Excitation of Baroreceptors Depresses A- and C-Components of the Somato-Cardiac Sympathetic Reflex in Anesthetized Rats

Wei Min Li, Xia LIU*, Mamoru KUMADA†, and Akio SATO‡

Department of Molecular and Cellular Physiology, Faculty of Medicine, The University of Tokyo, Tokyo, 113–0033 Japan; *Department of Cardiology, Rui Jin Hospital, Shanghai Second Medical University, Shanghai 200025, China; †Division of Basic Medical Sciences, St. Luke’s College of Nursing, Tokyo, 104–0044 Japan; and ‡Department of the Autonomic Nervous System, Tokyo Metropolitan Institute of Gerontology, Tokyo, 173–0015 Japan

Abstract: The effect of baroreceptor activation on somato-cardiac sympathetic reflex discharges was examined in urethane-anesthetized, vagotomized, and artificially ventilated rats. Single shock stimulation of myelinated (A) and unmyelinated (C) fibers in the tibial nerve of the left hindlimb elicited two separate excitatory reflex discharge components in a branch of the cardiac sympathetic nerve. They are termed the A- and C-components of the somato-cardiac sympathetic reflex discharges. When aortic nerves (AN) and carotid sinus nerves (CSN) were intact, a sudden increase in mean arterial blood pressure to about 150 mmHg induced by i.v. injection of phenylephrine (50 μg/kg) depressed the A- and C-components by up to $47 \pm 5.4$ and $37 \pm 7.7\%$ of the control values, respectively. However, bilateral sino-aortic denervation completely abolished the pressure-induced depression of both components. We conclude that baroreceptor afferent signals from the AN and CSN inhibit both A- and C-components of the excitatory somato-cardiac sympathetic reflex discharges. This and other previous evidence mentioned in the text indicate that inhibitory cardiac sympathetic reflexes originating from arterial baroreceptors and excitatory ones originating from somatic afferents interact, probably at the brainstem. [Japanese Journal of Physiology, 48, 261–266, 1998]

Key words: somatic afferent, cardiac sympathetic nerve, somato-sympathetic reflex, arterial baroreceptors, anesthetized rats.

The spontaneous (or tonic) discharge activity of the sympathetic nervous system has become well known since the original finding by Adrian et al. in 1932 [1]. This spontaneous discharge activity is reflexly inhibited by excitation of baroreceptor afferent nerves [2, 3].

Stimulation of somatic afferent nerves produces excitatory reflex discharges in sympathetic efferent nerves in anesthetized animals (see reviews by Sato and Schmidt [4]; Sato et al. [5]). The excitatory reflex components of the sympathetic efferent nerves evoked by stimulation of myelinated (A) and unmyelinated (C) fibers can be separated into A- and C-sympathetic reflex discharge components if a somatic afferent nerve has sufficient length, as seen in a limb nerve for isolation of action potentials of both myelinated and unmyelinated fibers [6–8]. The A-sympathetic reflex discharge has a short latency (41 ± 2 ms), and the C-sympathetic reflex discharge has a long latency (210 ± 13 ms) [9]. Electrical stimulation of hindlimb afferent nerve, when used for stimulation, elicits reflex discharges in a cardiac sympathetic nerve mainly via the reflex center of the supraspinal structures, and this reflex is referred to as the supraspinal reflex (see reviews by Sato and Schmidt [4]; Sato et al. [5]). The supraspinal excitatory reflex discharges in sympa-
thetic efferent nerves elicited by electrical stimulation of myelinated afferent fibers of somatic nerves (i.e., A-reflex) have already been shown to be inhibited by stimulation of baroreceptor afferent nerves [10–14]. It is an interesting question to ask whether baroreceptor afferent stimulation can inhibit the somatically-induced C-sympathetic reflex as the A-sympathetic reflex was inhibited.

The present experiments aimed to examine if excitation of baroreceptor afferent nerves following an increase in arterial blood pressure resulting from an intravenous injection of phenylephrine could depress supraspinal C-sympathetic reflex discharges in comparison with supraspinal A-sympathetic reflex discharges in a cardiac sympathetic nerve elicited by single electrical shock of a hindlimb nerve in anesthetized rats.

METHODS

Wistar rats (n=38) weighing 350–470 g were used for the present study. The rats were initially anesthetized with an intraperitoneal injection of urethane (1 g/kg). During the experimental course, an additional dose (about 0.1 g/kg) was injected intravenously (I.V.) when necessary. Vagal nerves were cut bilaterally at the cervical level. The trachea was intubated and connected to a respiratory pump (683, Harvard, USA) for artificial ventilation with the room air. The end-expiratory CO2 level, which was monitored with a gas monitor (1H26, NEC San-ei, Tokyo), was maintained at about 3.0%. Catheters were inserted into the right femoral artery for monitoring arterial blood pressure, and also into the right femoral vein for drug administration and fluid infusion (saline and Ficoll solution purchased from Otsuka Pharmaceutical Co., Ltd., Tokyo, and Pharmacia, Uppsala, Sweden, respectively). After insertion of catheters, the rat was immobilized with I.V. gallamine triethiodide (20 mg/kg) and fixed in a prone position on a table. Body temperature, monitored in the rectum, was maintained at 37.0–37.5°C by using a thermostatically regulated heating pad and an infrared lamp (ATB-1100, Nihon Kohden, Tokyo).

The left tibial nerve was dissected free from the surrounding tissue and cut approximately 1 cm proximal to the ankle joint. The central segment of the tibial nerve was placed on a pair of bipolar platinum iridium wire electrodes covered with paraffin oil for electrical stimulation. To elicit the somato-sympathetic reflex, a single square pulse of 0.5 ms duration and 20 V intensity (supramaximal intensity for the tibial nerve in rats) was applied every 3 s by a digital electrical stimulator (SEN-7130, Nihon Kohden).

After cutting off the left second costal bone, the left inferior cardiac sympathetic nerve was dissected and cut as close to the heart as possible. The central segment of the inferior cardiac nerve was placed on another pair of bipolar platinum iridium wire electrodes covered with paraffin oil for recording tonic background activity and somato-sympathetic reflex discharges. Reflex discharges elicited by electrical stimulation of the left tibial nerve were amplified by a preamplifier (S-0476, Nihon Kohden) with a time constant of 0.3 s, averaged (20 trials) by a computer (ATAC 3700, Nihon Kohden), displayed on an oscilloscope, stored on floppy disks, and recorded on an X-Y plotter (7440A, Hewlett-Packard, USA). Tonic background activity of the cardiac sympathetic nerve was amplified with a time constant of 0.01 s of the preamplifier and fed into a window discriminator that eliminated the background noise. Spike activity was counted every 5 s by using a computer (ATAC-3700, Nihon Kohden).

Phenylephrine was dissolved in saline at concentrations of 50 and 6.8 μg/ml. To activate arterial baroreceptors near-maximally, the mean arterial blood pressure (MAP) was maintained at about 150 mmHg by an initial bolus injection of 0.4 ml of phenylephrine solution of 50 μg/ml, followed by a continuous injection (lasting for 1 min) of 1 ml of phenylephrine solution of 6.8 μg/ml.

In 22 rats out of 38, the aortic and carotid sinus nerves were bilaterally cut during the experiment.

Data after the injection of phenylephrine were expressed by percentages (mean±SEM) recorded before the injection. Data before and after the injection of phenylephrine were compared by using raw data values, not percentage values. Statistical analysis was performed by using one-way ANOVA, followed by the Bonferroni/Dunn test for critical differences.

RESULTS

Effect of activation of arterial baroreceptors on the excitatory somato-sympathetic reflex discharges in rats with intact aortic nerves and carotid sinus nerves

At the beginning of each experiment, we confirmed that the A- and C-sympathetic reflex discharge components were elicited in a cardiac sympathetic efferent nerve by stimulation of myelinated A- and unmyelinated C-afferent fibers in the tibial nerve, respectively, in anesthetized rats, as reported by Adachi et al. [15].

In 8 rats with intact aortic nerves (AN) and carotid sinus nerves (CSN), MAP was at approximately 70–80 mmHg levels. Arterial baroreceptors were acti-
Baroreceptors and Somatic-Sympathetic Reflex

Fig. 1. The original specimens of somato-sympathetic cardiac A- and C-reflexes and mean arterial blood pressure (MAP) recorded before and after an i.v. injection of phenylephrine. The upper panels present the A- and C-reflexes recorded before (control) and after the drug injection at 0.25, 1.75, 3.25, and 4.75 min, respectively. Each specimen is an average (20 trials, over 1 min) of A- and C-reflex components evoked by electrical stimulation of the left tibial nerve. ▲ represents the time when the electrical stimulus at 20 V was applied. The lower curve shows a simultaneous response of MAP to injection of the drug. Time intervals (1 min) of the MAP change during which the reflexes were averaged are marked with columns on the curve.

vated near maximally by a sudden increase in MAP induced by an i.v. injection of phenylephrine. Subsequent to the injection, MAP rose sharply, reached a peak level at about 20 s, and, concomitantly, A- and C-sympathetic reflex discharge responses were markedly diminished. MAP and sympathetic response both returned to their original levels within 5 min (Fig. 1). Fifteen seconds after the injection, MAP rose to 198±16% of the preinjection level (expressed as 100%), and the A- and C-reflex responses were reduced to 47±5.4 and 37±7.7% of the preinjection control (expressed as 100%), respectively. At 1.75 min after the injection, MAP was 155±14% of control, whereas A- and C-reflex sympathetic responses were 69±4.7 and 64±4.9% of control, respectively. The baroreceptor-induced depression of A- and C-reflex responses disappeared at 3.25 min after the injection of phenylephrine, although MAP had not yet returned to its control level (Fig. 2A and B). When the magnitude of A- and C-reflex responses was plotted against various values of MAP measured at the four different times noted on the axis abscissae of Fig. 2A and B following i.v. injection of phenylephrine, there were MAP-dependent increases in the depression of both A- and C-reflexes, especially when an increase in MAP was between 125 and 200% (Fig. 2C).

Effect of activation of arterial baroreceptors on excitatory somato-sympathetic reflex discharges in sinoaortic denervated rats

The effect of baroreceptor activation on somato-sympathetic reflexes was examined in 12 rats after bilateral denervation of the AN and CSN. In these denervated rats, an injection of phenylephrine raised MAP to 202±9.3, 147±4.1, 125±5, and 117±4.5% at 0.25, 1.75, 3.25, and 4.75 min, respectively, after drug application (Fig. 3B). However, the depression of A- and C-reflex responses observed in rats with intact AN and CSN was completely abolished (Fig. 3A). In the preparation with the AN and CSN bilaterally denervated, there was no more change in the magnitude of A- and C-reflex responses, as shown in Fig. 3C, when plotted against MAP at four different times noted on axis abscissae of Fig. 3A and B following an i.v. injection of phenylephrine.

Effects of activation of baroreceptors on tonic activity of the inferior cardiac sympathetic nerve in rats with and without AN and CSN innervation

To identify the influence of baroreflex-induced depression of sympathetic efferent tonic activity on the somato-sympathetic reflexes, spike number counting was undergone by recording tonic background activity of the sympathetic cardiac nerve without stimulation of the somatic tibial afferent nerve fibers before and after an i.v. injection of phenylephrine. Figure 4 summarizes the effect of a sudden increase in MAP following an injection of phenylephrine on tonic activity of the inferior cardiac sympathetic nerve. An arithmetical average spike number, determined by counting for 1 min before the injection of phenylephrine, was expressed as the control value. The following responsive arithmetical average spike numbers, which were counted for the same duration as control was after the injection of phenylephrine, were expressed as...
Fig. 2. Effects of i.v. injection of phenylephrine on somato-sympathetic cardiac A- and C-reflexes (A, n=8) and MAP (B, n=8), and the relationship between the reflexes and the MAP (C) with the AN and CSN intact. The 100% represents the mean amplitude of the reflexes (A, C) and of the MAP (B, C) before the drug injection (control), and all subsequent changes after the injection are expressed as percentages of the control. The bars indicate SEM. The * and ** indicate significant differences at the p<0.05 and p<0.01 levels, respectively.

percentages of the control. In six rats tested with innervation of the AN and CSN, the average spike number of sympathetic cardiac nerves was significantly depressed to 25±3.1, 36±10, 40±15, and 53±19% of the control at 0.25, 1.75, 3.25, and 4.75 min after an injection of phenylephrine (Fig. 4A). However, this depression on the tonic activity was completely abolished after bilateral AN and CSN denervation (Fig. 4B).

DISCUSSION

The present study shows that activation of the arterial baroreceptors elicited by intravenous injection of phenylephrine causes an inhibition of both excitatory somato-sympathetic A- and C-reflex discharge components in anesthetized rats. This inhibition of the reflex is blood-pressure dependent. After severing arterial baroreceptor afferent nerves bilaterally, i.e., sinus and depressor nerves, the inhibition of the sympathetic A- and C-reflex discharges completely disappeared, even though the increase in systemic arterial blood pressure remained following the intravenous injection of phenylephrine. Thus the inhibition of the excitatory sympathetic A- and C-reflex discharges following an intravenous injection of phenylephrine were suggested to be a reflex response whose afferent pathway is included in sinus and depressor nerves from the baroreceptors.

The inhibition of the excitatory somato-sympathetic A-reflex discharge elicited by stimulation of baroreceptor afferents has already been reported by Baum and Becker [10], Baum and Shropshire [11], Coote and Downman [12], Kirchner et al. [13], and Koizumi
Baroreceptors and Somato-Sympathetic Reflex

Fig. 4. Effects of an i.v. injection of phenylephrine on tonic sympathetic cardiac nerve background activity without stimulation of the tibial afferent nerve fibers with (A) and without (B) innervation of the AN and CSN (n=6). The average spike number before the drug injection is expressed as 100% (control), and the subsequent average spike numbers at 0.25, 1.75, 3.25, and 4.75 min after the drug injection are expressed as percentages of the control. The bars indicate SEM. The * and ** indicate significant differences at the \( p<0.05 \) and \( p<0.01 \) levels, respectively.

and this inhibition was suggested to have occurred within the brainstem, not in the spinal cord, because the segmental somato-sympathetic A-reflex discharge of spinal origin was not inhibited by the excitation of baroreceptor afferent nerves, but the supraspinal somato-sympathetic A-reflex discharge was strongly inhibited by the excitation of baroreceptor afferent nerves. It is most likely that both A- and C-excitatory reflex discharges in the cardiac sympathetic nerve elicited by electrical stimulation of a hindlimb nerve shown herein are of supraspinal origin, as reported by Kimura et al. [9] and Sato [16] (see reviews by Sato and Schmidt [4]; Sato et al. [5]). Therefore the inhibition of the present excitatory somato-sympathetic A- and C-reflex discharges appears to be produced at the supraspinal level, probably within the brainstem, by the stimulation of baroreceptor afferent nerves. In another word, somatic excitatory afferent inputs may interfere with baroreceptor inhibitory afferent inputs in the brainstem and thereby influence sympathetic efferent nerve activity.

There was a barodependent correlation between the degree of inhibition of cardiac sympathetic tonic activity (Fig. 4A) and the degree of the A- and C-cardiac sympathetic reflex discharges (Fig. 2A) when baroreceptors were stimulated. This evidence suggests that the baroreceptor-induced inhibition of sympathetic tonic activity and the somatically induced excitatory A- and C-reflexes do interfere. However, a minor discrepancy was found between these baroreceptor induced tonic and reflex responses, as seen by comparing the results in Figs. 2A and 4A at 3.25 min after an i.v. injection of phenylephrine. This may suggest that either central pathways or central sensitivities of both baroreceptor-induced inhibitions of the sympathetic nerve and somatosympathetic reflexes are not fully in accord with each other.

The possibility of a direct inhibitory effect by phenylephrine on sympathetic neurons must be considered. However, after severing arterial baroreceptor afferent nerves bilaterally, both sinus and depressor nerves, no inhibitory effect of intravenously injected phenylephrine on the sympathetic efferent tonic activity could be demonstrated. Therefore the possibility of a direct inhibitory effect on the sympathetic neurons of phenylephrine at the dose used in this study can be eliminated. Thus we concluded that the inhibition of the excitatory somato-sympathetic A- and C-reflex discharges elicited by an i.v. injection of phenylephrine was due to a reflex response elicited by excitation of baroreceptor afferent nerves.

It has been well known that spontaneous activity of a sympathetic efferent nerve is inhibited by the excitation of baroreceptor afferent nerves [2, 3]. Further, as shown in more detail in the present experiments, sympathetic excitatory reflex discharges, both A- and C-reflex discharge components elicited by stimulation of somatic myelinated and unmyelinated afferent fibers, were also inhibited by an excitation of baroreceptor afferent nerves. Spontaneous activity of the cardiac sympathetic efferent nerve is reflexly increased by the stimulation of chemoreceptor afferent nerves [17], and the stimulation of chemoreceptor afferents can facilitate the excitatory sympathetic A- and C-reflex discharges [18]. Therefore the excitatory sympathetic A- and C-reflex discharges elicited by stimulation of somatic myelinated and unmyelinated afferent nerves can be modulated in an excitatory or inhibitory manner, depending on what kinds of visceral afferents, e.g., chemoreceptor or baroreceptor afferent, are stimulated.

Recently Li et al. [18] have shown that both excitatory A- and C-reflex discharges in cardiac sympathetic nerves were facilitated by a stimulation of chemoreceptor afferent nerves that originated in the arterial chemoreceptors. The stimulation of other visceral afferents, for example, glucose sensitive afferents and bladder afferents, may interfere with the so-
matically induced excitatory sympathetic A- and C-reflex discharges. Conversely, the viscerally induced sympathetic reflex responses might be influenced by somatic afferent stimulation. Sympathetic efferent nerve activity seems to be reflexly integrated probably at the brainstem; this observation is based not only on visceral afferent information, but also on somatic afferent information. It is evident from the present study that visceral and somatic afferent information interact, probably at the brainstem, to influence sympathetic efferent neuronal activity. This suggests that some sympathetic neurons are under the strong reflex control of visceral and somatic afferent information.

The authors express their thanks to Dr. Brian Budgell for his reading and comments on this paper. This work was encouraged by a Research Award to W. M. Li, from Leica Inc., Japan.

REFERENCES

1. Adrian ED, Bronk DW, and Phillips G: Discharges in mammalian sympathetic nerves. J Physiol (Lond) 74: 115–133, 1932