Effects of aortic nerve stimulation on discharges of sympathetic neurons innervating rat tail artery and vein

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Effects of aortic nerve stimulation on discharges of sympathetic neurons innervating rat tail artery and vein. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R942–R949, 1998.—Activity was recorded from postganglionic sympathetic neurons (PSNs) innervating either the caudal ventral artery (CVA) or a lateral vein (LV) of the tail circulation of anesthetized rats. The study sought to determine whether sympathetic activity directed at the CVA and LV was influenced by cardiovascular mechanoreceptor afferents and whether this effect was differential. Cardiac rhythmicity was not a robust component of either CVA PSN activity or LV PSN activity. Stimulation of an aortic nerve with short trains was followed by a decreased probability of discharge in both CVA and LV PSNs that was followed by a series of peaks that showed a constant periodicity that was not significantly different from that revealed by autocorrelogram analysis over the same data set. The latter dominant periodicity is referred to in this and related previous publications as the T rhythm. Furthermore, blood volume expansion and long-train aortic nerve stimulation produced a significant decrease in the frequency of the T rhythm. It is concluded that the CVA and LV sympathetic activity can be influenced by inputs from cardiovascular mechanoreceptors and that this effect is mediated in part by a modulation of the T rhythm.

baroreceptor; sympathetic rhythms; baroreflex

THE ABILITY OF THE sympathetic nervous system to differentially control sympathetic outflows in response to reflex inputs or as part of complex behaviors is well established (9). Consequently, it follows that different postganglionic sympathetic neurons (PSNs) can be regulated at least in part by separate and/or different combinations of antecedent neurons. Recently, we have begun to examine the possibility that the sympathetic activity supplying resistance and capacitance vessels, within the same vascular bed, can be controlled differentially. Information on this subject should provide further insights into the organization of the nervous control of cardiovascular function.

A number of studies have addressed indirectly the question of whether or not different vessels in the same vascular bed are controlled differentially by the sympathetic nervous system (6, 7). However, it is only by recording sympathetic activity supplying identified blood vessels that a definitive answer to this question can be obtained. This can be achieved by using a focal recording technique (12, 13). With use of this technique it has been shown that the discharges of single PSNs innervating the caudal ventral artery (CVA) and those innervating the lateral vein (LV) of the rat tail (both are part of a thermoregulatory cutaneous circulation (22)) have similar frequencies, patterns, and rhythmical components (the dominant rhythm revealed by autocorrelation analysis has been termed the T rhythm (12, 13), approximately 0.8 Hz under resting conditions) and that these are influenced similarly by whole body warming and hypocapnic apnea (9, 12, 13). The T rhythm is not “driven” by afferent feedback relayed via sinus, vagus, and aortic nerves and can have a different frequency to that of central respiratory drive (12). These similar characteristics of the discharges of CVA and LV PSNs are consistent with the idea that sympathetic neurons regulating these two vessels are at least in part under the control of common pools of neurons and/or equivalently controlled pools of neurons.

O’Leary and Johnson (19) demonstrated that in the anesthetized rat, tail vascular conductance was influenced by manipulations of blood pressure. The focus of the present study was to determine whether sympathetic activity directed at the CVA and that directed at the LV is differentially influenced by cardiovascular mechanoreceptor afferents, in particular those projecting through the aortic nerves (arterial baroreceptors (20, 21)).

In extensive investigations in rats and cats putative sympathetic vasoconstrictor fibers (cutaneous vasoconstrictors (CVCs)) recorded from nerves projecting to hairy or hairless skin in a minority of cases were observed to display strong cardiac-related activity [an indicator of beat-to-beat arterial baroreflex modulation (5, 8, 18)]. Furthermore, Johnson and Gilbey (9) reported that robust cardiac-related modulation was absent from ongoing activity recorded from sympathetic fibers picked from the ventral collector nerve of the rat tail (n = 3) and from activity focally recorded from the CVA (n = 6). In contrast, Yusof and Coote (23) reported cardiac-related activity in CVCs in the rat. Jänig and colleagues have suggested that CVCs with strong cardiac-related activity might innervate resistance vessels, whereas those with an absence of such patterning might innervate peripheral cutaneous veins (5, 8).

In their study on the cat CVCs, Michaelis et al. (18) determined that there was a correlation between the degree of cardiac rhythmicity of the discharges and their susceptibility to inhibition after pressure increases in a carotid blind sac. In contrast, in the rat most CVCs had their activity potentely decreased during an increase in systemic blood pressure (5). Thus PSNs may be influenced by cardiovascular mecha-
Aortic nerve stimulation and sympathetic activity

METHODS

Anesthesia and general animal maintenance. Experiments were conducted on 42 male Sprague-Dawley rats (220–310 g) under Project and Personal Licences issued by the Home Office. Animals were anesthetized with pentobarbital sodium (60 mg/kg ip). The depth of anesthesia was monitored continuously, and supplements of α-chloralose (10–30 µg/kg iv) were given when required as judged from 1) the stability of heart rate, blood pressure, phrenic nerve activity, or respiratory movements; 2) the size of pupils; and 3) palpebral and paw-pinch reflexes. Animals were paralyzed (gallamine triethiodide, 16 mg·kg$^{-1}$·h$^{-1}$ iv) during periods of data collection. During periods of paralysis the depth of anesthesia was assessed by monitoring heart rate, blood pressure, and phrenic nerve discharge. The animals were killed by an intravenous overdose of pentobarbital sodium.

A femoral artery and vein were cannulated to monitor arterial blood pressure and to administer drugs, respectively. The trachea was cannulated low in the neck, and the animals were given a pneumothorax and ventilated artificially (O$_2$-enriched room air). Peak expiratory CO$_2$ was monitored continuously, and arterial blood samples (75 µl) were taken regularly. During normocapnia arterial pH and gas tensions were kept within the following ranges: pH 7.30–7.45, P$_{CO_2}$ 38–50 mmHg, and P$_{O_2}$ 100–200 mmHg. Esophageal temperature was monitored and maintained at 37.0 ± 0.5°C. The bladder was cannulated to allow free passage of urine. For further details see Johnson and Gilbey (9, 12).

Preparation of nerves. The preparation of the nerves for stimulation and/or recording has been described previously, as have recording techniques (9, 12, 13). Neuronal activities were recorded from the PSNs supplying these two vessels (12, 13). Abstracts of this work have been published previously (10, 11).

RESULTS. Controls consisted of periods (301 s) immediately before and after each test run (trigger with no aortic nerve stimulation). The effect on PSN activity was examined using peristimulus time histograms and autocorrelograms (50-ms bins). The former was used to examine the effect of aortic nerve stimulation on the probability of firing of PSNs and the latter the effect on the frequency of the T rhythm. The onset of depression of PSN activity due to aortic nerve stimulation was calculated by measuring the time from the trigger point.
of data in each instance.

Blood pressure was checked for up to 3 min after.

The seven PSNs recorded from the CVA were investigated in five animals. Two PSNs on two occasions were recorded from the same animal at different times: 1 h was left between repeats of the protocol, and blood pressure was checked for stability.

Cardiac Rhythmicity in PSN Discharges

CVA. Of 16 CVA PSNs studied in animals with vagi cut (MABP $93 \pm 3$ mmHg) none showed cardiac rhythmicity. To determine if cardiac rhythmicity would be markedly different, 15 CVA PSNs were recorded in animals with vagi intact (MABP $96 \pm 6$ mmHg). Cardiac rhythmicity was present in only three (no significant difference between occurrences of cardiac rhythmicity in vagi cut and intact groups, Fishers exact test, Fig. 1). Thus CVA PSNs in animals with vagi intact do not frequently have robust cardiac rhythmicity compared with putative muscle vasoconstrictors [Häbler et al. (5)]. Lumbar sympathetic chain activity in all cases tested (3 vagi intact and 3 vagi cut preparations) had cardiac rhythmicity although none was present in simultaneously recorded CVA PSN activity (Fig. 1, Ab and Ac).

LV. Because there was no substantial difference between the cardiac rhythmicity of CVA PSNs recorded in animals with vagi intact compared with those recorded from animals with vagi cut, LV PSNs were only examined for cardiac rhythmicity in animals with vagi cut. As with CVA PSNs recorded in the same type of preparation no cardiac rhythmicity was found in the activity of the 17 LV PSNs. The MABP ($96 \pm 3$ mmHg) was not significantly different from vagi cut preparations in which activity to the CVA was recorded (unpaired Student's t-test).

Influence of Short-Train Aortic Nerve Stimulation

Effects on CVA activity. Data were taken from seven PSNs (5 animals with vagi cut). Results from a typical experiment are shown in Figs. 2 and 3. The peritrigger histograms accumulated during short-train aortic nerve stimulation revealed a period of depression of PSN activity, beginning at 590 ms poststimulus, after which PSN activity was reduced by 50–100% (Fig. 2Ab). No such depression was found in relation to phrenic events (Fig. 2Bb). In contrast, peritrigger histograms of both PSN activity and phrenic events taken during control periods (no aortic nerve stimulation) were essentially flat (see Fig. 2, Aa, Ac, Bb, and Bc). The histograms of PSN activity accumulated during aortic nerve stimulation also showed clear posttrigger peaks that were of a constant period (Fig. 2Ab). The frequency of this rhythm ($0.73 \pm 0.03$ Hz) was not
significantly different (paired Student’s t-test) from the T rhythm (compare Figs. 2Ab to 3Ab).

Autocorrelation analysis revealed that a T rhythm was present in all PSNs throughout experiments (mean frequency of 0.74 ± 0.03 Hz during control and recovery) and did not show a change in frequency during the period of short-train aortic nerve stimulation (0.73 ± 0.03 Hz, ANOVA; e.g., Fig. 3A). The frequency of phrenic rhythm during control and recovery (0.62 ± 0.06 and 0.66 ± 0.07 Hz, respectively) was also not significantly affected by stimulation (0.65 ± 0.06 Hz, ANOVA; see Fig. 3B).

MABP fell from 107 ± 5 mmHg during control to 95 ± 6 mmHg during aortic nerve stimulation and recovered to 104 ± 4 mmHg poststimulation (no significant change, ANOVA).

The mean rate of PSN activity during control and recovery (2.41 ± 0.25 and 2.45 ± 0.23 impulses/s, respectively) was not significantly affected in the intervening period of aortic nerve stimulation (2.26 ± 0.21 impulses/s, ANOVA).

Effects on LV activity. Results from a typical experiment are shown in Figs. 4 and 5. The peritrigger histograms accumulated during short-train aortic nerve stimulation revealed a period of depression of PSN activity, beginning at 520 ± 20 ms poststimulus, after which PSN activity was reduced by 50–100% (8 PSNs recorded from 6 animals with vagi cut, Fig. 4A). There was no concurrent decrease in the number of phrenic events (Fig. 4B). In six of these eight PSNs the histograms accumulated during aortic nerve stimulation also showed clear posttrigger peaks that were of a

Fig. 2. Peritriggered histograms of caudal ventral artery (CVA) PSN and phrenic nerve events during (bin width 50 ms) intermittent (every 7 s) short-train aortic nerve stimulation. Forty-three triggers per histogram. Trigger point indicated by arrow. A: PSN events. B: phrenic events (i.e., one phrenic event was triggered off the rising phase of each phrenic burst). a: without aortic nerve stimulation (i.e., trigger only). b: with aortic nerve stimulation (i.e., trigger plus stimulus to aortic nerve). Note that aortic nerve stimulation resulted in depression of PSN activity and subsequent rhythmicity, which may reflect a resetting of T rhythm (see DISCUSSION). Note lack of effect on phrenic events. c: recovery without aortic nerve stimulation (i.e., trigger only).

Fig. 3. Autocorrelograms of CVA PSN activity recorded from same unit and phrenic events (bin width 50 ms) during same 301-ms period shown in Fig. 2. A: PSN events. B: phrenic events (i.e., 1 phrenic event was triggered off the rising phase of each phrenic burst). a: in absence of aortic nerve stimulation. b: during short-train aortic nerve stimulation. c: recovery, after aortic nerve stimulation. It can be seen that during short-train stimulation of aortic nerve (1 train every 7 s) the periodicity of the sympathetic rhythm (and phrenic rhythm), as revealed by autocorrelogram analysis, is not obviously different from those seen before and after period of stimulation.
constant rhythm (Fig. 4A). The frequency of this rhythm (0.73 ± 0.07 Hz) was not significantly different (paired Student’s t-test) from the T rhythm (see below, compare Figs. 4A with 5A).

Autocorrelation analysis revealed that a T rhythm was present in all PSNs throughout experiments, the mean frequency of which [before (0.69 ± 0.06 Hz), during (0.72 ± 0.06 Hz), and after (0.72 ± 0.06 Hz) aortic nerve stimulation] was similar (ANOVA; Fig. 5A). Autocorrelograms of phrenic events revealed that frequency of the phrenic rhythm during control and recovery (0.72 ± 0.05 and 0.69 ± 0.02 Hz, respectively) was not affected by aortic nerve stimulation (0.69 ± 0.02 Hz, ANOVA, Fig. 5B).

MABP fell from 97 ± 3 to 93 ± 4 mmHg during stimulation (n = 8), but this difference was not significant (ANOVA) and recovered to 95 ± 3 mmHg after stimulation.

The mean rate of PSN firing during control and recovery (1.43 ± 0.27 and 1.50 ± 0.30 impulses/s, respectively) was not significantly affected during aortic nerve stimulation (1.46 ± 0.27 impulses/s, ANOVA, n = 8).

Influence of blood volume expansion on T rhythm. For this series of experiments activity was only recorded from PSNs supplying the CVA. The T rhythm was present in seven of seven PSNs during control conditions (Fig. 6, Ba and Bb, mean frequency of 0.85 ± 0.07 Hz). After infusion of 0.5 ml of Ficoll MABP rose from 99 ± 3 to 120 ± 5 mmHg 3 min after infusion. For 3 min after the infusion of plasma expander rhythmicity in all PSNs was lost (Fig. 6Bc). By the 3rd min after infusion rhythmicity was regained in five of seven PSNs (Fig. 6Bd) and was significantly slower than control (0.62 ± 0.07 Hz, P < 0.05, paired Student’s t-test). The response in a representative experiment is shown in Fig. 6. In two PSNs rhythmicity (0.68 and 0.72 Hz) was lost after infusion and did not recover within 5 min.

The rate of PSN activity slowed from 2.00 ± 0.46 impulses/s during the last minute of control to 1.52 ± 0.43 impulses/s in the 1st min after infusion, and then recovered to 1.76 ± 0.47 impulses/s in the 3rd min.
Influence of continuous train stimulation of aortic nerve.

To establish whether the above observations were consistent with the effects of arterial baroreceptor stimulation three CVA and three LV PSNs were recorded during 60 s of continuous 50 Hz stimulation using a voltage of twice threshold for the blood pressure response (see METHODS). The response was assessed by comparing PSN activity during stimulation with that during a control period (60 s) immediately before the stimulation. For the CVA the mean frequency of the T rhythm was \(0.52 \pm 0.06\) Hz [phrenic burst frequency (calculated from phrenic burst interval) \(0.44 \pm 0.08\) Hz] during the 1st min of aortic nerve stimulation compared with \(0.70 \pm 0.07\) Hz (phrenic burst frequency \(0.58 \pm 0.11\) Hz) before stimulation. For the LV the corresponding values were \(0.84 \pm 0.02\) Hz (phrenic \(0.70 \pm 0.02\) Hz) for control and \(0.74 \pm 0.04\) Hz (0.49 \(\pm 0.07\) Hz) for test. Because aortic nerve stimulation slowed the T rhythm frequency in all PSNs, whether recorded from CVA or LV, for statistical purposes CVA and LV PSNs were combined. In this manner it was found that the frequency of the T rhythm to these vessels was significantly slowed during aortic nerve stimulation compared with control (paired Student’s t-test, \(n = 6\)).

**DISCUSSION**

Cardiac Rhythmicity

In the present study we have confirmed and extended the findings of Jänig and co-workers and Johnson and Gilbey (5, 8, 9, 18) that sympathetic activity recorded from PSNs innervating cutaneous vascular beds does not commonly demonstrate robust cardiac rhythmicity. Furthermore, we have shown for the first time that this is true for the activity of sympathetic PSNs innervating an artery (CVA) and those innervating a capacitance vessel (LV). These observations appear to remove the possibility that the presence or absence of cardiac rhythmicity in sympathetic neurons innervating cutaneous blood vessels (see the introduction) can be explained totally on the basis of different target vessels (i.e., vein compared with artery). It is a conundrum why a few PSNs innervating the CVA displayed cardiac rhythmicity, whereas most did not. A difference in blood pressure leading to greater input from baroreceptors does not appear to be a likely explanation because in all cases resting blood pressure was similar. It is possible that only under certain unidentified conditions does input from arterial baroreceptors lead to a stable cardiac-related phasic modulation of PSN activity.

Influence of Aortic Nerve Stimulation

It was shown that sympathetic activities supplying the CVA and LV are decreased and a rhythmic component influenced in response to stimulation of an aortic nerve. Consequently, it is clear that any differential control of these two vessels by aortic afferents, if present, is subtle.

It was shown that sympathetic activities supplying both the CVA and LV decreased and a stimulus-synchronous component was generated in response to short-train stimulation of an aortic nerve. Therefore, as demonstrated previously (see the introduction), lack of cardiac rhythmicity in sympathetic discharges is not necessarily indicative of an absence of input from arterial baroreceptor afferents to the circuitry regulating such discharges. There are at least two explanations for the lack of cardiac rhythmicity in such cases: 1) there is an absence of robust tonic baroreceptor modulation of the particular sympathetic outflow under the conditions examined and 2) the influence of cardiovascular mechanoreceptor afferents are not manifest on a beat-to-beat basis but rather reflected in a steady tonic inhibition or through an influence on the output of central rhythm generators (3, 17).
Resetting of Rhythmic Discharges

In our experiments we revealed, using peristimulus time histogram analysis, that short-train stimulation of an aortic nerve, besides decreasing the discharges of PSNs innervating the two vessels, was followed by a rhythmic discharge. The periodicity revealed in such histograms was not significantly different from that of the T rhythm revealed by autocorrelation analysis of PSN activity (12, 13). We therefore suggest that the transient inhibition or disfacilitation of a neuron or neuronal networks produced after stimulation of the aortic nerve leads to a resetting (15) of the T rhythm. Importantly, the chosen stimulus intensity failed to reset the phrenic rhythm in a similar manner, thus showing once again that T rhythm generator(s) and respiratory rhythm generator(s) can be influenced differentially (12). It is possible that any input that leads to transient inhibition or excitation of the neuronal substrate responsible for the T rhythm will cause a resetting (e.g., somatic afferent stimulation). Such inputs could thus lead to sympathetic burst generation.

Slowing of T Rhythm

Consistent with the findings reported previously, that aortic nerve input can access the “T rhythm generators,” was the observation that both long-train stimulation of the aortic nerve and blood volume expansion [the latter would be expected to activate a gamut of cardiovascular mechanoreceptors (19)] led to a slowing of the frequency of the T rhythm. The similar effects produced by long-train stimulation and volume expansion indicate that the slowing of the T rhythm recorded from both CVA and LV PSNs after long-train stimulation are likely to be of functional importance. Such slowing of the frequency of the T rhythm might lead to consequent changes in neuroeffector transmission and relaxation of vascular smooth muscle (12, 17). Thus it appears that with respect to control of the tail circulation in the rat, cardiovascular mechanoreceptors can influence sympathetic drive by influencing the output of rhythm generators that regulate the discharge patterns of PSNs (2).

Our observations, taken in a wider context, are consistent with the idea that physiological inputs, such as cardiovascular mechanoreceptors, influence rhythm-generating neurons and/or networks and can thereby influence the pattern, in addition to the frequency, of PSN discharges regulating cardiovascular function. Furthermore, recent observations from our laboratory indicate that the population of PSNs innervating the same vessel are associated with multiple T rhythm generators that can be phase-locked (synchronous) or asynchronous and that the condition can be influenced by changing the physiological state, e.g., increasing central respiratory drive (2 and H.-S. Chang J. L. Smith, K. Staras, and M. P. Gilbey, unpublished data). Thus, taken together, these studies reveal some general principles that might underlie changes in burst amplitude and burst frequency that are often seen in the discharges of whole sympathetic nerves in response to a variety of perturbations in humans and other animals (17). For example, some perturbations might act to synchronize the discharges of individual PSNs and thereby produce an increase in whole nerve burst amplitude, whereas other factors might influence the frequencies of bursts. Some inputs might influence both burst amplitude and burst frequency.

Perspectives

Although sympathetic activity to the CVA and LV is important in controlling tail blood flow and capacitance in relation to thermoregulatory demands it also appears to be influenced by input from cardiovascular mechanoreceptor afferents. The present findings are consistent with those of O’Leary and Johnson (19) who assessed sympathetic vasconstrictor activity indirectly in the rat tail circulation. In addition, they are consistent with findings from human studies where skin blood flow has been found to change coincident with manipulations that change the inputs to both arterial baroreceptors and cardiopulmonary receptors (14, 16). However, robust cardiac-related activity was rarely seen in the sympathetic discharges in the current experiments and this is similar to observations in humans (1). Our data demonstrate that the input from cardiovascular mechanoreceptor afferents can influence the frequency of the T rhythm (12). We suggest that as a general principle through modulatory influences on slow rhythms (bursts of sympathetic activity) baroreceptor inputs can modulate sympathetic activity to vascular targets, perhaps tonically, in addition to or as an alternative to “modulating cardiac-related rhythms.”

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