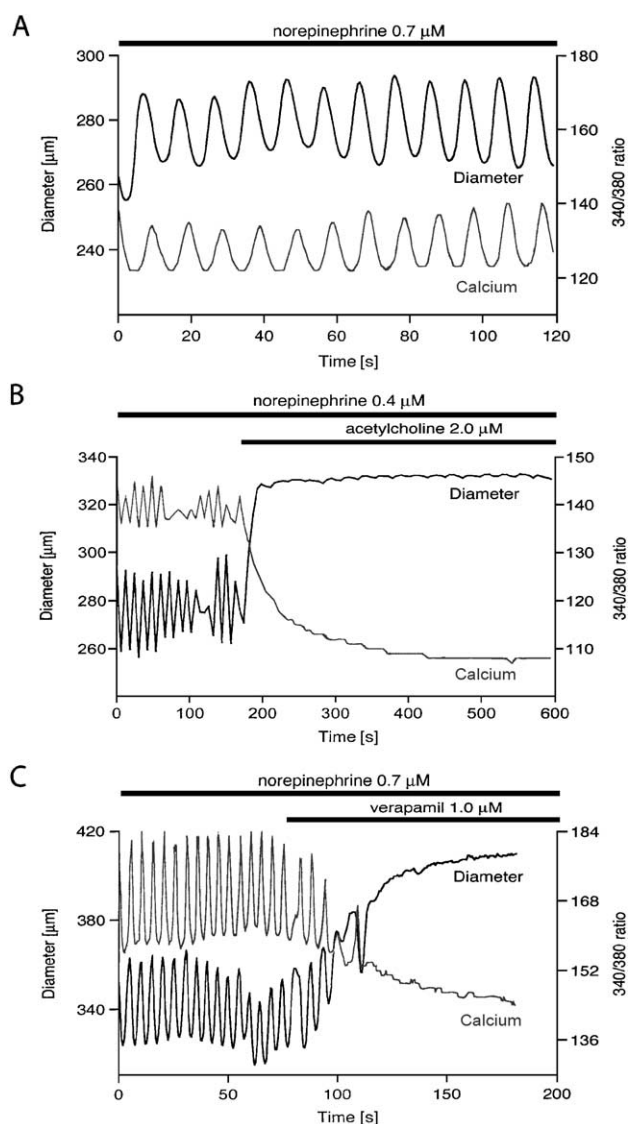
Fig. 4. Simultaneous measurement of external vessel diameter change and smooth muscle cells calcium level (Fura-2 ratio 340/380 in an arbitrary scale) during vasomotion induced by norepinephrine (A) and effect of acetylcholine (B) and verapamil (C) applied during vasomotion. The ratio are expressed as pixel gray scale levels of the ratio image.

(1 μM) caused vasorelaxation of the perfused artery (Fig. 4B). Simultaneously, smooth muscle cell cytosolic free calcium was decreased in parallel with vasodilation.

An inhibitor of L-type calcium channels, which are voltage dependent, verapamil (1 μM) applied during vasomotion caused by NE dilated the artery by $22 \pm 2\%$ (n = 4; mean $\pm$ SE) and diminished the amplitude of smooth muscle cell cytosolic free calcium oscillations by $93 \pm 2.5\%$ (n = 4; mean $\pm$ SE). Simultaneously, the amplitude of vasomotion was inhibited by $72 \pm 9\%$ (n = 4; mean $\pm$ SE) and the frequency by $80 \pm 4\%$ (n = 4; mean $\pm$ SE).

Discussion

Our results show that vasomotion induced by norepinephrine is accompanied by membrane potential oscillations. This oscillation does not exhibit the time course of an action potential, particularly the depolarization is slower than in an action potential. In addition, the amplitude of the oscillations is not all or none. Therefore this oscillation could be described as a slow wave. In addition, this result confirms the previous report that NE induced membrane potential depolarization [7,10].

In small arteries, not only voltage-operated calcium channels (VOCC) but also intracellular calcium stores play an important role in vasomotion evoked by NE [4]. In many previous reports, it was observed that NE depolarized the VSMC [7,10]. In our experiment, NE also depolarized the resting membrane potential in VSMC. It could result from the opening of VOCC and increase of intracellular calcium concentration in VSMC. Furthermore, increase of calcium due to VOCC might accelerate the release of calcium from intracellular stores via calcium-induced calcium release.

These mechanisms of increasing calcium induce vasoconstriction. This increase in cytosolic calcium concentration, whatever its source, intra- or extracellular, triggers vasomotion. In our study, cytosolic free calcium in VSMC oscillated in parallel with vasomotion. This observation is coherent with the concept that calcium oscillations are responsible for vasomotion. The more likely explanation of vasomotion is that the oscillations in the membrane potential might control the oscillations in cytosolic free calcium by alternatively increasing and decreasing the opening probability of the VOCC. The abolishment of the calcium oscillations simultaneously with vasomotion by inhibition of the VOCC using verapamil supports this hypothesis.

In this study, slow wave oscillations were 6 times out of 18 times synchronous with vasomotion. This phase shift can be explained by the observation that the entire vessel segment did not always contract synchronously over its entire length. Some times, wave propagations were observed. These waves did not always originate at the same places, could travel in one direction, and a few moments thereafter, in the opposite direction, were sometimes colliding and passing then through each other almost without any apparent deformation. Smooth muscle cell penetration for membrane potential measurements was done close to the perfusion tubing where the vessel did not move. On the other hand, vessel diameter was measured at the middle of the perfused segment. Indeed these two regions did not always contract simultaneously. However, when the entire vessel contracted synchronously (6 times out of 18), we observed a perfect synchronism between electrical slow waves and vasomotion. In addition, the observation that the inhibition of vasomotion by ouabain and Ach was accompanied by the suppression of the membrane potential oscillation supports a link from cause to effect between these parameters.

The Na^+–K^+ pump is electrogenic and seems to play an important role in the generation of rhythmic contractions in small arteries [3]. Our observation that a concentration of 10^{-5} M ouabain decreases the amplitude of slow waves without changing its frequency suggests that the Na^+–K^+ ATPase could not be essential for the generation of rhythmic membrane potential oscillations. The fact that a concentration of 1 mM ouabain suppresses vasomotion could not prove that the sodium pump is implicated, because in the millimolar range, ouabain is no longer specific. Indeed, 1 mM ouabain also attenuates cell-to-cell coupling via gap junctions in rat mesenteric artery [11] and the coupling between smooth muscle cells is necessary for vasomotion [12]. Whatever, 1 mM ouabain would suppress the membrane potential slow waves, this shows that an oscillation in the membrane potential is necessary to produce calcium oscillations and thus vasomotion. In this situation, the observation that acetylcholine is still able to relax

the strip shows that 1 mM ouabain does not suppress the slow wave by inhibiting the electromechanical coupling.

The EDHF evoked by agonists (e.g. ACh) cause membrane hyperpolarization and vasodilation in the rat mesenteric artery [9,13]. Hence, ACh, an endothelium-dependent smooth muscle cell hyperpolarizing vasodilator, in the rat vessel, was used to confirm that the different types of membrane potential changes observed during vasomotion originate from smooth muscle cell conductivity changes and do not result from a movement artifact. In the present study, ACh induced hyperpolarization and vasodilation of the vessel, whether membrane potential was oscillating or constant. These results demonstrate that the recorded cells were working, since the cells were responsive to ACh.

Cytosolic free calcium in smooth muscle cells was determined in arterial wall under isometric conditions and on pressurized vessels [5,6,14]. At our knowledge, calcium concentration and membrane potential in smooth muscle cells were never observed with vessel diameter on perfused pressurized rat mesenteric artery during vasomotion. The interest in this approach was first to measure these parameters on the same preparation and second, to determine them on a controlled pressurized perfused vessel so in conditions closed to the physiological one.

Conclusion

In conclusion, membrane potential changes during vasomotion induced by NE is a slow wave type oscillation in cannulated rat mesenteric artery. This oscillation, by rhythmically gating voltage-dependent calcium channels, is responsible for the oscillation of intracellular calcium and thus vasomotion. Although the Na^+-K^+ ATPase was considered as part of the oscillator, our results do not confirm that this is the case and the mechanism of oscillation seems to result from interplay between a membrane and an intracellular calcium oscillator [15].

Acknowledgements

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