Conducted vasoconstriction in rat mesenteric arterioles: role for dihydropyridine-insensitive Ca\(^{2+}\) channels

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The aim of this study was to evaluate the role of voltage-operated Ca\(^{2+}\) channels in the initiation and conduction of vasoconstrictor responses to local micropipette electrical stimulation of rat mesenteric arterioles (28 ± 1 \(\mu\)m, \(n = 79\)) in vivo. Local and conducted (600 \(\mu\)m upstream from the pipette) vasoconstriction was not blocked by TTX (1 \(\mu\)mol/l, \(n = 5\)), nifedipine, or nimodipine (10 \(\mu\)mol/l, \(n = 9\)). Increasing the \(K^+\) concentration of the superfusate to 75 mmol/l did not evoke vasoconstriction, but this depolarizing stimulus reversibly abolished vasoconstrictor responses to current stimulation (\(n = 7\)). Addition of the T-type Ca\(^{2+}\) antagonist mibefradil (10 \(\mu\)mol/l, \(n = 6\)) to the superfusate reversibly blocked local and conducted vasoconstriction to current stimulation. With the use of RT-PCR techniques, it was demonstrated that rat mesenteric arterioles <40 \(\mu\)m do not express mRNA for L-type Ca\(^{2+}\) channels (\(\alpha_{1C}\)-subunit), whereas mRNA coding for T-type subunits was found (\(\alpha_{1G}\) and \(\alpha_{1H}\)-subunits). The data indicate that L-type Ca\(^{2+}\) channels are absent from rat mesenteric arterioles <40 \(\mu\)m. Rather, the vasoconstrictor responses appear to rely on other types of voltage-gated, dihydropyridine-insensitive Ca\(^{2+}\) channels, possibly of the T-type.

L-type Ca\(^{2+}\) channels; T-type Ca\(^{2+}\) channels; mibefradil; Ca\(^{2+}\) channel antagonists; RT-PCR

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channels (2). Furthermore, the expression of mRNA coding for pore-forming subunits of these channels was evaluated in mesenteric arterioles and small arteries by using RT-PCR analysis.

METHODS

Animal Preparation and Intravital Videomicroscopy

The experimental setup, which was approved by the National Research Animal Committee, has been described previously (10). In 55 male Sprague-Dawley rats (344–397 g), halothane anesthesia was induced in a chamber containing 5% halothane in a 35% O2-65% N2 mixture followed by administration of 2% halothane in a 35% O2-65% N2 mixture with a mask. Catheters were inserted in the left jugular vein for infusion, and a catheter was inserted into the right carotid artery for continuous blood pressure measurement. After the initial surgery, halothane anesthesia was replaced by an intravenous infusion of pentobarbital sodium (120–150 μg/min). A median laparotomy was performed, and a loop of the small intestine with mesentery was exteriorized for vital microscopy. The mesentery was superfused with a 37°C physiological saline solution (PSS) at a rate of 3 ml/min [PSS contained (in mmol/l) 140 NaCl, 5.4 KCl, 1.0 MgCl2, 1.8 CaCl2, and 5.0 HEPES; pH 7.4]. The preparation holds ~3 ml of PSS. Arterioles located in the transparent part of the mesentery were observed by using a ×20 water-immersion objective on an upright microscope (BX50WI, Olympus) mounted on a motorized moveable stage. These arterioles stem from first- or second-order branches of the superior mesenteric artery, and they give rise to small capillary networks that supply the mesentery. The field was viewed on a monitor and recorded on videotape. The final magnification of the image on the monitor was ×700, which corresponded to a spatial resolution of ~0.5 μm.

Local Electrical Stimulation and NE Application

Arteriolar vasoconstriction was induced by local current stimulation as described by Steinhausen et al. (28). Glass pipettes filled with 2 mol/l NaCl (outer tip diameter 6 μm, resistance 1 MΩ) were placed in an electrode holder, mounted on a micromanipulator, and connected to the negative pole of an isolation unit controlled by a Grass stimulator. A platinum wire placed in the tissue served as a reference electrode. The pipette tip was placed 1–2 μm above the selected arteriole, and vasoconstriction was induced by a continuous train of unipolar pulses (frequency 10 Hz, pulse duration 2 ms, amplitude –70 V unless otherwise stated; see Fig. 1). The local response was defined as the stable maximal response for a particular stimulation. This stable local response was apparent after ~15 s. During ongoing stimulation, the microscope was then moved to the upstream position, and the conducted response was recorded. Subsequently, the microscope was returned to the local site to ensure that the local response was unchanged. Therefore, the length of the stimulus train varied slightly among experiments, but the entire procedure was completed within 40–60 s. In experiments where only local responses were measured, a fixed stimulus time of 30 s was used. A stable local response was always induced within this time period. Retracting the pipette 5–10 μm from the vessel wall abolished the vasoconstrictor response.

In another series of experiments, local and conducted vasoconstriction were induced by application of 0.1 mmol/l NE (10 nl/min) using micropipettes connected to a microperfusion pump.

Experimental Protocols

Current stimulation. The effect of adding the following compounds to the superfusing solution on baseline arteriolar diameters and on local and conducted vasoconstriction to current stimulation was tested: TTX (1 μmol/l, n = 5), phenolamine (10 μmol/l, n = 7), Nω-nitro-L-arginine-methyl ester (L-NAME; 10–50 μmol/l, n = 6), nifedipine or nimodipine (10 μmol/l, n = 9), high-K+ PSS (75 mmol/l K+ made by equimolar substitution of Na+ by K+ in the PSS solution, n = 7), NiCl2 (1 mmol/l, n = 5), and mibebradil (10 μmol/l, n = 6). Local and conducted vasoconstrictor responses were measured at baseline and again 15 min after changing the superfusing solution. The effects of high-K+ PSS were tested after 2–5 min.

In a separate series of experiments, the effect of micropipette application of mibebradil (10 μmol/l; 20 nl/min, n = 3) either at the site of current stimulation or 400 μm upstream from the stimulating pipette was tested.

Norepinephrine. In the experiments where vasoconstriction was induced by NE, the effect of the following compounds in the superfusate was tested: nimodipine (n = 2), mibebradil (n = 8), and high-K+ PSS (n = 5).

Data Acquisition and Analysis

Off-line analysis of the recorded experiments was performed using computer-assisted tracking of the endothelial edges. The responses at the local site and 600 μm upstream from the pipette were calculated as the relative change in internal diameter of the arteriole: (dpre – dpost)/dpre × 100%, where dpre and dpost are the diameters before and after treatment, respectively.

RT-PCR Analysis of Ca2+ Channel Subunit Expression in Mesenteric Arterioles and Arteries

RT-PCR. In these experiments, mRNA expression of three types of subunits from voltage-gated Ca2+ channels was tested: α1C (pore-forming subunit from L-type channels) and α1D and α1H (pore-forming subunits from T-type channels). Male Sprague-Dawley rats weighing 280–350 g (n = 5) were anesthetized by an intraperitoneal injection of pentobarbital sodium (350 μl; 50 mg/ml). A loop of the small intestine with its mesentery was exteriorized, and the arterioles (<40 μm) were removed and placed in ice-cold PSS. Furthermore, mesenteric resistance arteries (second-order branches of the superior mesenteric artery, internal diameter 200–300 μm) were excised. The vessels were gently dissected free of fat and connective tissue under a stereomicroscope and transferred to an Eppendorf tube containing RLT buffer (Qiagen, Germany) to which β-mercaptoethanol (1%) had been added. Samples were frozen at ~80°C until the time of RNA extraction.

RNA was extracted using the protocol described by Chomczynski and Sacchi (7). RT-PCR was performed as described previously (14). PCR primers were the following (DNA Technology, Aarhus, Denmark) for α1C: forward 5′-GACGTTGAG-GCCAAAGGT 3′, reverse 5′-GCTTGTATGCGTTCCTCC 3′, covering bases 3,910–4,130, 221 bp (GenBank accession no. AF027984); for α1D: forward 5′-TGAAGACAAGACGGTCTC 3′, reverse 5′-GGACGATCTCGGGAC 3′, covering bases 37–334, 297 bp of mouse partial sequence (Genbank accession no. M59786). Linker sequences were added to introduce EcoR1 and BamH1 restriction sites for cloning. β-Actin primers

CONDUCTED VASOCONSTRICTION AND Ca2+ CHANNELS

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were copied from Yu et al. (34). cDNA equivalents to a vessel length of 1 mm (\(\alpha_1\)-subunits) or 0.2 mm (actin) were used. Negative controls were \(H_2O\) instead of cDNA. The rat \(\alpha_1\)-subunit amplification products were cloned in vector pSP73 (Promega) by standard methods (22). The inserts were sequenced using SP6- and T7-specific primers.

**Southern blotting.** DNA probes were synthesized with 1 \(\mu\)Ci/\(\mu\)l \([\alpha-\text{32P}]dCTP\) (Amersham Pharmacia Biotech) and 0.05 \(U/\mu\)l Klenow enzyme by standard methods (22). DNA was transferred by capillary blotting (22) to a Zeta Probe GT membrane (Bio-Rad, Copenhagen, Denmark), and hybridization of the radioactive probe was allowed overnight at 42°C. The membrane was washed, and autoradiography was performed for 2–4 h.

**Statistics**

Values are means \(\pm\) SE. Vasoconstrictor responses were compared by Student’s \(t\)-test or ANOVA. A \(P\) value \(<0.05\) was considered significant.

**RESULTS**

Mean arterial blood pressure was 109 \(\pm\) 1 mmHg. Superfusion of the mesentery with high-\(K^+\) PSS resulted in an immediate drop in blood pressure of 5–10 mmHg, but blood pressure returned to baseline within 1–2 min (i.e., before new vasomotor responses were measured) in all animals. The remaining compounds when added to the superfusion solution did not affect mean arterial blood pressure. Resting diameter of the arterioles studied ranged from 15.7 to 44.9 \(\mu\)m with a mean value of 28.4 \(\pm\) 0.9 \(\mu\)m (\(n\) = 79).

**Responses to Current Stimulation**

Local electrical stimulation consistently induced a local vasoconstrictor response, which propagated upstream and downstream along the vessel. The time course for development of the propagated vasoconstrictor response to current stimulation is shown in Fig. 1. In this experiment, where the two observation points were interspaced by 800 \(\mu\)m, it was not possible to detect a delay between the vasoconstrictor responses at the two observation points. We reasoned that a delay >1 s would have been detectable and, therefore, the propagation velocity must have exceeded 800 \(\mu\)m/s.

A stimulus-response curve displaying the local contraction induced by 30 s of electrical stimulation (pulse duration 2 ms, frequency 10 Hz) is presented in Fig. 2. From this curve, it is evident that the stimulus protocol used in the present experiments (amplitude \(-70\) V) induces an approximately half-maximal vasoconstriction in these vessels. The effect of varying the pulse duration from 2 ms to direct current (continuous stimulation) with a fixed amplitude of \(-70\) V is shown in Fig. 3. Vasoconstrictor responses decreased with increasing pulse duration (\(P < 0.01\), ANOVA), and compared with the experiments using 2 ms of pulse duration, responses were significantly decreased when the pulse duration exceeded 100 ms. Vasoconstriction could not be induced by continuous stimulation (direct current).

To evaluate the role of perivascular nerve activation during micropipette electrical stimulation, local and conducted vasoconstriction was induced before and after addition of TTX (1 \(\mu\)mol/l) or phentolamine (10 \(\mu\)mol/l) to the superfusing solution for 15 min. Neither TTX nor phentolamine altered arteriolar baseline diameters (27.1 vs. 28.4 \(\mu\)m and 26.9 vs. 28.8 \(\mu\)m, respectively).

![Fig. 1. Time course for development of vasoconstrictor responses to current stimulation in 1 arteriole (pulse duration 2 ms, frequency 10 Hz, amplitude \(-70\) V, duration of stimulus train 20 s). Initially, the constrictor response 200 \(\mu\)m upstream from the pipette was recorded. After recovery of the vessel, vasoconstriction was induced again, and the response 1,000 \(\mu\)m upstream from the pipette was recorded. Two different stimulations were necessary as 800 \(\mu\)m exceeds the visual field on the monitor. Arrow indicates onset of current application.](http://ajpheart.physiology.org/)

![Fig. 2. Stimulus-response curve for local vasoconstriction induced by micropipette electrical stimulation with various voltage amplitudes (\(n = 5\) vessels). Pulse duration was 2 ms, and stimulus frequency was 10 Hz for all experiments.](http://ajpheart.physiology.org/)
Neither occlusion of the arteriole (data not shown) nor L-NAME (10–50 μmol/l) had any effect on the local and conducted vasoconstriction after electrical stimulation. L-NAME (Table 1) had no significant effect on the local and conducted responses to current stimulation (n = 6; Table 1). To establish whether other types of Ca2+ channels were involved in the vasoconstrictor responses, we tested the effect of NiCl2 (1 mmol/l) and mibebradil (10 μmol/l), both of which are known to block T-type voltage-activated Ca2+ channels. Superfusion with either NiCl2 or mibebradil did not change baseline arteriolar diameter. Superfusion with NiCl2 abolished, within minutes, both local and conducted vasoconstrictor responses to current stimulation (n = 5; Table 1), an effect that was reversed after 20 min of PSS superfusion (data not shown). Similarly, the addition of mibebradil to the superfusate almost completely eliminated all responses to current stimulation in a reversible fashion (Table 1 and Fig. 5). The maximal effect of mibebradil was apparent within 1–2 min after the superfusion was started. When mibebradil was applied by a micropipette directly on the site of current stimulation and 200 ms, frequencies of 5 and 3.5 Hz were used, respectively. Likewise, local and conducted vasoconstrictor responses to micropipette electrical stimulation were readily inducible during superfusion with either TTX and phentolamine used in the present series completely blocked the local vasoconstriction induced by current stimulation and pulse duration in 7 arterioles. Voltage amplitude was −70 V in all experiments. Stimulus frequency was 10 Hz for duration <100 ms. For experiments using a pulse duration of 100 and 200 ms, frequencies of 5 and 3.5 Hz were used, respectively. DC, direct current (continuous stimulation). *P < 0.05 compared with control Vasoconstriction, μm

<table>
<thead>
<tr>
<th>Control Vasoconstriction, μm</th>
<th>During Drug Superfusion Vasoconstriction, μm</th>
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<tr>
<td>Local 600 μm</td>
<td>Local 600 μm</td>
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<tr>
<td>TTX (1 μmol/l, n = 5)</td>
<td>10.9 ± 0.8 1.1 11.6 ± 1.0 41 ± 5% 11.7 ± 0.9 41 ± 5% 12.2 ± 1.1 41 ± 6%</td>
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<tr>
<td>Phentolamine (10 μmol/l, n = 7)</td>
<td>9.6 ± 1.0 37 ± 5% 10.0 ± 1.2 34 ± 4% 9.3 ± 0.6 37 ± 5% 10.4 ± 1.1 38 ± 4%</td>
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<tr>
<td>L-NAME (10–50 μmol/l, n = 6)</td>
<td>11.1 ± 1.0 37 ± 4% 8.5 ± 1.1 27 ± 5% 10.8 ± 1.7 36 ± 5% 8.2 ± 1.2 27 ± 5%</td>
</tr>
<tr>
<td>DHPs (10 μmol/l, n = 6)</td>
<td>10.0 ± 1.2 43 ± 5% 8.5 ± 0.6 36 ± 4% 9.7 ± 1.1 41 ± 5% 9.7 ± 0.7 39 ± 5%</td>
</tr>
<tr>
<td>NiCl2 (1 mmol/l, n = 5)</td>
<td>12.7 ± 1.8 45 ± 7% 11.8 ± 1.3 40.0 ± 4% 0.1 ± 0.3* 0.2 ± 1% −0.3 ± 0.2* −1 ± 1%</td>
</tr>
<tr>
<td>Mibebradil (10 μmol/l, n = 6)</td>
<td>14.9 ± 1.9 48 ± 6% 15.1 ± 2.6 46 ± 7% 0.7 ± 0.4* 2 ± 1% 0.5 ± 0.4* 1 ± 1%</td>
</tr>
<tr>
<td>High-K' PSS (75 mmol/l, n = 7)</td>
<td>13.7 ± 1.4 49 ± 6% 12.3 ± 1.2 44 ± 5% 0.2 ± 0.1* 1 ± 0% 0.0 ± 0.2* 0 ± 1%</td>
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Data are absolute and relative reductions in internal diameter are presented as means ± SE; n is the number of arterioles studied. Control experiments were conducted during superfusion with physiological saline solution (PSS). Stimulus protocol was the following: amplitude −70 V, pulse duration 2 ms, and frequency 10 Hz. TTX, tetrodotoxin; DHPs, dihydropyridines. *P < 0.01 when compared with the corresponding vasoconstriction under control conditions.

![Fig. 3. Relationship between vasoconstrictor response to electrical stimulation and pulse duration in 7 arterioles. Voltage amplitude was −70 V in all experiments. Stimulus frequency was 10 Hz for duration <100 ms. For experiments using a pulse duration of 100 and 200 ms, frequencies of 5 and 3.5 Hz were used, respectively. DC, direct current (continuous stimulation). *P < 0.05 compared with 2 ms.](http://ajpheart.physiology.org/DownloadedFrom)
ulation, it likewise completely blocked both local and conducted vasoconstriction (data not shown; $n = 5$).

However, when mibefradil was applied to a site 400 μm upstream from the stimulation site, the only effect was to block the conducted vasoconstriction at the application site (Table 2). Thus vasoconstriction was unchanged both proximal and distal to the site where mibefradil was applied (Table 2).

Unexpectedly, increasing the $K^+$ concentration of the superfusing solution to 75 mmol/l did not change arteriolar diameter in any of the 13 vessels studied (31.1 ± 2.2 vs. 31.1 ± 2.1 μm). These arterioles all contracted vigorously to topical application of 0.1 mmol/l NE during superfusion with high-$K^+$ PSS, indicating that there was no unspecific effect on the vascular contractility of high-$K^+$ PSS. In some vessels, high-$K^+$ PSS superfusion induced a reduction in intravascular flow velocity in the arteriole under study. Presumably, this effect was due to constriction in parent vessels. These vessels cannot be visualized in this preparation because they are covered by mesenteric fat cells. Similarly, the arterioles studied were unaffected by micropipette delivery of a solution containing 150–500 mmol/l KCl ($n = 4$; data not shown). Local and conducted vasoconstriction to current stimulation during treatment with high-$K^+$ PSS was tested in seven arterioles. In all vessels, both local and conducted responses were abolished a few seconds after replacing normal PSS with high-$K^+$ PSS (Table 1). This effect was fully reversible after 3–4 min of superfusion with normal PSS.

**Responses to NE Application**

Local vasoconstrictor responses to NE were larger than those induced by current stimulation (51 ± 3 vs. 42 ± 2%, $P = 0.03$). In contrast, local NE application induced a smaller conducted vasoconstriction 600 μm upstream than current stimulation (22 ± 2 vs. 38 ± 2%, $P < 0.001$). Vasoconstriction to topical application of 0.1 mmol NE was readily induced during superfusion with nimodipine (data not shown; $n = 3$). Local and conducted vasoconstrictor responses to micropipette application of NE (0.1 mM) during nimodipine superfusion amounted to 48 ± 4 and 16 ± 1%, respectively ($n = 2$). Local vasoconstriction to micropipette

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**Fig. 4.** Local and conducted vasoconstriction to local current stimulation (pulse duration 2 ms, frequency 10 Hz, voltage amplitude ~70 V) during superfusion with either 10 μmol/l nifedipine ($n = 5$) or 10 μmol/l nimodipine ($n = 4$).

**Fig. 5.** Influence of mibefradil (10 μmol/l) in the superfusing solution on of local (A) and conducted (B) vasoconstriction to local electrical stimulation in 6 arterioles. PSS, physiological saline solution.
The application of NE was not significantly altered by mibefradil (52 ± 6 vs. 45 ± 6%, n = 8; Fig. 6). Mibefradil did, however, clearly attenuate vasoconstrictor responses 600 μm upstream from the NE pipette (26 ± 7 vs. 10 ± 2%, P < 0.01, n = 8). Local and conducted vasoconstrictor responses to NE were not significantly altered by substituting the normal superfusing solution with high-K⁺ PSS (52 ± 4 vs. 48 ± 5% and 17 ± 3 vs. 23 ± 5%, respectively, n = 5).

RT-PCR Analyses

RT-PCR analysis of isolated mesenteric arterioles <40 μm (n = 13 vessels from 5 rats) showed mRNA expression for the T-type channel α₁G-subunit, whereas expression of mRNA for the L-type channel α₁C-subunit was not detected (Fig. 7). In mesenteric arteries, mRNA for both α₁C- and α₁G-subunits was found (n = 8 vessels from 5 rats; Fig. 7). Also, mRNA coding for the α₁H-subunit (T-type channels) was found both in mesenteric arterioles and arteries (data not shown). Sequencing of the cloned α₁C-, α₁G-, and α₁H-subunit amplification products revealed 100% homology with the respective sequences obtained from GenBank.

DISCUSSION

The most important new finding of the present study is that voltage-operated L-type Ca²⁺ channels appear not to be expressed in mesenteric arterioles, 40 μm. In accordance with this finding, the functional data show that L-type channels do not play a role in the local and conducted vascular responses after local stimulation of mesenteric arterioles with either current or NE. Furthermore, the results suggest that another voltage-operated, dihydropyridine-insensitive Ca²⁺ channel is necessary for induction of vasoconstriction in mesenteric arterioles after local electrical stimulation. On the basis of results obtained using the T-type Ca²⁺ channel blockers mibefradil and Ni²⁺, it is suggested that the channel involved may be a T-type channel.

Role of Perivascular Nerves

The present study shows that local application of a train of electrical impulses via a micropipette in rat mesenteric arterioles reproducibly, and in a dose-dependent manner, induces local and propagated vasoconstriction. Unlike results previously reported for arterioles from hamster skeletal muscle (27), the responses in the rat mesentery did not rely on activation of perivascular nerve fibers, because vasoconstriction was readily induced during superfusion with TTX. The dose of TTX used in our experiments has previously been shown to block vasoconstrictor responses to conventional “macroscopic” perivascular field stimulation in other vascular preparations (15). Further evidence for the absence of a role for perivascular nerves was offered by the finding that vasoconstriction to current stimulation was unaffected by superfusion with phentolamine. This treatment abolishes vasoconstrictor responses to nerve stimulation by conventional field
stimulation in the rat mesentery (9), consistent with the finding that almost all nerve fibers in the rat mesentery are noradrenergic (3).

**Role of Voltage-Operated Ca\(^{2+}\) Channels in Local Responses**

It is well documented that L-type Ca\(^{2+}\) channels are present and of major importance in the regulation of vasomotor tone in several vascular beds, for instance, in the larger resistance arteries upstream from the arterioles investigated in the present study (20, 30). In accordance with these reports, we found mRNA expression for the pore-forming subunit of L-type channels in mesenteric resistance arteries (diameter 200–300 μm). We therefore considered the possibility that the local constrictor response in arterioles to electrical stimulation was due to an effect on the VSMC membrane potential (depolarization) induced by the electrical field at the pipette tip and subsequent opening of L-type Ca\(^{2+}\) channels. However, the results of the RT-PCR analyses revealed that mRNA for the pore-forming subunit of VSMC L-type Ca\(^{2+}\) channels (α1C) is not expressed in small mesenteric arterioles. In accordance with this finding, the local response to current or NE was not affected by the addition of nifedipine or nimodipine to the superfusing solution. Furthermore, the L-type Ca\(^{2+}\) antagonists did not have any effect on baseline arteriolar diameter, which is consistent with the results of a previous study (1) in the rat mesentery using verapamil. In this context, it should be noted that the tone in small rat mesenteric arterioles during anesthesia is only ~10% (17) and, therefore, only minor effects of any vasodilator could be expected. Vasconstriction induced by depolarization with high-K\(^+\) solutions is believed to be exclusively due to activation of potential-dependent Ca\(^{2+}\) channels (5). Thus the finding that high-K\(^+\) PSS and microapplication of KCl consistently failed to induce vasoconstriction, together with the results mentioned above, strongly support that L-type Ca\(^{2+}\) channels do not play a role in regulating arteriolar tone in this microvascular bed. In line with the results obtained with KCl, it was shown that phasic depolarization using electrical stimulation (particularly with a pulse duration <100 ms; Fig. 3) was effective in inducing vasoconstriction, whereas tonic depolarization (direct current; Fig. 3) was not.

A recently published patch-clamp study of isolated VSMC from differently sized arterioles and arteries from the hamster mesenteric circulation has demonstrated an inverse relationship between the VSMC L-type Ca\(^{2+}\) channel current and vessel diameter. Hence, Morita et al. (19) reported that the voltage-sensitive Ca\(^{2+}\) membrane current in VSMC from hamster intestinal submucosal arterioles (<40–100 μm) was almost 100% nifedipine insensitive. The absence of functional L-type Ca\(^{2+}\) channels (and a poor vasoconstrictor response to depolarization with high-KCl solutions) has been reported also for the renal efferent arteriole (4, 16), whereas L-type channels play a major role in the regulation of the tone of the afferent arteriole. To our knowledge, the present study is the first to demonstrate that L-type Ca\(^{2+}\) channels are absent from the terminal arterioles of the rat mesentery.

In addition to dihydropyridine-sensitive L-type channels, some VSMC express at least one other voltage-operated Ca\(^{2+}\) channel: the transient (or T-type) channel, the physiological role of which remains uncertain (2, 13). This channel is characterized by a low voltage activation threshold and rapid inactivation during depolarization. In the present study, mRNA for pore-forming subunits of two types of T-type channels was shown to be expressed in small mesenteric arterioles. While no entirely selective blocker is currently available, relative selective blockade can be obtained by both inorganic compounds (e.g., NiCl\(_2\)) and organic drugs (e.g., mibefradil). Mibefradil in the concentration used in this study has been reported to block the T-type current completely while only inhibiting the L-type current by ~65% in VSMC (18). However, L-type blocking effects do not appear to be a concern in the present study, because this channel type was not found to be expressed in the mesenteric arterioles, as discussed above.

In the present study, NiCl\(_2\) and mibefradil reversibly blocked vasoconstrictor responses to electrical stimulation. The effect of mibefradil and NiCl\(_2\) on vascular contractility was not unsplicable, because these compounds did not reduce vasoconstrictor responses to topical or local NE stimulation. Furthermore, when the mesentery was superfused with high-K\(^+\) PSS, a condition that will cause persistent membrane depolarization, all responses to electrical stimulation were abolished, whereas the responses to NE were unaffected. This suggests that the putative channel involved inactivates during prolonged membrane depolarization. This possibility is further supported by the finding that electrical pulses of longer duration (>50 ms; Fig. 3) gave rise to only minor vasoconstrictor responses. On the basis of these findings, we suggest that local vasoconstriction to current stimulation, but not to NE stimulation, in rat mesenteric arterioles involves activation of a voltage-operated, fast-inactivating Ca\(^{2+}\) channel that is sensitive to mibefradil and NiCl\(_2\). The data are compatible with the hypothesis that this channel could be a T-type channel or another (yet unresolved) voltage-operated, fast-inactivating Ca\(^{2+}\) channel, as recently proposed for the hamster intestinal circulation (19).

**Electrotonic Propagation, Ca\(^{2+}\) Channels, and Conducted Vasoconstrictor Response**

The mechanisms underlying conducted vascular responses remain elusive. We (10) have previously provided evidence against significant upstream diffusion or venous convection of NE as an explanation for conducted responses in the mesenteric circulation. The present study extends this by demonstrating that propagation is not due to the remote effects of a change in blood flow caused by the local constriction. Thus con-
ducted responses could be observed in arterioles where blood flow had been interrupted by occlusion of an up- or downstream segment. Similar observations have been reported in hamster cheek pouch arterioles (21, 25).

Although a study (27) has suggested that perivascular nerves could be of importance in mediating remote vasoconstrictor responses to local electrical stimulation, it seems unlikely that nerves play any major role in the mesenteric circulation because neither TTX nor phentolamine had any effect on the remote vasoconstriction. Taken together, these observations strongly suggest that the propagation must depend on the spread of one or more signals between the cells of the vascular wall, i.e., the VSMC and/or the endothelial cells.

The majority of the studies on the cellular mechanism of vascular conducted responses have utilized the hamster cheek pouch preparation. In this microvascular bed, there is strong evidence to support that electrotonic spread of a localized change in the membrane potential through endothelial or smooth muscle gap junctions play a pivotal role in mediating conducted vascular responses (31, 33). L-type voltage-operated Ca\(^{2+}\) channels then establish the coupling between the change in membrane potential and the change in the tone of the vascular smooth muscle cells through changes in Ca\(^{2+}\) inflow (26, 32). However, it seems difficult to reconcile the present observations with such a mechanism. With the use of RT-PCR techniques, we were not able to detect mRNA for the \(\alpha_{\text{c}}\)-subunit of L-type Ca\(^{2+}\) channels. Superfusion with either nifedipine or nimodipine was without effect on the propagated responses to both local electrical stimulation and NE. On the basis of these findings, it seems reasonable to conclude that L-type Ca\(^{2+}\) channels do not play a role in mediating the propagated responses.

Superfusing the preparation with high-K\(^{+}\) PSS had no effect on the vascular diameters, showing that in these small mesenteric arterioles, prolonged changes in the membrane potential are unable to produce changes in the vascular tone, a prerequisite for the electrotonic theory. Furthermore, high-K\(^{+}\) PSS was without effect on conducted vasoconstriction after local NE stimulation. Because the high levels of extracellular K\(^{+}\) will depolarize both the smooth muscle cells and the endothelial cells, this strongly argues against a role for an electronic spread of a local depolarization as an explanation for the propagated response. One would anticipate that any voltage-sensitive step would already be fully activated under these circumstances. Recently published data on conducted vasodilation to micropipette application of acetylcholine in feed arteries in hamster skeletal muscle also conflict with the electrotonic theory (27). In these experiments, vasodilation propagated in principle infinitely, which is compatible with some form of regenerative spread of impulses but not with electronic spread of the local hyperpolarization induced in the endothelium and/or VSMC. Hence, also in this vasculature, mechanisms other than electrotonic propagation must be operating.

It should be emphasized that these results do not suggest that electrotonic spread of local perturbations in the membrane potential are without significance for vascular propagated responses. Rather, the results suggest that there could be several mechanisms underlying vascular propagated responses and that their significance may vary between different vascular beds. Although the present data do not support a role for L-type Ca\(^{2+}\) channels in propagated vasoconstriction in mesenteric arterioles, it cannot be excluded that other voltage-activated Ca\(^{2+}\) channels could play a role. Mibefradil induced an incomplete, but significant, blockade of the remote vasoconstriction to NE, suggesting that T-type Ca\(^{2+}\) channels could play a role in either the propagation process or in the upstream excitation-contraction coupling. However, these possibilities are, to some extent, contradicted by the finding that conducted vasoconstrictor responses to NE were unaffected by depolarization with high-K\(^{+}\) PSS, a condition that would have led to inactivation of any T-type Ca\(^{2+}\) channel. During stimulation with current, local application of mibebradil to a distant site abolished the propagated vasoconstriction in this specific part of the vessel. However, local mibebradil application was unable to block the propagation itself, because vasoconstriction was evident upstream from the site of mibebradil application. This would suggest that mibebradil acts to block at least a part of the excitation-contraction coupling in the propagated response without directly interfering with the propagation process itself. However, a word of caution is needed. Mibefradil, although believed to be a relatively specific blocker of voltage-activated Ca\(^{2+}\) channels, has been reported to have other actions, including inhibition of receptor-operated Ca\(^{2+}\) channels in VSMC (6, 12). Thus it is possible that the effects on propagated vasoconstriction could be due to actions other than inhibition of voltage-activated Ca\(^{2+}\) channels. This appears to be an interesting and important area for future studies.

In conclusion, we have demonstrated that dihydropyridine-insensitive but mibebradil-sensitive Ca\(^{2+}\) channels, possibly of the T-type, play a major role in the initiation of local and propagated vasoconstrictor responses to local current stimulation in the terminal microcirculation of the rat mesentery. Furthermore, the molecular and functional data indicate that functional L-type Ca\(^{2+}\) channels are not present in this microvascular bed at all. Finally, we have provided evidence that, at least in this vasculature, mechanisms other than electrotonic propagation of localized depolarization can be responsible for the conducted vasoconstrictor response.

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