Life Sciences 149 (2016) 72–78

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Systematic understanding of acute effects of intravenous guanfacine on rat carotid sinus baroreflex-mediated sympathetic arterial pressure regulation

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ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form 5 February 2016
Accepted 12 February 2016
Available online 13 February 2016

Keywords:
Guanfacine
Carotid sinus baroreflex
Open-loop systems analysis
Sympathetic nerve activity
Alpha-2 agonist

ABSTRACT

Aims: To assess the acute effects of intravenous guanfacine, an α_{2A}-adrenergic agonist, on sympathetic outflow from the central nervous system and on sympathetic arterial pressure (AP) response.
Main methods: In anesthetized Wistar Kyoto rats, carotid sinus baroreceptor regions were isolated. Changes in electrical sympathetic nerve activity (SNA) and AP in response to a baroreceptor pressure input were examined before and after an intravenous administration of a high dose (100 μg/kg, $n = 7$) or a low dose (20 μg/kg, $n = 5$) of guanfacine.
Key findings: The higher dose of guanfacine significantly narrowed the range of the AP response (86.8 ± 6.4 to 38.4 ± 12.9 mm Hg, $P < 0.01$) but increased the minimum AP (79.3 ± 7.5 to 93.2 ± 8.7 mm Hg, $P < 0.05$). In the neural arc, guanfacine reduced both the response range (90.4 ± 2.3 to 33.4 ± 10.7%, $P < 0.01$) and the minimum SNA (11.4 ± 1.9 to 2.6 ± 1.5%, $P < 0.01$). In the peripheral arc, guanfacine increased the intercept (67.6 ± 7.1 to 92.8 ± 8.5 mm Hg, $P < 0.01$) without a significant effect on the slope. The lower dose of guanfacine weakened the effects on both the neural and peripheral arcs.
Significance: Guanfacine suppressed SNA without a significant reduction of AP, which may be attributable to the peripheral vasoconstrictive effect. Reducing the dose of acutely administered intravenous guanfacine does not aid in separating the central sympathoinhibitory effect from the peripheral vasoconstrictive effect on AP in anesthetized rats in vivo.

1. Introduction

Guanfacine, an α_{2A}-adrenergic agonist, exerts an antihypertensive effect by suppressing electrical sympathetic nerve activity (SNA) via a mechanism of presynaptic inhibition of adrenergic neurons in the central nervous system [1–3]. Guanfacine can also function peripherally to inhibit norepinephrine release in response to adrenergic nerve stimulation in an isolated rabbit heart preparation [4]. On the other hand, a previous study from our laboratory indicated that intravenous guanfacine increased vagal acetylcholine release with little effect on sympathetic norepinephrine release in the atrial myocardial interstitium in anesthetized rabbits [5]. In the same study, intravenous guanfacine decreased heart rate (HR) but did not significantly affect arterial pressure (AP).

Several factors need to be considered to reconcile the above-mentioned diverse experimental results. First, background sympathetic tone was not intentionally increased in our previous study [5], which might have made it difficult to demonstrate sympathoinhibitory and hypotensive effects of guanfacine. However, this limitation is not always evident because intravenous medetomidine, another α_2-adrenergic agonist, reduced myocardial norepinephrine release and decreased AP in the same experimental setting [6]. Medetomidine is different from guanfacine in that it may induce a hypotensive effect via imidazoline receptors because medetomidine is an imidazole [7]. Second, while peripheral vasoconstriction is predominantly mediated by postsynaptic α_1-adrenergic receptors, postsynaptic α_2-adrenergic receptors also contribute to vasoconstriction in several vascular beds [8]. Guanfacine can induce pressor responses in pithed rats by acting on α_2-adrenergic receptors at low doses (in the range less than or equal to 10 μg/kg) and by additionally stimulating α_1-adrenergic receptors at high doses (in the range greater than or equal to 30 μg/kg) [9]. These peripheral vasoconstrictive effects might have counteracted the centrally-mediated hypotensive effect of guanfacine. Finally, under baroreflex closed-loop conditions, changes in SNA and AP by a given drug are attenuated by a negative-feedback mechanism. For instance, if AP is

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http://dx.doi.org/10.1016/j.lfs.2016.02.051
0024-3205/

decreased via a central sympathoinhibitory effect of guanfacine, the decrease in AP acts to increase SNA via the arterial baroreflex, counteracting any sympathoinhibitory effect.

In order to more systematically understand the total picture of the effects of guanfacine on SNA and AP, this study employed a framework of open-loop analysis of the carotid sinus baroreflex where the carotid sinus baroreceptor regions were isolated from systemic circulation [10–14]. In this method, the baroreceptor input pressure is controlled externally independent of changes in AP, which enables the examination of the effect of intravenous guanfacine at different levels of SNA. Furthermore, this method enables the separate estimation of the characteristics of the two principal arcs of the arterial baroreflex system, i.e., the neural and peripheral arcs [15,16]. The neural arc represents the input-output relationship between baroreceptor input pressure and SNA, whereas the peripheral arc represents the input-output relationship between SNA and AP. The central effect through presynaptic α_2-adrenergic receptors has been the major focus for the usage of guanfacine because a vasoconstrictive effect via postsynaptic α_2-adrenergic receptors may be regarded as secondary compared with that via postsynaptic α_1-adrenergic receptors [17]. We hypothesized, however, that the peripheral vasoconstrictor effect of guanfacine may be stronger than generally understood and cannot be ignored in the determination of AP during the acute intravenous administration of guanfacine.

2. Materials and methods

2.1. Surgical preparation

The animal experiments in this study were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocols were reviewed and approved by the Animal Subject Committee of the National Cerebral and Cardiovascular Center. Twelve male Wistar Kyoto (WKY) rats (394 ± 29 g, mean ± SD) were artificially ventilated after inducing anesthesia with a peritoneal injection of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml) at a dose of 2 ml/kg. To maintain a suitable level of anesthesia, the anesthetic mixture was diluted by physiological saline to a one-eighteenth concentration and continuously administered at a rate of 1 ml/kg through a venous catheter inserted into the right femoral vein. Another venous catheter inserted into the left femoral vein was used to administer test drugs. An arterial catheter was introduced from the right femoral artery to measure AP and HR. A pair of stainless steel wire electrodes were attached to a postganglionic branch of the splanchnic sympathetic nerve to record SNA [10,13]. Briefly, a biosignal amplifier (AB-610J, Nihon Kohden, Japan) magnified the nerve activity by 200,000 times (i.e., 1 V/5 μV) with a bandpass filter between 150 and 1000 Hz. The signal was then fed into an analog circuit for full-wave rectification and low-pass filtering with a cut-off frequency of 30 Hz to quantify SNA. Bilateral carotid sinus baroreceptor regions were isolated [18,19] to control carotid sinus pressure (CSP). Bilateral vagal and aortic depressor nerves were sectioned to minimize any confounding effects through reflexes from the cardiopulmonary region and aortic arch. Open-loop static characteristics of the carotid sinus baroreflex were estimated as follows. CSP was first decreased to 60 mm Hg for 5 min, then increased in a staircase manner from 60 to 180 mm Hg in increment of 20 mm Hg. Each step was maintained for 60 s. Carotid sinus baroreceptors were exposed to non-pulsatile pressure [10–14].

2.2. Protocols

In Protocol 1 (high dose, $n = 7$), guanfacine was intravenously administered as a bolus (100 μg/kg). Guanfacine was dissolved in physiological saline at a concentration of 100 μg/ml, and the solution was injected at a dose of 1 ml/kg over a duration of approximately 10 s. In our previous study, this dose of guanfacine effectively increased cardiac vagal acetylcholine release with little effect on sympathetic norepinephrine release in the rabbit atrial myocardium [5]. This dose was above the dose (approximately 30 μg/kg) that caused a 50% increase in AP relative to the maximum response in pithed rats [9]. Twenty minutes later, when hemodynamic changes reached a new steady state, the baroreflex responses were compared with those acquired before administering guanfacine. The effect of an injected volume (1 ml/kg) was assumed to be minimal as we waited for 20 min after guanfacine administration to analyze the data. The control data were acquired without a vehicle (physiological saline) pretreatment. At the end of the protocol, hexamethonium bromide was intravenously administered at a dose of 60 mg/kg to block the sympathetic ganglionic transmission and record the noise level of SNA.

In Protocol 2 (low dose, $n = 5$), the dose of guanfacine was reduced to 20 μg/kg and the effect on baroreflex responses was examined. Guanfacine was dissolved in physiological saline at a concentration of 20 μg/ml, and the solution was injected at a dose of 1 ml/kg over a duration of approximately 10 s. This dose was below the dose (approximately 30 μg/kg) that caused a 50% increase in AP relative to the maximum response in pithed rats [9]. This protocol was added based on the results of Protocol 1 to test the hypothesis that the lower dose could be effective in avoiding a peripheral vasoconstrictive effect while maintaining the central sympathoinhibitory effect of guanfacine (see Discussion section).

2.3. Data analysis

The staircase-wise input cycles are referred to as S1 through S7 (Fig. 1). Guanfacine was administered as a bolus 1 min after the completion of S2. The baroreflex responses obtained from the S2 cycle were treated as control, and those obtained from the S5 cycle were used to evaluate the effects of guanfacine. In each input cycle, data averaged for the last 10 s at each CSP level were used to quantify static characteristics of the carotid sinus baroreflex. SNA was normalized in each animal so that the SNA value at a CSP of 60 mm Hg under the control condition became 100% and that after ganglionic blockade became 0%. The input-output data for the relationships between CSP and AP (total reflex arc), between CSP and SNA (neural arc), and between CSP and HR were analyzed using a four-parameter logistic function, and the response range (P_1), slope coefficient (P_2), midpoint input pressure (P_3), and minimum value (P_4) were estimated [12,20]. As for the relationship between SNA and AP (peripheral arc), a linear regression analysis was used to obtain the intercept and slope of the relationship [12]. The operating point of the carotid sinus baroreflex was calculated from a baroreflex equilibrium diagram [12,16,21].

2.4. Statistical analysis

Data are presented as means ± standard error. Fitted parameter values were compared before and after administering guanfacine using a paired t-test in each protocol [22]. The differences were considered statistically significant when $P < 0.05$.

3. Results

Shown in Fig. 1 are the representative time series of the high dose protocol. Gray lines in the AP and HR panels indicate 200-Hz resampled data. Gray lines in the SNA panels indicate 10-Hz resampled data. Black lines in AP, SNA, and HR panels indicate 2-s moving averaged data. Before administering guanfacine, an increase in CSP decreased AP, SNA, and HR, reflecting the negative feedback operation of the carotid sinus baroreflex. Guanfacine gradually decreased the maximum AP, but it increased the minimum AP at the same time. Guanfacine acutely suppressed SNA, and the suppression persisted until the end of the protocol. The maximum HR gradually decreased after guanfacine. In