EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles

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Hoepl, Bernd, Barbara Rodenwaldt, Ulrich Pohl, and Cor de Wit. EDHF, but not NO or prostaglandins, is critical to evoke a conducted dilation upon ACh in hamster arterioles. Am J Physiol Heart Circ Physiol 283: H996–H1004, 2002. First published May 23, 2002; 10.1152/ajpheart.01082.2001.—Vasomotor reactions upon focal stimulation of arterioles have been shown to be conducted along the vascular wall. Such a conduction, which is assumed to reflect the spread of electrical signals, may contribute to coordination of responses within a vascular segment. We aimed to identify which endothelial autacoid(s) act as mediators of the local and conducted dilator responses, respectively. To this end, arterioles in the hamster cremaster microcirculation were locally stimulated with endothelium-dependent [acetylcholine (ACh)] or endothelium-independent dilators [sodium nitroprusside (SNP)], and the resulting changes in diameter were measured using a videomicroscopy technique at the site of application and up to 1.4 mm upstream at distant sites. Experiments were also performed after blockade of nitric oxide (NO) synthase, cyclooxygenase, P-450 monooxygenase, or K+ channels. Dilations upon ACh (71 ± 3%) were conducted rapidly (<1 s) upstream sites (at 1.4 mm: 37 ± 5%). Although the NO donor SNP induced a similar local dilation (71 ± 7%), this response was not conducted. Maximal amplitudes of ACh-induced dilations were not attenuated after inhibition of NO synthase and cyclooxygenase at the local and remote sites. However, additional treatment with a P-450 monooxygenase blocker (sulfaphenazole) strongly attenuated the local response (from 62 ± 9 to 17 ± 5%) and abrogated dilations at distant sites (at 0.67 mm: from 23 ± 4% to 4 ± 3%). Likewise, 17-octadecenoic acid strongly attenuated local and remote responses. Blockers of Ca2+-dependent K+ channels (charybdotoxin or iberiotoxin) attenuated dilations at the local and remote sites after focal application at the ACh stimulation site. In marked contrast, treatment of the upstream site with these blockers was without any effect. We conclude that upon local stimulation with ACh, a cytochrome P-450 monooxygenase product is generated that induces local dilation via the activation of Ca2+-dependent K+ channels and initiates conduction of the dilation. In contrast to the local site, neither activation of these K+ channels nor the synthesis of NO or prostaglandins is necessary to dilate the arterioles at remote, distant sites. This suggests that endothelium-derived hyperpolarizing factor serves as an important mediator to initiate conducted dilations and, by doing so, may act as a key player in the coordination of arteriolar behavior in the microcirculatory network.

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polarizing factor (EDHF) (4, 5, 17, 23, 26). However, it is not clear whether autacoids and which one in particular is the key mediator to initiate a propagated response in the hamster cremaster microcirculation. Because EDHF, by definition, acts by a change of the membrane potential, we hypothesized that EDHF is the crucial factor to initiate a dilation upon ACh that conducts along the arteriole. This hypothesis was tested by studying the effect of sequential inhibition of the synthesis of NO, prostaglandins, and EDHF on local and remote dilations initiated by ACh. If indeed EDHF evokes the local signal that is transmitted along the vascular wall, it does not necessarily imply that EDHF itself is also the mediator of the dilation at distant sites. This distant dilation could be induced either by a hyperpolarization per se or by a secondary release of autacoids. To address this issue, adequate blockers of K+ channels were applied in a focal manner, either at the site of ACh stimulation or at distant sites. Moreover, local microcirculatory hemodynamics were measured to study a possible involvement of flow-induced dilations during responses at local and remote sites.

METHODS

Animal preparation. The care of the animals and the conduct of the experiments were in accordance with the rules of the German animal protection law. Male golden Syrian hamsters (80–150 g body wt) were anesthetized by intraperitoneal injection of pentobarbital sodium (75 mg/kg). After cannulation of the right jugular vein with a polyethylene catheter, anesthesia was maintained by infusion of either pentobarbital (5–10 mg·kg⁻¹·h⁻¹) or a combination of droperidol (0.1 mg/kg), fentanyl (0.1 mg/kg), and midazolam (2 mg/kg) at a rate of 0.2–0.3 ml·min⁻¹·kg⁻¹. Arterial pressure was measured continuously via a catheter placed in the right carotid artery by means of a pressure transducer (Statham; Costa Mesa, CA). Heart rate was calculated from the oscillations of the blood pressure signal by means of an electronic frequency counter. These data were sampled at a rate of 2 Hz by an analog-to-digital board, processed with a data acquisition system (ONLINE), and stored on computer disk for later analysis. The animals were artificially ventilated (7025 Rodent Ventilator, Hugo Sachs Elektronik; Freiburg, Germany) to maintain PO₂ and PCO₂ at physiological values (85 and 40 mmHg, respectively), as confirmed by blood gas analysis. The right cremaster muscle was prepared as previously described (7).

Experimental setup. The cremaster muscle was superfused with warm (34°C) bicarbonate-buffered salt solution at a rate of 8 ml/min. The superfusion fluid had a pH of 7.4, a PO₂ of ~30 mmHg, and a PCO₂ of ~38 mmHg as measured in samples taken from the surface of the muscle. One or two arterioles were studied in each animal and monitored by means of a microscope (Metallux, Leitz; Wetzlar, Germany) equipped with a videocamera (charge-coupled device, Computer Optics; Weilheim, Germany). The microscopic images were acquired using a ×32 objective (numerical aperture: 0.40) and displayed on a video monitor at a 1,000-fold magnification as well as recorded on videotape (S-VHS, Sony) for off-line measurements of luminal diameters (video dimension analyser, IPM). Data from the dimension analyser were also sampled at 2 Hz by an analog-to-digital board and stored on computer disk.

Experimental protocols. After the preparation was allowed to stabilize from surgery for 30 min before an experimental protocol was started. To study the conduction of vascular responses, a micropipette was positioned in the close vicinity of an arteriole. In some experiments, the pipette was repositioned and placed directly into the tissues to check for diffusion and/or convection of the vasoactive substance after pressure ejection. Pipettes were pulled using a Brown-Flaming puller (model P-97, Sutter) from borosilicate glass (Hilgerberg, Germany; outer diameter 1 mm, inner diameter 0.5 mm). Tip opening was 1–2 μm. ACh (10 μmol/l) or sodium nitroprusside (SNP; 10 μmol/l) was applied by a pressure pulse (60–180 kPa for a period of 100–600 ms). Vascular diameters were monitored continuously from 30 s before until 90 s after the stimulation. If a response at the site of stimulation (local) was obtained, the same pulse stimulation was used, and vasomotor responses were studied at sites located between 0.67 and 1.40 mm upstream. In some experiments, centerline red blood cell (RBC) velocity was measured continuously by means of a modified dual-slit cross-correlation method, as described previously (6). Thereafter, NO synthase and cyclooxygenase were blocked by the addition of a combination of Nω-nitro-L-arginine (l-NNa; 30 μmol/l) and indomethacin (Indo; 3 μmol/l) to the superfusion fluid. These inhibitors were added 30 min before the response upon local ACh stimulation was restudied. Because pentobarbital narcosis affects the release and/or the efficacy of EDHF (7), this protocol was performed not only in animals anesthetized with pentobarbital but instead with a combination of droperidol, fentanyl, and midazolam. The latter anesthesia was used in all further groups. In these groups aimed to study the role of EDHF, control dilations upon ACh at the local and an upstream site (0.67 mm) were obtained in the presence of l-NNa and Indo. Thereafter, sulphaphenazole (10 μmol/l) (16) or 17-octadecenoic acid (ODYA; 50 μmol/l) was added to the superfusion fluid. While the blocker of cytochrome P-450 (P-450) monoxygenases, sulphaphenazole, was continuously present, the suicide inhibitor of P-450 monoxygenases, ODYA (36), was added to the superfusion fluid for 30 min and washed out before reexamination of the arteriolar responses. In other experimental groups, dilations were studied before and after application of specific blockers of Ca²⁺-dependent K⁺ channels [charrybotoxin (ChTX) or iberiotoxin (IbTX)] (18, 19). These blockers were applied via a micropipette either at the site of ACh stimulation (local) or at the remote site. The topical application of these blockers was done by repeated pressure ejections (130 kPa, 200 ms, 10–20 times). The efficacy of the K⁺ channel blockade was verified by checking the dilation in response to ACh applied locally at the same location. In a separate experimental group (n = 8), which did not receive any treatment, RBC velocity was measured simultaneously with diameter measurements at the local and, subsequently, at remote sites before and during stimulation of the vessel. The experiments lasted typically between 3 and 6 h. The maximal diameter of the investigated arterioles was measured by the superfusion of a combination of different vasodilators [adenosine (100 μmol/l), SNP (10 μmol/l), and papaverine (10 μmol/l)] at the end of the experimental protocol, and the animal was euthanized by an overdose of anesthesia.

Solutions and drugs. The salt buffer used for superfusion had the following composition (in mmol/l): 143 Na⁺, 6 K⁺, 2.5 Ca²⁺, 1.2 Mg2⁺, 128 Cl⁻, 25 HCO3⁻, 1.2 SO4²⁻, and 1.2 HPO4²⁻. ACh, SNP, ChTX, IbTX, and sulphaphenazole were purchased from Sigma (Deisenhofen, Germany). SNP was dissolved in 1 mmol/l Na-acetate at the day of experiment and stored in the dark. Stock solutions of ChTX and IbTX were prepared with 1 mmol/l Na-acetate.
were prepared in water and stored at −20°C until use. Sulfaphenazole was dissolved in 60% ethanol at a concentration of 10 μmol/1 at the day of experiment and further diluted using the superfusion buffer. For all other solutions and further dilutions, freshly prepared superfusion buffer (see above) was used.

Statistics and calculations. Vascular tone is expressed as quotient of the resting diameter of the vessel divided by its maximal diameter. Changes of the inner diameter of the vessels were normalized to the maximal possible constriction or dilation according to the relationship

\[
\text{Percentage of maximal response} = \frac{(D_{r} - D_{c})/(D_{m} - D_{c})} \times 100
\]

where \(D_{r}\) is the diameter observed after treatment and \(D_{c}\) is the control diameter before treatment. \(D_{m}\) is (for dilator responses) the diameter at maximal dilation or (for constriction) the minimal luminal diameter (zero). This normalization allows the comparison of vessels of different tone and size. The temporal nature of the responses on local stimulations were analyzed by determining the “time to peak response,” representing the interval between stimulus application and the attainment of peak diameter. The “response duration” was taken as the interval between stimulus application and 50% of recovery to resting diameter. Mean RBC velocity (\(V_{M}\)) was calculated from the measured centerline velocity divided by an empirical correction factor (1.6), and, from these corrected velocity values and simultaneously obtained diameter values (\(D\)), wall shear rate (WSR) was calculated according to the formula \(W_{SR} = 8 \times V_{M}/D\). Comparisons within groups were performed using paired t-tests, and, for multiple comparisons, P values were corrected according to Bonferroni. Data between groups were compared by ANOVA followed by post hoc analysis of the means. Differences were considered significant at a corrected error probability of \(P < 0.05\). Means ± SE of all data are presented.

RESULTS

Basal data. A total of 71 arterioles was studied in 58 hamsters. The animals exhibited a mean arterial pressure of 70 ± 2 mmHg, which was virtually stable throughout the experiment. Mean arterial pressure was not different in animals anesthetized with either pentobarbital (n = 21, 71 ± 3 mmHg) or a combination of droperidol, fentanyl, and midazolam (n = 37, 67 ± 2 mmHg, \(P = 0.16\)). The arterioles studied were of varying size and branching order, and their maximal diameter ranged from 25 to 85 μm (mean value: 50 ± 2 μm). The resting tone of the arterioles varied in untreated preparations from 0.40 to 0.94. The mean value in these vessels (n = 34) amounted to 0.56 ± 0.03. Arteriolar resting tone was not obtained in every single experiment, because in some experimental protocols the treatment with inhibitors of endothelial autacoid synthesis was started without prior diameter measurements. Arteriolar tone in these vessels (n = 32) amounted to 0.43 ± 0.02 in the presence of L-NNA and Indo (\(P < 0.05\) vs. untreated arterioles).

Local stimulation and conduction of vasodilator responses. Local stimulation of the arterioles with a short pulse (130 kPa for 20–100 ms) of the endothelium-dependent vasodilator ACh induced a dilation that peaked within 14 ± 1 s to a maximum of 71 ± 3% at the stimulation site (local, \(n = 32\)). The dilation was transient; the response duration (50% recovery to the initial diameter) amounted to 36 ± 3 s. Within 66 ± 4 s, the arterioles completely returned to their initial diameters. This local dilation was rapidly conducted to distant upstream sites; however, the amplitude was diminished. At a distance of 660 ± 10 μm, the maximal amplitude amounted to 42 ± 5% (\(P < 0.05\) vs. the local site) and was reached within 7 ± 1 s (\(P < 0.05\) vs. the local site). The time to peak and the amplitude were similar at more distant sites (1,400 ± 110 μm: 7 ± 1 s, 37 ± 5%). When the ACh pipette was placed in the tissue at a distance of 0.67 mm from the arteriole, only a small dilation of 6 ± 2% was observed after 18 ± 6 s. The application of the exogenous NO donor SNP (\(n = 5\)) induced a local dilation of a similar amplitude (71 ± 7%, \(P = 0.50\) vs. ACh). The time to maximum amplitude after SNP was longer (24 ± 3 s, \(P < 0.05\)) and the dilation lasted longer (response duration: 71 ± 8 s, \(P < 0.05\)) compared with ACh stimulation. Although the local dilation upon SNP in the same arterioles was of a similar amplitude, only a small dilation of 5 ± 2% (\(P = 0.07\)) was observed at upstream sites. This small peak was reached only after 25 ± 5 s (\(P < 0.05\) vs. ACh). Figure 1 compares local and remote dilations upon ACh and SNP application in the same arterioles.

To determine the influence of preconstriction on the conduction of dilations, the arterioles were grouped according to their tone (constriction level calculated as resting diameter divided by maximal diameter). Some of these otherwise-untreated vessels were preconstricted with norepinephrine (0.1 μmol/1, \(n = 7\)) to study vessels with a preconstriction level comparable with vessels after treatment with l-NNA and Indo. Vessels with highest tone (\(n = 14, 0.38 ± 0.02\)) tended to dilate less at the local and remote site (60 ± 4% and 34 ± 4%) than vessels with intermediate tone (\(n = 12, 0.54 ± 0.06,\) local: 66 ± 4%, remote: 35 ± 5%) or those with low tone (\(n = 9, 0.64 ± 0.01,\) local: 72 ± 5%, remote: 40 ± 4%). However, these small differences did not achieve level of significance (local: \(P = 0.17,\) remote: \(P = 0.53,\) ANOVA). Therefore, varying tone does not affect the conduction of the response.

Changes of wall shear stress during conducted vasodilations. To study the role of flow-induced dilation in inducing responses at upstream sites, RBC velocity was measured during application of ACh (\(n = 8\)). Local diameter increased from 30 ± 3 μm to a maximum of 52 ± 4 μm within 15 ± 3 s after stimulation with ACh. RBC velocity decreased from 3.6 ± 0.5 mm/s to a minimum of 1.6 ± 0.3 mm/s after 23 ± 4 s. Because of the increase of diameter and the concomitant decrease of RBC velocity, the calculated WSR decreased sharply from 630 ± 90 1/s to a minimum of 168 ± 34 1/s (Fig. 2). At a distance of 1,330 ± 500 μm, the arterioles dilated from 29 ± 2 to 37 ± 2 μm. At this remote site, the RBC velocity increased initially from 4.0 ± 0.6 to a peak of 4.5 ± 0.7 mm/s (observed 4 s after stimulation, \(P < 0.05\)). However, this increase in velocity was not accompanied by an increase of calculated WSR due to
the simultaneously developing dilation (WSR: 690 ± 95 vs. 675 ± 92 1/s before stimulation and at peak RBC velocity, respectively). Thus WSR remained initially stable, and an increase was never observed (Fig. 2). Subsequently, WSR even decreased and reached a minimum (531 ± 69 1/s) at 11 ± 2 s after stimulation with ACh.

Role of endothelial autacoids in the initiation of conducted vasodilations. The blockade of endothelial NO synthase (30 μmol/l L-NNA) and prostaglandin synthesis (3 μmol/l Indo) reduced resting arteriolar diameters from 25 ± 2 to 21 ± 2 μm (−16 ± 4%, P < 0.05). In animals anesthetized with pentobarbital (n = 7), the maximal amplitude of the dilation upon ACh at the local site was significantly reduced after treatment with L-NNA and Indo (from 68 ± 4% to 36 ± 5%; Fig. 3). This smaller peak was reached earlier (8 ± 1 vs. 13 ± 1 s after stimulation, P < 0.05), and the dilation was shorter (response duration: 19 ± 2 vs. 29 ± 2 s). The inhibition of NO synthase and cyclooxygenase also attenuated the dilation at the distant site (0.67 mm; Fig. 3). The dilation was reduced from 29 ± 7% to 13 ± 3% (P < 0.05), but the time to peak and response duration remained unaffected (data not shown). Because pentobarbital attenuates EDHF-mediated dilations (7), an additional set of experiments was performed in which the animals were anesthetized with a combination of droperidol, fentanyl, and midazolam (neuroleptanalgesia, n = 4). Under these experimental conditions, L-NNA and Indo did not reduce the maximal amplitude of the dilation upon ACh application at the local site (58 ± 6% vs. 52 ± 3%, control vs. L-NNA + Indo, respectively) or at the distant site (0.67 mm: 40 ± 6% vs. 36 ± 8%). Only the sustained phase of the local dilation was diminished after L-NNA and Indo (Fig. 3).

To further elucidate the role of EDHF in these ACh-induced responses, a blocker of the P-450 monoxygenase, sulfaphenazole (1 μmol/l), was applied in animals anesthetized with the same regimen. In arterioles pretreated with L-NNA and Indo, the dilation in response to ACh at the local site was severely impaired after sulfaphenazole (Fig. 4). The peak dilation was reduced from 62 ± 9% to a small remaining amplitude of 17 ± 5%. Sulfaphenazole virtually abrogated the dilation at the remote site (Fig. 4). A small remaining dilation of 4 ± 3% at this site (0.67 mm) did not reach the level of significance (P = 0.20). To test for potential effects of sulfaphenazole on gap junction conductivity, the spread of vasoconstrictions initiated by local K⁺ depolarization (3 M delivered via a micropipette, 130 kPa

![Fig. 1. Dilator response to local stimulation by ACh (10 mmol/l) or the nitric oxide (NO) donor sodium nitroprusside (SNP; 10 mmol/l) in the same arterioles (n = 5). Although in both cases the local dilation was of a similar magnitude (A), only the response to ACh was conducted to the upstream remote site (B). Arrows, ejection pulse of the stimulatory compound.](http://ajpheart.physiology.org/)

![Fig. 2. Changes in arteriolar diameter, red blood cell (RBC) velocity, and calculated wall shear rate (WSR) during stimulation with ACh (10 mmol/l) at the local (A) and upstream, remote site (B). At the local site, RBC velocity declined with developing dilation, which led to a sharp decrease of WSR. In contrast, the RBC velocity increased initially at the remote site, reaching a peak after 4 s. However, due to the simultaneous diameter increase, WSR did not exceed the control level. Thereafter, RBC velocity regained control levels accompanied by further dilation, both leading to a decrease of WSR. Data were obtained in 8 arterioles. For absolute values, see text.](http://ajpheart.physiology.org/)
Fig. 3. Effect of the inhibition of NO synthase \(30 \mu\text{mol/l} \ \text{N}^\text{G}\)-nitro-L-arginine (L-NNA) and cyclooxygenase (3 \mu\text{mol/l} \text{indomethacin} (Indo)) on dilations initiated by ACh at the local (A) and remote site (0.67 mm; B) in animals anesthetized with pentobarbital \((n = 7; \text{top})\) or a combination of droperidol, fentanyl, and midazolam \((n = 4; \text{bottom})\). In the presence of pentobarbital, L-NNA and Indo reduced the local as well as the remote dilation. However, in animals receiving neuroleptanalgesia, the initial dilation at the local site remained unaffected by L-NNA and Indo, and the dilation at the conducted site was not attenuated under these conditions. *Significant difference of the maximal amplitude of the dilation between groups \((P < 0.05)\). For absolute values, see Table 1.

Table 1. Diameter of arterioles stimulated with ACh before and after treatment

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Local Site</th>
<th>Remote Site</th>
<th>Maximum Diameter, μm</th>
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<tbody>
<tr>
<td></td>
<td>Resting Diameter, μm</td>
<td>Peak Diameter, μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (Pento)</td>
<td>31 ± 2</td>
<td>48 ± 2*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo (Pento)</td>
<td>25 ± 1</td>
<td>37 ± 3*</td>
<td></td>
</tr>
<tr>
<td>Control (Neurolept)</td>
<td>20 ± 2</td>
<td>29 ± 1*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo (Neurolept)</td>
<td>18 ± 2</td>
<td>27 ± 1*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo</td>
<td>25 ± 2</td>
<td>44 ± 4*</td>
<td></td>
</tr>
<tr>
<td>+ Sulfaphenazole</td>
<td>27 ± 3</td>
<td>32 ± 2*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo</td>
<td>15 ± 3</td>
<td>37 ± 2*</td>
<td></td>
</tr>
<tr>
<td>+ ODYA</td>
<td>20 ± 2</td>
<td>24 ± 3*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo</td>
<td>15 ± 3</td>
<td>27 ± 5*</td>
<td></td>
</tr>
<tr>
<td>+ ChTX (local)</td>
<td>18 ± 2</td>
<td>22 ± 3*</td>
<td></td>
</tr>
<tr>
<td>+ ChTX (remote)</td>
<td>23 ± 1</td>
<td>32 ± 2*</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo (remote)</td>
<td>23 ± 3</td>
<td>34 ± 3*</td>
<td></td>
</tr>
<tr>
<td>+ IbTX (local)</td>
<td>28 ± 4</td>
<td>29 ± 4</td>
<td></td>
</tr>
<tr>
<td>L-NNA/Indo</td>
<td>18 ± 2</td>
<td>24 ± 2*</td>
<td></td>
</tr>
<tr>
<td>+ IbTX (remote)</td>
<td>17 ± 2</td>
<td>22 ± 2*</td>
<td></td>
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</tbody>
</table>

Values are means ± SE; \(n\) = no. of arterioles. Arteriolar diameters were measured at the application site (local) and at an upstream site (remote; distance; 0.67 mm) shortly before ACh application (resting). Peak values represent maximal diameters after ACh application. For every treatment group, the respective control values are shown. Maximum diameter represents the diameter determined at the end of the experiment during superfusion of a combination of adenosine, sodium nitroprusside, and papaverine \((10 \mu\text{mol/l} \text{each})\). Pento and Neurolept, pentobarbital anesthesia and neuroleptanalgesia, respectively; L-NNA, \text{N}^\text{G}-\text{nitro-L-arginine}; Indo, indomethacin; ODYA, 17-octadecynoic acid; ChTX, charybdotoxin; IbTX, ibiotixin. *Significant difference \((P < 0.05)\) from resting diameter. †\(P = 0.09\).
anesthetized by neuroleptanalgesia. To block NO synthase and cyclooxygenase, L-NNA and Indo were added to the superfusion fluid in these experiments. The application of ChTX at the site of ACh ejection attenuated the peak dilation from 46 ± 9% to 19 ± 5% (P < 0.05, n = 4; Fig. 5). The time to peak remained unaltered (9 ± 1 vs. 11 ± 1 s, P = 0.25). At a distance of 0.67 mm upstream, the dilation was also attenuated (from 29 ± 4% to 10 ± 5%, P < 0.05) without an effect on time to peak (10 ± 1 vs. 8 ± 1 s, P = 0.33). If ChTX was applied at a distant site (0.67 mm), the dilation upon ACh ejection remained unaltered at the local (45 ± 4% vs. 47 ± 8%, L-NNA + Indo vs. ChTX, respectively, P = 0.83) and remote sites (33 ± 4% vs. 30 ± 6%, P = 0.71, n = 6; Fig. 5). To check for the efficacy of the applied ChTX at the remote site, ACh was also ejected directly at this site. In marked contrast to the remote response, the dilation upon ACh ejected at the site treated with ChTX was significantly attenuated (from 51 ± 8% to 24 ± 5%, P < 0.05). However, all sites treated with ChTX dilated close to maximal (77 ± 6%) upon superfusion of SNP and adenosine (each 10 μmol/l).

In a further experimental series, IbTX was used instead of ChTX. The application of IbTX at the site of ACh stimulation significantly reduced the dilation from 45 ± 4% to 12 ± 6% (P < 0.05, n = 6). In contrast, treatment of the remote site with IbTX (distance from ACh pipette: 0.67 mm) did not alter the response at this site (30 ± 8% vs. 23 ± 5%, P = 0.53, n = 6, L-NNA + Indo and IbTX, respectively). Figure 6 depicts the responses before and after treatment with IbTX. However, IbTX did not affect dilation upon SNP and adenosine (85 ± 15%).

**DISCUSSION**

The results of this study are consistent with the hypothesis that the release and action of EDHF is a prereq-
Table 1. Site of action of EDHF, Ca^{2+}, and conducted dilations in these animals. To pinpoint the (sulfaphenazole and ODYA) strongly attenuated local different inhibitors of the On the other hand, the superfusion of two chemically not interfere with the slight if compounds were used for anesthesia that do cyclooxygenase during pentobarbital anesthesia, but only sions. The conduction of a dilation after endothelial stim-

ation was affected by inhibitors of NO synthase and tions obtained in this study are in line with these conclu-

sions. The conduction of a dilation after endothelial stim-

ulation was affected by inhibitors of NO synthase and cyclooxygenase during pentobarbital anesthesia, but only slightly if compounds were used for anesthesia that do not interfere with the P-450 monooxygenase pathway. On the other hand, the superfusion of two chemically different inhibitors of the P-450 monooxygenase pathway (sulfaphenazole and ODYA) strongly attenuated local and conducted dilations in these animals. To pinpoint the site of action of EDHF, Ca^{2+}-dependent K^+ channels were blocked by topical application of ChTX or IbTX. Applying these blockers at a conducted site did not alter the local or conducted dilation. In contrast, topical blockade at the ACh stimulation site severely attenuated the local and also the conducted response. Thus the signal transmitted along the vascular wall after stimulation with ACh is most likely a hyperpolarization induced by the opening of K^+ channels generated by a P-450 monooxygenase product at the stimulation site. Accordingly, the dilations at upstream sites were not preceded or accompanied by increases in WSR, thereby excluding a flow-induced dilation. Even more convincing is the fact that dilations were observed at remote sites at a distance of 0.67 mm with a time delay of <1 s, which excludes diffusion of mediators and can virtually only be achieved by a signal that is transmitted electrotonically. Therefore, we conclude that endothelial stimulation with ACh elicits a local hyperpolarization by the release and action of EDHF at the local site. The data suggest that this hyperpolarization is conducted to upstream sites via the endothelial and/or smooth muscle layer to induce a dilation along the arteriole. At remote sites, release and action of all endothelial factors studied are not required.

The conduction of signals along arterioles is classically studied by focal, transient application of vasomotor stimuli in the vicinity of arterioles either on isolated vessels (11, 13, 34, 35) or in the intact microcirculation (28, 31–33). In the hamster cremaster muscle microcirculation, microejection of ACh evoked not only local but also upstream vasodilations (Fig. 1). In contrast to ACh, the NO donor SNP did not initiate a remote dilation, although the local dilation was of a similar amplitude. This demonstrates that a local dilation per se is not sufficient to elicit a conducted response and the divergence between these two dilators rules out that distant responses were due to diffusion of the locally ejected vasodilator. Distal dilation might increase flow at upstream sites giving rise to flow-induced dilation, which is elicited by increases of wall shear stress (9). A comparable local dilation does not necessarily imply that local hemodynamic changes were similar in both groups. To estimate a possible contribution of flow-induced dilation at the conducted site, RBC velocities were measured simultaneously with diameter recordings. Under the assumption of an unchanged flow profile, WSR was calculated from these data. These calculated WSR values were never found to exceed control levels before or during ACh-induced dilations at remote sites (Fig. 2). Similar observations were obtained in hamster feeding arteries and in the microcirculation of rats and hamsters by other inves-

uisite to initiate a vasodilation that conducts along the vascular wall in the hamster cremaster microcirculation. Most importantly, the present data show that activation of Ca^{2+}-dependent K^+ channels, presumably by EDHF, is necessary only at the site of vessel stimulation but not at remote, distant sites. Other endothelial autacoids, namely NO and prostaglandins, are less important to initiate a conducting dilation; however, they play a role in pentobarbital-anesthetized animals. Several observations obtained in this study are in line with these conclu-

Fig. 6. Effect of regional blockade of Ca^{2+} channels by iberiotoxin (IbTX) applied in the vicinity of the arteriole either at the local (A) or the remote site (B). IbTX treatment is indicated by the gray area in the insets, which also denote the observed site of the arteriole and the site of ACh stimulation (pipette). Similar to ChTX, the application of IbTX at the ACh stimulation site abrogated the dilation at the local site (n = 6). However, the injection of IbTX at the remote site, performed in separate experiments, did not alter the conducted dilation at this site (n = 6). Thus the ability of IbTX and ChTX to affect the dilations was virtually similar, but IbTX was more effective. *Significant difference (P < 0.05) of the maximal amplitude. All experiments were done in the presence of L-NNA (30 µmol/l) and Indo (3 µmol/l) in animals anesthetized by neuroleptanalgesia. For absolute values, see Table 1.

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tigators (12, 24, 31). Because wall shear stress is directly proportional to WSR (as long as viscosity remains unchanged), it follows that the conducted responses in this setting were not evoked by mechanisms related to changes of flow or shear stress within the microcirculation.

The endothelium releases several mediators upon ACh in this preparation (7, 10). Because other endothelium-dependent vasodilators are also capable of initiating a conducted response [e.g., bradykinin, (8)], the mechanism of action is likely related to the release of an endothelial autacoid. Therefore, we used selective blockers of all known endothelial autacoids to identify their potential mediator roles. Blockade of NO synthase and cyclooxygenase diminished the local as well as remote responses upon ACh stimulation in animals anesthetized with pentobarbital. However, we have demonstrated previously that, under these conditions, the release of EDHF, which is presumably a P-450 monoxygenase product in the hamster microcirculation, is compromised (7). Therefore, an anesthetic regimen was used in another experimental series that does not inhibit the synthesis of EDHF. In this group, the blockade of NO and prostaglandin synthesis diminished the local as well as remote sites, although the response declined earlier. Furthermore, a NO donor did not elicit conducted responses, supporting the view that NO was only of minor importance; however, prostaglandins may have a role. Also, in other vascular beds [hamster cheek pouch (27)] or in experiments on isolated arteries, NO and prostaglandins (13, 33) were not crucial as mediators to evoke a conducted vasodilation. To further clarify the role of P-450 monoxygenase, this pathway was blocked by superfusing sulfaphenazole (16, 17) or ODYA. These treatments strongly attenuated the local dilation upon ACh stimulation, confirming our previous findings that EDHF is likely to be a product of the P-450 monoxygenase pathway in the hamster microcirculation (7) and isolated small arteries (4). Moreover, the data show that EDHF formation is necessary to initiate a dilation at remote sites as these responses were completely abrogated by sulfaphenazole (Fig. 4) and strongly reduced by ODYA. It is unlikely that the action of sulfaphenazole was due to blockade of gap junctions, because the spread of vascular constriction initiated by local K+ depolarization was unhindered in the presence of this compound. The crucial role of EDHF is supported by the observation that the arteriole dilates nearly synchronously at local and remote sites. Only changes of membrane potential (evoked by EDHF) are conducted sufficiently fast for this synchronicity to be observed. And, indeed, we have previously shown that in hamster arterioles, a hyperpolarization was induced by ACh but not NO (3).

In the cremaster muscle of mice and in the hamster cheek pouch microcirculation, EDHF was also involved in the generation of conducted vasodilations (22, 33). This was deduced from the inhibitory action of a P-450 monoxygenase blocker (17-ODYA). In extension of these studies, we now show that EDHF is, however, not the mediator of the dilation at remote sites. This was achieved by applying blockers of Ca2+-dependent K+ channels in a topical manner, which have been shown to be a target of EDHF in this (7) and other preparations (21, 25). The application of ChTX at the stimulation site strongly attenuated not only the local dilation but also the dilatory response at the conducted site (Fig. 5). This suggests that EDHF is released at the site of ACh application and induces, at this site, a hyperpolarization of the endothelial and/or vascular smooth muscle cells. In the absence of an activation of Ca2+-dependent K+ channels at the stimulation site, a conduction of the response was not found. On the contrary, the blockade of these channels at the remote site did not affect the dilation at this site and the local dilatory response was also preserved (Fig. 5). The inability of ChTX to block remote dilations after topical application at these sites was not due to an insufficient blockade. A sufficient K+ channel blockade was clearly demonstrated by the strong attenuation of the dilation in response to ACh directly applied at this site. Virtually similar results were obtained with IbTX (Fig. 6), a more potent and more specific blocker of the large-conductance Ca2+-dependent K+ channel (19), further emphasizing the critical role of the activation of Ca2+-dependent K+ channels at the site of ACh stimulation.

The fact that the dilations at distant sites are not diminished despite at least partial blockade of Ca2+-dependent K+ channels as well as sufficient inhibition of NO synthase and cyclooxygenase raises the question of how this remote dilation is accomplished. The present data suggest that hyperpolarization is conducted via the smooth muscle and/or endothelial layer to these sites and is sufficient to induce a dilatory response. In very recent studies, it has been elegantly demonstrated that local electrical activation and hyperpolarization of the endothelium is sufficient to induce a dilation that conducts along the vascular wall (14, 15). For the signal transmission along the arterioles and possibly amplification, the activation of inward rectifier K+ channels has been shown to be crucial in coronary arterioles (29). Such a mechanism may also be involved in this preparation. However, the initial local hyperpolarization necessary to evoke a signal that travels along the arteriole seems to be achieved by the focal EDHF release and action as shown here. Whether EDHF hyperpolarizes not only the smooth muscle but also the endothelium remains to be elucidated.

In summary, we have shown that a dilator that evokes release of a factor(s) activating Ca2+-dependent K+ channels elicited conducted vasodilations. The activation of these K+ channels at distant sites, however, was not necessary for this response to be observed. We conclude that a local change of membrane potential is required to initiate a remote vasomotor response, which, in the case of ACh, is generated by EDHF at the ACh application site. This suggests that the role of EDHF is not only to act as a compensatory mechanism, e.g., in case of impaired NO formation, but also serves as a critical endothelial autacoid to coordinate arteriolar behaviour in the microcirculatory network. Inasmuch as skeletal muscle con-
traction may release EDHF or other hyperpolarizing factors, this may explain conducted dilation as observed after skeletal muscle fiber activation (2) and contribute to active hyperemia.

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