

EXPERIMENTAL STUDY

The effects of dexmedetomidine on human internal mammary artery and saphenous vein grafts under hypothermia and normothermia

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OBJECTIVES: The purpose of this study was to determine the effects of hypothermia and normothermia on the isolated human saphenous vein (SV) and internal mammary artery (IMA) responses to dexmedetomidine.

METHODS: The response of human IMA and SV strips with (E+) and without (E-) endothelium subjected to cumulative concentrations of (10^{-9} , 0^{-6} M) dexmedetomidine were recorded at 37 °C and at 28 °C. OnE-way ANOVA was used for analysis. A $p < 0.05$ was considered significant.

RESULTS: At 37°C dexmedetomidine resulted in similar significant concentration-dependent contractions in both E+ and E- SV strips ($p < 0.05$). At 37 °C dexmedetomidine resulted in significant concentration-dependent contractions in E+ IMA strips, these contractions were significantly lower at all concentrations of dexmedetomidine in E- compared to E+ IMA strips ($p < 0.05$). When results between similar groups of SV and IMA strips were compared, the contractions were significantly higher in the IMA strips in E+ and E- at 37 °C and also E- 28 °C groups compared to SV ($p < 0.05$).

CONCLUSION: In conclusion, dexmedetomidine causes in vitro vasoconstriction in human IMA and SV grafts. These contractions are greater in IMA compared to SV grafts. Endothelium-derived pathways are possibly involved in the contractile responses of IMA. Moderate hypothermia augments vasoconstriction in SV grafts (*Fig. 3, Ref. 27*). Text in PDF www.elis.sk.

KEY WORDS: dexmedetomidine, hypothermia, normothermia, in vitro, internal mammary artery, saphenous vein.

Introduction

Dexmedetomidine is a highly selective α_2 -adrenergic receptor agonist. Its $\alpha_2:\alpha_1$ adrenoreceptor specificity ratio is five to ten times that of clonidine (1,600 : 1) (1). This characteristic makes dexmedetomidine primarily sedative-anxiolytic with analgesic properties (2). Dexmedetomidine has a short half-life of six minutes, which makes it an ideal drug for intravenous titration. Dexmedetomidine is currently approved by the Food and Drug Administration (FDA) for sedation and mechanical ventilation and monitored anesthesia care in adults (3). Perioperative use of dexmedetomidine is becoming popular in cardiac anesthesia with reports of reduced incidence of early postoperative delirium, ventricular tachycardia and atrial fibrillation, and earlier postoperative extubation (4–6). Use of

dexmedetomidine in cardiac surgery is not free of risks. Dexmedetomidine activates α_2 -adrenergic receptors at lower concentrations and α_1 -adrenergic receptors at higher concentrations causing vasoconstriction of human vessels (7). Saphenous vein (SV) and internal mammary artery (IMA) are used as bypass conduits during coronary artery bypass graft (CABG) surgery. The success of surgery depends on the patency of the conduits. Besides injury of the graft during harvest, pressure and rate of blood flow through the conduit, endogenous and exogenous vasoconstrictors may contribute to poor graft function after surgery (8). Vasospasm of the graft can be lethal (9). Hypothermia has been shown to induce significant changes in responsiveness of vascular smooth muscle cells to various drugs (10, 11, 12). Previous studies on the effects of hypothermia concerning adrenergic drugs were done mainly with noradrenaline, which has a high affinity for α_1 -receptors. Information collected from preclinical experimental studies shows that due to the differences in α_1 and α_2 adrenergic receptors of different vessels, their response to adrenergic drugs and hypothermia may be different (13–15). Hence, it may be essential to know the direct effects of dexmedetomidine on SV and IMA under hypothermic and normothermic conditions. This in vitro study was designed to assess the vascular effects of dexmedetomidine on isolated human IMA and SV grafts during normothermia and moderate hypothermia.

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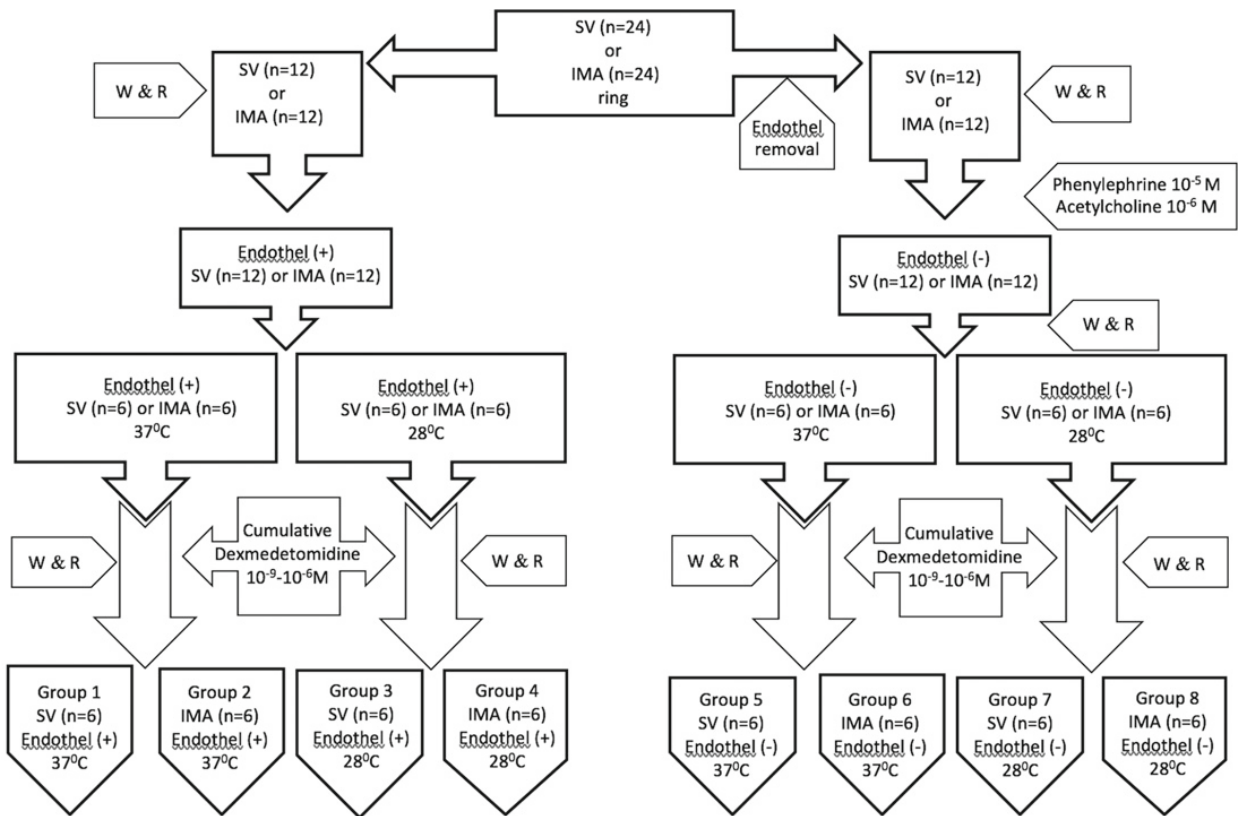


Fig. 1. Flow chart of experimental procedures. SV – saphenous vein, IMA – internal mammary artery, W&R – wash and rest.

Materials and methods

The Selcuk University Ethics Committee approved the study (201–3188). After receiving written and signed informed consent, otherwise discarded saphenous vein and internal mammary artery segments were obtained from patients undergoing coronary artery bypass surgery. SV and IMA segments were obtained from 24 patients; 17 males, 7 females with a mean age of 63.2 ± 8.9 years (range 43–74 years). Vessel segments from patients with a history of diabetes were not included. Preoperative medicines, such as calcium channel blockers, ACE inhibitors, and nitrates were discontinued at 24 h before surgery. SV segments were kept in heparinized blood until transferred to the laboratory in cold Krebs–Henseleit Solution (KHS). Once in the laboratory, excess fat and connective tissue were removed from the vessel, and cut into spiral strips 8–10 mm in length. Strips were mounted in 20 mL organ baths containing KHS at 37 °C continuously gassed with 95 % O₂, 5 % CO₂. The strips were allowed to equilibrate for 60 minutes under a resting tension of 1.5 g for IMA and 1 g for SV with repeated washing every 15 minutes. The strips were connected directly to an isometric force displacement transducer. Smooth muscle contractions were recorded with a digitized data acquisition system (MP35, BIOPAC, Goleta, CA, USA).

Endothelium-denuded tissue strips were obtained by removing the inner surface of the strips with a cotton swab. Removal of the endothelium was confirmed by pre-contracting the rings with phenylephrine (10⁻⁵ M), then adding acetylcholine (10⁻⁶ M) before each experiment. Endothelium-denuded vessels (E-) contracted in response to acetylcholine, whereas endothelium-intact (E+) vessels relaxed. The strips were rewashed and allowed to equilibrate.

Experimental protocols are summarized in Figure 1.

The experiments were conducted in eight groups of vessels; group 1: SV (E+) normothermia (37 °C) (n = 6), group 2: IMA (E+) normothermia (n = 6), group 3: SV (E+) hypothermia (28 °C) (n = 6), group 4: IMA (E+) hypothermia (28 °C) (n = 6), group 5: SV (E-) normothermia (37 °C) (n = 6), group 6: IMA (E-) normothermia (37 °C) (n = 6), group 7: SV (E-) hypothermia (28 °C) (n = 6), group 8: IMA (E-) hypothermia (28 °C) (n = 6). In each group of vessels; after the equilibration period, 0.1 mL of phenylephrine (10⁻⁵ M) was added to the tissue bath, and control contractions were obtained. The tissues were rewashed and were allowed to equilibrate. After the equilibration period, cumulative doses of dexmedetomidine (10⁻⁹, 10⁻⁸, 10⁻⁷ and 10⁻⁶ M) were administered in a volume of 0.1 mL to the organ bath, and the contraction responses were recorded. Contractions induced by dexmedetomidine were expressed as the percentage of phenylephrine-induced control contraction.

Drugs: KH solution was prepared in the laboratory with composed of (in mM) NaCl 119; KCl 4.7; MgSO₄ 1.5; KH₂PO₄ 1.2; CaCl₂ 2.5; NaHCO₃ 25; glucose 11. Dexmedetomidine was obtained from Kocak Farma (Istanbul, Turkey). Acetylcholine and phenylephrine were obtained from Sigma-Aldrich (St. Louis, MO, USA) and diluted in distilled water. All concentrations are expressed as final molar concentrations (M).

Statistical analysis was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± SD. One-way ANOVA (Bonferroni Analysis) was used for Intergroup and intragroup comparison. A value of $p < 0.05$ was considered statistically significant.

Results

Under normothermic conditions (37°C), cumulative dexmedetomidine (10⁻⁹ M, 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M) resulted in similar significant concentration-dependent contractions in both E+ and E- SV strips ($p < 0.05$). Cooling the E+ SV to 28 °C significantly augmented the dexmedetomidine elicited contractions at the lower three concentrations of dexmedetomidine (10⁻⁹ M, 10⁻⁸ M, and 10⁻⁷ M) ($p < 0.05$). Cooling the E- SV also significantly augmented these contractions at the lower two concentrations of dexmedetomidine (10⁻⁹ M, and 10⁻⁸ M) ($p < 0.05$). Cumulative dexmedetomidine elicited contractions at 28 °C were similar between E+ and E- SV strips ($p > 0.05$) (Fig. 2).

Under normothermic conditions, while cumulative dexmedetomidine resulted in significant concentration-dependent contractions

in E+ IMA strips ($p < 0.05$), it resulted in similar contractions in E- IMA strips ($p > 0.05$), these contractions were significantly lower at all concentrations of dexmedetomidine in E- compared to E+ IMA strips ($p < 0.05$). At 28 °C, cumulative dexmedetomidine caused contractions which were not concentration-dependent in both E+ and E- IMA strips ($p > 0.05$), these contractions were similar between all concentrations of dexmedetomidine in E- compared to E+ IMA strips ($p > 0.05$). Cooling did not cause significant changes in cumulative dexmedetomidine-induced contractions in E- IMA strips ($p > 0.05$) (Fig. 3).

When the contractions elicited by dexmedetomidine between similar groups of SV and IMA strips are compared, the contractions were significantly higher in the IMA strips in E+ (E_{max} : 85.0 ± 7.0 % vs 105.0 ± 5.8 %) and E- (E_{max} : 81.3 ± 14.2 % vs 96.2 ± 8.2 %) at 37 °C and also E- (E_{max} : 87.5 ± 8.5 % vs 98.8 ± 1.3 %) 28 °C groups compared to SV ($p < 0.05$). Contractions were found similar between E+ 28 °C groups (E_{max} : 91.0 ± 15.5 % vs 99.0 ± 1.4 %) ($p > 0.05$).

Discussion

This study provides new information suggesting that at clinically relevant concentrations, dexmedetomidine elicits vasoconstriction in human SV and IMA grafts (16). While moderate hypothermia augments dexmedetomidine elicited vasoconstriction in SV grafts, it does not affect the IMA. Under normothermia removal of the endothelium reduces these contractions in IMA grafts.

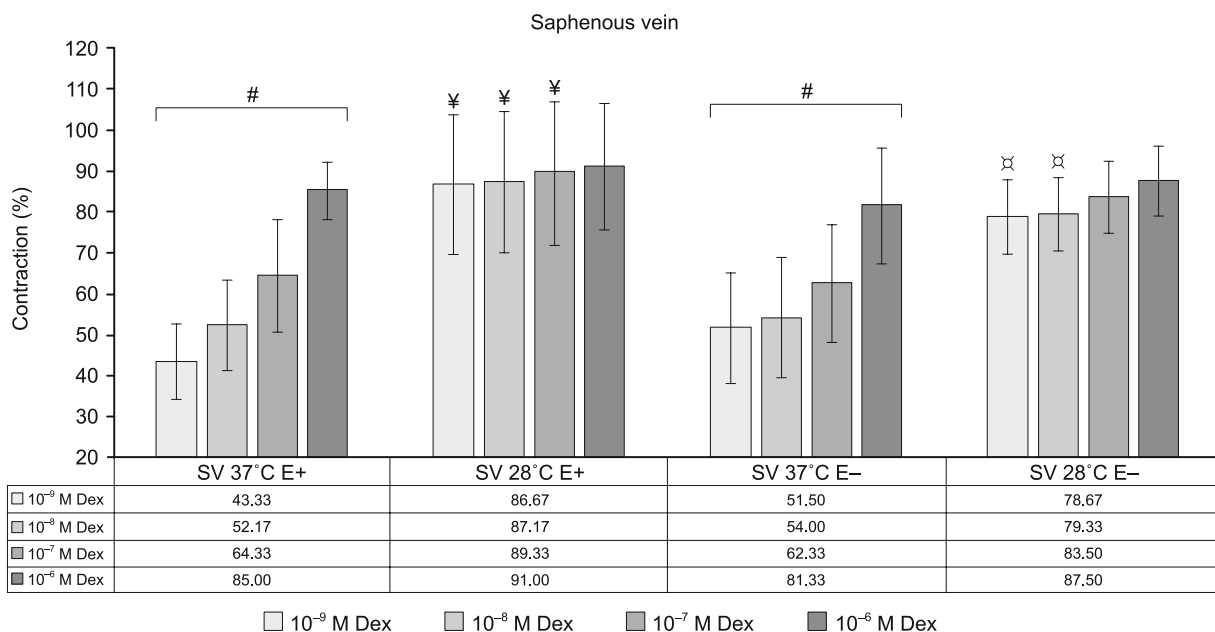


Fig. 2. Effects of cumulative dexmedetomidine induced contractions in human saphenous vein. # ($p < 0.05$) compared to other concentrations of dexmedetomidine, ¥ ($p < 0.05$) compared to same concentration of dexmedetomidine at 37 °C (E+), □ ($p < 0.05$) compared to same concentration of dexmedetomidine at 37 °C (E-). Dex – dexmedetomidine (10⁻⁹–10⁻⁶ M).

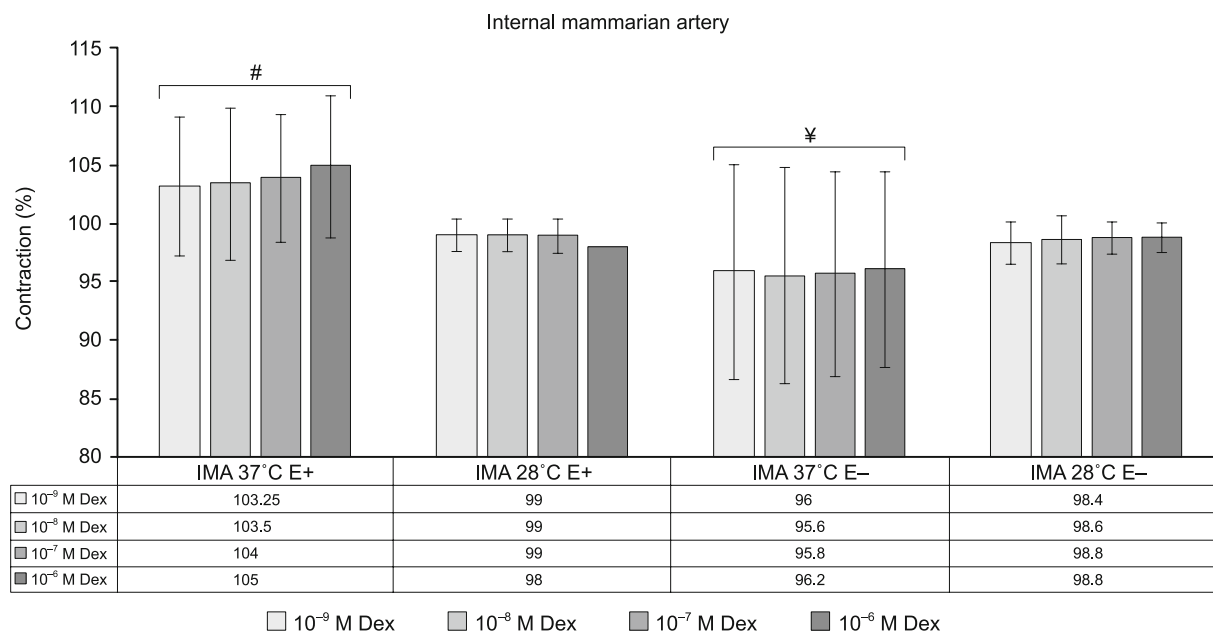


Fig. 3. Effects of cumulative dexmedetomidine induced contractions in human internal mammary artery. # ($p < 0.05$) compared to other concentrations of dexmedetomidine, ¥ ($p < 0.05$) compared to E+ IMA in same concentration of dexmedetomidine at 37 °C.

There is considerable clinical evidence that α_2 adrenergic agonists reduce myocardial ischemia, mortality and myocardial infarction following vascular surgery (17). These potential benefits of dexmedetomidine make the drug a convenient adjunct to general anesthetic drugs for cardiac surgery which employs hypothermia during cardiopulmonary bypass. There is little information on the direct effects of dexmedetomidine on human vessels and no information on how hypothermia might change these effects. Previously Yildiz et al have shown that at normal body temperature dexmedetomidine elicits concentration-dependent vasoconstriction in human internal mammary arteries in vitro. Lower concentrations of dexmedetomidine activated α_2 -adrenoceptors and higher concentrations also activated α_1 -adrenoceptor activity (7). In the present study similar to Yildiz et al., at 37 °C dexmedetomidine caused concentration-dependent contractions in both endothelium-intact IMA and SV. Compared to the SV vasoconstriction was much pronounced in the IMA which predominantly contains α_1 -adrenoceptors with little α_2 -adrenoceptor function (18, 19). Variations in the response of arteries and veins to vasoactive drugs are due to biological heterogeneity of different vessels (20). Our study design did not aim to assess the activity of subtypes of adrenoceptors, but still, phenylephrine caused a contraction in both IMA and SV suggesting strong α_1 -adrenoceptor activity in both vessels. In Weinstein et al. study α_2 -adrenoceptor antagonist caused little changes in contractile response in IMA but marked changes in SV suggesting that α_2 -adrenoceptors are present in SV (19). From previous work, it is known that α -adrenoceptors are composed of α_1 and α_2 subtypes which both mediate contraction in smooth muscle. The predominance of adrenoceptor subtypes

varies from one vessel to another. In vivo, adrenoceptors are not the only determinant of vasoconstriction of vessels. Vasoreactivity is a balance between various vasoconstrictors including thromboxane A_2 , prostaglandin $F_{2\alpha}$ and endothelin and vasodilators such as nitric oxide, prostacyclin and the endothelium-derived hyperpolarizing factor which are released from the endothelium (13). Removal of the endothelium caused a significant reduction in contraction of the IMA at normothermia suggesting a considerable contribution of endothelium-derived vasoconstrictors in the human IMA. Endothelial factors did not seem to affect the results of the SV.

Temperature is an important factor in cardiovascular surgery which employs mild hypothermia (32–35 °C), moderate hypothermia (28–32 °C) or deep hypothermia (< 28 °C) (21). It is known that pharmacokinetic and pharmacodynamic processes are temperature-dependent. Experimental evidence shows that hypothermia alters vascular reactivity to adrenergic drugs which may be different depending on the species and the structure of the vessel (14, 22). Flavahan et al have shown that in canine vessels, hypothermia augmented contractions to norepinephrine in SV, but caused depression in femoral veins (15). An in-vivo study in cats has shown that vasoconstriction elicited with noradrenaline stays intact during moderate hypothermia (23).

From previous studies, it is also known that the basal tone is present in isolated resting human SV segments at 37 °C. This basal tone is decreased by local cooling and enhanced by local warming and is not dependent on the presence of the endothelium (24). Similar to our results, in a previous study by Bodellson et al hypothermia diminished the resting tension of the IMA and the contraction to noradrenaline. In contrast, in the saphenous vein,

the contraction to noradrenaline was augmented by hypothermia. Thus, the authors concluded that hypothermia augments the receptor-mediated contraction in saphenous vein but depresses it in the internal mammary artery (25). In the present study cooling from 37 °C to 28 °C also resulted in opposite responses in IMA and SV segments with endothelium. While hypothermia augmented vasoconstriction in SV grafts, it did not affect the vasoconstriction in IMA grafts. These results suggest that the differential sensitivity of SV and IMA to cooling may result from differences in the efficiency of α_1 and α_2 -adrenoceptor response coupling. In the saphenous vein, there is a large α_1 -adrenoceptor reserve which buffers the α_1 -adrenergic response from the inhibitory influence of cooling. This together with a cooling-induced increase in α_2 -adrenoceptor affinity and α_2 -adrenoceptor agonist dexmedetomidine results in increased contraction. In the IMA, there is no α_1 -adrenoceptor reserve and cooling, therefore, depresses α_1 -adrenergic responses (25). In a study by Gomez et al dose-response curves for noradrenaline, phenylephrine and clonidine were determined from human skin arteries at 24 °C and compared to 37 °C. Noradrenaline induced dose-dependent contraction, and the sensitivity was increased during cooling. Phenylephrine and clonidine caused dose-dependent contraction, and the sensitivity of the arteries was augmented at 24 °C. The arteries also showed a lower maximal contraction to the adrenergic agonists used and KCl (50 mM) during cooling. The authors concluded that cooling: (a) increases the sensitivity of post junctional α_1 - and α_2 -adrenoceptors in human skin arteries and (b) depresses contractility of these arteries to α -adrenergic stimulation and direct activation of vascular smooth muscle (26).

There are limitations to this study. This is an in vitro study conducted with a limited number of remnant grafts. In vitro analyses of drugs may have different results compared to clinical use which is affected from various factors. Spasm of the coronary artery bypass graft conduits can occur both during harvesting and after the graft is connected. It is essential to know the major causes of spasm of the vascular graft in order to use the most appropriate way to prevent spasm. In vitro measurements of vasoreactivity of arteries and vein segments allow more precise analysis of the efficacy of various conditions and drugs. But because in vivo measurements are conducted in a controlled environment without blood flow, shear stress, hormonal and extrinsic neural activity, they can only help us to predict what can happen in vivo. It is known that a combination of factors including surgical trauma, locally released vasoconstrictors, neural factors, and circulating hormones are likely to cause abnormal constriction activity in the graft (27). Nevertheless, our in vitro results imply that the clinical use of dexmedetomidine during coronary artery bypass surgery may carry a risk of vasoconstriction of the SV and IMA grafts.

In conclusion, dexmedetomidine causes in vitro vasoconstriction in human IMA and SV grafts. These contractions are greater in IMA compared to SV grafts. Endothelium-derived pathways are possibly involved in the contractile responses of IMA. Moderate hypothermia further augments dexmedetomidine elicited vasoconstriction in SV grafts. Studies are warranted to elucidate

the in vivo effects of dexmedetomidine-induced contractions and hypothermia on human graft vessels.

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