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Potassium-induced intermittent vasomotion in rat isolated pulmonary artery

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Abstract

A novel intermittent vasomotion induced by potassium in rat pulmonary artery was investigated with a view to characterize the ion channel mechanisms governing such secondary oscillatory activity. Isometric force was recorded from ring preparations of rat isolated pulmonary arteries incubated in a modified Krebs buffer containing K⁺ 15–18 mM and nitro-L-arginine methyl ester (10 μM). Tissues exhibited a stable pattern of on-off vasomotion consisting of intermittent contractile wave (ICW) activity with a periodicity of 7–8/hr and a rising phase of oscillatory ramping-up of contractile tone at 7 cycles/min. L-channel antagonists arrested (nicardipine; 3nM) or retarded (verapamil, 30 nM) ICW activity with a concomitant wave asynchronizat ion or decrease in amplitude. Mibefradil (30–100 nM) inhibited ICW ramping-up without affecting ICW period. Niflumic acid (1.0–3.0 μM) exerted dual actions on ICW amplitude but arrested ICW cycling at 10 μM. K⁺-channel blockers produced shortening of ICW period (4-aminopyridine, Ba²⁺ 30 μM; Cs⁺ 3.0–6.0 mM) and increase (tetraethylammonium; 1.0 mM) or decrease (Ba²⁺, 100 μM) in amplitude. Cyclopiazonic acid caused ICW asynchronization (0.3 μM) or cessation (1.0 μM) of ICW cycling. Fasudil retarded ramping-up contractile oscillations without changing ICW period. The inhibitory effects of nicardipine, niflumic acid and cyclopiazonic acid were partially surmounted by small additional increments in [K⁺]. Our findings support the concept that a secondary vasomotive oscillator operates in rat pulmonary artery which enables the activity of the primary oscillator to be regulated in a cyclic manner via sarcolemmal L-type Ca²⁺ channels and an array of K conductances.

Key words: intermittent contractile wave, ion channels, rhythmogenesis, oscillator, vascular smooth muscle

Introduction

Under appropriate in vivo or in vitro conditions, diverse vascular beds manifest vasomotion in the form of rhythmic contraction and relaxation cycles. While it has been suggested that oscillation in vascular tone can promote oxygen delivery, the exact physiological role of this extensively studied phenomenon remains to be elucidated (Nilsson and Aalkjær, 2003; Aalkjær and
Vasomotor oscillations are known to occur at different frequencies comprising “slow waves” at 1–3 cycles per minute (Seifert et al., 1988; Kastrup et al., 1989; Bollinger et al., 1991) and “fast waves” at 10–25 cycles per minute (Colantuoni et al., 1985; Meyer et al., 1987; Slaaf et al., 1987; Kastrup et al., 1989).

The sarcoplasmic reticular (SR) Ca pump and myoendothelial signaling are considered to play integral roles in vasomotor rhythmogenesis. However, important inter-species and regional differences have been noted. For instance, in rat mesenteric artery, vasomotion is thought not to require Ca-pumping by the SR and to involve an interaction between endothelium and vascular myocytes via gap junctions (Rahman et al., 2007). In some arterial blood vessels vasomotion entails changes in membrane potential (Peng et al., 2001; Oishi et al., 2002), while in others voltage independence has been inferred (Haddock et al., 2002). In human pial arteries, vasomotion can be initiated by exposure to high extracellular potassium ([K+]e) (66 mM) followed by a return to normal [K+]e. This maneuver has been reported to bring about spontaneous changes in membrane potential of 5 to 15 mV, and an accompanying vasomotion that was inhibited by the L-type Ca channel antagonist, nifedipine (Gokina et al., 1996).

In myocytes of rat main pulmonary artery, oscillation in intracellular Ca2+ is presumed to be linked to vasomotion, with extracellular Ca2+ making a very limited contribution (Hyvelin et al., 1998). As shown in the present investigation, vasomotion in the same blood vessel displays a higher order slow periodicity which does not appear to have been reported previously. In this exploratory study, we have examined the roles played by different ion channels (L-type Ca, K and Cl), Rho-dependent kinase and a functional SR in the sequence of events associated with this secondary cycling process.

**Material and Methods**

All procedures on animals were carried out in accordance with the guidelines and approval of the Animal Care Committee, Memorial University, St. John’s, Newfoundland and Labrador, Canada.

**Tissue isolation**

Male Sprague-Dawley rats (260–300 g) were anaesthetized with sodium pentobarbital (65 mg/kg). The main left and right pulmonary arteries were isolated, dissected free of connective tissue and separated from the pulmonary trunk at room temperature in Krebs buffer with the following composition (in mM): NaCl, 130; KCl, 4.0; glucose, 11; MgCl2, 1.2; CaCl2, 2.5; KH2PO4, 1.2; NaHCO3, 12.5; EDTA, 0.1. The pH of the buffer following saturation with a 95% O2 : 5% CO2 gas mixture was 7.4 at 36 ± 1°C.

**Experimental protocol**

Mechanical force measurements were made from ring preparations of right and left main pulmonary arteries (~2 mm in length) which were mounted in 20 mL organ baths at 36–37°C under a force of 1.0 g and gassed continuously with a mixture of 95% O2 : 5% CO2 (Bieger et al., 2006). The tissues were equilibrated for 45–55 min and isometric tension was measured by means
of force displacement transducers (Model FT03, Grass Instruments Co., MA, U.S.A.) connected to a polygraph (Model 7PCPB, Grass Instruments Co., MA, U.S.A.). Elevation in [K\(^{+}\)]\(_{e}\) was achieved by the replacement of regular Krebs solution with the experimental buffer containing 15 mM [K\(^{+}\)]\(_{e}\). The increase in the amount of [K\(^{+}\)]\(_{e}\) was compensated for by removal of an equivalent amount of [Na\(^{+}\)]\(_{e}\). Further elevation of [K\(^{+}\)]\(_{e}\) by 1–2 mM was achieved by direct addition of KCl to the tissue bath. Since endothelial nitric oxide production can be expected to impair intermittent wave generation, the nitric oxide synthase inhibitor N\(^{\omega}\)-nitro-L-arginine methyl ester (10 \(\mu\)M) was routinely added to the bathing solution.

Once the periodic vasomotion was initiated, blood vessels were allowed to cycle for at least 1 hr before the addition of vehicle or drugs. Thereafter, vehicle (twice distilled water, DMSO, absolute alcohol) or various drugs were introduced into the organ bath over the course of 5–6 hr while mechanical activity was continuously recorded. The effects of the following agents were examined: absolute alcohol (4, 14, 40 \(\mu\)L), 4-aminopyridine (4-AP; 10, 30 100 \(\mu\)M; K-channel blocker), BaCl\(_{2}\) (10, 30, 100 \(\mu\)M; K-channel blocker), CsCl (6.0 mM; K-channel blocker), ceramide (0.3, 1, 10 \(\mu\)M; inhibitor of diacylglycerol kinase), cyclopiazonic acid (0.1, 0.3, 1.0 \(\mu\)M; inhibitor of Ca\(^{2+}\)-ATPase in the sarcoplasmic reticulum); deltamethrin (10, 30, 100 nM; inhibitor of calcineurin phosphatase), double distilled water (2.0, 4.0, 14 \(\mu\)L), DMSO (2.0, 4.0, 14 \(\mu\)L), fasudil (1.0, 3.0, 10 \(\mu\)M; Rho-dependent kinase inhibitor), nicardipine (1.0, 3.0 nM; L-type Ca-channel antagonist), niflumic acid (1.0, 3.0, 10 \(\mu\)M; Cl-channel blocker), mibefradil (10, 30, 100 nM; T-type Ca-channel antagonist), tetraethylammonium (TEA; 0.1, 0.3, 1.0 mM; K channel blocker) and verapamil (3.0, 10, 30 nM; Ca-channel antagonist).

Data and statistical analysis

The parameters employed to analyze the pattern of the on-off vasomotion or intermittent contractile wave (ICW) activity are illustrated schematically in Fig. 1A. Specifically, time for slow contractions to rise (ramp-up) to peak (P\(_{T}\)), time to relaxation to baseline (R\(_{T}\)), period duration (D\(_{P}\)), rest period, and amplitude of contraction were measured, where rest period equals \([D_{P}] – (P_{T} + R_{T})\]. The term asynchronization denotes the splitting up of the waves into low amplitude wavelets.

One-way analysis of variance was used for statistical evaluation of the data. The Bonferroni test was used for comparisons between control and treatment within groups, and the Student-Newman-Keuls multiple range test was used for comparison between groups. For all cases, a probability of error of less than 0.05 was selected as the criterion for statistical significance. Values presented are mean ± S.E.M. unless otherwise stated.

Chemicals

Solutions of all drugs were made in double-distilled water with the following exceptions: stock solutions of 0.1 M ceramide and 0.001 M deltamethrin were prepared in absolute alcohol, that of 0.01 M cyclopiazonic acid in DMSO. All drugs were purchased from RBI/Sigma (Oakville, Ontario, Canada).
Results

Intermittent vasomotion was initiated in the isolated pulmonary artery within 2 min following the increase in [K+]e to 15–17 mM. Intermittent contractile wave (ICW) activity persisted at 5–6 cycles per hr for 5–6 hr without obvious signs of fatigue. The example shown in Figure 1B demonstrates that the rising phase of each wave consisted of fast rhythmic activity of small amplitude at an average frequency of approximately 7.8 ± 1.2 cycles per min (mean ± SD; n = 9 animals). However, in other tissues producing typical ICW activity the underlying fast rhythmic contractions were more or less dampened such that the mechanogram presented a smooth trace. During the pre-drug control phase, time of rise to peak (PT) averaged 2.9 ± 0.8, time to relaxation (RT) 4.9 ± 1.0 and period duration (DP) 10.9 ± 1.3 min, with a peak amplitude of 171.3 ± 8.0 mg, (mean ± SD; n = 45 animals). The apparent rest period between waves averaged 3.2 ± 0.25 min (mean ± SD; n = 45 animals). Addition of twice distilled water in time parallel groups had no effect on PT, RT, DP, or the peak of the amplitude of these oscillations (Table 1). However, the addition of alcohol and DMSO significantly reduced time of contraction to peak (PT) without affecting any other parameter (Table 3).

Effects of Ca channel antagonists and niflumic acid

The vascular selective Ca-channel antagonist, nicardipine, inhibited periodic vasomotion. At the lower concentration tested it did not significantly affect rise time to peak (PT), time to relaxation (RT) or period duration (DP) but significantly reduced amplitude (Table 1). In the presence of nicardipine 3.0 nM, on-off intermittent activity ceased completely; however, further
Vasomotion of pulmonary artery

Table 1. The effect of various drugs on intermittent vasomotion initiated by elevation in $[K^+]_e$ (15–17 mM) in isolated rat pulmonary artery. Time intervals (min) are listed for rise to peak ($P_T$), time to relax ($R_T$) and duration of period ($D_P$); wave amplitude is expressed as % contraction of control ($H_c$). Each value is mean ± S.E.M of 5–6 experiments.

| Groups             | $P_T$   | $R_T$   | $D_P$   | $H_c$  |
|--------------------|---------|---------|---------|--------|
| Distilled H$_2$O ($\mu$L) |         |         |         |        |
| Control            | 2.3 ± 0.2 | 3.9 ± 0.3 | 9.6 ± 1.2 | 100    |
| 2                  | 2.4 ± 0.3 | 4.3 ± 0.4 | 9.2 ± 0.9 | 127 ± 15 |
| 4                  | 2.7 ± 0.4 | 3.7 ± 0.4 | 9.1 ± 1.2 | 127 ± 15 |
| 14                 | 1.9 ± 0.2 | 3.6 ± 0.4 | 9.9 ± 1.2 | 113 ± 11 |
| Nicardipine (nM)   |         |         |         |        |
| Control            | 2.8 ± 0.2 | 4.9 ± 0.4 | 10.2 ± 1.7 | 100    |
| 1                  | 2.8 ± 0.1 | 3.9 ± 0.2 | 11.4 ± 1.8 | 71 ± 5$^a$ |
| 3                  | Ø       | Ø       | Ø       | Ø      |
| Verapamil (nM)     |         |         |         |        |
| Control            | 3.0 ± 0.2 | 5.1 ± 0.5 | 10.5 ± 0.7 | 100    |
| 3                  | 2.8 ± 0.3 | 4.9 ± 0.2 | 9.2 ± 0.6 | 157 ± 21 |
| 10                 | 2.3 ± 0.4$^a$ | 4.5 ± 0.1 | 9.0 ± 0.5 | 119 ± 25 |
| 30                 | 1.6 ± 0.5$^a$ | 3.7 ± 0.4 | 24.1 ± 7.0$^a$ | ASY    |
| Mibefradil (nM)    |         |         |         |        |
| Control            | 2.8 ± 0.3 | 5.9 ± 0.4 | 11.9 ± 1.0 | 100    |
| 10                 | 2.6 ± 0.2 | 5.2 ± 0.4 | 11.2 ± 0.8 | 115 ± 13 |
| 30                 | 1.9 ± 0.2$^a$ | 5.2 ± 0.6 | 10.4 ± 0.7 | ASY    |
| 100                | 1.8 ± 0.2$^a$ | 4.0 ± 0.3 | 10.2 ± 1.7 | ASY    |
| Niflumic acid ($\mu$M) |         |         |         |        |
| Control            | 2.5 ± 0.5 | 5.0 ± 0.5 | 10.7 ± 0.6 | 100    |
| 1                  | 2.7 ± 0.5 | 5.00 ± 0.3 | 11.6 ± 0.6 | 191 ± 35$^c$ |
| 3                  | 1.85 ± 0.2 | 4.8 ± 0.5 | 25.6 ± 8.0$^d$ | 142 ± 47 |
| 10                 | Ø       | Ø       | Ø       | Ø      |

$a$, significantly different from respective control; $b$, significantly different from respective normalized ($%H_c$) time-control; $c$, significantly different from higher concentrations; $d$, significantly different from lower concentration; Ø, not determinable due to absence of intermittent contractile wave; ASY, asynchronization.

Elevation of $[K^+]_e$ (final concentration 18.0–19.5 mM) re-established wave activity at a decreased frequency in two tissues ($P_T$: 1.9 min; $R_T$: 2.6 min; $D_P$: 25 min; amplitude of 77.0 mg; resting period of 21 min).

Verapamil disrupted cycling as evidenced by asynchronization of the ICW cycles, and at the higher concentrations it significantly shortened the rise time to peak without affecting time to relaxation. However, it significantly increased period duration in parallel with the reduction in $P_T$ (Table 1).

Mibefradil, a T-type Ca channel antagonist, caused asynchronization of the waves as evidenced by a decrease in frequency of fast rhythmic contractions during the ramping-up phase. Although it progressively and significantly reduced rise time to peak (~35%), it failed to alter either time to relaxation or period duration of the contractile pattern (Fig. 2; Table 1). Niflumic acid had a biphasic effect on the amplitude of ICW. At the lowest concentration, it significantly increased amplitude, at the higher concentration it exerted an inhibitory effect (Table 1). Addition of niflumic acid (3.0 $\mu$M) increased period duration without change in rise time to peak or time to relaxation.
Niflumic acid, at 10 \( \mu \text{M} \), arrested wave activity, which was however re-instated in three tissues by further raising the \([K^+]_e\) (P\(_T\): 1.2 ± 0.3 min; R\(_T\): 3.4 ± 0.3 min; D\(_P\): 17.0 ± 3.8 min; amplitude of 262.0 ± 27.9 mg; resting period 14.7 ± 4.2 min) (Fig. 3).

**Effect of K channel blockade**

TEA significantly increased ICW amplitude at 1.0 mM but not at 0.1 or 0.3 mM, while exerting little effect on duration or frequency (Table 2). In contrast, 4-AP, did not alter either amplitude, or rise time to peak (P\(_T\)) or time to relaxation (R\(_T\)) at any of the concentrations tested but caused a significant increase in frequency (+48%) of activity (Table 2). Ba\(^{2+}\) also significantly increased frequency at higher concentrations (+67%) and significantly reduced rise time to peak (–30%) without affecting time to relaxation (Table 2). Ba\(^{2+}\) significantly attenuated amplitude at 100 \( \mu \text{M} \) but not at 10 or 30 \( \mu \text{M} \) (Table 2). Cs\(^+\) (3.0–6.0 mM) eliminated ICW activity within 5–15 min and caused continuous fast oscillation of tone which lacked a temporal pattern and were of low irregular amplitude. This effect was accompanied by a rise in baseline tone.
Fig. 3. The effect of niflumic acid on intermittent vasomotion in isolated rat main pulmonary artery produced by elevation in [K⁺], (15.0 mM). Panel A: control; B: 1.0 μM; C: 3.0 μM; D: 10 μM; E: 10 μM + additional (↓) KCl (21 mM final).

Table 2. The effect of various drugs on intermittent vasomotion initiated by elevation in [K⁺], (15–17 mM) in isolated rat pulmonary artery. Time intervals (min) are listed for rise to peak (P), time to relax (R) and duration of period (D); wave amplitude is expressed as % contraction of control (H). Each value is mean ± S.E.M of 5–6 experiments.

| Groups      | PT    | RT    | DP    | % Hc  |
|-------------|-------|-------|-------|-------|
| **TEA (mM)** |       |       |       |       |
| Control     | 3.2 ± 0.3 | 4.8 ± 0.4 | 12.3 ± 1.3 | 100   |
| 0.1         | 3.1 ± 0.4 | 4.9 ± 0.4 | 12.1 ± 0.6 | 111 ± 16 |
| 0.3         | 2.4 ± 0.1 | 4.9 ± 0.3 | 11.1 ± 0.6 | 158 ± 16 |
| 1.0         | 2.7 ± 0.3 | 4.8 ± 0.4 | 10.4 ± 0.2 | 193 ± 19b |
| **4-AP (μM)** |       |       |       |       |
| Control     | 2.8 ± 0.3 | 5.0 ± 0.6 | 11.7 ± 0.8 | 100   |
| 30          | 2.2 ± 0.3 | 4.7 ± 0.3 | 8.1 ± 0.6a | 119 ± 13 |
| 100         | 2.0 ± 0.3 | 4.2 ± 0.2 | 8.1 ± 0.4a | 121 ± 11 |
| 300         | 2.4 ± 0.5 | 4.2 ± 0.2 | 8.0 ± 0.5a | 122 ± 9  |
| **Barium (μM)** |       |       |       |       |
| Control     | 3.6 ± 0.2 | 4.3 ± 0.5 | 11.4 ± 0.7 | 100   |
| 10          | 2.6 ± 0.3a | 4.7 ± 0.6 | 10.0 ± 0.8 | 94 ± 21 |
| 30          | 2.5 ± 0.3a | 3.9 ± 0.4 | 9.1 ± 0.8a | 111 ± 32 |
| 100         | 2.7 ± 0.4a | 4.1 ± 0.4 | 8.0 ± 0.7a | 65 ± 8  |

a, significantly different from respective control; b, significantly different from respective normalized (%Hc) time-control; TEA, tetraethylammonium; 4-AP, 4-aminopyridine.
Cyclopiazonic acid exerted inhibitory effects; at 0.3 μM, ICW underwent asynchronization into complex wavelets (Fig. 4). At 1.0 μM, cycling ceased in all tissues. However, after addition of KCl (final concentration (16.5–18 mM), 3 out of 7 tissues reverted to ICW activity (P<sub>T</sub>: 1.0 ± 0.2 min, R<sub>T</sub>: 3.1 ± 0.5 min, D<sub>P</sub>: 7.2 ± 2.2 min and amplitude of 224.0 ± 31.0 mg; resting period of 4.4 ± 1.0 min).

Exposure to fasudil resulted in reduction in amplitude and a concomitant slowing of the fast oscillatory activity of the rising phase to the point where individual repetitive contractions became discernible. Although this effect appeared concentration dependent, it faded within 10–15 min (Fig. 5). Nonetheless, the pattern of ICW activity remained evident at the highest concentration tested in 3 out of 5 tissues. The addition of ceramide or deltamethrin had negligible effects (Table 3).

**Discussion**

The pattern of vasomotion investigated in this study supports the concept of two distinct
oscillator mechanisms existing in the rat pulmonary arterial bed. The first, or primary, oscillator manifests itself in the initial ramping-up of the activity and shows features typical of vasomotion described in various arterial blood vessels. The secondary oscillator reveals itself in on-off cycling of vasomotion occurring at frequencies in the range of 7–8 per hr. As discussed below, the presence of a secondary oscillator mechanism accords with pharmacological characteristics demonstrating that certain agents (nicardipine and 4-AP) appeared to preferentially affect slow oscillations, while others (mibefradil and fasudil) preferentially interfered with the fast oscillator. Nevertheless, some of the pharmacological actions observed (i.e., niflumic acid, Ba²⁺), particularly on ICW amplitude, suggest a lack of selectivity with regard to both oscillator mechanisms. Remarkably, relaxation time was unaffected by any of the agents studied, suggesting that changes in wave configuration reflect changes in its rising phase.

**Role of sarcolemmal Ca channels**

Our findings imply that ICW generation critically depends on an influx of Ca²⁺ through voltage gated L-type Ca channels. Since this activity was initiated and maintained by elevation of [K⁺]ₑ, the underlying mechanism very likely involves the depolarization of the membrane potential of vascular myocytes. It is pertinent to note that in rat thoracic aorta, spontaneous rhythmic
contractions with a frequency of 4.7 to 9.6 cycles/min were associated with small changes in membrane potential of a magnitude of 2–5 mV peak-to-peak (Hayashida et al., 1986). As expected, nicardipine was most effective in suppressing the ICW activity. Moreover, a partial reversal in Ca-channel antagonism could be achieved by additional increases in [K+]o, probably because the expected greater degree of membrane depolarization would lead to an increase in the probability of opening of more voltage-gated Ca channels, thus surmounting the antagonism by nicardipine.

Interestingly, the actions of verapamil, a so called “cardiac selective” Ca-channel antagonist (Triggle, 1992), appeared different from those of nicardipine. Verapamil caused severe asynchronization of activity without consistently reducing amplitude. These observations may suggest that vascular Ca channels (probably with low activation threshold) are not affected in the same manner or that other membrane actions are responsible for disrupting the coordinated

Table 3. The effect of various drugs on intermittent vasomotion initiated by elevation in [K+]o (15–17 mM) in isolated rat pulmonary artery. Time intervals (min) are listed for rise to peak (Pf), time to relax (Rf) and duration of period (Dp); wave amplitude is expressed as % contraction of control (Hc). Each value is mean ± S.E.M of 5–7 experiments (*, except n = 3)

| Groups                  | Pf     | Rf     | Dp     | % Hc  |
|-------------------------|--------|--------|--------|-------|
| Absolute Alcohol (µL)   |        |        |        |       |
| Control                 | 3.6 ± 0.3 | 5.0 ± 0.3 | 11.6 ± 0.9 | 100   |
| 4                       | 2.3 ± 0.1 | 5.0 ± 0.3 | 11.7 ± 0.5 | 94 ± 11 |
| 14                      | 2.5 ± 0.1 | 5.4 ± 0.5 | 11.6 ± 0.7 | 111 ± 12 |
| 40                      | 2.4 ± 0.2 | 4.7 ± 0.3 | 10.9 ± 0.5 | 109 ± 12 |
| DMSO (µL)               |        |        |        |       |
| Control                 | 3.4 ± 0.2 | 5.2 ± 0.4 | 11.3 ± 0.9 | 100   |
| 2                       | 3.3 ± 0.4 | 4.8 ± 0.3 | 10.4 ± 0.9 | 117 ± 14 |
| 4                       | 2.5 ± 0.2 | 5.1 ± 0.5 | 10.4 ± 1.0 | 136 ± 13 |
| 14                      | 2.0 ± 0.3 | 4.7 ± 0.3 | 11.0 ± 1.8 | 140 ± 9  |
| Cyclopiazonic acid (µM) |        |        |        |       |
| Control                 | 3.1 ± 0.3 | 4.6 ± 0.3 | 11.0 ± 0.7 | 100   |
| 0.1                     | 2.8 ± 0.4 | 4.4 ± 0.5 | 11.4 ± 1.0 | 127 ± 16 |
| 0.3                     | 2.2 ± 0.4 | 4.6 ± 0.7 | 13.5 ± 3.0 | ASY   |
| 1.0                     | Ø      | Ø      | Ø      | Ø     |
| Fasudil (µM)            |        |        |        |       |
| Control                 | 3.0 ± 0.4 | 4.9 ± 0.5 | 9.6 ± 1.2 | 100   |
| 1.0                     | 2.3 ± 0.4 | 4.5 ± 0.2 | 11.2 ± 1.2 | 73 ± 20 |
| 3.0                     | 1.7 ± 0.4 | 3.8 ± 0.7 | 10.2 ± 1.6 | 69 ± 22 |
| 10*                     | 2.0 ± 0.5 | 3.8 ± 1.0 | 14.5 ± 2.2 | 65 ± 14 |
| Deltamethrin (nM)       |        |        |        |       |
| Control                 | 3.5 ± 0.4 | 4.6 ± 0.3 | 12.1 ± 0.9 | 100   |
| 10                      | 3.4 ± 0.4 | 5.0 ± 0.3 | 10.7 ± 1.0 | 131 ± 26 |
| 30                      | 2.8 ± 0.4 | 4.1 ± 0.3 | 11.5 ± 1.0 | 140 ± 30 |
| 100                     | 2.6 ± 0.6 | 5.1 ± 0.6 | 11.4 ± 1.1 | 130 ± 36 |
| Ceramide (µM)           |        |        |        |       |
| Control                 | 2.6 ± 0.3 | 5.0 ± 0.3 | 10.6 ± 0.9 | 100   |
| 0.3                     | 2.6 ± 0.3 | 5.0 ± 0.4 | 11.2 ± 0.9 | 124 ± 12 |
| 1.0                     | 2.2 ± 0.2 | 5.0 ± 0.4 | 9.6 ± 0.9  | 131 ± 21 |
| 3.0                     | 2.0 ± 0.3 | 4.8 ± 0.3 | 10.9 ± 1.0 | 121 ± 17 |

a, significantly different from respective control; Ø, not determinable due to absence of intermittent contractile wave; ASY, asynchronization.
oscillatory activity of myocytes.

Among the Ca-channel antagonists mibefradil exerted the most selective action on the ramping-up phase of the ICW owing to its ability to retard the underlying fast oscillation. Thus it seems likely that T-type Ca channels have a supportive role in generating the fast oscillatory activity. More to the point, the actions of this blocker reveal a relative degree of dissociation between fast and slow oscillatory mechanisms.

**Role of Cl channels**

ICW generation was also inhibited by the Ca$^{2+}$-activated chloride channel antagonist, niflumic acid. Calcium-activated chloride channels are expressed in the plasma membrane of different types of smooth muscle cells (Chipperfield and Harper, 2000). A large body of evidence suggests that stimulation of Ca$^{2+}$-activated chloride current is a major contributor in the process of excitation-contraction coupling in vascular muscle (Criddle et al., 1996, 1997; He and Tabrizchi, 1997; Parai and Tabrizchi, 2005). The opening of Ca$^{2+}$-activated chloride channels is thought to be the essential link to chloride efflux leading to membrane depolarization of vascular muscle and subsequent opening of L-type Ca channels (Large and Wang, 1996). However, activation of such current is associated with receptor-mediated excitation-contraction coupling in vascular muscle while KCl-induced contractions are insensitive to the actions of compounds such as niflumic acid (Criddle et al., 1996; Tabrizchi and Duggan 2000). Conceivably ICW generation is, in part, the result of a release of endogenous mediators (noradrenaline from pulmonary arterial nerves) induced by high [K$^+$]$_e$ with consequent activation of niflumic acid-sensitive pathways. However, it was apparent that the inhibitory effect of niflumic acid could be reversed in the same manner as that of nicardipine, *i.e.* by further elevation in [K$^+$]$_e$. Thus, the initial modest elevation in [K$^+$]$_e$ would probably cause only small scale depolarization of myocyte membrane potential, yet permitting sufficient Ca$^{2+}$ influx *via* L-type channels leading to opening of chloride channels. In this scenario, the niflumic acid effect would resemble that of nicardipine as observed here. Chloride ions have been suggested to be critical for oscillations of both membrane potential and intracellular Ca$^{2+}$ during fast vasomotion (Boedtkjer et al., 2008). Arguably, the initial fast oscillatory ramp-up constitutes the underlying mechanism of ICW generation in the rat pulmonary artery.

Paradoxically, niflumic acid augmented the amplitude of contractions in the main pulmonary arteries at the lowest concentration. In rat pulmonary artery smooth muscle cells, niflumic acid has been noted to cause Ca$^{2+}$ release from intracellular stores (Cruickshank et al., 2003). However, the stimulatory effect seen here may not be due to such a mechanism since the concentration of niflumic acid utilized by these authors was of the order of 50 µM, a much higher concentration than the one employed here. An alternative explanation would be the paradoxical stimulatory effects of niflumic acid on the Ca$^{2+}$-activated chloride channels. This effect has been found to occur at µM and mM concentrations leading to a two-fold increase in activity of the Ca$^{2+}$-activated chloride channels (Ledoux et al., 2005). Enhanced activation of Ca$^{2+}$-activated chloride channels would be expected to produce a greater degree of depolarization resulting in additional recruitment of voltage-sensitive Ca channels, and consequently leading to a greater influx of Ca$^{2+}$ into the vascular myocytes hence augmentation of mechanical force generation.
Role of K channels

Based on electrophysiological studies, multiple forms of K channels have been identified in vascular muscle including, the noninactivating TEA-sensitive, rapidly inactivating 4-AP-sensitive, and slowly inactivating TEA-insensitive channels (Cox, 2005). Inhibition of the K channels would be expected to remove a braking influence on ICW generation. Consistent with this idea TEA significantly increased the amplitude of waves (reflecting a disinhibition of the ramping up phase), whereas 4-AP produced an increase in their frequency by a virtually selective action. In view of the partially overlapping activity profiles of these two K-channel blockers, it would seem likely that Ca\(^{2+}\)-activated K\(^+\) currents (TEA-sensitive) play a predominant role in restraining the ramping-up phase, while the voltage-gated 4-AP-sensitive K\(^+\) conductances contribute to the regulation of the ICW rhythm.

The effects of barium on wave generation are consistent with its known blocking actions on K channels. Its effectiveness in increasing frequency parallels that of 4-AP; however, its ability to diminish rise time to peak, analogous to that of mibefradil and verapamil, would suggest impairment of Ca\(^{2+}\) influx. Likewise its dampening effects on amplitude at higher concentration may reflect interference with the process of excitation-contraction coupling. The inhibitory effect of cesium on wave generation adds further support to the involvement of K conductances yet to be fully characterized. Beside various K channels, this ion is also capable of blocking certain two-pore domain K (TASK1) conductances as previously reported in *Xenopus* oocytes (O’Connell et al., 2005).

Role of SR

Cyclopiazonic acid effectively disrupted generation of ICW, as evidenced by disintegration into complex wavelets, ultimately progressing to complete inhibition of cycling events. Accordingly, a functional SR would appear necessary for generating this pattern. Cycling and release of intracellular Ca\(^{2+}\) are deemed to be necessary for vasomotion (Hyvelin et al., 1998; Haddock et al., 2002). Lee and colleagues (2001) strongly suggest that Ca\(^{2+}\) release from the SR and refilling of the SR Ca\(^{2+}\) stores through the SR Ca-ATPase are required for generation of repetitive Ca\(^{2+}\) waves. However, in rat mesenteric artery a fast rhythmic oscillation in membrane potential and tone can be generated in the presence of cyclopiazonic acid (Rahman et al., 2007), while in rabbit mesenteric artery mechanical oscillations similarly are resistant to blockade by ryanodine and cyclopiazonic acid (Omote et al., 1993; Omote and Mizusawa, 1993). In the present study, some treated preparations did not become quiescent or if so responded to increase in [K\(^+\)]\(_e\) with a resumption of ICW activity of modified configuration.

Other signaling pathways

Rho-dependent kinase seems to play an important role in the process of ICW production. KCl-evoked contraction has been linked to the activation of Rho kinase (Urban et al., 2003). Moreover, Rho kinase has also been implicated in events leading to Ca\(^{2+}\) entry in vascular muscle distinct from voltage- or store-operated channels in rat aorta and mesenteric arteries (Ghisdal et al., 2003). The effect of Rho-kinase inhibition by fasudil was characterized by slowing down of the fast vasomotion (*i.e.*, the ramping-up portion), which appears to account for the resulting changes in
wave shape (decreased $P_T$ and amplitude). The persistence of the wave pattern in this situation was all the more noteworthy. Thus calcium sensitization by Rho-kinase seems to be one of the prerequisites for fast oscillations in the rat pulmonary artery. The inhibitory effects of fasudil may not have been solely due to an attenuation of $Ca^{2+}$ sensitization process but also resulted from perturbation in $Ca^{2+}$ entry into the vascular myocyte leading to a disruption in periodic vasomotion in the pulmonary artery. The lower concentration of fasudil employed in the present study is approximately 2.5 times the IC$_{50}$ against Rho kinase (Tamura et al., 2005). However, at the higher concentration, it is possible that fasudil could inhibit other kinases involved in the process of excitation-contraction coupling (Tamura et al., 2005).

Based on the ineffectiveness of any effect of ceramide and deltamethrin, it would appear that the involvement of diacylglycerol kinase and calcineurin type phosphatases, respectively, were minimal in modulating periodic vasomotion.

Outlook
The methodological conditions of the present work (i.e. use of ring preparation and high [K$^+$]e) are apt to raise questions about the physiological relevance of our data. In preliminary work we have been able to observe, by means of in vitro pressure recordings from a preparation consisting of the trunk and two main pulmonary arteries, the intermittent vasomotor activity pattern described here in ring preparations. As we recently reported this same pattern of vasomotion can be induced by application of agonist (Burke et al., 2010).

In broad terms, the physiological role of vasomotion is not yet well understood and remains to be fully defined. Based on theoretical assumptions and modeling, it has been postulated that in situ vasomotion may participate in oxygen delivery to tissues (Tsai and Intaglietta, 1993). Moreover, this seems to hold true for oscillations that occur at slow frequency and are of high amplitude (Goldman and Popel, 2001). Therefore, it is possible that the slow periodic vasomotion described here for the main pulmonary artery is a subtle means by which blood delivery to the lungs can be modified. In essence, the motion would intermittently complement right ventricular contractions in the delivery of blood to the lungs. Considering that the normal heart rate and fast vasomotor rhythm are orders of magnitude higher than the frequency of periodic vasomotion, it is the summated amplitude of the periodic vasomotion that perhaps matters functionally.

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References
Aalkjær, C. and Nilsson, H. (2005). Vasomotion: cellular background for the oscillator and for the synchronization of smooth muscle cells. Br. J. Pharmacol. 144: 605–616.
Bieger, D., Parai, K., Ford, C.A. and Tabrizchi, R. (2006). $\beta$-Adrenoceptor mediated responses in rat pulmonary artery: putative role of TASK-1 related K channels. Naunyn-Schmiedeberg's Arch.
Boedtkjer, D.M., Matchkov, V.V., Boedtkjer, E., Nilsson, H. and Aalkjær, C. (2008). Vasomotion has chloride-dependency in rat mesenteric small arteries. *Pflügers Arch.* 457: 389–404.

Bollinger, A., Hoffmann, U. and Franzeck, U.K. (1991). Evaluation of flux motion in man by the laser Doppler technique. *Blood Vessels* 28 Suppl 1: 21–26.

Burke, M.M., Bieger, D. and Tabrizchi, R. (2010). Agonist-induced periodic vasomotion in rat isolated pulmonary artery. *Fund. Clin. Pharmacol.* doi: 10.1111/j.1472-8206.2010.00878.x. (Epub ahead of print).

Chipperfield, A.R. and Harper, A.A. (2000). Chloride in smooth muscle. *Prog. Biophys. Mol. Biol.* 74: 175–221.

Colantuoni, A., Bertuglia, S. and Intaglietta, M. (1985). Variations of rhythmic diameter changes at the arterial microvascular bifurcations. *Pflügers Arch.* 403: 289–295.

Cox, R.H. (2005). Molecular determinants of voltage-gated potassium currents in vascular smooth muscle. *Cell Biochem. Biophys.* 42: 167–195.

Criddle, D.N., de Moura, R.S., Greenwood, I.A. and Large, W.A. (1996). Effect of niflumic acid on noradrenaline-induced contractions of the rat aorta. *Br. J. Pharmacol.* 118: 1065–1071.

Criddle, D.N., de Moura, R.S., Greenwood, I.A. and Large, W.A. (1997). Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat. *Br. J. Pharmacol.* 120: 813–818.

Cruickshank, S.F., Baxter, L.M. and Drummond, R.M. (2003). The Cl− channel blocker niflumic acid releases Ca2+ from an intracellular store in rat pulmonary artery smooth muscle cells. *Br. J. Pharmacol.* 140: 1442–1450.

Ghisdal, P., Vandenberg, G. and Morel, N. (2003). Rho-dependent kinase is involved in agonist-activated calcium entry in rat arteries. *J. Physiol. (Lond.)* 551: 855–867.

Gokina, N.I., Bevan, R.D., Walters, C.L. and Bevan, J.A. (1996). Electrical activity underlying rhythmic contraction in human pial arteries. *Circ. Res.* 78: 148–153.

Goldman, D. and Popel, A.S. (2001). A computational study of the effect of vasomotion on oxygen transport from capillary networks. *J. Theor. Biol.* 209: 189–199.

Haddock, R.E., Hirst, G.D. and Hill, C.E. (2002). Voltage independence of vasomotion in isolated irideal arterioles of the rat. *J. Physiol. (Lond.)* 540: 219–229.

Hayashida, N., Okui, K. and Fukuda, Y. (1986). Mechanism of spontaneous rhythmic contraction in isolated rat large artery. *Jpn. J. Physiol.* 36: 783–794.

He, Y. and Tabrizchi, R. (1997). Effects of niflumic acid on alpha1-adrenoceptor-induced vasoconstriction in mesenteric artery in vitro and in vivo in two-kidney one-clip hypertensive rats. *Eur. J. Pharmacol.* 328:191–199.

Hyvelin, J.M., Guibert, C., Martha, R. and Savineau, J.P. (1998). Cellular mechanisms and role of endothelin-1-induced calcium oscillations in pulmonary arterial myocytes. *Am. J. Physiol.* 275: L269–L282.

Kastrup, J., Bülow, J. and Lassen, N.A. (1989). Vasomotion in human skin before and after local heating recorded with laser Doppler flowmetry. A method for induction of vasomotion. *Int. J. Microcirc. Clin. Exp.* 8: 205–215.

Large, W.A. and Wang, Q. (1996). Characteristics and physiological role of the Ca2+- activated Cl− conductance in smooth muscle. *Am. J. Physiol.* 271: C435–C454.

Ledoux, J., Greenwood, I.A. and Leblanc, N. (2005). Dynamics of Ca2+-dependent Cl− channel modulation by niflumic acid in rabbit coronary arterial myocytes. *Mol. Pharmacol.* 67: 163–173.

Lee, C.H., Poburko, D., Sahota, P., Sandhu, J., Ruehlmann, D.O. and van Breemen, C. (2001). The mechanism of phenylephrine-mediated [Ca2+]i oscillations underlying tonic contraction in the rabbit inferior vena cava. *J. Physiol. (Lond.)* 534: 641–650.

Meyer, J.-U., Lindbom, L. and Intaglietta, M. (1987). Coordinated diameter oscillations at arteriolar
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bifurcations in skeletal muscle. *Am. J. Physiol.* **253**: H568–H573.

Nilsson, H. and Aalkjær, C. (2003). Vasomotion: mechanisms and physiological importance. *Mol. Interv.* **3**: 79–89.

O’Connell, A.D., Morton, M.J., Sivaprasadarao, A. and Hunter, M. (2005). Selectivity and interactions of Ba\(^{2+}\) and Cs\(^+\) with wild-type and mutant TASK1 K\(^+\) channels expressed in *Xenopus* oocytes. *J. Physiol. (Lond.)* **562**: 687–696.

Oishi, H., Schuster, A., Lamboley, M., Stergiopulos, N., Meister, J.J. and Bény, J.L. (2002). Role of membrane potential in vasomotion of isolated pressurized rat arteries. *Life Sci.* **71**: 2239–2248.

Omote, M., Kajimoto, N. and Mizusawa, H. (1993). The ionic mechanism of phenylephrine-induced rhythmic contractions in rabbit mesenteric arteries treated with ryanodine. *Acta Physiol. Scand.* **147**: 9–13.

Omote, M. and Mizusawa, H. (1993). The role of sarcoplasmic reticulum in endothelium-dependent and endothelium-independent rhythmic contractions in the rabbit mesenteric artery. *Acta Physiol. Scand.* **149**: 15–21.

Parai, K. and Tabrizchi, R. (2005). Effects of chloride substitution in isolated mesenteric blood vessels from Dahl normotensive and hypertensive rats. *J. Cardiovasc. Pharmacol.* **46**: 105–114.

Peng, H., Matchkov, V., Ivarsen, A., Aalkjær, C. and Nilsson, H. (2001). Hypothesis for the initiation of vasomotion. *Circ. Res.* **88**: 810–815.

Rahman, A., Hughes, A., Matchkov, V., Nilsson, H. and Aalkjær, C. (2007). Antiphase oscillations of endothelium and smooth muscle [Ca\(^{2+}\)]\(_i\) in vasomotion of rat mesenteric small arteries. *Cell Calcium* **42**: 536–547.

Seifert, H., Jäger, K. and Bollinger, A. (1988). Analysis of flow motion by the laser Doppler technique in patients with peripheral arterial occlusive disease. *Int. J. Microcirc. Clin. Exp.* **7**: 223–236.

Slaaf, D.W., Tangelder, G.J., Teirlinck, H.C. and Reneman, R.S. (1987). Arteriolar vasomotion and arterial pressure reduction in rabbit tenuissimus muscle. *Microvasc. Res.* **33**: 71–80.

Tabrizchi, R. and Duggan, J.A. (2000). The interrelationship between chloride ions and endothelium on alpha-adrenoceptor-mediated contractions in aortic rings from Dahl normotensive and hypertensive rats. *Cardiovasc. Res.* **48**: 393–401.

Tamura, M., Nakao, H., Yoshizaki, H., Shiratsuchi, M., Shigyo, H., Yamada, H., Ozawa, T., Totsuka, J. and Hidaka, H. (2005). Development of specific Rho-kinase inhibitors and their clinical application. *Biochim. Biophys. Acta* **1754**: 245–252.

Triggle, D.J. (1992). Biochemical and pharmacological differences among calcium antagonists: Clinical implications. In: Calcium Antagonists in Clinical Medicine, ed. by M. Epstein, Hanley & Belfus Inc., Philadelphia, pp. 1–27.

Tsai, A.G. and Intaglietta, M. (1993). Evidence of flowmotion induced changes in local tissue oxygenation. *Int. J. Microcirc. Clin. Exp.* **12**: 75–88.

Urban, N.H., Berg, K.M. and Ratz, P.H. (2003). K depolarization induces RhoA kinase translocation to caveolae and Ca\(^{2+}\) sensitization of arterial muscle. *Am. J. Physiol.* **285**: C1377–C1385.