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Impaired coronary contraction to phenylephrine after cardioplegic arrest in diabetic patients

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ABSTRACT

Background: We have previously found that hyperkalemic cardioplegic arrest in the setting of cardiopulmonary bypass (CP/CPB) is associated with impairment of the coronary arteriolar response to phenylephrine in nondiabetic (ND) patients. We hypothesized that diabetes may alter coronary arteriolar response to alpha-1 adrenergic agonist in the setting of CP/CPB. In this study, we further investigated the effects of diabetes on the altered coronary arteriolar response to phenylephrine in patients undergoing cardiac surgery.

Methods: Coronary arterioles (90-150 μm in diameter) were harvested pre- and post-CP/CPB from the ND and diabetic mellitus (DM) patients ($n = 8$/group) undergoing cardiac surgery. In-vitro microvascular reactivity was examined in response to phenylephrine. The protein expression/localization of the alpha-1 adrenergic receptors in the atrial myocardium was measured by Western blotting and immunohistochemistry.

Results: Phenylephrine (10^{-9} to 10^{-4} M) induced a dose-dependent contractile response in both ND and DM vessels pre- and post-CP/CPB. There was no significant difference in the pre-CP/CPB contractile responses to phenylephrine between ND and DM groups. The post-CP/CPB contractile response was significantly diminished in both ND and DM groups compared with the respective pre-CP/CPB response ($P < 0.05$ *versus* pre-CP/CPB). This diminished contractile response was more pronounced in vessels from DM patients compared with vessels from ND patients ($P < 0.05$ *versus* ND). There were no significant differences in the protein expression of alpha-1A and alpha-1B receptors in the atrial myocardium between the ND and DM groups or tissue harvested pre- or post-CP/CPB.

Conclusions: Diabetes is associated with a decreased contractile response of coronary arterioles to phenylephrine in the setting of CP/CPB *versus* that observed in ND patients. This alteration may contribute to the vasomotor dysfunction of coronary microcirculation seen early after CP/CPB in patients with diabetes.

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Background

Cardiac surgery, especially that involving cardioplegia (CP) and cardiopulmonary bypass (CPB), is associated with significant changes in vasomotor regulation[1,2] and subsequent organ injury. New q waves on electrocardiogram tracings are frequently observed after cardiac surgery, even when no hemodynamic changes or other signs of myocardial injury are observed.[3] Previous studies from our laboratory suggest that the response of coronary microvasculature to alpha-adrenergic stimulation is significantly reduced after CP/CPB and cardiac surgery in a porcine model.[4-6] Other studies confirmed that the diminished vasoconstriction in response to phenylephrine is also observed in human coronary microvessels after cardiac surgery and that the vasoconstriction to phenylephrine is mediated by protein kinase C (PKC)-α.[7] Furthermore, the effect of alpha-1 adrenergic stimulation on coronary microvessels may be increased in states of hypoperfusion,[8] such as during CPB.

Diabetes mellitus (DM) is associated with severe autonomic dysfunction and vasomotor dysregulation.[1,9-11] DM has been associated with increased morbidity and mortality in patients undergoing any cardiac surgical procedures[12,13] and following coronary artery bypass grafting specifically.[14,15] Many of the microvascular and macrovascular complications of diabetes are related to increased oxidative/nitrosative stress, hyperglycemia, and changes in vascular signaling.[9,11] Recently, we reported differential microvascular regulation before and after CP/CPB, correlating to the extent of serum glucose control.[9,11]

Alterations in vasomotor regulation can lead to vasoplegia, a common complication of CP/CPB seen in up to 25% of patients. Vasoplegia manifests with decreased systemic vascular resistance and hypotension.[16] These patients are at increased risk of morbidity and mortality following cardiac surgery and CP/CPB. The incidence of postoperative vasodilatory shock is higher in patients with diabetes for a number of reasons. Patients with diabetes are more likely to be prescribed angiotensin-converting enzyme inhibitors for both the antihypertensive and the renoprotective effects.[17] Furthermore, angiotensin-converting enzyme inhibitors have been identified as independent predictors of vasodilatory shock[18] due to the chronic blunting of the renin-angiotensin-aldosterone system and attenuation of adrenergic responsiveness.[19] Vasoplegia has traditionally been treated with alpha-1 adrenergic vasoconstrictors such as phenylephrine and norepinephrine. These medications must be administered carefully to avoid potentially dangerous side effects, including peripheral ischemia of the extremities and mesenteric ischemia, leading to tissue necrosis, mucosal injury, and metabolic acidosis.[16] In addition, peripheral vascular responses to vasoactive agents such as phenylephrine may affect the coronary circulation in a differential manner from the rest of the body by increasing systemic blood pressure suddenly while reducing coronary artery blood flow.[20] A better understanding of the regulation of the microvasculature may lead to improved outcomes in the patients with and without diabetes.

The purpose of this study is to investigate the effects of diabetes on coronary arteriolar response to the alpha-1 adrenergic agonist phenylephrine in the setting of CP/CPB and cardiac surgery.

Methods

Human subjects and tissue harvesting

Patients were divided into two groups based on their hemoglobin A1c (HbA1c) and medical history. Patients with an HbA1c $\geq$ 8.5% or a documented history of DM were placed into the DM group. Patients with a normal range HbA1c ($\leq$6.2%) and no known history of diabetes were placed in the nondiabetic (ND) group. Patients were excluded from the study if they experienced an aortic cross-clamp time longer than 120 min or a CPB time longer than 180 min.

All procedures were approved by the institutional review board of Rhode Island Hospital and Brown Medical School, and informed consent was obtained from all enrolled patients as required by the institutional review board.

During coronary artery bypass grafting, two samples were taken from the right atrial appendage using a double-purse-string technique with 3-0 polypropylene sutures. The first tissue sample was collected during the placement of the venous cannula in the right atrium before initiation of CPB. During collection, the superior suture was tightened to secure the cannula, while the inferior suture remained loose. This allowed the atrium between the sutures to be exposed to the CP solution, the effects of CPB, and the effects of reperfusion. The second sample was collected between the two purse-string sutures, approximately 10 to 15 min after cross-clamp removal. Harvested tissue was either frozen in liquid nitrogen for immunoblotting studies, fixed in 10% formalin for 24 h followed by paraffinization and sectioning for immunofluorescence studies, or stored in cold Krebs buffer for in-vitro microvessel analysis. This tissue collection method is similar to previous studies of human coronary arterioles.[11,21,22]

A hypothermic blood-based CP solution (8°C, 4:1 mixture of oxygenated blood and hyperkalemic crystalloid solution) (CAPS, Lanham, MD) was used for CP. An initial 650 to 1000 mL of hyperkalemic (K^+ 25 mmol/L) CP solution was delivered in an antegrade fashion into the aortic root, followed by 200-500 mL of CP solution (K^+ 8 mmol/L) every 15-20 min until the cross clamp was removed. The CPB circuit included a Medtronic affinity integrated hollow fiber oxygenator/cardiotomy reservoir with trillium coating (Medtronic, Minneapolis, MN), and an arterial 38-micron filter (Medtronic affinity) with trillium coating. The CP perfusion system (Medtronic Myotherm 4:1 system) with trillium coating was used.

Microvessel constriction studies

Coronary arterioles ranging from 90 to 150 μm in internal diameters were carefully dissected from pre- and post-CP/CPB samples of atrial tissue with the use of a 10× to 60× dissecting microscope. Microvascular reactivity studies were conducted in-vitro using an organ bath chamber and video