Role of Nitric Oxide and Ca$^{++}$-Dependent K$^+$ Channels in Mediating Heterogeneous Microvascular Responses to Acetylcholine in Different Vascular Beds$^1$

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ABSTRACT

Endothelium-derived relaxing factors may differentially modulate vascular tone in relaxation from arteries of specific vascular beds. We evaluated the role of nitric oxide (NO), prostacyclin (PGI$_2$) and endothelium-derived hyperpolarizing factor in determining basal tone and acetylcholine (ACh)-induced relaxation of coronary (Cor), skeletal muscle (Ske) and mesenteric (Mes) small arteries (150–250 $\mu$m) isolated from male Golden Syrian hamsters (16–17 weeks). Intraluminal diameter (ID) was recorded in vessels maintained at a constant pressure of 40 mm Hg. Charybdotoxin (0.1 $\mu$M), a blocker of large Ca$^{++}$-dependent K$^+$ channels (BK$_{Ca}$), decreased base-line ID by 33 $\pm$ 4% and 15 $\pm$ 4% in Cor and Mes small arteries, respectively. Neither the nitric oxide synthase (NOS) inhibitor, $N$-nitro-$\omega$-arginine (LNA, 0.1 mM), indomethacin (10$^{-5}$ M) nor apamin (0.5 $\mu$M), which blocks small Ca$^{++}$-dependent K$^+$ channels (SK$_{Ca}$), affected ID. Maximal relaxation to ACh was significantly reduced by LNA in Cor arteries preconstricted with thromboxane A$_2$ analog, U46619. LNA shifted the dose-response curve to the right without altering maximal relaxation to ACh in Mes arteries and had no effect on relaxation to ACh in Ske arteries relaxation. A high extracellular K$^+$ concentration (25–50 mM) largely reduced relaxation to ACh in Ske and Mes and abolished relaxation in Cor arteries, whereas indomethacin had no effect on any vessel. Blockade of both BK$_{Ca}$ and SK$_{Ca}$ channels with a combination of charybdotoxin and apamin abolished relaxation to ACh in Cor, but had no effect in Mes or Ske arteries. Collectively, these results indicate that ACh-induced relaxation is mediated by both NO and an endothelium-derived hyperpolarizing factor that opens K$^+$ channels independently of NO or PGI$_2$ in Cor and Mes arteries. Relaxation of Ske arteries is completely due to a NO and PGI$_2$-independent opening of K$^+$ channels. Relaxation to ACh is mediated by K$_{Ca}$ channels in Cor arteries, and by other types of K$^+$ channels in Ske and Mes arteries. Additionally, BK$_{Ca}$ channels regulate basal tone in Cor and Mes, but not Ske arteries. These results indicate that arteries of similar size use different mechanisms of endothelium-dependent regulation of vascular tone and relaxation which are dependent on the vascular bed.

The endothelium is an important regulator of vascular tone due to synthesis and release of vasodilatory substances including NO, PGI$_2$ and EDHF (Moncada and Vane, 1979; Furchgott and Zawadzki, 1980; Feletou and Vanhoutte, 1988). Acetylcholine is generally used to induce production of EDRFs and may also stimulate release of vasoconstrictor cyclooxygenase products from endothelial cells (Miller and Vanhoutte, 1985; Luscher and Vanhoutte, 1990). After stimulation of muscarinic receptors, endothelial NOS converts $\omega$-arginine to NO (Palmer et al., 1988). NO may produce relaxation by decreasing smooth muscle cell Ca$^{++}$ levels through a cGMP-dependent pathway or through hyperpolarization due to increased conductance of K$^+$ channels (Gruetter et al., 1981; Murphy and Brayden, 1995a; Corriu et al., 1996b). PGI$_2$ is produced by the action of prostacyclin synthase on endoperoxides, which are produced by cyclooxygenase. $\omega$-Arginine analogs such as LNA inhibit NOS activity, whereas Indo inhibits cyclooxygenase. The enzyme responsible for EDHF production is not known. However, EDHF may be arachidonic acid metabolites of the cytochrome P$_450$ pathway such as epoxyeicosatrienoic acids (Campbell et al., 1996). Relaxation to EDHF has been mediated through increased conductance of Ca$^{++}$-dependent K$^+$ channels and ATP-sensitive K$^+$ channels (Cowan et al., 1993; Campbell et al., 1996).

Studies have demonstrated that arteries exhibit heterogeneity in endothelial and smooth muscle cell shape and func-

ABBREVIATIONS: ACh, acetylcholine; AP, apamin; BK$_{Ca}$, large Ca$^{++}$-dependent K$^+$ channel; CTX, charybdotoxin; Cor, coronary; EDHF, endothelium-derived hyperpolarizing factor; EDRF, endothelium-derived relaxing factor; ID, intraluminal diameter; Indo, indomethacin; LNA, $N$-nitro-$\omega$-arginine; Mes, mesenteric; NO, nitric oxide; NOS, nitric oxide synthase; PGI$_2$, prostacyclin; SK$_{Ca}$, small Ca$^{++}$-dependent K$^+$ channel; Ske, skeletal muscle.
tation (Gumkowski et al., 1987; Archer et al., 1996). Heterogeneous vascular relaxation in vessels of different sizes has also been demonstrated (Galle et al., 1993; Archer et al., 1996). An example of functional heterogeneity of vascular responsiveness is the defense reaction in which renal and mesenteric vascular beds vasoconstrict, whereas the skeletal vascular bed vasodilates (Abrahams et al., 1960). Studies examining heterogeneity in isolated vessels have been performed in large arteries such as the carotid artery, femoral artery and aorta (Nagao et al., 1992; Cowan et al., 1993; Ferrer et al., 1995). However, heterogeneity in the mechanisms mediating endothelium-dependent relaxation in small arteries has not been determined. Small arteries contribute to vascular resistance and may exhibit different mechanisms of endothelium-dependent relaxation than large arteries. The present study was designed to examine the roles of NO, cyclooxygenase products and EDHF in ACh-induced vascular responses in Cor, Ske and Mes small arteries (150–250 μm diameter).

Methods

Male Golden Syrian hamsters (16–17 weeks old) were obtained from Biobreeders, Fitchburg, MA. Hamsters were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and heparin (100 U) was administered into the left ventricle of the heart. The skin over the abdomen was removed and the abdominal skeletal muscle was removed to isolate second-order branching sections of the superior epigastric artery. A section of the small intestine about 2 cm below the stomach was clamped and removed with the mesentery intact for isolation of a third-order branch of the mesenteric artery. The heart was removed for dissection of a second-order branch of the left main coronary artery. All tissues were placed in chilled, oxygenated (20% O₂, 5% CO₂, balance N₂) Krebs-Ringer bicarbonate solution (mM, composition: NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25; dextrose, 11.1).

Ske, Cor and Mes small arteries (150–250 μm diameter) were dissected by use of an Olympus dissection scope. Segments about 1 to 2 mm in length were isolated and mounted in a vessel bath between two glass micropipettes (70 μm diameter tip) with 10–0 silk ophthalmic suture. The lumen of the vessel was filled with Krebs' buffer through the micropipette and maintained at a constant pressure of 40 mm Hg. Vessels were monitored under a Olympus inverted microscope connected to a video monitor. Intraluminal diameter was continually measured by a video dimension analyzer (Living Systems Instrumentation, Burlington, VT) and recorded on a Grass polygraph.

Protocol. After equilibration at 37°C in oxygenated Krebs' buffer for at least 30 min, Ske, Cor and Mes small arteries were preconstricted to 35 to 55% of resting diameter with the thromboxane A₂ analog (U46619) and allowed to stabilize. Endothelium-dependent relaxation was assessed by performing a dose-response curve to ACh (10⁻⁸ to 3 × 10⁻⁶ M). To determine the role of vasoactive prostanoids in the response to ACh, vessels were pretreated with Indomethacin (10⁻⁵ M), an inhibitor of cyclooxygenase, for 20 min before performing a dose-response curve to ACh. To determine the role of NO in the response to ACh, vessels were pretreated with L-NAME (0.1 mM), an inhibitor of NO synthase, for 20 min before performing a dose-response curve to ACh. To determine the role of Ca⁺⁺ channels (Corriu et al., 1996), an inhibitor of Kᵦ⁺ channels (Corriu et al., 1996), and an inhibitor of BK Ca and AP (0.5 M) to inhibit SKCa. These agents were combined because of the finding of another study that both large and small channels had to be blocked to prevent relaxation mediated by Kᵦ⁺⁺ channels (Corriu et al., 1996a). When possible, more than one Ske, Cor or Mes artery was obtained from the same hamster, but a single experiment was performed only once in the same hamster. Additionally, only one dose-response curve was performed per vessel.

Results

Base-line ID after equilibration at 40 mm Hg intraluminal pressure and the percent preconstriction produced by U46619 or KCl in Ske, Cor and Mes small arteries are shown in table 1. IDs were within the range of 150 to 250 μm and the level of preconstriction was in the range of 35 to 55%. Base-line ID was unaffected by pretreatment with Indo, L-NAME or KCl. CTX caused a 33 ± 4% and 15 ± 4% contraction from baseline in Cor and Mes small arteries, respectively. CTX had no effect on base-line ID in Ske small arteries.

Response to ACh. Dose-response curves to ACh in isolated Ske, Cor and Mes small arteries are shown in figure 1. ACh produced dose-dependent relaxation with a maximum of approximately 100% in vessels from each vascular bed. Relaxation to ACh was significantly reduced at a dose of 10⁻⁵ M in Cor compared with Ske or Mes small arteries.

Effect of inhibition of NOS on the response to ACh.

Dose-response curves to ACh in the absence and presence of L-NAME are demonstrated in figure 2. Relaxation to ACh was unaffected by the presence of L-NAME in Ske small arteries. However, L-NAME significantly lowered relaxation to ACh in Cor and Mes small arteries. Additionally, in the presence of L-NAME, relaxation to ACh was less in Cor than in Mes small arteries. Maximum relaxation to ACh was not altered by L-NAME in Ske or Mes small arteries, but was significantly reduced from 97 ± 2% to 49 ± 11% in Cor small arteries.

| TABLE 1 |
| Base-line IDs and % preconstriction values for Mes, Ske, and Cor small arteries* |
| Base line | Preconstriction |
| U46619 | KCl |
| mm | % | mm | % |
| Mes (n = 68) | 236 ± 4 | 43 ± 2 | 54 ± 2 |
| Ske (n = 82) | 188 ± 2 | 49 ± 2 | 43 ± 1 |
| Cor (n = 81) | 189 ± 4 | 38 ± 1 | 42 ± 2 |

| * Values represent mean ± S.E.M. |
Role of K⁺ channels in ACh-induced relaxation.

Dose-response curves to ACh in Ske, Cor and Mes small arteries preconstricted with KCl are demonstrated in figure 3. Blockade of K⁺ efflux with high extracellular K⁺ significantly inhibited relaxation to ACh in Cor, Ske and Mes small arteries. Additionally, constriction to ACh at concentrations of 10⁻⁶ to 3 × 10⁻⁵ M was observed in Cor small arteries. In the presence of high extracellular K⁺, dose-dependent relaxation to ACh at the concentrations of 3 × 10⁻⁷ to 3 × 10⁻⁵ M was significantly lower in Cor than in Ske or Mes small arteries, and was similar in Mes and Ske small arteries.

The percent maximal relaxation and EC₅₀ values for the response to ACh are summarized in figure 4. The dose-response curve to ACh was shifted to the right as indicated by a significant increase in EC₅₀ in Cor compared with Ske or Mes small arteries. Indo did not significantly alter the EC₅₀ or the maximum relaxation to ACh in Ske, Cor or Mes small arteries. In the presence of LNA, the EC₅₀ was increased in

Fig. 1. Dose-response curves to ACh in Ske, Cor and Mes small arteries preconstricted with U46619. Values represent mean ± S.E.M. * P < .05 vs. Ske or Mes.

Fig. 2. Dose-response curves to ACh in the absence and presence of LNA in Ske, Cor and Mes small arteries preconstricted with U46619. Values represent mean ± S.E.M. * P < .05 vs. respective untreated control; t P < .05 vs. Ske-LNA; # P < .05 vs. Mes-LNA.

Fig. 3. Dose-response curves to ACh in Ske, Cor and Mes small arteries preconstricted with either U46619 or KCl. Values represent mean ± S.E.M. * P < .05 vs. respective U46619-preconstricted group; t P < .05 vs. Ske-KCl or Mes-KCl.
Mes compared with Ske small arteries and was further enhanced in Cor small arteries, which had a significantly greater EC_{50} than Mes or Ske small arteries. Maximal relaxation to ACh in Cor, Ske and Mes small arteries was significantly decreased by a high extracellular K^{+} concentration, whereas EC_{50} values in Ske and Mes small arteries were significantly higher than the respective U46619-preconstricted controls.

**Role of vasoconstrictor cyclooxygenase products in relaxation to ACh.** The role of cyclooxygenase products in masking relaxation to ACh in Mes and Ske small arteries and in producing contraction of Cor small arteries in the presence of high extracellular K^{+} levels was determined. Pretreatment of KCl-preconstricted Cor or Ske small arteries with Indo did not significantly alter relaxation to ACh. However, Indo significantly enhanced relaxation to ACh in KCl-preconstricted Mes small arteries (fig. 5). This component of the ACh-induced relaxation was reversed by pretreatment of vessels with both Indo and LNA, which indicated that it was mediated by NO. Some relaxation to ACh remained in the presence of LNA, Indo and a high extracellular K^{+} concentration.

**Effect of blockade of BKCa and SKCa channels on relaxation to ACh.** Dose-response curves to ACh in Ske, Cor and Mes small arteries pretreated with a combination of CTX and AP in the presence of Indo are shown in figure 6. The combination of CTX and AP had no effect on ACh-induced relaxation of Mes small arteries, caused a moderate decrease in relaxation in Ske small arteries and completely abolished relaxation in Cor small arteries. The effect of CTX/AP on the response to ACh in Cor small arteries was very similar to the effect of increasing the extracellular K^{+} concentration (fig. 3). In both cases contraction to ACh was observed. The EC_{50} value and % maximum relaxation to ACh in Ske and Mes small arteries were not altered by CTX/AP (fig. 7). In the presence of CTX/AP, relaxation to ACh was significantly less in Cor than in Ske or Mes small arteries. Because these vessels were pretreated with Indo, it can be concluded that cyclooxygenase products do not mediate the ACh-induced contraction.

**Discussion**

Several endothelium-derived vasoactive substances can modulate vascular tone of small arteries. The present study has shown that inhibition of NOS or cyclooxygenase activity, or blockade of SKCa channels, has no significant effect on tone of isolated Ske, Cor or Mes arteries. Conversely, BKCa channels appear to be open under similar conditions and modulate basal tone of Cor and Mes, but not Ske, isolated small arteries. In other studies, CTX was found to produce a moderate contraction of isolated porcine coronary arteries.
and a dose-dependent contraction of carotid, femoral and superior mesenteric arteries of Wistar Kyoto and spontaneously hypertensive rats (Asano et al., 1993; O'Rourke, 1996). Mesenteric veins of Sprague-Dawley rats were found to contract in response to CTX, but not AP (Winquist et al., 1989). CTX had no effect on basal tone of the rabbit superior mesenteric artery (Khan et al., 1993). Therefore, the role of K Ca channels in determining basal tone appears to depend on both the species and the vascular bed and size.

ACh induced similar quantitative relaxation of isolated Mes and Ske small arteries, and relaxation of Cor small arteries was slightly less than Mes or Ske small arteries. Additionally, heterogeneity existed in the mechanisms mediating ACh-induced relaxation among arteries of similar size from different vascular beds. It has been well documented that ACh stimulates NO production and release in large arteries such as aorta, in which inhibitors of NOS activity nearly abolish relaxation. (Nagao et al., 1992; Wu et al., 1993). In contrast, heterogenous responses based on arterial diameter within the rat pulmonary vascular bed have been observed where the role of NO in endothelium-dependent relaxation was found to be enhanced in conduit compared with resistance rat pulmonary artery rings (Archer et al., 1996). Others have observed that NO-mediated relaxation is enhanced with increases in vessel size (Hwa et al., 1994). Our study indicates that ACh-induced release of NO does not contribute to Ske small artery relaxation, plays a moderate role in relaxing Mes small arteries and significantly contributes to relaxation of Cor small arteries. Cyclooxygenase products were not found to contribute to ACh-induced relaxation in any vascular bed in this study.

Both EDHF and NO are capable of opening vascular smooth muscle K+ channels (Murphy and Brayden, 1995a; Campbell et al., 1996; Corriu et al., 1996b). Endothelium-dependent vascular relaxation which remains in the presence of NOS inhibitors can be blocked by a high [K+]o (Cowan et al., 1993; Corriu et al., 1996b). In the present study, relaxation to ACh was largely inhibited in all vascular beds by a high [K+]o, and contraction to ACh was observed in Cor small arteries. Because LNA and Indo had little effect on relaxation in Mes and no effect in Ske vessels, these results indicate that opening of K+ channels, mediated by an EDHF that is independent of NO or PGI2, is required for relaxation to ACh in Ske and Mes small arteries. Conversely, Cor small arteries appear to depend on both NO and a hyperpolarizing factor other than NO or PGI2, because Indo had no effect, LNA significantly impaired relaxation and a high [K+]o nearly abolished relaxation.

A bioassay study showed that an EDHF is a product of the vascular endothelium, independent of PGI2 and NO, which acts to produce hyperpolarization and relaxation of smooth muscle cells (Mombouli et al., 1996). Unlike NO, the role of EDHF in mediating relaxation has been shown to be enhanced with decreasing vessel size (Hwa et al., 1994). This hypothesis is supported by our finding that ACh-induced relaxation in Mes small arteries was largely mediated by K+ channels, and the finding of others that ACh-induced relaxation of the rat main superior mesenteric artery was not prevented by a high [K+]o (Chen and Cheung, 1996). Some studies indicate that EDHF is a product of the cytochrome P450 pathway derived from arachidonic acid (Bauersachs et al., 1994; Campbell et al., 1996; Chen and Cheung, 1996).
However, studies on endothelium-dependent relaxation to bradykinin in porcine coronary arteries suggest that EDHF is produced by a pathway independent of cytochrome P450, but reliant on arachidonic acid (Weintraub et al., 1995), whereas ACh-induced relaxation of guinea pig carotid artery is not mediated by lipooxygenase or cytochrome P450 (Corriu et al., 1996a). Studies in bovine coronary arteries suggest that epoxyeicosatrienoic acids are EDHF (Campbell et al., 1996), whereas a study in the rat hepatic artery refutes this result (Zygmunt et al., 1996).

Relaxation to EDHF has been shown to be mediated primarily through opening of K<sub>Ca</sub> and K<sub>ATP</sub> channels of vascular smooth muscle cells (Cowan et al., 1993; Bauersachs et al., 1994; Campbell et al., 1996). In the present study, heterogeneity in the type of K<sup>-</sup> channels mediating relaxation to ACh was observed. K<sub>Ca</sub> channels do not mediate endothelium-dependent relaxation to ACh in Mes, minimally contribute to relaxation in Ske and largely mediate relaxation in Cor small arteries. Additionally, a contraction in response to ACh was observed after inhibition of K<sub>ca</sub> channels with CTX and AP, similar to that observed in the presence of high [K<sup>-</sup>]. In other studies, the ACh-induced hyperpolarization of vascular smooth muscle cells which remained after inhibition of NO and cyclooxygenase was inhibited by AP in rabbit mesenteric arteries and by a combination of AP and CTX in guinea pig carotid arteries (Murphy and Brayden, 1995b; Corriu et al., 1996b).

Although vasodilatory cyclooxygenase products do not appear to contribute to ACh-induced relaxation of small arteries from the three vascular beds studied, release of a vasoconstrictor cyclooxygenase product masked relaxation to ACh in Mes, but not Ske or Cor small arteries, which indicates heterogeneity among vascular beds. The component of relaxation masked by vasoconstrictor cyclooxygenase products was mediated by NO as indicated by its reversal by LNA. Because a high [K<sup>-</sup>], was present in this experiment, relaxation to NO could not have been mediated through an increase in K<sup>+</sup> channel flux, and was most likely caused by a decrease in smooth muscle cell intracellular Ca<sup>2+</sup> mediated by cGMP. The mechanism mediating contraction to ACh in Cor small arteries cannot be identified from the results of this study. However, it can be concluded that contraction is not mediated by a cyclooxygenase product. Other possibilities include ACh-induced release of endothelin or direct stimulation of smooth muscle cell muscarinic receptors.

Although the causes of the differential vascular responses cannot be determined from the results of this study, they may be related to a heterogeneity in the types and numbers of K<sup>-</sup> channels present in vascular tissue. Archer et al. (1996) demonstrated a higher number of K<sub>Ca</sub> channels in sheep pulmonary conduit than in resistance arteries, whereas a higher number of delayed rectifier K<sup>-</sup> channels where observed in resistance than in conduit arteries (Archer et al., 1996). An altered sensitivity to NO could also contribute to heterogeneity and has been observed in a comparison of smooth muscle of rabbit aorta, mesenteric and femoral arteries (Galle et al., 1993).

Heterogeneity of vascular reactivity is important for physiological responses such as the defense reaction. Additionally, adequate perfusion of individual vascular beds depends on heterogeneity in the responsiveness of vessels of different size. However, it is important to note that heterogeneity in the mechanisms mediating relaxation in arteries from different vascular beds may contribute to the vascular patterns associated with development of diseases such as atherosclerosis (Verbeuren et al., 1986). Several studies have demonstrated that responsiveness is altered in selective vascular beds in diseases such as hypertension and congestive heart failure (Wright and Fozard, 1990; Galle et al., 1991; O’Murchu et al., 1994; Fuchs, 1996). O’Murchu et al. (1994) suggested that the selective increase in endothelium-dependent relaxation in coronary arteries of dogs with congestive heart failure may contribute to preserving coronary blood flow. The results of this study clearly indicate that the role of NO and Ca<sup>2+</sup>-dependent K<sup>-</sup> channels in regulation of basal tone and mechanisms mediating ACh-induced relaxation of isolated Cor small arteries are different from those of Ske or Mes arteries.

References


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