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Characterization of vasoconstrictor-induced relaxation in the cerebral basilar artery

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ABSTRACT

The vascular endothelium regulates vascular smooth muscle functions by releasing endothelium-derived vasoactive substances. To identify physiological mechanisms mediating the inhibitory effect of the endothelium on vasoconstrictors, ring preparations of the basilar artery isolated from Wistar rats were used in an organ bath study. In the intact basilar artery (with endothelium), 100 nM serotonin (5-HT) induced phasic contraction (28.7 ± 4.1% of 60 mM of KCl) followed by profound time-dependent relaxation at 3 min (3.8 ± 0.4%). In the denuded basilar artery (without endothelium), the 5-HT-induced contraction was enhanced (51.7 ± 16.1%), while time-dependent relaxation was abolished. In the intact basilar artery, the contraction was facilitated and the amplitude of the phasic contraction was significantly enhanced (70.1 ± 10.3%), but time-dependent relaxation was still manifest at 3 min (25.7 ± 10.0%) in the presence of Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) and indomethacin. Time-dependent relaxation induced by 5-HT was abolished in Ca^{2+}-free and in K^+-free Krebs-Henseleit Buffer (KHB). Furthermore, the 5-HT-induced contraction was enhanced by treatment with ouabain (105.6 ± 11.8%), tetraethylammonium chloride (133.2 ± 7.9%), charybdotoxin with apamin (145.4 ± 6.4%) or $BaCl_2$ (72.2 ± 13.8%) at 3 min; also, time-dependent relaxation was abolished by these blockers in the presence of L-NAME and indomethacin. U46619 (100 nM) induced sustained contraction without time-dependent relaxation in normal KHB, but charybdotoxin with apamin did not affect the contraction. The results suggest that time-dependent relaxation is modulated by endothelial Na^+/K^+-ATPase and Ca^{2+}-activated K^+ channel (K_{Ca}) activity, especially small and intermediate conductance K_{Ca}-prominent ionic mechanisms of the so-called endothelium-derived hyperpolarizing factor.

Keywords: basilar artery, time-dependent relaxation, Na^+/K^+-ATPase, Ca^{2+}-activated K^+ channels, EDHF

1. Introduction

Cerebrovascular disease is probably the result of hemodynamic disturbance in cerebral circulation that leads to mortality or disability (Vanhoutte et al., 2009). The study of endothelium-dependent and -independent factors that regulate blood flow through cerebral circulation is conducive to understanding the physiological characteristics of cerebrovascular functions. The vascular endothelium regulates vascular tone by releasing endogenous factors such as endothelium-derived relaxing factor (EDRF) and contracting factors that include nitric oxide (NO) (Furchgott and Zawadzki, 1980), prostacyclin (PGI_2) (Moncada and Vane, 1979), endothelium-derived hyperpolarizing factor (EDHF) (Chen et al., 1988), endothelin and prostaglandin $F_{2\alpha}$, all of which regulate homeostasis and vascular tone through multiple mechanisms. Currently, unlike NO and PGI_2, relatively little is known about the biochemical, physiological or pharmacological aspects of EDHF, which is now considered one of the most important regulators of cerebrovascular tone (Kitazono et al., 1995; Schildmeyer and Bryan, 2002). The role of EDHF in the modulation of vasoconstrictor-induced contractile response of the basilar artery has not been clarified. As one of the endothelium-dependent factors, K^+ is an important regulator in cerebral vessels, where hyperpolarization of vascular smooth muscle cells through K^+ channel activation promotes relaxation (Faraci and Sobey, 1998).

The removal and dysfunction of the vascular endothelium have been known to increase contraction induced by various stimulants such as serotonin (5-HT), thromboxane A_2 (TXA_2) and noradrenaline, suggesting that the endothelium regulates contraction by releasing EDRF, including NO and PGI_2 (Vanhoutte and Mombouli, 1996). Vasoconstrictor-induced responses usually comprise phasic and tonic contraction periods. The amplitude of tonic contraction, whether sustained or relaxed, depends on the vasoconstrictor. The contractile response to 5-HT comprises a phasic contraction followed by time-dependent relaxation. The manifestation of time-dependent relaxation can be mediated by NO and PGI_2 (Bruning et al., 1993; Diaz et al., 2008; Henrion et al., 2001), and the

influence of EDHF (Berhane et al., 2008) has recently been demonstrated in cerebral arteries (Wackenfors et al., 2006). The mechanism of EDHF in the cerebral artery induced by different agonists remains unclear (Bryan et al., 2005; Petersson et al., 1995; Wackenfors et al., 2006), and the underlying mechanism of time-dependent relaxation in cerebral circulation is not well understood, particularly with regard to the roles of Na^+/K^+-ATPase and K^+ channels. The present study was designed to investigate the role of the endothelium in the regulation of contractile responses to 5-HT and U46619 in the basilar artery. Understanding the mechanisms of time-dependent relaxation would contribute to the clinical treatment of cerebrovascular disease.

2. Materials and Methods

2.1. Animals

All protocols involving animals were approved by the Guidelines for Animal Experimentation of Shimane University and complied with the Guidelines for Animal Experimentation required by the Japanese Association for Laboratory Animal Science. All the male and female Wistar rats, 17-24 weeks old, weighing 260-390 g and used in this study were maintained under the following controlled environmental conditions: 23 ± 2°C, 50 ± 10% relative humidity, 12 / 12 h light/dark cycle, 13-15 cycles of air exchange / h, and given food and water *ad libitum*.

2.2. Basilar artery ring preparation and organ bath setup

The rats were anesthetized with diethyl ether (Wako Pure Chemical Industries, Osaka, Japan), then transcardially perfused with 50 ml Krebs-Henseleit Buffer (KHB) containing 118 mM of NaCl, 4.5 mM of KCl, 2.5 mM of $CaCl_2$, 1.2 mM of KH_2PO_4, 1.5 mM of $MgSO_4$, 25 mM of $NaHCO_3$, and 11 mM glucose, pH 7.4, and aerated with 95% O_2 / 5% CO_2. To prepare the denuded basilar artery, the transcardial perfusion included 20 ml of 0.07% saponin for 10 s, followed by 50 ml KHB to abolish endothelial function, as detailed by Samata et al (1986). The whole brain was quickly removed, and the basilar artery isolated from the brain was gently cleaned of any connective tissue in KHB and cut into 2 ring preparations (each 2.5-3 mm long) under a dissecting microscope.

2.3. Experimental protocols

The cleaned preparations were placed in a chamber (UFER, Medical Kishimoto, Japan) containing KHB at 37 ± 0.5°C. Two fine tungsten wires (Ø 50 μm) were then passed through the lumen of the basilar artery, with one end of each wire connected to an isometric transducer (T7-8-240, ORIENTEC, Tokyo, Japan) and the other attached to the holder; the isometric tension was then recorded on a

polygraph (RECTIGRAPH-8K, San-ei, Tokyo, Japan). The experiments were monitored by a computer-based analysis system in Mac-Lab and Chart 4.1 software (AD Instruments, Inc., Colorado Springs, CO, USA).

The basilar artery preparations were loaded with 140-150 mg tension (the optimal tension that induces a constant contractile response to 60 mM KCl) and allowed to equilibrate for 40-50 min while being washed with KHB every 15 min. The preparations were then exposed to 60 mM of KCl, which caused contraction, and washed with KHB three times. The KCl-induced contraction was established as a standard contractile response for relative comparison with subsequent agonist applications, as described in our previous study (Enkhjargal et al., 2008). An inhibitor was added 15-20 min before agonist application in the organ bath.

2.4. Chemicals

Serotonin (5-HT), Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), tetraethylammonium chloride (TEA), and ouabain [(-)-ouabain octahydrate] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Charybdotoxin, and apamin were from Calbiochem, (Germany), and $BaCl_2$ was from Nakarai Chemicals Ltd., Japan. The drugs dissolved in distilled water were stocked as 1-10 mM solutions. U46619 [9,11-dideoxy-9a,11a-methanoepoxy-Prostaglandin $F_{2\alpha}$] (Cayman Chemical, USA) was dissolved in ethanol (99.5%) and stocked as 10 mM solutions and then diluted with distilled water immediately before use. Indomethacin purchased from Wako Pure Chemical Industries (Osaka, Japan) was dissolved in DMSO and stocked as 10 mM solutions. The final concentration of DMSO was less than 0.1%.

All chemicals and materials of the highest grade available commercially were used at the following final concentrations: 5-HT (100 nM); U46619 (100 nM); ouabain (17 μM); TEA (1 mM);

charybdotoxin (100 nM); apamin (100 nM); $BaCl_2$ (8 μM); L- NAME (100 μM); indomethacin (10 μM).

2.5. Statistical analysis

Results are expressed as means ± S.E.M. The responses calculated from different treatments were averaged by group and treatment and compared by one-way ANOVA, followed by the LDS test for post hoc analysis. Differences in the contractile responses between the two groups were determined by independent sample t-tests. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Baseline contractile responses to KCl

The resting tension was significantly greater in the denuded basilar artery (247.0 ± 19.9 mg) than in the intact basilar artery (153.3 ± 5.0 mg). The mean standard contractile response to 60 mM of KCl of the denuded basilar artery (258.5 ± 18.0 mg) was not significantly different from that of the intact basilar artery (269.6 ± 18.5 mg, n = 20) (Fig. 1-A). All results are shown as a percentage (%) of the 60 mM of KCl in the intact and denuded basilar artery. To assess the effectiveness of endothelium removal, preparations preconstricted by 100 nM of 5-HT were challenged with 1 μM acetylcholine. The relaxation induced by acetylcholine was detected in the intact basilar artery, but not in the denuded basilar artery (Fig. 1-A).

3.2. Contractile responses to 5-HT in the presence or absence of L-NAME and indomethacin

The 5-HT-induced contraction comprised an initial fast contraction (phasic component) followed by a sustained contraction (tonic component). In the intact basilar artery, the tonic component decreased gradually and its amplitude decreased time dependently (Fig. 1-A and B). The phasic component (28.7 ± 4.1%) was followed by relaxation (3.8 ± 0.4%) at 3 min, and its amplitude was significantly enhanced (70.1 ± 10.3%), but the time-dependent decrease in the tonic component (called time-dependent relaxation) was still manifest (25.7 ± 10.0%) at 3 min in the presence of L-NAME and indomethacin. In the denuded basilar artery, the phasic component was significantly enhanced (51.7 ± 16.1%) compared with that in the intact basilar artery, and the tonic component was high (42.2 ± 14.9%) in the absence of L-NAME and indomethacin at 3 min. The tonic component was significantly enhanced (77.5 ± 17.0%) in the presence of L-NAME and indomethacin, compared with that in their absence, at 3 min (Fig. 1-B). Acetylcholine-induced relaxation was observed in the intact basilar artery, but not in the denuded basilar artery (Fig. 1 middle panel).

3.3. Contractile responses to U46619 in the presence or absence of L-NAME and indomethacin

The contractile response to U46619 was significantly greater in the denuded basilar artery than in the intact basilar artery (Fig. 2-A and B). In the intact basilar artery, the slight tonic component (1.7 ± 0.6%) in the absence of L-NAME and indomethacin, increased slightly but significantly (6.0 ± 0.7 %) at 3 min in the presence of L-NAME and indomethacin. In the denuded basilar artery, the tonic component (29.2 ± 9.5%) was the same in the presence or absence of L-NAME and indomethacin. Acetylcholine-induced relaxation was observed in the intact basilar artery, but not in the denuded basilar artery (Fig. 2 -A middle panel).

3.4. Effects of Na^+/K^+-ATPase inhibition on contractile responses

In K^+-free KHB and the presence of L-NAME and indomethacin, the resting tension of the intact basilar artery increased markedly at 10 min, then decreased slowly at 30 min (Fig. 3-A) and that of the denuded basilar artery initially increased at 10 min but continued to decrease after 30 min. Overall, the difference of resting tension between the intact and denuded basilar arteries was abolished in K^+-free KHB after 40 min; thereafter, no differences in contractile responses to 5-HT were observed between the two arteries. Relaxation induced by the addition of 4.5 mM of KCl to the precontracted basilar artery with 5-HT after exposure to K^+-free KHB was observed in both the intact and denuded basilar arteries in the presence of L-NAME and indomethacin. The relaxation induced by the restoration of KCl concentration in KHB was of a longer duration in the intact than in the denuded basilar artery. A re-contractile response was subsequently observed in the denuded basilar artery at 29.4 ± 1.5 min, but not in the intact basilar artery during the 40 min period of observation (Fig. 3A).

Similar results were observed from U46619-induced contractile responses in K^+-free KHB and the presence of L-NAME and indomethacin (Fig. 3-B). The re-contractile response was observed in the

denuded basilar artery at 26.7 ± 1.6 min, but not in the intact basilar artery during the 40 min period of observation.

In the presence of L-NAME and indomethacin, the 5-HT-induced contraction comprised a phasic component followed by time-dependent relaxation (Fig. 4-A). Time-dependent relaxation (25.7 ± 10.0%) was blocked by treatment with ouabain, and the tonic component (105.6 ± 11.8%) was observed at 3 min. The U46619-induced tonic contraction (5.4 ± 0.8%) was enhanced (85.9 ± 14.2%) at 3 min by treatment with ouabain (Fig. 4-B).

3.5. Effects of K^+-channel blockers on the contractile responses to 5-HT and U46619 in the presence of L-NAME and indomethacin

In the presence of L-NAME and indomethacin, time-dependent relaxation induced by 5-HT in the intact basilar artery was blocked by treatment with TEA (a Ca^{2+}-activated K^+ channel (K_{Ca}) blocker) and the tonic component appeared (133.2 ± 7.9%) at 3 min (Fig. 5-A). The contractions were also enhanced by treatment with charybdotoxin (an intermediate and large conductance K_{Ca} blocker), apamin (a small conductance K_{Ca} blocker) (145.4 ± 6.4%) and $BaCl_2$ (an inward rectifier (K_{IR}) channel blocker) (72.2 ± 13.8%) at 3 min (Fig. 5-A).

The U46619-induced contraction was also strongly enhanced by the treatment with TEA (129.5 ± 5.8%) and with $BaCl_2$ (63.4 ± 15.5%) at 3 min (Fig. 5-B). Characteristically, the contraction induced by U46619 (5.6 ± 0.8%) was relatively unchanged by the treatment with charybdotoxin and apamin (8.3 ± 1.3%).

3.6. Role of Ca^{2+} in the manifestation of time-dependent relaxation in intact basilar artery in the presence of L-NAME and indomethacin

The amplitude of the 5-HT-induced contraction after incubation with Ca^{2+}-free KHB for 30 min

was significantly lower than that after incubation with normal KHB, but similar in the intact (6.3 ± 1.3%) and denuded (5.6 ± 1.9%) basilar artery (Fig. 6-A and B). After adding 2.5 mM Ca^{2+} to restore to normal KHB, and in the presence of 5-HT, the phasic contraction increased significantly in both the intact (124.6 ± 5.3%) and the denuded (163.7 ± 15.3%) basilar arteries. The tonic contraction reverted to the level of time-dependent relaxation in the intact basilar artery (1.6 ± 1.6 %), but time-dependent relaxation was not detected in the denuded basilar artery (128.5 ± 13.9 %) in the presence of 5-HT at 3 min.

The amplitude of the U46619-induced contraction after incubation with Ca^{2+}-free KHB for 30 min was similar to that in the intact (11.0 ± 1.0%) and the denuded (18.2 ± 5.0%) basilar artery (Fig. 6-A and B). After adding Ca^{2+} to restore to normal KHB, the phasic component of the U46619-induced contraction increased significantly (117.4 ± 5.7%) and time-dependent relaxation became manifest (-0.8 ± 3.7%) in the intact basilar artery. The tonic component of the contraction increased (171.9 ± 37.9%) and time-dependent relaxation was affected slightly (100.7 ± 13.9%) in the denuded basilar artery.

4. Discussion

In the intact basilar artery, the contractile response to 5-HT comprised a phasic contraction followed by time-dependent relaxation; however, removal of the endothelium augmented the phasic component of the 5HT-induced contraction and markedly attenuated time-dependent relaxation. Nonetheless, time-dependent relaxation was still detected in the presence of L-NAME and indomethacin in the intact basilar artery, suggesting that the endothelium-dependent relaxation is partly mediated by NO, either alone or in concert with a relaxant cyclooxygenase product.

The main novel characteristics of time-dependent relaxation observed in the presence of L-NAME and indomethacin were its dependence on: 1) the endothelium 2) intracellular Ca^{2+} increase and K_{Ca} function in the endothelium, and 3) the activity of Na^+/K^+-ATPase and the K_{IR} channel. The long duration of K^+-induced relaxation was produced by the activation of Na^+/K^+-ATPase in the intact basilar artery. The Na^+/K^+-ATPase and Na^+/Ca^{2+} exchanger plays an important role in modulating vascular contraction. Na^+/K^+-ATPase inhibition affects the synthesis or release of EDRF more than it does its effector pathway (Woolfson and Poston, 1991). Na^+/K^+-ATPase activation inhibits Ca^{2+} mobilization in endothelial cells and, thereby, endothelium-dependent relaxation (Seol et al., 2004). Therefore, the difference in the duration of K^+-induced relaxation may be dependent on the activity of Na^+/K^+-ATPase in endothelial cells. The manifestation of re-contraction might be related to the Na^+/Ca^{2+} exchanger system in smooth muscles. Ouabain is a well known Na^+/K^+-ATPase inhibitor in arterioles (Johnson et al., 1998; McCarron and Halpern, 1990; Webb and Bohr, 1978). Time-dependent relaxation is probably mediated by an increase in Ca^{2+} influx in both the intact and denuded basilar arteries attributed mainly to the inhibition of electrogenic Na^+/K^+-ATPase in smooth muscles. In endothelial and vascular smooth muscle cells, an increase in intracellular Ca^{2+} by contractile agonists stimulates K_{Ca} channels. The K_{IR} channels, which are strongly voltage-dependent, shut down upon membrane depolarization (Edwards et al., 1988). The

subsequent increase in extracellular K^+ activates K_{IR} and Na^+/K^+-ATPase in the membrane of the vascular smooth muscle, resulting in smooth muscle hyperpolarization. Although we observed no contraction of the basilar artery during treatment with any of the blockers in this study, contraction was induced in both the intact and denuded basilar arteries by the removal of K^+ from KHB. The activation of Na^+/K^+-ATPase was associated with the endothelium for the resting tension in the intact basilar artery.

Ba^{2+} (< 50 μM) is currently the most selective and effective inhibitor of K_{IR} channels (Quayle et al., 1993). PCR products corresponding to mRNA for K_{IR} are present in the basilar artery, aorta, and brain (Zaritsky et al., 2000). Thus, data confirming the expression of K_{IR} in the intact basilar artery and the inhibitory effects of Ba^{2+} on time-dependent relaxation of the 5-HT-induced contraction are consistent with the role of K_{IR} channels in the functional effects of K^+ in cerebral circulation (Bradley et al., 1999). Several studies have demonstrated that EDHF relaxation is triggered in the endothelium, and that hyperpolarization of the endothelium by the activation of K^+ channels is requisite for agonist-induced EDHF relaxation responses (Brahler et al., 2009; Dora et al., 2008; Edwards et al., 1998; Eichler et al., 2003; Jiang et al., 2000). The vasodilation induced by 5-HT has been shown to depend on EDHF in several vascular beds (Berhane et al., 2008; Tschudi et al., 1991).

To be considered relaxation through EDHF, time-dependent relaxation must be consistent with the following criteria: 1) require the endothelium, 2) be distinct from NO or PGI_2 3) involve K_{Ca} channels, and 4) cause relaxation by hyperpolarizing the vascular smooth muscle, as reported in previous studies (Bryan et al., 2005; Schildmeyer and Bryan, 2002). In our study, time-dependent relaxation was completely abolished and the tonic contraction was enhanced to the same extent by ouabain, a non-selective K_{Ca} blocker (TEA) or an intermediate-conductance K_{Ca} (IK_{Ca}) blocker (charybdotoxin) with a small-conductance K_{Ca} (SK_{Ca}) blocker (apamin). Moreover, time-dependent relaxation was completely inhibited by the K_{IR} blocker, $BaCl_2$. Thus, our results are in agreement with those of

other EDHF studies (Ding et al., 2000; Edwards et al., 1998; Gluais et al., 2005).

Both IK_{Ca} and SK_{Ca} channels have been described in the endothelium (McNeish et al., 2006), demonstrating that activation of TXA_2 receptors in the rat middle cerebral artery inhibits the SK_{Ca} input to endothelium-dependent hyperpolarization when NO synthesis is inhibited. Based on our current results, the U46119-induced contraction was not affected by channel blockers charybdotoxin and apamin, which might be related to the inhibitory effect of U46119 on IK_{Ca} and SK_{Ca}.

EDHF-mediated relaxation could be induced by direct activation of IK_{Ca} followed by subsequent endothelial hyperpolarization without any increase in endothelial Ca^{2+} in the middle cerebral artery (Marrelli et al., 2003). 5-HT might activate the EDHF system in a K_{Ca}-dependent manner, regardless of substantial differences in K_{Ca} type (You et all., 1999) and the transduction system in time-dependent relaxation (Ng et all., 2008). EDHF is a term that represents multiple factors, and the endothelium-dependent hyperpolarizing system may mediate a collaboration of K^+ channels that supports the autoregulation mechanisms of time-dependent relaxation in the basilar artery.

We did not measure smooth muscle membrane potential alongside our measurements of contractility. Indeed, doing so would be a prohibitive technical challenge. Therefore, we were not able to obtain direct evidence suggesting that time-dependent relaxation insensitivity to L-NAME and indomethacin is directly associated with hyperpolarization. Nonetheless, our studies are the first on time-dependent relaxation through EDHF in the basilar artery.

In conclusion, our study provides an important model and perspective for the investigation of cerebral circulation, especially with respect to elucidating time-dependent relaxation mechanisms.

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References

Berhane, Y., Bailey, S.R., Putignano, C., Elliott, J., 2008. Characterization of agonist-induced endothelium-dependent vasodilatory responses in the vascular bed of the equine digit. J. Vet. Pharmacol. Ther. 31, 1-8.

Bradley, K.K., Jaggar, J.H., Bonev, A.D., Heppner, T.J., Flynn, E.R., Nelson, M.T., Horowitz, B., 1999. Kir2.1 encodes the inward rectifier potassium channel in rat arterial smooth muscle cells. J. Physiol. 515 (Pt 3), 639-651.

Brahler, S., Kaistha, A., Schmidt, V.J., Wolfle, S.E., Busch, C., Kaistha, B.P., Kacik, M., Hasenau, A.L., Grgic, I., Si, H., Bond, C.T., Adelman, J.P., Wulff, H., de Wit, C., Hoyer, J., Kohler, R., 2009. Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. Circulation 119, 2323-2332.

Bruning, T.A., Chang, P.C., Blauw, G.J., Vermeij, P., van Zwieten, P.A., 1993. Serotonin-induced vasodilatation in the human forearm is mediated by the "nitric oxide-pathway": no evidence for involvement of the 5-HT3-receptor. J. Cardiovasc. Pharmacol. 22, 44-51.

Bryan, R.M., Jr., You, J., Golding, E.M., Marrelli, S.P., 2005. Endothelium-derived hyperpolarizing factor: a cousin to nitric oxide and prostacyclin. Anesthesiology 102, 1261-1277.

Chen, G., Suzuki, H., Weston, A.H., 1988. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br. J. Pharmacol. 95, 1165-1174.

Diaz, J., Ni, W., Thompson, J., King, A., Fink, G.D., Watts, S.W., 2008. 5-Hydroxytryptamine lowers blood pressure in normotensive and hypertensive rats. J. Pharmacol. Exp. Ther. 325, 1031-1038.

Ding, H., Kubes, P., Triggle, C., 2000. Potassium- and acetylcholine-induced vasorelaxation in mice lacking endothelial nitric oxide synthase. Br. J. Pharmacol. 129, 1194-1200.

Dora, K.A., Gallagher, N.T., McNeish, A., Garland, C.J., 2008. Modulation of endothelial cell

KCa3.1 channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. Circ. Res. 102, 1247-1255.

Edwards, F.R., Hirst, G.D., Silverberg, G.D., 1988. Inward rectification in rat cerebral arterioles; involvement of potassium ions in autoregulation. J. Physiol. 404, 455-466.

Edwards, G., Dora, K.A., Gardener, M.J., Garland, C.J., Weston, A.H., 1998. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 396, 269-272.

Eichler, I., Wibawa, J., Grgic, I., Knorr, A., Brakemeier, S., Pries, A.R., Hoyer, J., Kohler, R., 2003. Selective blockade of endothelial Ca^{2+}-activated small- and intermediate-conductance K^+-channels suppresses EDHF-mediated vasodilation. Br. J. Pharmacol. 138, 594-601.

Enkhjargal, B., Hashimoto, M., Kinoshita, H., Sakai, Y., 2008. Characteristics of contractile activity in the renal artery of ovariectomized rats. J. Smooth Muscle Res. 44, 17-28.

Faraci, F.M., Sobey, C.G., 1998. Role of potassium channels in regulation of cerebral vascular tone. J. Cereb. Blood Flow Metab. 18, 1047-1063.

Furchgott, R.F., Zawadzki, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373-376.

Gluais, P., Edwards, G., Weston, A.H., Falck, J.R., Vanhoutte, P.M., Feletou, M., 2005. Role of SK(Ca) and IK(Ca) in endothelium-dependent hyperpolarizations of the guinea-pig isolated carotid artery. Br. J. Pharmacol. 144, 477-485.

Henrion, D., Kubis, N., Levy, B.I., 2001. Physiological and pathophysiological functions of the AT(2) subtype receptor of angiotensin II: from large arteries to the microcirculation. Hypertension 38, 1150-1157.

Jiang, F., Li, C.G., Rand, M.J., 2000. Mechanisms of nitric oxide-independent relaxations induced by carbachol and acetylcholine in rat isolated renal arteries. Br. J. Pharmacol. 130, 1191-1200.

Johnson, T.D., Marrelli, S.P., Steenberg, M.L., Childres, W.F., Bryan, R.M., Jr., 1998. Inward rectifier

potassium channels in the rat middle cerebral artery. Am. J. Physiol. 274, R541-547.

Kitazono, T., Faraci, F.M., Taguchi, H., Heistad, D.D., 1995. Role of potassium channels in cerebral blood vessels. Stroke 26, 1713-1723.

Marrelli, S.P., Eckmann, M.S., Hunte, M.S., 2003. Role of endothelial intermediate conductance KCa channels in cerebral EDHF-mediated dilations. Am. J. Physiol. Heart Circ. Physiol. 285, H1590-1599.

McCarron, J.G., Halpern, W., 1990. Potassium dilates rat cerebral arteries by two independent mechanisms. Am. J. Physiol. 259, H902-908.

McNeish, A.J., Sandow, S.L., Neylon, C.B., Chen, M.X., Dora, K.A., Garland, C.J., 2006. Evidence for involvement of both IKCa and SKCa channels in hyperpolarizing responses of the rat middle cerebral artery. Stroke 37, 1277-1282.

Moncada, S., Vane, J.R., 1979. The role of prostacyclin in vascular tissue. Fed. Proc. 38, 66-71.

Petersson, J., Zygmunt, P.M., Brandt, L., Hogestatt, E.D., 1995. Substance P-induced relaxation and hyperpolarization in human cerebral arteries. Br. J. Pharmacol. 115, 889-894.

Quayle, J.M., McCarron, J.G., Brayden, J.E., Nelson, M.T., 1993. Inward rectifier K^+ currents in smooth muscle cells from rat resistance-sized cerebral arteries. Am. J. Physiol. 265, C1363-1370.

Samata, K., Kimura, T., Satoh, S., Watanabe, H., 1986. Chemical removal of the endothelium by saponin in the isolated dog femoral artery. Eur. J. Pharmacol. 128, 85-91.

Schildmeyer, L.A., Bryan, R.M., Jr., 2002. Effect of NO on EDHF response in rat middle cerebral arteries. Am. J. Physiol. Heart Circ. Physiol. 282, H734-738.

Seol, G.H., Ahn, S.C., Kim, J.A., Nilius, B., Suh, S.H., 2004. Inhibition of endothelium-dependent vasorelaxation by extracellular K(+): a novel controlling signal for vascular contractility. Am. J. Physiol. Heart Circ. Physiol. 286, H329-339.

Tschudi, M., Richard, V., Buhler, F.R., Luscher, T.F., 1991. Importance of endothelium-derived nitric oxide in porcine coronary resistance arteries. Am. J. Physiol. 260, H13-20.

Vanhoutte, P.M., Shimokawa, H., Tang, E.H., Feletou, M., 2009. Endothelial dysfunction and vascular disease. Acta Physiologica 196, 193-222,

Vanhoutte, P.M., Mombouli, J.V., 1996. Vascular endothelium: vasoactive mediators. Prog. Cardiovasc. Dis. 39, 229-238.

Wackenfors, A., Vikman, P., Nilsson, E., Edvinsson, L., Malmsjo, M., 2006. Angiotensin II-induced vasodilatation in cerebral arteries is mediated by endothelium-derived hyperpolarising factor. Eur. J. Pharmacol. 531, 259-263.

Webb, R.C., Bohr, D.F., 1978. Potassium-induced relaxation as an indicator of Na^+-K^+ ATPase activity in vascular smooth muscle. Blood Vessels 15, 198-207.

Woolfson, R.G., Poston, L., 1991. Effect of ouabain on endothelium-dependent relaxation of human resistance arteries. Hypertension 17, 619-625.

Zaritsky, J.J., Eckman, D.M., Wellman, G.C., Nelson, M.T., Schwarz, T.L., 2000. Targeted disruption of Kir2.1 and Kir2.2 genes reveals the essential role of the inwardly rectifying K(+) current in K(+)-mediated vasodilation. Circ. Res. 87, 160-166.

Figure legends

Fig. 1.

Contractile responses to KCl and to 5-HT in the intact basilar artery and the denuded basilar artery in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin.

A. Typical recordings of contraction in response to 60 mM of KCl or 100 nM of 5-HT followed by relaxation (time-dependent relaxation) in the intact basilar artery, and effects of 1 μM acetylcholine (ACh) on these responses in the intact and the denuded basilar artery in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin (INDO).

B. Summary of contractile responses to 100 nM of 5-HT in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin (INDO). Values are means ± S.E.M. * Significantly different from the intact basilar artery. $P < 0.05$. # Significantly different from the intact basilar artery in the presence of 100 μM of L-NAME and 10 μM indomethacin. $P < 0.05$.

Fig. 2.

Contractile responses to 60 mM of KCl or 100 nM of U46619 in the intact and denuded basilar artery in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin.

A. Typical recordings of contraction in response to 60 mM of KCl or 100 nM of U46619 in the intact basilar artery, and effects of 1 μM of acetylcholine (ACh) on these responses in the intact and denuded basilar artery rings in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin (INDO).

B. Summary of contractile responses to 100 nM of U46619 in the intact and denuded basilar artery rings in the absence and presence of 100 μM of L-NAME and 10 μM indomethacin (INDO). Values are means ±S.E.M. * Significantly different from the intact basilar artery preparations. $P < 0.05$. # Significantly different from the intact basilar artery in the presence of 100 μM of

L-NAME and 10 μM indomethacin. $P < 0.05$.

Fig. 3.

Effects of Na^+/K^+-ATPase inhibition on basilar artery contractile responses.

A. Effects of K^+-free KHB on the resting tension in the intact and denuded basilar artery. Contractile responses to 5-HT in K^+-free KHB and effects of adding 4.5 mM of KCl on the contraction in the intact and denuded basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery. $P < 0.05$.

B. Effects of K^+-free KHB on the resting tension in the intact and denuded basilar artery. Contractile responses to U46619 in K^+-free KHB and effects of adding 4.5 mM of KCl on the contraction in the intact and denuded basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery. $P < 0.05$.

Fig. 4.

Effects of ouabain on contraction in the intact basilar artery in the presence of 100 μM of L-NAME and 10 μM indomethacin (INDO).

A. Effects of ouabain on time-dependent relaxation induced by 5-HT in the intact basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery in the absence of 17 μM ouabain. $P < 0.05$.

B. Effects of ouabain on the contraction induced by U46619 in the intact basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery in the absence of ouabain. $P < 0.05$.

Fig. 5.

Effects of K^+-channel blockers on contraction in the intact basilar artery in the presence of 100 μM of L-NAME and 10 μM indomethacin.

A. Effects of K^+-channel blockers on 5-HT-induced contraction and time-dependent relaxation in the intact basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery. $P < 0.05$.

Effects of K^+-channel blockers on U46619-induced contraction in the intact basilar artery. Values are means ± S.E.M. * Significantly different from the intact basilar artery. $P < 0.05$. Indomethacin (INDO); charybdotoxin (ChTX).

Fig. 6.

Role of Ca^{2+} in the manifestation of time-dependent relaxation in the intact basilar artery in the presence of L-NAME and indomethacin.

A. Typical recordings of contraction induced by 5-HT or U46619 in Ca^{2+}-free KHB, and effects of adding 2.5 mM of $CaCl_2$ on time-dependent relaxation in the intact and denuded basilar artery.

B. Summary of the amplitude of tension induced by 5-HT or U46119 in Ca^{2+}-free KHB (open), phasic tension induced by adding 2.5 mM of $CaCl_2$ (gray), and tonic tension 3 min after addition of Ca^{2+} (dotted). Values are means ± S.E.M. * Significantly different from the intact basilar artery.