Bradykinin Preconditioning Preserves Coronary Microvascular Reactivity During Cardioplegia–Reperfusion

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Background. **Alterations of microvascular reactivity reduce myocardial perfusion after ischemic cardioplegia. We hypothesized that bradykinin preconditioning (BKPC) would preserve endothelium-dependent microvascular responses and improve myocardial function after cardioplegic ischemia–reperfusion.**

Methods. **Rabbit hearts were perfused with Krebs-Henseleit buffer (KHB). The hearts were arrested for 60 minutes with moderately cold (25°C) crystalloid cardioplegia (MCCP, n = 8) or with cold (0° to 4°C) crystalloid cardioplegia (CCCP) (n = 6). The BKPC hearts received a 10-minute coronary infusion of 10^{-8} M BK-enriched KHB, followed by a 5-minute recovery period, and then were arrested for 60 minutes with MCCP (BKPC + MCCP, n = 8) or with CCCP (BKPC + CCCP, n = 6). The hearts were reperfused for 30 minutes with KHB. Six control hearts were perfused with KHB for 90 minutes without cardioplegia-ischemia. Left ventricle performance was measured, and in vitro relaxation responses of precontracted coronary arterioles (internal diameter, 80 to 150 μm) were obtained in a pressurized no-flow state.**

Results. **Ischemic arrest with MCCP or CCCP markedly reduced endothelium-dependent relaxation to adenosine 5′-diphosphate, substance P, and calcium ionophore (A23187). Both MCCP and CCCP significantly enhanced contractile responses to U46619 (10^{-7} M), a thromboxane A_2 analogue, compared with control ($p < 0.05$). In contrast, BKPC significantly improved the recovery of endothelium-dependent relaxation to adenosine 5′-diphosphate, substance P, and A23187 compared with MCCP or CCCP, respectively. BKPC reduced the contractile responses to U46619 compared with MCCP or CCCP. BKPC also improved postischemic performance compared with MCCP or CCCP alone ($p < 0.05$).**

Conclusions. **BKPC preserves endothelium-dependent microvascular responses and prevents the hypercontractility to U46619. These effects may provide increased coronary perfusion and prevent arteriolar spasm after open heart surgery. They suggest that BK preconditions the coronary microvasculature during cardiovascular surgery.**

(Ann Thorac Surg 2005;79:911–6)

Ischemic preconditioning (IPC) has been defined as an adaptive mechanism induced by a brief period of nonlethal ischemia–reperfusion (IR) increasing the heart's resistance to a subsequent ischemia [1]. The protective effects of IPC have been demonstrated in all animal species including humans, resulting in the endogenous form of protection against infarct size, cardiac dysfunction, and arrhythmias [1–5]. We, and others, have found that IPC protects coronary microvessels against endothelial dysfunction and preserves normal microvascular regulation [6–8].

Although the molecular mechanisms responsible for IPC are incompletely understood, the induction of the preconditioned state by pharmacologic agents may be an important strategy for reducing myocardial dysfunction after cardioplegic arrest [9–11]. Several triggers or mediators released from the ischemic heart during IR, including adenosine, bradykinin (BK), and nitric oxide (NO), can induce the preconditioned state when given exogenously before a period of prolonged ischemia [3, 4, 12–15].

Recent investigations have shown that BK pretreatment improves recovery of left ventricular (LV) function after normothermic ischemia and reperfusion [16–18]. These observations suggest that pharmacologic BKPC may be an important new strategy for improving myocardial protection during heart surgery. The present study tested the hypotheses that pharmacologic BKPC would also preserve microvascular responses and improve endothelial function after a period of cardioplegic ischemia in an isolated rabbit heart preparation.

Material and Methods

Experimental Model

Animals were cared for in accordance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* (National Institutes of

Accepted for publication Sept 2, 2004.

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Published by Elsevier Inc

0003-4975/05/$30.00
doi:10.1016/j.athoracsur.2004.09.017

Abbreviations and Acronyms

ADP = adenosine 5′-diphosphate
ACE = angiotensin-converting enzyme
BK = bradykinin
BKPC = bradykinin preconditioning
CF = coronary flow
CCCP = cold crystalloid cardioplegia
KHB = Krebs-Henseleit buffer
IPC = ischemic preconditioning
IR = ischemia–reperfusion
LV = left ventricle
LVDP = left ventricular developed pressure
LVEDP = left ventricular end diastolic pressure
LVSP = left ventricular systolic pressure
MCCP = moderately cold crystalloid cardioplegia
NO = nitric oxide
RP = reperfusion
SD = standard deviation
SNP = sodium nitroprusside
SEM = standard error of the mean

Health publication No.5377-3 1996). The Institutional Animal Care and Use Committee of Beth Israel Deaconess Medical Center and Harvard Medical Area Standing Committee approved the protocols used in this study.

New Zealand white rabbits (1.5 to 2.5 kg) were used in this study (Millbrook Farm, Amherst, MA). Rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (2.5 mg/kg, intramuscularly) and anticoagulated with heparin (2000 U/kg, intravenously). The heart was rapidly exposed, the aorta was cannulated, and the heart was retrogradely perfused in situ to avoid ischemia.

The heart was excised and mounted in an organ chamber on a Langendorff perfusion system. The heart was retrogradely perfused at 70 mm Hg with a modified Krebs-Henseleit buffer (KHB) composed of 118 mmol/L NaCl , 25 mmol/L $NaHCO_3$, 1.2 mmol/L $KHPO_4$, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.8 mmol/L $CaCl_2$, and 11.0 mmol/L 11.0 glucose. The KHB was equilibrated with 95% O_2 and 5% CO_2, adjusted to a pH of 7.35 to 7.4 at 37°C, and filtered with a 5 μm filter (Gilman Scientific, Inc., Ann Arbor, MI). The right ventricular myocardial temperature was measured with a thermistor needle probe (Mallinckrodt, Inc, St. Louis, MO) and was maintained at 37°C during the periods of KHB perfusion and reperfusion by regulation of the organ chamber temperature. Our Langendorff apparatus permits instantaneous change of the perfusion fluids from standard KHB to one containing different pharmacologic substances or cardioplegia solution by adjusting an inlet valve to the aortic perfusion cannula.

Measurements

Isovolumetric measurement of LV performance was made using a compliant latex balloon connected to a pressure transducer that was inserted in the LV across the mitral valve. A calibrated syringe attached to the pressure transducer system was used to fill the balloon with a volume of saline needed to maintain a LV end diastolic pressure (LVEDP) of 5 mm Hg during the measurement of the baseline LV performance. This same balloon volume was used for subsequent measurements of LV performance after reperfusion.

LV performance was assessed by measurement of LV systolic pressure (LVSP) and LVEDP. LV developed pressure (LVDP) = LVSP − LVEDP. Positive and negative first derivatives of LVSP (+dP/dt and −dP/dt, mm Hg/s) were calculated as indices of ventricular contractility and compliance respectively.

Analog pressure data from the LV balloon were amplified and converted to a digital signal for on-line data recording and computation (Gould-PONEMAN, Gould, Valley View, OH). Continuous pressure measurements were sampled at specific time points in each experiment. Coronary flow (mL/min) was measured by the timed collection of effluent from the right ventricle exiting the heart from the severed pulmonary artery. Hearts that failed to generate a LVDP greater than 80 mm Hg or a coronary flow of less than 25 mL/min during the stabilization phase of the experiment were excluded from further study.

Experimental Protocols

After 30 minutes of equilibration, the hearts were divided into three groups. Six hearts (control group) were further buffer-perfused for 60 minutes without cardioplegic ischemia. In crystalloid cardioplegia (CCP) groups, the hearts were arrested for 60 minutes with moderately cold (25°C) CP (MCCP, n = 8) or with cold (0° to 4°C) CP (CCCP, n = 6). MCCP or CCP was reinfused every 20 minutes during 60 minutes of hypothermic ischemia. In BKPC groups, eight hearts received a 10-minute coronary infusion of 10^{-8} M BK-enriched KHB, followed by a 5-minute recovery period and then were arrested for 60 minutes with MCCP (BKPC + MCCP, n = 8) or CCCP (BKPC + CCCP, n = 6). The hearts from MCCP, CCCP, BKPC + MCCP, and BKPC + CCCP groups were reperfused for 30 minutes with KHB. The composition of the crystalloid cardioplegic solution was 121 mmol/L NaCl, 25 mmol/L KCl, 12 mmol/L $NaHCO_3$, and 11.1 mmol/L glucose. The pH was 7.6, and the partial pressure of oxygen range was 180 to 300 mm Hg.

After 60 minutes of cardioplegia arrest, the hearts were reperfused for 30 minutes with KHB. In all groups, the hearts were excised and one piece of LV tissue was immersed in cold KHB buffer for in vitro microvessel study.

In Vitro Coronary Microvessel Studies

Coronary artery microvessels (80 to 150 μm in internal diameter) from the LV free wall were dissected under a ×10 to 60 magnification microscope (Olympus Optical, Tokyo, Japan). Microvessels were placed in a microvessel chamber, cannulated with dual glass micropipettes (40 to 80 μm in diameter), and secured with 10-0 nylon monofilament sutures (Ethicon, Inc, Somerville, NJ). Oxygenated (95% oxygen and 5% carbon dioxide) KHB warmed