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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 α_2 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 α_{2B} antagonist, and prazosin, an α_1 antagonist. The results suggest that dexmedetomidine acts on α_2 adrenergic receptors at sympathetic nerve terminals to suppress the release of norepinephrine. In addition, the findings suggest that dexmedetomidine directly affects α_1 adrenoceptors and/or α_{2B} 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.

1. Introduction

Dexmedetomidine is a selective and specific agonist for pre- and post-synaptic α_2 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 α_2 adrenergic receptors have been identified ($\alpha_{2A/D}$, α_{2B}, and α_{2C}) on the basis of pharmacological analysis and

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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 α_2 *versus* the α_1 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 (1,15,16). 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 K_{ATP} 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 Ca^{2+}-free Tyrode solution for 5 min. Subsequently, the perfusate was switched to Ca^{2+}-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; $CaCl_2$ 1.8; $MgCl_2$ 0.53; NaH_2PO_4 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; Na_2ATP 4; $MgCl_2$ 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 KH_2PO_4, 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,