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Nonuniformity in the von Bezold-Jarisch reflex

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Salo LM, Woods RL, Anderson CR, McAllen RM. Nonuniformity in the von Bezold-Jarisch reflex. *Am J Physiol Regul Integr Comp Physiol* 293: R714–R720, 2007. First published June 13, 2007; doi:10.1152/ajpregu.00099.2007.—The von Bezold-Jarisch reflex (BJR) is a vagally mediated chemoreflex from the heart and lungs, causing hypopnea, bradycardia, and inhibition of sympathetic vasomotor tone. However, cardiac sympathetic nerve activity (CSNA) has not been systematically compared with vasomotor activity during the BJR. In 11 urethane-anesthetized (1–1.5 g/kg iv), artificially ventilated rats, we measured CSNA simultaneously with lumbar sympathetic activity (LSNA) while the BJR was evoked by right atrial bolus injections of phenylbiguanide (0.5, 1.0, 1.5, and 2 μg). Nerve and heartbeat responses were analyzed by calculating normalized cumulative sums. LSNA and heartbeats were always reduced by the BJR. An excitatory "rebound" component often followed the inhibition of LSNA but never outweighed it. For CSNA, however, excitation usually (in 7 of 11 rats) outweighed any initial inhibition, such that the net response to phenylbiguanide was excitatory. The differences in net response between LSNA, CSNA, and heartbeats were all significant ($P < 0.01$). A second experimental series on seven rats showed that methyl atropine (1 mg/kg iv) abolished the bradycardia of the BJR, whereas subsequent bilateral vagotomy substantially reduced LSNA and CSNA responses, both excitatory and inhibitory. These findings show that, during the BJR, *1*) CSNA is often excited, *2*) there may be coactivation of sympathetic and parasympathetic drives to the heart, *3*) divergent responses may be evoked simultaneously in cardiac vagal, cardiac sympathetic, and vasomotor nervous pathways, and *4*) those divergent responses are mediated primarily by the vagi.

sympathetic nerve activity; cardiac; vasomotor; lumbar; phenylbiguanide

THE VON BEZOLD-JARISCH REFLEX (BJR) is evoked from cardiopulmonary chemoreceptive afferents and causes hypopnea, bradycardia, and vasodilatation (4, 8, 10, 11, 17). The cardiovascular actions (bradycardia and systemic vasodilatation) are attributable primarily to activation of unmyelinated vagal afferents (4, 9, 10) with terminals in the left ventricle, which are accessible from the coronary circulation (9, 29). The BJR may be activated by a variety of chemical stimuli delivered by the circulation to the heart, including veratrum alkaloids (9, 10), nicotine (12), and 5-HT_3 receptor agonists such as serotonin (42, 44) and phenylbiguanide (PBG) (1, 4, 8, 11, 15). It has been suggested that the BJR is activated by ischemic metabolites during myocardial ischemia and infarction (particularly inferoposterior) and plays a cardioprotective role by reducing the workload of the heart (17, 27, 37).

The reflex vasodilatation of the BJR is due to inhibition of sympathetic vasoconstrictor tone (4, 10), which is considered to be generalized (43) and has been measured in adrenal (7), lumbar (35, 44), splanchnic (35), splenic (45), and renal (4, 39, 41, 45) sympathetic nerves. With respect to the cardiac sympathetic nerves, the evidence is less clear. A strong parasympathetic bradycardia predominates, and most workers have found that this is essentially abolished by atropine (7, 8, 44). In some studies, however, a component of the bradycardia attributable to sympathetic withdrawal has been inferred from a residual heart rate response after muscarinic blockade (11, 39), which was then abolished by β-adrenergic antagonists (2, 49). Cardiac sympathetic nerve activity (CSNA), which has rarely been recorded during the BJR, is reported to be inhibited (1, 38).

Reflexes may often have discordant actions on cardiac and vasomotor neural drives (24, 34), but no study has yet systematically compared the relative effects of the BJR on cardiac and vascular sympathetic nerves. We set out to establish whether the sympathoinhibition of the BJR was distributed uniformly or whether this putatively cardioprotective reflex might perhaps inhibit CSNA preferentially. We therefore measured CSNA simultaneously with a representative sympathetic vasomotor outflow (lumbar sympathetic nerve activity; LSNA) and heart rate in anesthetized rats, whereas the BJR was evoked by PBG delivered intravenously to the right atrium.

MATERIALS AND METHODS

Animals. Eighteen adult male Sprague-Dawley rats (320–625 g) were used in this study. All procedures conformed to National Health and Medical Research Council guidelines and were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

Surgical preparation. In all experiments, initial anesthesia was induced by pentobarbital sodium (45 mg/kg ip). Animals were then tracheotomized and artificially ventilated with 2% isoflurane (Rhodia Australia) in 100% O_2 throughout surgical preparation. Airway pressure was monitored by a transducer attached to the inspiratory line. The right femoral artery and vein were cannulated to measure arterial pressure and to administer drugs, respectively. The arterial catheter was filled with heparinized saline (20 U/ml) and connected to a pressure transducer. A continuous intravenous infusion of Haemaccel (Hoechst, Melbourne, Australia; 1–2 ml/h) was given throughout the experiment to help maintain the animal's cardiovascular condition (32). Rectal temperature was monitored and maintained between 36.3 and 37.9°C with a heating blanket. A catheter was passed down the right jugular vein until its tip lay at the right atrial-vena caval junction for PBG administration. The bladder was cannulated suprapubically and allowed to drain.

A dorsal incision was made, and a clamp was applied to the first thoracic spinal process. This was used to elevate and support the animal's thorax as it lay on its right side for dissection and nerve recording. To expose the left cardiac sympathetic nerves, an incision

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was made at the base of the scapula, which was freed from underlying muscles and reflected rostrally. The heads of the first three ribs were exposed, and the head of the second rib was removed with rongeurs. Gentle dissection exposed the cardiac nerve or nerves as they left the stellate ganglion en route for the heart. The most suitable nerve was then cut as close as possible to the pleura. A small pressure foot was positioned below the nerve to reduce cardiac and respiratory displacements. To further reduce movement, a unilateral pneumothorax was made via a lower thoracic intercostal space and kept patent with a short polyethylene tube. The left lumbar sympathetic trunk was approached retroperitoneally via a flank incision. The trunk was exposed by retraction, cut as distally as convenient (usually between the T_5 and T_6 ganglia) and dissected free from the surrounding fascia. In seven rats (second experimental series), the vagi were identified in the neck and loosely looped with silk sutures ready for later section.

After surgical procedures were completed, animals were given urethane (1–1.5 g/kg iv over ~30 min), and isoflurane was discontinued.

Nerve recording. Both CSNA and LSNA were recorded by placing the central cut end of the nerves over silver bipolar hook recording electrodes under pools of mineral oil. The efferent nerve activity was amplified (10,000×) and filtered (bandpass 10–1,000 Hz for LSNA, 300–1,000 Hz for CSNA) (Neurolog; Digitimer, Harlow, UK). The nerve signals were then sent to an oscilloscope, an audio monitor, and a computer interface (CED Power1401; CED, Cambridge, UK), along with blood and ventilatory pressures. Signals were recorded for computer analysis with Spike2 software (CED).

Experimental protocol. All experimental protocols were carried out under urethane anesthesia, without any paralyzing agent. In the first experimental series (11 rats), PBG (Sigma; 100 μg/ml), a 5-HT_3 receptor agonist, was administered in bolus doses (0.5, 1.0, 1.5, and 2.0 μg) to the right atrium via the jugular cannula to evoke the BJR. Doses were given in ascending order, allowing at least 10 min between injections to avoid tachyphylaxis, and the full dose range was repeated three times in each animal. In a second experimental series (7 rats), PBG was given as a single dose (1.5 μg) three times before and after methyl atropine (Sigma; 1 mg/kg iv) was given and again three times after bilateral section of the vagi, allowing at least 10 min between injections. At the beginning of the experiment, these animals also each received two doses of phenylephrine (Sigma) and sodium nitroprusside (DBL; 3 μg iv in each case) to test nerve responses to raising and lowering of blood pressure.

At the end of all experiments, hexamethonium (Aldrich; 50 mg/kg iv) was given to demonstrate that recordings were from postganglionic sympathetic fibers and to establish the level of baseline noise. Animals were killed with an overdose of pentobarbital sodium (>100 mg/kg iv). Postmortem examination confirmed that the tip of the jugular cannula was sited within 2 mm of the right atrial inlet in all animals.

Analysis. All analyses were done offline with the use of Spike2 software. Both CSNA and LSNA were rectified, and the respective noise levels (measured after hexamethonium) were subtracted from each signal. Mean arterial pressure and heart rate were calculated from the blood pressure recording. Records spanning from 30 s before (control period) until 30 s after each stimulus were extracted for further analyses. For each 1-s period in the record, mean rectified CSNA, mean rectified LSNA, mean arterial pressure, and the number of heartbeats were calculated. These data were normalized with respect to their 30-s prestimulus control period.

To assess barosensitivity, mean arterial pressure and rectified CSNA and LSNA responses to phenylephrine and sodium nitroprusside were measured in five rats from the second series (where both CSNA and LSNA were recorded). One-second values of CSNA, LSNA, and mean arterial pressure were smoothed by a three-point running mean. Responses to sodium nitroprusside were calculated as the mean value between 15 and 30 s after the injection. Because the action of phenylephrine was more transient, readings were taken of peak arterial pressure and the minima of CSNA and LSNA. Data measured from individual animals were pooled to calculate group means and SE.

To quantify and compare sympathetic nerve and heart rate responses to PBG, cumulative sum (CUSUM) values were calculated for CSNA, LSNA, and heartbeats from their respective normalized records. The CUSUM (18) measures the cumulative deficit or excess of each variable over each second after the stimulus. In its normalized form, the CUSUM value tracks the number of seconds' worth of added (or missing) activity that has occurred since the stimulus. A negative CUSUM slope denotes continuing inhibition, whereas a positive slope indicates a period of activity greater than the prestimulus control level. A slope of zero indicates activity equal to the control level. CUSUMs of repeat responses to the same PBG dose in the same animal were averaged.

Statistical analysis. The net response of CSNA, LSNA, or heartbeats was defined as its CUSUM value 30 s after the stimulus (30-s CUSUM). In the first experimental series, the significance of differences in net response with respect to neural outflow (CSNA, LSNA, or heartbeats), PBG dose, and animal was investigated by three-way ANOVA. The significance of pair-wise differences between CSNA, LSNA, and heartbeat responses was then assessed by Tukey's post hoc test. The inhibitory component of each response was defined as the most negative CUSUM value after the stimulus. This response component was analyzed in the same manner by three-way ANOVA and the Tukey's post hoc test. The excitatory component of a response (when present) was defined as the difference between the most negative point and the most positive subsequent CUSUM value. These data, however, were not normally distributed, precluding the use of ANOVA. The Wilcoxon signed rank test was therefore used to determine the significance of differences between the excitatory components of CSNA and LSNA to each PBG dose.

To assess the successive effects of methyl atropine and bilateral vagotomy on CSNA, LSNA, and heartbeat responses to PBG (in the second experimental series), the 30-s CUSUM was calculated for each variable. These 30-s CUSUMs were then compared before and after atropine and before and after vagotomy. For LSNA and heartbeats, 30-s CUSUMs were all negative, and the significance of the effects of atropine and vagotomy were compared by paired *t*-test. In the case of CSNA, five animals showed a negative and two animals a positive 30-s CUSUM. We therefore used the Wilcoxon signed rank test to determine whether the magnitudes of those responses (positive and negative) were shifted toward zero after atropine or vagotomy.

To test for differences in barosensitivity between CSNA and LSNA, the responses of these nerves to phenylephrine and sodium nitroprusside in the same animal were compared by paired *t*-test.

In all tests, $P < 0.05$ was considered significant. In the text and illustrations, CUSUM responses to each PBG dose were averaged across animals and given as mean ± SE.

RESULTS

Both LSNA and CSNA showed ongoing activity, which was strongly modulated by the arterial pulse, inhibited by phenylephrine-induced rises in blood pressure, and excited by sodium nitroprusside-induced falls in blood pressure (data not shown). In five rats tested, there was no difference between the CSNA and LSNA responses to phenylephrine (−92.5 ± 3.9% and −83.8 ± 6.5% of control activity, respectively, for a peak increase in mean arterial pressure of 40.0 ± 3.6 mmHg; $P = 0.23$) or sodium nitroprusside (+29.8 ± 10.5% and +34.7 ± 13.0% above control values, respectively, for a fall in mean arterial pressure of 42.7 ± 5.6 mmHg; $P = 0.59$).

In experimental *series 1*, PBG injections to the right atrium caused a dose-related bradycardia and hypotension in all animals ($n = 11$), confirming published findings (11, 21). The

threshold dose was always ≤1.0 μg. In response to four PBG doses (0.5, 1.0, 1.5, and 2.0 μg), the maximum falls in heart rate were 61.9 ± 3.0, 197.2 ± 8.6, 282.7 ± 6.9, and 318.0 ± 5.6 beats/min, respectively, and the maximum falls in mean arterial pressure were 12 ± 0.8, 30.0 ± 1.1, 40.2 ± 1.2, and 44.5 ± 0.9 mmHg, respectively. Baseline values were 374.3 ± 3.5 beats/min and 86.3 ± 1.0 mmHg.

In confirmation of previous reports (35, 44), LSNA was always inhibited by PBG doses above threshold. CSNA, however, gave more variable responses. Two examples, taken from different rats, are illustrated in Fig. 1. The chart record in Fig. 1*A* shows a case where LSNA and CSNA were simultaneously inhibited, to a similar degree. In the case shown in Fig. 1*B*, however, CSNA was not inhibited but excited. Inhibition of CSNA followed by excitation was also commonly seen.

To quantify and compare the profiles of sympathetic nerve and heart rate responses to PBG, we calculated normalized CUSUM values (see MATERIALS AND METHODS). The CUSUMs of individual LSNA and CSNA responses are shown at Fig. 1, *bottom*: note the overlapping CUSUM lines in Fig. 1*A* and divergent lines in Fig. 1*B*. The CUSUMs of group mean responses are shown in Fig. 2. The CUSUMs of CSNA, LSNA, and heartbeats show quite distinct time profiles. In Fig. 2*B*, the continuing downward trends of the CUSUMs show that the bradycardia persisted for the full 30-s poststimulus period at all but the lowest dose of PBG. LSNA, however (Fig. 2*A*), showed inhibition followed by a degree of "rebound" activation, with periods of greater than baseline activity being shown by a small positive CUSUM slope. CSNA (Fig. 2*C*) showed a much greater tendency than LSNA to rebound; indeed, 4 of 11 rats showed pure excitatory responses (as in Fig. 1*B*).

The contrasting time profiles of CSNA, LSNA, and heartbeat responses are shown most clearly when their CUSUMs are overlaid. Figure 2*D* (*top trace*) shows these for the same submaximal dose of PBG (1.5 μg). The CSNA profile diverges from the others 1 s after the stimulus, whereas those for LSNA and heartbeats separate after 7 s (Fig. 2*D*, arrowheads). For comparison, mean arterial pressure (not its CUSUM) is shown on the same time scale (Fig. 2*D*, *bottom trace*).

From the CUSUM measurements, we extracted net responses 30 s after the stimulus and maximum inhibitory and excitatory components (see MATERIALS AND METHODS). The net responses of CSNA, LSNA, and heartbeats are shown in Fig. 3. Three-way ANOVA showed that net responses to PBG were dose related ($P < 0.003$) and significantly different between CSNA, LSNA, and heartbeats ($P < 0.001$), as well as different between individual animals ($P < 0.001$). Post hoc testing further revealed that the pair-wise differences between CSNA, LSNA, and heartbeats responses were all significant ($P \leq 0.01$).

CSNA responses, in particular, were highly variable between animals. In four rats of experimental *series 1*, responses were purely excitatory; in three others, there was early inhibition followed by stronger excitation, whereas the remaining four rats showed predominant inhibition. To further examine the differences between CSNA, LSNA, and heartbeat responses, the inhibitory and excitatory components of each variable were separately analyzed (see MATERIALS AND METHODS). Three-way ANOVA applied to the inhibitory component of responses revealed that they were PBG dose related ($P < 0.001$) and that there were significant differences between neural responses (CSNA, LSNA, heartbeats; $P < 0.001$) and between individual rats ($P < 0.001$). Post hoc testing for

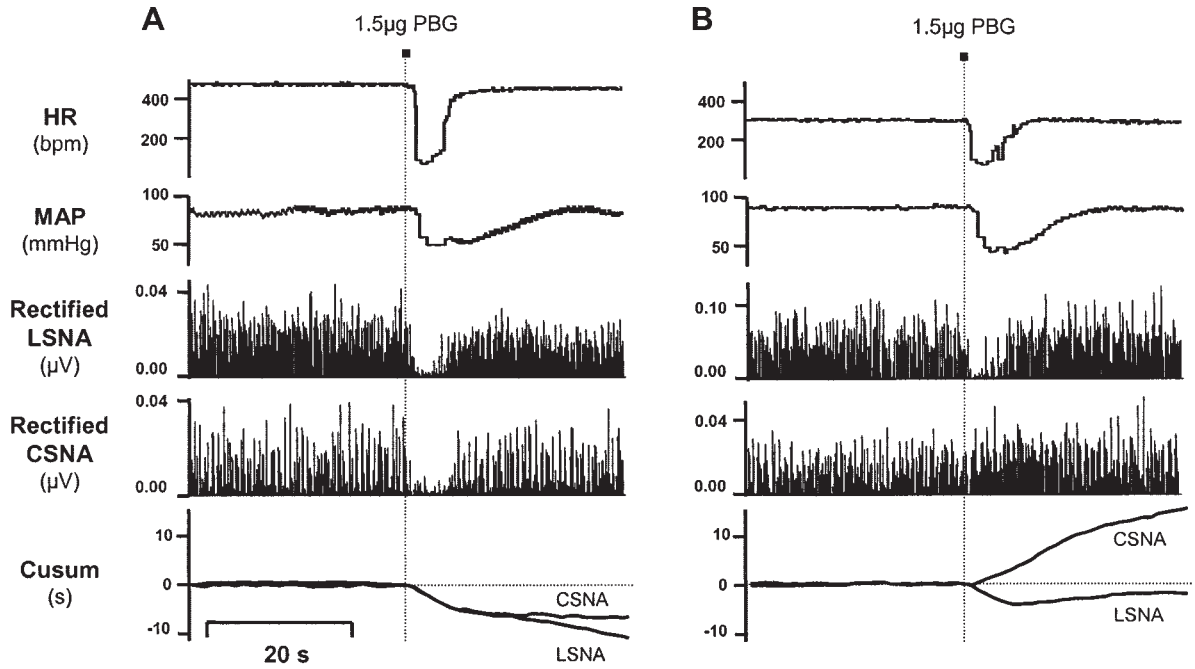

Fig. 1. Representative examples of cardiovascular and sympathetic nerve responses to 1.5 μg phenylbiguanide (PBG; given at the right atrial-vena caval junction at times indicated) (experimental *series 1*). Traces show heart rate [HR; beats/min (bpm)], mean arterial pressure (MAP), rectified lumbar (LSNA) and cardiac (CSNA) sympathetic nerve activities, and cumulative sums (CUSUMs) of LSNA and CSNA (horizontal dotted lines indicate zero level). *A*: example where CSNA was inhibited. *B*: example from another rat, where CSNA was excited.

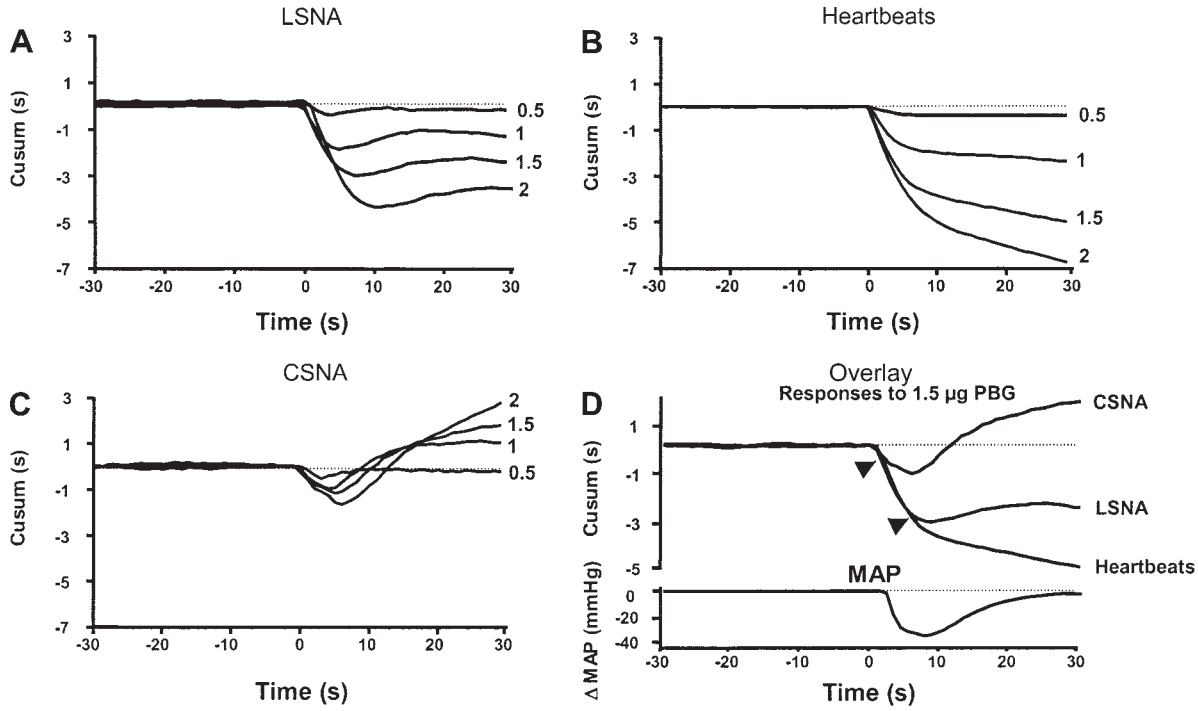

Fig. 2. CUSUMs of normalized, grouped mean data from 11 animals (experimental *series 1*) for LSNA (*A*), heartbeats (*B*), and CSNA (*C*) in response to 4 doses of PBG (in μg as indicated). *D*, *top*: overlaid CUSUMs of LSNA, CSNA, and heartbeat responses to 1.5 μg PBG; times where traces diverge significantly are indicated by arrowheads. *D*, *bottom*: MAP (not its CUSUM) on the same time scale. Horizontal dotted lines indicate zero level.

pair-wise differences showed no significant difference between the inhibitory component of CSNA and LSNA responses ($P = 0.071$), although both differed significantly from the heartbeat response ($P < 0.001$, for both). Data for the excitatory component failed normality testing and were analyzed nonparametrically. The excitatory components of CSNA and LSNA responses to the two highest PBG doses were significantly different ($P < 0.05$). There was no excitatory component in the heartbeat response.

These findings indicate that the differences between the overall BJR responses in CSNA and LSNA are largely attributable to the strength of the excitatory rebound, whereas stronger inhibition distinguished the heartbeat response from that of the two sympathetic nerves. To further investigate the mechanisms underlying the excitatory components of the CSNA and LSNA responses, we performed a second series of experiments on seven rats. In these, a standard dose of 1.5 μg PBG was administered before and after methyl atropine was given and again after bilateral vagotomy.

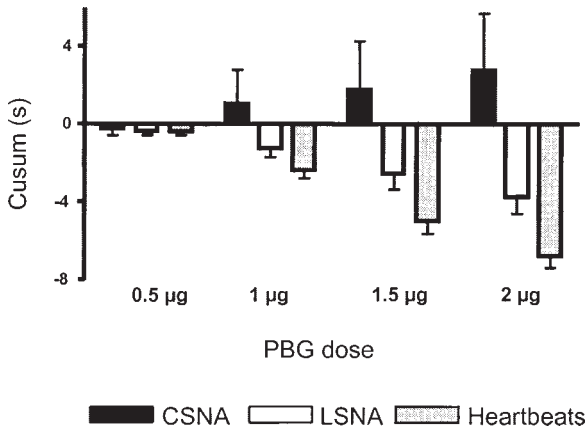

Fig. 3. Net responses of LSNA, CSNA, and heartbeats for each PBG dose (experimental *series 1*). Columns show grouped mean CUSUM values ($n = 11$ rats) taken 30 s after stimulus. Error bars indicate SE.

Figure 4 shows examples of the effects of methyl atropine and bilateral vagotomy on CSNA, LSNA, and heartbeat responses to 1.5 μg PBG in individual animals. Figure 4*A* shows an excitatory CSNA response (seen in 2 rats of this series), and Fig. 4*B* shows an inhibitory CSNA response (seen in 5 rats). Methyl atropine abolished the bradycardic response to PBG (Fig. 4*C* and Table 1) ($P < 0.05$). Methyl atropine also significantly reduced the fall in blood pressure after PBG ($P < 0.05$) but had no significant effect on CSNA and LSNA responses ($P > 0.1$ and $P = 0.89$, respectively) (Fig. 4, *A*, *B*, and *D*; Table 1). In the two rats with an excitatory CSNA response to PBG, muscarinic blockade of the vagal bradycardia did not unmask any measurable tachycardia (e.g., Fig. 4, *A* and *C*, which show data from the same animal).

Subsequent bilateral vagotomy substantially reduced the effect of PBG on LSNA (Fig. 4*D* and Table 1; $P < 0.05$). In the case of CSNA, a technical complication arose after vagotomy in three rats (one previously excited and two previously inhibited by PBG): vagotomy apparently released a large, transient signal burst in the CSNA recording 2–3 s after PBG injection. We remain uncertain how much that burst reflected real CSNA or a signal artifact from elsewhere; it was not associated with any obvious movement, but respiratory pressure and blood pressure were raised for ~2 s. Nevertheless, it was clear that bilateral vagotomy significantly attenuated the effects of PBG on CSNA, both excitatory and inhibitory (Fig. 4, *A* and *B*, Table 1; $P < 0.01$).

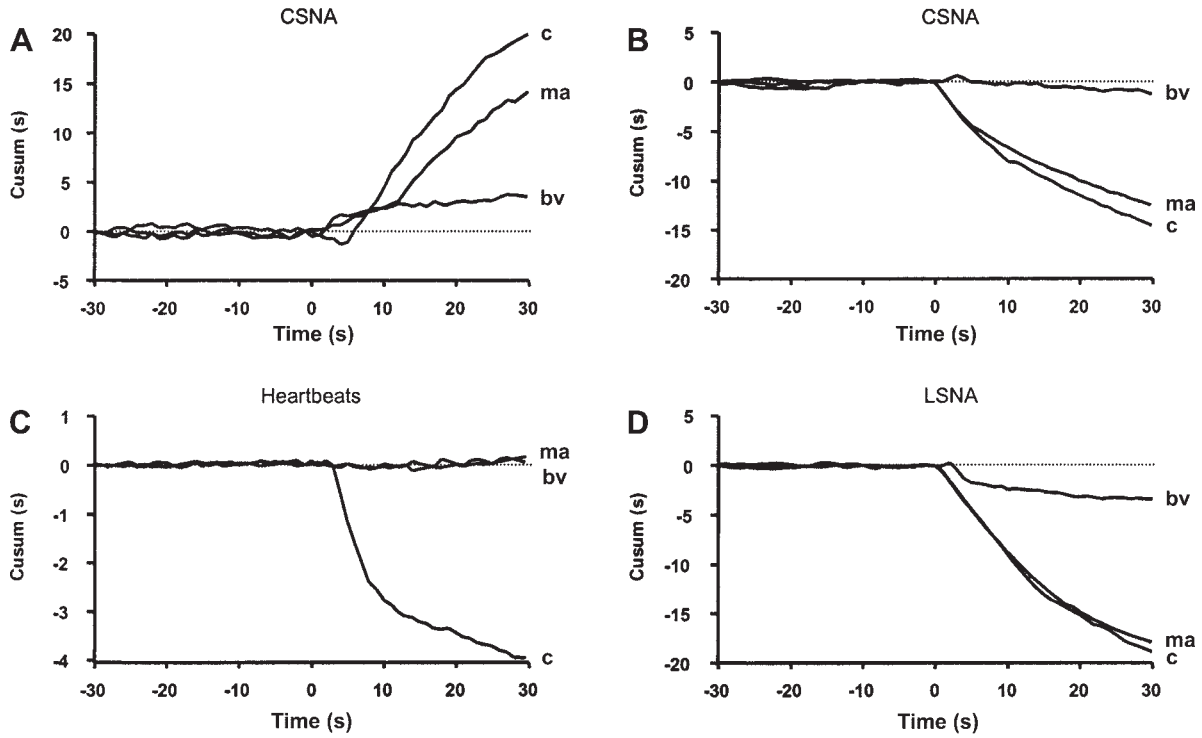

Fig. 4. CUSUMs of normalized mean data from individual animals (experimental *series 2*) in response to 1.5 μg of PBG during control tests (c), after treatment with methyl atropine (ma), and after bilateral vagotomy (bv). *A* and *B*: CSNA responses in different animals, one excitatory and one inhibitory. *C*: heartbeat responses from the same animal as *A*. *D*: LSNA responses from another rat. All CUSUM traces represent the mean of 3 responses to PBG. Horizontal dotted lines indicate zero level.

DISCUSSION

Our null hypothesis was that CSNA should be inhibited at least as much as LSNA and that heart rate should follow a similar inhibitory profile during activation of the BJR. What we found was quite different, and this finding showed three major points of interest. First, the BJR can excite cardiac sympathetic nerves, in contrast to the widely held belief that this is a uniformly sympathoinhibitory reflex. Second, this cardiac sympathetic nerve excitation occurs in the presence of the well-established vagally mediated bradycardia (7, 8, 44), providing evidence that this reflex evokes cardiac sympathovagal coactivation. Third, the BJR can produce discordant actions on cardiac and vasomotor sympathetic outflows, increasing the CSNA while decreasing LSNA.

Table 1. *The effect of methyl atropine and vagotomy on the CSNA, LSNA, heartbeat, and MAP responses to 1.5 μg PBG*

	Control	Methyl atropine	Vagotomy
ΔMAP, mmHg (n = 7)	−41.6±7.7	−25.9±6.2*	−8.5±2.6†
30-s CUSUM values			
Heartbeats (n = 7)	−4.2±0.8	−0.2±0.1*	0.2±0.1
LSNA (n = 5)	−8.2±3.1	−8.4±2.2	−1.1±1.4†
CSNA (inhibitory) (n = 5)	−10.7±2.0	−10.8±2.2	1.4±1.1
CSNA (excitatory) (n = 2)	15.1±4.8	13.6±0.5	4.5±0.9
CSNA (all, displacement from zero) (n = 7)	12.0±2.0	11.6±1.6	2.6±0.8††

Values are group means ± SE from experimental *series 2*. Data are taken from the mean responses to 3 doses of 1.5 μg PBG in each rat (n) before methyl atropine (control), after methyl atropine administration, and again after subsequent bilateral vagotomy. ΔMAP, change in mean arterial pressure; CUSUM, cumulative sum; LSNA, lumbar sympathetic nerve activity; CSNA, cardiac sympathetic nerve activity. *Significantly different from control values ($P < 0.05$, paired t-test). Significantly different from those before vagotomy: †$P < 0.05$, paired t-test and ††$P < 0.01$ (Wilcoxon signed rank test).

Excitatory effects. The frequent excitatory responses that we measured in CSNA during the BJR were a novel and unexpected finding. Although CSNA has rarely been directly measured during this reflex (1, 38), and in these cases was found to be inhibited, it has yet to be compared systematically with a simultaneously recorded sympathetic vasomotor nerve. Excitation of CSNA, however, has been measured during activation of the coronary hypertensive chemoreflex, the "mirror image of the Bezold-Jarisch reflex" (19) in dogs. This reflex may be induced by injection of serotonin into the left atrium or left anterior descending coronary artery (16). It always causes marked hypertension, with variable effects on the heart, attributable to coactivation of vagal and sympathetic cardiac nerves (16). Tachycardia and hypertension were also seen in response to intracoronary or intravenous PBG in ~50% of conscious sheep (40). The hypertensive reflex contrasts with the classic BJR reported in the present study, however, in which there were always hypotension, sympathetic vasomotor inhibition, and bradycardia.

It is worth emphasizing that the net heart rate response to right atrial PBG in our experiments was always bradycardia, notwithstanding any excitatory response in CSNA. This was almost entirely vagal because it was blocked by methyl atropine. This vagal bradycardia (7, 8, 44) could have masked cardiac sympathetic effects on heart rate, but we found no

supporting data for this. The fact that in the present study one left cardiac sympathetic nerve was cut (for recording) could have contributed to this outcome, although it should be noted that other cardiac nerves on the left and all of those on the right remained intact. Moreover, in most similar studies where cardiac nerves remained intact, the responses were always bradycardia (e.g., Refs. 3, 4, 8, 10, 11, 49). Measures of cardiac inotropy have also failed to detect any cardioexcitatory response during the BJR (2, 3, 49), although Barron and Bishop (2, 3) did find a positive inotropic response to intracoronary veratridine that was unmasked after cold block of the cervical vagi.

The pattern of response to PBG usually found here for LSNA and CSNA was that of an initial inhibition followed by a secondary excitatory phase. No secondary phase was apparent in the heart rate response; thus, by inference, the reflex caused a monotonic excitation of vagal cardioinhibitory neurons. The finding that methyl atropine abolished the reflex bradycardia confirms this view. Quantitatively, the magnitude of the secondary sympathoexcitatory phase was the main cause of differences between CSNA and LSNA. Possible causes include *1*) concomitant activation by PBG of nonvagal afferents such as cardiac "sympathetic afferents" (26, 45) or abdominal afferents (14, 45), which have generally cardiovascular excitatory actions (26, 45), in contrast to the predominantly sympathoinhibitory actions of vagal afferents (17); and *2*) unloading of arterial baroreceptors by the fall in blood pressure, which also has sympathoexcitatory consequences (33).

Considering the first of these possible mechanisms, many studies have shown that activation of cardiac "sympathetic afferents", by electrical stimulation of these afferent fibers (26, 31, 46) or by chemical stimuli such as bradykinin (45, 46), or coronary occlusion (13), has sympathoexcitatory effects. Those excitatory effects may be enhanced or unmasked by vagotomy (14, 31). With regard to any preferential excitatory action on CSNA, the evidence is limited. Malik and Minisi (25) found that either intracoronary injection of bradykinin or coronary occlusion caused a significantly stronger activation of CSNA than renal sympathetic nerve activity in baroreceptor-denervated dogs after bilateral vagotomy, and Weaver et al. (46) found that epicardially applied bradykinin produced equivalent excitation of splenic sympathetic nerve activity and CSNA, which was greater than that of renal sympathetic nerve activity. Such preferential cardio-cardiac actions could be a consequence of restricted segmental outflow in spinal reflex pathways (36). In fact, we found that excitatory CSNA responses to PBG were substantially reduced by vagotomy. Thus, although sympathetic afferents could have contributed, most of the excitatory component in CSNA seems to be dependent on vagal afferents.

If the second hypothetical mechanism (baroreceptor unloading) were responsible for sympathoexcitation in our experiments, this implies that CSNA responded more strongly to a fall in blood pressure than LSNA. On this point, the literature is inconclusive. Kamiya et al. (20) found equivalent excitatory and inhibitory responses of cardiac, renal, and muscle sympathetic nerve activity to steady-state (1 min average) falls and rises in pressure in anesthetized rabbits (20). On the other hand, Ninomiya et al. (28) found that CSNA was slightly more strongly inhibited than renal sympathetic nerve activity by transient blood pressure rises in conscious cats, although they did not measure the two nerves' responses to hypotension. When we measured CSNA and LSNA responses to baroreceptor unloading (sodium nitroprusside), we found no material difference between them. This and our finding that sympathoexcitatory responses to PBG were little changed after methyl atropine, which reduced the hypotension, suggest that baroreceptor unloading was not the primary cause of sympathoexcitatory responses and it could not explain the disparity between CSNA and LSNA responses to PBG.

Sympathovagal coactivation. Our direct recordings of CSNA at the same time as heart rate revealed that cardiac sympathovagal coactivation was apparently the rule rather than the exception during the BJR. Although it could be argued that eliciting the BJR with PBG may cause sympathovagal coexcitation as a consequence of activating more than one afferent type, sympathovagal coactivation has been reported to occur in response to "pure" physiological reflex stimuli. The latter includes mild hypoxia and hypercapnia (23) as well as nasopharyngeal afferent stimulation to elicit the diving response (30). Sympathovagal coactivation has also been suggested to occur during the oculocardiac reflex and in response to somatic noxious stimulation (30). Although its functional significance in the BJR remains uncertain, cardiac sympathovagal coactivation is now recognized as a common centrally generated autonomic motor pattern, and a series of plausible suggestions about its biological value have been advanced (30).

Differential control. Whatever the mechanism(s) behind their unequal responses to PBG, it is clear that distinct central neural pathways must control CSNA and LSNA (and vagal cardioinhibition). This view is consonant with the previous demonstration that cardiac and hindlimb muscle sympathetic nerves are driven by separate premotoneuron populations within the cat rostral ventrolateral medulla (RVLM) (5). Furthermore, Koganezawa and Terui (22) identified a distinct subpopulation of RVLM barosensitive bulbospinal neurons, which, like CSNA but unlike renal sympathetic nerve activity, are inhibited by hypoxia in rabbits (22). It is therefore plausible that distinct cardiac sympathetic premotoneurons exist within the RVLM. The RVLM has long been considered to be an essential synaptic relay in the central neural pathway mediating the sympathoinhibitory component of the BJR (41, 44). [Earlier synaptic relays include the nucleus tractus solitarii (15, 44), caudal ventrolateral medulla (rats and rabbits) (15, 35, 42, 44), or lateral tegmental field (cats) (1).] However, apart from a few cells that were transiently excited (44), RVLM sympathetic premotoneurons are held to be uniformly inhibited by the BJR (41, 44). If specific cardiac sympathetic premotoneurons do indeed exist in the RVLM, it seems unlikely that they would have been missed (41, 44).

An alternative possibility is that the excitatory components of cardiac (and lumbar) sympathetic responses to PBG are mediated not via the RVLM but by other neural pathways. In the case of cardiac sympathetic nerves, premotoneurons of the medullary raphé (6, 47, 48) are attractive candidates.

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GRANTS

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