Bidirectional Regulatory Effects of Dexmedetomidine on Porcine Coronary Tone *In Vitro*

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Source of support: This work was supported by Health and Family Planning Commission of Sichuan Province (No. 13018 to SZ Zhou) and partly by the National Natural Science Foundation of China (No. 31300948 to X. Tan)

Background: Studies *in vivo* have shown that dexmedetomidine (DEX) could protect the myocardium and modulate the coronary blood flow. This study aimed to investigate the direct and concentration-dependent effects of DEX on the tone of porcine coronary artery *in vitro* and the underlying mechanisms.

Material/Methods: Distal branches of the porcine anterior descending coronary arteries were dissected and cut into 3–5 mm rings. The tones of coronary rings in response to cumulative DEX were measured using the PowerLab system. Coronary rings were divided into three groups: 1) endothelium-intact coronary rings without drug pretreatment (control); 2) endothelium-intact coronary rings pretreated with either yohimbine, tetraethylamine (TEA) or NG-nitro-L-arginine methyl ester (L-NAME); and 3) endothelium-denuded coronary rings pretreated with either yohimbine or TEA.

Results: DEX induced coronary ring relaxation at lower concentrations (10^{-9} to 10^{-7} M) followed by constriction at higher concentrations (10^{-6} to 10^{-5} M). The coronary constrictive effect of higher DEX (10^{-5} M) was greater in the endothelium-denuded rings than in the endothelium-intact rings. Yohimbine reduced the coronary constrictive effect of DEX at higher concentrations (10^{-6} to 10^{-5} M). TEA and L-NAME significantly reduced the coronary relaxing effect of DEX at lower concentrations (10^{-9} to 10^{-7} M) in endothelium-intact rings. TEA attenuated the coronary relaxation induced by DEX in endothelium-denuded rings.

Conclusions: DEX exerts bidirectional effects on porcine coronary tone. The coronary relaxing effect of DEX at lower concentrations is likely associated with endothelium integrity, NO synthesis and BKCa channel activation, while the coronary constrictive effect of DEX at higher concentrations is mediated by α2 adrenoceptors in the coronary smooth muscle cells.

MeSH Keywords: Coronary Vessels • Dexmedetomidine • Large-Conductance Calcium-Activated Potassium Channels • Nitric Oxide

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/903501

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Background

Dexmedetomidine (DEX) is a α2 adrenoceptor (α2-AR) agonist and has been widely used in clinical anesthesia and intensive care units (ICU) because of its sedative and analgesic effects. Emily et al. [1] found that DEX at low concentration (10^{-9} to 3×10^{-6} M) has a vascular relaxation effect, while DEX at higher concentrations ($>3\times10^{-6}$ M) displays a vasoconstriction effect in isolated rat mesenteric artery rings. Research has shown that upon receiving intravenous DEX, healthy adults present a transient increase in blood pressure followed by a decrease in blood pressure and heart rate [2]. Studies also suggest that DEX could exert a myocardial protective effect via reducing the norepinephrine (NE) levels in areas with myocardial ischemia and myocardial oxygen consumption [3]. Therefore, DEX shows obvious advantages in cardiac surgery for its peri-operative sedation and analgesia [4–6].

Myocardial oxygen supply depends on normal coronary relaxation and contraction and is the determining factor for maintaining normal cardiac function. Research has shown that DEX at the recommended infusion rate reduces the myocardial blood flow (MBF) of healthy male participants by sympatholysis and reduction of heart work, and DEX at approximately three times the maximal recommended infusion rate does not further reduce the MBF [7]. However, another study indicated that DEX has no effect on coronary blood flow in conscious dogs [8].

Based on these studies, we speculated that the cardioprotective effect of DEX may be associated with the coronary relaxing effect of this drug and the subsequent increase of myocardial oxygen supply. To test this hypothesis, we investigated the mechanisms underlying the biphasic effects of DEX on coronary tone using variant blockers.

Material and Methods

Preparation and tension recording of porcine coronary artery rings

All experiments involving animals were approved by the Animal Investigation Committee of Southwest Medical University. Male pigs weighing 100–150 kg were sacrificed at Luzhou Changxing slaughter house. A total of 30 pig hearts were used in this study. The hearts were removed within 15 minutes and were immediately sent to the laboratory in ice-cold normal Tyrode's solution (in mM: NaCl 127, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.4, HEPES-Na 10, glucose 12, pH 7.4 with NaOH). The left anterior descending coronary artery (1–2 mm diameter) was separated. Fat and connective tissue in the artery were carefully removed under a microscope. The isolated coronary artery was cut into rings of approximately 2–3 mm in length, and perfused in oxygenated
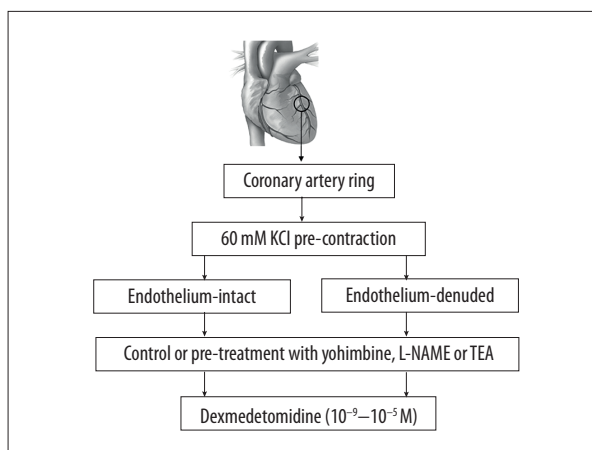
**Figure 1.** Schematic diagram of the experimental design.

(95% O_2, 5% CO_2) ice-cold Tyrode's solution at 37°C. The isometric tension of coronary rings was recorded with a force transducer connected to PowerLab system (AD Instruments, Australia). The coronary ring was given a 3 g pre-load and allowed to equilibrate for 60 minutes before the subsequent experiment. After the specimen was stabilized, 60 mM KCl was added to the bath to a final concentration of 40 mM to induce coronary ring constriction and stabilized for 10–15 minutes. The effects of DEX on vascular tone were then observed at this level. To remove the endothelium, the trimmed coronary ring was swabbed twice using a cotton swab that fit the diameter of the vessel. Endothelium removal was confirmed by observation of a <20% relaxation response to 10^{-5} M acetylcholine (ACh) of 60 mM KCl-preconstricted rings.

All study protocols were approved by the Ethics Committee of Southwest Medical University.

Experimental protocol

The accumulative dosing method was used to create an ascending final concentration of DEX in the bath at 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M, with a dosing interval of five minutes. Changes of vessel tensions were observed in KCl pre-constricted endothelium-intact and endothelium-denuded coronary rings, and the percentage changes of tension caused by DEX at different concentrations were calculated.

The experimental protocol was shown in Figure 1. To investigate the effect of DEX on the tension of coronary ring, endothelium-intact artery rings were first incubated with α2-AR antagonist yohimbine (10^{-5} M), large-conductance calcium-activated potassium (BKCa) channel inhibitor TEA (10^{-3} M) or NO synthase inhibitor L-NAME (10^{-5} M) for 20 minutes; endothelium-denuded artery rings were incubated with either yohimbine (10^{-5} M) or TEA (10^{-3} M) for 20 minutes, and then DEX was added to desired final concentration. The effects of
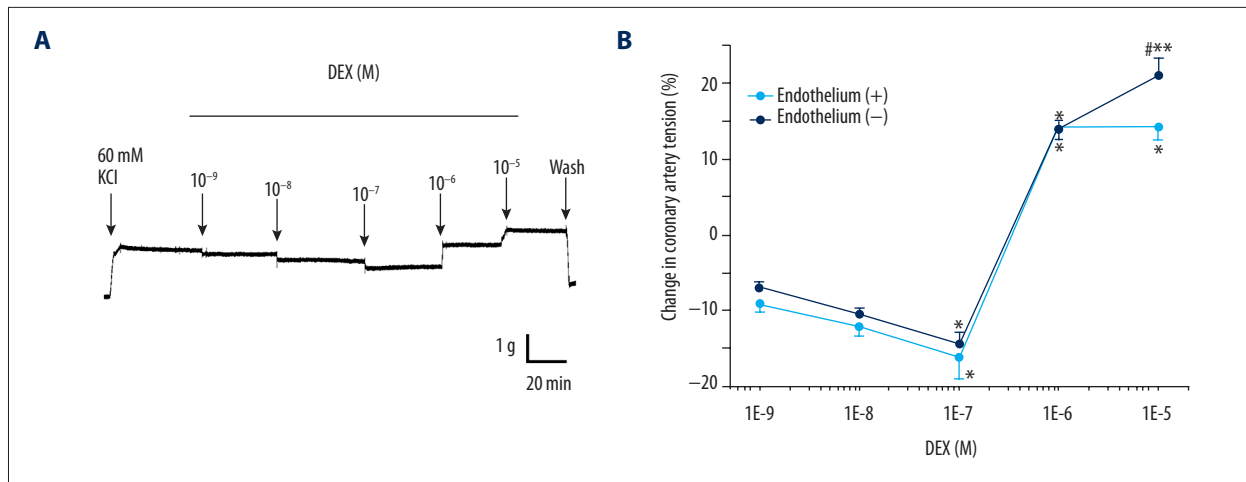
Figure 2. Effect of dexmedetomidine (DEX) on the tone of porcine coronary artery ring. (**A**) Representative traces of coronary tensions. (**B**) Statistical concentration-response curves of DEX-induced tension changes in porcine coronary rings with or without endothelium. * $p < 0.05$, ** $p < 0.01$ versus 10^{-9} M DEX. # $p < 0.05$ versus the endothelium-intact rings.

DEX at different concentrations on the coronary tone were then measured.

Drugs and reagents

Dexmedetomidine was purchased from Xinchen pharmaceutical company (Jiangsu Province, China, batch number: 11092034). Yohimbine, tetraethylammonium, and L-NAME were purchased from Sigma company (USA). All other reagents were purchased from domestic companies in China. Dexmedetomidine was dissolved in saline when use. L-NAME and yohimbine were dissolved and diluted in distilled water and the working concentrations in the bath were 10^{-5} M. The working concentration of TEA was 10^{-3} M.

Statistical analysis

Results were expressed as mean ±SEM. One-way analysis of variance (ANOVA) was used to compare among groups. Student's t tests were also performed for paired group comparisons. A value of $p < 0.05$ was considered statistically significant.

Results

Figure 2 shows the concentration (10^{-9} to 10^{-5} M) dependent effects of DEX on the tensions of coronary rings pre-constricted with 60 mM KCl. Figure 2A shows the representative original tension trace data and Figure 2B shows the statistical tension data. There was no difference if the endothelium was intact or denuded; lower DEX concentrations (10^{-9} to 10^{-7} M) caused a concentration-dependent relaxation ($p < 0.05$), while higher DEX concentrations (10^{-6} to 10^{-5} M) caused a concentration-dependent constriction. Furthermore, the coronary

constrictive effect of higher DEX (10^{-5} M) was stronger in endothelium-denuded coronary rings than that in endothelium intact rings ($p < 0.05$).

In endothelium-intact rings, yohimbine (10^{-5} M) significantly decreased the constrictive effect of higher DEX (10^{-6} and 10^{-5} M) ($p < 0.05$), but this did not alter the relaxation effect of DEX at lower concentrations (10^{-9} to 10^{-7} M) ($p > 0.05$) (Figure 3A). In contrast, TEA (10^{-3} M) significantly decreased the relaxing effect of lower DEX (10^{-9} to 10^{-7} M) ($p < 0.05$), but had no effect on the coronary constrictive effect of DEX at higher concentrations (10^{-6} and 10^{-5} M) ($p > 0.05$) (Figure 3B). L-NAME (10^{-5} M) reduced the coronary relaxation effect of DEX at lower concentrations (10^{-9} to 10^{-7} M) (Figure 3C).

In endothelium-denuded coronary rings pre-incubated with yohimbine (10^{-5} M), the coronary constriction efficiency of higher DEX (10^{-6} and 10^{-5} M) decreased significantly ($p < 0.05$), but yohimbine did not alter the coronary relaxation induced by lower DEX (10^{-9} to 10^{-7} M) (Figure 4A). TEA pretreatment significantly decreased the coronary relaxing effect of lower DEX (10^{-9} to 10^{-7} M), but did not affect the coronary constriction efficiency of higher DEX (10^{-6} and 10^{-5} M) (Figure 4B).

No significant difference was found in the DEX-induced tension changes between the endothelium-intact and endothelium-denuded rings pre-incubated with either yohimbine or TEA (10^{-5} M) ($p > 0.05$) (Figure 5A, 5B).

Discussion

The present study demonstrated that DEX exerted a bidirectional regulatory effect on the tensions of porcine coronary
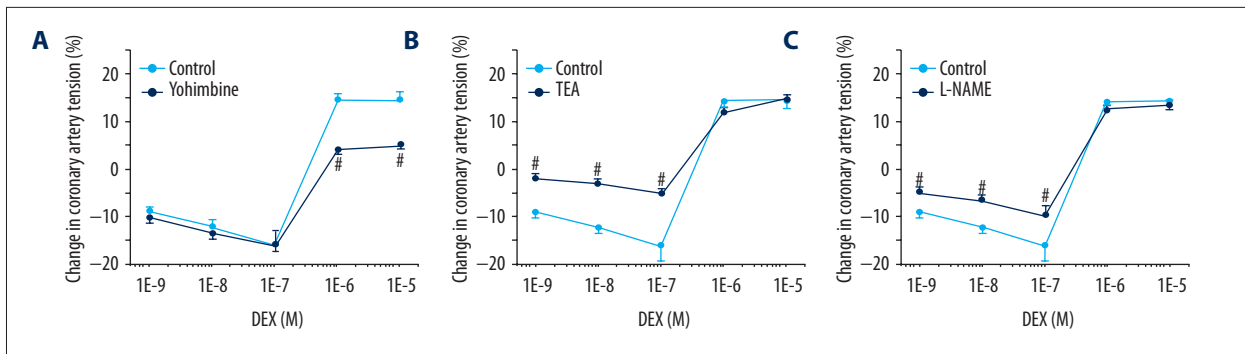
Figure 3. The concentration-response curves of DEX-induced coronary tone changes in endothelium-intact porcine coronary rings at different pretreatments. The concentration range of DEX used in this experiment was 10^{-9} to 10^{-5} M. (**A–C**), Pretreatment with yohimbine (10^{-5} M), TEA (10^{-5} M), and L-NAME, respectively. # $p<0.05$ versus control.
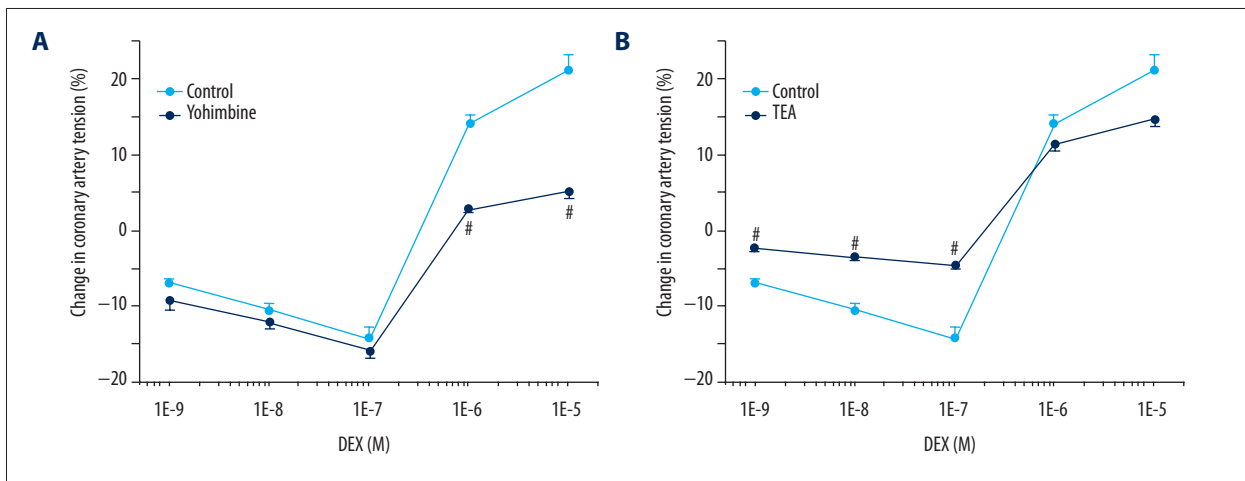
Figure 4. Concentration-response curves of DEX-induced coronary tone changes in endothelium-denuded porcine coronary artery rings at different pretreatments. (**A**) Pretreatment with yohimbine (10^{-5} M). (**B**) Pretreatment with TEA (10^{-5} M). # $p<0.05$ versus control.
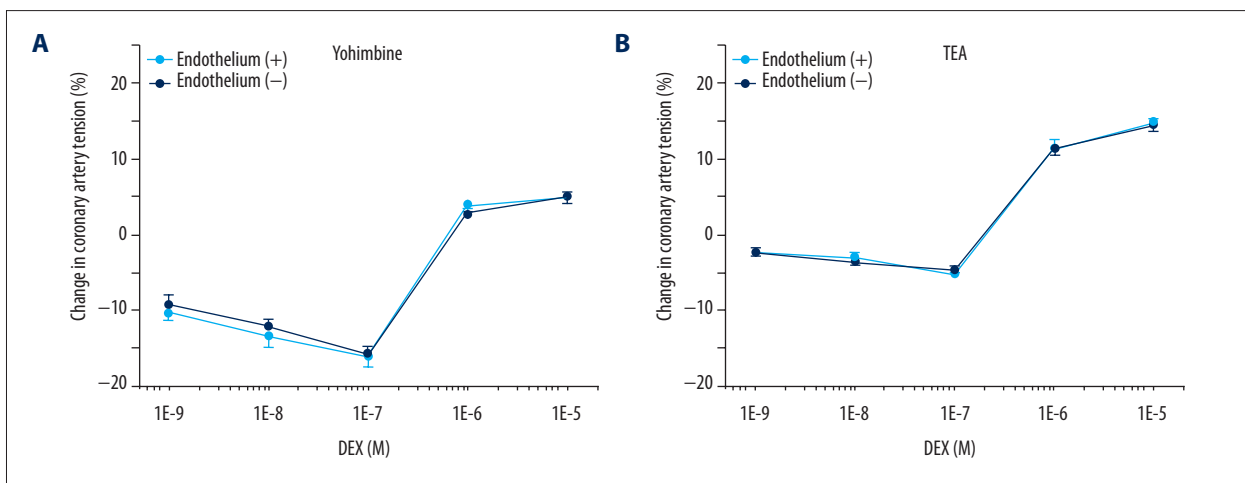
Figure 5. Role of endothelium integrity in the DEX-induced tension changes porcine coronary rings pretreated with different blockers. (**A**) Pretreatment with yohimbine (10^{-5} M). (**B**) Pretreatment with TEA (10^{-5} M). # $p<0.05$ versus control.

rings either with endothelium-intact or endothelium-denuded, i.e., DEX at lower concentrations (10^{-9} to 10^{-7} M) relaxed the coronary rings, while higher DEX (10^{-6} to 10^{-5} M) constricted the rings. In addition, the coronary constrictive effect of higher DEX (10^{-5} M) in endothelium-denuded rings was greater than that in the endothelium-intact rings. TEA and L-NAME significantly decreased the coronary relaxing effect of lower DEX; while yohimbine (10^{-5} M) significantly decreased the coronary constricting effect of higher DEX.

The relaxation and constriction of coronary arteries are the main factors determining the myocardial oxygen supply. Chilian et al. showed that α1-adrenoreceptors (α1-ARs) are distributed in proximal coronary arteries, and α2-ARs are expressed mainly in the distal resistance coronary arteries [9]. Research has shown that lower concentration of DEX reduced myocardial perfusion and myocardial oxygen demand, while higher DEX does not further reduce the MBF [7]. Kundra et al. found that DEX reduces the diameters of diseased and normal coronary arteries, but maintains the myocardial oxygen demand-supply ratio by decreasing the heart rate *in vivo* [10,11]. However, another study found that DEX did not affect coronary blood flow in conscious dogs [8]. Coughlan et al. found that higher DEX resulted in constriction of canine distal coronary artery by activation of peripheral α2-AR [12]. Another study also showed that DEX dosage administered in higher than clinical usage (>1.25 ng/mL) caused contraction of isolated human gastroepiploic arterial rings, while 0.36–1.25 ng/mL of DEX did not enhance the vasoconstriction caused by vasopressin in isolated human gastroepiploic arteries [13]. Our study present results observed in isolated porcine coronary arteries were consistent with these studies.

Based on our study result that DEX regulates coronary artery tension in a concentration dependent manner, we further investigated the roles of different signaling pathways involved in DEX-induced coronary tone change. We demonstrated that the constrictive effect of higher DEX (10^{-6} and 10^{-5} M) on coronary artery was significantly reduced by α2-AR antagonist yohimbine (10^{-5} M), and that this effect had no significant association with the endothelial integrity, suggesting that the coronary constricting effect of higher DEX was mediated by the α2-AR in the coronary smooth muscle cells. A previous study that used isolated human mesenteric arteries found that α1-AR was involved in the vasoconstrictive effect of DEX at higher concentration [14]. However, the role of α1-AR in the effect of DEX in porcine coronary needs further investigation. Other studies discovered that the constricting effect of DEX on vascular smooth muscle was due to increases in calcium sensitivity and calcium influx via stimulating the α2-AR [15,16].

BKCa channels are the main outward current channels expressed in vascular smooth muscle cells and play important

roles in maintaining the smooth muscle cell membrane potential, regulating intracellular Ca^{2+} level and reducing vascular tone [17]. Experiments have shown that, after pretreatment of rat aortic rings with TEA, 10^{-7} to 10^{-6} M DEX can significantly increase the contractility of endothelium-intact artery rings, suggesting that DEX may directly activate the BKCa channels of vascular smooth muscle cells or endothelial cells and cause vasorelaxation [18]. Using the inside-out patch-clamp technique, a previous study on the BKCa channels of rat mesenteric artery smooth muscle cells showed that DEX can increase BKCa channel open probability and therefore directly activate BKCa channels [19]. To further understand the effect of DEX on the BKCa channels of coronary artery smooth muscle cells, we observed the influence of TEA (10^{-3} M) on the coronary relaxing effect of lower DEX and show that TEA significantly reduced the vasorelaxing effect of DEX, indicating that DEX may directly activate BKCa channels of porcine arterial smooth muscle cells and relax the coronary arteries, and thus the effect was endothelium-independent.

NO is a potent vasodilator through various pathways [20]. In the current study, pre-incubation with L-NAME (10^{-5} M) reduced the relaxing effect of lower DEX (10^{-9} to 10^{-7} M) on coronary arteries, indicating that the coronary relaxing effect of lower DEX may be associated with NO synthesis through activation of α2-AR on endothelial cells. Other studies have also shown that DEX reduces 5-HT-induced vasoconstriction in isolated human pulmonary artery rings mainly by activating α2-AR and increasing NO release [21]. In addition, DEX is a strong NOS activator in human umbilical vein, and pertussis toxin can inhibit this effect, further confirming that the effect of DEX on NOS is mediated by G protein [22]. Thus, the vasorelaxing effect of DEX may be reduced in patients with atherosclerosis or with coronary endothelial impairment.

Conclusions

Our findings indicate that the effects of DEX on isolated porcine coronary artery rings are concentration-dependent and bidirectional. DEX at lower concentrations (10^{-9} to 10^{-7} M) induces coronary relaxation which may be associated with endothelium, activation of BKCa channels and NO synthesis; while DEX at higher concentrations (10^{-6} to 10^{-5} M) causes coronary constriction which may be due to a direct effect of DEX on the α2 receptors of vascular smooth muscle cells. Caution has to be taken when DEX is used for CABG sedation, especially the dosage of this drug.

Acknowledgements

References:

1. Wong ES, Man RY, Vanhoutte PM, Ng KF: Dexmedetomidine induces both relaxations and contractions, via different α2-adrenoceptor subtypes, in the isolated mesenteric artery and aorta of the rat. J Pharmacol Exp Ther, 2010; 335: 659–64

2. Mack PF, Perrine K, Kobylarz E et al: Dexmedetomidine and neurocognitive testing in awake craniotomy. J Neurosurg Anesthesiol, 2004; 16: 20–25

3. Yoshitomi O, Cho S, Hara T et al: Direct protective effects of dexmedetomidine against myocardial ischemia-reperfusion injury in anesthetized pigs. Shock, 2012; 38: 92–97

4. Okada H, Kurita T, Mochizuki T et al: The cardioprotective effect of dexmedetomidine on global ischaemia in isolated rat hearts. Resuscitation, 2007; 74: 538–45

5. Ibacache M, Sanchez G, Pedrozo Z et al: Dexmedetomidine preconditioning activates pro-survival kinases and attenuates regional ischemia/reperfusion injury in rat heart. Biochim Biophys Acta, 2012; 1822: 537–45

6. Ji F, Li Z, Nguyen H et al: Perioperative dexmedetomidine improves outcomes of cardiac surgery. Circulation, 2013; 127: 1576–84

7. Snapir A, Posti J, Kentala E et al: Effects of low and high plasma concentrations of dexmedetomidine on myocardial perfusion and cardiac function in healthy male subjects. Anesthesiology, 2006; 105: 902–10; quiz 1069–70

8. Schmeling WT, Kampine JP, Roerig DL, Warltier DC: The effects of the stereoisomers of the alpha 2-adrenergic agonist medetomidine on systemic and coronary hemodynamics in conscious dogs. Anesthesiology, 1991; 75: 499–511

9. Chilian WM: Functional distribution of alpha 1- and alpha 2-adrenergic receptors in the coronary microcirculation. Circulation, 1991; 84: 2108–22

10. Kundra TS, Kaur P: Effect of dexmedetomidine on normal coronary vessel diameter. Pharmacology, 2016; 98: 217–19

11. Kundra TS, Nagaraja PS, Singh NG et al: Effect of dexmedetomidine on diseased coronary vessel diameter and myocardial protection in percutaneous coronary interventional patients. Ann Card Anaesth, 2016; 19: 394–98

12. Coughlan MG, Lee JG, Bosnjak ZJ et al: Direct coronary and cerebral vascular responses to dexmedetomidine. Significance of endogenous nitric oxide synthesis. Anesthesiology, 1992; 77: 998–1006

13. Kim WS, Baek SH, Lee SK et al: Dexmedetomidine does not produce vasocontraction on human isolated gastroepiploic artery. Korean J Anesthesiol, 2011; 60: 428–33

14. Yildiz O, Ulusoy HB, Seyrek M et al: Dexmedetomidine produces dual alpha2-adrenergic agonist and alpha1-adrenergic antagonist actions on human isolated internal mammary artery. J Cardiothorac Vasc Anesth, 2007; 21(5): 696–700

15. Kim JG, Sung HJ, Ok SH et al: Calcium sensitization involved in dexmedetomidine-induced contraction of isolated rat aorta. Can J Physiol Pharmacol, 2011; 89: 681–89

16. Ok SH, Bae SI, Shim HS, Sohn JT: Dexmedetomidine-induced contraction of isolated rat aorta is dependent on extracellular calcium concentration. Korean J Anesthesiol, 2012; 63: 253–59

17. Yang Y, Cai F, Li PY et al: Activation of high conductance Ca(2+)-activated K(+) channels by sodium tanshinoneII-A sulfonate (DS-201) in porcine coronary artery smooth muscle cells. Eur J Pharmacol, 2008; 598: 9–15

18. Kim HJ, Sohn JT, Jeong YS et al: Direct effect of dexmedetomidine on rat isolated aorta involves endothelial nitric oxide synthesis and activation of the lipoxygenase pathway. Clin Exp Pharmacol Physiol, 2009; 36: 406–12

19. Zhou SZ, Zeng D, Li ML et al: [Effects of different concentrations of dexmedetomidine on BKCa channels in rat mesenteric arterial smooth muscle cells.] Chinese Journal of Anesthesiology, 2013; 33: 424–26 [in Chinese]

20. Oelze M, Mollnau H, Hoffmann N et al: Vasodilator-stimulated phosphoprotein serine 239 phosphorylation as a sensitive monitor of defective nitric oxide/cGMP signaling and endothelial dysfunction. Circ Res, 2000; 87: 999–1005

21. Nong L, Deng C, Kuang S et al: Dexmedetomidine inhibits 5-HT-induced intrapulmonary artery vasoconstriction. Nan Fang Yi Ke Da Xue Xue Bao, 2014; 34: 303–7

22. Joshi MS, Ferguson TB Jr., Johnson FK et al: Receptor-mediated activation of nitric oxide synthesis by arginine in endothelial cells. Proc Natl Acad Sci USA, 2007; 104: 9982–87

Conflict of interests

None.