

Heterogeneity of Endothelium-Dependent Vasodilation in Pressurized Cerebral and Small Mesenteric Resistance Arteries of the Rat¹

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ABSTRACT

We compared endothelial responses to calcium-mobilizing agents in mesenteric and cerebral resistance arteries of the rat. Middle cerebral and small mesenteric arteries were mounted in a pressure myograph, and myogenic responses were recorded. The effects of acetylcholine (ACh), bradykinin, substance P, histamine, A23187, cyclopiazonic acid (CPA), and sodium nitroprusside were investigated in both arteries with myogenic tone in the absence and presence of nitric oxide synthase and cyclooxygenase inhibitors. The effects of raised potassium, K⁺ channel blockers, and arachidonic metabolism inhibition were examined on the nitric oxide (NO) synthase/cyclooxygenase inhibitor-resistant dilation induced by ACh and CPA. Cerebral arteries display a high level of myogenic reactivity compared with mesenteric arteries. In cerebral arteries, only bradykinin and substance P induced endothelium-dependent dilation. The observed dilation was solely related to the activation of the NO

pathway. However, in mesenteric arteries, all of the vasoactive agents induced endothelium-dependent dilation. A combination of NO, cyclooxygenase-derived prostanoids, and a factor with endothelium-derived hyperpolarizing factor-like properties was responsible for the observed vasodilation. NO and cyclooxygenase derivatives were able to compensate for each other in the CPA-induced endothelium-dependent vasodilation when one of the two pathways was blocked. Moreover, small Ca²⁺-activated K⁺ channels and a combination of both large and small Ca²⁺-activated K⁺ channels were implicated in the endothelium-derived hyperpolarizing factor-like component of dilation to ACh and CPA, respectively. Finally, the results suggest that the pathway by which agonists raise intracellular calcium concentration may determine the nature of the endothelial secretory product.

Heterogeneity in the responsiveness of blood vessels is to a large extent related to the variability in the types and densities of pharmacological receptors and ion transport mechanisms of smooth muscle (Mulvany and Aalkjær, 1990). In addition, there are a number of reports indicating that endothelial secretions may vary, depending on the size and location of the artery (Hwa et al., 1994; Clark and Fuchs, 1997). The vascular endothelium can release contracting factors (Miller and Vanhoutte, 1985) and relaxing factors such as nitric oxide (NO), prostacyclin, and endothelium-derived hy-

perpolarizing factor (EDHF; Moncada et al., 1977; Furchgott and Zawadzki, 1980; Rubanyi and Vanhoutte, 1987). The release of EDHF from the endothelium is triggered by agonists such as acetylcholine (ACh), bradykinin (BK), histamine (Hist), or substance P (SP), and has been demonstrated in various arteries from different species (for a review, see Mombouli and Vanhoutte, 1997). The identity of EDHF has not yet been established, but its action is believed to occur via the activation of K⁺ channels in vascular smooth muscle cells. However, the types of K⁺ channels stimulated and the endothelial secretion responsible for this stimulation seem to vary according to the preparation investigated (for reviews, see Mombouli and Vanhoutte, 1997; Vanhoutte, 1998).

To further complicate matters, NO causes hyperpolarization in rabbit carotid artery (Cohen et al., 1997) and activates

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ABBREVIATIONS: NO, nitric oxide; 4-AP, 4-aminopyridine; A23187, calcium ionophore; ACh, acetylcholine; BK, bradykinin; BK_{Ca}, large conductance Ca²⁺-activated K⁺ channels; ChTX, charybdotoxin; COX, cyclooxygenase; CPA, cyclopiazonic acid; DHO, dihydroouabain; ER, endoplasmic reticulum; Hb, oxyhemoglobin; Hist, histamine; IbtX, iberiotoxin; Indo, indomethacin; [Ca²⁺]_i, intracellular calcium concentration; K_{ATP}, ATP-activated K⁺ channels; KPSS, high potassium physiological salt solution; EDHF, endothelium-derived hyperpolarizing factor; L-NAME, N^ω-nitro-L-arginine methyl ester; NOS, nitric oxide synthase; OOPC, oleyloxyethylphosphorylcholine; PE, phenylephrine; PSS, physiological salt solution; SK_{Ca}, small conductance Ca²⁺-activated K⁺ channels; SNP, sodium nitroprusside; SP, substance P; TEA, tetraethylammonium; 17-ODYA, 17-octadecyloxy acid.

large conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) in arterial smooth muscle cells (Bolotina et al., 1994; Weidelt et al., 1997) and ATP-activated K^+ channels (K_{ATP} ; Murphy and Brayden, 1995; Weidelt et al., 1997) in vascular smooth muscle. In the mesenteric vascular bed, including the main mesenteric artery and its smaller branches, the action of EDHF is not inhibited by K_{ATP} blockers (McPherson and Angus, 1991). The inhibitory influence of charybdotoxin (ChTX) suggests rather the involvement of BK_{Ca} (Hwa et al., 1994). Some studies performed in bovine, porcine, and rat coronary arteries suggest that EDHF may be a metabolite of arachidonic acid, derived from cytochrome P-450-dependent monooxygenase (Hecker et al., 1994). More recently, EDHF has been described as K^+ in small resistance arteries of rat (Edwards et al., 1998). These observations emphasize the complexity of vascular endothelial secretions as well as the need to further explore the contribution of EDHF when both the tissue and the agonist are varied. Another unresolved question is whether the same EDHF released from different arteries targets different K^+ channels depending on the location of the smooth muscle or whether there are multiple EDHFs, each targeting a specific K^+ channel.

Comparative studies on resistance arteries have revealed that cerebral arteries differ pharmacologically and electrophysiologically from arteries in other areas. For example, cerebral arteries are more sensitive than peripheral arteries to dihydropyridine Ca^{2+} antagonists and agonists (Asano et al., 1993) and feature prominent coupling between electrical and mechanical events (Harder and Waters, 1984). Studies examining heterogeneity in isolated vessels have mainly been performed in large systemic arteries. To date, no study has compared endothelial function in cerebral and peripheral resistance arteries.

Thus, experiments were designed to compare the endothelial responses of cerebral resistance and small mesenteric arteries to a range of vasoactive agents. The relative contribution of EDHF to the total endothelium-mediated relaxation as a function of the mechanism whereby the stimulus enhances the cytoplasmic calcium concentration is explored. A unique set of targets for EDHF released from the endothelium of small mesenteric arteries is identified based on the experiments performed by using different K^+ channel inhibitors. To recreate relevant physiological conditions, arteries were constricted by pressure rather than agonists. For this reason, it was important to initially compare the tone induced by pressure in both types of resistance vessels before studying the effects of different endothelial agonists.

The present study provides evidence for a role of EDHF in activating small conductance Ca^{2+} -activated K^+ channels (SK_{Ca}), BK_{Ca} , but not K_{ATP} .

Materials and Methods

Vessel Isolation and Cannulation. Male Sprague-Dawley rats (200–300 g) were anesthetized with i.p. injections of sodium pentobarbital (Somnotol; 30 mg/kg; MCT Pharmaceutical, Cambridge, Ontario, Canada) and heparin (Hepalean; 500 U/kg; Organon-Teknika, Toronto, Canada) and then sacrificed by decapitation. The brain or small intestine with attached mesentery was excised and transferred to a dissection dish filled with physiological salt solution (PSS) at 4°C. Distal middle cerebral or small mesenteric (third- or fourth-generation) arteries were dissected from surrounding connec-

tive tissues and transferred to the experimental chamber of an arteriograph filled with oxygenated PSS at 37°C.

Each vessel was tied onto a proximal glass microcannula with a tip diameter of 60 to 80 μm using single strands (20 μm) of 4-0 braided nylon suture; the perfusion pressure was then gently raised to clear the vessel of blood. The distal end of the artery was similarly mounted to the outflow microcannula. After several minutes of perfusion, the distal outflow cannula was closed, and the transmural pressure was slowly increased 80 mm Hg by using an electronic pressure servo system (Living Systems, Burlington, VT).

The PSS in the vessel chamber was continuously recirculated by superfusion around the pressurized artery at a flow of 20 to 25 ml/min passing through an external reservoir that was bubbled with a gas mixture of 95% O_2 /5% CO_2 . A heating pump connected to a glass heat exchanger warmed the PSS to 37°C, and a pH microprobe was positioned in the bath. pH was maintained at 7.4 ± 0.04 by adjustment of the reservoir gassing rate.

The arteriograph containing a cannulated pressurized artery was placed on the stage of an inverted microscope with a monochrome video camera attached to a viewing tube, and was allowed to equilibrate for 60 min. Arterial dimensions were measured using a video system that provides automatic continuous readout measurements of luminal diameter and wall thickness. The information is updated every 17 ms, and the precision of the diameter measurement is within 1%. A more technical description of the principle of the components of the system and a schematic drawing of the instrumentation are provided elsewhere (Halpern et al., 1984). Cerebral and mesenteric arterial myogenic tone developed spontaneously and consistently during equilibration, resulting in significantly reduced luminal diameter. Once attained, it remains stable for hours unless perturbed by changes in transmural pressure or the addition of vasoactive compounds (Skarsgard et al., 1997).

In some experiments, the endothelium was removed by intraluminal perfusion with 0.5% 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate for 30 s. The presence of functional endothelium was assessed in all preparations by the ability of 0.1 μM BK (cerebral arteries) or 1 μM ACh (small mesenteric arteries) to induce dilation.

Myogenic Tone. Microvessels with a diameter below 125 μm (70–120 μm) from cerebral or mesenteric beds were studied to identify region-specific differences in myogenic profile. After the development of myogenic tone during the equilibrium period (60 min), the relation between pressure and vessel diameter was studied. Intravascular pressure was decreased to 10 mm Hg and then raised in 10-mm-Hg steps from 10 to 120 mm Hg, while measuring corresponding changes in vessel diameter. At each step, diameter was monitored for 5 to 10 min until steady state was achieved. The protocol was repeated, and the results were averaged.

Endothelial Heterogeneity. To identify heterogeneity in receptor- and nonreceptor-mediated vasoactivity between cerebral and mesenteric vascular beds, responses to ACh (1 μM), BK (0.1 μM), SP (0.01 μM), Hist (3 μM), calcium ionophore (A23187; 0.1 μM), cyclopiazonic acid (CPA; 20 μM), an inhibitor of endoplasmic reticulum (ER) Ca^{2+} -ATPase, and sodium nitroprusside (SNP; 10 μM), an exogenous NO donor, were investigated. A pilot study determined that these concentrations yielded a maximum response in both cerebral and small mesenteric arteries. Vasoactive compounds were added to the circulating buffer at the final concentrations reported above, and changes in luminal diameter were measured. The choice and the order of vasoactive agent exposure to the vessels were randomized so that treatment of the vessel with a particular agent would not influence the subsequent response to another.

Signaling in Endothelial Heterogeneity. To test the role of NO in the vasodilation induced by various agents, N^{ω} -nitro-L-arginine methyl ester (L-NAME; 200 μM), a competitive inhibitor of constitutive and inducible nitric oxide synthase (NOS) isoforms, was added to the superfusing buffer and allowed to circulate for 20 min until a new steady-state diameter was reached. This was followed by reas-

assessment of the vasodilation due to the agents used. Otherwise, in the two preparations (cerebral and mesenteric arteries), the same vessels were challenged by two vasoactive agents in the absence and presence of L-NAME. Several vessels were used as controls (by omitting the addition of L-NAME in an otherwise identical protocol) to verify that the diameter responses to a vasoactive agent (ACh, BK, SP, Hist, A23187, CPA, SNP) did not decrease with repeated exposure or with time. The effects of NO scavenging were also assessed in vessels challenged with ACh or CPA using oxyhemoglobin (Hb; 10 μ M) at the concentration previously used in small mesenteric artery of the rat (Hwa et al., 1994).

Similarly, effects of cyclooxygenase (COX) blockade and depolarization [0 or 40 mM high-potassium physiological salt solution (KPSS)] were investigated in vessels challenged with ACh and CPA. Increasing concentrations of ACh (10^{-8} to 10^{-3} M) and CPA (10^{-8} to 10^{-3} M) were added to arteries in the absence and in the presence of the COX inhibitor indomethacin (Indo; 10 μ M), used at a maximally active concentration. ACh or CPA effects were also investigated in normal PSS, in K^+ -free PSS, or in 40 mM KPSS (i.e., in which NaCl was substituted for an equimolar concentration of KCl) in the presence of Indo plus L-NAME. K^+ -free or 40 mM KPSS causes a fixed depolarization of the vascular smooth muscle; this technique prevents cell hyperpolarization as a signaling event in ACh- or CPA-induced vasodilation.

Dilation Resistant to Indo plus L-NAME. The possible involvement of K^+ channels in the vasodilation induced by ACh and CPA in myogenically active vessels was assessed in normal PSS containing L-NAME and Indo. In these experiments, K^+ channel blockers were used at the concentrations known to selectively inhibit specific K^+ channels; tetraethylammonium (TEA; 3–5 mM), charybdotoxin (ChTX; 100–150 nM), and iberiotoxin (IbTX; 30 nM) inhibited BK_{Ca} , apamin (0.3 μ M) inhibited SK_{Ca} , glibenclamide (10 μ M) inhibited K_{ATP} , 4-aminopyridine (4-AP; 100 μ M) inhibited voltage-gated K^+ channels, and $BaCl_2$ (30 μ M) inhibited inward rectifier K^+ channels.

Similarly, possible roles of arachidonic acid metabolites and activation of Na^+ , K^+ -ATPase in the L-NAME plus Indo-resistant dilation induced by ACh and CPA were investigated using the following inhibitors: quinacrine (10–50 μ M) and oleyloxyethylphosphorylcholine (OOPC; 1–10 μ M) are inhibitors of phospholipase A_2 ; SKF 525a (10–100 μ M), clotrimazole (10–30 μ M), and 17-octadecynoic acid (17-ODYA; 20, 40, 50 μ M) are inhibitors of cytochrome P-450; and dihydroouabain (DHO; 30 μ M) is a Na^+ , K^+ -ATPase inhibitor.

To obtain a similar level of tone in myogenically active vessels (20–30% of decrease in diameter), phenylephrine (PE; 10^{-9} or 10^{-8} M) was added to some small mesenteric arteries when L-NAME was used in combination with Indo plus one or several of the inhibitors. In cerebral arteries, PE was not added. In addition, none of the preliminary studies of the vasoreactivity of different agents involves the use of PE in the two preparations. Parallel control experiments of the effects of CPA or ACh on myogenic tone (in small mesenteric arteries) in the presence of PE did not influence subsequent relaxation to these agents compared with those obtained in the absence of PE (myogenic tone solely). All the inhibitors were incubated with the myogenically active vessel for 20 min before ACh or CPA was added.

At the conclusion of each experiment, the superfusion solution was changed to a calcium-free PSS that contained 2 mM EGTA and no $CaCl_2$. Vessels were incubated for 20 min, and then the pressure steps were repeated to obtain the “passive” diameter of each vessel at each pressure value to calculate the percentage of myogenic constriction.

Expression of Results and Statistical Analysis. Myogenic tone at each pressure was expressed as percent decrease in diameter from the “passive” diameter or

$$\text{Percent constriction} = 100\% \times [(D_{Ca-free} - D_{PSS})/D_{Ca-free}] \quad (1)$$

where D is the arterial diameter in Ca^{2+} -free buffer or PSS.

Vasodilator responses were expressed as percent increase in di-

ameter from the initial diameter (due to myogenic tone) at the corresponding pressure.

$$\text{Percent dilation} = 100\% \times [(D_X - D_{mt})/(D_{Ca-free} - D_{mt})] \quad (2)$$

where D is the measured arterial diameter, and subscripts X, mt, and Ca-free denote arterial diameters at each dose of agonist (X), initially (mt), and in Ca^{2+} -free buffer (Ca-free).

Wall tension was calculated using the Laplace relation:

Wall tension = transmural pressure \times vessel radius,

$$\text{with } 1 \text{ mm Hg} = 1.33 \times 10^{-4} \text{ N/m}^2 \quad (3)$$

All results are expressed as mean \pm S.E.M of *n* experiments. One vessel was taken from each animal. Statistical evaluation was done (ANOVA). Mean values were considered significantly different when $P < .05$.

Drugs and Solutions. The ionic composition of the PSS consisted of 119 mM NaCl, 4.7 mM KCl, 1.18 mM KH_2PO_4 , 24 mM $NaHCO_3$, 1.17 mM $MgSO_4 \cdot 7H_2O$, 1.6 mM $CaCl_2$, 5.5 mM glucose, and 0.026 mM EDTA. ACh, A23187, 4-AP, barium chloride, BK, ChTX, clotrimazole, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate, DHO, glibenclamide, Hb, Hist, Indo, L-NAME, PE, TEA, 17-ODYA, and SKF 525a were purchased from Sigma (Ontario, Canada). Apamin, CPA, IbTX, OOPC, and SNP were purchased from Calbiochem (San Diego, CA). Quinacrine was purchased from Research Biochemicals, Inc. (Natick, MA). Stock solutions were diluted in deionized water (NANOpure). CPA, Indo, and glibenclamide were prepared in dimethyl sulfoxide. Clotrimazole, 17-ODYA, and SKF 525a were dissolved in absolute ethanol. Reduced Hb was prepared by treatment of Hb solution with sodium dithionite according to Martin et al. (1985). The effects of ethanol and other solvents were tested, and none of the vehicle solutions altered the pressure-diameter relation or the vascular responses to norepinephrine and ACh.

Results

Myogenic Tone of Middle Cerebral and Small Mesenteric Arteries. Cerebral arteries of all sizes developed graded myogenic constrictions over the physiological pressure range. The steady-state response of distal middle cerebral arteries (range, 70–120 μ m; mean, $96.1 \pm 18.2 \mu$ m; $n = 7$) to stepwise intraluminal pressure under zero-flow conditions is shown in Fig. 1A. A maximum constriction ($25.64 \pm 1\%$) was obtained at 60 mm Hg. Beyond this point, further increases in intravascular pressure (up to 120 mm Hg) did not significantly change the diameter. Thus, maximal myogenic responsiveness was identified at an intravascular pressure of approximately 60 mm Hg in cerebral arteries of the rat. No statistical difference was observed in the tone of middle cerebral arteries at 60 mm Hg in the absence ($26.8 \pm 4.92\%$) and presence ($25.6 \pm 1\%$) of the endothelium.

The steady-state response of mesenteric resistance-sized arteries (mean, $100.5 \pm 19.3 \mu$ m, $n = 7$) to stepwise intraluminal pressure is shown in Fig. 1A. Arteries constricted by $19.47 \pm 0.38\%$ when pressure was increased from 40 to 80 mm Hg. Further increases in pressure did not significantly enhance the diameter change, suggesting that maximal myogenic responsiveness occurs at an intravascular pressure of 80 mm Hg in these arteries. In small mesenteric arteries, the percentage of constriction induced at 80 mm Hg was not significantly different in vessels without endothelium ($21.1 \pm 7.2\%$) and with endothelium ($19.47 \pm 0.38\%$). The presence of 10 μ M tetrodotoxin (a highly selective blocker of Na^+ channels that is essential for propagation of neural impulses) did

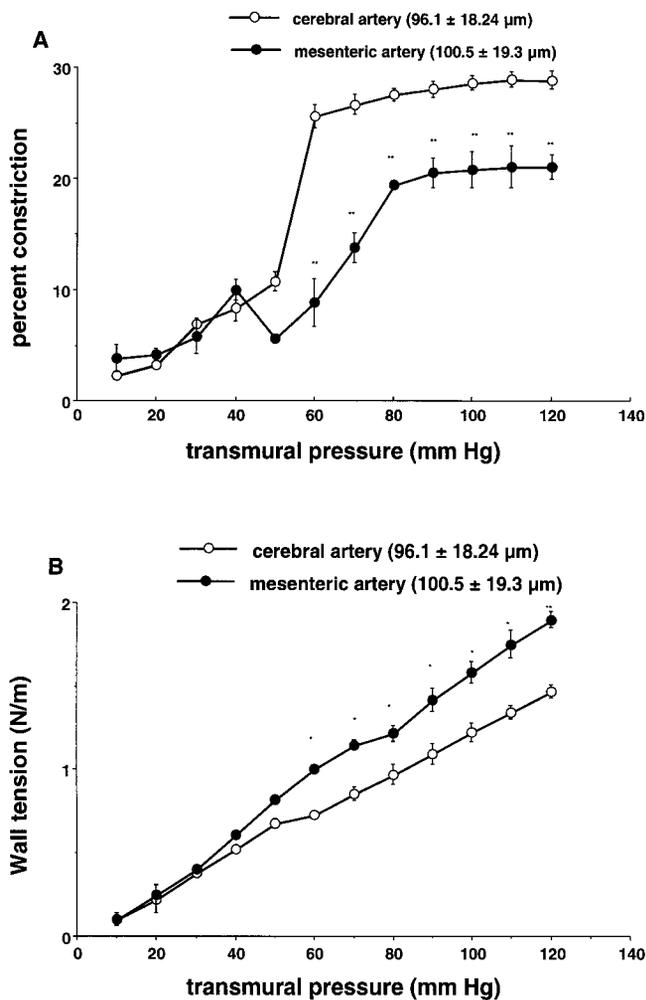


Fig. 1. Myogenic tone (A) and wall tension (B) in rat distal middle cerebral and small mesenteric resistance-sized arteries. The points are mean \pm S.E.M. (vertical bars) of 14 experiments. * $P < .05$, ** $P < .01$, *** $P < .001$, significantly different compared with distal middle cerebral arteries.

not affect pressure-dependent active tone in resistance (cerebral, $24.7 \pm 8.5\%$; mesenteric, $20.6 \pm 1.8\%$) arteries at 60 and 80 mm Hg, respectively.

These results were reinforced by expressing the wall tension as a function of transmural pressure (Fig. 1B). The results showed a significant difference in wall tension between cerebral and mesenteric arteries at pressures from 80 to 120 mm Hg. Wall tension was greater in mesenteric arteries at higher pressures compared with cerebral arteries; these results demonstrate greater myogenic responsiveness of cerebral arteries.

Vasomotion of Middle Cerebral and Small Mesenteric Arteries. Figure 2A shows the responses of cerebral arteries to several vasoactive agents. Only BK, SP, and SNP induced vasodilation; notably, ACh failed to induce significant dilation. Control experiments using a distal middle cerebral artery (approximately 100 μm) and proximal middle cerebral artery (approximately 300 μm) showed that the former was able to dilate modestly (0–5%) and the latter to a greater degree to ACh (1, 10 μM). However, in the same arteries, BK (0.1 μM) or SP induced somewhat greater dilation (20–50%). Hist, A23187, and CPA all caused vasoconstriction. In the presence of L-NAME, the dilations induced

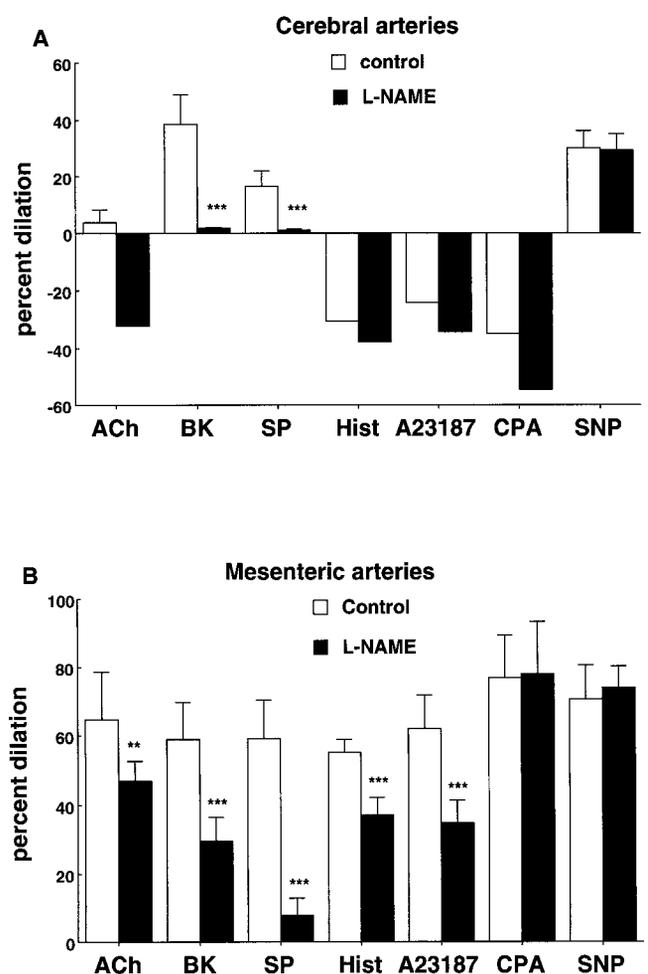


Fig. 2. Comparative effects of agonists and vasoactive agents in rat (A) distal middle cerebral and (B) small mesenteric arteries with myogenic tone. Histograms show the responses induced by ACh (1 μM), BK (0.1 μM), SP (0.01 μM), Hist (3 μM), A23187 (0.1 μM), CPA (20 μM), and SNP (10 μM) in the absence (open columns) and in the presence of 200 μM L-NAME (filled columns). The points are mean \pm S.E.M. (vertical bars) of seven experiments. ** $P < .01$, *** $P < .001$, significantly different from the percentage of the dilation obtained in the absence of L-NAME.

by BK and SP were abolished, whereas ACh induced a contraction. Hist, A23187, and CPA all caused greater vasoconstriction in the presence of L-NAME, whereas the SNP-induced dilation was not significantly different from control.

Figure 2B shows the effects of various vasoactive agents on myogenically active small mesenteric arteries. In contrast to the effects on cerebral arteries, all the agents tested induced dilation. L-NAME attenuated dilation to all agents except for CPA and SNP. However, the attenuation by L-NAME was only partial. Endothelium removal completely abolished ACh and CPA dilations (Table 2). Similar results were obtained with thapsigargin (10 μM), another inhibitor of sarcoplasmic reticulum Ca^{2+} -ATPase (not shown).

Vasodilation Resistant to NOS Inhibitor in Mesenteric Arteries. Because the L-NAME-resistant components of ACh and CPA vasodilation could be due to endothelial production of prostacyclin, Indo was used to inhibit COX (Figs. 3 and 4). L-NAME alone ($P < .01$) and Indo alone ($P < .05$) partially inhibited the dilation induced by ACh (Fig. 5A). The combination of L-NAME plus Indo is not greater than L-NAME alone or Indo alone (Table 1, Figs. 3 and 5A). In

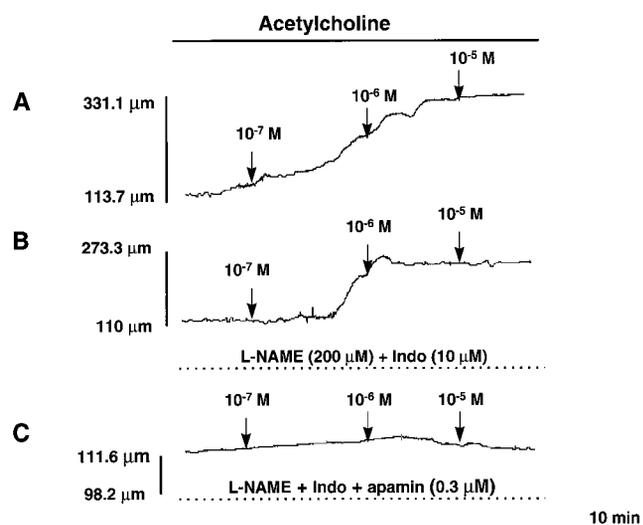


Fig. 3. Mechanisms of vasodilation independent of NO/COX pathway induced by ACh in rat small mesenteric arteries pressurized at 80 mm Hg. Representative diameter traces of five to seven experiments. ACh concentration-response curves (10^{-7} to 10^{-5} M) in small mesenteric arteries of the rat with myogenic tone at 80 mm Hg in the absence (A) and in the presence of either 200 μ M L-NAME plus 10 μ M Indo (B) or L-NAME plus Indo plus 0.3 μ M apamin (C).

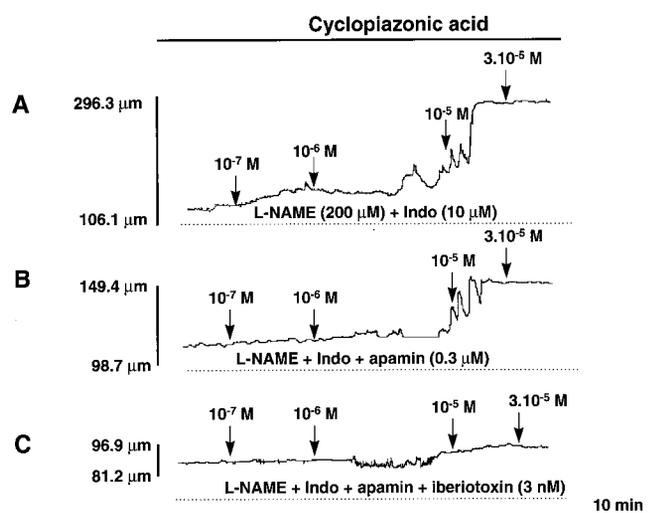


Fig. 4. Mechanisms of vasodilation independent of the NOS/COX pathways induced by CPA in rat small mesenteric arteries pressurized at 80 mm Hg. Representative diameter traces of five to seven experiments. CPA concentration-response curves (10^{-7} to 3.10^{-5} M) in rat small mesenteric arteries with myogenic tone at 80 mm Hg in the presence of 200 μ M L-NAME plus 10 μ M Indo (A), L-NAME plus Indo plus 0.3 μ M apamin (B), and L-NAME plus Indo plus apamin plus 30 nM IbTX (C).

contrast, Indo alone, L-NAME alone, Hb alone, or the combination of L-NAME plus Hb did not affect the dilation produced by CPA (Fig. 6A). Only the combination of Indo plus L-NAME partially affected CPA-induced dilation of small mesenteric arteries (Table 1 and Fig. 6A).

Vascular smooth muscle cell depolarization (0 or 40 mM KCl) has been used to determine whether this could abolish the dilatation resistant to NOS and COX inhibitors. Changes in K^+ concentration (4.7–40 mM) produced an increase in contraction of the arteries ($-26.66 \pm 7.63\%$; $n = 4$). Exposure to 0 or 40 mM KPSS in the continuous presence of L-NAME plus Indo abolished the vasodilation induced by ACh and CPA (Table 2). Likewise, removal of the endothelium also

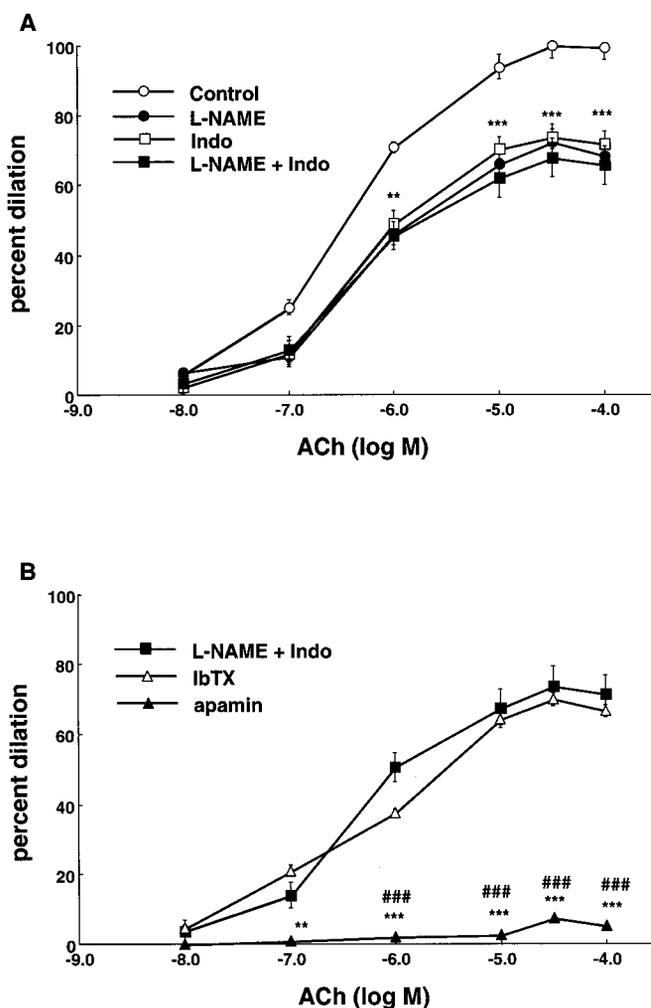


Fig. 5. Mechanisms of ACh-induced vasodilation resistant to L-NAME and Indo in myogenically active small mesenteric arteries of the rat. A, concentration-response curves to ACh in small mesenteric arteries of the rat pressurized at 80 mm Hg in the absence (\circ) and in the presence of 200 μ M L-NAME (\bullet), 10 μ M Indo (\square), and L-NAME plus Indo (\blacksquare). B, concentration-response curves to ACh in small mesenteric arteries of the rat pressurized at 80 mm Hg in the presence of L-NAME plus Indo (\blacksquare), L-NAME plus Indo plus 30 nM IbTX (\triangle), and L-NAME plus Indo plus 0.3 μ M apamin (\blacktriangle). The points are mean \pm S.E.M. (vertical bars) of five to seven experiments. $**P < .01$, $***P < .001$, significantly different compared with the percentage of the dilation induced by ACh in the absence of any drugs. $##P < .01$, $###P < .001$, significantly different compared with the percentage of the dilation induced by ACh in the presence of L-NAME plus Indo.

abolished this dilation (Table 2). The dilation induced by SNP ($70.78 \pm 9.9\%$, $n = 3$ in the presence of endothelium) was preserved after exposure to 40 mM KPSS ($66.01 \pm 3.32\%$, $n = 3$) or in the absence of endothelium ($72.26 \pm 7.9\%$, $n = 3$). In addition, agonists such as SP, BK, and Hist induced dilation of mesenteric resistance arteries preconstricted by 40 mM KPSS by $83.15 \pm 2.3\%$ ($n = 5$), $46.32 \pm 4.1\%$ ($n = 5$), and $29.3 \pm 2.8\%$ ($n = 5$), respectively.

EDHF Signaling. Blockade of the BK_{Ca} channel with IbTX did not abolish the L-NAME plus Indo-resistant vasodilation to ACh or CPA (Table 3, Figs. 5B and 6B). Similar results were obtained using other K^+ -channel blockers (TEA, ChTX, BaCl₂, 4-AP, glibenclamide; Table 3). Only apamin was effective in reducing the L-NAME plus Indo-resistant vasodilation induced by ACh (Figs. 3C and 5B) or CPA (Figs.

TABLE 1

Effects of NO, COX inhibition, and Hb on 20 μ M CPA and 1 μ M ACh-induced dilation in rat small mesenteric arteries with myogenic tone at 80 mm Hg

Treatment	CPA	ACh
	% dilation	
Control	96.87 \pm 3.21	96.83 \pm 3.01
+L-NAME (200 μ M)	96.74 \pm 2.98	66.53 \pm 8.01 ^b
+Indo (10 μ M)	95.6 \pm 3.23	67.92 \pm 10.56 ^a
+Hb (10 μ M)	95.34 \pm 2.66	
+L-NAME + Indo	74.31 \pm 12.11 ^b	62.18 \pm 3.73 ^c
+L-NAME + Hb	92.93 \pm 2.61	

Values are mean \pm S.E.M. of four to six experiments.

^a $P < .05$, ^b $P < .01$, ^c $P < .001$, significantly different compared with the corresponding control values.

4B and 6B); the combination of apamin and IbTX resulted in near-complete inhibition of vasodilation (Figs. 4C and 6B).

Quinacrine, OOPC, 17-ODYA, SKF 525a, clotrimazole, and DHO had no effect on the L-NAME plus Indo-resistant dilation to CPA. In small mesenteric arteries, 17-ODYA and OOPC blocked myogenic tone only at higher concentrations (data not shown). In this case, PE (10^{-8} to 10^{-7} M) was added in the superfusion bath to boost the tone to study the mechanisms of the dilation produced by ACh and CPA.

Discussion

Cerebral arteries of the rat display a higher level of myogenic reactivity compared with mesenteric arteries of comparable size. Johnson (1980) proposed that the stimulus for myogenic tone is wall tension rather than hydrostatic pressure. According to this hypothesis, myogenic constriction decreases wall tension and thus reduces the stimulus for further constriction. As shown in Fig. 1, the greater myogenic constriction in cerebral arteries was able to maintain a lower wall tension than was observed in the mesenteric arteries. The mechanism underlying this difference may be related to a higher state of activation of L-type voltage-dependent Ca^{2+} channels in cerebral arteries compared with peripheral arteries (Asano et al., 1993). Furthermore, in the cerebral circulation, both large and small arteries are important resistance vessels (Faraci, 1991). The marked myogenic tone coupled with the parallel arrangement of cerebral vessels would promote regional regulation of flow with minimal overall changes in blood volume. In contrast in the mesenteric vascular bed, resistance is mainly determined by the small arteries, which are arranged in series with the conduit arteries.

In myogenically active cerebral arteries, BK and SP produced endothelium-dependent dilation, which was completely inhibited by L-NAME, indicating the release of NO. This finding is consistent with previous studies performed in different types of cerebral arteries (Faraci and Brian, 1994; Zimmermann et al., 1997). In basilar artery of rat, Hist and A23187 induced endothelium-dependent dilation (Faraci and Brian, 1994), which was not the case in the present study. These results might indicate that there are regional differences in endothelial function within the same vascular bed. Alternatively, the observed discrepancy may be related to differences in smooth muscle sensitivity to the vasoactive agents.

For comparison with middle cerebral arteries, we chose small mesenteric arteries of similar size, which may also

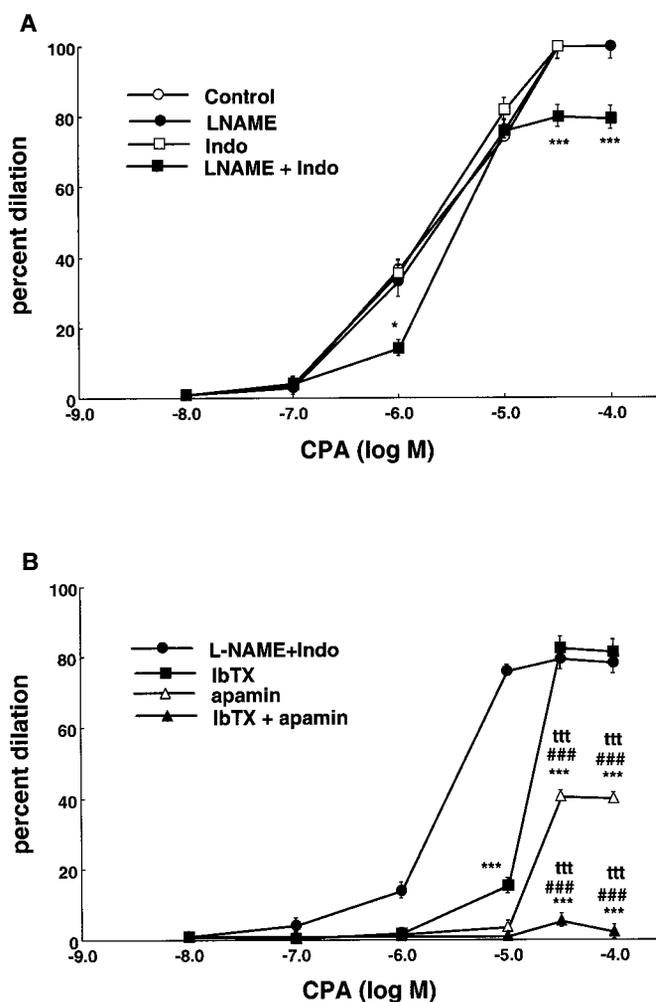


Fig. 6. Mechanisms of CPA-induced vasodilation resistant to L-NAME and Indo in rat small mesenteric arteries with myogenic tone at 80 mm Hg. A, concentration-response curves to CPA in small mesenteric arteries of the rat pressurized at 80 mm Hg in the absence (○) and in the presence of 200 μ M L-NAME (●), 10 μ M Indo (□), and L-NAME plus Indo (■). B, concentration-response curves to CPA in small mesenteric arteries of the rat pressurized at 80 mm Hg in the presence of L-NAME plus Indo (●), L-NAME plus Indo plus 30 nM IbTX (■), L-NAME plus Indo plus 0.3 μ M apamin (△), and L-NAME plus Indo plus IbTX plus 0.3 μ M apamin (▲). The points are mean \pm S.E.M. (vertical bars) of five to seven experiments. * $P < .05$, *** $P < .001$, significantly different compared with the percentage of the dilation induced by CPA in the absence of any drugs. ## $P < .01$, ### $P < .001$, significantly different compared with the percentage of the dilation induced by CPA in the presence of L-NAME plus Indo. ttt $P < .001$, significantly different compared with the percentage of the dilation induced by CPA in the presence of L-NAME plus Indo plus IbTX.

contribute to peripheral resistance. However, reports on their endothelial function are sparse and somewhat controversial (Chen and Cheung, 1997; Weidelt et al., 1997). Our results show that all the vasoactive agents tested produced endothelium-dependent dilations of small mesenteric arteries. As in cerebral arteries, SP-induced vasodilation appeared to be mediated by NO. However, dilation in response to CPA was due to NO-independent pathways, whereas responses to ACh, BK, Hist, and A23187 were due to activation of both NO-dependent and -independent pathways.

The existence of an NOS/COX inhibitor-resistant vasodilation in small mesenteric resistance arteries suggests an alternative pathway, as reviewed elsewhere (Moubouli and Vanhoutte, 1997). Although direct electrophysiological mea-

TABLE 2

Effects of K⁺-free PSS (0 mM KPSS), 40 mM KPSS, and the removal of endothelium (-E) on the L-NAME plus Indo-resistant effects of 20 μM CPA and 1 μM ACh in small mesenteric resistance arteries of the rat pressurized at 80 mm Hg

Treatment	CPA	ACh
	% dilation	
PSS (L-NAME + Indo)	74.31 ± 12.11	63 ± 3.55
+0 mM KPSS	1.67 ± 0.85*	-35 ± 10.03*
+40 mM KPSS	-44.55 ± 9.42*	-48.43 ± 2.71*
+(-E)	-36.41 ± 1.53*	-62.89 ± 3.6*

Values are mean ± S.E.M. of three to five experiments.

* *P* < .001 significantly different compared with control values. Negative values correspond to contraction.

TABLE 3

Effects of K⁺ channels inhibitors on 200 μM LNAME plus 10 μM Indo-resistant effects of 20 μM CPA and 1 μM ACh on myogenically active rat small mesenteric resistance arteries pressurized at 80 mm Hg

Treatment	CPA	ACh
	% dilation	
L-NAME + Indo	74.31 ± 12.11	62.18 ± 3.73
+Glybenclamide	74.11 ± 10.6	78.06 ± 14.34
+BaCl ₂	74.49 ± 18.44	
+4-AP	84.95 ± 7.29	
+TEA	70.67 ± 8.02	
+IbTX	81.1 ± 3.92	59.33 ± 1.15
+ChTX	82.97 ± 5.97	
+Apamin	16.42 ± 2.77 ^a	-50.98 ± 3.52 ^a
+Apamin + IbTX	2.5 ± 0.71 ^{a,b,c}	

Values are mean ± S.E.M. of three to six experiments.

^a *P* < .001, significantly different compared with the vasodilation obtained in the presence of L-NAME plus Indo.

^b *P* < .001, significantly different compared with the dilation obtained in the presence of L-NAME plus Indo plus IbTX.

^c *P* < .001, significantly different compared with the dilation obtained in the presence of L-NAME plus Indo plus apamin plus IbTX.

Negative values correspond to constriction.

surements have not been recorded, the following data strongly support the release of EDHF in the ACh- and CPA-induced vasodilation.

First, CPA- and ACh-induced vasodilation remaining in the presence of L-NAME plus Indo is abolished by high K⁺ solution. This finding indicates that hyperpolarization is likely responsible for these CPA- and ACh-induced dilations. It has been shown previously that exposure to high K⁺ concentrations does not affect NO-mediated relaxations in rat small mesenteric arteries (Fukao et al., 1995; Chen and Cheung, 1997) and does not impair the increase in tissue cGMP content (Kuhberger et al., 1994). A good correlation between ACh-induced hyperpolarization and relaxation (measured simultaneously) exists in the rat mesenteric vascular bed (McPherson and Angus, 1991). The present results indicate that L-NAME plus Indo-resistant ACh- and CPA-induced dilation of small mesenteric arteries do not involve K_{ATP}, voltage-gated K⁺ channels, and K_{ir}. These results are in contrast to those obtained in different vascular preparations (Cowan et al., 1993; Plane and Garland, 1993).

Second, relaxation induced by ACh, which was independent of the NO/COX pathway, was unaffected by IbTX but was abolished by apamin. This result suggests the participation of SK_{Ca} in ACh-induced dilation. This is in accordance with previous studies conducted in larger mesenteric arteries (Murphy and Brayden, 1995; Chen and Cheung, 1997). However, apamin had no effect on ACh-induced EDHF production in guinea pig coronary arteries (Eckmann et al., 1992).

A previous investigation in rat mesenteric preparations

reported that CPA and thapsigargin, both inhibitors of Ca²⁺ uptake by endothelial ER, are able to produce EDHF by increasing intracellular calcium concentration (Fukao et al., 1995). However, the nature of the K⁺ channels activated by EDHF is not known. Our results suggest a major contribution by SK_{Ca} in the CPA-induced dilation that is resistant to NOS and COX inhibition. Complete abolition of the dilation of arteries could be achieved only when the two types of K_{Ca} were blocked with apamin and IbTX. The current study provides evidence that BK_{Ca} and SK_{Ca} participate in the hyperpolarization and dilation responses induced by ACh and CPA on myogenically active small mesenteric resistance arteries of the rat. However, we cannot completely explain the difference effects of IbTX on dilations produced by ACh and CPA.

It appears that ACh is more similar to CPA than SP in terms of the relative quantities of NO and EDHF that may be generated. Moreover, the dynamics of intracellular calcium concentration ([Ca²⁺]_i) changes during relaxation induced by ACh and CPA may be quite similar (Wang et al., 1995; Rahimian et al., 1998); both vasodilators relaxed mesenteric arteries to the same extent in the presence of L-NAME plus Indo (Figs. 5B and 6B). Thus, it is likely that the difference observed between ACh and CPA in terms of the inhibitory effects of IbTX may be due to the release of more than one EDHF, and their cumulative effects are slightly different in the case of ACh. Different candidates for EDHF have been suggested (Mombouli and Vanhoutte, 1997; Edwards et al., 1998; Vanhoutte, 1998). Therefore, the release of different substances by these vasodilators (ACh and CPA) leads to activation of different types (or subtypes) of K⁺ channels; alternatively, differential mechanisms of EDHF-mediated K⁺ channel activation may explain the results.

Recent evidence suggests that EDHF could be a cytochrome P-450-derived arachidonic acid metabolite (Hecker et al., 1994). We used the suicide-substrate inhibitor 17-ODYA, two mechanistically different inhibitors of a large number of cytochrome P-450-dependent systems (SKF 525a, clotrimazole), and phospholipase A₂ inhibitors on the CPA-induced endothelium-dependent vasodilation. Our data strongly suggest that in the small mesenteric artery, EDHF is unlikely to be an epoxyeicosatrienoic acid. That is in accordance with similar results observed in guinea pig carotid arteries (Corriu et al., 1996). This finding contrasts, however, with observations made in other test systems (Hecker et al., 1994). The apparent contradictions in the literature may be due to liberation of different EDHFs as well as the different distributions of K⁺ channel types in various smooth muscle preparations.

It is known that an increase in [Ca²⁺]_i in the endothelial cells is an important stimulus not only for the formation of NO but also for the activation of the NO-independent pathways (Busse et al., 1993; Fukao et al., 1993). This seems to be true also in myogenically active small mesenteric arteries, because the agonists used in this study are able to activate NO-dependent and -independent pathways and are known to increase [Ca²⁺]_i in endothelial cells (Dinerman et al., 1993). The increase in [Ca²⁺]_i induced by agonists is due to inositol trisphosphate-induced release from the ER as well as by extracellular Ca²⁺ influx. A23187 increases Ca²⁺ permeability of both cell and ER membranes, whereas CPA depletes intracellular Ca²⁺ stores by selectively inhibiting sarcoplasmic/ER Ca²⁺-dependent ATPase in a variety of tissues (Sei-

dlar et al., 1989; Lagaud et al., 1999). The striking difference between CPA, which selectively releases EDHF, and SP, which releases only NO (no EDHF), strongly suggests that the mode of Ca^{2+} elevation determines the nature of the secretory product. Hence, we postulate that CPA, by slowly releasing Ca^{2+} from the ER through inhibition of Ca^{2+} re-uptake by sarcoplasmic/ER Ca^{2+} -dependent ATPase, stimulates synthesis of EDHF. On the other hand, SP may increase $[\text{Ca}^{2+}]_i$ mainly by activating Ca^{2+} influx and perhaps Ca^{2+} release from the superficial ER (Sharma and Davis, 1995), thus stimulating endothelial NOS localized near the cell membrane (Pollock et al., 1993). On the other hand, we found that ACh was closer to CPA than SP in terms of the relative quantities of NO and EDHF. We have shown previously that CPA and ACh are able to raise $[\text{Ca}^{2+}]_i$ in endothelial cells by releasing Ca^{2+} from intracellular stores and activating Ca^{2+} influx through receptor-operated and store-operated channels (Wang et al., 1995; Rahimian et al., 1998). Thus, although we intend to further investigate specific $[\text{Ca}^{2+}]_i$ patterns, it is possible that those due to ACh and CPA may be quite similar. Clearly, further investigation providing more precise information on the exact mechanisms are necessary to resolve this issue.

In summary, we have shown that cerebral arteries of the rat display a higher level of pressure-dependent active tone compared with mesenteric arteries, suggesting that the myogenic responsiveness depends in part on the vascular bed from which the vessel is isolated. We also showed that the mechanisms of endothelium-dependent agonist responses differ between the distal middle cerebral and small mesenteric arteries of the rat. In addition, we suggest that the pathway by which agonists raise $[\text{Ca}^{2+}]_i$ may determine the nature of the endothelial secretory product. Heterogeneity of vascular reactivity is important for the plasticity of physiological responses. Adequate perfusion of individual vascular beds depends on heterogeneity in the responsiveness of vessels of different size, and this report supports the concept that this is at least partly due to variability in endothelial signaling.

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