Life Sciences 92 (2013) 1202–1207

Contents lists available at SciVerse ScienceDirect

Life Sciences

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

Effects of cilnidipine on sympathetic outflow and sympathetic arterial pressure and heart rate regulations in rats

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ARTICLE INFO

Article history:
Received 29 November 2012
Accepted 8 May 2013

Keywords:
Cilnidipine
Carotid sinus baroreflex
Open-loop systems analysis
Sympathetic nerve activity

ABSTRACT

Aims: Cilnidipine is a unique Ca^{2+} channel blocker that inhibits both L-type and N-type Ca^{2+} channels. The present study aimed to assess the effects of intravenous cilnidipine on sympathetic outflow and sympathetic arterial pressure (AP) and heart rate (HR) regulations.
Main methods: Carotid sinus baroreceptor regions were isolated from the systemic circulation in anesthetized and vagotomized Wistar Kyoto rats. Changes in efferent sympathetic nerve activity (SNA), AP and HR in response to a stepwise input of carotid sinus pressure were examined before and during intravenous cilnidipine administration (30 μg/kg bolus + 100 μg kg^{-1} h^{-1} infusion, n = 6).
Key findings: Cilnidipine significantly reduced the AP response range (from 68.0 ± 10.2 to 34.6 ± 4.1 mmHg, P = 0.007) but did not affect the SNA response range (from 90.4 ± 10.3 to 84.7 ± 9.5%, P = 0.297) or the HR response range (from 50.4 ± 10.1 to 48.1 ± 6.2 beats/min, P = 0.719).
Significance: Cilnidipine, at a depressor dose used in the present study, does not acutely suppress sympathetic outflow from the central nervous system. Also, it spared the sympathetic HR response, suggesting that N-type Ca^{2+} channel blocking action at the cardiac sympathetic nerve endings may be a modest one.

Introduction

Calcium channel blockers are widely used to reduce arterial pressure (AP) for the treatment of hypertension. L-type Ca^{2+} channel blockers exert a strong AP-lowering effect via relaxation of vascular smooth muscles. The hypotensive state induced by L-type Ca^{2+} channel blockers may evoke baroreflex-mediated sympathetic excitation and vagal withdrawal, leading to increased heart rate (HR) known as "reflex tachycardia" (Leenen, 1996; Ruzicka and Leenen, 1996). Cilnidipine is a Ca^{2+} channel blocker used in Japan. This compound has unique property of inhibiting not only L-type Ca^{2+} channels but also N-type Ca^{2+} channels (Uneyama et al., 1997, 1999; Takahara et al., 2002, 2003). Although the property of sympathetic inhibition via the N-type Ca^{2+} channel blockade may be beneficial for preventing reflex tachycardia, effects of intravenous cilnidipine on sympathetic outflow from the central nervous system remain unknown. Neuroprotective action of cilnidipine against focal brain ischemia (Takahara et al., 2004) suggests possible central effects, but little information is available in literature whether cilnidipine crosses the blood–brain barrier in intact conditions. Moreover, even if a test substance does not cross the blood–brain barrier, there is also a possibility that the substance affects the sympathetic outflow by acting on those central structures which lack the blood–brain barrier (Simpson, 1981; Kawada et al., 2009). Therefore, direct measurements of efferent sympathetic nerve activity (SNA) are mandatory to determine the central effects of intravenous cilnidipine.

When the arterial baroreflex is normally operative, any change in AP induced by a test drug inevitably causes a reflex change in SNA. This closed-loop operation makes it difficult to assess the pure drug effect on SNA under in vivo conditions. To circumvent the problem, we employed a method of open-loop systems analysis where the baroreceptor input pressure was externally controlled independently of AP (Kawada et al., 2010). We also adopted a framework of dividing the arterial baroreflex system into neural arc and peripheral arc subsystems (Mohrman and Heller, 2006; Sato et al., 1999a). The neural arc represents the relationship between baroreceptor pressure input and efferent SNA, and the peripheral arc represents the relationship between efferent SNA and AP. The central and peripheral effects of a given drug can be assessed by changes in the neural and peripheral arcs, respectively. Using this framework, the first purpose of the present study was to examine the effects of intravenous cilnidipine on the sympathetic outflow from the central nervous system. The second purpose was to estimate a relative potency of a depressor dose of cilnidipine in suppressing sympathetic HR response. If a given dose of cilnidipine exerted significant inhibition of N-type Ca^{2+} channels at cardiac sympathetic nerve endings, the magnitude of sympathetic HR response might be reduced.

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http://dx.doi.org/10.1016/j.lfs.2013.05.004

Materials and methods

Surgical preparation

Animal care was provided in strict accordance with the *Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences*, approved by the Physiological Society of Japan. All protocols were reviewed and approved by the Animal Subject Committee of National Cerebral and Cardiovascular Center. Seventeen male Wistar Kyoto rats (330–395 g) were anesthetized by an intraperitoneal injection (2 mL/kg) of a mixture of α-chloralose (40 mg/mL) and urethane (250 mg/mL), and ventilated mechanically with oxygen-enriched room air. An appropriate depth of anesthesia was maintained by continuous intravenous infusion of a diluted solution of the above anesthetic mixture via the right femoral vein. Another venous catheter was prepared for infusing test drugs from the left femoral vein. An arterial catheter was inserted into the right femoral artery to measure AP. A cardiotachometer (AT-601G, Nihon Kohden, Tokyo, Japan) was used to detect HR from AP. Body temperature of the animal was maintained by a heating pad at approximately 38 °C.

A postganglionic branch of the splanchnic sympathetic nerve was exposed through a left flank incision. A pair of stainless steel wire electrodes (Bioflex wire, AS633, Cooner Wire, CA, USA) was attached to the nerve, and then secured and insulated with silicone glue (Kwik-Sil, World Precision Instruments, FL, USA). To quantify SNA, a preamplified nerve signal was band-pass filtered at 150–1000 Hz, and then full-wave rectified and low-pass filtered with a cut-off frequency of 30 Hz using analog circuits. At the end of the experiment, a ganglionic blocker hexamethonium bromide (60 mg/kg) was given in bolus to confirm the disappearance of SNA and to measure the noise level.

Bilateral vagal and aortic depressor nerves were sectioned at the neck region to avoid reflexes from the cardiopulmonary region and aortic arch. Carotid sinus baroreceptor regions were isolated from the systemic circulation according to previously reported procedures (Shoukas et al., 1991; Sato et al., 1999b). Carotid sinus pressure (CSP) was controlled using a servo-controlled piston pump. To estimate open-loop static input–output relationship of the carotid sinus baroreflex, CSP was first decreased to 60 mmHg for 4 min, and then increased stepwise from 60 to 180 mmHg in increments of 20 mmHg every minute (Kawada et al., 2009, 2010). The stepwise CSP input cycle was repeated throughout the protocol.

Protocol 1 ($n = 6$)

We dissolved 1-mg cilnidipine (courtesy of Ajinomoto Co., Inc., Japan and Mochida Pharmaceutical Co., Ltd., Japan) into 10-μL dimethyl sulfoxide (DMSO) and diluted it with polyethylene glycol 200 to a concentration of 1-mg/mL cilnidipine (1% v/v of DMSO). Then the solution was diluted with physiological saline before use. A bolus injection (30-μg/kg cilnidipine with 0.3-μL/kg DMSO) was given followed by continuous infusion (100-$\mu g \cdot kg^{-1} \cdot h^{-1}$ cilnidipine with 1-$\mu L \cdot kg^{-1} \cdot h^{-1}$ DMSO) via the venous catheter. The bolus dose was selected near the dose of maximum AP depression in rats based on a previous study (Takahara et al., 2002). The dose of continuous infusion was determined from a preliminary study so that the hypotensive effect was sustained until the end of the protocol.

Protocol 2 ($n = 5$)

Because the effects of cilnidipine on sympathetic HR response were modest in Protocol 1, it raised a question about the type of Ca^{2+} channels mediating norepinephrine release at cardiac sympathetic nerve endings in rats. To rule out the possibility that Ca^{2+} channels other than the N-type mediate the sympathetic HR response in rats, the effects of a selective N-type Ca^{2+} channel blockader ω-conotoxin GVIA (50 μg/kg) were examined (Akiyama et al., 2004). We dissolved ω-conotoxin GVIA into distilled water to a concentration of 100 μM. This stock solution was diluted with physiological saline before use.

Protocol 3 ($n = 6$)

Because DMSO per se has several hemodynamic effects (Santos et al., 2003), we examined the per se effects of DMSO (0.3-μL/kg bolus followed by 1-$\mu L \cdot kg^{-1} \cdot h^{-1}$ continuous infusion) on open-loop static characteristics of the carotid sinus baroreflex and HR regulation. We also examined the effects of 5-times higher dose of cilnidipine (bolus injection: 150-μg/kg cilnidipine with 1.5-μL/kg DMSO, followed by continuous infusion: 500-$\mu g \cdot kg^{-1} \cdot h^{-1}$ cilnidipine with 5-$\mu L \cdot kg^{-1} \cdot h^{-1}$ DMSO).

Data analysis

Data were sampled at 1000 Hz using a 16-bit analog-to-digital converter and stored on a dedicated laboratory computer system. To estimate the input–output relationship at steady state, mean SNA, AP and HR were obtained from the last 10-s data at each CSP level of the stepwise input. The values were averaged for two consecutive stepwise input cycles immediately before the drug administration, and were used as control data. Hemodynamics reached a new steady state within the first stepwise input cycle after the drug administration. The values averaged from the second and third stepwise input cycles following the drug administration were used to evaluate the drug effect. In each rat, SNA values were normalized using the noise level of SNA obtained after hexamethonium bromide administration as 0%, and the SNA value corresponding to CSP of 60 mmHg in the control data as 100%.

Static characteristics of the total-loop response (AP versus CSP), HR regulation (HR versus CSP) and neural arc (SNA versus CSP) showed an inverse sigmoid curve, and were quantified by fitting a four-parameter logistic function to the data (Kent et al., 1972) as follows.

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

where y denotes the output value (AP, HR or SNA); P_1 is the response range of y (i.e., the difference between the maximum and minimum values of y); P_2 is the slope coefficient; P_3 is the midpoint of the sigmoid curve on the CSP axis; and P_4 is the minimum value of y. The maximum gain or the maximum slope of the logistic function (G_{max}) was calculated as $P_1P_2/4$.

Static characteristics of the peripheral arc (AP versus SNA) approximated a straight line, and were quantified by linear regression as follows:

$$\mathrm{AP} = P_{\mathrm{slope}} \times \mathrm{SNA} + P_{\mathrm{intercept}}$$

where P_{slope} and $P_{intercept}$ represent the slope and intercept, respectively. The operating-point AP and SNA were determined by equilibrating the neural and peripheral arc characteristics on a pressure-SNA plane (Yamamoto et al., 2004; Kamiya et al., 2005).

Statistical Analysis

Data are presented as means ± SE. Drug effects were examined using paired t-test. The difference was considered significant at $P < 0.05$ (Glantz, 2002).

Results

Typical recordings of CSP, SNA, AP and HR obtained from one animal in Protocol 1 are shown in Fig. 1A. A stepwise elevation in CSP decreased SNA, AP and HR under control conditions. Administration of cilnidipine did not significantly affect SNA, and the SNA response to