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Ivabradine does not acutely affect open-loop baroreflex static characteristics and spares sympathetic heart rate control in rats

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ABSTRACT

Aims: To assess the acute effects of intravenous ivabradine, a selective bradycardic agent, on carotid sinus baroreflex-mediated sympathetic arterial pressure (AP) and heart rate (HR) responses.
Methods and results: In anesthetized and vagotomized Wistar–Kyoto rats ($n = 6$), carotid sinus baroreceptor regions were isolated. Changes in splanchnic sympathetic nerve activity (SNA), AP, and HR in response to a step-wise pressure input were examined before and after intravenous ivabradine (2 mg/kg). Ivabradine did not affect the response range of SNA (91.8 ± 6.5 vs. 93.5 ± 9.8%) or AP (89.6 ± 10.6 vs. 91.0 ± 9.7 mm Hg). Ivabradine significantly reduced the minimum HR from 369.4 ± 8.4 to 223.3 ± 13.2 ($P < 0.001$) but did not attenuate the HR response range (69.1 ± 7.0 vs. 82.5 ± 9.6 beats/min).
Conclusions: Ivabradine does not acutely affect baroreflex-mediated sympathetic AP regulation and also spares the magnitude of the sympathetic HR response, despite significant bradycardia. The preserved sympathetic HR response, which could not be afforded by beta-blockers, may contribute to some beneficial clinical effects of ivabradine.

1. Introduction

Selective bradycardic agents such as zatebradine and ivabradine slow heart rate (HR) by inhibiting the I_f current generated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in the sinoatrial node [1–3]. Among four HCN isoforms known, HCN4 is the most highly expressed in the sinoatrial node. Although HCN channels are also responsible for the I_h current in neuronal cells, ivabradine shows no significant effect on the I_h current in the central nervous system in clinical application, because it does not cross the blood–brain barrier. Mild to moderate visual side effects, however, are reported due to inhibition of the I_h current in the eye. In contrast, a high incidence of visual side effects was the reason that the clinical development of zatebradine was stopped [4].

Although the overall safety profile of ivabradine has been established [5], its effects on arterial baroreflex-mediated sympathetic arterial pressure (AP) and HR controls have not been fully quantified. A study by Dias da Silva et al. [6] demonstrated that administration of ivabradine increased efferent sympathetic nerve activity (SNA) in anesthetized rats. The increase in SNA after ivabradine was not observed in rats with sinoaortic baroreceptor denervation, suggesting that it was mediated by the arterial baroreflex. Although the study demonstrated the lack of a direct effect of ivabradine on SNA close to the normal operating pressure, its effects over the entire operating range of the arterial baroreflex system remain to be characterized.

To understand arterial baroreflex-mediated AP regulation in its entirety, a framework of open-loop systems analysis can be adopted [7–12]. In open-loop systems analysis, the arterial baroreflex system may be divided into two principal subsystems: a neural arc from pressure input to efferent SNA, and a peripheral arc from SNA to AP [13,14]. We hypothesized that intravenous ivabradine may not affect neural arc characteristics significantly because of its inability to cross the blood–brain barrier. Having said that, HCN channels are also expressed outside of the central nervous system such as the soma of sensory neurons and mechanosensitive fibers of the aortic depressor nerve [15]. We cannot rule out a possibility that intravenous ivabradine would modify the neural arc characteristics by acting on those HCN channels when examined comprehensively. On the other hand, we hypothesized that ivabradine would modify peripheral arc characteristics due to its significant bradycardic effect. In addition, we expected that

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intravenous ivabradine would attenuate the sympathetic HR response because, arguably, HCN channels primarily mediate tachycardia in response to sympathetic stimulation [3,16].

2. Materials and methods

2.1. Surgical preparation

Animal care was conducted in strict accordance with NIH guidelines ("Guide for the Care and Use of Laboratory Animals"). All protocols were reviewed and approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center (No. 16024).

Six Wistar–Kyoto rats weighing 349 to 384 g were anesthetized by intraperitoneal injection of a mixture of urethane (500 mg/kg) and α-chloralose (80 mg/kg), and were mechanically ventilated with room air supplemented with oxygen. A maintenance dose of the anesthetic mixture (urethane 27.8 $mg \cdot kg^{-1} \cdot h^{-1}$ and α-chloralose 4.44 $mg \cdot kg^{-1} \cdot h^{-1}$) was administered intravenously to provide an appropriate level of anesthesia, which was checked by the lack of an eye blink reflex. AP was measured using a fluid-filled pressure transducer (DX-200, Nihon Kohden, Japan) connected to polyethylene tubing (PE50, Becton Dickinson and Company, Sparks, MD) inserted into the right femoral artery. HR was derived from a body surface electrocardiogram. A postganglionic branch of the left splanchnic sympathetic nerve was exposed retroperitoneally, and SNA was measured from a pair of stainless steel wires (AS633, Cooner Wire, Chatsworth, CA) attached to the nerve. Silicone glue (Kwik-Sil, World Precision Instruments, Sarasota, FL) was used for insulation and fixation. The recorded signal included afferent impulses because the nerve distal to the electrode was not sectioned. However, judging from the near disappearance of burst activities after ganglionic blockade, the majority of recorded SNA might have originated from postganglionic efferent fibers. Bilateral carotid sinus baroreceptor regions were isolated [17,18] to control carotid sinus pressure (CSP). Bilateral aortic depressor nerves were sectioned to avoid confounding reflex effects from the aortic arch. Vagal nerves were also sectioned to avoid reflexes from cardiopulmonary regions, and to focus on sympathetic AP and HR control. After the end of the experiment, the animals were euthanized by overdose of intravenous pentobarbital administration.

2.2. Protocol

CSP was decreased to 60 mm Hg for 5 min, then increased step-wise up to 180 mm Hg in increments of 20 mm Hg/min. The step-wise CSP input was repeated and designated as S1 through S5, as shown in the top panel of Fig. 1. Ivabradine (2 mg/kg) was administered intravenously 1 min after the completion of S2. At the end of the protocol, the ganglionic blocker hexamethonium bromide (60 mg/kg) was administered intravenously.

2.3. Data analysis

Data were acquired using a 16-bit analog-to-digital converter [AD16-16(PCI)EV, Contec, Japan] at a sampling rate of 1000 Hz. Mean AP, systolic AP, diastolic AP, HR, and SNA values were averaged for the last 10 s at each CSP level. Data obtained during S2 were treated as a control, and those obtained during S5 were used to evaluate the effect of ivabradine. As the absolute amplitude of SNA varied among animals depending on the recording conditions, SNA was normalized in each animal so that the noise level after ganglionic blockade was zero and the value at a CSP of 60 mmHg under control conditions (S2) was 100%.

Open-loop static characteristics of the neural arc were quantified by fitting a four-parameter logistic function [19] to the CSP–SNA data:

$$\mathrm{SNA} = \frac{P_1}{1 + \exp[P_2(\mathrm{CSP} - P_3)]} + P_4 \quad (1)$$

where P_1 is the response range, P_2 is the slope coefficient, P_3 is the midpoint input pressure, and P_4 is the minimum SNA or the lower plateau of the logistic function. Changes in mean AP, systolic AP, diastolic AP, and HR as a function of CSP were likewise analyzed using the four-parameter logistic function. For the total reflex arc (the CSP–mean AP relationship), the maximum gain (G_{max}) was calculated from $P_1 \times P_2/4$. In addition, HR was converted into R–R intervals, and the relationship between CSP and the R–R interval was analyzed.

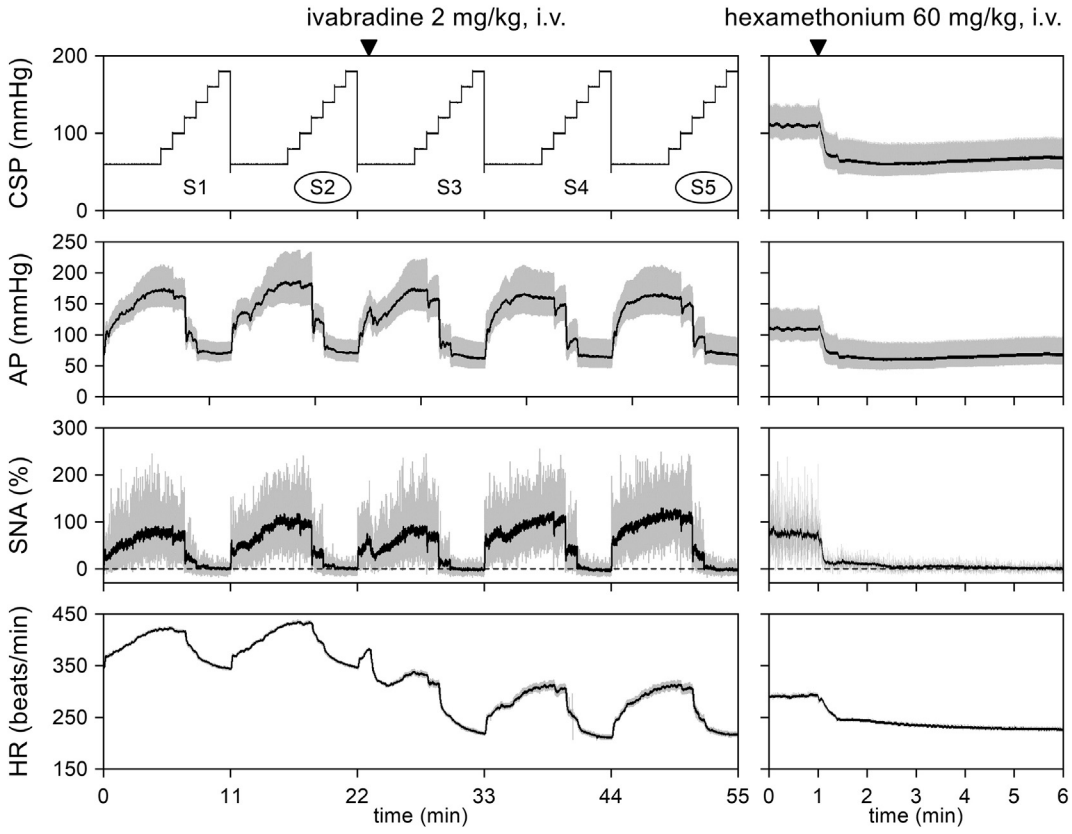

Fig. 1. Typical recordings of carotid sinus pressure (CSP), arterial pressure (AP), sympathetic nerve activity (SNA), and heart rate (HR). In the AP and HR plots, the gray and black lines indicate 200-Hz resampled signals and 2-s moving averaged signals, respectively. In the SNA plot, the gray and black lines indicate a 10-Hz resampled signal and a 2-s moving averaged signal, respectively. In the left panels, the step-wise CSP inputs were labeled S1 through S5. After the completion of S2, ivabradine was intravenously administered (2 mg/kg) as a bolus injection. The effects of intravenous ivabradine were assessed by comparing the baroreflex responses between S2 and S5. In the right panels, burst activities in SNA nearly disappeared after intravenous administration of hexamethonium bromide (60 mg/kg).