Use of intermediate/small conductance calcium-activated potassium-channel activator for endothelial protection

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Objectives: Endothelial dysfunction occurs in hypoxia-related states such as ischemic heart disease or heart surgery. Intermediate- and small-conductance calcium-activated potassium channels (IK_{Ca} and SK_{Ca}) are closely related to endothelium-dependent hyperpolarizing factor–mediated endothelial function. However, the status of these K_{Ca} under hypoxia is unknown. We investigated whether endothelial dysfunction under hypoxic state is related to the alterations of IK_{Ca} and SK_{Ca} and whether use of IK_{Ca}/SK_{Ca} activator may protect endothelium from hypoxia–reoxygenation injury.

Methods: Isometric tension measurement, patch-clamp technique, intracellular membrane potential recording, and molecular methods were used to study porcine coronary arteries and endothelial cells.

Results: Hypoxia–reoxygenation (60–30 minutes) decreased endothelium-dependent hyperpolarizing factor–mediated relaxation at normothermia in Krebs solution (43.3% ± 6.3% vs 82.3% ± 2.9%) and in St Thomas' Hospital cardioplegic solution (28.9% ± 1.8% vs 78.1% ± 3.0%) ($P < .001$) as well as at hypothermia in St Thomas' Hospital solution (43.1% ± 2.6%, $P < .001$). Hypoxia–reoxygenation markedly reduced endothelial IK_{Ca} (2.8 ± 0.6 vs 6.9 ± 0.6 pA/pF) and SK_{Ca} currents (1.5 ± 0.3 vs 4.3 ± 0.4 pA/pF) ($P < .05$) and downregulated endothelial IK_{Ca} expression. IK_{Ca}/SK_{Ca} activator 1-ethyl-2-benzimidazolinone enhanced K^+ current in endothelial cells that was blunted by hypoxia. Further, 1-ethyl-2-benzimidazolinone restored ($P < .001$) endothelium-dependent hyperpolarizing factor–mediated relaxation with hyperpolarization recovered from 6.0 ± 0.3 to 7.8 ± 0.4 mV ($P < .05$).

Conclusions: In porcine coronary arteries, hypoxia markedly reduced endothelial K^+ currents related to IK_{Ca} and SK_{Ca} with downregulation of protein expression and endothelium-derived hyperpolarizing factor function. IK_{Ca}/SK_{Ca} activator may preserve endothelium-dependent hyperpolarizing factor–mediated relaxation with enhancement of K^+ current in endothelial cells and cellular membrane potential hyperpolarization in smooth muscle cells and may become a new strategy to protect coronary endothelium in cardiac surgery or transplantation. (J Thorac Cardiovasc Surg 2011;141:501-10)

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Vascular endothelium releases nitric oxide (NO),[1,2] prostacyclin (PGI_2),[3] and endothelium-derived hyperpolarizing factor (EDHF)[4] to regulate blood flow. The EDHF-mediated relaxation and the associated hyperpolarization involve intermediate conductance calcium-activated K^+ channels (IK_{Ca}) and small conductance calcium-activated K^+ channels (SK_{Ca}) in the endothelial cell.[5,6] In the vascular smooth muscle, various ion channels such as Ba^{2+}-sensitive, inward rectifier K^+ channels (Kir), Na^+-K^+-adenosine triphosphatase,[7] and large conductance calcium-activated K^+ channels (BK_{Ca}) are involved.[8] Intraluminal application of blockers of IK_{Ca} and SK_{Ca}, charybdotoxin, and apamin blocked the EDHF-mediated function,[9] providing direct evidence for the involvement of endothelial IK_{Ca} and SK_{Ca} in this mechanism.

Ischemia–reperfusion (I-R) or hypoxia–reoxygenation (H-R) injury remains the major cause of cardiac dysfunction in ischemic heart disease and heart surgery including heart transplantation. I-R/H-R injury involves damage to myocytes as well as the coronary circulation (the vascular smooth muscle and the endothelium). Compared with vascular smooth muscle, endothelium is more vulnerable

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Abbreviations and Acronyms

ANOVA = analysis of variance
BK_{Ca} = large conductance calcium-activated K^+ channels
CI = confidence interval
1-EBIO = 1-ethyl-2-benzimidazolinone
EDHF = endothelium-derived hyperpolarizing factor
HBO = oxyhemoglobin
H-R = hypoxia–reoxygenation
IK_{Ca} = intermediate conductance calcium-activated K^+ channels
Indo = indomethacin
I-R = ischemia–reperfusion
K_{Ca} = calcium-activated potassium channels
Kir = inward rectifier K^+ channels
$_L$-NNA = N^G-nitro-L-arginine
NO = nitric oxide
PGI_2 = prostacyclin
SK_{Ca} = small conductance calcium-activated K^+ channels
ST solution = St Thomas Hospital cardioplegic solution

to I-R/H-R injury.[10,11] Previous studies have demonstrated that alteration of endothelial function under I-R/H-R is related not only to NO and PGI_2, but also to EDHF mechanism.[12-14] However, whether the change of EDHF-mediated function under I-R/H-R is related to the changes of endothelial IK_{Ca} or SK_{Ca} remains unexplored. We tested the hypothesis that endothelial dysfunction owing to H-R is related to the alterations of IK_{Ca} and SK_{Ca}.

Furthermore, the role of Ca^{2+}-activated K^+ channel (K_{Ca}) in ischemic preconditioning,[15] normal coronary arteriole,[16] or smooth muscle[17] has been reported. In one of our recent studies that mimics the clinical setting of heart surgery, we[18] observed the alterations of electrophysiologic properties and related function of smooth muscle K_{Ca} in coronary arteries exposed to ischemia or hyperkalemia. However, with regard to coronary endothelium, although it has been reported that cardioplegia may damage coronary endothelial function[19-22] and that microvascular dysfunction caused by cardioplegic arrest is likely in part owing to impaired function of IK_{Ca} and SK_{Ca} in the coronary microcirculation,[23] the electrophysiologic mechanisms responsible for endothelial IK_{Ca} and SK_{Ca} dysfunction in I-R or H-R states are still largely unclear.

Our previous studies further demonstrated that in both large and microcoronary arteries, the impaired endothelial function is mainly related to the EDHF pathway,[24-26] whereas NO release is not affected by K^+ at the concentration of 20 mmol/L.[27] Owing to the importance of endothelial IK_{Ca} and SK_{Ca} in the EDHF-mediated function, we therefore aimed to investigate whether endothelial dysfunction under hypoxic state is related to the alterations of IK_{Ca} and SK_{Ca} and whether use of IK_{Ca} and SK_{Ca} activator may protect porcine coronary endothelium from H-R injury. From this study, we suggested that a new strategy[28] targeting on the endothelial IK_{Ca} and SK_{Ca} may be beneficial to the protection of endothelial function during heart surgery or transplantation.

METHODS

Patch-Clamp Study of Endothelial IK_{Ca} and SK_{Ca} Currents

Isolation and culture of endothelial cells. Fresh porcine hearts from the hog (either sex) weighing about 30 kg, collected from a local slaughterhouse, were placed in a container filled with cold (4°C) Krebs solution and immediately transferred to the laboratory. In brief, porcine large coronary arteries were dissected into 4 × 4-mm strips and treated with 0.2% collagenase (type I; Sigma Chemical Company, St Louis, Mo) in phosphate-buffered saline solution for 25 minutes at 37°C. After the enzyme digestion, the suspension was centrifuged at 1600 rpm for 5 minutes. The cells were resuspended in 5 mL culture medium containing 90% Roswell Park Memorial Institute medium and 10% fetal bovine serum with 100 U/mL penicillin and 100 μg/mL streptomycin. After a 1-hour incubation at 37°C, the medium was replaced once to remove unattached cells. Attached endothelial cells were cultured in a humidified incubator with 5% CO_2 at 37°C. For maintaining electrophysiologic properties of isolated coronary endothelial cells, only primary cells were used for experiments.

Patch-clamp recording of IK_{Ca} and SK_{Ca} currents. K^+ currents in porcine primary endothelial cells were measured at room temperature (20°C–24°C) by whole-cell patch-clamp technique. Pharmacologic blockers, iberiotoxin for BK_{Ca}, charybdotoxin for IK_{Ca}, and apamin for SK_{Ca} were used to differentiate the role of BK_{Ca}, SK_{Ca} and IK_{Ca}.[29,30] The effect of IK_{Ca}/SK_{Ca} activator 1-ethyl-2-benzimidazolinone (1-EBIO) on the K^+ current was also examined.

Hypoxia exposure of endothelial cells. The primary culture of endothelial cells (seeded on glass coverslips) was placed in our renovated plastic cover–sealed myograph chamber filled with Krebs solution, and the solution was continuously bubbled with 95% N_2–5% CO_2[12] P_{O_2} change was monitored by an Oxygen Meter (model 781; Strathkelvin Instrument, Glasgow, Scotland, United Kingdom). The effectiveness of the device for hypoxia exposure has been demonstrated in our previous studies. The hypoxia in these experiments was attempted to approximate that in relaxation studies (1 hour). Endothelial cells were exposed to hypoxia before being transferred to the experimental chamber for current recording.