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Open-loop characteristics of the arterial baroreflex after blockade of unmyelinated baroreceptors with resiniferatoxin

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ABSTRACT

Arterial baroreceptors can be divided into two categories dependent on whether their axons are myelinated (A-fiber) or unmyelinated (C-fiber). We investigated the effect of periaxonal resiniferatoxin (RTX), a blocker of C-fiber baroreceptor activity, on the open-loop static characteristics of the arterial baroreflex. The baroreceptor region of the right aortic depressor nerve was isolated, and intra-baroreceptor region pressure (BRP) was changed from 60 to 180 mm Hg in 10 anesthetized Sprague–Dawley rats. Open-loop static characteristics of the neural arc from BRP to efferent sympathetic nerve activity (SNA), peripheral arc from SNA to arterial pressure (AP), and total reflex arc from BRP to AP were estimated. Although blocking C-fiber activity with RTX resulted in a lower response range ($33.7 \pm 4.6\%$ and $49.4 \pm 4.8\%$, $P < 0.01$) and higher minimum SNA ($78.0 \pm 4.7\%$ and $53.6 \pm 5.0\%$, $P < 0.001$) of the steady-state neural arc, the peak SNA response to BRP was greater at a BRP of 160 mm Hg ($-37.87 \pm 5.83\%$ and $-26.28 \pm 4.90\%$, $P = 0.01$). RTX also resulted in a lower response range (27.8 ± 5.0 mm Hg and 40.9 ± 5.2 mm Hg, $P < 0.01$) and higher minimum AP (92.4 ± 4.7 mm Hg and 79.1 ± 4.9 mm Hg, $P < 0.001$) of the total reflex arc. Despite these changes, the maximum slope of the neural arc and the maximum gain of the total reflex arc did not differ significantly after RTX. These data suggest that A-fiber baroreceptors can regulate AP and maintain the maximum gain when systemic AP is around the normal operating range. In contrast, C-fiber baroreceptors are critically important for reductions in SNA and AP when systemic AP is raised above the normal operating range.

1. Introduction

The primary neural regulator of arterial pressure (AP) is the arterial baroreflex. The sensors of the arterial baroreflex (baroreceptors) can be divided into two categories dependent on whether their axons are myelinated (A-fiber) or unmyelinated (C-fiber). A-fiber baroreceptors have low pressure threshold and high firing frequency characteristics, whereas C-fiber baroreceptors have high pressure threshold and low firing frequency characteristics (Thoren et al., 1999). Furthermore, Seagard and colleagues demonstrated that A-fiber baroreceptors are primarily involved in the dynamic (beat-to-beat) sensitivity of the arterial baroreflex and C-fiber baroreceptors are responsible for regulation of the set-point for mean AP (Seagard et al., 1993). We have previously demonstrated that A- and C-fiber central pathways from afferent nerve stimulation to efferent sympathetic nerve activity (SNA) reveal dynamic characteristics that are matched with respective fiber characteristics (Turner et al., 2014), i.e., the A-fiber central pathway contributes to AP regulation at higher modulation frequencies compared with the C-fiber central pathway. A potential limitation of our previous study is that the information obtained from electrical stimulation of afferent fibers needs to be translated into baroreflex function in terms of pressure input. The contribution of A- and C-fiber baroreceptors to the AP regulation during pressure input needs to be quantified.

In rats, C-fiber baroreceptors express transient receptor potential vanilloid-1 (TRPV1) ion channels. C-fiber baroreceptor activity can be selectively blocked by acute periaxonal application (Fan and Andresen, 1998; Reynolds et al., 2006) or ablated by intra-peritoneal injection (Sun et al., 2009) of the TRPV1 agonist capsaicin or its ultrapotent analog resiniferatoxin (RTX). According to the study by Sun et al. (2009), rats treated with an intra-peritoneal injection of RTX show a significant decrease in the ability of the arterial baroreflex to reduce renal SNA and heart rate (HR). While their study clearly demonstrates the importance of C-fiber baroreceptors in arterial baroreflex function, there are a couple of concerns. First, because they used pharmacological pressure intervention (sodium nitroprusside and phenylephrine), the input–output relationship of the total reflex arc between pressure input and AP was not determined. Conventional pharmacological pressure manipulation is closed-loop in nature, changes in SNA inevitably alter AP, and vice versa. An open-loop investigation of the arterial baroreflex is required to identify the true baroreceptor input pressure to SNA

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and AP characteristics. Furthermore, the two fiber types of baroreceptors affect AP and HR differently (Fan et al., 1999). Therefore, it is essential to estimate the AP response in addition to the HR response to understand the total picture of the arterial baroreflex control via the two fiber types of baroreceptors. Second, TRPV receptors are expressed not only in C-fiber baroreceptors but also in a large variety of unmyelinated neurons and other tissues (Caterina and Julius, 1999). Zahner et al. (2003) demonstrated preserved sympathetic activation in response to an intracerebroventricular administration of a $GABA_A$ receptor antagonist bicuculline in RTX-treated rats. Nevertheless, effects of RTX on arterial baroreflex function through mechanisms other than the ablation of C-fiber baroreceptors cannot be completely ruled out. For instance, TRPV ion channels are involved in osmotic activation of neurons in the organum vasculosum lamina terminalis (OVLT), and TRPV knockout mice show significantly attenuated water intake in response to a systemic hypertonic challenge (Ciura and Bourque, 2006). A secondary effect of RTX on drinking behavior could modify arterial baroreflex function indirectly through changes in body fluid balance and associated humoral factors.

Taking the above-mentioned concerns into consideration, the aim of the present study was to elucidate the contribution of C-fiber baroreceptors to AP regulation using an open-loop analysis of arterial baroreflex function (Kawada et al., 2010, 2014). We employed a method of isolating the baroreceptor region of the right aortic depressor nerve (ADN) (Sato et al., 1999a). The ADN traverses the neck region where we can apply RTX periaxonally to block C-fiber activity, thereby avoiding any systemic effects of RTX. We hypothesize that because C-fiber baroreceptors function at high input pressure levels, blocking C-fiber baroreceptor activity with RTX will result in a reduction in the response range and gain of open-loop characteristics of the total reflex arc.

2. Material and Methods

The study was performed on Sprague–Dawley rats (350–400 g). The animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, which has been approved by the Physiological Society of Japan. The experimental protocols were reviewed and approved by the Animal Subjects Committee at National Cerebral and Cardiovascular Center.

2.1. Animal Preparation

The animals ($n = 10$) were anesthetized with an intra-peritoneal injection (2 ml/kg) of urethane (250 mg/ml) and α-chloralose (40 mg/ml). After the induction of anesthesia, the trachea was intubated for mechanical ventilation with 100% oxygen mixed with room air. A 20-fold diluted solution of the above anesthetic mixture was continuously administered via a venous catheter inserted in the right femoral vein. Depth of anesthesia was monitored by testing the withdrawal and blink reflexes and rate of anesthetic mixture infusion (2–3 ml/kg/h) was adjusted to maintain surgical levels of anesthesia. An additional venous catheter was inserted in the left femoral vein for later administration of hexamethonium. An arterial catheter was inserted in the right femoral artery to measure AP, from which HR was determined by a cardiotachometer. A postganglionic branch of the splanchnic sympathetic nerve was exposed through a left flank incision, and SNA was recorded (Turner et al., 2014). Preamplified nerve signals were 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 to quantify SNA. At the end of the experiment, hexamethonium bromide (60 mg/kg) was intravenously administered in bolus, and the noise level of SNA was measured.

Both vagus and carotid sinus nerves, and the left ADN were sectioned to avoid confounding reflex effects. The baroreceptor region of the right ADN was isolated from the systemic circulation (Sato et al., 1999a). The intra-baroreceptor region pressure (BRP) was controlled by a servo-controlled piston pump.

2.2. Protocol

A staircase-wise input protocol was used to estimate the open-loop static characteristics of the baroreflex (Kawada et al., 2010, 2014). BRP was first decreased to 60 mm Hg for 4 min, and then increased from 60 to 180 mm Hg in increments of 20 mm Hg per minute. Although our isolation procedure can measure stable baroreflex responses to changes in BRP for the duration of the protocol (Sato et al., 1999a), a stabilization period of 30-minutes (3 cycles of the staircase-wise input protocol) was monitored before collecting control data and rats were excluded from data analysis if the response to BRP diminished within this period. After obtaining control data, 1 μM of RTX (LC Laboratories, MA, USA) was periaxonally applied to the right ADN. RTX was applied to the ADN with a small solution-soaked cotton pledget for the remainder of the protocol. This dose of RTX can irreversibly block C-fiber action potential conduction without significantly interfering with A-fiber conduction (Reynolds et al., 2006). We have also previously shown that responses in SNA and AP from selective electrical stimulation of A-fiber baroreceptors in the left ADN were unaffected by the application of RTX. Responses in SNA and AP from the selective electrical stimulation of C-fiber baroreceptors were eliminated after RTX (Turner et al., 2014). Twenty-minutes later, the staircase-wise input protocol was repeated to examine the effects of RTX on the aortic baroreflex function.

2.3. Data Analysis

Data were sampled at 200 Hz using a 16-bit analog-to-digital. For each input cycle, seven data points of mean SNA, AP, and HR were calculated from the data recorded during the last 10 s at each BRP level. In addition, the peak SNA response was quantified by averaging the first 2 s directly following a step in BRP and subtracting it from the preceding final SNA response. Furthermore, the change in final SNA response was calculated by subtracting the value recorded during the last 10 s at each BRP level from the last 10 s of the BRP level directly preceding. The noise level of SNA was determined by averaging the SNA signal after the hexamethonium administration for 10 s and was defined as zero. The SNA value at the BRP level of 60 mm Hg of the control condition was defined as 100% in each animal.

The total reflex arc can be divided into the neural arc from BRP to SNA and the peripheral arc from SNA to AP (Mohrman and Heller, 2010; Sato et al., 1999b). Open-loop static characteristics of the total reflex arc (BRP–AP) were described by fitting the following four-parameter logistic function to the seven data points (Kent et al., 1972):

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

where y denotes the output; P_1 is the response range of y; P_2 is the slope coefficient; P_3 is the midpoint pressure on the BRP axis; and P_4 is the lower plateau of y. The maximum gain or the maximum slope was calculated as P_1P_2 / 4. Open-loop static characteristics of the neural arc (BRP–SNA) and those of HR control (BRP–HR) were also described by using the four-parameter logistic function as shown above.

The peripheral arc (SNA–AP) showed a linear relationship and open-loop static characteristics were analyzed using a linear regression analysis (Glantz, 2002) as follows:

$$AP = P_A \times SNA + P_B \quad (2)$$

where P_A and P_B represent the slope and intercept, respectively.