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Short communication

Guanfacine enhances cardiac acetylcholine release with little effect on norepinephrine release in anesthetized rabbits

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ABSTRACT

An α_{2A}-adrenergic agonist guanfacine improves autonomic imbalance in attention-deficit hyperactivity disorder, suggesting that it may be useful to correct autonomic imbalance in chronic heart failure (CHF) patients. To investigate the effects of guanfacine on cardiac autonomic nerve activities, a microdialysis technique was applied to anesthetized rabbit heart. Acetylcholine (ACh) and norepinephrine (NE) concentrations in atrial dialysates were measured as indices of cardiac autonomic nerve activities. Guanfacine at a dose of 100 μg/kg significantly decreased heart rate and increased dialysate ACh concentration without decreasing sympathetic NE release. Guanfacine may be useful for vagal activation therapy in CHF patients.

1. Introduction

Autonomic imbalance with activation of sympathetic nerve system and suppression of vagal nerve system causes progression of heart failure. Vagal activation has recently become a therapeutic option to correct autonomic imbalance in patients with chronic heart failure (CHF) (De Ferrari and Schwartz, 2011). Currently a clinical trial of electrical vagal nerve stimulation (VNS) for CHF is on-going (Hauptman et al., 2012). We have already demonstrated that an α_2-adrenergic agonist, medetomidine, activates cardiac vagal nerve (Shimizu et al., 2012), suggesting that a class of α_2-adrenergic agonists may correct the autonomic imbalance in CHF patients. However, medetomidine also has a sedative anesthetic effect. This may prevent widespread clinical use of medetomidine or dexmedetomidine in CHF treatment. Furthermore, severe hypotension during medetomidine treatment may also limit its clinical use.

Guanfacine, a selective α_{2A}-adrenergic agonist, has recently been approved for the treatment of attention-deficit hyperactivity disorder (ADHD) (Biederman et al., 2008). A systematic review suggests that children with unmedicated ADHD experience lower levels of cardiac vagal control than healthy controls, and guanfacine partly corrects this autonomic imbalance in ADHD patients (Rash and Aguirre-Camacho, 2012). Furthermore, Yamazaki et al. (2005) have reported that guanfacine improves sympathovagal imbalance related to rapid-eye-movement (REM)/non-REM ultradian sleep rhythm in CHF patients. Thus, guanfacine may be a potential pharmacological agent for vagal activation therapy in CHF patients. To clarify the effects of guanfacine on cardiac autonomic nerve activities, we applied a microdialysis technique to rabbit heart.

2. Materials and methods

2.1. Surgical preparation

Animal care was provided in accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences* published by the Physiological Society of Japan. All protocols were approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Seven Japanese white rabbits weighing 2.4 to 2.8 kg were used in this study. Anesthesia was initiated by an intravenous injection of pentobarbital sodium (50 mg/kg) via the marginal ear vein, and then maintained at an appropriate level by continuous intravenous infusion of α-chloralose and urethane (16 $mg \cdot kg^{-1} \cdot h^{-1}$ and 100 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively). Adequate anesthesia level was confirmed by loss of the ear pinch response. The animals were ventilated mechanically with a mixture of room air and oxygen (respiratory rate, 30 cycles/min; volume, 15 ml/kg). A fluid-filled catheter was inserted

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into the femoral artery to monitor systemic arterial pressure. Esophageal temperature was maintained between 38 and 39 °C using a heating pad.

With the animal in supine position, a right lateral thoracotomy was performed and the right 3rd to 5th ribs were partially resected to expose the heart. After pericardium incision, a dialysis probe was implanted as described in *Dialysis Technique* below. Three stainless steel electrodes were attached around the thoracotomy incision for monitoring body surface electrocardiogram (ECG). The ECG was connected to a cardiotachometer and heart rate was recorded.

At the end of the experiment, the animal was euthanized by injecting an overdose of pentobarbital sodium. In the postmortem examination, the inside of the resected atrial wall was observed macroscopically to confirm that the dialysis membrane was implanted totally within the atrial myocardium.

2.2. Dialysis technique

The materials and properties of the dialysis probe have been described previously (Shimizu et al., 2009, 2010). A dialysis fiber of semipermeable membrane (length 4 mm, PAN-1200; Asahi Chemical, Tokyo, Japan) was attached at both ends to polyethylene tubes (length 25 cm). The dialysis probe was implanted into the right atrial myocardium near the sinoatrial node, and was perfused with Ringer's solution containing a cholinesterase inhibitor, eserine (100 μM), at a speed of 2 μl/min using a microinjection pump (CMA/102, Carnegie Medicin, Sweden). Experimental protocol was started 2 h after implantation. Eight microliters of phosphate buffer (pH 3.5) was added to each sample tube before dialysate sampling, and each dialysate sampling period was set at 20 min (1 sample volume = 40 μl). Dialysate acetylcholine (ACh) and norepinephrine (NE) concentrations were analyzed separately by high performance liquid chromatography (Akiyama et al., 1991, 1994).

2.3. Experimental protocols

We investigated the effects of intravenous guanfacine on vagal ACh and sympathetic NE releases into the myocardium. Baseline dialysate samples were collected over 20 min before the injection of guanfacine. A low dose (10 μg/kg) of guanfacine (Sigma-Aldrich Co. LLC., St. Louis, MO, USA) was injected intravenously via the femoral vein. After approximately 20-min hemodynamic stabilization, dialysate was sampled for 20 min (40 μl). Thereafter, a high dose (100 μg/kg) of guanfacine was injected intravenously and another 20-min dialysate sample was collected after 20-min hemodynamic stabilization. Finally, bilateral cervical vagotomy was performed and a 20-min dialysate sample was collected 5 min after vagotomy taking into account the dead space between the dialysate membrane and the sample tube.

2.4. Statistical analysis

All data are presented as mean ± standard error. Heart rate and mean arterial pressure were compared by one-way repeated measures analysis of variance (ANOVA) followed by a Dunnett's test against baseline. After logarithmic transformation, dialysate ACh and NE concentrations were also compared by one-way repeated measures ANOVA followed by a Dunnett's test against baseline. Differences were considered significant at $P < 0.05$.

3. Results

Intravenous guanfacine at a dose of 10 μg/kg did not affect heart rate (264 ± 8 bpm at baseline to 243 ± 7 bpm, not significant) and mean arterial pressure (88 ± 2 mm Hg at baseline to 77 ± 2 mm Hg, not significant) (Fig. 1A and B). Dialysate ACh and NE concentrations at baseline were 6.7 ± 1.2 nM and 193 ± 22 pM, respectively (Fig. 2A and B). Intravenous injection of 10 μg/kg of guanfacine did not affect dialysate ACh and NE concentrations (7.3 ± 1.2 nM and 156 ± 23 pM, respectively).

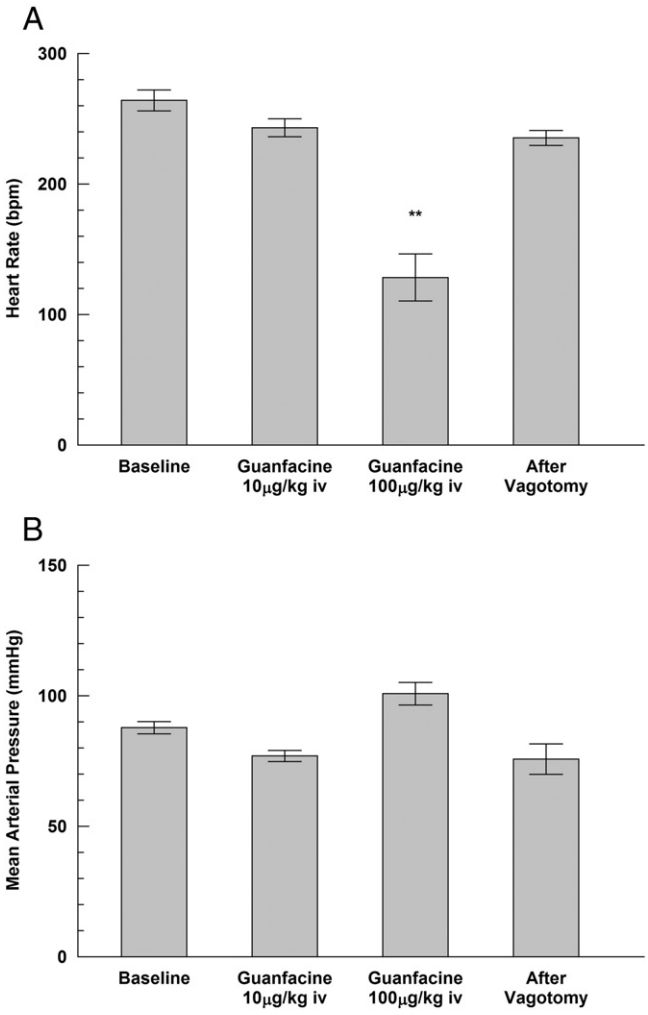

Fig. 1. Heart rate (A) and mean arterial pressure (B) at baseline, after intravenous injection (iv.) of guanfacine, and after bilateral cervical vagaotomy. **, $P < 0.01$ by Dunnett's test against baseline.

Intravenous guanfacine at a dose of 100 μg/kg significantly decreased heart rate to 128 ± 18 bpm ($P < 0.01$ vs. baseline), but had no effect on mean arterial pressure (101 ± 4 mm Hg, not significant vs. baseline) (Fig. 1B). Intravenous injection of 100 μg/kg of guanfacine significantly increased dialysate ACh concentration to 41.7 ± 8.4 nM ($P < 0.01$ vs. baseline) (Fig. 2A), whereas this dose of guanfacine did not affect dialysate NE concentration (172 ± 36 pM, not significant vs. baseline) (Fig. 2B). Heart rate and dialysate ACh concentration recovered to the baseline levels immediately after vagotomy (235 ± 6 bpm and 7.8 ± 1.2 nM, respectively).

4. Discussion

Guanfacine, a selective α_{2A}-adrenergic agonist, was previously used as a centrally acting antihypertensive drug because study indicated that guanfacine acted on the central nervous system and suppressed sympathetic nerve activity (Scholtysik, 1986). Although α_{2A}-adrenergic receptor subtype plays a principal role in central hypotensive effects of α_2-adrenergic agonists (MacMillan et al, 1996), the sympatholytic effect of guanfacine seems to be weaker than those of other α_2-adrenergic agonists. Our previous study demonstrated that 10 and 100 μg/kg of medetomidine, another α_2-adrenergic agonist, significantly decreased sympathetic NE release to the heart (Shimizu et al., 2012).