学 位 論 文 の 要 旨

氏名　Budbazar Enkhjargal

学　位　論　文　名	Characterization of Vasoconstrictor-Induced Relaxation in the Cerebral Basilar Artery
発　表　雑　誌　名 （巻，初頁～終頁，年）	European Journal of Pharmacology (2010, in press)
著　　者　　名	Budbazar Enkhjargal, Michio Hashimoto, Yasushi Sakai, Osamu Shido

論 文 内 容 の 要 旨

INTRODUCTION

The vascular endothelium regulates vascular tone by releasing endothelium-derived vasoactive substances such as endothelium-derived relaxing and contracting factors that include nitric oxide (NO), prostacyclin (PGI_2), endothelium-derived hyperpolarizing factor (EDHF), endothelin and prostaglandin $F_{2\alpha}$, all of which regulate homeostasis and vascular tone through multiple mechanisms. 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. The contractile response to serotonin (5-HT) comprises a phasic contraction followed by time-dependent relaxation. The present study was designed to investigate the role of the endothelium in the regulation of contractile responses to 5-HT and U46619, a thromboxane A_2 agonist, in the cerebral basilar artery.

MATERIALS AND METHODS

All the male and female Wistar rats were anesthetized with diethyl ether, then transcardially perfused with 50 ml Krebs-Henseleit Buffer (KHB). To prepare the denuded basilar artery, the transcardial perfusion included 20 ml of 0.07% saponin for 10 s, followed by 50 ml of KHB to abolish endothelial function. The cleaned preparations of basilar artery (each 2.5-3 mm long) were placed in a chamber containing KHB at $37 \pm 0.5^{\circ}C$, pH 7.4, and aerated with 95% O_2 / 5% CO_2. The isometric tension was recorded on a polygraph and monitored by a computer-based analysis system in Mac-Lab and Chart 4.1

software. The basilar artery preparations were loaded with 140-150 mg tension 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 all results are shown as a percentage (%) of the 60 mM of KCl-induced contraction in the intact and denuded basilar artery. All chemicals were used at the following final concentrations: 5-HT (100 nM); U46619 (100 nM); ouabain (17 μM), Na^+/K^+-ATPase blocker; tetraethylammonium chloride (TEA) (1 mM), a non-selective Ca^{2+}-activated K^+ channel (K_{Ca}) blocker; charybdotoxin (100 nM), an intermediate (IK_{Ca}) and large conductance K_{Ca} blocker; apamin (100 nM), a small conductance K_{Ca} (SK_{Ca}) blocker; $BaCl_2$ (8 μM), an inward rectifier (K_{IR}) channel blocker; Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) (100 μM), a non-selective NO synthase inhibitor; indomethacin (10 μM), a non-selective cyclooxygenase inhibitor. Results are expressed as means ± S.E.M. *P*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

In the intact basilar artery (with endothelium), 5-HT induced phasic contraction (28.7 ± 4.1% of 60 mM KCl) followed by profound time-dependent relaxation at 3 min (3.8 ± 0.4%). In the denuded artery (without endothelium), the 5-HT-induced contraction was enhanced (51.7 ± 16.1%), while the 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 L-NAME and indomethacin. Acetylcholine-induced relaxation was observed in the intact basilar artery, but not in the denuded basilar artery. Similar results were observed from U46619-induced contractile responses in the presence or absence of L-NAME and indomethacin. These results suggest that the endothelium-dependent relaxation of basilar artery is partly mediated by NO, either alone or in concert with a relaxant cyclooxygenase product.

In K^+-free KHB and the presence of L-NAME and indomethacin, no differences in contractile responses to 5-HT were observed between the intact and denuded basilar arteries. 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 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. 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. Similar results were observed from U46619-induced contractile responses in K^+-free KHB and the presence of L-NAME and indomethacin. These results suggest that the long duration of K^+-induced relaxation was produced by the activation of Na^+/K^+-ATPase in the intact basilar artery.

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 (133.2 ± 7.9%), charybdotoxin with apamin (145.4 ± 6.4%) and $BaCl_2$ (72.2 ± 13.8%) at 3 min. Characteristically, the contraction induced by U46619 was relatively unchanged by the treatment with charybdotoxin and apamin. 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. The tonic contraction reverted to the level of time-dependent relaxation in the intact basilar artery (1.6 ± 1.6 %), but it was not detected in the denuded basilar artery (128.5 ± 13.9 %) in the presence of 5-HT at 3 min. Similar results were observed in the U46619-induced contractile responses in Ca^{+2}-free KHB and the presence of L-NAME and indomethacin. These results suggest that time-dependent relaxation is probably mediated by an increase in Ca^{2+} influx in both the intact and denuded basilar artery attributed mainly to the inhibition of electrogenic Na^+/K^+-ATPase in smooth muscle. In endothelial and vascular smooth muscle cells, an increase in intracellular Ca^{2+} by contractile agonists stimulates K_{Ca} channels. 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.

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. 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}.

CONCLUSION

The present results indicate that the time-dependent relaxation is a major regulation mechanism via EDHF, involving collaboration of K^+ channels potassium channel in the basilar artery. Our study provides an important model and perspective for the investigation of cerebral circulation, especially with respect to elucidating time-dependent relaxation mechanisms.