Age-related effects of dexmedetomidine on myocardial contraction and coronary circulation in isolated guinea pig hearts

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Abstract

Dexmedetomidine is a selective α2 adrenergic agonist. Although dexmedetomidine is widely used for sedation and analgesia, it frequently produces hypotension and bradycardia. The present study aimed to evaluate the effects of dexmedetomidine on cardiac function and coronary circulation using Langendorff-perfused guinea pig hearts. Coronary perfusion pressure (CPP) and left ventricular pressure (LVP) were continuously monitored, and electric field stimulation (EFS) was applied to stimulate sympathetic nerve terminals. Dexmedetomidine almost completely inhibited the EFS-induced increase in LVP at all ages. The effect of dexmedetomidine on coronary artery resistance varied according to postnatal age, i.e., dexmedetomidine had little effect on CPP in young hearts (<4 weeks) but increased CPP by 10 mmHg at 4–8 weeks and by 15 mmHg at >8 weeks. The increase in CPP in adult hearts was inhibited by imiloxan, an α2B antagonist, and prazosin, an α1 antagonist. The results suggest that dexmedetomidine acts on α2 adrenergic receptors at sympathetic nerve terminals to suppress the release of norepinephrine. In addition, the findings suggest that dexmedetomidine directly affects α1 adrenoceptors and/or α2B adrenoceptors on coronary smooth muscles to increase CPP. The age-related changes in α adrenoceptor subtypes may be linked to the cardiodepressant effects of dexmedetomidine.

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1. Introduction

Dexmedetomidine is a selective and specific agonist for pre- and post-synaptic α2 adrenergic receptors, and is widely used, not only as an adjunct to anesthesia, but also as a sedative during mechanical ventilation in the intensive care unit (1–4). Although adverse effects, such as bradycardia and hypotension, are observed in up to 30% of patients, they are generally easily managed with intravenous fluids and atropine (2). In rare cases, however, dexmedetomidine causes profound left ventricular dysfunction and refractory shock (5–8). Tanaka et al. (9) reported that dexmedetomidine-related severe cardiovascular cases could be divided into two groups, i.e., fatal cardiovascular collapse preceded by hypotension, and severe bradycardia resulting in asystole with no other signs of circulatory depression immediately before the event. Multiple factors that can affect cardiac function and hemodynamics seem to be involved in these clinical cases. For example, patients in intensive care units have likely received sedative drugs such as propofol, fentanyl, and other anesthetics besides dexmedetomidine, and patient background characteristics, such as cardiovascular disease or disease severity, are not identical. Therefore, it is difficult to definitively state that dexmedetomidine is the cause of cardiac arrest (9). Animal studies have also shown dexmedetomidine-related cardiovascular effects, but the results are not always consistent among various studies. Lin et al (10) reported vasopressor effects and a decrease in heart rate and cardiac output induced by dexmedetomidine during propofol or isoflurane anesthesia, but they were within clinically acceptable ranges. Augmented bradycardia and a decrease in cardiac output have been reported in the presence of fentanyl (11) or isoflurane (12). Pascoe (12) reported that a high dose of dexmedetomidine (3 μg/kg) produced significant increases in systemic arterial pressure, central venous pressure, and pulmonary occlusion pressure, accompanied by decreases in heart rate, cardiac output, and oxygen delivery in the presence of isoflurane.

Three subtypes of α2 adrenergic receptors have been identified (α2A, α2B, and α2C) on the basis of pharmacological analysis and...
molecular cloning. The three subtypes are widely expressed in different tissues and organs, and they mediate many different physiological and pharmacological effects in the cardiovascular system, including both vascular constriction and dilatation (13,14). Although dexmedetomidine possesses a high specificity for the α2 versus the α1 receptor, it does not show significant differences in affinity for the three adrenoceptor subtypes (13). It is thus possible that dexmedetomidine mediates both sympatholytic and vasoconstrictive hemodynamic effects (1.5,16). Recent studies have suggested that clinically relevant genetic polymorphisms, as well as patient traits/characteristics and pharmacokinetics in critically ill patients, could affect the response to dexmedetomidine (17,18).

In addition to the above receptor-mediated actions, it has also been reported that dexmedetomidine has direct effects on various ion channels, such as vascular KATP Channels, hyperpolarization-activated cation currents, and the neuronal delayed-rectifier K+ current and Na+ current (19–22). All these pharmacological actions should be examined to clarify the mechanisms underlying the severe adverse effects of dexmedetomidine observed in human and animal studies.

In the present study, we aimed to evaluate the effects of dexmedetomidine on cardiac parameters, using Langendorff-perfused hearts of guinea pigs of various ages. We found that the effect of dexmedetomidine on coronary artery resistance varied according to postnatal age.

2. Materials and methods

2.1. Measurement of left ventricular pressure and coronary perfusion pressure

All animal experiments were approved by The Animal Ethics Committee of Akita University School of Medicine. Left ventricular pressure (LVP) and coronary perfusion pressure (CPP) were measured using the previously reported method (23). In brief, guinea pigs were anesthetized with pentobarbital (50 mg/kg, i.p.), and the heart was rapidly excised and ascending aorta cannulated through the aorta. The excised heart was then mounted on a Langendorff perfusion device (Fig. 1A), and was continuously perfused with oxygenated Tyrode solution containing 5.5 μM atropine (37 °C). The flow rate of the perfusate was adjusted to obtain a perfusion pressure of approximately 50 mmHg, and kept constant throughout the experiment. The flow rate was maintained at 6–12 mL/min in the present study. A fluid-filled balloon was inserted into the left ventricle via the left atrium, and the balloon volume was adjusted to maintain the end-diastolic pressure at 10 mmHg. LVP and CPP were recorded using a PowerLab (AD Instruments, Colorado Springs, CO, USA) at a sampling frequency of 1 kHz. Electrofield stimulation (EFS) was applied via metal electrodes placed at both sides of the heart in the chamber to stimulate the sympathetic nerve terminals (Fig. 1). A train of four electrical field pulses of 1 ms duration with a 3 ms interval was repeated at 4 Hz, and usually 5 s stimulation was sufficient to achieve a saturated response to a given intensity of stimulation (24). Although the EFS sometimes triggered ventricular extrasystole, it was only transient and did not affect measurement of LVP and CPP during the course of the study.

2.2. Isolation of ventricular myocytes and patch clamping

Ventricular myocytes were isolated from guinea pig hearts by enzymatic dissociation as previously described (23). Briefly, a Langendorff perfusion system was used to perfuse the heart with Ca2+-free Tyrode solution for 5 min. Subsequently, the perfusate was switched to Ca2+-free Tyrode solution containing 0.02% collagenase (Wako, Osaka, Japan), and the heart was digested for approximately 30 min. The heart was then rinsed with high K+/low Cl− solution before the left ventricle was dissected and stored in the same solution at 4 °C until required. A small piece of ventricular tissue was dissected and gently agitated in the recording chamber (0.5 mL volume) containing normal Tyrode solution. Once the cells had settled on the base of the recording chamber, they were perfused with normal Tyrode solution at 2–3 mL/min. All experiments were performed at 36–37 °C on rod-shaped quiescent single cells that had clear sarcomere striations.

Cells were patch-clamped to record whole-cell currents using a patch-clamp amplifier (EPC-7; List, Darmstadt, Germany). Patch pipettes were prepared from glass capillaries (Warner Instrument Co., Hamden, CT, USA) using a micropipette puller (Model P-97; Sutter Instrument Co., Novato, CA, USA). Electrode resistance ranged from 3 to 5 MΩ. Data were recorded at 2–10 kHz on a personal computer using pClamp software (Axon Instruments, Foster City, CA, USA).

2.3. Solutions and drugs

Tyrode solution comprised (in mM): NaCl 136.9; KCl 5.4; CaCl2 1.8; MgCl2 0.53; Na2HPO4 0.33; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 5.0; and glucose 5.5 (pH 7.4, adjusted with NaOH). The pipette solution for recording action potentials contained (in mM): K-aspartate 110; KCl 20; Na2ATP 4; MgCl2 2; HEPES 5; and EGTA 10 (pH 7.0, adjusted with KOH). The high K+ low Cl− solution for storage of the isolated cardiomyocytes comprised 10 mM taurine, 10 mM oxalic acid, 70 mM l-glutamic acid, 25 mM KCl, 10 mM KH2PO4, 0.5 mM EGTA, 11 mM glucose, and 10 mM HEPES (pH 7.4, adjusted with KOH).

Dexmedetomidine, clonidine, yohimbine, and prazosin were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.4. Statistical analysis

The peak LVP, heart rate, CPP and coronary flow were measured and the differences between the presence and absence of dexmedetomidine were calculated. Results are expressed as mean ± SEM. Comparisons of more than three groups were conducted by one-way repeated-measures analysis of variance with the Tukey–Kramer HSD test. Comparisons of two groups were conducted by a paired t-test. A P-value < 0.05 was considered to indicate statistical significance. All statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Effects of dexmedetomidine on CPP, LVP, and heart rate in hearts of different ages

The excised heart was mounted on a Langendorff perfusion device and was continuously perfused with oxygenated Tyrode solution containing 5.5 μM atropine (37 °C). The flow rate of the perfusate was adjusted to approximately 50 mmHg. After the CPP, LVP, and heart rate had stabilized for more than 20 min, 10 nM and 100 nM dexmedetomidine were applied via the coronary arteries. Fig. 2A shows representative recordings of CPP, LVP, dp/dt, and heart rate of hearts of guinea pigs aged ≤4 weeks (left), 4–8 weeks (middle) and >8 weeks (right). LVP, dp/dt, and heart rate were little affected by dexmedetomidine in any age of heart examined, indicating that dexmedetomidine had no direct effect on cardiac contractility and sino-atrial node function. In contrast, the CPP varied significantly depending on age, i.e., dexmedetomidine had little effect on CPP in young hearts while, at concentrations >10 nM,
Fig. 1. Measurement of LVP, CPP and HR in isolated heart of guinea pigs. (A) An excised guinea pig heart was mounted on a Langendorff perfusion device. A fluid-filled balloon catheter connected to a pressure transducer was inserted into the left ventricle via the left atrium. The balloon volume was adjusted to maintain the end-diastolic pressure at approximately 10 mmHg. The heart was continuously perfused with oxygenated Tyrode solution (37 °C) at a constant flow (6–8 mL/min) using a peristaltic pump. Representative raw traces for LVP and CPP are shown at the right panel. (B) EFS was applied to stimulate the sympathetic nerve terminals (delay: 4 ms; duration: 1 ms for 5 s). LVP was increased by EFS. LVP: Left ventricular pressure; CPP: Coronary perfusion pressure; HR: Heart rate; EFS: Electrical field stimulation.

Fig. 2. Age-dependent effect of dexmedetomidine on CPP. (A) Representative traces showing the effects of dexmedetomidine on CPP and LVP in hearts of guinea pigs of various ages. Left, < 4 weeks; middle, 4–8 weeks; right, >8 weeks. Note that dexmedetomidine markedly increased CPP at ages >4 weeks. (B–D) Age-dependent effects of dexmedetomidine on peak LVP (B), HR (C) and CPP (D). *P < 0.05 (vs. age < 4 weeks); **P < 0.01 (vs. age < 4 weeks).
it increased CPP in adult hearts. The effects on the peak LVP, heart rate, and CPP are summarized in Fig. 2B–D. There was no significant difference in the peak LVP before and after addition of 10 or 100 nM dexmedetomidine, while dexmedetomidine significantly increased CPP in older hearts (age <4 weeks vs. age 4–8 weeks: \( P < 0.05 \); age <4 weeks vs. age >8 weeks: \( P < 0.01 \); Fig. 2D). We also examined the effect of a non-selective \( \alpha_2 \) adrenoceptor agonist clonidine on CPP at 4–8 weeks guinea pigs. The CPP was increased by 3.1 ± 1.4 mmHg at 10 nM (\( p = 0.157 \)), and 3.3 ± 1.9 mmHg at 100 nM clonidine (\( p = 0.051 \)), and these values were not statistically significant.

To confirm that coronary vascular resistance is increased in response to dexmedetomidine in older guinea pigs, dexmedetomidine was applied during constant CPP. In Fig. 3, CPP was maintained constant at 50 mmHg and the effects of dexmedetomidine on coronary flow and LVP were examined in hearts from guinea pigs >8 weeks old. Coronary flow was found to decrease in a concentration-dependent manner. In contrast, the peak LVP was little affected by dexmedetomidine (Fig. 3C).

3.2. Mechanism of the response of coronary artery resistance to dexmedetomidine

To investigate the mechanisms underlying the dexmedetomidine-induced increase in coronary artery resistance in adult guinea pigs, effects of an \( \alpha_2B \) adrenoceptor inhibitor, imiloxan, were examined. Imiloxan (3 µM) suppressed the effect of 10 nM dexmedetomidine, but not that of 100 nM dexmedetomidine (Fig. 4A). The effect of prazosin, a potent \( \alpha_1 \) antagonist, was also investigated. Prazosin (1 µM) completely inhibited the increase in CPP induced by 10 nM dexmedetomidine, and its effect was slightly attenuated when the dexmedetomidine concentration was increased to 100 nM.

3.3. Inhibitory effects of dexmedetomidine on LVP with EFS

In the experiment shown in Fig. 5A, a heart isolated from a guinea pig >8 weeks old was mounted on a Langendorff apparatus and was perfused with Tyrode solution. After the CPP, LVP, and heart rate had stabilized for >20 min, 25V EFS was applied for 5 s, resulting in a significant increase in LVP accompanied by a biphasic change in CPP, i.e., a transient increase was followed by sustained decrease. Dexmedetomidine inhibited the EFS-induced changes in LVP, and increased CPP in a concentration-dependent manner (Fig. 5A). The inhibitory effect of dexmedetomidine on the EFS-induced increase in LVP was also examined at various EFS amplitudes (Fig. 5B). The relationship between the concentration of dexmedetomidine and the increase in LVP showed a half-maximal concentration at approximately 0.2 nM (Fig. 5C). Similar results were observed in hearts isolated from guinea pigs <4 weeks and 4–8 weeks old (data not illustrated).

3.4. Effects of dexmedetomidine on the action potential of ventricular myocytes

Effects of dexmedetomidine on the action potentials of isolated ventricular myocytes were examined. Action potentials were elicited every 10 s under conventional whole-cell clamp conditions, and 100 nM dexmedetomidine was applied (Fig. 6). Dexmedetomidine had no effect on action potential duration or amplitude, or on the resting potential. Similar results were obtained in three other ventricular cells.

4. Discussion

The present study demonstrated that dexmedetomidine by itself had little effect on heart rate and on the dp/dt of the LVP, but
inhibited the EFS-induced increase in dp/dt of the LVP. Also, the effect of dexmedetomidine on CPP differed depending on postnatal age, i.e., it had little effect on CPP in young hearts (<4 weeks) but increased CPP by 10 mmHg in 4–8-week-old hearts and by 15 mmHg in >8-week-old hearts. This is the first study to demonstrate an age-dependent effect of dexmedetomidine on coronary vascular resistance.

There are three subtypes of \( \alpha_2 \) adrenoceptor, namely \( \alpha_{2A/D} \), \( \alpha_{2B} \), and \( \alpha_{2C} \) (13,14). Most of the classical functions mediated by \( \alpha_2 \) adrenergic receptors, such as hypotension, sedation, analgesia,
hypothemia, and anesthetic-sparing effect, are mediated primarily by the α2A/D subtype. The α2B subtype is the principal mediator of the hypertensive response to α2 agonists. The α2C subtype is involved in many central nervous system processes such as the sympathetic nerve terminals by dexmedetomidine inhibits inhibition of neurotransmitter release is already present immediately after birth (27). Activation of the α2A/D and α2C receptors in the sympathetic nerve terminals by dexmedetomidine inhibits voltage-dependent Ca++ channels and resulting norepinephrine release (28). In support of this, pharmacological block of N-type Ca++ channels, which play a major role in the sympathetic nerve terminals (29), were shown to inhibit the EFS-induced increase in LVP (24), similar to the present result (Fig. 6).

In contrast, the effect of dexmedetomidine almost completely inhibited the increase in LVP induced by EFS from hearts of guinea pigs of all ages. This is consistent with the previous findings that the α2A/D and α2C subtypes are important in the presynaptic inhibition of norepinephrine release (26), and that α2A/D-mediated auto-inhibition of neurotransmitter release is already present immediately after birth (27). Activation of the α2A/D and α2C receptors in the sympathetic nerve terminals by dexmedetomidine inhibits voltage-dependent Ca++ channels and resulting norepinephrine release (28). In support of this, pharmacological block of N-type Ca++ channels, which play a major role in the sympathetic nerve terminals (29), were shown to inhibit the EFS-induced increase in LVP (24), similar to the present result (Fig. 6).

In contrast, the effect of dexmedetomidine on CPP differed depending on postnatal age, i.e., dexmedetomidine did not affect CPP in young hearts (<4 weeks) but increased CPP by 10–15 mmHg in older hearts. The dexmedetomidine-induced elevation of CPP was suppressed by imiloxan, indicating that this was mediated by α2B receptors. The inhibitory effect of imiloxan was attenuated by increasing the dexmedetomidine concentration from 10 to 100 nM. This suggests that there was competitive antagonism between dexmedetomidine and imiloxan at the α2B receptor. Essentially similar effects were observed for prazosin. Although prazosin is known as an α1 receptor antagonist (K< 1 nM), a radioligand binding study showed that Ki values were significantly lower (30 nM) for α2B receptors than for α2A receptors (K< 1000 nM) (30). It was also reported that the Ki values for imiloxan were 1750 nM and 50 nM for α2A receptors and α2B receptors, respectively (30). Thus prazosin appears to be more specific and effective in inhibiting α2B receptors than imiloxan.

As adrenoceptor activation of vascular postsynaptic receptors can cause both vasoconstriction and vasodilation, the overall effects of α2 receptor activation are complex, and may differ among various vascular preparations. It has been reported that multiple α2 receptors can cause vasoconstriction in most vascular beds (31). In general, α2A receptors are expressed exclusively in large arteries, whereas α2B receptors contribute more to vascular tone in small arteries and veins (31). Nonselective activation of α2 receptors usually leads to a biphasic blood pressure response: a short hypertensive phase that is induced by α2B Receptors followed by long-lasting hypotension mediated by α2A receptors (32). In the coronary circulation, α1 and α2 adrenoceptors contribute to coronary vasoconstriction. While α1 receptors induce vasoconstriction of epicardial conduit vessels and participate in microvascular vasoconstriction of resistive vessels, α2 receptors predominantly mediate microvascular resistive vessel constriction (33), although the specific receptor subtypes mediating this constriction have not been defined. It has been reported that intracoronary infusion of the selective α2 agonist xylometazoline increased coronary resistance by a maximum of 60% (33). When assessed with 15O-labeled water and positron emission tomography in healthy male subjects, coronary vascular resistance increased in response to dexmedetomidine infusion (34). Coronary artery resistance also increased with dexmedetomidine at doses of 1 μg/kg or higher in anesthetized goats (35). All these findings, together with the present results that dexmedetomidine-induced elevation of CPP was suppressed by imiloxan, support the view that dexmedetomidine-induced elevation of CPP probably results from activation of α2B receptors. The lack of effects of clonidine, a non-selective α2 agonist, on CPP is also consistent with this view, since dexmedetomidine was approximately 10 times more potent for α2 receptors than clonidine (36).

The reason why dexmedetomidine did not increase CPP in young guinea pig hearts is not known at present. We speculate that the expression of α2 receptor subtypes may change during the course of development. An alteration in α-adrenergic receptor-mediated effects has been reported in various organs and tissues. For example, ontogenetic profiles differed substantially between two α adrenergic receptor types in rat hearts, i.e., α1 receptors showed a striking increment during the first 2 weeks postpartum, while α2 receptors declined markedly during the first week and remained low thereafter (37). In cerebellar Purkinje cells, inhibitory effects of α1 receptors are absent until the second postnatal week, and became predominant thereafter (38). Further studies including the immunohistochemistry and the measurement of mRNA level or protein levels of adrenoceptor subtypes in cardiac ventricles and
coronary artery in different ages are necessary to elucidate the mechanisms of age-dependent effects of dexmedetomidine on coronary resistance. Also, it is important to know whether dexmedetomidine-induced elevation of CPP was observed in knock-out mice with deletion of the α2B adrenoceptor.

The response of CPP to EFS was characterized by a biphasic change in CPP, i.e., a transient increase was followed by sustained decrease (Fig. 5). The transient increase is probably due to α2B receptor activation, and the sustained decrease might be derived from β receptor activation. It has been also known that locally produced vasoactive hormones (i.e. adenosine, bradykinin) are capable of inducing vasorelaxation of coronary resistance arteries via the endothelium. Thus, it is also possible that the increased contractility in response to EFS facilitated the production of these vasoactive hormones, thereby causing the sustained decrease of CPP. In any cases, dexmedetomidine attenuated both responses via the suppression of norepinephrine release from the sympathetic nerve terminals.

Dexmedetomidine is approved by the Food and Drug Administration (FDA) for sedation of mechanically ventilated patients at a dose of 1 μg/kg administered over 10 min followed by infusion of 0.2–0.7 μg/kg/h. The concentration of dexmedetomidine may reach 10 nM after an initial bolus of 6 μg/kg/h for 10 min, which is recommended by the FDA. The present study demonstrated that dexmedetomidine at >10 nM increased CPP and almost completely inhibited sympathetic stimulation. Therefore, more cautious administration of dexmedetomidine may be warranted in patients with parasympathetic dominance in cases where drugs that stimulate parasympathetic nerves, such as certain anesthetic agents, are used [7, 39]. Also, a combination of dexmedetomidine with other cardiodrepressant drugs, high sympathetic stimulation, or subclinical coronary artery disease and endothelial dysfunction, could augment the adverse effects of dexmedetomidine. In addition, the present study suggests that the increase in coronary vascular resistance may be another factor related to the adverse effects of dexmedetomidine, particularly in adults. It has been reported that a variation in the α2B adrenoceptor gene was associated with an increased risk of fatal acute myocardial infarction and sudden cardiac death, possibly mediated by coronary vasospasm [40]. It is possible that, in certain genetically predisposed individuals, stimulation of α2B receptors by dexmedetomidine may lead to increased coronary vasoconstriction, thereby leading to myocardial ischemia and myocardial dysfunction.

In conclusion, the present study showed that dexmedetomidine inhibits the increase in left ventricular pressure induced by sympathetic stimulation. In addition, dexmedetomidine increases CPP in hearts of guinea pigs aged >4 weeks. The dexmedetomidine-induced increase in CPP in adult guinea pig hearts appears to be mediated by α2B receptors.

Conflicts of interest
None of the authors have any conflicts of interest associated with this study.

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