Continual recordings of cardiac sympathetic nerve activity in conscious sheep

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Jardine, David L., Christopher J. Charles, Ian C. Melton, Clive N. May, Melanie D. Forrester, Christopher M. Frampton, Sinclair I. Bennett, and Hamid Ikram. Continual recordings of cardiac sympathetic nerve activity in conscious sheep. Am J Physiol Heart Circ Physiol 282: H93–H99, 2002.—Cardiac sympathetic nerve activity (CSNA) is of major importance in the etiology of heart disease but is impossible to measure directly in humans. Ovine and human cardiovascular systems are similar; therefore, we have developed a method for the daily recording of CSNA in conscious sheep. After thoracotomy, electrodes were glued into the left thoracic cardiac nerve and CSNA, blood pressure (BP), and heart rate were recorded daily. Satisfactory recordings ≥7 days of CSNA were obtained in 11 of 28 sheep (40%).

Methods. First, baroreflex-modulated sympathetic activity represents only one part of the control of CSNA, which is also affected by higher functions, respiratory pathways, and chemoreceptors (12). Second, anesthesia decreases RSNA and CSNA (8, 27); and third, control of CSNA may be different to that of RSNA and MSNA (17).

We have attempted to overcome these problems by implanting long-term microneurographic recording electrodes in the cardiac sympathetic nerves to measure efferent, postganglionic CSNA continually in conscious sheep. This technique requires cardiac nerves of sufficient size to be easily identified and dissected at operation. The sheep heart is similar to that of the human in many ways, including dimensions of the chambers, coronary anatomy, and magnitude of hemodynamic variables such as blood pressure (BP), heart rate (HR), and cardiac output (22). Sympathetic innervation of the heart is bilateral and from the upper thoracic roots is similar to that of the human (24). Our laboratory has undertaken extensive cardiac neuroendocrine studies in sheep because of these advantages (3).

We wanted to know 1) whether it is possible to record CSNA in conscious animals each day; 2) the median duration of the recordings; 3) the proportion of thora-tomized animals in which recordings are possible for 1 wk or longer; 4) how CSNA changes after thoracot-
omy and if so, whether this is modulated by baroreflex activity; and 5) whether low frequency HR variability (LFHRV) correlates with CSNA.

METHODS

All procedures were undertaken in accordance with local animal ethics committee approval.

Electrode manufacture. A stainless steel insect pin (0.1 mm diameter \times 10 mm length) was etched in acid to a fine point at both ends. One end was embedded in a 25-strand stainless steel wire-connecting lead and glued with cyanoacrylate leaving only a 1.5-mm recording tip protruding. The connecting lead measured 40 cm in length and was Teflon coated (Ben Clark; North Liberty, IA).

Thoracotomy and electrode placement. Twenty-eight adult Coopworth sheep were starved for 48 h and water restricted for 24 h before surgery. The well-established method of recording RSNA in sheep (23) was modified for the purposes of recording from the thoracic cardiac nerve. Surgical preparation was conducted with the animal under standard thiopentone (17 mg/kg), halothane, and nitrous oxide-oxygen anesthesia. With the animal in the left lateral position, the left forelimb was manipulated to provide access to the body of the fifth rib inferior to the scapula. A left thoracotomy incision (\approx 20 cm across) exposed the muscles deltoideus, trapezius, and omotransversarius, which were split and retracted to provide access to the rib. The periosteum was incised and the rib removed. The thorax was clamped open, the parietal pleura was opened, and the lung was retracted using wet packs. The thoracic cardiac nerve(s) was then identified running obliquely across longus colli and the esophagus to form an arcade on the superior margin of the azygus vein, receiving branches from adjacent thoracic ganglia before reaching the base of the heart where it formed the cardiac ganglion (Fig. 1) (24). After the nerve was dissected clear of fat and tissue, the exposed tips of the electrodes were pushed obliquely through the nerve sheath, ensuring they were positioned in the center of the nerve. Up to five electrodes were implanted and glued in place with cyanoacrylate glue (Selley's superglue) and the connecting leads were exteriorized through the thoracotomy wound. A Camino arterial catheter (Medtronic AVE) was placed in the left common carotid for continuous BP monitoring (model 110-4 Camino; San Diego, CA), and a central venous line was positioned in the internal jugular vein. Subcutaneous electrodes were applied to each limb quarter for electrocardiogram (ECG) recordings. The intercostal space and split muscle were repaired using synthetic nonabsorbable suture. A drain tube was placed in the thorax to reestablish negative intrathoracic pressure postoperatively. The skin was closed with silk sutures and string ties attached to the wool. The drain was generally removed 24 h postoperatively and intramuscular pethidine (50 mg) was administered for postoperative analgesia.

CSNA recordings. Sympathetic recordings were made from the electrodes via a preamplifier with an active probe (model DAM-80; World Precision Instruments). The raw signal was processed by a nerve traffic analyzer and stored continuously with on-line BP and ECG in a dedicated personal computer using locally written software. The raw signal was filtered between 300 and 3,000 Hz and integrated using a time constant of 100 ms. Postganglionic efferent sympathetic nerve activity was identified by the following characteristics: 1) bursts were synchronized to the arterial pulse; 2) bursts decreased during a hexamethonium infusion (2 mg/kg over

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**Fig. 1.** Cardiac nerves and related ganglia of sheep; left lateral view. Thoracic cardiac nerve(s) (13) run obliquely across longus colli (B) and the esophagus (C) to form an arcade on the superior margin of the azygus vein, receiving branches from adjacent thoracic ganglia (3b–3h) before reaching the base of the heart where it forms the cardiac ganglion (6). The recording electrodes are glued into the cardiac nerve(s) and the connecting leads run from the thoracotomy wound as indicated by the arrows. [Adapted from McKibben and Getty (24)].
2 h, performed in two animals only; 3) there was an inverse relationship between burst area and diastolic BP, most obvious during baroreflex tests performed on all animals (see below) (37). Only recordings with a signal-to-noise ratio of >2 were analyzed. CSNA was quantified by: 1) counting the number of bursts per minute (burst frequency); 2) counting the number of bursts per 100 heartbeats (burst incidence); and 3) measuring the area under the integrated signal per minute (burst area).

Baseline recordings. All recordings were undertaken by the same technician in a dedicated room between 9:00 and 11:00 AM each day while the animals were standing in cages. After the recordings, the animals were disconnected for the remaining 22 h of the day. Care was taken to ensure the animals were not disturbed, and recordings were only used if the variables were stable over the preceding 10 min. Simultaneous BP, HR, and CSNA levels were averaged each minute during a 5-min recording interval every day.

Baroreflex control of CSNA and HR. After baseline measurements of BP, HR, and CSNA, BP was raised with an intravenous bolus of phenylephrine (150 µg) to achieve a maximum increase in diastolic BP of 10 mmHg over 1 min. One minute later, intravenous nitroprusside was given (150 µg), to induce a 20 mmHg ramp decrease in diastolic BP over the second minute (Fig. 2). The area under the integrated nerve signal and the duration of the R-R intervals were expressed as percentages of baseline levels and averaged every 5 s for 120 s. These values were logged and plotted against percent change in diastolic and systolic BP to calculate baroreflex control of CSNA and HR on days 3 and 6 after thoracotomy, respectively.

HR variability. All sinuses beats from the 5-min interval were analyzed from the ECG signal recorded at the same time as CSNA was measured. Frequency domain analysis of HR variability was undertaken using the fast Fourier transform method. The current guidelines for HRV are empirically derived, mainly from human studies and no frequency domain analysis has been undertaken on sheep (2, 38). We measured spectral power in two frequency domains: LFHRV 0.06–0.1 Hz and high-frequency HRV (HFHRV, 0.15–0.4 Hz). We used absolute LFHRV power (ms²) and normalized LFHRV power (normalized units, nu) to assess sympathetic and parasympathetic effects on the sinus node (17, 21). HFHRV is thought to represent mainly parasympathetic cardiac activity but it is subject to respiratory rate and tidal volume, both of which are variable in sheep after thoracotomy (38). In one animal, we measured the effects of β-blockade on LFHRV and atropine on HFHRV as a means of validating the frequency domains used.

Statistics. Within-group comparisons were made using ANOVA for repeated measures and where time effects were indicated, Fisher’s least-significant difference test was used to compare specific time points with baseline. Individual correlations between variables were made using Pearson’s correlation coefficient. Group correlations between CSNA and LFHRV were made using a general linear model, which allows for differences between animals.

RESULTS

Continuous recordings of CSNA were possible for over a week in 11 of 28 sheep. Nine recordings lasted between 7 and 14 days. Two recordings lasted over 30 days and an example is shown (Fig. 3). Mean length of recording was 10.6 days (range 7–47). In the remaining 17 animals, there were five perioperative deaths and 12 unsatisfactory recordings. Unsatisfactory recordings included 10 sheep with absent recording fields from day 1, and two sheep with recordings lasting <7 days. CSNA burst area decreased during hexamethonium infusion in both sheep tested, and an example is shown in Fig. 4. MBP and CSNA burst area decreased from 92 to 65 mmHg and from 35 to 6 U/min. HR increased from 110 to 135 beats/min. LFHRV increased from 0.16 ms² at baseline to 0.2, 10 min after atropine 0.4 mg iv and decreased from 0.16 ms² to 0.12, 20 min after atenolol (1 mg iv).

Changes in MBP, HR, CSNA (bursts/min and burst area/min) are shown for the 11 sheep during the first 7 days after thoracotomy (Fig. 5). CSNA burst frequency (P = 0.001) and incidence (P = 0.01) changed with time, whereas burst area (P = 0.3) was not significantly changed. MBP changed with time (P = 0.02), whereas HR was stable (P = 0.1). CSNA burst frequency gradually decreased from baseline at 78 ± 8 bursts/min to be lower by day 5 (60 ± 7 bursts/min, P = 0.02) and remained low on days 6 and 7 (57 ± 7 bursts/min, P = 0.008 and 54 ± 6 bursts/min, P = 0.003). CSNA burst area tended to decrease from 52 ± 7 U/min at baseline to 40 ± 6 U/min on day 3 (P = 0.2) and remained at a similar level, thereafter (38 ± 12 U/min on day 7, P = 0.29). CSNA burst incidence decreased from baseline at 76 ± 9 to 57 ± 7 bursts/100 beats on day 7 (P = 0.04). MBP decreased gradually during the first 7 days from 102 ± 4 mmHg at baseline
to be lower by day 7 (94 ± 4 mmHg, \( P = 0.03 \)), whereas HR tended to decrease from 105 ± 6 to 97 ± 4 beats/min (\( P = 0.11 \)). Absolute LFHRV power decreased with time (\( P = 0.02 \)), whereas normalized LFHRV remained stable (\( P = 0.43 \)). Absolute LFHRV power decreased from 0.12 ms\(^2\) at baseline to be lower on day 6 (0.06 ± 0.02 ms\(^2\), \( P = 0.004 \)) and remained at 0.06 ± 0.02 ms\(^2\) on day 7 (\( P = 0.009 \)) (Fig. 6). Normalized LFHRV tended to decrease gradually from 0.19 nu at baseline to 0.14 nu on day 7 (\( P = 0.15 \)). No overall correlation was found between daily measures of LFHRV and CSNA bursts/min (\( R^2 = 0.02, P = 0.5 \)) or any other CSNA parameters. Analysis of limited data from five animals recorded beyond 7 days did not show progressive decrease in CSNA, MBP, HR, or LFHRV during the second week and subsequent weeks, although there was no significant correlation between CSNA and LFHRV. BP and HR followed a similar pattern, consistent with this observation. Baroreflex control of CSNA and HR remained constant implying that the decrease in CSNA was modulated by other mechanisms.

**DISCUSSION**

Satisfactory recordings of CSNA were achieved each day for a mean duration of 10.6 days after implantation of electrodes in 40% of sheep studied. We believe this to be the longest duration of CSNA recordings undertaken in conscious animals to date. CSNA and LFHRV decreased during the first week after thoracotomy and appeared to stabilize during the second week and subsequent weeks, although there was no significant correlation between CSNA and LFHRV. BP and HR followed a similar pattern, consistent with this observation. Baroreflex control of CSNA and HR remained constant implying that the decrease in CSNA was modulated by other mechanisms.

**Advantages of conscious CSNA recordings.** The recording of CSNA in conscious animal models promises to be the most direct and sensitive method for assessing changes in cardiac sympathetic control (12, 25). CSNA is easily quantifiable by measuring burst frequency, incidence, and area from the integrated waveform, but the field must be verified daily using standard criteria for sympathetic microneurography (37). We were also able to construct baroreflex slopes for CSNA and BP similar to those reported in RSNA studies (6–8, 15, 40). By measuring CSNA at the same time each day under stable conditions, we were able to demonstrate changes that occurred independently of baroreflex activity. Consistent daily recording times are important, because both absolute sympathetic activity and baroreflex slopes may be subject to circadian variation (26, 36). Reproducible resting recordings give additional information that may be more relevant to the conscious human than baroreflex ramp tests under general anesthesia or immediately after surgery.

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*Fig. 3. Conscious recordings averaged from five 1-min samples between 9:00 and 11:00 AM each day for 32 days after thoracotomy in a single animal. CSNA is measured in bursts per minute (b/min). MBP, Mean blood pressure; LFHRV, low-frequency HR variability.*

*Fig. 4. Decrease in BP and CSNA observed after hexamethonium 120 mg iv over 60 min in one animal. MBP decreased from 92 to 65 mmHg; CSNA burst area decreased from 35 to 6 U/min; and HR increased from 110 to 135 beats/min.*
ever, the finding that CSNA baroreflex activity remained constant for at least 7 days after surgery is reassuring for the interpretation of the RSNA studies. We hypothesize that CSNA is increased by the stress of thoracotomy and takes at least 5 days to return to its steady-state level. Therefore, measurements taken during the first week in postoperative animal models may be difficult to interpret (11, 27).

Most of the recent data on sympathetic activity and cardiac function comes from RSNA and CSNA recordings in anesthetized animals (7, 11, 20, 39, 40). Unfortunately, it has been clearly demonstrated that general anesthesia decreases RSNA in the rabbit and CSNA in the cat (8, 27). Therefore, measurement of CSNA in conscious animals may be more applicable to clinical studies of heart failure in humans (5, 14). In addition, CSNA should be measured rather than RSNA or MSNA because CSNA increases before RSNA in heart failure, and there is differentiation of sympathetic activity between vascular beds (18, 29, 33).

**HRV and CSNA.** The simultaneous measurement of LFHRV and CSNA in the conscious animal is a novel approach to the assessment of HR variability as a noninvasive index of cardiac sympathetic activity (9). Because the HR of the sheep is similar to that of the human, the LF peak of spectral power (0.06–0.1 Hz) is also likely to be similar and was, therefore, chosen for this experiment. LF power is thought to represent baroreflex feedback on the sinus node in response to sympathetic effects on the vasculature, and we observed appropriate changes during parasympathetic and sympathetic inhibition. We demonstrated that although LFHRV and CSNA decrease in parallel during the week after thoracotomy, there was no clear correlation between the two. This is consistent with the human studies, in which no correlations were observed in normal subjects between baseline values of MSNA and LFHRV (30, 31, 34). In addition, heart failure studies have shown decreased LFHRV when cardiac sympathetic activity is increased (9, 19, 35). Although LFHRV has been shown to correlate with MSNA dur-
ing sympathetic stimulation, it is probably determined by both sympathetic and parasympathetic effects on the sinus node. We conclude that absolute indices of sympathetic activity, including MSNA and CSNA are not comparable to oscillatory indices such as LFHRV and should not be used interchangeably. We were only able to show a significant decrease in absolute power LFHRV, because HFHRV was extremely variable between animals, and this would have affected normalized LFHRV measurements. We suspect this is because parasympathetic control of HR is closely linked to respiratory rate and tidal volume, both of which were variably affected by thoracotomy.

Limitations of the study. Unfortunately, the manufacture and placement of the recording electrodes is technically demanding, and the recordings are difficult to maintain. In addition to our validation techniques, postganglionic CSNA should be confirmed by ganglion blockade in all animals but we were concerned that this would cause prolonged hypotension and distress, even with phenylephrine cover. We had to strike a balance between validation of CSNA and preservation of the recordings. We had no way of predicting which electrodes would produce the recordings of longest duration and in over 50% of animals used, no recordings were possible. This was probably because the electrodes tended to pull from the nerves as the animal recovered after surgery. On the basis of limited data from five animals during the second week, it appeared that CSNA did not decrease further with time. Regular validation of the nerve signal was required to ensure that any apparent decrease was not secondary to loss of the recording field. We recorded multifiber postganglionic activity from the nerves supplying only the left side of the heart. Although the same cardiothoracic nerve was used in most animals, up to five electrodes were placed, and in some animals, more than one nerve was used (24). Detailed studies on cardiac sympathetic nerves in dogs have shown that they receive input from a number of thoracic ganglia apart from those in the paravertebral sympathetic chain, and there is considerable overlap from ipsilateral and contralateral nerves within most portions of the heart (1, 32). As in the human, the sympathetic nerves on the left predominantly supply the left ventricle and those on the right contribute more to the sinus node. This may be a source of variability of CSNA measurement between animals and may also explain the lack of correlation between CSNA and LFHRV. We did not measure respiratory rate and so cannot confirm our suggestion that this may have caused major variation in HFHRV and normalized LFHRV. The fast Fourier method for HRV requires stationarity for calculating spectral power and at least 264 heartbeats. We were, therefore, unable to measure changes in spectral power during the nitropressive BP ramps as a means of validating the LF and HF bands. For practical reasons, it was only possible to record during 2 h of each day. We attempted to decrease sampling variation by ensuring the animals were hemodynamically stable and the recordings were made between 9:00 and 11:00 AM. Human studies have demonstrated circadian variation of MSNA baroreflex slopes and MSNA variation during sleep (26, 36).

In summary, we have demonstrated it is possible to undertake daily CSNA recordings in conscious sheep and that CSNA decreases during the first week after thoracotomy. As expected, this change does not appear to be modulated by baroreflexes. After thoracotomy, LFHRV does not correlate with CSNA. Daily analysis of resting CSNA in the conscious animal may provide new insights in the investigation of cardiac disease.

Allastair McGill, Medical Illustration Department, Christchurch Hospital, prepared the figures.

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REFERENCES


