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Calcium-activated potassium channels contribute to human skeletal muscle microvascular endothelial dysfunction related to cardiopulmonary bypass

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Abstract

Background—We investigated the role of calcium-activated potassium (K_{Ca}) channel activity in human skeletal muscle microvascular function in the setting of cardiopulmonary bypass (CPB).

Methods and Results—Human skeletal muscle arterioles (80- to 180 μm in diameter) were dissected from tissue harvested before and after CPB. In vitro relaxation responses of precontracted arterioles in a pressurized no-flow state were examined in the presence of K_{Ca} channel activators/blockers and several other vasodilators. Post-CPB responses to the activator of intermediate (IK_{Ca}) and small conductance (SK_{Ca}) K_{Ca} channels, NS309, to the endothelium-dependent vasodilator adenosine 5′-diphosphate (ADP), and to substance P were reduced compared with pre-CPB responses ($P < .05$), respectively, whereas responses to the activator of large conductance (BK_{Ca}) K_{Ca} channels, NS1619, and to the endothelium-independent vasodilator, sodium nitroprusside (SNP) were unchanged. Endothelial denudation decreased NS309-induced relaxation and abolished that induced by ADP or substance P ($P< .05$), but had no effect on relaxation induced by either NS1619 or SNP. Polypeptide levels of BK_{Ca}, IK_{Ca}, and $SK3_{Ca}$ were not altered post-CPB.

Conclusion—IK/SK-mediated relaxation is predominantly endothelium dependent, whereas BK-mediated relaxation seems to be largely independent of endothelial function in human skeletal muscle microvasculature. CPB-associated microvascular dysfunction likely arises in part from impaired function of endothelial SK and IK channels in the peripheral microvasculature.

Cardiopulmonary bypass (CPB) induces a systemic inflammatory response, resulting in various degrees of organ dysfunction in multiple systems.[1] We have shown previously that CPB elicits complex, multifactorial vasomotor disturbances that vary according to the affected organ beds.[1-4] Specifically, CPB was associated with reduced vascular resistances in the skeletal muscle and peripheral circulation, and increased propensity to spasm in the cardiac, pulmonary, mesenteric, and cerebral vascular beds. Smooth muscle, but not endothelium, has been found to be responsible for this CPB-mediated vasomotor dysfunction.[1-4] In contrast, previous studies have shown that CPB-injured endothelium and impaired microvascular endothelial function in heart and other peripheral organs/tissues by showing decreased responses to endothelial-dependent relaxation.[1-3] Therefore, many procedures, such as heparin-coated

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circuits, systemic cooling, complement inhibition, and neutrophil depletion have been used in the CPB circuit to abrogate CPB-mediated inflammatory responses and peripheral endothelial dysfunction.

Endothelium has been found to release 3 major endothelium-dependent relaxing factors, namely, nitric oxide, prostacylin, and endothelium-dependent hyperpolarization factor.[5] Endothelium-dependent hyperpolarization factor dilates arteries via opening of calcium-activated potassium channels in endothelial and smooth muscle cells[5]; however, the role of K_{Ca} channel activation in CPB-related microvascular endothelial dysfunction in human peripheral resistance arterioles and the regulatory properties of these K_{Ca} channels in this vascular bed have been little investigated. This study was designed to examine the effect of CPB on vascular responses of human skeletal muscle microvessels to K_{Ca} channel openers and other vasoactive substances and to correlate these responses to possible alterations in expression of K_{Ca} polypeptides in human skeletal muscle tissue.

MATERIALS AND METHODS

Human subjects and tissue harvesting

Samples of skeletal muscle from the left internal mammary artery bed were harvested before and after CPB. The pre-CPB specimen was taken after cannulation, and the post-CPB specimen was collected from a different location in the left internal mammary artery bed after removal of the aortic cross-clamp and weaning from CPB. Tissue samples for immunoblot analysis assay were immediately frozen in liquid nitrogen. Tissue for microvascular studies was placed in cold (5°C to 10°C) Krebs buffer solution. All procedures were approved by the Institutional Review Board of Beth Israel Deaconess Medical Center, Harvard Medical School, and informed consent was obtained from all enrolled patients as required by the Institutional Review Board.

Microvessel relaxation studies

Skeletal muscle arterioles (80 to 180 μm internal diameters) from the left internal mammary artery bed were dissected before and after CPB. Microvessel studies were performed by in vitro organ bath videomicroscopy as described previously.[6] After vessel precontraction with U46619, endothelium-independent and -dependent relaxation to sodium nitroprusside (SNP), adenosine 59-diphosphate (ADP), and substance P were examined.

Immunoblot

Small arteries were dissected, cleaned of connective tissues, and solubilized in SDS-PAGE buffer. Total protein (40 μg) was fractionated on an 8% to 16% SDS-PAGE, then transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore Corporation, Bedford, Mass) as described previously.[3] Membranes were incubated for 1 hour at room temperature with 1:200 dilutions of individual rabbit polyclonal primary antibodies to BK_{Ca}-β_1 (Santa Cruz Biotechnology, Santa Cruz, Calif.), IK_{Ca} or $SK3_{Ca}$ (Alomone Labs Ltd, Jerusalem, Israel). The membranes were then incubated for 1 hour with horse-radish peroxidase-conjugated secondary anti-immunoglobulin washed 3 times in Tris saline buffer, and processed for chemiluminescent detection (Pierce, Rockford, Ill) on x-ray film (Kodak, Rochester, NY). Band intensity was measured by densitometric analysis of autoradiograph films using NIH Image J 1.33.

Chemicals

NS309, NS1619, U46619, SNP, ADP, and substance P were obtained from Sigma. SNP, ADP, and substance P were dissolved in ultrapure distilled water and prepared on the day of the study. U46619 was dissolved in ethanol to make a stock solution. NS309 and NS1619 were dissolved

in dimethylsulfoxide to make a stock solution. All stock solutions were stored at -20°C. All dilutions were prepared daily.

Data analysis

Data are presented as mean values and standard error of the mean. The relaxation responses were expressed as the percentage of relaxation of the U46619-preconstricted diameter of the microvessels. Repeated-measures ANOVA and the Student *t* test were used to compare variables among or between vessels. The treatment effects were statistically examined by the paired or independent 2-tailed Student *t* test. Statistical significance was taken at a probability value of <.05.

RESULTS

Patient characteristics

Tissue samples from 27 patients were studied. Twenty-seven patients (mean age, 67 ± 6 years) underwent coronary artery bypass grafting with duration of cardioplegic arrest of 58 ± 4 minutes and CPB time of 71.0 ± 18 minutes. Twenty-one patients were male and 6 were female. Of the 27 patients, 22 carried a preoperative diagnosis of hypertension. All patients with preoperative hypertension were on medication (β-blocker, calcium channel blocker, or angiotensin-converting enzyme inhibitor), and received perioperative β-blockade. Diabetes mellitus (type 1 or 2) was present in 5 of 27 patients.

Vessel characteristics

There were no significant differences in the values of vessel diameters among groups (Table). Similar U46619 concentrations were required to produce adequate precontraction in both groups.

Responses to NS309 and NS1619

Both NS309 (Fig 1, *A*) and NS1619 (Fig 2, *A*) induced dose-dependent relaxation of skeletal muscle arterioles. CPB impaired the relaxation response to NS 309, compared with pre-CPB responses ($P < .05$; Fig 1, *A*). In contrast, the responses to NS1619 were unchanged pre- and post-CPB (Fig 2, *A*). Pretreatment with TRAM34/apamin abolished vessel relaxation induced by NS309 ($P < .001$; Fig 1, *B*), and NS1619-induced relaxation was inhibited by pretreatment with iberiotoxin ($P < .001$; Fig 2, *B*). Furthermore, NS309-mediated vasorelaxation of the skeletal muscle arterioles was decreased by removal of the endothelium ($P < .001$; Fig 1, *C*), whereas responses to NS1619 were unaffected (Fig 2, *C*).

Responses to ADP, substance P, and SNP

ADP, substance P, and SNP induced a dose-dependent relaxation of coronary arterioles (Fig 3, *A*-*C*). CPB markedly decreased the relaxation responses both to ADP and to substance P compared with pre-CPB responses ($P < .05$; Fig 3, *A, B*). In contrast, the responses to SNP were unchanged pre- and post-CPB (Fig 3, *C*). Endothelium denudation abolished responses to both ADP and substance P ($P < .001$;Fig 3, *A, B*), but left intact SNP-induced relaxation.

Effect of CPB on levels of K_{Ca} polypeptides

Pre-CPB skeletal muscle artery levels of the K_{Ca} channel polypeptides BK_{Ca}-β_1, IK_{Ca}, and $SK3_{Ca}$ were unchanged in the post-CPB period as detected by immunoblot (Fig 4).

DISCUSSION

We have shown previously that relaxation responses of porcine skeletal muscle arterioles to the endothelium-dependent vasodilator acetylcholine are decreased after hypothermic CPB.[2,3] The present study, indicating that relaxation responses of human skeletal muscle arterioles to the endothelium-dependent vasodilators ADP and substance P are impaired post-CPB, suggests that CPB is associated with endothelial dysfunction of human peripheral microvasculature, and is consistent with the earlier study in porcine arterioles. The vascular endothelium is important not only for regulation of permeability, but also as the site of interaction with circulating leukocytes in the inflammatory response associated with CPB. Thus, CPB-induced activation of complement and neutrophils, release of proinflammatory cytokines and free radicals, and excessive adrenergic stimulation may all contribute to increased vascular permeability and endothelial swelling, resulting in peripheral microvascular endothelial dysfunction.

There are 3 types of vascular K_{Ca} channels—BK_{Ca}, IK_{Ca}, and SK_{Ca}.[7,8] In the present study, relaxation responses to NS309 were blocked completely in the presence of the IK_{Ca} blocker TRAM 34 and the SK_{Ca} inhibitor apamin, consistent with the proposed specificity of NS309 as a IK_{Ca}/SK_{Ca} activator. In contrast, relaxation responses to NS1619 were abolished in the presence of the BK_{Ca} blocker iberiotoxin, indicating specificity of NS1619 as a specific BK_{Ca} activator. Endothelium denudation significantly diminished NS309-induced relaxation, but failed to affect the response to NS1619, suggesting that relaxation induced by the IK_{Ca}/SK_{Ca} opener NS309 is endothelium dependent. Remarkably, CPB impaired the dose-dependent vasodilatation induced by the IK_{Ca}/SK_{Ca} channel activator NS309 but not that induced by the BK_{Ca} opener NS1619, suggesting that IK_{Ca}/SK_{Ca} inactivation may contribute to endothelial dysfunction of human skeletal muscle microvasculature. The molecular mechanism of CPB-related IK_{Ca}/SK_{Ca} dysfunction is unclear. CPB may modulate activity of serine and/or tyrosine kinases, leading to possible downregulation of IK_{Ca}/SK_{Ca} channels.[1,4,9] Alternatively or additionally, release of free radicals and/or cytokines during CPB might inhibit IK_{Ca}/SK_{Ca} channel activity of endothelium.[1,10]

To further test the presence in human skeletal muscle microvasculature of IK_{Ca}/SK_{Ca} and BK_{Ca} polypeptides, we performed immunoblot analyses to detect these channel proteins. BK_{Ca}, IK_{Ca}, and SK_{Ca} polypeptides were highly expressed in human skeletal muscle microvessels. CPB did not, however, alter total microvessel polypeptide abundance of any of these channels, suggesting that brief hypothermic ischemia and reperfusion modifies channel activity without change in channel polypeptide abundance. Recently, we also found that the responses to NS309 were significantly impaired in the coronary microvasculature after cardioplegic arrest and reperfusion, suggesting that SK and IK may be responsible for coronary microvascular endothelial dysfunction after cardiac operations.[11]

In conclusion, this study demonstrated that K_{Ca} channels are expressed in human skeletal muscle microvasculature. Inactivation of IK/SK channels may contribute to CPB-related microvascular endothelial dysfunction of human peripheral tissue.

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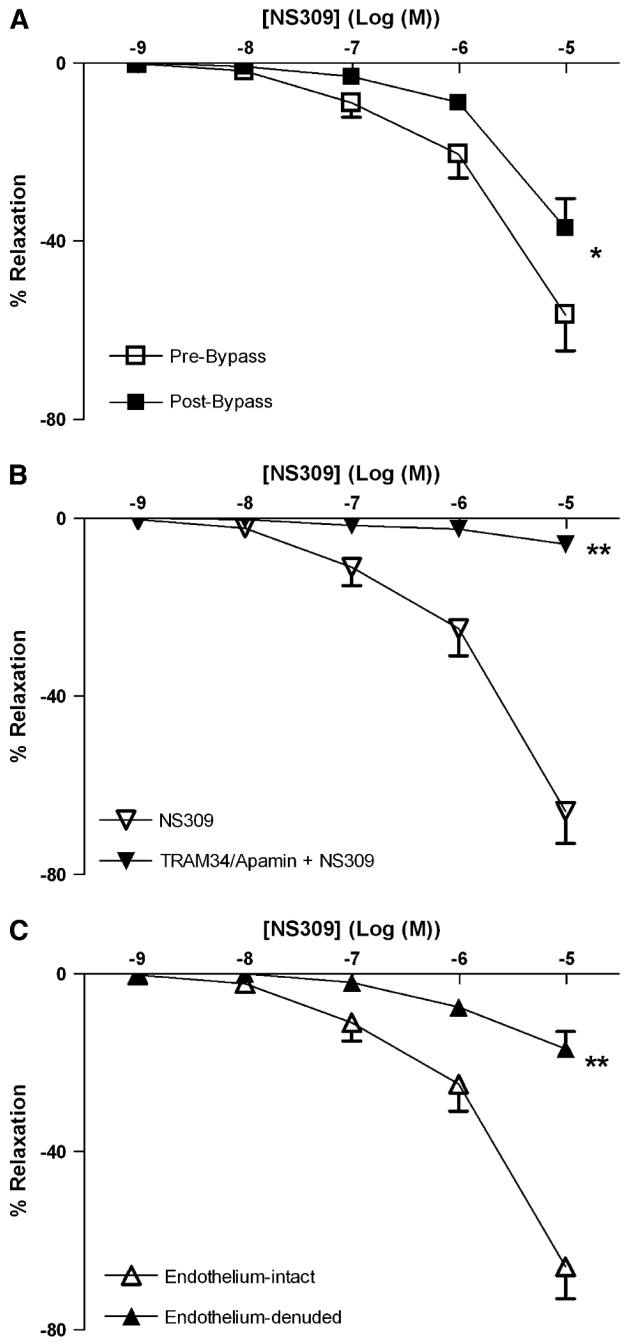

Fig 1.

Vasoreactivity of human skeletal muscle arterioles to the IK/SK channel activator, NS309. **A**, Dose-dependent relaxation of human skeletal muscle arterioles ($n = 8$) in response to NS309 pre- and post-CPB. *$P < .05$. **B**, Dose-dependent, NS309-mediated relaxation of pre-CPB human skeletal muscle arterioles pretreated in the absence or presence of TRAM34 and apamin ($n = 6$). **$P < .001$. **C**, Dose-dependent, NS309-mediated relaxation of pre-CPB human skeletal muscle arterioles ($n = 6$) with intact or denuded endothelium. **$P < .001$. Data are presented as percent relaxation after preconstriction with U46619.

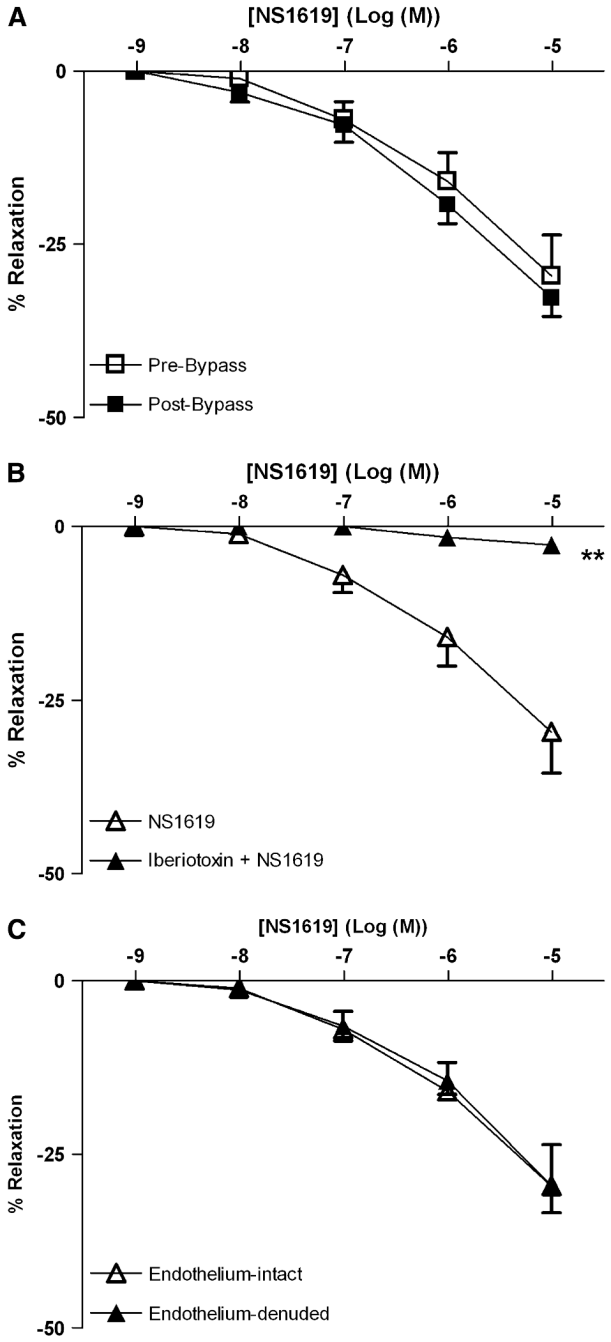

Fig 2.

Vasoreactivity of human skeletal muscle arterioles to the BK channel activator NS1619. **A**, Dose-dependent relaxation of human skeletal muscle arterioles in response to NS1619 either pre- or post-CPB (n = 8 per group). **B**, Dose-dependent, NS1619-mediated relaxation of pre-CPB human skeletal muscle arterioles pretreated in the absence or presence of iberiotoxin (n =6; $^{**}P < .001$). **C**, Dose-dependent, NS1619-induced relaxation of pre-CPB human skeletal muscle arterioles with intact or denuded endothelium (n = 6 per group). Data are presented as percent relaxation after preconstriction with U46619.

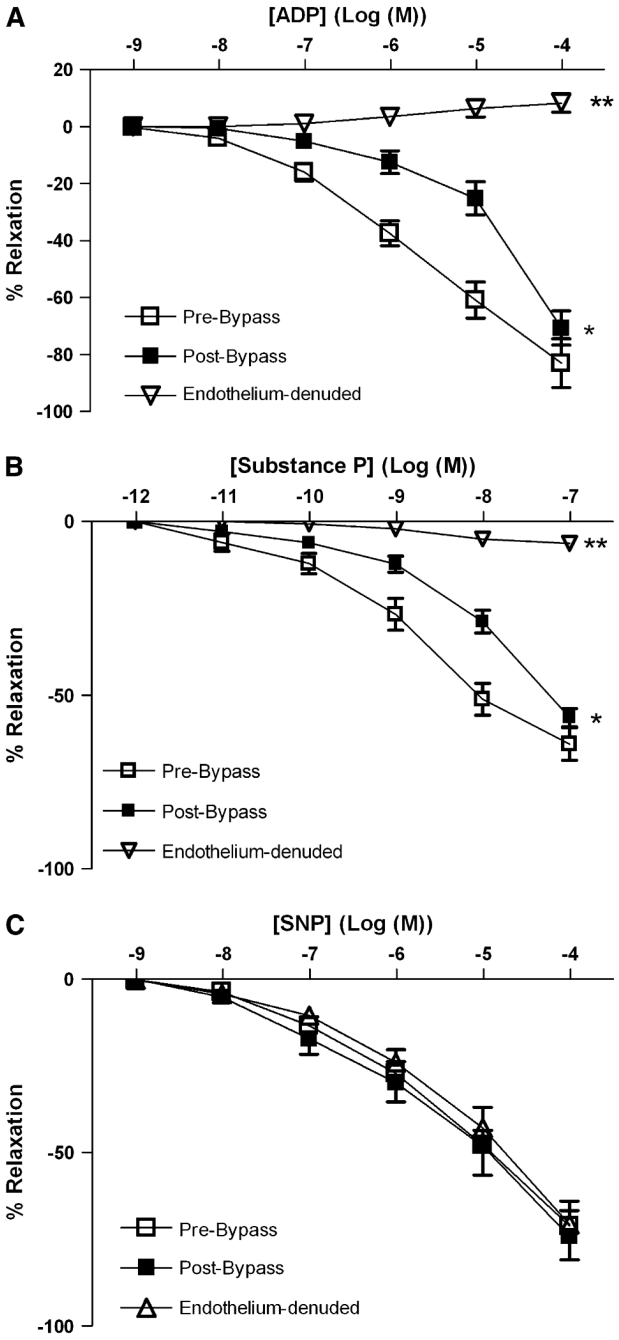

Fig 3.

In vitro response of preconstricted human skeletal muscle arterioles to endogenous vasodilators. Arterioles were compared pre- and post-CPB (n = 8 per group). Pre-CPB arterioles were compared with intact endothelium and after endothelial denudation (n =6 per group) as indicated. **A**, Response to the endothelium-dependent vasodilator, ADP. *P < .05 versus pre-CPB; **P < .001 versus intact pre-CPB. **B**, Response to substance P. *P < .05 versus pre-CP-Rep; *P <.001 versus pre-CP-Rep. **C**, Response to the endothelium-independent vasodilator, SNP.

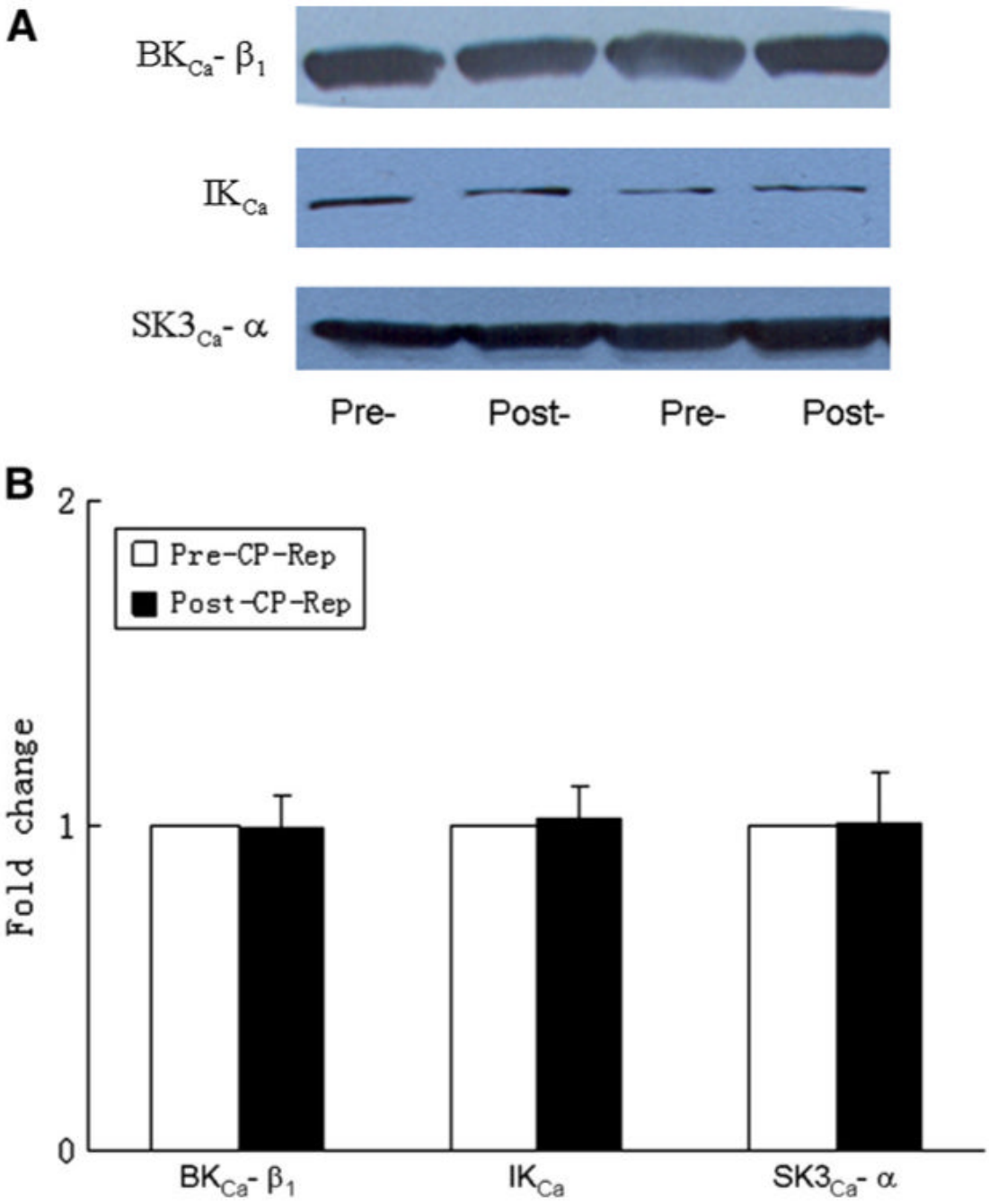

Fig 4.

A, Representative immunoblots of human skeletal muscle microvessels (40 μg loaded protein) developed with antibodies to BK-β_1,IK_{Ca}, and $SK3_{Ca}$ polypeptides. **B**, Densitometric evaluation of immunoblot band intensity normalized to pre-CPB values shows unaltered levels of K_{Ca} polypeptides after CPB (n = 6 per group).

Table

Vessel diameters (μm, mean ± SEM)

	Prebypass	*Postbypass*
NS309	146 ± 10	135 ± 10
TRAM34/apamin + NS309	157 ± 9	
Endothelium-denudation + NS309	150 ± 15	
NS1619	162 ± 16	148 ± 12
Iberiotoxin + NS1619	168 ± 6	
Endothelium-denudation + NS 1619	167 ± 5	
ADP	146 ± 11	151 ± 8
Endothelium-denudation + ADP	158 ± 8	
Substance P	149 ± 9	157 ± 6
Endothelium-denudation + substance P	158 ± 8	
SNP	137 ± 14	145 ± 9
Endothelium-denudation + ADP	163 ± 7	

ADP, adenosine 5′-diphosphate; SNP, sodium nitroprusside.