ACETYLCHOLINE-INDUCED RELAXATIONS OF RABBIT SMALL MESENTERIC ARTERIES: ROLE OF ARACHIDONIC ACID METABOLITES AND K+  

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Running title: Acetylcholine-induced relaxation in rabbit mesenteric arteries  

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ABSTRACT

Acetylcholine-induced endothelium-dependent relaxation in rabbit small mesenteric arteries is resistant to N-nitro-L-arginine (L-NA) and indomethacin but sensitive to high K⁺, indicating the relaxations are mediated by endothelium-derived hyperpolarizing factors (EDHFs). The identity of the EDHFs in this vascular bed remains undefined. Small mesenteric arteries pretreated with L-NA and indomethacin, were contracted with phenylephrine. Acetylcholine (10⁻¹⁰-10⁻⁶M) caused concentration-dependent relaxations that were shifted to the right by lipoxygenase inhibition, the K⁺_Ca channel inhibitors apamin (100 nM) or charybdotoxin (100 nM), and eliminated by the combination of apamin plus charybdotoxin. Relaxations to acetylcholine were also blocked by a combination of barium (200 µM) and apamin, but not barium plus charybdotoxin. Addition of K⁺ (10.9 mM final concentration) to the preconstricted arteries elicited small relaxations. K⁺ addition prior to acetylcholine restored charybdotoxin-sensitive component of relaxations to acetylcholine. K⁺ (10.9 mM) also relaxed endothelium-denuded arteries, and the relaxations were inhibited by barium, but not by charybdotoxin and apamin. Using whole-cell patch-clamp analysis, acetylcholine (10⁻⁷M) stimulated voltage-dependent outward K⁺ current from endothelial cells, which was inhibited by charybdotoxin indicating K⁺ efflux. Arachidonic acid (10⁻⁷-10⁻⁴M) induced concentration-related relaxations that were inhibited by apamin, but not by charybdotoxin and barium. Addition of arachidonic acid after K⁺ (10.9 mM) resulted in more potent relaxations to arachidonic acid compared to control without K⁺ (5.9 mM). These findings suggest that in rabbit mesenteric arteries, acetylcholine-induced, L-NA and indomethacin-resistant relaxation is mediated by endothelial cell K⁺ efflux and arachidonic acid metabolites, and a synergism exists between these two separate mechanisms.
**Key words:** acetylcholine, potassium channels, lipoxygenase, endothelium-derived hyperpolarizing factor
INTRODUCTION

Stimulation of the vascular endothelium with acetylcholine, bradykinin and increases in flow induces vasodilation by releasing soluble factors such as nitric oxide (NO), prostacyclin, as well as endothelium-derived hyperpolarizing factors (EDHFs) (1,5,10,20). The action of EDHFs has been demonstrated in a number of vascular beds, where a significant portion of endothelium-dependent relaxation persists in the presence of NO synthase (NOS) and cycloxygenase inhibitors such as N-nitro-L-arginine (L-NA) and indomethacin. This EDHF-mediated relaxation is associated with smooth muscle K⁺ channel activation and hyperpolarization and is sensitive to inhibition to high extracellular K⁺ or K⁺ channel blockers (1). The mediators of EDHF vary with species, vascular bed and vascular size. In bovine, porcine and canine coronary arteries, cytochrome P450 metabolites of arachidonic acid, epoxyeicosatrienoic acids (EETs) act as EDHFs (3,8,11,23), whereas K⁺ ion may represent EDHF in rat hepatic arteries (7). In addition, H₂O₂ and myoendothelial gap junctions might mediate smooth muscle hyperpolarization and EDHF response in some murine vascular beds (16,26).

The EDHF-mediated relaxations involve different subtypes of K⁺ channels. Blockade of the large-conductance Ca²⁺-activated K⁺ channel (BKCa) blocker inhibits EDHF-mediated relaxations to acetylcholine in bovine coronary arteries (3), whereas the inhibition of intermediate-conductance KCa (IKCa) channel is sufficient to block EDHF-mediated relaxation in rat cerebral arteries (14). In rat hepatic arteries, stimulation of endothelial cells leads to K⁺ efflux into the myoendothelial space, which causes relaxation by activating inward-rectifying K⁺ (Kir) channels and Na⁺/K⁺-ATPase on the smooth muscle (7). In many other arteries, simultaneous application of inhibitors for both small-conductance KCa (SKCa) and IKCa channels is required to block EDHF response (1).
In rabbit small mesenteric arteries, acetylcholine-induced relaxation is resistant to inhibition by L-NA and indomethacin but sensitive to high K⁺, indicating the activity of EDHF (9). The identity of EDHFs in this vasculature remains elusive. Recently, we reported that the lipoxygenase metabolites of arachidonic acid, trihydroxyeicosatrienoic acids (THETAs), activate apamin-sensitive or SKCa currents on vascular smooth muscle. These lipoxygenase metabolites serve as EDHFs and mediate non-NO and non-prostacyclin relaxations to arachidonic acid and acetylcholine in rabbit aorta and rabbit mesenteric arteries (4,13,31). The present study evaluated the effects of various K⁺ channel blockers on acetylcholine-induced relaxations of rabbit small mesenteric arteries. Based on the results with K⁺ channel blockers, the potential roles of arachidonic acid metabolites and K⁺ efflux as EDHFs were explored.

**MATERIALS AND METHODS**

**Wire myograph.** Experiments were performed on isolated mesenteric arteries of 4-6 week old, male New Zealand White rabbits (New Franken Research Rabbits, New Franken, WI). The experimental protocol was approved by the Animal Care Committee of the Medical College of Wisconsin. Second or third order branches from the superior mesentery arteries (200-300 μm) were isolated, and placed in HEPES solution consisting of (in mM): NaCl 150, KCl 5.0, CaCl₂ 1.8, MgCl₂ 1.0, HEPES 10, and glucose 5.5, pH 7.4. Arterial segments (1.5 mm long) were threaded on two stainless steel wires (40 μm diameter) and mounted on a 4-chamber wire myograph (model 610M, Danish Myo Technology A/S). Arteries were equilibrated at 37°C for 30 min in physiological saline solution (PSS) containing (in mM): NaCl 119, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.17, NaHCO₃ 24, KH₂PO₄ 1.18, EDTA 0.026, and glucose 5.5, bubbled with 95% O₂/5% CO₂ (30). The resting tension was set at 1 millinewton (mN). Arteries were stimulated 2
times with KCl (60 mM) plus phenylephrine (10 µM) for 3-5 min at 10 min intervals before the initiation of experimental protocols.

**Vascular relaxation responses.** A submaximal concentration of phenylephrine (0.5 – 2 µM) was added to the bath to precontract the arteries to 50-75% of maximal phenylephrine response. After the contraction reached steady state, cumulative concentration-response to acetylcholine (10^{-10}-10^{-6} M) were determined. Relaxations were examined as paired rings or before and after the application of inhibitors. To examine the role of NO and cyclooxygenase metabolites, arteries were pretreated for 15-30 min with the endothelial NO synthase (NOS) inhibitor L-NA (30 µM) or the cyclooxygenase inhibitor, indomethacin (10 µM). To examine the contribution of smooth muscle hyperpolarization to the relaxation responses, arteries were preconstricted with KCl (60 mM) and the responses to acetylcholine was determined. Where indicated, the endothelium was removed by gently rubbing the intimal surface of the artery with a human hair. The endothelium was considered intact if acetylcholine (1 µM) caused >80% relaxation of the phenylephrine-precontracted arteries and effectively denuded if acetylcholine induced less than 10% relaxation.

All subsequent experiments on EDHF-mediated relaxations were performed in the presence of L-NA (30 µM) and indomethacin (10 µM). The determine the contribution of lipoxygenase metabolites, arteries were pretreated for 15-30 min with cinnamyl-3,4-dihydroxy-a-dyanocinnamate (CDC, 1 µM), nordihydroguaiaretic acid (NDGA, 1 µM) or ebselen (1 µM). Stepwise addition of KCl (2.5 mM, 8.4-18.4 mM final concentration) were added to determine the concentration-dependent relaxations to K^+. To characterize the K^+ channels that mediate the relaxations to acetylcholine, arteries were pretreated for 15-30 min with the following agents
alone or in combination: charybdotoxin (100 nM), an IKCa channel blocker, apamin (100 nM), a SKCa channel blocker, barium (200 µM), a KIr channel blocker, iberiotoxin (100 nM) a BKCa channel blocker or ouabain (10 or 100 µM), a Na+/K+-ATPase inhibitor. To examine the role of K+ efflux and arachidonic acid metabolites as EDHFs, vascular responses to a low concentration of K+ (5 mM, 10.9 mM final), arachidonic acid (10⁻⁷-10⁻⁴ M) and THETAs (0.1-1x concentration) were examined in the absence and presence of the various K+ channel blockers. To examine the interaction of K+ efflux and arachidonic acid metabolites, vascular responses to arachidonic acid or THETAs were determined in the presence of KCl (5 mM, final concentration 10.9 mM).

**Patch-clamp recording.** Whole-cell recordings of K+ currents were obtained in freshly isolated mesenteric endothelial cells using a modification of published methods (12,24). In brief, rabbit small mesenteric arteries were dissected and incubated with low calcium (0.1 mM) PSS containing 1 mg/ml albumin followed by sequential incubation at 37°C with papain (1 mg/ml) and dithiothreitol (0.5 mg/ml) for 15 min, then collagenase (2.5 mg/ml), trypsin inhibitor (1 mg/ml), and elastase (0.5 mg/ml) for 15-20 min. All enzymes were purchased from Sigma Chemical. Endothelial cells were dislodged first with gentle triturations. The remaining tissue was removed and the cells were placed on ice. Endothelial cells were placed in a 1 ml patch-clamp chamber on an inverted microscope (Olympus IX70). Currents were recorded with an Axopatch 200B amplifier (Axon Instruments) and pClamp 8 software (Axon instruments). Cells were dialyzed with a pipette solution composed of (in mM) 145 K+ glutamate, 1 MgCl₂, 10 HEPES, 1 EGTA, 1 Na₂ATP, 0.05 Na₂GTP and 100 nM ionized Ca²⁺ (pH 7.2), and perfused with a bath solution composed of (in mM) 145 NaCl, 4 KCl, 1 MgCl₂, 10 glucose, 10 HEPES and 2 CaCl₂.
(pH 7.4) at room temperature. Pipette tip resistance averaged 4-8 MOhm. Macroscopic $K^+$ currents were generated by progressive, stepwise, 10-mV depolarizing steps (500-ms duration and 5-second intervals) from a constant holding potential of –60 mV. Currents were sampled at 3 kHz and filtered at 1 kHz. After control currents were recorded, acetylcholine ($10^{-7}$ M) was applied in the absence and presence of charybdotoxin (100 nM) or apamin (100 nM). The effect of charybdotoxin on control currents was also determined. Indomethacin (10 $\mu$M) was present in all perfusate solutions. Recordings were performed in triplicate and averaged to estimate $K^+$ current density. The membrane capacitance of each cell was estimated by integrating the capacitative current generated by a 10-mV hyperpolarizing pulse after electronic cancellation of pipette-patch capacitance. Current density is expressed as picoamperes per picofarad (pA/pF) to account for differences in cell membrane area.

**Materials.** Phenylephrine, acetylcholine, arachidonic acid (sodium salt), L-NA, indomethacin, NDGA, ebselen, apamin, charybdotoxin, iberiotoxin, barium, and ouabain were purchased from Sigma Chemical. CDC was obtained from Bio-Mol Research laboratories. 11,12,15-THETA was synthesized and isolated as previously described (31).

**Data analysis.** Relaxation responses are expressed as a percentage relaxation relative to phenylephrine- or KCl-precontraction, with 100% relaxation representing basal tension. Current density is expressed as normalized current density with maximum control current at +60 mV representing 100%. Data are presented as mean±SEM. Significance of differences between mean values was evaluated by Student $t$ test or ANOVA followed by the Student-Newman-Keuls multiple comparison test. $P<0.05$ was considered statistically significant.
RESULTS

We first determined the contribution of three major relaxing factors, i.e. NO, prostacyclin, and EDHFs in acetylcholine relaxation responses. In phenylephrine-contracted arteries, acetylcholine ($10^{-10}$-$10^{-6}$ M) elicited concentration-dependent relaxations, with a maximal relaxation of 99±0.3% (Fig. 1A). Pretreatment of arteries with L-NA (30 µM) caused a rightward shift of the concentration-response curve to acetylcholine (maximal relaxation of 99±0.2%; Fig. 1B). In contrast, indomethacin (10 µM) was without effect (Fig. 1B). L-NA plus indomethacin had no significant effect on basal tension. High K+ (60 mM) also shifted acetylcholine responses to the right (maximal relaxation of 89±0.3%; Fig. 1C). Removal of the endothelium eliminated acetylcholine-induced relaxations (Fig. 1D).

To further define the role of EDHFs in acetylcholine-induced relaxations, arteries in all subsequent experiments were treated with L-NA (30 µM) and indomethacin (10 µM) to block NO and prostacyclin pathways. In the presence of L-NA and indomethacin, a significant portion of the relaxation to acetylcholine remained (maximal relaxation of 99±0.2%), which was blocked by high K+ (60 mM KCl) indicating the involvement of K+ channels and EDHF (Fig. 2A). The L-NA- and indomethacin-resistant relaxation was eliminated by a combination of the SKCa channel blocker apamin (100 nM) and IKCa channel blocker charybdotoxin (100 nM). Apamin and charybdotoxin alone shifted the acetylcholine responses to the right without affecting maximal relaxations (Fig. 2B). Barium (200 µM), a KIr channel blocker, caused a rightward shift of the concentration-response curve to acetylcholine. The combination of barium and apamin blocked the acetylcholine responses, whereas barium plus charybdotoxin had no further inhibition compared to the blockers alone (Fig. 2C). Relaxations to acetylcholine were not affected by 10 µM ouabain, a Na+/K+-ATPase inhibitor but were significantly inhibited by 100
μM ouabain (Fig. 2D). The indomethacin and L-NA resistant relaxations to acetylcholine were not altered by iberiotoxin (Fig. 2E) and the combination of apamin plus iberiotoxin inhibited the relaxations to similar extent as apamin alone. These studies suggest the involvement of two parallel pathways in the relaxations to acetylcholine: (1) a SKCa, apamin-sensitive component and (2) an IKCa and KIr component sensitive to charybdotoxin and barium. Iberiotoxin sensitive BKCa channels are not involved in the responses.

Previous studies on the rabbit aorta indicate that THETAs, lipoxygenase metabolites of arachidonic acid, mediate a portion of the relaxations to acetylcholine by activation of apamin-sensitive K+ channels (4,13). To determine if lipoxygenase metabolites contribute to the apamin-sensitive component of acetylcholine-induced relaxations, arteries were treated with the lipoxygenase inhibitors, CDC, NDGA and ebselen (Fig 3A-C). CDC, NDGA and ebselen inhibited the relaxations to acetylcholine and shifted the concentration-response to the right. This concentration of the inhibitors blocked the conversion of 14C-arachidonic acid to 14C-HEETAs and -THETAs by 50-70% (data not shown)(31). However, higher concentrations of the lipoxygenase inhibitors inhibited phenylephrine contractions in these arteries and therefore could not be tested on acetylcholine-induced relaxations. These findings indicate that THETAs contribute to the relaxations to acetylcholine in rabbit mesenteric arteries.

In L-NA and indomethacin treated, preconstricted rings, stepwise increases in extracellular K+ caused concentration-related relaxations (Fig. 4A). Maximal relaxations were 64±4% at 13.4 mM K+. Increases in K+ above 15.9 mM K+ resulted in constriction. In endothelium-denuded arteries, 10.9 mM K+ induced sustained relaxations that were inhibited by barium, but not by charybdotoxin or apamin (Fig 4B). Thus, in rabbit mesenteric arteries, K+ alone causes
relaxation independent of NO, cyclooxygenase metabolites and the endothelium. The relaxations to K⁺ are inhibited by barium implicating Kᵢᵣ channels in the response.

The role of K⁺ efflux via IKCa channels in EDHF-mediated relaxations to acetylcholine was examined. Arteries were treated with charybdotoxin to block K⁺ efflux. Addition of a low concentration of K⁺ (10.9 mM, final concentration) to mimic K⁺ efflux elicited small sustained relaxations. K⁺ addition prior to acetylcholine restored charybdotoxin-sensitive component of relaxations to acetylcholine (Fig. 4C).

Patch-clamp studies were performed to confirm K⁺ efflux via IKCa channels in vascular endothelial cells (Fig. 5). Whole-cell K⁺ currents of freshly isolated endothelial cells were elicited by 10-mV depolarizing steps from −60 to +60 mV. Capacitance of the cells averaged 15.7±2.2 pF. Perfusion of the endothelial cells with acetylcholine (10⁻⁷ M) resulted in a significant increase in outward K⁺ currents. This increase was subsequently inhibited by charybdotoxin (100 nM) (Fig. 5A) but not apamin (100 nM) (Fig. 5B). Under control conditions, charybdotoxin decreased the outward K⁺ currents by 30%.

To examine the contribution of arachidonic acid metabolites to the EDHF response in rabbit small mesenteric arteries, the effect of K⁺ channel inhibitors were examined on arachidonic acid-induced relaxations. As shown in Fig. 6, arachidonic acid (10⁻⁷-10⁻⁴ M) caused concentration-dependent relaxations in small mesenteric arteries contracted with phenylephrine in the presence of L-NA and indomethacin. The maximal relaxation to arachidonic acid was 94±2%. The relaxations to arachidonic acid were markedly inhibited by apamin (maximal relaxation of 45±10%). In contrast, charybdotoxin and barium did not have significant effect on relaxation responses. The combination of apamin and charybdotoxin or barium had similar inhibition on arachidonic acid-induced relaxations as apamin alone. Since both arachidonic acid
and 10.9 mM K+ relax mesenteric arteries, we tested the interaction of these two relaxing factors. Addition of 10.9 mM K+ potentiated the relaxations to arachidonic acid when compared to control relaxations with arachidonic acid (Fig. 6C).

We also examined the effects of 11,12,15-THETA on the vascular tone of rabbit small mesenteric arteries (31). 11,12,15-THETA (0.1-1x concentration) relaxed preconstricted, denuded arteries in a concentration-dependent manner (Fig. 7A). These relaxations were blocked by apamin. Similar to arachidonic acid and K+, K+ enhanced the relaxations to 11,12,15-THETA (Fig. 7B).

**DISCUSSION**

In the present study, we characterized relaxation responses to acetylcholine in isolated rabbit small mesenteric arteries. We found that acetylcholine induced a potent endothelium-dependent relaxation, which is resistant to L-NA and indomethacin. The acetylcholine-induced, NO- and prostacyclin-independent relaxation is eliminated in arteries depolarized by high K+, thus confirming the previous findings that acetylcholine-induced relaxation is mediated by membrane-potential sensitive mechanisms or EDHFs (9). With regard to the mediators of this EDHF response, we provided first evidence that the EDHF-dependent relaxation response to acetylcholine is mediated by two parallel mechanisms (i.e., K+ efflux and arachidonic acid metabolites) and a synergism exists between these two mechanisms.

EDHF-mediated relaxations may involve different subtypes of K+ channels in various vascular beds (1,3,7,14). The present study shows that in rabbit mesenteric arteries, apamin and charybdotoxin partially reduced acetylcholine-induced relaxation and a combination of both toxins abolished the response. In contrast, apamin plus iberiotoxin, a selective BKCa channel blocker, did not further inhibit acetylcholine-induced relaxation compared to apamin alone.
These results indicate that the EDHF response involves the activation of SK Ca and IK Ca channels in rabbit mesenteric arteries. Additionally, acetylcholine-induced relaxation was abolished by a combination of barium and apamin, whereas the relaxation was only partially inhibited by barium alone. This suggests that Kir channels are also involved in the EDHF response. Since barium plus charybdotoxin elicited a similar inhibition on acetylcholine-induced relaxation compared to either toxin alone, it is likely that acetylcholine stimulates a sequential activation of IK Ca and Kir channels. Alternatively, ouabain inhibited the acetylcholine relaxations only at the concentration of 100 µM. Because of non-specific actions of ouabain at high concentrations, the significance of this result remains inconclusive (15). Taken together, our results suggest that the EDHF-dependent relaxation to acetylcholine involves two parallel pathways, i.e., the activation of IK Ca and Kir channels in one pathway and the activation of SK Ca channels in another.

Previous studies have suggested that IK Ca channels are restricted to the endothelium, and K+ efflux via these channels may serve as an EDHF in some arteries (2,7,17,27). In contrast, K+ does not appear to significantly contribute to the EDHF response in arteries such as porcine coronary, guinea-pig carotid and mouse mesenteric artery (6,25). K+ release into the myoendothelial space relaxes the adjacent smooth muscle by activating smooth muscle Kir channels and possibly Na+/K+-ATPase (7,22,29). In the present study, acetylcholine-induced EDHF-dependent relaxations were inhibited by charybdotoxin. Raising bath K+ concentration to 10.9 mM significantly enhanced the EDHF-dependent relaxations to acetylcholine. Additionally, K+ (10.9 mM) induced relaxation in denuded mesenteric arteries and these relaxations were inhibited by barium but not charybdotoxin. K+ efflux via IK Ca channels, but not SK Ca channels, was confirmed by whole-cell patch-clamp of isolated mesenteric endothelial cells. In those cells, acetylcholine elicited a significant increase in outward currents and this increase was
subsequently inhibited by charybdotoxin but not apamin. Based on these results, we suggest that K⁺ efflux via endothelial IKCa channels contributes to EDHF-dependent relaxation responses to acetylcholine in rabbit small mesenteric arteries. K⁺ induces relaxation in these arteries which is mediated by activation of smooth muscle Kᵢp channels.

Maximum relaxation to exogenous K⁺ (13.4 mM) averaged 64%, whereas maximum indomethacin-, L-NA- and lipoxygenase-resistant relaxation to acetylcholine were 90-100%. Similarly, maximal indomethacin-, L-NA- and apamin-resistant relaxation to acetylcholine were 90-100%. The reason for the discrepancy is unclear. It is possible that exogenous K⁺ may not be as efficacious as local release of endogenous K⁺. Additionally, because higher concentrations of the lipoxygenase inhibitors could not be used in these arteries, residual relaxations by lipoxygenase metabolites may occur.

As discussed previously, our data indicate that the activation of apamin-sensitive SKCa channels represents another pathway in acetylcholine-induced EDHF-dependent relaxations. This is consistent with a previous report by Murphy and Brayden that apamin inhibits the non-NO and non-prostanoid-dependent hyperpolarization to acetylcholine in rabbit mesenteric arteries (21). With regard to the possible mechanisms responsible for SKCa channel activation, we reported that lipoxygenase metabolites of arachidonic acid, 11,12,15-THETA activates apamin-sensitive or SKCa currents on vascular smooth muscle, thereby acting as an EDHF in acetylcholine-induced relaxations in rabbit aorta (13). In the current study, arachidonic acid-induced relaxations were markedly inhibited by apamin. Similarly, 11,12,15-THETA also relaxed the rabbit mesenteric arteries, which were inhibited by apamin. Therefore, arachidonic acid metabolites could serve as mediators of the SKCa channel activation and relaxations to acetylcholine in rabbit small mesenteric arteries. The SKCa channels responsible for
acetylcholine-induced relaxations are probably present on vascular smooth muscle cells, since apamin did not affect the outward $K^+$ currents activated by acetylcholine in mesenteric endothelial cells.

It is important to note that $K^+$ and arachidonic acid acted synergistically in inducing relaxations. Addition of arachidonic acid after 10.9 mM $K^+$ elicited more potent relaxations compared to control relaxation with arachidonic acid. There is also greater relaxation to 11,12,15-THETA plus 10.9 mM $K^+$ compared to either agents alone. These results provide further support for two parallel pathways in relaxation responses of rabbit small mesenteric arteries. A similar scheme of EDHF-dependent relaxations involving parallel pathways has been recently reported in porcine coronary arteries (28) and rat cremaster arteries (18). In these arteries, bradykinin hyperpolarizes smooth muscle cells by endothelial cell hyperpolarization and gap junctions as one mechanism and endothelial production of EETs and $BK_{Ca}$ channel action as another mechanism.

In summary, the data from the present study indicate that acetylcholine-induced EDHF-dependent relaxation in rabbit small mesenteric arteries involves two separate parallel mechanisms, i.e. $K^+$ efflux and arachidonic acid metabolites (Fig. 8). The opening of endothelial $IK_{Ca}$ channels in response to acetylcholine induces $K^+$ efflux, which activates smooth muscle $K_{ir}$ channels. In another pathway, acetylcholine stimulates the metabolism of arachidonic acid in endothelial cells by the lipoxygenase pathway and these metabolites diffuse into the myoendothelial space and activate smooth muscle $SK_{Ca}$ channels. The two mediators have a synergistic effect to cause relaxation. The co-release of $K^+$ and arachidonic acid lipoxygenase metabolites mediate the EDHF response in rabbit mesenteric arteries.
ACKNOWLEDGMENTS

This work was supported by a grant from the National Heart, Lung, and Blood Institute (HL-37981). Dr. Zhang is a postdoctoral fellow of the American Heart Association, Greater Midwest Affiliate, and a recipient of the Jenkins Cardiovascular Research Fellowship. The authors thank Gretchen Barg for secretarial assistance.
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FIGURE LEGENDS

Fig. 1. Effects of L-NA, indomethacin, high K⁺, and endothelial removal on acetylcholine-induced relaxation of rabbit small mesenteric arteries. Arteries were pretreated with L-NA (30 
µM) (A) or indomethacin (10 µM) (B), or removed of the endothelium (D). Phenylephrine (A, B and D) or KCl (60 mM) (C) was used to contract arteries. Values are mean±SEM; n=4-8. *, P<0.05 vs. control.

Fig. 2. Effects of high extracellular K⁺ and K⁺ channel blockers on acetylcholine-induced relaxations of rabbit small mesenteric arteries in the presence of L-NA and indomethacin. A: Arteries were contracted KCl (60 mM). B: Arteries were pretreated with apamin (100 nM), charybdotoxin (ChTX, 100 nM), or a combination of apamin and charybdotoxin. C: Arteries were pretreated with barium (200 µM) alone or in combination with charybdotoxin (ChTX, 100 nM) or apamin (100 nM). D: Arteries were pretreated with ouabain (10 µM or 100 µM). E: Arteries were pretreated with iberiotoxin (100 nM) or the combination of iberiotoxin and apamin. Phenylephrine was used to contract arteries unless otherwise stated. Values are mean±SEM; n=4-18. *, P<0.05 vs. control.

Fig. 3. Effect of lipoxygenase inhibitors and K channel inhibitors on acetylcholine-induced relaxations in mesenteric arteries. A: Effect of CDC (1 µM), miconazole (2 µM), charybdotoxin (100 nM) or barium (200 µM) on relaxations. B: Effect of NGDA (1 µM) on relaxations. C: Effect of ebselen (1 µM) on relaxations. All vessels were pretreated with L-NA and indomethacin and preconstricted with phenylephrine. Values are mean±SEM; n=7-12. *, P<0.05 vs. control.
Fig. 4. Effect of K⁺ on relaxations of rabbit small mesenteric arteries. A: Relaxation response to stepwise increased in K⁺ (2.5 mM) B: Effect of charybdotoxin, apamin and barium on 10.9 mM K⁺-induced relaxations of denuded arteries. Arteries were pretreated with vehicle (C), charybdotoxin (ChTX, 100 nM), apamin (100 nM) or barium (200 µM). C: Effect of K⁺ on acetylcholine responses. Arteries were pretreated with charybdotoxin (100 nM) in the presence of L-NA and indomethacin, and KCl (5 mM; 10.9 mM final) was added before the determination of acetylcholine responses. Phenylephrine was used to contract arteries. Values are mean±SEM; n=7-23. *, P<0.05 vs. control.

Fig. 5. Effect of acetylcholine on whole-cell outward K⁺ current of isolated mesenteric endothelial cells. A: Whole-cell current densities of cells perfused with vehicle and acetylcholine (ACh), 0.1 µM) in the absence or presence of charybdotoxin (CBTX, 100 nM). B: Whole-cell current densities of cells perfused with vehicle and acetylcholine in the absence or presence of apamin (100 nM). Values are mean ± SEM; n=9-15. *, P<0.05 vs. control **, P<0.05 vs. ACh.

Fig. 6. Arachidonic acid-induced relaxation of rabbit small mesenteric arteries in the presence of L-NA and indomethacin. A: Arteries were pretreated with apamin (100 nM), charybdotoxin (ChTX, 100 nM), or a combination of apamin and charybdotoxin. B: Arteries were pretreated with barium (200 nM) alone or in combination with apamin (100 nM). C: KCl (5 mM; 10.9 mM final) was added before the determination of arachidonic acid responses. Phenylephrine was used to contract arteries. Values are mean±SEM; n=7-20. *, P<0.05 vs. control.

Fig. 7. Relaxation of rabbit small mesenteric arteries by 11,12,15-THETA. A: Concentration-
related relaxations to 11,12,15-THETA in control arteries or arteries pretreated with apamin (100 nM). B: Relaxations to KCl (5 mM; 10.9 mM final), and 11,12,15-THETA (0.5x) alone or in combination. Experiments were performed on denuded arteries and phenylephrine was used to contract arteries. Values are mean±SEM; n=3-6. *, P<0.05 vs. KCl or THETA alone.

Fig. 8. Acetylcholine-induced EDHF-dependent relaxations involve two parallel mechanisms, namely K+ efflux and arachidonic acid metabolite release. K+ efflux via endothelial IKCa channels activates smooth muscle Kir channels, whereas the arachidonic acid metabolite, 11,12,15-THETA diffuses to the smooth muscle cells to activate SKCa channels.
Fig. 2
Fig. 3

Copyright Information
Fig. 4

A

% Relaxation

KCl (mM)

B

% Relaxation

C ChTX

C Apamin

C Barium

C

ChTX

KCl (10.9 mM)

+ChTX

C

KCl

Acetylcholine (Log M)

C

Control

ChTX

KCl (10.9 mM)

+ChTX

Fig. 4
Fig. 5

A

- Control
- ACh
- CBTX
- ACh + CBTX

B

- Control
- ACh
- ACh + Apamin

Normalized Current Density vs. Membrane Potential (mV)
Fig. 6
Fig. 7
Fig. 8