Release of Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor (EDHF) in Porcine Coronary Arteries Exposed to Hyperkalemia: Effect of Nicorandil

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Background. **Although the detrimental effect of hyperkalemia on coronary endothelium has been reported, there is no direct evidence regarding the effect of hyperkalemic exposure on nitric oxide (NO) release from the coronary endothelium. In addition, it is unclear whether nicorandil, a K_{ATP} channel opener, used as hyperpolarizing cardioplegia or added in hyperkalemic cardioplegic solution may protect endothelial function during cardiac surgery. The present study was designed to clarify NO release and the function of endothelium-derived hyperpolarizing factor (EDHF) in coronary circulation with respect to the effect of hyperkalemia and nicorandil.**

Methods. **Nitric oxide was measured by using a NO-specific electrode, and EDHF-mediated relaxation was investigated in a myograph. Substance P- and calcium ionophore A_{23187}-induced NO release was compared in porcine left circumflex coronary arteries before and after 1-hour exposure to 20 mM potassium (K^+) at 37°C. In coronary microarteries (diameter 200 to 450 μm), precontracted with U_{46619}, in the presence of indomethacin (7 μM), N^G-nitro-L-arginine (300 μM), and oxyhemoglobin (20 μM), EDHF-mediated relaxation was induced by bradykinin (−10 to −6.5 log M) after incubation with Krebs (control) or 20 mM K^+ with or without 10 μM nicorandil at 37°C for 1 hour.**

Results. **Neither substance P (58.8 ± 5.0 versus 66.2 ± 7.2 nmol/L) nor A_{23187} (86.6 ± 9.0 versus 82.4 ± 9.2 nmol/L in control) induced NO release was altered by hyperkalemic exposure ($p > 0.05$). In contrast, EDHF-mediated relaxation was decreased from 84.2% ± 3.8% to 42.3% ± 6.0% ($p < 0.001$) that was partially restored by nicorandil (50.7% ± 5.5%, $p < 0.05$).**

Conclusions. **Exposure to potassium at 20 mM does not affect NO release but impairs EDHF-mediated relaxation in coronary arteries. Supplementation of nicorandil in hyperkalemic cardioplegia may provide a protective effect on EDHF-related endothelial function.**

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Hyperkalemic cardioplegia has been widely used in cardiac surgery. As a major ion component, potassium (K^+) at high concentrations (hyperkalemia) induces immediate cardiac arrest, which lowers energy demand and conserves the myocardial energy reserves. This energy preservation ultimately leads the heart to better tolerance to ischemia during the operation [1, 2].

Although the general strategy of myocardial protection in cardiac surgery is still using hyperkalemia in either blood or crystalloid cardioplegia, a number of studies in the last decades have indicated the unfavorable effect of hyperkalemic solutions on vascular endothelial function [3–5].

The endothelial damage due to hyperkalemia has been reported to be associated with altered vascular resistance, blood flow, and vasodilatation [3–5]. Investigations from our laboratory on individual endothelium-derived relaxing factors (EDRFs) demonstrated the susceptibility of endothelium-derived hyperpolarizing factor (EDHF) to high concentrations of K^+ [6–9]. In contrast, although there are a number of studies [3–5] that reported the effect of hyperkalemia on the endothelial function as a whole, the direct effect of hyperkalemia on the release of nitric oxide (NO), the other major EDRF, has not been reported. Therefore, the first objective of the present study was to investigate the effect of the high concentration of K^+ used in cardioplegia on the endothelium-derived NO release. A direct and sensitive method—electrochemical measurement of the NO concentration—was used.

Further, because the coronary endothelium largely contributes to the entire cardiac performance, how to protect the function of endothelium, in addition to myocardium, has attracted great attention among cardiac

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Abbreviations and Acronyms

ANOVA = analysis of variance
EC_{50} = 50% effective concentration
EDHF = endothelium-derived hyperpolarizing factor
EDRF = endothelium-derived relaxing factors
Hb = hemoglobin
NO = nitric oxide
$_L$-NNA = N^G-nitro-L-arginine
HbO = oxyhemoglobin
K^+ = potassium
PGI_2 = prostacyclin

surgeons. Supplementation with a K^+ channel opener, such as aprikalim, in hyperkalemic cardioplegia, not only improves ventricular contractility but also preserves EDHF-mediated coronary relaxation [10, 11]. Nicorandil is a hybrid K^+ channel opener that also possesses NO-release property. Use of nicorandil shows potent cardioprotective effect in rabbits [12]. When nicorandil is used as a component of St. Thomas Hospital [13] or blood [14] cardioplegia, replacing potassium, it improves preservation of energetics and function in pig hearts. It has been also reported that when added in cold hyperkalemic solution, nicorandil is beneficial to the indomethacin and N^G-nitro-L-arginine-resistant endothelial function in the pig [15]. More importantly, nicorandil is the only K^+ channel opener used clinically as cardioplegia to protect the myocardial function, and a recent clinical study in coronary surgery by Hayashi and colleagues [16] suggests that nicorandil administration during cardiopulmonary bypass provides enhanced myocardial protective effects against ischemia-reperfusion in patients undergoing coronary artery bypass grafting.

With the recognition that coronary microcirculation is crucial for myocardial perfusion, the second part of the present study was designed to investigate the effect of nicorandil on endothelial function in porcine coronary microarteries with regard to EDHF. This objective was fulfilled by evaluating the effect of nicorandil and the effect of nicorandil as an additive to hyperkalemic solution.

Material and Methods

General

Fresh porcine hearts collected from a local slaughterhouse were placed in a container filled with cold Krebs solution and immediately transferred to the laboratory. Upon receipt of the heart, left circumflex coronary arteries (for NO measurement) and intramyocardial coronary microarteries, usually the tertiary branches of the left anterior descending artery, with diameter ranging from 200 to 450 μm (for isometric force study) were carefully dissected out with great caution to protect the endothelium. The Krebs solution was preaerated with a gas mixture of 95% O_2 to 5% CO_2 at 37°C and had the following composition (in mM): 144 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 128.7 Cl^-, 25 HCO_3^-, 1.2 SO_4^{2-}, 1.2 $H_2PO_4^-$, and 11.0 glucose.

NO Measurement

Nitric oxide was directly measured electrochemically. A membrane-type NO-sensitive electrode (ISO-NOP; World Precision Instrument, Sarasota, FL) and isolated NO meter (ISO-NO Mark II; World Precision Instrument) were used to measure the NO generated by vascular endothelium as in our previous studies in both animal and human vessels [17–20]. The detection of NO is an electrochemical method in which a potential is applied to the measuring electrode relative to the reference electrode, and the resulting current due to the electrochemical oxidation of NO is monitored. The membrane-type NO sensitive electrode consists of a working electrode covered by a gas-permeable polymeric membrane. The NO diffuses through the selective membrane or coatings and is oxidized on the surface of the prepolarized electrode, resulting in an electrical current. The magnitude of the redox current is in direct proportion to the concentration of NO in the sample and is amplified by the NO meter and registered by a computer (Duo · 18 data recording system; World Precision Instrument). The ISO-NOP has an inherently high selectivity due to its electrodes being separated from the sample in which measurements are being made by gas-permeable hydrophobic membranes. This excludes interference from solution or dissolved species other than gas [7–20].

The selectivity of the NO-sensitive electrode was tested in connection with calibration, where a lack of response to strong saline solution (3 moles/L) or sodium nitrite up to 100 μmol/L was taken as evidence for an intact coating of the electrode. The electrodes did not respond to acetylcholine (10 μmol/L), bradykinin (1 μmol/L), indomethacin (7 μmol/L), N^G-nitro-L-arginine ($_L$-NNA, 300 μmol/L), and oxyhemoglobin (HbO, 20 μmol/L) that were added into the calibration glass vial.

The membrane-type electrode is calibrated by chemical titration based on the following equation:

$$2KNO_2 + 2KI + 2H_2SO_4 \rightarrow 2NO + I_2 + 2H_2O + 2K_2SO_4, \quad (1)$$

where a known amount of KNO_2 is added to produce a known amount of NO. The quantity (and so the concentration) of NO generated can be calculated directly from the stoichiometry if the concentrations of the reagents are known [19, 20].

The calibration was performed daily before the experiment. The NO-sensitive electrode was inserted into the organ chamber vertically and placed as close to the endothelial surface as possible by means of a micromanipulator (WR-6; Narishige International, Tokyo, Japan). The NO electrode was connected to the amplifier (NO meter ISO-NO Mark 2; World Precision Instrument) and the signals were recorded. The NO concentration measured with the NO sensitive electrode reflects the NO released from the endothelium minus the NO clearanced by degradation and diffusion [19, 20].