Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure

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Submitted 10 May 2007; accepted in final form 22 January 2008

Cassaglia PA, Griffiths RI, Walker AM. Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure. Am J Physiol Regul Integr Comp Physiol 294: R1255–R1261, 2008. First published January 23, 2008; doi:10.1152/ajpregu.00332.2007.—Sympathetic vasoconstriction of cerebral vessels has been proposed to be a protective mechanism for the brain, limiting cerebral perfusion and microcirculatory pressure during transient increases in arterial pressure. To furnish direct neural evidence for this proposition, we aimed to develop a method for recording cerebral sympathetic nerve activity (SNA) from the superior cervical ganglion (SCG). We hypothesized that SNA recorded from the SCG increases during imposed hypertension, but not during hypotension. Lambs (n = 11) were anesthetized (x-chloralose, 20 mg·kg⁻¹·h⁻¹) and ventilated. SNA was measured using 25-μm tungsten microelectrodes inserted into the SCG. Arterial blood pressure (AP) was pharmacologically raised (adrenaline, phenylephrine, or ANG II, 1–50 μg/kg iv), mechanically raised (intravascular balloon in the thoracic aorta), or lowered (sodium nitroprusside, 1–50 μg/kg iv). In response to adrenaline (n = 10), mean AP increased 135 ± 10% from baseline (mean ± SE), and the RMS value of SNA (Square Root of the Mean of the Squares, SNARMS) increased 255 ± 120%. In response to mechanically induced hypertension, mean AP increased 43 ± 3%, and SNARMS increased 53 ± 13%. Generally, (9 of 10 animals), SNARMS did not increase, as AP was lowered with sodium nitroprusside. Using a new model for direct recording of cerebral SNA from the SCG, we have demonstrated that SNA increases in response to large induced rises, but not falls, in AP. These findings furnish direct support for the proposed protective role for sympathetic nerves in the cerebral circulation.

cerebral circulation; hypertension; sympathetic nervous system; adrenaline; lamb

CEREBRAL VESSELS HAVE A RICH sympathetic innervation distributed widely throughout the cerebral circulation, including cerebral arteries, arterioles and, to a lesser extent, veins (10, 12). Cerebral sympathetic innervation originates in the superior cervical ganglion (SCG), with minor contributions from the stellate ganglia and possibly an intrinsic central adrenergic pathway (1, 11, 13). Electrical stimulation of the efferent pathway (2, 7, 40) and application of adrenergic agonists to cerebral vessels (3, 39) both elicit powerful cerebral vasoconstriction, particularly during early life.

Despite these intensive investigations, the exact functional role of the sympathetic nervous system in the regulation of the cerebral circulation remains an issue of debate (32). Sympathetic vasoconstriction of cerebral vessels has been proposed to be a protective mechanism for the brain, limiting cerebral perfusion and microcirculatory pressure during transient increases in arterial pressure (4, 14, 22). Stimulation of the sympathetic trunk immediately caudal to the SCG during periods of acute hypertension limits the resulting increase in cerebral blood flow in several studies (6, 22, 36).

Large natural increases in arterial pressure can occur during sympathetically dominated conditions such as during weightlifting, sexual activity (30), and, as recently recognized, during REM sleep (35), and REM sleep disrupted by apnea (34). Cerebral sympathetic activity appears to limit cerebral perfusion during normal sleep, as baseline cerebral blood flow, as well as the blood flow surges occurring during the natural transient blood pressure surges of REM sleep are augmented after cervical (SCG) sympathectomy in sleeping lambs (27).

As yet, there is no animal model available to directly assess possible protective roles for sympathetic activity during natural increases of cerebral perfusion pressure. To date, direct neural recordings have been limited to single-unit recordings from SCG neurons in anesthetized preparations (26, 29). Although this method has provided important insights into SCG neuron discharge patterns, they are not practicable in anesthetized, freely moving animals.

Accordingly, our aim was to develop a method for recording cerebral sympathetic nerve activity (SNA) in the anesthetized lamb that could be adapted for subsequent use in the conscious lamb. Additionally, we aimed to use this model to further examine the protective role of cerebral SNA first proposed by Busija et al. (6). We hypothesized that cerebral SNA recorded from the SCG increases during periods of induced hypertension, but not hypotension, and we tested the hypothesis during pharmacological and mechanical manipulations of systemic arterial pressure.

MATERIALS AND METHODS

General Procedures

Studies were conducted on eleven Merino/Border-Leicester lambs (1–2 wk old, 4–8 kg body wt). Anesthesia was induced with ketamine (5 mg/kg iv) and α-chloralose (40 mg/kg iv loading dose) and maintained for the duration of the experiment by infusion of α-chloralose (20 mg·kg⁻¹·h⁻¹ iv) (Sigma, St. Louis, MO). All surgical and experimental procedures were performed in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes established by the National Health and Medical Research Council of Australia and were approved by the Monash University-Monash Medical Centre Committee on Ethics in Animal Experimentation.

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Lambs were placed supine and artificially ventilated with oxygen-enriched room air through an endotracheal tube. Arterial oxygen saturation (Nellcor Pulse Oximeter, Hayward, CA), end tidal CO2, endotracheal pressure, and rectal temperature (U-lab Instruments, Port Louis, Mauritius) were monitored to ensure adequate anesthesia. Arterial blood pressure was monitored continuously through a radial artery catheter connected to a calibrated strain gauge manometer (Cobe CDX III, Cobe Laboratories, Lakewood, CO) referenced to the lamb’s midthoracic level. Pressure signals were low-pass filtered at 100 Hz.

An intra-arterial balloon (Fogarty Dilation Atrialseptostomy Catheter, 5F; Baxter Healthcare, Santa Ana, CA) was surgically inserted via the femoral artery into the caudal abdominal aorta.

**Sympathetic Nerve Recording**

The SCG was accessed via a midline neck incision made caudal to the level of the jaw. The vagus nerve was located and followed to the point at which the cervical sympathetic trunk visibly separated to continue on to the SCG. The SCG was located, and a pair of lightweight, Teflon-coated tungsten microelectrodes (25-μm shaft diameter, 1 mm bare tip) (California Fine Wire, Grover Beach, CA) was inserted into its cranial aspect. A separate stainless steel electrode was attached onto nearby tissue as a ground reference electrode.

The neural signal was amplified (25,000 times) and to minimize low-frequency noise filtered (100–2000 Hz) using Cyberamp (Axon Instruments, Foster City, CA) and together with pressure signals was digitized and recorded at a sampling frequency of 10 kHz, using a data acquisition-recording system (Powerlab, ver. 5.3, ADI Instruments, Carrollton, TX). Chart ver. 5.3 was used for storage and on-line analysis, including power spectral analysis, which was performed on the RMS (Root Mean Square) of the neural signal.

**Experimental Protocol**

Acute hypertension was induced pharmacologically (adrenaline, phenylephrine, or ANG II, Sigma) with a range of doses (1–50 μg/kg iv) or mechanically with inflation of the intra-arterial balloon. Hypertension was induced pharmacologically with a vasodilator (sodium nitroprusside, 1–50 μg/kg iv, Sigma). At least 5 min were allowed between pressor and depressor tests for blood pressure to return to baseline levels.

While recording SNA, animals were killed at the end of each experiment with an overdose of pentobarbital sodium (150 mg/kg iv).

**Data Analysis**

Arterial blood pressure (AP) was averaged in second-by-second segments. The RMS of the raw SNA signal (Square Root of the Mean of the Squares, SNA_RMS) was calculated second by second using Chart ver. 5.3 (ADI Instruments).

Responses to vasoconstrictor drug injections and balloon inflations are expressed as peak % change from a baseline value averaged over 30 s before each test. The onset of the mean AP (MAP) increase was defined as the first of three consecutive increases immediately following drug injection or balloon inflation. The onset of SNA_RMS change was defined as the first of three consecutive time points, which exceeded 5% of the baseline value following onset of the MAP increase. To examine the relationship between SNA_RMS and MAP, data from repeated tests in each lamb were expressed as % changes, averaged for each lamb, and then assigned into bins of 25% across the MAP range. SNA_RMS changes after sodium nitroprusside, intra-arterial balloon deflation, or post mortem were obtained by averaging over 10 s when AP reached its minimum value. A trendline was fitted through pharmacological data and intra-arterial balloon data using a fourth-order polynomial (Excel, Microsoft Corp, Redmond WA).

**Statistics**

Values are reported as means ± SE. Changes of SNA from baseline were compared using a paired Student’s t-test or a Wilcoxon signed-ranks test if data were not normally distributed (SigmaStat for Windows, Systat Software Inc., Richmond VA). P < 0.05 was considered significant.

**RESULTS**

**Animal Characteristics**

Recordings from the SCG were obtained in five male and six female lambs. At the beginning of the experiment MAP was 77.5 ± 4.4 mmHg, body weight was 61. ± 0.4 kg, and mean age was 10.1 ± 1.4 days. Body temperature and arterial blood gases were close to normal physiological levels (temperature, 39.0 ± 0.4°C; arterial Po2, 157.0 ± 7.2 mmHg; PaCO2, 34.4 ± 2.2 mmHg; and pH, 7.4 ± 0.0).

**Responses to Hypertension and Hypotension**

**Adrenaline.** An example of the pattern of the change in SNA during adrenaline-induced hypertension is illustrated in Fig. 1. Increased AP was associated with an increase in SNA amplitude (μV) beginning when AP had increased by 50 mmHg above a baseline level of 70 mmHg (top). After reaching its peak, cyclic AP modulation associated with the ventilator cycle was reflected in a clear modulation of the SNA trace. SNA spectral power (μV2) in the range of 0–10 Hz (bottom) was ~200 times greater at peak AP than at baseline AP. The ventilator-related modulation evident in the raw neural signal was reflected in a spectral peak at the ventilator frequency (0.4 Hz). There was a very small contribution to total SNA power at heart frequency (~3–4 Hz).

Grouped responses to adrenaline (n = 10) are shown in Fig. 2. Onset of increase in SNA_RMS was evident when MAP increased by 56 ± 7% (40 ± 9 mmHg) from the baseline value of 71 ± 4 mmHg. Peak SNA_RMS increased by 255 ± 120% from baseline (P < 0.05); the associated increase in MAP was 135 ± 10%.

**Phenylephrine and ANG II.** SNA responses similar to those occurring in response to adrenaline-induced hypertension were evident with other hypertensive drugs, though ventilatory modulation was not found. As shown in Fig. 3A (phenylephrine, 10 μg/kg iv) and 3B (ANG II, 10 μg/kg iv), SNA increased shortly after AP was elevated, when MAP had reached 35 ± 5 mmHg and 45 ± 10 mmHg above baseline, respectively. With phenylephrine injection (n = 5), peak SNA_RMS increased by 126 ± 10% (P < 0.05) in association with a 96 ± 9% increase in MAP. With ANG II (n = 4), peak SNA_RMS increased 134 ± 11% (P < 0.05) associated with an increase in AP of 48 ± 19%.

**Sodium nitroprusside.** Typically, no change occurred in SNA with nitroprusside-induced hypotension (eight of nine animals), as illustrated in Fig. 3C. The average fall in MAP (n = 8) was 48 ± 2%; the associated change in SNA_RMS was 2 ± 2% (P = nonsignificant). In a single lamb, nitroprusside-induced hypotension resulted in an increase in SNA (Fig. 4A). In this lamb, by contrast with the other lambs studied, SNA was decreased, not increased, when AP was raised with adrenaline (Fig. 4B).

**Mechanically induced hypertension.** Hypertension was induced by intra-arterial balloon inflation in a total of 10 tests in...
three lambs. As illustrated in Fig. 5, AP rapidly increased from 95 mmHg to 137 mmHg, coincident with an increase in SNA.

Grouped responses to mechanically induced hypertension are shown in Fig. 6. Baseline MAP was 79 ± 3 mmHg. With rapid balloon inflation, both MAP and SNA increased coincidently from baseline values. The onset of increase in SNA was evident when MAP increased by 45 ± 3%, and the associated peak of SNA was 53 ± 13% (P < 0.05). During the period of balloon inflation, there was a slow decline in MAP, which was closely followed by a fall in SNA. On deflation of the balloon, MAP rapidly dropped to levels below baseline before recovering. As MAP declined, SNA largely followed but did not fall below baseline values nor increased when MAP fell below its baseline level.

SNA vs. MAP. The relationship between SNA over the entire range of MAP studied, including post mortem values, is shown in Fig. 7. SNA increased when MAP was elevated 40–60% from baseline with either adrenaline (n = 10), ANG II (n = 3), or inflation of the intra-arterial balloon (n = 3). No change of SNA was observed when MAP was lowered with nitroprusside injection (n = 8) nor when MAP fell below baseline transiently following deflation of IA balloon (n = 3). Post mortem, there was a small reduction from baseline SNA of 15 ± 8% (n = 8, P < 0.05). Post mortem there was an intravascular pressure of ~10 mmHg referenced to heart level; at this time, there was background electrical activity of 15 ± 8% (n = 8, P < 0.05) below the baseline SNA.

This activity was a measure of the background system noise in the electrode recordings. As illustrated in Fig. 1 (bottom), system noise was ~1/250 the level evident at high blood pressure and ~1/10 the level at baseline pressure.

DISCUSSION

In the present study, we have developed a novel method for multiunit recording of sympathetic nerve activity from the superior cervical ganglion in the anesthetized animal. Here, we report the first direct evidence that cerebral SNA increases with imposed elevations of arterial pressure.

An increase in SNA was consistently recorded from the SCG in response to a range of vasoconstrictor pharmacological agents, comprising adrenaline, phenylephrine, and ANG II. SNA also increased when arterial pressure was raised mechanically by inflation of an intra-arterial balloon. A variety of methods of raising arterial pressure were examined to exclude the possibility that pharmacological stimulation of known receptors within the SCG was involved in mediating the SNA response. ANG II receptors, as well as α-adrenoceptors, are found on sympathetic neurons and have been shown to mediate synaptic transmission (8, 37, 38); hence, SNA increases recorded during hypertension were potentially due to ANG II receptor or vascular α-adrenoceptor stimulation. However, as SNA also increased with hypertension induced by intra-arterial balloon inflation, receptor stimulation cannot account for the increases in SNA observed (Fig. 7).
With the exception of one lamb, all methods of raising AP induced SNA increases in a similar pattern, with the augmentation of SNA beginning at an apparent AP threshold, rather than after a fixed delay following the onset of AP increase. Threshold values of AP averaged 40 mmHg (56%) above baseline with adrenaline injections (Fig. 2); the corresponding values were 36 mmHg and 40% in balloon inflations (Fig. 6). The time of SNA increase was evidently related to the slope of the AP increase: being delayed when AP rose slowly, as exemplified in Fig. 1 or prompt when AP rose rapidly as with balloon inflation (Fig. 5). Further evidence for the presence of a threshold was provided by the respiratory modulation of SNA that was present at elevated AP but not at baseline pressure (Fig. 1).

Arterial baroreceptors are the likely candidates for receptors that would signal a sudden increase in AP above a set threshold and trigger a reflex increase in cerebral SNA. Previously, a baroreceptor mechanism has been demonstrated to mediate SNA modulation arising from cyclic blood pressure variations associated with artificial ventilation (17). The basis of the ventilatory modulation of SNA observed in our study is also likely to be explained by baroreceptor stimulation, as the modulating effect was most prominent at high levels of AP and as baroreceptors are involved in the translation of ventilatory activity to modulation of AP (17, 26). Ventilatory modulation of SNA was infrequent in our study, at variance with a

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previous study in which it was present in most fibers of the sympathetic trunk of rats (17) but in keeping with another in which it was rare in neurons supplying the hind limb (18). Possibly, ventilatory modulation of SNA in our study may have been increased if the cyclic variation of AP was enhanced, as has been found in the rat (19).

Threshold values for the onset of $$\text{SNARMS}$$ were reached when AP was increased by 40–60% above baseline (Fig. 7), equivalent to a mean arterial pressure of 100–115 mmHg. This threshold value is similar to the upper limit of cerebral blood flow autoregulation in the lamb of 100 mmHg (23, 31). Speculatively, exceeding the upper AP limit of the autoregulation curve may also be the trigger for a reflex increase in cerebral sympathetic vasoconstrictor activity. The functional significance of a threshold for augmented SNA may be protection of the cerebral microvasculature against high perfusion pressure.

Importantly, we found essentially no evidence for activation of cerebral SNA in response to systemic hypotension. In the majority of studies, SNA activity increased as pressure rose but did not increase as arterial pressure was lowered, suggesting that the population of SCG neurons that we sampled was activated in a pressure-protective pattern specific to the brain and not as a component of a widespread, systemic reflex restoration of arterial pressure. Exceptionally, in a single lamb, we observed a reversed pattern of SNA responses to AP variation. In this lamb, hypertension resulted in a decrease of SNA, while hypotension did elicit an increase in SNA, a pattern that would be typical of neurons involved in systemic pressure regulation.

With the intention of mainly sampling neurons innervating cerebral vessels, we inserted electrodes into the cranial aspect of the SCG, as this region predominantly contains neurons projecting to the brain. Of the total population of neurons in this region of the ganglion, as many as 75% project to the internal carotid nerve (ICN) (5, 15, 21, 28). Of the ICN-derived fibers, the overwhelming majority (90%) are likely to innervate cerebral vessels, based on the much greater mass and blood flow of the brain compared with the other tissues innervated by the ICN, principally skin (33). Though we did not ascertain the structures innervated by the recording neurons, in the exceptional lamb in which an increase in SNA was observed with hypotension, it is likely that the microelectrodes were sampling the remaining 25% of neurons innervating extra-cerebral structures, such as the eye, which respond to hypotension with vasoconstriction (9).

In developing the SNA recording method, we excluded movement artifact as the primary source of the SNA signal by filtering low frequencies, carefully securing electrodes, and by performing measurements during brief interruptions and modifications of lung ventilation, the principal source of movement. Significant SNA power was observed at 0–10 Hz, consistent with previous descriptions of the action potential firing rates of sympathetic neurons, which are typically <10 Hz (25, 29). In a control study, SNA power was 200-fold greater at elevated levels of blood pressure than at baseline pressure and promptly fell to zero when the animal was killed, while the lung continued to be ventilated (Fig. 1). The disappearance of the electrical signal post mortem excludes ventilator-induced movement (and amplifier noise) as the primary source of the SNA signal. Additionally, the time delay between the onset of AP elevation and the rise in SNA, together with the small amount of spectral power in the range of heart frequencies, excludes pulsatile movement artifact from nearby arteries as a contaminant of SNA.

**Fig. 6.** Coherent averages ± SE of MAP and $$\text{SNARMS}$$ during intra-arterial balloon inflation (dashed box, $$n = 3$$). Note the close correspondence of $$\text{SNARMS}$$ with MAP. The onset of $$\text{SNARMS}$$ increase occurs when MAP increases to 38% above baseline.

![Coherent averages ± SE of MAP and SNARMS during intra-arterial balloon inflation](image-url)
Tonic SNA was present in these preparations, as demonstrated by a slight (15%) decrease in the SNA signal post mortem. Previous studies have shown the presence of tonic SNA from single-unit recordings in the SCG (24, 29). As some anesthetic agents have been shown to suppress autonomic nervous activity (20), tonic SNA may be more pronounced in conscious animals. Supporting this suggestion, SCG ablation increased baseline cerebral blood flow by ~1/3 in lambs undergoing spontaneous sleep-wake cycles in the study of Loos et al. (27).

These new data provide additional support for the concept that the sympathetic nervous system plays a protective role in the cerebral circulation by preventing excessive perfusion. Stimulation of the sympathetic trunk during acute hypertension limits increases in cerebral blood flow (4, 6, 41). Cervical ganglionectomy has been shown to result in elevated baseline CBF, as well as greater CBF increases associated with natural blood pressure surges (27). Until the present study, no evidence based on direct neural recordings of SNA during potentially damaging elevations in AP was available. Together, these studies suggest that natural sympathetic activation may protect the brain from excessive blood flow, raised microcirculatory pressure, edema, and hemorrhage.

**Perspectives and Significance**

SNA directed to cerebral vessels increases with acute hypertension, but not with hypotension, suggesting that it serves a protective function for the cerebral microcirculation, and not a regulatory role for maintenance of systemic arterial pressure. Measurement of cerebral SNA in a conscious, spontaneously behaving animal would enable the “protective hypothesis” to be examined during natural increases of cerebral perfusion pressure, such as those that occur commonly in REM sleep and sleep disrupted by conditions such as obstructive sleep apnea (16, 34).

**ACKNOWLEDGMENTS**

We express our gratitude to Vojta Brodecky and Dr. Susan Feng for expert technical assistance.

**GRANTS**

This work was supported by the National Health and Medical Research Council of Australia.

**REFERENCES**


