

Patterning of somatosympathetic reflexes

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Kerman, I. A., and B. J. Yates. Patterning of somatosympathetic reflexes. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R716–R724, 1999.—In a previous study, we reported that vestibular nerve stimulation in the cat elicits a specific pattern of sympathetic nerve activation, such that responses are particularly large in the renal nerve. This patterning of vestibulosympathetic reflexes was the same in anesthetized and decerebrate preparations. In the present study, we report that inputs from skin and muscle also elicit a specific patterning of sympathetic outflow, which is distinct from that produced by vestibular stimulation. Renal, superior mesenteric, and lumbar colonic nerves respond most strongly to forelimb and hindlimb nerve stimulation (~60% of maximal nerve activation), whereas external carotid and hypogastric nerves were least sensitive to these inputs (~20% of maximal nerve activation). In contrast to vestibulosympathetic reflexes, the expression of responses to skin and muscle afferent activation differs in decerebrate and anesthetized animals. In baroreceptor-intact animals, somatosympathetic responses were strongly attenuated (to <20% of control in every nerve) by increasing blood pressure levels to >150 mmHg. These findings demonstrate that different types of somatic inputs elicit specific patterns of sympathetic nerve activation, presumably generated through distinct neural circuits.

cutaneous input; muscle input; vestibular system; exercise; pain

SOMATOSYMPATHETIC REFLEXES (SSR) elicited by inputs from *group III* and *IV* skin and muscle afferents have been studied for over a hundred years (for review see Refs. 15, 28, 30) and are mediated by both spinal and supraspinal circuits (15, 28, 30). The spinal component of SSR is mediated by polysynaptic circuitry and is expressed as short-latency (~25 ms) excitation (15, 28, 30). The supraspinal component is mediated by circuitry that includes the rostral ventrolateral medulla (RVLM; see Refs. 22 and 23) and, due to the length and slow conduction velocity of ascending and descending projections, is expressed as a long-latency (~100 ms) excitation (15, 28, 30). The spinal response is segmentally arranged and is largest when recorded from sympathetic efferents arising from the spinal cord segments that receive the stimulated somatic afferents (29). In contrast, the supraspinal component of SSR has a wider distribution and can be recorded from sympathetic efferents arising from many levels of the spinal cord (29). These findings have led to the idea that muscle and cutaneous inputs can preferentially activate sympathetic nerves located at different levels of

the neuraxis via spinal circuitry (28, 30). Although previous investigators have suggested that the supraspinal component of SSR may be preferentially expressed within certain sympathetic nerves (15), there is no definitive evidence to support this notion.

In a previous study (12), we explored the patterning of sympathetic activity that occurs in response to activation of another type of somatic afferents, those from the vestibular labyrinth. Vestibulosympathetic reflexes have been suggested to play a specific role in blood pressure maintenance during postural changes (34). Vestibulosympathetic reflexes, like long-latency components of SSR, are mediated through brain stem circuits that include the RVLM (35). However, existing data suggest that vestibular stimulation may elicit a more precise pattern of activation of sympathetic efferents than is produced by muscle or cutaneous inputs. For example, the relative size of vestibulosympathetic reflexes (as a fraction of the maximal nerve response elicited by stimulation of the preganglionic fibers) differs from nerve to nerve; these responses are larger in the renal nerve than in other sympathetic nerves in the neck, abdomen, and pelvis (12). In addition, the relative sizes of sympathetic nerve responses to vestibular stimulation were not affected by decerebration, suggesting that vestibulosympathetic reflexes can be generated entirely by brain stem circuitry. Furthermore, the magnitude of vestibulosympathetic reflexes is markedly reduced by increasing blood pressure, indicating that these responses are largely the result of activation of vasoconstrictor efferents (12). These findings suggest that vestibular stimulation only affects specific components of the sympathetic nervous system and does not globally and nonspecifically alter the excitability of sympathetic efferents throughout the body.

The purpose of the present study was to determine the pattern of activation of sympathetic efferents produced by stimulation of skin plus muscle afferents and to compare this response pattern with that elicited by vestibular inputs (12). Such a comparison is useful to determine whether vestibulosympathetic reflexes serve a specific physiological purpose (e.g., maintenance of cardiovascular function during postural changes). If this is the case, then vestibular stimulation should elicit a distinct response pattern that is different from that produced by activation of other somatic afferents. The specific goals of this study were to determine 1) whether differences in the size of supraspinal reflex responses to activation of skin plus muscle afferents exist among different sympathetic nerves previously shown to mediate vestibulosympathetic responses, 2) whether expression of SSR is similar in decerebrate and anesthetized animals, 3) whether the patterning of SSR is similar to that of vestibulosympathetic reflexes,

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and 4) whether SSR are similar to vestibulosympathetic reflexes in being mediated by vasoconstrictor sympathetic efferents.

METHODS

All of the procedures used in this study conformed to the American Physiological Society's *Guiding Principles for the Care and Use of Laboratory Animals* and were approved by the University of Pittsburgh's Animal Care and Use Committee.

General surgical procedures. Two groups of adult cats (supplied by Liberty Laboratories, Waverley, NY) were employed in these experiments. In the first group of animals ($n = 8$), anesthesia was induced with 1.5–2.5% halothane vaporized in O_2 and N_2O . After bilateral ligation of the common carotid arteries, animals were rendered decerebrate at the midcollicular level, and anesthesia was removed. In another group of 16 animals, cats were premedicated with a mixture of ketamine (15–20 mg/kg im) and xylazine (0.1–0.2 mg/kg im) and were anesthetized with a mixture of α -chloralose and urethan (initial dose of 40 mg/kg α -chloralose and 200 mg/kg urethan iv, supplemented every 2 h with an injection of 10% of the original dose). Core body temperature was monitored and maintained near 38°C using a heating pad and heat lamp. Blood pressure was monitored using a Millar (Houston, TX) transducer inserted in the femoral artery. Ringer lactate or an 8% solution of the α -adrenergic-agonist Aramine (metaraminol bitartrate; Merck Sharpe & Dohme) was administered (iv) to maintain blood pressure >100 mmHg when necessary. Before the start of recording sessions, animals were paralyzed with gallamine triethiodide (Sigma, St. Louis, MO) administered intravenously (10 mg/kg initial dose, supplemented by hourly doses of 5 mg/kg) and were artificially ventilated. End-tidal CO_2 was monitored and maintained between 3.5 and 4.0%. Before paralysis, animals were monitored to assure that the anesthetic dose was adequate to maintain areflexia; after a deep level of anesthesia was initially obtained, our schedule of providing supplementary drug injections every 2 h was always sufficient. Thus we are confident that animals remained deeply anesthetized throughout the experiment.

After decerebration or attainment of a surgical level of anesthesia (evaluated by the absence of withdrawal reflexes and responses to pinch of the toe pad), the abdominal cavity and/or sympathetic innervation of the neck was exposed via a midline incision. Sympathetic postganglionic nerves innervating the external carotid artery (external carotid nerve, ECN), the kidney (renal nerve, REN), the gut (superior mesenteric nerve, SMN, and lumbar colonic nerve, LCN), and the bladder (hypogastric nerve, HGN) were dissected and prepared for monopolar recordings. Up to five postganglionic nerves were dissected in each animal. Nerves (cervical sympathetic chain, thoracic splanchnic nerves, and lumbar splanchnic nerves) carrying preganglionic fibers were prepared for stimulation.

In two animals, a small laminectomy was performed to expose the C1 spinal segment. This cord segment was then transected with the use of a blunt spatula so that we could determine which components of SSR were elicited through supraspinal circuitry.

Stimulation and recording procedures. Skin flaps surrounding the dissection area were pulled up and sutured to a metal frame to create a mineral oil pool. Monopolar recordings of sympathetic nerve activity were performed under oil using low-resistance silver hook electrodes. The signal was amplified (factor of 10^3 – 10^5), filtered with a bandpass of 10–10,000

Hz, full-wave rectified, integrated (1-ms time constant), and digitized at a sampling rate of 1,000 Hz, using a 1401-Plus analog-to-digital converter manufactured by Cambridge Electronic Design (CED), Cambridge, UK. Digitized data were acquired by a Macintosh Quadra 800 computer and were averaged online using the Spike2 software package (CED). Blood pressure measurements were also digitized at a sampling rate of 100 Hz and were collected to allow a correlation between levels of blood pressure and sympathetic nerve activity. In some animals, needle electrodes were placed on each side of the chest for recording of the electrocardiogram (ECG), which was sampled at 1,000 Hz. Heart rate was determined from peak-to-peak intervals in the ECG or blood pressure waves.

Nerves carrying preganglionic sympathetic fibers were stimulated using bipolar silver hook electrodes. Preganglionic nerves were stimulated to produce maximal activation of the postganglionic nerves, so that the magnitude of SSR could be quantified (see below). In experiments in which the REN and SMN were studied, all of the thoracic splanchnic nerves entering the superior mesenteric and celiac ganglia (which are fused with each other) were stimulated together by laying the nerves across the leads of the same stimulating electrode. In experiments in which activity of the LCN and HGN were recorded, a similar procedure was used to simultaneously stimulate all of the lumbar splanchnic nerves entering the inferior mesenteric ganglion. Maximal activation of ECN was produced by stimulation of the cervical sympathetic trunk.

Stimulation of somatic afferents. Muscle and skin afferents were activated by placing a femoral ($n = 21$) or a brachial plexus ($n = 4$) nerve on a bipolar silver hook electrode (in 2 animals, both a femoral and a brachial plexus nerve were prepared for stimulation). Sixteen of the 24 animals were also prepared for electrical stimulation of vestibular afferents using a previously described method (12, 35). Responses to vestibular stimulation in these animals were reported previously (12).

Experimental design. To elicit SSR, brachial plexus and femoral nerve afferents were stimulated using five-shock trains (0.2-ms shock duration, 3-ms intershock interval) delivered every 1–2 s. A wide range of stimulation intensities (between 0.1 and 4 mA) was used to determine maximal responses to somatic nerve stimulation. Nerves carrying preganglionic fibers (cervical sympathetic trunk, thoracic and lumbar splanchnic nerves) were stimulated with single 0.2-ms-wide square-wave pulses, delivered every 1–2 s and at intensities of 10–1,500 μA .

Nerve responses were averaged online over several stimulus trials (10–40 for preganglionic stimulation, 80–300 for somatic stimulation). Response sizes were determined by measuring area under the response waveform (i.e., the combined area of positive and negative deflections from baseline), which was divided by baseline nerve activity. Standardizing response area measurements to baseline activity was performed to account for possible baseline fluctuations during the recording session. To enable comparisons of the size of SSR recorded from different nerves, maximal sympathetic nerve responses to somatic stimulation were expressed as a percentage of the maximal nerve activation elicited by preganglionic stimulation. The same quantification procedure was performed for the measurement of vestibulosympathetic reflexes in the earlier study (12).

To study the relationship between blood pressure levels and the magnitude of SSR, somatic stimulation was delivered at normal resting levels of blood pressure and during periods of high blood pressure produced by intravenous infusions of Aramine or lactated Ringer solution. Alternatively, somatic

stimulation was triggered off the QRS complex of the ECG, and the delay was set so that the stimulus was delivered during systole, diastole, or at intermediate blood pressure levels. Fluid or Aramine infusions were also used in some cases to increase systolic blood pressure levels during trials in which the stimulus was triggered from the ECG. Influences of blood pressure on the magnitude of SSR were only studied in animals anesthetized using chloralose/urethan, as ligation of the common carotid arteries in decerebrate animals (which was necessary to prevent bleeding from the brain transection) may have altered the gain of the baroreceptor reflexes. However, three of the anesthetized animals were baroreceptor denervated (by bilateral cervical vagotomy and cauterizing around the bifurcation of the common carotid arteries) to verify that the observed effects of high blood pressure on the expression of SSR were the result of baroreceptor inputs.

Statistical analysis. Differences in the magnitude of responses recorded from different sympathetic nerves were evaluated using an analysis of covariance (ANCOVA) model (SPSS for Windows 6.1 software). In this model, recording site (different sympathetic nerves), laterality of stimulus (ipsilateral vs. contralateral), and preparation (decerebrate vs. anesthetized) were included as factors, whereas baseline nerve activity was used as a covariate. A significant effect among nerve responses was evaluated post hoc with Student's *t*-tests. The relationship between blood pressure and the magnitude of SSR was evaluated using a least-squares linear regression and Pearson's correlation analyses (Excel 97 software). Results were considered statistically significant at $P < 0.05$. Data are expressed as means \pm SE.

RESULTS

Sympathetic nerve responses to muscle and skin afferent stimulation. All of the nerves in which activity was sampled in the present study responded to somatic nerve stimulation. Because the individual stimulation periods were quite short (15-ms duration) and were separated by up to 2 s, no changes in blood pressure or heart rate were elicited during SSR. All of the sympathetic nerves responded with a qualitatively similar pattern that consisted of one or more excitatory components followed by a prolonged decrease in nerve activity (see Figs. 1 and 4 for examples). In some responses, a short-latency (~ 25 ms), short-duration (~ 50 ms) excitation preceded the longer-latency (~ 100 ms) excitation that was present in every case. Table 1 indicates the latency and duration of the short- and long-latency components of SSR for each nerve included in this study. Previous work demonstrated that the early excitation is elicited through spinal circuits, whereas the later excitatory period is produced via supraspinal circuitry (15, 28, 30). This presumption was confirmed in two animals in which the spinal cord was transected at the level of the first cervical vertebra. In both animals, this transection resulted in abolition of the late excitatory component in all nerves recorded: REN ($n = 2$), LCN ($n = 2$), SMN ($n = 1$), and HGN ($n = 1$). In one animal, the early excitatory component was present in all nerves before the transection, and this response was unaffected by this procedure. In the other animal, the short-latency component was absent before the spinal cord transection but appeared after the lesion.

Table 1. Onset latencies and duration of the short- and long-duration components of SSR recorded from different nerves

	Component 1			Component 2		
	Onset latency, ms	Duration, ms	<i>n</i>	Onset latency, ms	Duration, ms	<i>n</i>
ECN	24 \pm 6	44 \pm 10	4	84 \pm 7	174 \pm 17	12
REN	23 \pm 2	60 \pm 4	5	98 \pm 4	113 \pm 8	11
SMN	25 \pm 3	56 \pm 4	3	108 \pm 7	156 \pm 18	9
LCN	23 \pm 3	55 \pm 3	7	116 \pm 8	171 \pm 12	14
HGN	28 \pm 2	58 \pm 5	11	134 \pm 8	168 \pm 10	13

Values are expressed as means \pm SE; *n*, no. of animals. ECN, external carotid nerve; REN, renal nerve; SMN, superior mesenteric nerve; LCN, lumbar colonic nerve; HGN, hypogastric nerve; SSR, somatosympathetic reflexes. Measurements were made for responses elicited by a train of 5 shocks; latencies were measured from the first shock in the stimulus train. Early (*component 1*) responses were not present in every case.

Quantitative differences in response sizes. The magnitude of sympathetic nerve responses to skin plus muscle stimuli was quantified in 8 decerebrate and 16 chloralose/urethan-anesthetized cats. Within each experiment, the area under the response waveform was measured and standardized to baseline nerve activity. These measurements were then expressed as a fraction of maximal nerve activation due to preganglionic activation (see Fig. 1 for examples). This technique was used previously (12) and allows comparisons of experimental results across different nerves and across different experiments. Typically, stimulation of preganglionic nerves resulted in massive excitation recordable from the postganglionic nerves that was considerably greater in size than responses to somatic stimulation (Fig. 1). Maximal responses to somatic stimulation were determined by evaluating response sizes at several different stimulation intensities. Only responses that were demonstrated to be maximal in size were included in this analysis.

As a first step, we determined whether preparation type (decerebrate vs. anesthetized) influenced expression of SSR by comparing responses recorded in anesthetized and decerebrate cats. The relative magnitude of SSR was measured in REN ($n = 8$ in anesthetized group, $n = 3$ in decerebrate group), LCN ($n = 8$ anesthetized, $n = 5$ decerebrate), and HGN ($n = 8$ anesthetized, $n = 5$ decerebrate). Amplitudes of responses ranged between 10 and 80% of maximal nerve activation due to preganglionic stimulation for all the nerves across both preparations (Fig. 2). Statistical analysis using ANCOVA demonstrated a significant effect ($P < 0.05$) due to preparation type. Post hoc analysis revealed statistically significant differences (Student's *t*-test, $P < 0.05$) in the relative amplitude of responses of LCN (77 \pm 17% anesthetized, 20 \pm 7% decerebrate) and HGN (24 \pm 5% anesthetized, 8 \pm 2% decerebrate; Fig. 2). Differences in the size of responses of REN (52 \pm 7% anesthetized, 73 \pm 18% decerebrate) did not reach statistical significance (Fig. 2). Thus the patterning of sympathetic outflow in response to limb

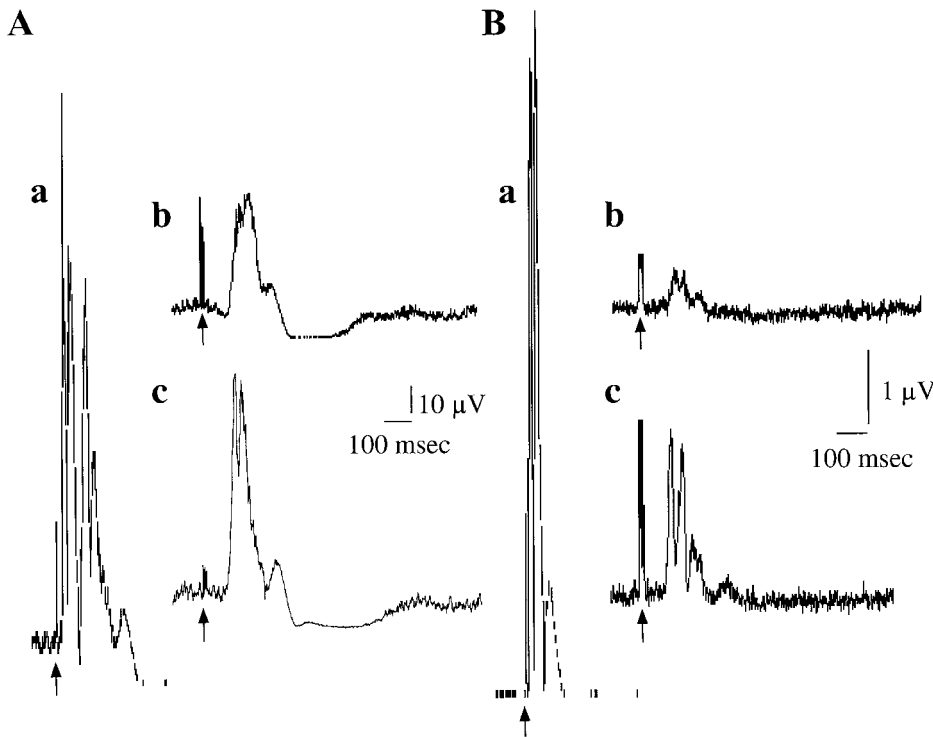


Fig. 1. Magnitude of somatosympathetic reflexes (SSR) compared with maximal nerve activation. Nerves carrying preganglionic fibers were stimulated to produce maximal nerve activation of renal (REN; *Aa*) and external carotid (ECN; *Ba*) nerves. These responses were compared with those produced by stimulation of femoral (*Ab* and *Bb*) and brachial plexus (*Ac* and *Bc*) afferents. All recordings were made in the same preparation; stimulation intensities as follows: 1.0 mA (*Aa*, *Ba*, *Ab*, and *Bb*) and 1.5 mA (*Ac* and *Bc*). Stimulus onset is indicated by arrows.

nerve stimulation appears to be different in decerebrate and intact anesthetized cats.

Because the expression of SSR appeared to differ in decerebrate and anesthetized animals, data collected from these two preparations were not pooled, and subsequent experiments were performed in intact anesthetized animals. In this preparation, the amplitude of the supraspinal component of SSR was quantified relative to maximum nerve activation in the following

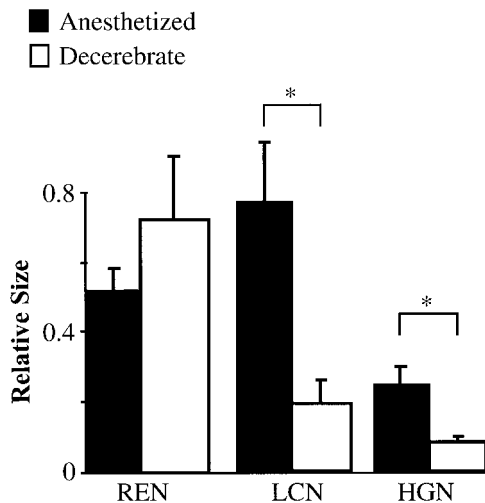


Fig. 2. Comparison of relative magnitude of sympathetic nerve responses to activation of muscle and skin afferents recorded in decerebrate animals and animals anesthetized using chloralose/urethan. Responses are expressed as fractions of maximal nerve activation due to preganglionic stimulation. LCN, lumbar colonic nerve; HGN, hypogastric nerve. Preparation type (decerebrate vs. anesthetized) had a statistically significant effect [analysis of covariance (ANCOVA), $P < 0.05$] on the size of SSR. * Results of statistically significant post hoc comparisons (Student's *t*-test, $P < 0.05$).

nerves: ECN ($n = 11$), REN ($n = 8$), SMN ($n = 7$), LCN ($n = 8$), and HGN ($n = 8$). Statistical analysis with ANCOVA revealed significant ($P < 0.05$) variability in the magnitude of responses recorded from different nerves, whereas effects due to animal's sex, stimulus laterality, and baseline nerve activity did not reach significance. Post hoc analyses (Student's *t*-tests) revealed statistically significant differences ($P < 0.05$) between response sizes of REN ($52 \pm 7\%$), SMN ($49 \pm 9\%$), LCN ($78 \pm 17\%$), and those of ECN ($17 \pm 6\%$) and HGN ($24 \pm 5\%$; Table 2).

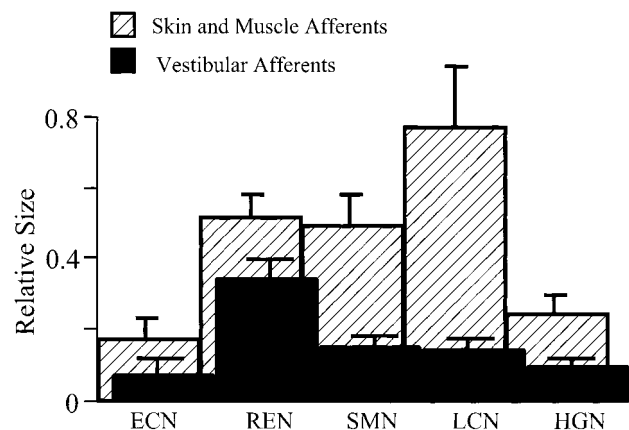


Fig. 3. Comparison of the size of SSR and vestibulosympathetic reflexes in different sympathetic nerves. Responses to activation of muscle and skin afferents were quantified and compared with those elicited by stimulation of vestibular afferents in anesthetized animals. Two types of inputs led to distinct patterning of sympathetic outflow. Activity of LCN, superior mesenteric nerve (SMN), and REN was most powerfully affected by skin plus muscle afferent activation (ANCOVA, $P < 0.05$; post hoc analyses presented in Table 2). In response to vestibular stimulation, REN responses were larger than those of the other nerves (data from Ref. 12).

Table 2. Results of post hoc analyses of differences in the magnitude of SSR

	REN	SMN	LCN	HGN
ECN	*	*	*	0.4
REN		0.8	0.2	*
SMN			0.2	*
LCN				*

After initial analysis of covariance, between-nerve comparisons in the anesthetized group were made using Student's *t*-test. *Comparisons that produced statistically significant differences of response sizes ($P < 0.05$). *P* values of comparisons that did not reach statistical significance are reported.

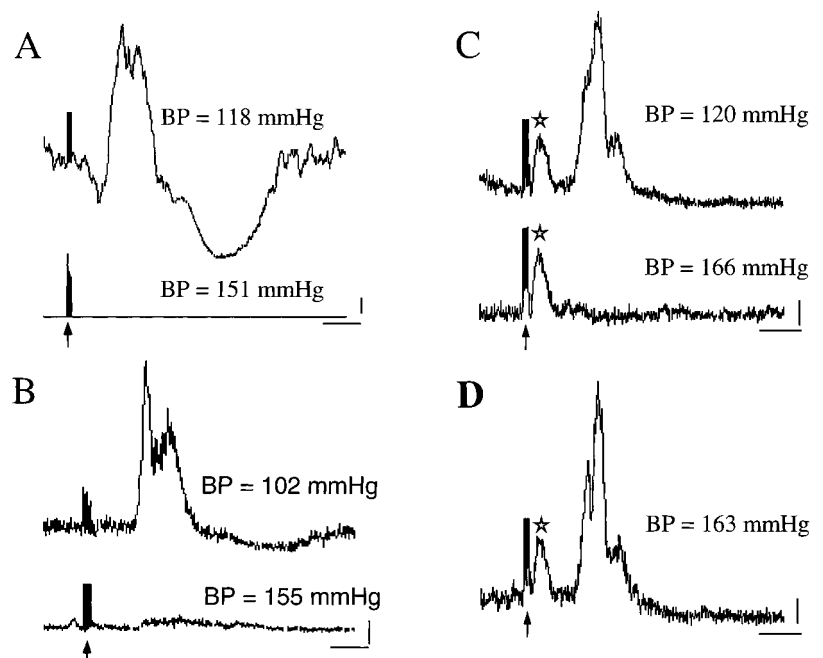
The next goal of our study was to compare the patterning of SSR with that which occurs in response to vestibular stimulation (12). Because patterning of SSR but not vestibul sympathetic reflexes (12) is different between anesthetized and decerebrate animals, only SSR recorded from anesthetized animals were included in this analysis. On average, responses to skin plus muscle inputs were larger than those to vestibular stimulation ($42 \pm 5\%$ vs. $18 \pm 3\%$), which may reflect activation of a greater number of afferents during stimulation of limb nerves than during stimulation of the labyrinth. The relative amplitude of SSR and vestibul sympathetic reflexes also differed between sympathetic nerves. Responses of REN to vestibular inputs were larger than those of the other nerves. In contrast, responses to stimulation of skin plus muscle afferents were equally prominent in REN, SMN, and LCN (Fig. 3). Thus stimulation of muscle and cutaneous afferents appears to elicit prominent changes in a larger number of sympathetic outflows than does vestibular stimulation, at least in animals anesthetized using chloralose/urethan.

Effects of blood pressure on expression of SSR. Activity of vasoconstrictor sympathetic efferents, but not

that of efferents with noncardiovascular functions (e.g., those regulating gastrointestinal motility), is silenced by blood pressure increases (1, 11). To determine whether SSR were mediated by vasoconstrictor efferents, we took advantage of this observation by eliciting SSR at multiple blood pressure levels. These experiments were performed in nine chloralose/urethan-anesthetized animals in which the common carotid arteries remained patent, and effects of baroreceptor loading and unloading on the expression of SSR could be evaluated. In two animals, somatic stimulation was locked to the cardiovascular cycle by using the peak of the QRS complex of the ECG to trigger the stimulus. The latency was set to deliver stimulation during either diastole or systole. This method was also combined with fluid or Aramine infusion to further increase systolic blood pressure. In two other animals, Aramine infusion was used to raise the mean blood pressure levels, whereas somatic stimulation was delivered randomly with respect to the cardiovascular cycle. In five animals, both of these methods of manipulating blood pressure during somatic stimulation were employed in separate trials.

In all cases, raising blood pressure levels (using either method) led to strong attenuation of the supraspinal excitatory component of the SSR (see Fig. 4 for examples). This was true for all sympathetic nerves included in the study and in response to both forelimb (4 animals) and hindlimb (6 animals) afferent stimulation. In contrast, the magnitude of the spinally mediated early excitatory component was not affected by blood pressure increases in all of the nerves from which this component was recorded: REN ($n = 1$), SMN ($n = 5$), LCN ($n = 2$), and HGN ($n = 2$; Fig. 4C). To verify that the effects of altering blood pressure on SSR were due to specific activation of baroreceptor afferents, in three animals sympathetic nerve responses to skin plus

Fig. 4. Effects of manipulating blood pressure (BP) on expression of SSR. To compare responses elicited at different levels of blood pressure, the femoral (A) or brachial plexus (B-D) nerve were stimulated during diastole or systole (arrows). Aramine infusion was used to further increase blood pressure during systolic stimulation. In C and D, recordings were performed before and after baroreceptor denervation in the same animal. Strong attenuation of the supraspinal responses by blood pressure increases (A-C, bottom traces) suggest that they are mediated by vasoconstrictor efferents. Attenuation of the supraspinal component was abolished after baroreceptor denervation (D), suggesting that this effect of increased blood pressure was mediated by baroreceptor afferents. Note that the supraspinal component of responses was attenuated by increasing blood pressure, whereas the spinal component (stars in C and D) was unaffected by this manipulation. Stimulus intensities as follows: 1.0 mA (A) and 3.0 mA (B-D). Vertical scale bars: 5 μ V; horizontal scale bar: 100 ms.



muscle inputs were recorded at high blood pressure before and after baroreceptor denervation. Before baroreceptor denervation, the animals maintained a blood pressure of 111 ± 15 mmHg and a heart rate of 226 ± 4 beats/min. After baroreceptor denervation, the blood pressure increased slightly to 114 ± 6 mmHg, whereas the heart rate increased to 240 ± 0.02 beats/min. This difference in heart rate reached statistical significance ($P < 0.05$, paired Student's *t*-test). In two of these animals, REN sympathetic activity was recorded and increased by 67% and 131% in the two cases after baroreceptor denervation. When baroreceptors were intact in these two animals, REN activity was silenced when blood pressure was elevated 50 mmHg during intravenous Aramine infusion. However, after the baroreceptor denervation, REN activity was unaffected by a similar change in blood pressure, suggesting that this approach successfully removed baroreceptor inputs. In all three animals, the supraspinal excitation was strongly attenuated by increasing blood pressure when baroreceptors were intact (Fig. 4C). After baroreceptor denervation, raising blood pressure had no effect on the magnitude of SSR (Fig. 4D).

To quantify the effects of blood pressure on the size of SSR, we measured peak-to-trough amplitude of sympa-

thetic nerve responses recorded at different blood pressure levels. These values were expressed as a percentage of the largest response recorded from each nerve and were plotted as a function of blood pressure. Pooled data are illustrated in Fig. 5. Linear regression analysis revealed statistically significant ($P < 0.05$) correlations between response amplitude and blood pressure levels for ECN, REN, LCN, SMN, and HGN. For all nerves, increasing blood pressure to >150 mmHg attenuated the size of sympathetic nerve responses to $<20\%$ of the magnitude when stimulation was presented at resting blood pressure levels (Fig. 5).

DISCUSSION

The present study supports the finding that both spinal and supraspinal circuitry contributes to producing SSR and provides quantitative evidence to demonstrate that, although many sympathetic nerves respond to somatic stimuli, the supraspinal component of SSR is differentially expressed among sympathetic nerves. In anesthetized animals, stimulation of skin plus muscle afferents leads to more powerful activation of renal and intestinal sympathetic efferents than fibers innervating the bladder and the external carotid

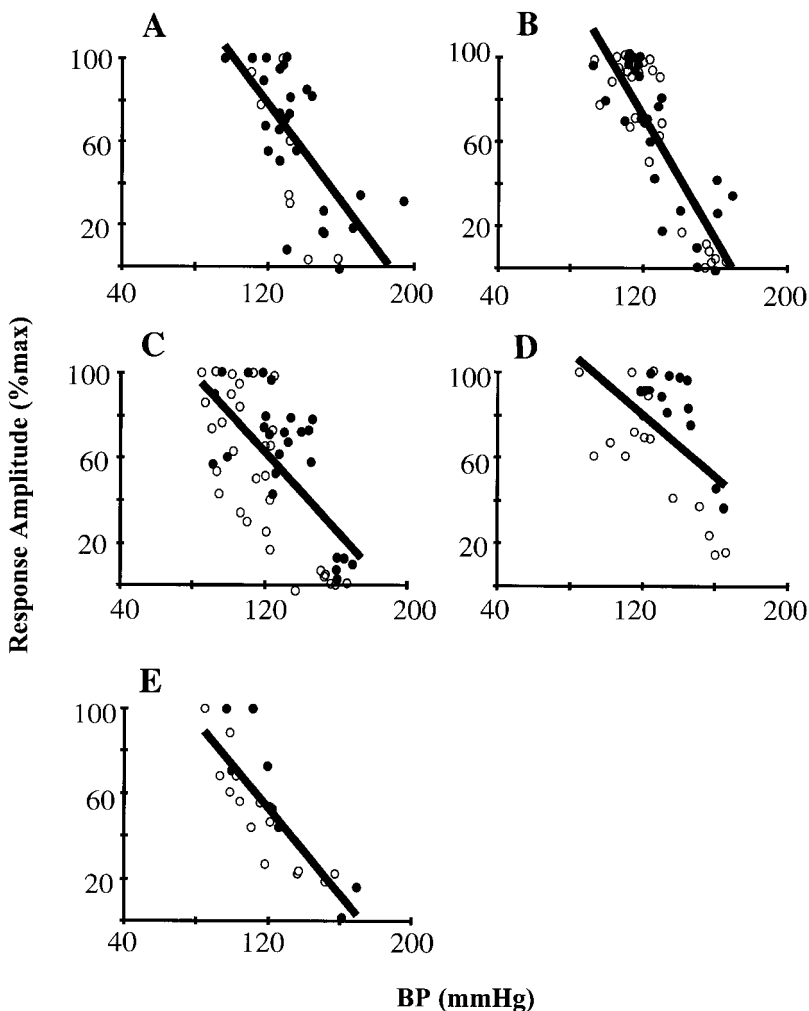


Fig. 5. Relationship between blood pressure and expression of SSR. Strong negative relationship between blood pressure levels and the size of responses to muscle and skin afferent stimulation was present in all nerves. Open circles, responses to brachial plexus nerve stimulation; filled circles, responses to femoral nerve stimulation. Results of Pearson's correlations demonstrated that the relationship between blood pressure and SSR amplitude was statistically significant ($P < 0.01$) for every nerve: A: ECN, $r^2 = 0.47$ ($n = 3$); B: REN, $r^2 = 0.71$ ($n = 4$); C: SMN, $r^2 = 0.45$ ($n = 4$); D: LCN, $r^2 = 0.27$ ($n = 3$); E: HGN, $r^2 = 0.72$ ($n = 3$).

artery. These findings extend the results of a recent study, which suggested that the effects of limb nerve stimulation on REN and adrenal nerve activity are larger than on that of cardiac, hepatic, and splenic nerves in dogs (14). Furthermore, the method for measuring response magnitude (determining area of the response waveform) in this study is an improvement on the method (measuring amplitude of the peak response) employed in the previous study, which may have underestimated responses of some of the nerves (14). Thus the present findings indicate that activity of a subset of sympathetic nerves is preferentially influenced by stimulation of muscle plus cutaneous afferents.

Previous work on the organization of SSR demonstrated that selective electrical stimulation of cutaneous or muscle afferents can produce increases in sympathetic nerve activity. For example, cutaneous afferent activation at stimulation intensities high enough to activate type IV afferents generates pressor responses in decerebrate (6) and chloralose/urethan-anesthetized (27) cats. These blood pressure increases are accompanied by tachycardia and increased firing of the inferior cardiac nerve (27). Likewise, selective stimulation of type IV muscle afferents leads to sympathetically mediated pressor responses and tachycardia (6, 27). In the present study, skin and muscle afferents were simultaneously activated with high-intensity stimulation of mixed somatic nerves. Thus the relative contribution of skin vs. muscle inputs to the observed patterning of sympathetic outflow cannot be determined from our results.

The patterning of sympathetic outflow in response to simultaneous activation of cutaneous and muscle afferents was affected by the type of preparation (anesthetized vs. decerebrate). One possible explanation for this observation is that forebrain circuitry is necessary for processing of inputs from skin and muscle and for generating appropriate sympathetic patterning. An alternative explanation is that chloralose/urethan anesthesia alters the pattern of sympathetic nerve responses to skin plus muscle afferent activation. This possibility is minimized by the observation that the pattern of sympathetic nerve responses to another somatic signal, vestibular inputs, does not appear to be affected by chloralose/urethan anesthesia (12). Nonetheless, the prospect that our results are due to the actions of anesthesia on brain stem circuits that generate SSR cannot be dismissed on the basis of current data.

Patterning of sympathetic outflow in response to stimulation of skin plus muscle afferents is different than that which occurs in response to vestibular stimulation (12). Specifically, expression of SSR, but not that of vestibulosympathetic reflexes, is affected by preparation type (decerebrate vs. anesthetized). Additionally, vestibular stimulation elicits particularly strong responses in REN (12), whereas several abdominal sympathetic nerves are activated to a similar degree during the supraspinal component of SSR. Taken together, these observations suggest that different somatic in-

puts lead to distinct patterns of sympathetic activation, which may be mediated by separate central pathways.

This study also examined the interaction of somatic and baroreceptor inputs in control of sympathetic nerve activity; although this issue has been investigated previously, the interpretation of prior studies is limited due to methodological constraints (3, 13, 16, 17). In some of the previous experiments, baroreceptor afferent activation was achieved by sudden and maintained stretch of the carotid sinus (3, 16), which is a poor representation of physiological blood pressure oscillations. In others, recordings of sympathetic activity were made from nerves composed exclusively of sympathetic efferents with cardiovascular functions (i.e., the REN; see Refs. 3, 13, 17), making it difficult to determine whether efferents with noncardiovascular functions are activated by somatic stimulation. In the present study, we increased blood pressure to activate baroreceptor afferents. Although this stimulus has the potential to activate a variety of stretch receptors, including those in the heart and in the venous circulation (18), the source of major inhibitory input to vasoconstrictor efferents originates from the arterial baroreceptors (4). Our results are in agreement with those of previous studies and show that expression of the supraspinal component of SSR is impaired by baroreceptor afferent input during periods of high blood pressure (3, 13, 16, 17) but that the spinal component is not affected by altering blood pressure (13, 16). Pressor responses to muscle stretch and bilateral carotid occlusion have previously been suggested to share much of the same central circuitry (24, 25). One cardiovascular regulatory region in which somatic and cardiovascular inputs are known to converge is the RVLM (4); spinally projecting vasomotor cells in this area may have been so powerfully inhibited when blood pressure was high that they were unable to transmit somatic signals to the spinal cord.

It is more difficult to account for the fact that the spinal component of SSR was unaffected by blood pressure increases. In the case of sympathetic nerves that innervate both vascular and nonvascular smooth muscle (e.g., HGN), it is possible that the spinal component is mediated by nonvasoconstrictor fibers (whose activity is not affected by increasing blood pressure). However, raising blood pressure does not alter the magnitude of the spinal component of SSR in the REN (13), which is composed predominantly of vasoconstrictor fibers (5). One possibility is that spinal pathways are capable of exciting vasomotor sympathetic preganglionic neurons even in the absence of tonic excitatory inputs to these cells from the RVLM. Thus the present data do not show unequivocally that spinal cord pathways provide skin plus muscle inputs to only sympathetic preganglionic neurons that affect vascular smooth muscle. However, it appears that descending pathways selectively relay somatic signals to components of the sympathetic nervous system that regulate blood pressure.

One limitation of the type of stimulation employed in the current study (e.g., high-intensity electrical stimu-

lation) is that it nonspecifically activates a large number of afferents. Nevertheless, the present findings show that even such a large stimulus only affects the excitability of a limited number of sympathetic efferents. It is likely that patterning of SSR reflects sympathetic activation that occurs in response to a variety of physiological stressors. Selective renal and intestinal vasoconstriction, which occurs in response to activation of skin plus muscle inputs, is a hallmark of the defense reaction (8, 19). This is a behavioral response that is triggered by threatening and painful stimuli, which prepares the animal for "fight-or-flight" (8, 9). Both the behavioral and autonomic changes are coordinated by forebrain structures, including the hypothalamus (10) and the periaqueductal gray (2). In our experiments, we found that patterning of SSR was different in anesthetized animals and animals in which connections between the forebrain and brain stem were interrupted by decerebration. Because our peripheral nerve stimulation activated pain fibers (among other afferents), it is possible that patterning of sympathetic outflow as part of SSR represents a component of the defense reaction in response to painful inputs.

The stimuli employed in this study also presumably activated afferents from metabo- and mechanoreceptors in muscle. During exercise, inputs from these receptors trigger changes in sympathetic nervous system activity and lead to an increase in blood pressure (7). Inputs from mechanoreceptors produce cardiovascular responses during static exercise and elicit changes in muscle vasoconstrictor, renal, cardiac, and adrenal sympathetic nerve activity (20, 21, 32). Metaboreceptor inputs are evoked by the presence of glycogen breakdown products (26) and influence the activity of muscle vasoconstrictor, renal, and cardiac sympathetic nerves during rhythmic exercise (31, 33). Although inputs from contracting muscles are known to affect sympathetic nervous system activity, the pattern of activation of different sympathetic nerves during exercise has not been explored. Based on the present findings, it seems possible that afferent input from contracting muscles may lead to preferential activation of renal and intestinal sympathetic efferents. This hypothesis remains to be examined directly.

Perspectives

This study demonstrated that stimulation of skin plus muscle afferents leads to specific patterning of sympathetic nerve activity. Vasoconstrictor efferents appear to be powerfully activated during the supraspinal components of SSR, particularly those vasoconstrictor fibers in sympathetic nerves innervating the kidney and intestine. However, the patterning of SSR differs between decerebrate and anesthetized animals, which is in contrast to vestibulosympathetic responses that are largest when recorded from the REN and that are unaffected by decerebration (12). These findings suggest that different types of somatic inputs lead to specific patterning of sympathetic nerve activity; each pattern presumably subserves a specific physiological role. The central autonomic circuitry required to gener-

ate such specific patterns of sympathetic outflow is likely to be in part unique for particular types of somatic inputs.

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