Am J Physiol Heart Circ Physiol
280: H1272–H1277, 2001.

Endothelial cell protein kinase G inhibits release of EDHF through a PKG-sensitive cation channel

K. A. DORA,[1] C. J. GARLAND,[1] H. Y. KWAN,[2] AND X. YAO[2]
[1]*Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY, United Kingdom; and* [2]*Department of Physiology, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong, China*

Received 21 August 2000; accepted in final form 17 October 2000

Dora, K. A., C. J. Garland, H. Y. Kwan, and X. Yao. Endothelial cell protein kinase G inhibits release of EDHF through a PKG-sensitive cation channel. *Am J Physiol Heart Circ Physiol* 280: H1272–H1277, 2001.—The release of dilator agents from vascular endothelial cells is modulated by changes in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). In this study, we demonstrate the presence of a Ca^{2+}-permeable cation channel in inside-out membrane patches of endothelial cells isolated from small mesenteric arteries. The activity of the channel is increased by KT-5823, a highly selective inhibitor of protein kinase G (PKG), while it is decreased by direct application of active PKG. Application of KT-5823 induces Ca^{2+} influx in the endothelial cells isolated from small mesenteric arteries, and it also causes endothelium-dependent relaxations in isolated small mesenteric arteries. KT-5823-induced relaxations in small mesenteric arteries are greatly reduced by 35 mM K^+ or 50 nM charybdotoxin + 50 nM apamin, suggesting that endothelium-derived hyperpolarizing factor (EDHF) is the participating dilator. The involvement of EDHF is further supported by experiments in which the relaxations of small mesenteric arteries are shown to be accompanied by membrane repolarization. These data strongly argue for a major role of a PKG-sensitive cation channel in modulating the release of EDHF from endothelial cells in rat small mesenteric arteries.

intracellular calcium; nonselective cation channel; resistance arteries; endothelium-derived hyperpolarizing factor

ENDOTHELIAL CELLS MODULATE smooth muscle tone in rat mesenteric arteries through the release of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) (15, 17, 27). EDHF hyperpolarizes vascular smooth muscle cells by opening K^+ channels and, consequently, closing voltage-dependent Ca^{2+} channels, thus causing relaxation (22). It appears that the importance of EDHF varies along the vascular tree. While the role of NO-mediated relaxations decreases with vessel size, the importance of EDHF increases (1, 17, 27, 30). The importance of EDHF in smaller arteries may thus explain why impairment of EDHF-mediated relaxations appears to contribute to hypertension (10, 32).

Little is known about the control and regulation of EDHF synthesis and/or release, mainly because the chemical identity of EDHF has not been characterized. Nevertheless, it is believed that the release of EDHF, like that of NO, follows an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) within the endothelial cells (6, 21). Extracellular Ca^{2+} influx and intracellular Ca^{2+} release have been shown to stimulate EDHF release (6, 11, 23, 25). Recently, a Ca^{2+}-permeable nonselective cation channel was identified in rat aortic endothelial cells (35). The activity of this channel was inhibited by cGMP via a protein kinase G (PKG)-dependent phosphorylation pathway (35). It is possible that the Ca^{2+} influx through this channel may elevate $[Ca^{2+}]_i$ and stimulate EDHF release.

In the present study, we used KT-5823, a highly specific inhibitor of PKG (13), to activate the PKG-sensitive nonselective cation channel. We found that KT-5823 treatment evoked Ca^{2+} influx in the endothelial cells isolated from small mesenteric arteries. In isolated small mesenteric arteries, KT-5823 was able to produce endothelium-dependent vasorelaxations. The relaxant responses to KT-5823 in small mesenteric arteries were sensitive to the inhibition of EDHF pathway, suggesting that EDHF was the participating vasodilator.

MATERIALS AND METHODS

Measurement of $[Ca^{2+}]_i$ and Ca^{2+} current in isolated endothelial cells. Male Sprague-Dawley or Wistar rats were stunned and killed by exsanguination. Segments of the superior mesenteric artery and smaller branches were removed and cleaned of adherent fat and connective tissue. The arteries were cut into small sheets and placed in 0.2% collagenase (Sigma Chemical) in PBS in a shaking incubator at 37°C for 45 min. After the enzyme digestion, the suspension was centrifuged at 800 *g* for 5 min. Cells were then resuspended in 90% RPMI 1640 and 10% fetal bovine serum (GIBCO-BRL) and kept in an incubator at 37°C with 5% CO_2. Confluent cell monolayers were passaged using 0.25% trypsin (Sigma Chemical) containing 2.5 mM EDTA. Only cells from the first two passages were used. The identity of the endothelial cells was confirmed by immunostaining. After fixation

Address for reprint requests and other correspondence: X. Yao, Dept. of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong (E-mail: yao2068@cuhk.edu.hk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

 0363-6135/01 $5.00 Copyright © 2001 the American Physiological Society

Downloaded from ajpheart.physiology.org on December 15, 2010

(4% formaldehyde in PBS for 2 h) and blocking (with 1% BSA in PBS for 30 min), the cells were stained with a polyclonal antibody against human von Willebrand factor (DAKO; diluted 1:400 in PBS with 1% BSA) overnight at 4°C. The slides were washed in PBS and then incubated with FITC-labeled goat anti-rabbit IgG (DAKO) for 1 h. For controls, some slides were incubated in 1% BSA in PBS without the primary antibody. Some slides were counterstained with 0.00003% 4,6-diamidino-2-phenylindole in 0.9% NaCl. After they were washed in PBS, the slides were mounted in glycerol and examined under a fluorescence microscope. More than 98% of the cells were positively stained by the antibody against von Willebrand factor, indicating that they were of endothelial origin. In another control experiment, antibody against von Willebrand factor did not stain smooth muscle cells in segments of rat aorta.

Single-channel currents in isolated endothelial cells were measured by standard methods (19, 35) with an EPC-9 patch-clamp amplifier. The signal was sampled at 5.0 kHz and filtered at 1 kHz for data analysis with TAC and TAC-fit software. The probability of the channel being open (NP_o) was estimated from the total time spent in the open state divided by total time of the record (5 continuous seconds). In cell-attached patches, the patch membrane potential was displaced −40 mV from its resting potential. In inside-out mode, the patch potential was held at −100 mV. The bath solution contained sodium glutamate-saline (in mM: 142.5 sodium glutamate, 1 EGTA, 10 HEPES, pH 7.4), and the pipette solution contained $CaCl_2$-saline (in mM: 100 $CaCl_2$, 10 HEPES, pH 7.4). In experiments involving active PKG, the channel was first activated by application of negative suction (−20 mmHg) through the pipettes to prestretch the membrane patches. This maneuver enabled the effect of PKG to be assessed at a single-channel level. Active PKG was the bovine recombinant isoform-1α (Calbiochem). Vehicle containing 10 μM cGMP, 10 μM ATP, and 7 mM $MgCl_2$ was added before addition of active PKG, inasmuch as the vehicle was required for PKG activation (3).

$[Ca^{2+}]_i$ was measured after endothelial cells were loaded with the Ca^{2+}-sensitive fluorescent dye fluo 3-AM, as described previously (20). Before use, cells were incubated for 1 h at room temperature in medium containing fluo 3-AM (10 μM) and Pluronic F127 (0.02%; Molecular Probes) in normal physiological saline solution, which contained (in mM) 140 NaCl, 1 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 5 HEPES, pH 7.4. After they were washed in normal physiological saline solution, the circular disks containing the cells were pinned in a specially designed chamber, which was placed on the stage of an inverted microscope (Diaphot 200, Nikon). Cells were then exposed to KT-5823 (1 μM), and the fluorescence signal at 488 nm was monitored and recorded using an MRC-1000 laser scanning confocal imaging system with MRC-1000 software. Data analysis was performed with Confocal Assistant and Metaflour.

Measurement of tension and membrane potential in isolated arteries. Segments of the superior mesenteric artery (~1 mm diameter, large artery) or secondary and tertiary branches (diameter = 150–250 μm, small arteries) were dissected, and the surrounding connective tissue was carefully removed. The arteries were cut into rings ~2 mm long. Superior mesenteric arteries were mounted in 10-ml organ baths, and isometric tension was measured with an FT03 force-displacement transducer (Grass Instruments). Small mesenteric arteries were mounted in a Mulvary-Halpern myograph (model 400A, J. P. Trading) under a normalized tension, as previously described (15). Before commencement of the experiments, all artery segments were allowed to equilibrate for ~60 min at 37°C. In some experiments, the endothelium was mechanically disrupted with a small piece of plastic tubing (large arteries) or with a human hair (small arteries). Successful removal of the endothelial cell layer was verified by the lack of any relaxant response to ACh (1 μM).

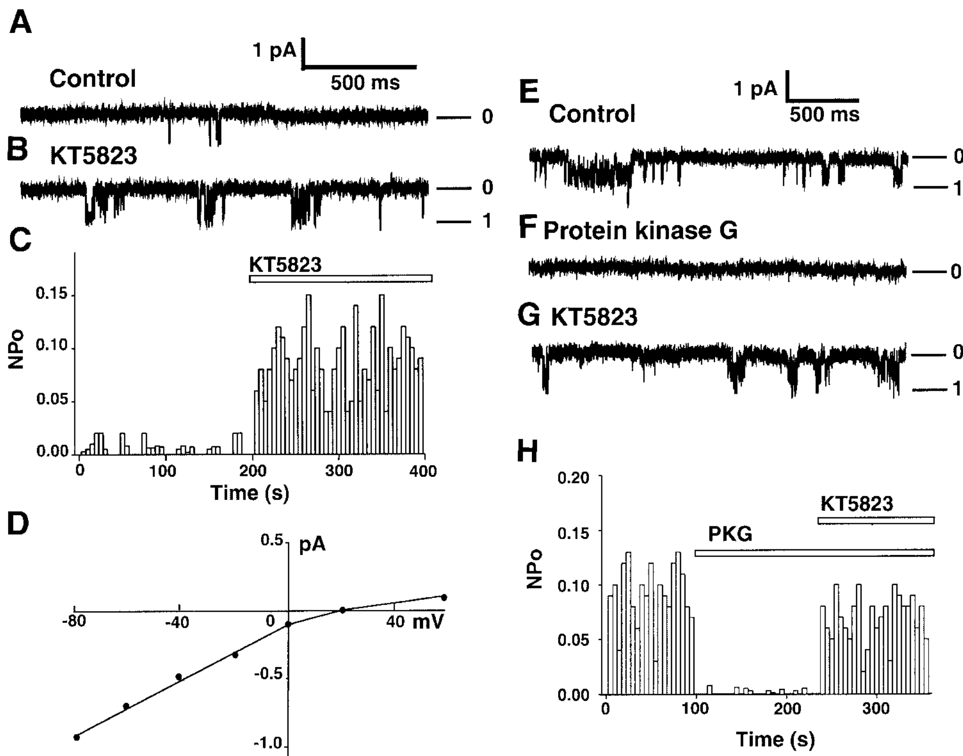

Fig. 1. Effect of KT-5823 on a Ca^{2+}-permeable channel in isolated endothelial cells. *A–C*: experiment on a cell-attached membrane patch from an endothelial cell isolated from superior mesenteric artery. Patch potential was −40 mV displaced from its resting potential. KT-5823 (1 μM) markedly increased single-channel activity (*B* and *C*). NP_o, probability that the channels are open. *D*: single-channel current-voltage relationship of KT-5823-activated channel. *E–H*: experiment on an inside-out patch from an isolated endothelial cell. Patch potential was held at −100 mV. Membrane patch was prestretched to increase single-current activity. Protein kinase G (PKG, 10 nM) abolished this activity (*F* and *H*), and 1 μM KT-5823 restored it (*G* and *H*). Pipette/bath solution contained Ca^{2+} saline/sodium glutamate. NP_o values were measured at 5-s intervals.

Downloaded from ajpheart.physiology.org on December 15, 2010

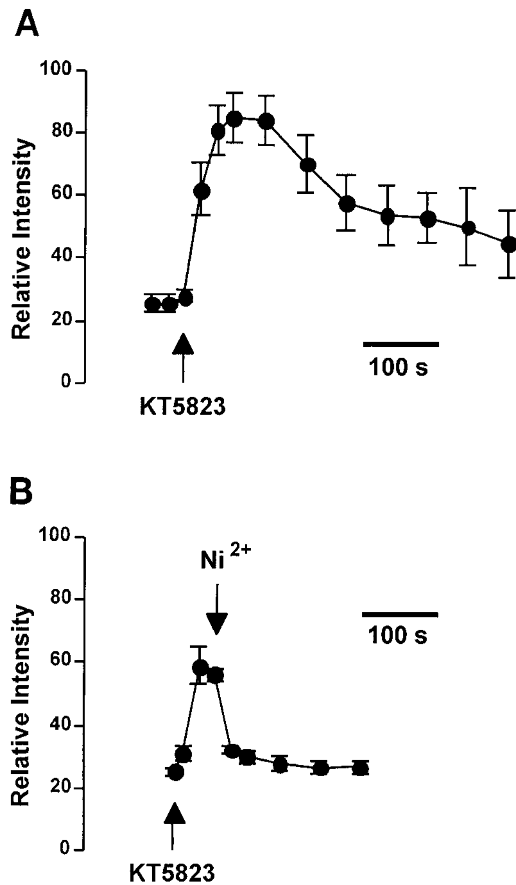

Fig. 2. Effect of KT-5823 on isolated endothelial cell intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Relative fluorescence intensity was monitored in fluo 3-AM-loaded endothelial cells isolated from small mesenteric arteries. Cells were placed in normal physiological saline solution, which contained 1 mM $CaCl_2$. *A*: 1 μM KT-5823 alone. *B*: KT-5823 followed by 3 mM Ni^{2+}. Values are means ± SE (*n* = 5–7).

All agents were added directly to the bath and rapidly mixed by gassing. Since phenylephrine-induced contraction was rhythmic, the mean value taken from each oscillation was used to indicate the contraction force in that particular time period.

In a separate series of experiments for simultaneously measuring tension and membrane potential, the artery segment was superfused (at 3.5–4.0 ml/min) with Krebs solution that had been gassed (and mixed) with 95% O_2-5% CO_2 and warmed to 37°C. Phenylephrine was added to the Krebs solution reservoir, whereas KT-5823 (Calbiochem) and ACh were bolus additions to the reservoir, with an approximate dilution of 1:2 at the artery segment. Smooth muscle membrane potential was measured with a glass microelectrode advanced through the adventitial surface of the arterial segment. The electrodes were filled with 2 M KCl and had resistances of 80–150 MΩ. Membrane electrical events were recorded through a high-impedance direct-current preamplifier (Neurolog 102G). A sudden voltage decrease was taken to indicate smooth muscle cell penetration.

All experiments were performed at 37°C in Krebs solution (in mM: 118.0 NaCl, 25.0 $NaHCO_3$, 3.6 KCl, 1.2 $MgSO_4 \cdot 7H_2O$, 1.2 KH_2PO_4, 11.0 glucose, 2.5 $CaCl_2$). Indomethacin (2.8 μM; Sigma Chemical) was present in solution. The Krebs solution was continuously aerated with 95% O_2-5% CO_2. Since the relaxations evoked by KT-5823 resulted in desensitization, a fresh artery was used for each treatment, and each *n* value indicates a separate animal. In some experiments, arteries were incubated in the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical) and/or apamin + charybdotoxin (synthetic forms; Latoxan) for 15–20 min before addition of phenylephrine (at a concentration to match the control level of contraction). Data were stored at 2 Hz using a MacLab data acquisition system (model 4e) coupled to a Macintosh Performa 475.

RESULTS AND DISCUSSION

KT-5823-induced Ca^{2+} influx through a nonselective cation channel. KT-5823 is a highly specific inhibitor for PKG. It is membrane permeable. The IC_{50} for PKG is 0.234 μM. For a structurally very similar enzyme, protein kinase A, its IC_{50} is >10 μM (13). In cell-attached membrane patches, treatment of isolated endothelial cells with KT-5823 (1 μM) activated a Ca^{2+}-permeable channel (Fig. 1, *B* and *C*). The activity of the channel could be recorded in endothelial cells isolated from rat aorta or from rat small mesenteric arteries. Application of KT-5823 increased the mean NP_o of the channel from 0.01 ± 0.01 to 0.10 ± 0.03 ($n = 8$, $P < 0.05$). NP_o was increased by mechanosuction through the pipettes (Fig. 1*E*). Subsequent application of active PKG to the cytoplasmic side of excised membrane

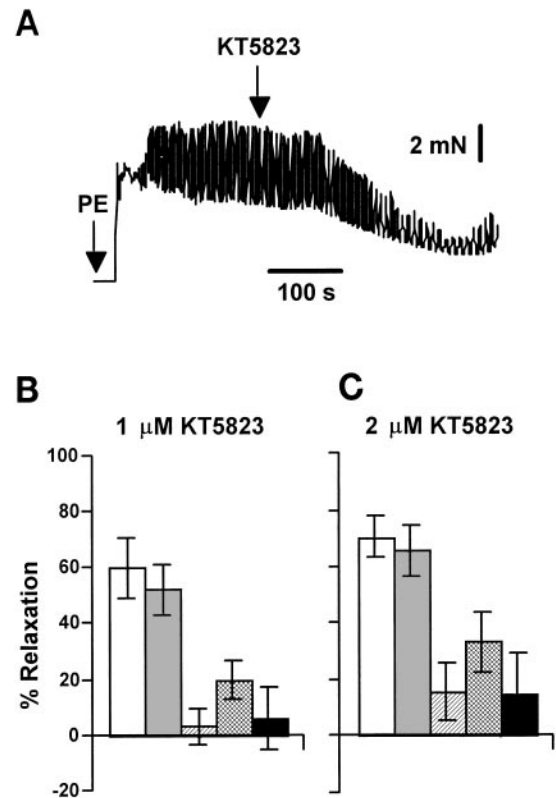

Fig. 3. Effect of KT-5823 on phenylephrine (PE)-induced tension in small mesenteric arteries. *A*: representative trace showing relaxation evoked by 1 μM KT-5823. Summary of responses to 1 μM (*B*) or 2 μM (*C*) KT-5823 is shown under control conditions (open bars), after treatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME, stippled bars), L-NAME + 35 mM K^+ (hatched bars), and L-NAME + apamin + charybdotoxin (cross-hatched bars), and in endothelium-denuded arteries (solid bars). Values are means ± SE (*n* = 3–7).

Downloaded from ajpheart.physiology.org on December 15, 2010

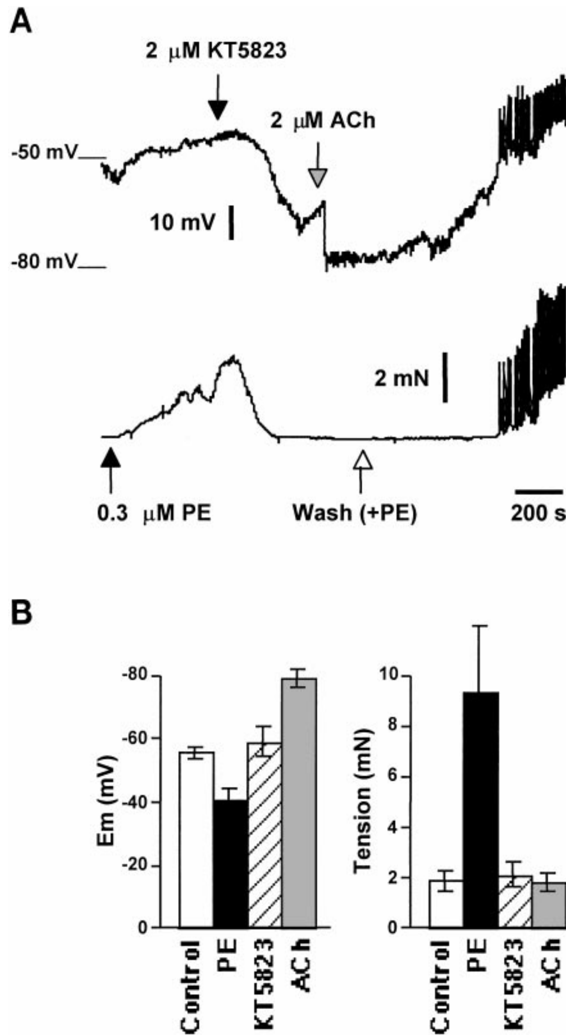

Fig. 4. Effect of KT-5823 on membrane potential. *A*: representative traces of membrane potential (Em; *top trace*) and tension (*bottom trace*) in a small mesenteric artery. Agents were not directly added to the myograph bath, causing a delay in responses. *B*: summary of peak responses to agents each added in the same sequence as shown in *A*. Values are means ± SE (n = 4).

patches abolished the channel activity (Fig. 1, *F* and *H*), with mean NP_o from six different patches reduced from 0.12 ± 0.05 to 0.001 ± 0.001 (n = 6, $P < 0.05$). Among these six patches, three had long-lasting tight seals, which made the application of KT-5823 feasible. KT-5823 reversed the channel inhibition due to PKG (Fig. 1, *G* and *H*), with mean NP_o increased from 0.001 ± 0.001 to 0.06 ± 0.04 (n = 3, $P < 0.05$). Current-voltage relationships of this channel were generated from inside-out membrane patches when the pipette/bath contained Ca^{2+} saline/sodium glutamate solution (Fig. 1*D*). On the basis of the current-voltage relationship curves, the channel reversed at 22 ± 4 mV (n = 5) and the single-channel conductance is estimated to be 10 ± 3 pS (n = 5). When the pipette/bath solution contained NaCl/KCl-saline, the reversal potential was 2 ± 4 mV and the single-channel conductance was 30 ± 4 pS (n = 6). With the use of the constant field equation (34), the relative permeability (P) ratio of the channel was calculated to be 5:1:1 P_{Ca}-P_{Na}-P_K.

We then tested the effect of KT-5823 on $[Ca^{2+}]_i$ in the endothelial cells isolated from mesenteric arteries. In cells isolated from the superior mesenteric arteries or the smaller branches, application of KT-5823 (1 μM) elicited a significant increase in $[Ca^{2+}]_i$ (n = 7–18; Fig. 2*A*). The KT-5823-evoked increase in $[Ca^{2+}]_i$ was rapidly blocked by 3 mM Ni^{2+}, indicating an influx of extracellular Ca^{2+} (Fig. 2*B*). KT-5823 was unable to initiate any rise in $[Ca^{2+}]_i$ if 3 mM Ni^{2+} was continuously present in the medium (n = 4). Ni^{2+} alone had no significant effect on resting Ca^{2+} concentration over the time course of the experiments. These experiments demonstrated that KT-5823, possibly through its action on a PKG-sensitive cation channel, could stimulate Ca^{2+} influx in the endothelial cells isolated from small mesenteric arteries. This hypothesis assumes the existence of PKG activity in isolated endothelial cells, which was indeed demonstrated in our previous study (20) as well as in other reports (9).

Role of the EDHF pathway. Phenylephrine (3 μM) induced rhythmic smooth muscle contractions in small,

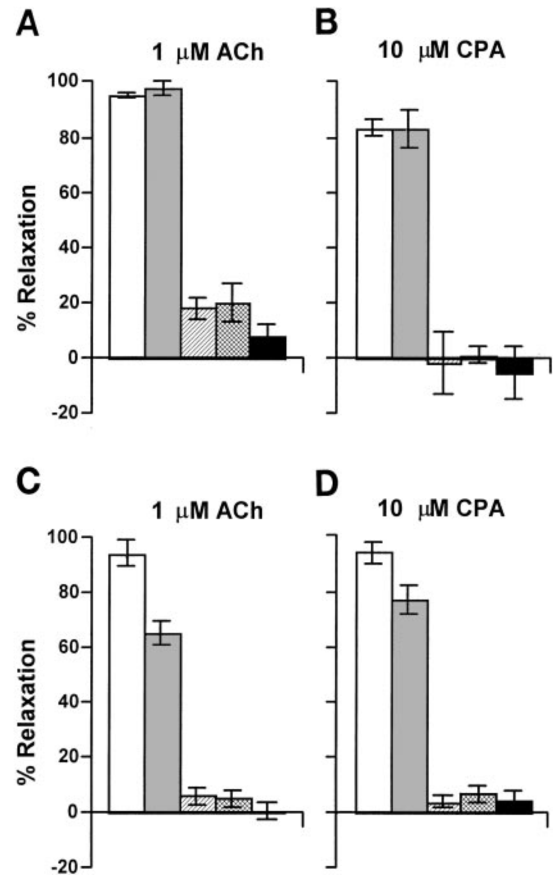

Fig. 5. Effect of ACh and cyclopiazonic acid (CPA) on phenylephrine-induced tension in mesenteric arteries. Summary of responses to 1 μM ACh and 10 μM CPA in small (*A* and *B*) and superior (*C* and *D*) mesenteric arteries is shown under control conditions (open bars), after treatment with L-NAME (stippled bars), L-NAME + 35 mM K^+ (hatched bars), and L-NAME + apamin + charybdotoxin (cross-hatched bars), and in endothelium-denuded arteries (solid bars). Values are means ± SE (n = 3–9).

Downloaded from ajpheart.physiology.org on December 15, 2010

endothelium-intact mesenteric arteries. Subsequent addition of 1 or 2 μM KT-5823 stimulated a marked and sustained relaxation (59.7 ± 10.5 or 70.5 ± 7.1%, n = 7; Fig. 3) that was not reversible. PKG is known to be present in vascular endothelial cells and vascular smooth muscle (2, 9, 28). Nevertheless, it appeared that the relaxant responses to KT-5823 were caused by the action of KT-5823 on endothelial cell PKG, rather than on smooth muscle PKG. One reason was that KT-5823 had no effect in precontracted but endothelium-denuded vessels (6.1 ± 11.5%, n = 4). Another reason was that an inhibitory action on smooth muscle PKG would lead to constriction, rather than dilation, since activation of smooth muscle PKG should dilate blood vessels via stimulation of Ca^{2+}-activated K^+ currents (4, 12), inhibition of Ca^{2+} channels (18, 26), and stimulation of Ca^{2+}-ATPase (36).

The relaxant responses to KT-5823 (1 μM) were not significantly affected by L-NAME (100 μM), an NO synthase inhibitor (Fig. 3). This may indicate a lack of involvement by NO in the KT-5823-induced relaxations. On the other hand, the relaxations to KT-5823 were blocked by 35 mM K^+ (3.2 ± 6.5% maximal relaxation, n = 4) or by the Ca^{2+}-activated K^+ channel blockers charybdotoxin and apamin (both at 50 nM, 19.5 ± 6.8% maximal relaxation, n = 4; Fig. 3, *B* and *C*). It is well documented that treatment by >25 mM K^+ (5, 7, 21, 33) or by the combination of charybdotoxin and apamin (8, 14, 16) can effectively abolish the action of EDHF. Our results, therefore, suggest that KT-5823-induced relaxations in small mesenteric arteries are mediated by EDHF.

This hypothesis was further supported by the membrane hyperpolarizations of smooth muscle in response to KT-5823 treatment. Figure 4 showed experiments in which vessel tension and smooth muscle membrane potential were measured simultaneously. Phenylephrine depolarized the smooth muscle cells in these arteries from −55.8 ± 2.0 to −40.8 ± 3.6 mV (n = 4). KT-5823 (1 μM) evoked sustained repolarizations that were slow in onset (18.3 ± 2.8 mV, n = 4) and gradually returned toward the resting membrane potential (Fig. 4). The subsequent application of ACh (1 μM) stimulated further hyperpolarizations so that the membrane potentials approached K^+ equilibrium potential (−79.1 ± 3.2 mV, n = 4).

Relationship between artery size and KT-5823-evoked responses. While KT-5823 induced endothelium-dependent relaxations in small mesenteric arteries (100–300 μm diameter), it had no effect on the superior mesenteric artery (~1 mm diameter). In contrast, Fig. 5 showed that ACh and cyclopiazonic acid (CPA) evoked maximal relaxations in large and small mesenteric arteries. In small mesenteric arteries, ACh- or CPA-induced relaxant responses resembled the KT-5823-induced response, in that L-NAME had no effect but inhibition of the EDHF pathway completely abolished the response. These results support the notion that EDHF is the main vasodilator in rat small mesenteric arteries. The relaxant responses to ACh or CPA in superior mesenteric arteries, however, exhibited different properties. In these large superior mesenteric arteries, ACh- or CPA-induced relaxations were sensitive to inhibition of the NO pathway as well as the EDHF pathway. Figure 5 showed that L-NAME reduced the relaxations to ACh or CPA by 20–30%. Further inhibition of EDHF pathways almost abolished the relaxant responses to ACh and CPA (Fig. 5). This result is consistent with numerous previous reports in which the contribution of NO has been shown to decrease and that of EDHF to increase as vessel size decreases (27, 29, 31).

It appears that KT-5823 is less effective in releasing vasodilators from endothelial cells than ACh or CPA. KT-5823 was not able to induce relaxation in the large superior mesenteric arteries. In small mesenteric arteries, KT-5823 could cause only 60–70% (Fig. 3) relaxation in phenylephrine-contracted arteries. In addition, the relaxant responses developed with a slow time course (6.0 ± 0.7 min, n = 7; Fig. 3). This was different from the response to ACh or CPA, in which the relaxations were complete and the responses appeared immediately after addition of the chemicals. One possible explanation for these discrepancies is that KT-5823 is able to stimulate only a small Ca^{2+} influx; as a consequence, the amount of vasodilators released from endothelial cells may be low. The released vasodilators may not be sufficient to cause measurable relaxations in large arteries that contain many smooth muscle layers. In contrast, ACh or CPA not only stimulates Ca^{2+} influx but also evokes Ca^{2+} release from intracellular Ca^{2+} stores (24). The quantity of vasodilators released may be relatively higher. Consequently, these dilators are able to elicit fast and complete relaxations in small mesenteric arteries. For the same reason, they may relax the large superior mesenteric arteries.

In conclusion, we have demonstrated that a highly selective PKG inhibitor, KT-5823, can induce endothelium-dependent relaxations in isolated rat small mesenteric arteries. The relaxations are mediated by EDHF. A likely scenario is that KT-5823 activates Ca^{2+} influx across the plasma membrane through its action on a PKG-sensitive cation channel. The subsequent rise in intracellular Ca^{2+} may stimulate the production/release of EDHF, which then causes the endothelium-dependent relaxations. Our data suggest that the release of EDHF can be regulated indirectly by the activity of endothelial PKG through a PKG-sensitive cation channel.

We thank Kong Sum Yi for technical assistance.

This study was supported by Hong Kong Research Council Grant CUHK4079/00M and by British/Hong Kong Research Council Grant JS 99/25.

REFERENCES

1. **Adeagbo AS and Triggle CR.** Varying extracellular [K+]: a functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J Cardiovasc Pharmacol* 21: 423–429, 1993.
2. **Archer AL, Huang JMC, Hampl V, Nelson DP, Shultz PJ, and Weir EK.** Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K+ channel by cGMP-

Downloaded from ajpheart.physiology.org on December 15, 2010

dependent protein kinase. *Proc Natl Acad Sci USA* 91: 7583–7587, 1994.
3. **Beebe SJ and Corbin JD.** Cyclic nucleotide-dependent protein kinases. In: *The Enzymes,* edited by Boyer PD and Krebs EG. New York: Academic, 1986, vol. 17, p. 43–106.
4. **Carrier GO, Fuchs LC, Winecoff AP, Giulumian AD, and White RE.** Nitrovasodilators relax mesenteric microvessels by cGMP-induced stimulation of Ca^{2+}-activated K^+ channels. *Am J Physiol Heart Circ Physiol* 273: H76–H84, 1997.
5. **Chen G and Suzuki H.** Some electrical properties of endothelium-dependent hyperpolarization recorded from rat arterial smooth muscle cells. *J Physiol (Lond)* 410: 91–106, 1989.
6. **Chen G and Suzuki H.** Calcium dependency of the endothelium-dependent hyperpolarization in smooth muscle cells of the rabbit carotid artery. *J Physiol (Lond)* 421: 521–534, 1990.
7. **Corriu C, Feletou M, Canet E, and Vanhoutte PM.** Inhibitors of the cytochrome *P*-450-monooxygenase and endothelium-dependent hyperpolarization in the guinea-pig isolated carotid artery. *Br J Pharmacol* 117: 607–610, 1996.
8. **Corriu C, Feletou M, Canet E, and Vanhoutte PM.** Endothelium-derived factors and hyperpolarizations of isolated carotid artery of the guinea-pig. *Br J Pharmacol* 119: 959–964, 1996.
9. **Draijer R, Vaandrager AB, Nolte C, De Jonge HR, Walter U, and van Hinsbergh VWM.** Expression of cGMP-dependent protein kinase I and phosphorylation of its substrate, vasodilator-stimulated phosphoprotein, in human endothelial cells of different origin. *Circ Res* 77: 897–905, 1995.
10. **Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Takata Y, and Fujishima M.** Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ Res* 70: 660–669, 1992.
11. **Fukao M, Hattori Y, Kanno M, Sakuma I, and Kitabatake A.** Sources of Ca^{2+} in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. *Br J Pharmacol* 120: 1328–1334, 1997.
12. **Fukao M, Mason HS, Britton FC, Kenyon JL, Horowitz B, and Keef KD.** Cyclic-GMP-dependent protein kinase activates cloned BK_{Ca} channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J Biol Chem* 274: 10927–10935, 1999.
13. **Gadbois DM, Crissman HA, Tobey RA, and Morton Bradbury E.** Multiple kinase arrest points in the G_1 phase of nontransformed mammalian cells are absent in transformed cells. *Proc Natl Acad Sci USA* 89: 8626–8630, 1992.
14. **Garcia-Pascual A, Labadia A, Jimenez E, and Costa G.** Endothelium-dependent relaxation to acetylcholine in bovine oviductal arteries: mediation by nitric oxide and changes in apamin-sensitive K^+ conductance. *Br J Pharmacol* 115: 1221–1230, 1995.
15. **Garland CJ and McPherson GA.** Evidence that nitric oxide does not mediate the hyperpolarization and relaxation of acetylcholine in the rat small mesenteric artery. *Br J Pharmacol* 105: 429–435, 1992.
16. **Garland CJ and Plane F.** Relative importance of endothelium-derived hyperpolarizing factor for relaxation of vascular smooth muscle in different arterial beds. In: *Endothelium-Dependent Hyperpolarizing Factor*, edited by Vanhoutte PM. Amsterdam: Harwood Academic, 1996, p. 173–181.
17. **Garland CJ, Plane F, Kemp BK, and Cocks TM.** Endothelium-derived hyperpolarization: a role in the control of vascular tone. *Trends Pharmacol Sci* 16: 23–30, 1995.
18. **Godfraind T.** EDRF and cGMP control gating of receptor-operated calcium channels in vascular smooth muscle. *Eur J Pharmacol* 126: 341–343, 1986.
19. **Hamill OP, Marty A, Neher E, Sakmann B, and Sigworth FJ.** Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch* 391: 85–100, 1981.
20. **Kwan HY, Huang Y, and Yao X.** Store-operated calcium entry in vascular endothelial cells is inhibited by cGMP via a protein kinase G-dependent mechanism. *J Biol Chem* 275: 6758–6763, 2000.
21. **Nagao T and Vanhoutte PM.** Hyperpolarization is a mechanism for endothelium-dependent relaxation in porcine coronary artery. *J Physiol (Lond)* 445: 355–367, 1992.
22. **Nagao T and Vanhoutte PM.** Endothelium-derived hyperpolarization factor and endothelium-dependent relaxations. *Am J Respir Cell Mol Biol* 8: 1–63, 1993.
23. **Nilius B, Viana F, and Droogmans G.** Ion channels in vascular endothelium. *Annu Rev Physiol* 59: 145–170, 1997.
24. **Parekh AB and Penner R.** Store depletion and calcium influx. *Physiol Rev* 77: 901–930, 1997.
25. **Plane F, Pearson T, and Garland CJ.** Multiple pathways underlying endothelium-dependent relaxation in the rabbit isolated femoral artery. *Br J Pharmacol* 115: 31–38, 1995.
26. **Ruiz-Velasco V, Zhong J, Hume JR, and Keef KD.** Modulation of Ca^{2+} channels by cyclic nucleotide cross activation of opposing protein kinases in rabbit portal vein. *Circ Res* 82: 557–565, 1998.
27. **Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M, and Takeshita A.** The importance of the hyperpolarizing mechanism increases as the vessel size decreases in the endothelium-dependent relaxations in rat mesenteric circulation. *J Cardiovasc Pharmacol* 28: 703–711, 1996.
28. **Soff GA, Cornwell TL, Cundiff DL, Gately S, and Lincoln TM.** Smooth muscle cell expression of type I cyclic GMP-dependent protein kinase is suppressed by continuous exposure to nitrovasodilators, theophylline, cyclic GMP, and cyclic AMP. *J Clin Invest* 100: 2580–2587, 1997.
29. **Tomioka H, Hattori Y, Fukao M, Sato A, Liu M, Sakuma I, Kitabatake A, and Kanno M.** Relaxation in different-sized rat blood vessels mediated by endothelium-derived hyperpolarizing factor: importance of processes mediating precontractions. *J Vasc Res* 36: 311–320, 1999.
30. **Tschudi M, Richard V, Bahler FR, and Lescher TF.** Importance of endothelium-derived nitric oxide in porcine coronary resistance arteries. *Am J Physiol Heart Circ Physiol* 260: H13–H20, 1991.
31. **Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, and Takeshita A.** Importance of endothelium-derived hyperpolarization factor in human arteries. *J Clin Invest* 100: 2793–2799, 1997.
32. **Van de Voode J, Vanveel B, and Leusen I.** Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. *Circ Res* 70: 1–8, 1992.
33. **Waldron GJ and Garland CJ.** Contribution of both nitric oxide and a change in membrane potential to acetylcholine-induced relaxation in the rat small mesenteric artery. *Br J Pharmacol* 112: 831–836, 1994.
34. **Yamamoto Y, Chen G, Miwa K, and Suzuki H.** Permeability and Mg^{2+} blockade of histamine-operated cation channel in endothelial cells of rat intrapulmonary artery. *J Physiol (Lond)* 450: 395–408, 1992.
35. **Yao X, Kwan HY, Chan FL, Chan NWK, and Huang Y.** A protein kinase G-sensitive channel mediates flow-induced Ca^{2+} entry into vascular endothelial cells. *FASEB J* 14: 932–938, 2000.
36. **Yoshida Y, Toyosato A, Islam MO, Koga T, Fujita S, and Imai S.** Stimulation of plasma membrane Ca^{2+}-pump ATPase of vascular smooth muscle by cGMP-dependent protein kinase: functional reconstitution with purified proteins. *Mol Cell Biochem* 190: 157–167, 1999.

Downloaded from ajpheart.physiology.org on December 15, 2010