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Local electric stimulation causes conducted calcium response in rat interlobular arteries

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Salomonsson, Max, Finn Gustafsson, Ditte Andreasen, Boye L. Jensen, and Niels-Henrik Holstein-Rathlou. Local electric stimulation causes conducted calcium response in rat interlobular arteries. *Am J Physiol Renal Physiol* 283: F473–F480, 2002. First published April 10, 2002; 10.1152/ajprenal.00247.2001.—The purpose of the present study was to investigate the conducted Ca^{2+} response to local electrical stimulation in isolated rat interlobular arteries. Interlobular arteries were isolated from young Sprague-Dawley rats, loaded with fura 2, and attached to pipettes in a chamber on an inverted microscope. Local electrical pulse stimulation (200 ms, 100 V) was administered by means of an NaCl-filled microelectrode (0.7–1 MΩ) juxtaposed to one end of the vessel. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with an image system at a site ~500 μm from the location of the electrode. The expression of mRNA for pore-forming units $Ca_V3.1$ and $Ca_V3.2$ of voltage-sensitive T-type channels was investigated by using RT-PCR. Current stimulation elicited a conducted $[Ca^{2+}]_i$ response. A positive electrode (relative to ground) increased $[Ca^{2+}]_i$ to 145 ± 7% of baseline, whereas the response was absent when the electrode was negative. This response was not dependent on perivascular nerves, because the conducted response was unaffected by TTX (1 μM). The conducted $[Ca^{2+}]_i$ response was abolished by an ambient Ca^{2+} free solution and blunted by nifedipine (1 μM). Rat interlobular arteries exhibited conducted $[Ca^{2+}]_i$ response to current stimulation. This response was dependent on Ca^{2+} entry. L-type Ca^{2+} channels may play a role in this process.

microcirculation; vascular smooth muscle; hemodynamics; nifedipine; mibefradil; calcium channels

PROPAGATED VASOMOTOR RESPONSES (VMR) are believed to be of importance in the coordination of microvascular tone (10). Already in 1922, Krogh et al. (19) found that application of silver nitrate to a vessel between two toes of a frog caused vasodilation to a vessel between two neighboring toes. This reaction was interpreted as being caused by a local nerve reaction (axon reflex), because in this experimental setting the connection to the central nervous system was cut. In 1970, Duling and Berne (3) showed that localized application of acetylcholine caused remote vasodilation in hamster cheek pouch arterioles. Since then, conducted VMR have been demonstrated in several vascular beds as a response to local micropipette application of different vasoactive substances and current stimulation (see Ref. 10 for a recent review).

The mechanisms behind propagated vasoconstriction in resistance vessels are not clear and seem to differ among preparations from different vascular beds (9, 33, 36). The electrotonic spread of a depolarization wave with a subsequent opening of L-type voltage-sensitive Ca^{2+} channels has been suggested as an explanation for vessels from hamster cheek pouch (36). However, in a recent study, we found that the conducted vasoconstriction in response to local application of norepinephrine (NE) or local current stimulation in mesenteric arterioles was insensitive to the L-type channel blocker nifedipine (9). Using RT-PCR on microdissected mesenteric arterioles, we were not able to detect mRNA for the $Ca_V1.2$ subunit, corresponding to the L- type Ca^{2+} channel. On the other hand, we found mRNA for the $Ca_V3.1$ and $Ca_V3.2$ subunits that corresponds to the voltage-sensitive T-type Ca^{2+} channels (9). Indeed, the responses to NE and current were blunted by the application of the T-type channel blocker mibefradil.

Renal preglomerular arteries and arterioles show pronounced propagated VMR to both KCl and current stimulation (33, 34). Because the existence of L-type Ca^{2+} channels is well established in renal preglomerular resistance vessels (6, 25), we tested whether these channels were involved in the conducted vasomotor response in these vessels. We also wanted to establish the existence of a propagated Ca^{2+} response because, to our knowledge, no direct measurements of intracellular cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) have been performed in association with the conducted vasomotor response. We used microdissected fura 2 loaded interlobular arteries from Sprague-Dawley rats to investigate the distant (500 μm) $[Ca^{2+}]_i$ response to electrical stimulation. Nifedipine and mibefradil were

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used to block L- and T-type Ca^{2+} channels, respectively. We also used the RT-PCR technique to analyze the expression of mRNA for the $Ca_V3.1$ and the $Ca_V3.2$ (previously α_{1G} and α_{1H}, respectively) pore-forming subunits of the voltage-sensitive T-type Ca^{2+} channels.

METHODS

Isolation and fura 2 loading of preparation. Interlobular arteries were microdissected from Sprague-Dawley rats (250–300 g) using regular methods for microdissection described previously (25). Slices (0.5- to 1-mm thick) of the kidney were cut from the midregion and transferred to a dissection dish containing a physiological salt solution (PSS) with bovine serum albumin (Sigma) added to a final concentration of 0.5 g/dl. The isolation procedure was performed under a microscope (×9–120 magnification). The interlobular artery was localized at its origin from an arcuate artery, and the tubular structures were carefully stripped away with sharpened forceps. If no preparation was obtained during the first 120 min of dissection, the kidney was discarded. When the dissection procedure was completed, the vessel was loaded with fura 2 AM for 45–60 min in the dark at room temperature. Fura 2-AM (Molecular Probes) was dissolved in DMSO (1 mM) and mixed with PSS to give a final concentration of 5 μM. Pluronic F127 (0.01%; Sigma) was added to facilitate uptake of fura 2. After the loading, the vessel was transferred to a chamber containing PSS on the stage of an inverted microscope (Olympus) by using a micropipette. Both ends of the vessel were then aspirated into glass holding pipettes with syringes (Fig. 1).

Measurements of $[Ca^{2+}]_i$. For measurements of $[Ca^{2+}]_i$, a ×40 quartz oil immersion objective was used. The first part of this study was performed with an intensified video camera and Image-1 software (Universal Imaging). For the second part, a digital video camera (SensiCam) and Image Workbench software (Axon) were used. The experimental procedure was similar for the two systems. The vessel was visualized on a computer screen by using the video camera and software. An area for measurement of $[Ca^{2+}]_i$ was then encircled by using a software-based routine. A monochromator controlled by the software was used for excitation with ultraviolet light of alternating 340- and 380-nm wavelengths. The fluorescent emission was detected by the digital video camera and stored on the computer. $[Ca^{2+}]_i$ was calculated with the Grynkiewicz equation (8)

$$[Ca^{2+}]_i = K_d \cdot [(R - R_{min})/(R_{max} - R)] \cdot (S_f/S_b)$$

in which K_d is the dissociation constant of fura 2 for Ca^{2+}; S_f and S_b are the 380-nm fluorescence at zero and saturating Ca^{2+} concentrations, respectively; and R_{min} and R_{max} are values of R (fluorescence ratio 340/380) at zero and at saturating (39 μM) Ca^{2+} concentration, respectively. Values for K_d, R_{min}, R_{max}, S_f, and S_b were determined in vitro as previously described (14, 27, 28).

Artificial solutions. The PSS solution contained the following (in mM): 135 NaCl, 5.0 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 10 HEPES, and 5.0 D-glucose. The Ca^{2+}-free solution was obtained by adding 1 mM EGTA to PSS and replacing $CaCl_2$ with NaCl. The solution high in potassium (K100) was obtained by replacing NaCl with KCl to a final K^+ concentration of 100 mM. The pH of all solutions was adjusted to 7.35–7.45.

Drugs. Nifedipine (Sigma) was dissolved in DMSO and diluted in PSS to a final concentration of 10^{-6} M, a concentration known to inhibit L-type Ca^{2+} channels (4). The T-type blocker mibefradil was dissolved in PSS to a final concentration of 10^{-7} or 10^{-6} M. These concentrations are reported to inhibit T-type channels with minor influence on L-type channels (21). NE and TTX (Sigma) were dissolved in PSS to final concentrations of 5×10^{-6} M and 10^{-6} M, respectively.

Electrical stimulation. Local electrical stimulation was performed as previously described (9, 33). Glass pipettes, pulled to an outer tip diameter of 8–10 μm, were filled with NaCl (2 M; resistance 0.7–1 MΩ) and placed in an electrode holder attached to a micromanipulator. Current pulses were obtained from an isolation unit controlled by a Grass stimulator. The pipette tip was positioned close to the vessel, ~500 μm proximal to the area where $[Ca^{2+}]_i$ was measured. When the electrode was placed at the same distance (500 μm) from the area of measurements, but removed from the vessel, the $[Ca^{2+}]_i$ response to electrical stimulation was abolished (Fig. 2). This indicates that the distant response is due to spread along the vessel wall and not to a generalized electrical field. Unless otherwise stated, the vessel was stimulated by a train of continuous unipolar current pulses (2.5 Hz frequency, 200-ms pulse duration, +100 V amplitude).

Experimental protocol. The experimental solutions were added in a volume large enough to allow total exchange of the fluid in the experimental chamber several times. The volume in the experimental chamber was maintained constant during replacement of fluids by a vacuum suction system. The viability of the preparation was assessed by visual observation and stimulation with the K100 solution. In the series wherein NE was used, the viability was tested with this drug. Vessels not responding promptly with an increase in $[Ca^{2+}]_i$ were discarded. These vessels were almost always visibly mechanically damaged by dissection procedures, and/or the cells of the vessel were clearly swollen. The increase in $[Ca^{2+}]_i$ was accompanied by a visible contraction of the vessel. The preparation was then stimulated with a current pulse for at least 1 min, and the change in $[Ca^{2+}]_i$ was recorded at the distant site. Thereafter, the preparation was allowed to recover for ~5 min. The solution in the experimental chamber was then replaced with a solution containing TTX, Ca^{2+}-free solution, nifedipine, or mibefradil. After ~3-

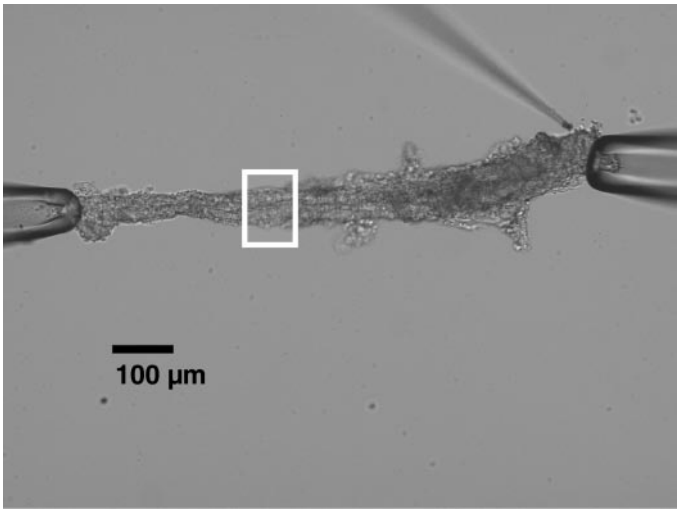

Fig. 1. Micrograph depicting the interlobular artery preparation. The vessel is held at both ends with glass pipettes. The stimulation electrode is positioned close to the *right* holding pipette, and the area for intracellular cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) measurement is marked.

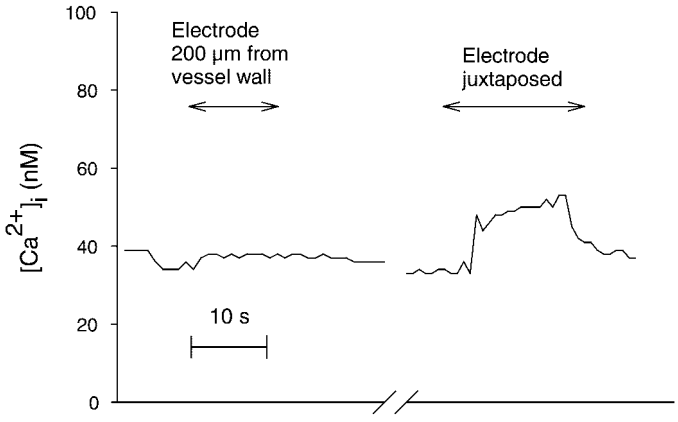

Fig. 2. Original recording of $[Ca^{2+}]_i$ in interlobular arteries depicting the stimulatory effect of local electric stimulation with the electrode retracted from or juxtaposed to the vessel, respectively. $[Ca^{2+}]_i$ is measured 500-μm distal to the position of the juxtaposed electrode. Note that only when the electrode is juxtaposed is there a conducted $[Ca^{2+}]_i$ response. Arrows, stimulation with a train of current pulses (+100 V, 200 ms).

min exposure to the pretreatment solution, the preparation was again challenged with a train of current pulses. The preparation was thereafter allowed to recover in control solution for ~5 min before a final stimulation with current. The second current stimulation under control conditions was performed to certify that the action of the pretreatment was reversible. In separate experiments, we investigated whether treatment with the Ca^{2+}-free solution depleted the $[Ca^{2+}]$ stores. In these experiments, the vessels were pretreated with the Ca^{2+}-free solution and challenged with 5×10^{-6} M of NE. This response was compared with the NE response in the control situation.

RT-PCR analysis of Ca^{2+} channels. mRNA expression of the pore-forming $Ca_V3.1$ and $Ca_V3.2$ subunits from T-type voltage-gated Ca^{2+} channels was tested on interlobular arteries from Sprague-Dawley rats. The vessels were isolated as described above. The vessels were then transferred to an Eppendorf tube containing RLT buffer (Qiagen) and 1% β-mercapto-ethanol. The samples were stored at −80°C until the analysis. RNA extraction and RT-PCR was performed as described previously (1, 15). The following PCR primers were used (DNA Technology, Aarhus, Denmark): $Ca_V3.1$, forward 5′GAA CGT GAG GCC AAG AGT 3′, reverse 5′GCT TGT ATG CGT TCC CCT 3′, covering bases 3910–4130, 221 bp (GenBank accession no. AF027984); and $Ca_V3.2$, forward 5′ GCT CTC ACC CGT CTA CTT CG 3′, reverse 5′ AGA TAC TTT TCG CAC GAC CAG G 3′, covering bases 5549–5795, 256 bp (GenBank accession no. AF290213). To introduce *Eco*RI and *Bam*HI restriction sites for cloning, linker sequences were added. β-actin primers were copied from Yu et al. (38). cDNA equivalent to 1-mm vessel length was used. For negative controls, we omitted RT in the reverse transcription reaction on interlobular arteries. Standard methods (29) were used to clone the amplification products of α_1-pore forming subunits in vector pSP73 (Promega). SP6 and T7-specific primers were used to sequence inserts.

Statistical analyses. Data are presented as means ± SE. SigmaStat (Jandel Scientific/SPSS) software was used for data analysis. Statistical significance was evaluated by Student's paired *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Measurement of $[Ca^{2+}]_i$. The baseline $[Ca^{2+}]_i$ in 22 interlobular arteries from 19 rats averaged 67 ± 7 nM. Current stimulation with the polarity of the electrode held positive relative to the ground elicited a conducted $[Ca^{2+}]_i$ response at a site ~500-μm distal from the point of stimulation that reached 91 ± 8 nM ($P < 0.001$, $n = 22$). This response was sensitive to the polarity of the electrode, because a negative electrode did not elicit any response (99 ± 4% of baseline, $P = 1$, $n = 7$; Fig. 3). When the $[Ca^{2+}]_i$ response was measured simultaneously at 400 and 500 μm from the electrode, it was found that the response was initiated at the same time at both sites. Because the time resolution of the system is ~1 s, we concluded that the velocity of the conducted response must be >100 μm/s (Fig. 4).

Effect of TTX. The conducted $[Ca^{2+}]_i$ response was not dependent on remaining perivascular nerves in the adventitial layer of the preparation, because it was unaffected by pretreatment with TTX (1 μM; Fig. 5). Under control conditions, current stimulation increased distant $[Ca^{2+}]_i$ from 81 ± 27 to 107 ± 29 nM ($n = 4$, $P < 0.01$). Pretreatment with TTX in the same vessels did not ($P = 0.38$) influence the response to current stimulation (from 77 ± 31 to 100 ± 29 nM, $P < 0.01$).

Ca^{2+} entry vs. mobilization. The effects of current stimulation were assessed with and without a Ca^{2+}-free bath solution containing EGTA (1 mM) to evaluate the relative importance of extracellular Ca^{2+} entry and mobilization from intracellular stores for the current-induced $[Ca^{2+}]_i$ response. We found that the conducted $[Ca^{2+}]_i$ response was totally abolished by the ambient Ca^{2+}-free solution (Fig. 6). Current stimulation under control conditions elevated distant $[Ca^{2+}]_i$ from 66 ± 18 to 87 ± 16 nM ($n = 6$, $P < 0.05$). These results indicate that the conducted $[Ca^{2+}]_i$ response is totally

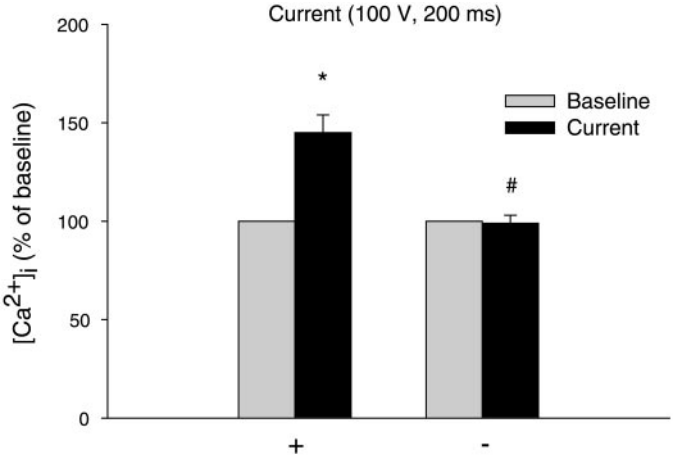

Fig. 3. Conducted $[Ca^{2+}]_i$ response after local electric stimulation with the polarity of the electrode held negative (−) or positive (+). Values are percentage of baseline $[Ca^{2+}]_i$ ± SE. *$P < 0.05$ vs. baseline; #$P < 0.05$ vs.local electrical stimulation with positive electrode.

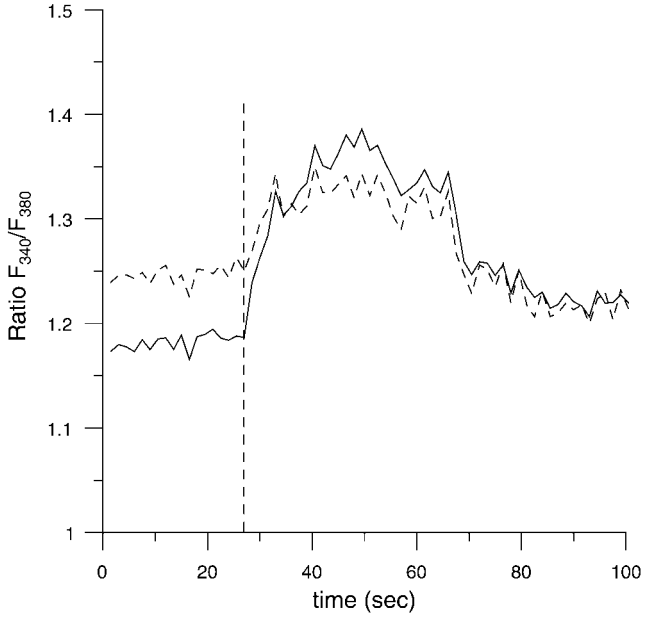

Fig. 4. Original recording of $[Ca^{2+}]_i$ in interlobular arteries showing the $[Ca^{2+}]_i$ response measured simultaneously at 400 and 500 μm from the electrode. Initiation of current stimulation is indicated by the dashed vertical line. It was found that the response was initiated at the same time at both sites. Because the time resolution of the system is ~1 s, it is concluded that the velocity of the conducted response is >100 μm/s. F_{340}/F_{380}, fluorescence ratio.

dependent on Ca^{2+} entry from the extracellular space. To exclude the possibility that the lack of response under Ca^{2+}-free conditions was due to depletion of intracellular stores, we stimulated the vessels with NE after pretreatment with the Ca^{2+}-free solution. In control experiments, 5×10^{-6} M NE caused a peak increase in $[Ca^{2+}]_i$ from 49 ± 6 to 134 ± 12 nM. The plateau amounted to 89 ± 11 nM. After pretreatment with the Ca^{2+}-free solution, NE induced a transient peak response from 40 ± 6 to 85 ± 12 nM ($n = 4$, $P <$ 0.01). This finding indicates that $[Ca^{2+}]$ stores are not depleted under these conditions.

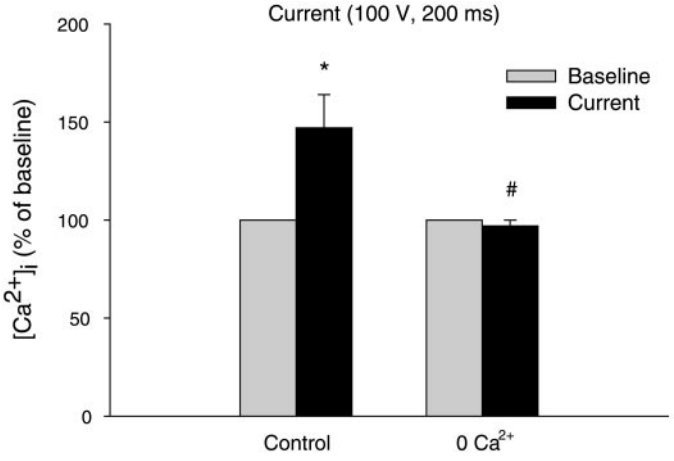

Fig. 6. Effects of local electrical stimulation before and after pretreatment with a nominally Ca^{2+}-free solution containing 1 mM EGTA. $[Ca^{2+}]_i$ is depicted as percentage of baseline in the presence or absence of the pretreatment solution. Values are means ± SE (n = 6). *$P < 0.05$ vs. baseline; #$P < 0.05$ vs. local electrical stimulation without treatment.

Involvement of voltage-operated L-type Ca^{2+} channels. Because we found a strong dependency on Ca^{2+} entry, we next investigated the involvement of voltage-gated L-type Ca^{2+} channels. The dihydropyridine nifedipine (10^{-6} M) was used to antagonize the current-induced $[Ca^{2+}]_i$ response. In control recordings, current stimulation caused a rise in $[Ca^{2+}]_i$ from 65 ± 13 to 96 ± 16 nM ($n = 6$, $P < 0.05$). Pretreatment with nifedipine significantly attenuated the $[Ca^{2+}]_i$ response to electrical stimulation (from 48 ± 10 to 59 ± 12 nM, $P < 0.05$; Fig. 7). The nifedipine-induced attenuation of the response was typically reversible after removal of nifedipine.

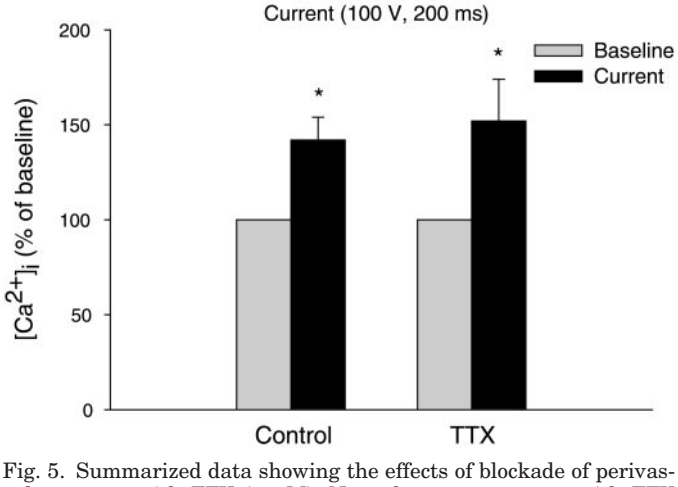

Fig. 5. Summarized data showing the effects of blockade of perivascular nerves with TTX (1 μM). Note that pretreatment with TTX does not affect the conducted $[Ca^{2+}]_i$ response to local electrical stimulation. Values are percentage of baseline $[Ca^{2+}]_i$ ± SE. *$P <$ 0.05 vs. baseline ($n = 4$).

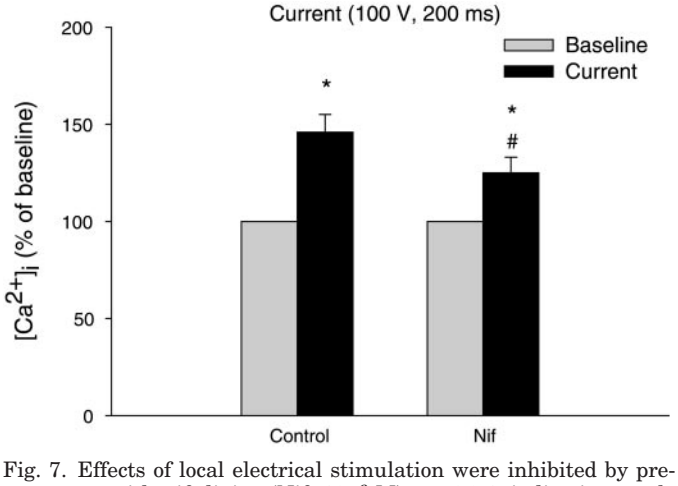

Fig. 7. Effects of local electrical stimulation were inhibited by pretreatment with nifedipine (Nif; 10^{-6} M) to ~50%, indicating a role for L-type Ca^{2+} channels in the conducted $[Ca^{2+}]_i$ response. Values are percentage of baseline $[Ca^{2+}]_i$ ± SE. *$P < 0.05$ vs. baseline; #$P <$ 0.05 vs. local electrical stimulation without nifedipine treatment ($n = 6$).

Involvement of voltage-operated T-type Ca^{2+} channels. To test whether part of the Ca^{2+} entry could be mediated by T-type Ca^{2+} channels, we investigated the effect of the T-type channel antagonist mibefradil. It was found that the $[Ca^{2+}]_i$ response to local current stimulation was not affected by mibefradil treatment (Fig. 8). In the presence of mibefradil (10^{-7} M), $[Ca^{2+}]_i$ increased from 72 ± 12 to 94 ± 15 nM ($n = 6$, $P < 0.01$). Under control conditions, the corresponding increase was from 73 ± 10 to 96 ± 12 nM ($P < 0.01$). We wished to exclude the possibility that this concentration of mibefradil was too small to evoke an attenuating effect on the response to current. Therefore, we performed a series of experiments in which we utilized a higher concentration of this compound (10^{-6} M). With this higher concentration of mibefradil, there was no significant effect on the current-induced distant $[Ca^{2+}]_i$ response. In control conditions, $[Ca^{2+}]_i$ increased from 70 ± 7 to 92 ± 5 nM ($n = 5$), and after mibefradil treatment, the rise in $[Ca^{2+}]_i$ was similar (from 70 ± 8 to 89 ± 7 nM, $P < 0.01$).

RT-PCR analysis. To test whether the lack of effect by mibefradil was due to the absence of gene products for the T-type channel $Ca_V3.1$ and $Ca_V3.2$ pore-forming units, the presence of mRNA was investigated in interlobular artery preparations. Our RT-PCR analysis of interlobular vessels from three rats, however, showed expression of mRNA for both these subunits (Fig. 9).

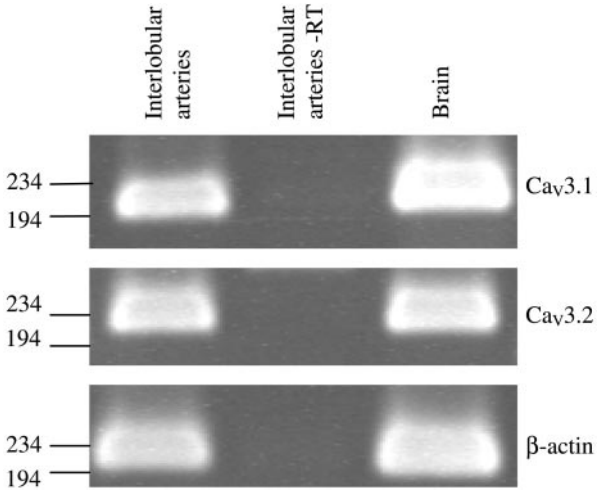

Fig. 9. Representative electrophoresis gel of RT-PCR product. PCR products for $Ca_V3.1$ (*top*), $Ca_V3.2$ (*middle*), and β-actin (*bottom*). *Lane 1*: PCR products with cDNA from interlobular arteries as template. *Lane 2*: negative controls in which RT was omitted in the reverse transcription step. *Lane 3*: positive controls with cDNA from brain as template.

DISCUSSION

In this study, a conducted $[Ca^{2+}]_i$ response is, to our knowledge, shown in a resistance vessel as a response to distant local electrical stimulation for the first time. Few other studies have dealt with conducted VMR in renal resistance vessels (13, 17, 33, 34). In one study, an indication for a functional coupling along the renal vascular tree was found where synchronized oscillations in proximal tubular pressure were recorded in nephrons originating from the same interlobular artery (13). In a more recent study, utilizing the juxtamedullary nephron preparation, it was found that the vasoconstriction caused by application of KCl to an afferent arteriole was rapidly conducted to neighboring afferent arterioles (34).

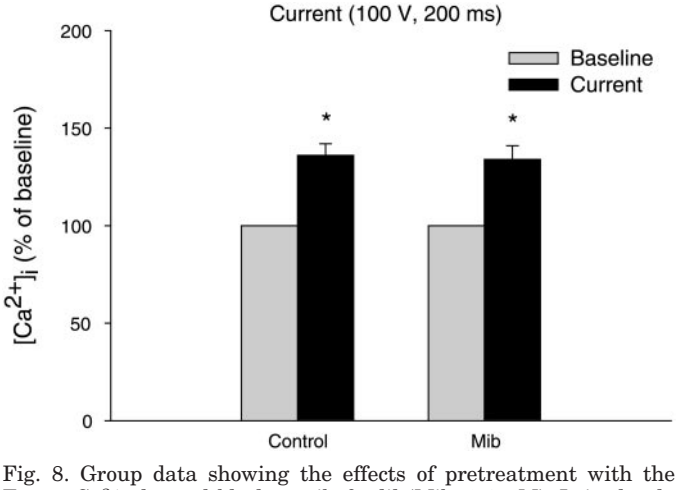

Fig. 8. Group data showing the effects of pretreatment with the T-type Ca^{2+} channel blocker mibefradil (Mib; 0.1 μM). It is clearly shown that pretreatment with mibefradil does not affect the conducted $[Ca^{2+}]_i$ response to local electrical stimulation. Values are means ± SE of percentage of baseline $[Ca^{2+}]_i$. *$P < 0.05$ vs. baseline ($n = 4$).

Using the larger isolated renal interlobar-arcuate arteries, Goligorsky et al. (6) found that stimulation with 50 mM K^+ initially increased the $[Ca^{2+}]_i$ at branching points of vessels. This increase gradually spread both distally and proximally. The rate of propagation of this wave was as low as 2 μm/s. This is of the same order of magnitude as previously found for the propagation of intercellular Ca^{2+} waves via gap junctions in astrocytes (2). Intercellular spread of a Ca^{2+} wave has been documented both in whole organs and in several cell culture systems (24). This wave might originate from the diffusion of Ca^{2+} over gap junctions, which in turn activates intracellular ryanodine receptors to release Ca^{2+} from intracellular stores by means of Ca^{2+}-induced Ca^{2+} release (18, 31). Another possibility is that inositol trisphoshate (IP_3) diffuses across the gap junction and subsequently activates IP_3 receptors in the sarcoplasmatic reticulum, thus giving rise to $[Ca^{2+}]$ release (32). However, intercellular Ca^{2+} waves typically have a velocity of around 25 μm/s (24). Our observations indicated that the velocity of the conducted $[Ca^{2+}]_i$ response was at least 100 μm/s. Therefore, it is less likely that the mechanism behind the observed conducted $[Ca^{2+}]_i$ response is relying on intercellular Ca^{2+} waves mediated by cell-to-cell Ca^{2+} or IP_3 signaling.

Krogh et al. (19) and, later, Duling and Berne (3) speculated that the conducted VMR were dependent on perivascular nerve action. A role for perivascular nerve stimulation has also recently been suggested for the conducted constriction in response to local electrical

stimulation in hamster skeletal muscle (30). It seems unlikely that such a mechanism is of importance in the present preparation because the $[Ca^{2+}]_i$ response was unaffected by the addition of TTX in a concentration of 10^{-6} M. This is a concentration known to block the activity of perivascular nerves after local and field electrical stimulation (20, 30).

The present results indicate that the conducted $[Ca^{2+}]_i$ response to local electrical stimulation is dependent on Ca^{2+} entry from the extracellular space, because the response is absent when the ambient solution was Ca^{2+} free. We cannot exclude the possibility that release of Ca^{2+} from intracellular stores might be of some importance, because exposure to a Ca^{2+}-free medium could deplete these stores of Ca^{2+}. However, this seems unlikely because we found a transient increase in $[Ca^{2+}]_i$ in response to NE even after pretreatment of rat preglomerular vessels with a Ca^{2+}-free solution. This is supported by observations from previous studies (25, 26). Furthermore, contraction of isolated rabbit afferent arterioles has been found in response to NE after 5 min in a nominally Ca^{2+}-free solution (16).

In several vascular beds, the available evidence suggests that conducted VMR are due to the electrotonic spread of a local de- or hyperpolarization through gap junctions between the smooth muscle cells and/or the endothelial cells (12, 35, 36). The distant change in membrane potential can then modify vascular tone by opening or closing voltage-operated Ca^{2+} channels. In accord with this theory, the conducted vasoconstriction in renal preglomerular vessels has been reported to decay exponentially with a length constant that is consistent with that predicted for electrotonic spread (33, 34). In the split hydronephrotic kidney, Steinhausen et al. (33) showed that local electric stimulation of the interlobular artery caused a propagated VMR at a distant site (up to ~600 μm). In accord with the findings of the present study, the polarity of the electrode was of importance for the nature of the conducted VMR. It was reported that stimulation with a depolarizing current caused a distant vasoconstriction, whereas a hyperpolarizing current caused a distant vasodilation. In our hands, only a positive electrode elicited a distant increase in $[Ca^{2+}]_i$. Unfortunately, the paper by Steinhausen et al. does not contain information on the polarity of the electrodes used in the experiments. The current from a positive electrode will hyperpolarize the cell membrane at the entry site, whereas it will depolarize the membrane at the exit sites (5). The entry site will only be a narrow region close to the tip of the pipette, whereas the exit region, because of the intra- and intercellular spread of the current (via gap junctions), will be much larger. We hypothesize that the latter will dominate; therefore, the overall effect of using a positive electrode for the stimulation will be a depolarization of the cell membrane. This is reinforced by the finding that the response evoked by a positive electrode could partly be antagonized by nifedipine, which blocks the activation of voltage-sensitive L-type Ca^{2+} channels. In the present study, an electrode that was negative with respect to ground was not able to elicit a consistent change in distant $[Ca^{2+}]_i$. This finding does not exclude the possibility that conducted hyperpolarization might play a role in conditions other than those in the present study (e.g., when $[Ca^{2+}]_i$ is increased). Because conducted dilation is dominant in other vascular beds (e.g., skeletal muscle), the present observation might indicate interorgan heterogeneity in this respect (30).

The increase in $[Ca^{2+}]_i$ from 67 to 91 nM in response to current stimulation is rather small. These absolute values should be considered with some caution. Even if we normally could not detect any fluorescence from the endothelial cells, we cannot totally exclude the possibility that fura 2 loading of these cells might affect the apparent magnitude of the response. The increase is, however, >50% of the absolute plateau increase after stimulation with 5 μM NE, a stimulation known to substantially affect renal preglomerular resistance. Also, the increase in $[Ca^{2+}]_i$ was typically accompanied by a visible contraction of the vessel. We therefore think it likely that the observed increase in $[Ca^{2+}]_i$ corresponds to physiologically relevant change in vessel tone.

In a recent study, we found that in small (<40 μm) mesenteric arterioles, the conducted vasoconstrictor response to local current stimulation and topical administration of NE did not decay measurably between the site of stimulation and a site ~600 μm up- or downstream from that point (9). Furthermore, the responses were not affected by addition of nifedipine, an inhibitor of L-type voltage-sensitive Ca^{2+} channels. Also, application of a high-K^+ solution to the arteriole did not elicit any vasoconstriction. Instead, this maneuver inhibited the conducted VMR to local electrical stimulation. Thus these findings are not in accord with an electrotonic spread of a depolarizing current and subsequent opening of L-type Ca^{2+} channels. On the other hand, in the mesenteric arterioles, we found several lines of evidence for the participation of T-type voltage-sensitive Ca^{2+} channels in the VMR (9).

In contrast to these findings, the present study shows that in interlobular arteries, pretreatment with the dihydropyridine nifedipine attenuated the distant $[Ca^{2+}]_i$ response to local electrical stimulation by >50%. This observation indicates that L-type Ca^{2+} channels, at least partly, participate in the $[Ca^{2+}]_i$ response to the conducted signal. The existence of these channels in rat preglomerular resistance vessels is also supported by previous findings (6, 25). The fact that low extracellular Ca^{2+} inhibited the increase in $[Ca^{2+}]_i$ more strongly than nifedipine implicates a second Ca^{2+} entry pathway. Previous studies provide further support for the participation of more than one Ca^{2+} entry pathway in smooth muscle cells from rat preglomerular vessels, as well as in vascular smooth muscle preparations of other origins (7, 11, 25, 26). The details of this alternative Ca^{2+} entry pathway still have to be determined. It is not clear whether the nondihydropidine-sensitive pathway is voltage sensitive or not. Consequently, from the present result, it is

not possible to conclude whether the conducted $[Ca^{2+}]_i$ response in rat interlobular arteries is mediated exclusively by an electrotonic spread of a change in membrane potential or whether other intercellular signaling pathways participate in the conducted response.

Using the RT-PCR technique, we demonstrated the presence of the gene product for the $Ca_V3.1$ and $Ca_V3.2$ pore-forming units of T-type Ca^{2+} channels. The result from the functional studies, however, did not support any significant role of these channels in the conducted $[Ca^{2+}]_i$ response. We found that adding the T-type channel antagonist mibefradil at concentrations of 10^{-7} M (shown not to affect L-type channels but to block ~50% of the T-type channels) or 10^{-6} M did not attenuate the conducted $[Ca^{2+}]_i$ response (21). Furthermore, in preliminary experiments, we found that the longer the duration of the current pulse, the stronger the $[Ca^{2+}]_i$ response. This is characteristic for Ca^{2+} entry through L-type channels, because they inactivate more slowly than T-type channels. With a pulse duration of 200 ms, as used in this study, the T-type channels should be inactivated (22). The role of T-type channels in this preparation therefore remains elusive. Another possibility is that the membrane potential of the smooth muscle cells is in the range wherein the T-channels are inactivated. In pressurized vessels, the membrane potential might reach these values. In our nonpressurized preparation, this is, however, less likely. Available results on renal hemodynamic parameters using mibefradil are difficult to interpret because of the lack of specificity of this blocker (23).

In the present study, we are not able to determine whether the conducted response utilizes a smooth muscle or an endothelial pathway. This issue remains controversial (35, 37). In the literature, there is, however, support for the notion that conducted vasoconstriction is transmitted by means of the spread of depolarization in the smooth muscle layer only (35).

In summary, we have, for the first time, demonstrated a conducted $[Ca^{2+}]_i$ response in an intact vessel as a response to local electrical stimulation. This response was not dependent on the action of perivascular nerves, because the conducted response was unaffected by TTX. The conducted $[Ca^{2+}]_i$ response was absent in a Ca^{2+}-free environment, implying a pivotal role of Ca^{2+} entry. Blockade of L-type Ca^{2+} channels attenuated the response by ~50%. This indicates that at least part of the response is due to electrotonic spread of membrane depolarization. T-type Ca^{2+} channels are likely to be expressed, because we found the gene product for these channels. However, they seem not to play any role in the conducted $[Ca^{2+}]_i$ response, because this mechanism was not affected by mibefradil, a blocker of these channels. Therefore, the second pathway for Ca^{2+} entry remains elusive and is thus an important subject for future studies.

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