Published in final edited form as:

J Surg Res. 2018 October ; 230: 80–86. doi:10.1016/j.jss.2018.04.045.

Impaired Coronary Contraction to Phenylephrine after Cardioplegic Arrest in Diabetic Patients

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Abstract

Introduction: 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 non-diabetic (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 μ m in diameter) were harvested pre- and post-CP/CPB from the ND and diabetic (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 were measured by Western blotting and immunohistochemistry.

Results: Phenylephrine (10^{-9} - 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 to the respective pre-CP/CPB response ($P < 0.05$ vs. pre-CP/CPB). This diminished contractile response was more pronounced in vessels from DM patients compared to vessels from ND patients ($P < 0.05$ vs. 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.

Conclusion: Diabetes is associated with a decreased contractile response of coronary arterioles to phenylephrine in the setting of CP/CPB versus that observed in nondiabetic patients. This

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Authors Contribution: Each of the authors listed below have made substantial contributions in each of these three areas: (1) conceiving and designing the study; or collecting the data; or analyzing and interpreting the data;(N.S.; C.G.; I.L.; A.G.; L.A.S.; J.G.F. F.W.S.; J.F.); (2) writing the manuscript or providing critical revisions that are important for the intellectual content; (N.S.; L.A.S.; F.W.S.; J.F.) and (3) approving the final version of the manuscript (N.S.; C.G.; I.L.; A.G.; L.A.S.; J.G.F.; F.W.S.; J.F.)

Presented at the 13th Annual Academic Surgical Congress (ASC), Jan 30-Feb. 1, 2018, Jacksonville, FL

Conflict of interest
None declared

alteration may contribute to the vasomotor dysfunction of coronary microcirculation seen early after CP/CPB in patients with diabetes.

Keywords

phenylephrine; alpha-adrenergic receptors, microvascular reactivity; coronary microcirculation; cardioplegia; diabetes, vasomotor tone

INTRODUCTION

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 (CABG) 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 (ACE-I) for both the antihypertensive and the renoprotective effects.¹⁷ Furthermore, ACE-Is have been identified as independent predictors of vasodilatory shock¹⁸ 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 HbA1C and their medical history. Patients with an HbA1C $\geq 8.5\%$ or a documented history of diabetes mellitus were placed into the diabetic (DM) group. Patients with a normal range HbA1C ($< 6.2\%$) and no known history of diabetes were placed in the non-diabetic (ND) group. Patients were excluded from the study if they experienced an aortic cross-clamp time longer than 120 minutes or a CPB time longer than 180 minutes.

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 (CABG), 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 2 purse-string sutures, approximately 10 to 15 minutes after cross clamp removal. Harvested tissue was either frozen in liquid nitrogen for immunoblotting studies, fixed in 10% formalin for 24 hours 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 (8C, 4:1 mixture of oxygenated blood and hyperkalemic crystalloid solution) (CAPS, Lanham, MD) was used for cardioplegia. An initial 650 to 1000ml of hyperkalemic (K^+ 25mmol/L) CP solution was delivered in an antegrade fashion into the aortic root, followed by 200-500ml of CP solution (K^+ 8 mmol/L) every 15-20 minutes until the cross clamp was removed. The CPB circuit included a Medtronic Affinity integrated hollow fiber oxygenator/cardiectomy reservoir with trillium coating (Medtronic, Minneapolis, MN), and an arterial 38mg-filter (Medtronic Affinity) with trillium coating. The cardioplegia perfusion system (Medtronic Myotherm 4:1 system) with trillium coating was used.

Microvessel Constriction Studies

Coronary arterioles ranging from 90 to 150 micrometer in internal diameters were carefully dissected from pre- and post-CP/CPB samples of atrial tissue with the use of a 10 to 60x dissecting microscope. Microvascular reactivity studies were conducted in-vitro with the use of an organ bath chamber and video microscopy in a manner similar to that reported previously.^{11,21,22} Microvessels were cannulated using glass micropipettes which ranged from 40 to 80 μ m in diameter and secured with 10-0 nylon monofilament sutures. Oxygenated (95% oxygen/5% carbon dioxide) Krebs buffer solution maintained at 37°C was continuously circulated throughout the microvessel chamber and reservoir. The arterioles were pressurized to 40 mmHg in a no-flow state using a burette manometer filled with Krebs solution. An inverted microscope (40 to 200X, Olympus CK2, Olympus Optical, Tokyo, Japan) connected to a video camera was used to display the vessel image onto a monitor. Internal luminal diameter of the vessels was measured using an electronic dimension analyzer (Living Systems, Burlington, VT). After allowing the vessels to bathe in the microvessel chamber of Krebs buffer for at least 30 minutes, phenylephrine (10^{-9} - 10^{-4} M) was applied to the vessel in increasing concentrations. After each administration, the vessel diameter was measured and recorded.

Immunoblotting Studies

Arterioles were dissected from atrial myocardium of eight patients per group and cleaned of connective tissues. The arterioles were then solubilized in a SDS-PAGE buffer. Total protein (μ g) was fractionated on an 8-16% SDS-PAGE gel and subsequently transferred to a polyvinylidene difluoride membrane (Millipore Corporation, Bedford, MA) as previously described.^{23,24} Each membrane was incubated for 1 hour in a 1:400 dilution (2.5 μ g/ml) of individual antibodies to anti- α -1A and anti- α -1B receptors (Abcam, Cambridge, MA) at room temperature.

Immunofluorescence Photomicroscopy Studies

Paraffin-embedded atrial tissue sections from five patients per group were de-paraffinized in xylene, then rehydrated in graded ethanol and phosphate-buffered saline solution (PBS) and the antigen was unmasked using sodium citrate (10 mmol/L, pH = 6.0) at 90-100°C for 30 minutes. The samples were then washed using more PBS and blocked with 2% bovine serum albumin in PBS for 2 hours at room temperature. After another PBS wash, sections were incubated overnight with anti- α -1A and anti- α -1B antibodies (each used at 1.1 μ g/ml; Abcam) and smooth muscle actin antibody (2.5 μ g/ml) at 4°C as in previous studies.^{11, 22, 23} Sections were then washed in PBS and incubated with the applicable Alexa-fluor secondary antibodies and mounted using a fluorescent mounting medium (Vector Labs, Burlingame, CA). Fluorescent microscopy was conducted using a Zeiss LSM510 confocal microscope system (Carl Zeiss MicroImaging, Inc., Thornwood, NY) in a similar fashion to previous studies.²³⁻²⁵ Negative controls were produced by labeling tissues with only the anti-actin antibodies along with the secondary antibodies.

Chemicals

Phenylephrine was obtained from Sigma-Aldrich and dissolved in ultrapure distilled water on the day of the study.

Data Analysis

Data are presented as the mean $\pm$ standard deviation (SD). Categorical data of patient characteristics were analyzed with Fisher's exact test. P values < 0.05 were considered significant. Microvessel responses are expressed as percent contraction of the baseline diameter. Microvascular data were analyzed using 2-way repeated-measures ANOVA with a post-hoc Bonferroni test. Clinical and Western blot data were analyzed by Kruskal-Wallis tests followed with Dunn multiple comparison test (GraphPad Software, Inc, San Diego, CA).

RESULTS

Patient Characteristics

Patient characteristics are displayed in Table 1. There were 8 patients in each group. The HbA1C in the ND group was $5.6 \pm 0.2\%$, while the HbA1C in the DM group was $9.2 \pm 0.4\%$. All patients with hypertension were on antihypertensive medications (β -blocker, aspirin, calcium channel blocker, or angiotensin-converting enzyme inhibitor) and all patients with diabetes were on oral anti-diabetic medications and/or insulin.

Microvascular Reactivity

Phenylephrine (10^{-9} - 10^{-4} M) induced concentration-dependent contractile responses in both ND and DM vessels pre- and post-CP/CPB (Figure 1). There was no significant difference in the contractile response of vessels to phenylephrine between pre-CP/CPB-ND and pre-CP/CPB-DM groups ($P > 0.05$ versus pre-CP/CPB-ND, Figure 1). The post-CP/CPB contractile response was significantly diminished compared to the pre-CP/CPB response in the two groups ($P < 0.05$ vs. pre-CP/CPB, Figure 1). This diminished contractile response was more pronounced post-CP/CPB in vessels harvested from DM vs. ND patients ($P < 0.05$ vs. post-CP/CPB-ND).

Effect of CP/CPB on the Expression of Alpha-1 Adrenergic Receptors

On Western blot analysis, there was no significant difference in the protein expression of alpha-1A or alpha-1B receptors in the atrial myocardium between the ND and DM tissue. Likewise, no change in expression was seen in either group after CPB (Figure 2, $P > 0.05$).

Vascular Distribution of Alpha-1 Adrenergic Receptors

There was no significant difference in the localization of alpha 1A (red) and alpha-1B adrenergic receptors (green) in the atrial myocardium or atrial microvascular tissue between the ND and DM groups or between pre-CP/CPB and post-CP/CPB tissue (Figure 3).

DISCUSSION

In our current study of the coronary arteriolar reactivity to phenylephrine and alpha-1 adrenergic receptors, the novel findings can be summarized as follows: (1) phenylephrine induced a concentration-dependent contractile response which was not significantly different between vessels from DM and ND groups before CP/CPB; (2) the responses to phenylephrine in both DM and ND groups were decreased after CP/CPB, but this decrease was more pronounced in the DM group; (3) there was no difference in the expression of alpha-1 receptor proteins in the atrial myocardium between DM and ND groups or between the groups before and after CP/CPB; (4) nor were there any significant differences in the localization of alpha-1 receptors in the atrial myocardium between the DM and ND groups or between pre-CP/CPB and post-CP/CPB tissue.

The findings suggest that diabetes is associated with a decreased contractile response of coronary arterioles to phenylephrine in the setting of CP/CPB. The fact that the protein expression as well as the receptor distribution was not significantly altered on Western blots and immunofluorescence respectively, indicate that this altered response is via post-translational modification. Thus, the cellular mechanisms responsible for diabetic- and CP/CPB downregulation of alpha adrenergic receptors including internalization need to be further investigated. Despite the fact that the number of patients was small, the groups were well matched. Therefore, the differences in response to phenylephrine must have been due to diet-induced diabetes or some factor related to it. These factors could include chronic hyperglycemia, diet-induced hyperlipidemia, increased oxidative stress, advanced glycosylation end products, or diabetes-induced dyslipidemia. We did not examine the specific changes related to the fat-fed model of metabolic syndrome. It is likely that all of these could have contributed to the change in vascular reactivity.

We know from previous studies that alpha-adrenergic signaling, specifically in response to phenylephrine, can be impaired in the coronary microvasculature following CP/CPB through impaired function of PKC.⁷ Alteration to the PKC- pathway can result in impairment of microvessel contraction to other substances such as endothelin-1.²¹ It is believed that this impairment in patients with diabetes is due to increased PKC/NOX expression/activation, increased oxidative/nitrosative stress, enhanced myocardial apoptosis, and increased vascular permeability in the setting of CP/CPB and cardiac surgery.^{9, 10, 25-27}

Given the alteration of alpha1-adrenergic signaling seen in patients with diabetes in this study, practitioners should be extra vigilant to watch for vasoplegia. Also, based on our results, physicians should exercise caution when dosing an alpha-1 adrenergic agonist, especially phenylephrine, for patients with diabetes after CP/CPB. Vasopressin is occasionally used in place of phenylephrine or norepinephrine. However, the vasoconstrictive effects of vasopressin are also altered after myocardial ischemia.²⁸ Physicians may even want to consider alternative drugs such as methylene blue which acts by inhibiting guanylate cyclase and thereby lessening the vasoplegic effect,²⁹ although the effects have not been studied extensively in this population.

In conclusion, the present study is the first to indicate that diabetes is associated with a further decreased contractile response of coronary arterioles to phenylephrine in the setting of CP/CPB versus that observed in non-diabetic patients. The lack of changes in alpha-1 adrenergic receptor expression suggests that the impairment in response to alpha-1 adrenergic stimulation is post-translational. Further research is warranted to explore the effects CP/CPB on the coronary microvasculature in the setting of diabetes in order to mitigate the deleterious effect that it may have on the outcome of patients undergoing cardiac surgery.

Acknowledgment

We would like to thank all the nurses, physician assistants and perfusionists in the cardiac operating rooms at Lifespan Hospitals for collecting the tissue samples and data regarding patient characteristics.

Funding

This research project was supported by NIH R01-HL-46716 and RO1HL128831, and U54GM115677 to F.W.S.; This project was also supported by the NIH1R01HL127072-01A1, 1R01 HL136347-01, and AHA-Grant-in-Aid (#15GRNT25710105) to J.F.; and Summer Assistantship Award through Basic and Translational Research (BTR) Program (NIH T35HL094308) to N.S., C.G. and A.G, and the NIH 5T32GM065085-13 grant to L.A.S.

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Figure 1.

Dose dependent contractile response to phenylephrine (10^{-9} - 10^{-5} M) in the in-vitro coronary arterioles; A, coronary arteriolar contractile response to phenylephrine in the non-diabetic (ND) patients before (pre-) and after (post-) CP/CPB; B, Contractile response to phenylephrine in the diabetic (DM) patients before (pre-) and after (post-) CP/CPB; C, Contractile response to phenylephrine in ND and DM patients at baseline (pre-CP/CPB); Contractile response to phenylephrine in ND and DM patients post-CP/CPB; n = 8/group, mean $\pm$ SD, *P<0.05 vs. pre-CP/CPB; or ND.

Figure 2. Representative immunoblots of human atrial tissue samples for alpha-1A (A) and alpha-1B (C) receptors, harvested pre- and post-CP/CPB from patients with or without diabetes. B and D, Densitometric analysis shows no significant differences in alpha-1A and alpha-1B receptor expression between pre- and post-CP/CPB or between ND and DM groups, $P > 0.05$ vs. pre-CP/CPB or ND; mean $\pm$ SD, $n = 6$ /group.

Figure 3.
Immuno-localization of alpha 1A (red) and alpha-1B (green) receptors in the coronary microvessels.

Table 1:

Patient Characteristics

Patient Characteristics	ND	DM	P value
Age (y) *	70 ± 8	68 ± 12	0.53
Male N (%)	6 (75%)	7 (87.5%)	1.00
HbA1c (%) *	5.6 ± 0.2	9.2 ± 0.4 [#]	0.0001
Patient Blood Glucose (mg/dL, pre-CP/CPB) *	112 ± 9	214 ± 23 [#]	0.0001
Patient Blood Glucose (mg/dL, during CP/CPB) *	142 ± 16	193 ± 14 [#]	0.0008
Pre-operative Insulin N (%)	0	5 [#] (62.5%)	0.47
Intra-operative Insulin N (%)	1 (12.5%)	8 [#] (100%)	0.001
Obesity (BMI>30) N(%)	4 (50%)	5 (62.5%)	1.00
Hypercholesterolemia N(%)	8 (100%)	8 (100%)	1.00
Hypertension N(%)	7 (87.5%)	8 (100%)	1.0
Atrial fibrillation N(%)	0 (0%)	0 (0%)	1.00
Duration of CPB (min) *	116 ± 30	114 ± 33	0.35
Cross-clamp time (min) *	97 ± 22	93 ± 21	0.41
CABG only N(%)	6 (75%)	6 (75%)	1.00
CABG plus AVR or MVR N (%)	2 (25%)	2 (25%)	1.00
Oral-anti-hyperglycemic medications N(%)	8 (100%)	8 [#] (100%)	1.00
Angiotensin Converting Enzyme Inhibitor (ACEI) N (%)	3 (37.5%)	4 (50%)	0.61
Angiotensin II receptor blocker (ARB) N(%)	5 (62.5%)	5 (62.5%)	1.00
Calcium channel blocker N (%)	7(78.5 %)	6 (75%)	0.52
Beta blocker N(%)	2 (25%)	3 (37.5%)	0.60
Aspirin N(%)	8 (100%)	8 (100%)	1.00

* = mean ± SD,

[#] P<0.05 vs. ND = non-diabetes; DM = diabetes, CABG = coronary artery bypass grafting, AVR = aortic valve replacement, MVR = mitral valve replacement.