Effects of Spinal Section and of Positive-Feedback Excitatory Reflex on Sympathetic and Heart Rate Variability

Nicola Montano, Chiara Cogliati, Valdo Jose Dias da Silva, Tomaso Gnecchi-Ruscone, Marcello Massimini, Alberto Porta, Alberto Malliani

Abstract—The sympathetic outflow appears to be capable of displaying a rhythmicity synchronous with cardiovascular Mayer’s waves even after spinal section. To test the hypothesis that spinal sympathetic low frequency (LF) oscillation can be enhanced during sympathetic excitation, we recorded cardiac sympathetic nerve activity (SNA), R-R interval, arterial pressure, and ventilation in 9 unanesthetized decerebrate-vagotomized cats before and after C1 spinal section. LF and high frequency (HF) components were detected in the variability of SNA, R-R interval, and systolic arterial pressure both before and after spinal section. In this latter condition, a significant coherence between LFSNA and LF R-R was present in 5 animals, whereas HF SNA and HF R-R were correlated in 4 animals. During an excitatory sympathetic spinal reflex elicited by aortic constriction, the efferent sympathetic firing was markedly enhanced (from 7 to 33 spikes/s); concomitantly, the powers of both LFSNA and HF SNA were also increased. Coherence between LFSNA and LF R-R became significant in all cases, whereas HF SNA and HF R-R became correlated in 6 animals. In 3 animals, the reflex sympathetic excitation was no longer elicitable after interrupting a vast contingent of sympathetic afferents by means of thoracic dorsal root section. We report for the first time that LF and HF oscillations are detectable in SNA, R-R interval, and systolic arterial pressure variabilities of decerebrate-vagotomized spinal cats and that an excitatory spinal reflex is capable of increasing the power of both SNA spectral components. (Hypertension. 2000;36:1029-1034.)

Key Words: autonomic nervous system ■ sympathetic nervous system ■ heart ■ reflex

The role of cardiovascular sympathetic afferent fibers1 in mediating excitatory reflexes with positive feedback characteristics has been demonstrated in both anesthetized cats2 and conscious dogs.3 Furthermore, sympathetic excitatory reflexes may play a major role in determining the sympathetic overactivity that often accompanies acute myocardial ischemia4 and heart failure.5,6 Spinal sympathetic excitatory reflexes, in addition, may represent the major mechanism leading, in tetraplegic patients, to dramatic hypertensive crises.1,6 On the other hand, Fernandez de Molina and Perl7 were the first to report that experimental animals with cervical spinal section could exhibit oscillations of the sympathetic efferent discharge in phase with Mayer’s waves. However, in the absence of spectral methodology, their description was merely qualitative. Recently, it was found that tetraplegic patients can have low frequency (LF) oscillations, now quantified with spectral techniques,,8-10 in their heart period (R-R interval) and systolic arterial pressure (SAP) variabilities.11,12 These findings are in keeping with an LF oscillation originating, at least in part, at the spinal level. This rhythmicity is likely to arise from the contribution of both peripheral13 and central14 mechanisms according to physiological closed-loop conditions.8,15

The purpose of this study was to investigate whether a positive-feedback spinal reflex mediated by cardiovascular sympathetic afferent fibers was capable of increasing spinal rhythmicity. The major novel findings that we report here are that (1) in decerebrate-vagotomized cats with a subsequent cervical spinal section, LF and high frequency (HF) spectral components are present in sympathetic nerve activity (SNA), R-R interval, and SAP variability; (2) the powers of LFSNA and HF SNA are markedly increased during a sympathetic excitation obtained with a positive-feedback reflex mechanism elicited by aortic constriction.

Methods

All experiments were approved by the Animal Care Committee of the Italian Health Institute.

General Surgical Procedure

Successful experiments were performed on 9 cats (2.5 to 3.5 kg). Transient anesthesia was induced with intramuscular injection of

Received May 11, 2000; first decision June 1, 2000; revision accepted June 20, 2000.
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ketamine (10 mg/kg) and propiomazine (4 mg/kg) to decerebrate the animals by midcollicular transection, after which the forebrain was removed by suction. The decerebration procedure allowed us to carry out the rest of the experiment without the depressive influence of anesthesia on neural structures.

The trachea was cannulated and artificial ventilation was performed while maintaining end-tidal CO₂ within physiological range. Bilateral cervical vagotomy was performed in all animals to abolish cardiac vagal modulation and to restrict cardiac innervation to sympathetic circuits while leaving the arterial baroreflex mechanisms qualitatively unaltered.

A polyethylene catheter was inserted into the thoracic aorta and a Swan-Ganz catheter introduced into the inferior vena cava. After paravertebral abdominal incision, the aorta was exposed and isolated: A ligature that was passed around the upper part of its abdominal segment made it possible to obtain gradual constrictions whenever necessary. Inflations of the Swan-Ganz balloon were used to reduce venous return and, consequently, arterial pressure.

The left stellate ganglion and its branches were exposed retropleurally. From the cut central end of the third white ramus communicans, known to contribute importantly to the efferent innervation of the heart, preganglionic fibers were isolated and filaments that were responsive to baroreflex mechanisms were selected by means of either increases or decreases in aortic pressure. SNA, ECG, thoracic aortic pressure, and ventilation were recorded and stored as previously reported. While the same nerve recording was maintained, each animal underwent a subsequent spinal section at C1 level, without additional anesthesia, which was made unnecessary by previous decerebration. The completeness of both neural transections was confirmed at autopsy.

In 3 decerebrate-vagotomized animals, before the spinal section and isolation of the sympathetic nerve filament, a dorsal laminectomy from C6 to T8 vertebral segments was carried out to isolate the dorsal roots from C7 to T7.

Experimental Protocol
Recordings corresponding to baseline conditions (10 minutes) were obtained at least 2 hours after decerebration and vagotomy and again 3 to 4 hours after spinal section. In this latter condition, recordings (5 minutes) were also obtained during aortic constriction, eliciting sympathetic excitation. To demonstrate the reflex nature of this response, the recordings were repeated in 3 experiments after cutting the dorsal roots C7 to T7.

Data Processing and Analysis
All signals were A/D converted and sampled at a frequency of 3 kHz. Sympathetic multiunit discharge signal was assessed by a
Spectral Measurements of SNA, R-R Interval, and SAP Variabilities in Decerebrate-Vagotomized Cats (n=9) Before and After Spinal Section

<table>
<thead>
<tr>
<th>Variables</th>
<th>Decerebration Baseline</th>
<th>Spinal Section Baseline</th>
<th>Aortic Constriction</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, spikes, s</td>
<td>36.9±7.7</td>
<td>7.6±4.7*</td>
<td>33.6±7.1†</td>
</tr>
<tr>
<td>Variance, (spikes/s)^2</td>
<td>215.6±57.4</td>
<td>9.9±3.4*</td>
<td>203.6±75.7†</td>
</tr>
<tr>
<td>LF, Hz</td>
<td>0.1±0.01</td>
<td>0.09±0.01*</td>
<td>0.09±0.01*</td>
</tr>
<tr>
<td>LF (spikes/s)^2</td>
<td>96.3±4.5</td>
<td>2.4±0.8*</td>
<td>65.4±26.4†</td>
</tr>
<tr>
<td>HF, Hz</td>
<td>0.33±0.01</td>
<td>0.33±0.01</td>
<td>0.34±0.01</td>
</tr>
<tr>
<td>HF (spikes/s)^2</td>
<td>71.7±20.7</td>
<td>2.4±0.8*</td>
<td>70.3±27.6†</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.4±0.2</td>
<td>1.5±0.4</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>R-R</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean, ms</td>
<td>349±19</td>
<td>499±37*</td>
<td>474±35*</td>
</tr>
<tr>
<td>Variance, ms^2</td>
<td>0.4±0.2</td>
<td>1.1±0.4</td>
<td>2.1±0.8</td>
</tr>
<tr>
<td>LF, Hz</td>
<td>0.09±0.01</td>
<td>0.07±0.01*</td>
<td>0.07±0.01*</td>
</tr>
<tr>
<td>LF, ms^2</td>
<td>0.3±0.2</td>
<td>0.3±0.1</td>
<td>1.3±0.8</td>
</tr>
<tr>
<td>LF, nu</td>
<td>42.4±12.1</td>
<td>52.6±6.8</td>
<td>60.7±8.5</td>
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<tr>
<td>HF, Hz</td>
<td>0.32±0.01</td>
<td>0.33±0.01</td>
<td>0.33±0.01</td>
</tr>
<tr>
<td>HF, ms^2</td>
<td>0.04±0.01</td>
<td>0.1±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>LF/HF</td>
<td>17.2±3.1</td>
<td>20.7±4.6</td>
<td>19.9±4.8</td>
</tr>
<tr>
<td>LF/HF</td>
<td>3.9±2.3</td>
<td>4.6±2.4</td>
<td>15.9±8.6</td>
</tr>
</tbody>
</table>

| SAP        |          |            |             |
| Mean, mm Hg | 119±5 | 122±3 | 151±8 |
| Variance, mm Hg^2 | 14.1±8.3 | 21.7±11.8 | 24.0±9.6 |
| LF, Hz     | 0.08±0.01 | 0.05±0.01* | 0.08±0.01 |
| LF, mm Hg^2 | 1.5±0.4 | 2.2±1.5 | 10.3±7.7 |
| LF, nu     | 25.6±3.9 | 33.8±9.0 | 27.9±12.9 |
| HF, Hz     | 0.33±0.01 | 0.33±0.01 | 0.32±0.01 |
| HF, mm Hg^2 | 8.3±5.3 | 7.3±4.8 | 8.9±6.3 |
| HF, nu     | 55.5±5.9 | 57.9±8.0 | 51.3±8.5 |
| LF/HF      | 0.6±0.1 | 1.1±0.5 | 1.6±1.2 |

\(^*P<0.05 \text{ (vs baseline decerebration+vagotomy)}; \dagger P<0.05 \text{ (after spinal section, baseline vs aortic constriction).}\)

digital spike counter every 20 ms. Neural counts were then low-pass filtered by means of an FIR filter with cutoff frequency at 1 Hz. The neural signal was sampled in correspondence of each R peak. Beat-by-beat series were analyzed by autoregressive parametric spectral and cross-spectral analysis. The squared coherence function ($K^2$) was used to determine whether spectral components found in the SNA variability were correlated to those present in R-R interval variability and to ventilation. Only values of $K^2 > 0.5$ were considered significant according to the original work by de Boer et al. An average $K^2$ value was calculated among significant cases only.

Statistical Analysis

Differences between various conditions were assessed by 1-way ANOVA for repeated measures followed by Newman-Keuls test when the variables were normally distributed or by Friedman’s test when a skewed distribution was present; a level of $P<0.05$ was considered significant.

Results

In unanesthetized decerebrate-vagotomized animals, mean SNA was 36.9±7.7 spikes/s, R-R interval was 349±19 ms, and SAP was 119±5 mm Hg (Figure 1 and Table). Spectral profiles of SNA, R-R, and SAP variabilities were characterized by LF and HF components (Figure 2 and Table) as previously reported for decerebrate unanesthetized cats without vagotomy. A significant coherence between LF SNA and LF R-R was present in 7 cases ($K^2=0.72±0.07$), whereas the coherence between HF SNA and HF R-R ($K^2=0.86±0.05$) and HF SNA and ventilation ($K^2=0.94±0.02$) was significant in all 9 animals.

Three to 4 hours after spinal section, mean SNA was markedly reduced to $7.6±4.7$ spikes/s ($P<0.01$) (Figure 1 and Table). R-R interval was significantly increased to 499±37 ms ($P<0.001$), whereas SAP was unchanged (122±3 mm Hg). Also in this condition, spectral profiles of SNA, R-R, and SAP variabilities were characterized by LF and HF oscillations (Figure 2 and Table). However, LF SNA and HF SNA were markedly reduced in their absolute values, reflecting the drastic reduction of variance, whereas their normalized values and the LF/HF ratio were not significantly modified. Interestingly, a significant decrease in LF central frequency was observed after spinal section in all variability signals (Table). Coherence analysis revealed a significant correlation between LF SNA and LF R-R in 5 cases ($K^2=0.65±0.05$). In 4 animals, a significant coherence was present between HF SNA and HF R-R ($K^2=0.67±0.03$) and between HF SNA and ventilation ($K^2=0.62±0.11$).

During aortic constriction, SAP raised to 151±8 mm Hg ($P<0.01$) and mean SNA markedly increased to 33.6±7.1 spikes/s ($P<0.001$), whereas R-R interval was unchanged (Table). The marked excitation of SNA was associated with a similar increase in its variance and in the absolute powers of both LF and HF components (Figure 2 and Table). However, the fractional distribution of power in SNA variability was unmodified and, accordingly, LF normalized units (nu), HF nu, and LF/HF ratio were unchanged. Notably, LF SNA and LF R-R became coherent in all cases ($K^2=0.71±0.05$), whereas HF SNA became correlated with HF R-R ($K^2=0.72±0.06$) and with ventilation ($K^2=0.81±0.06$) in 6 animals. Figure 3 exemplifies an experiment in which LF SNA and LF R-R were not correlated in baseline conditions, whereas a significant coherence was present during aortic constriction.

The reflex nature of the sympathetic excitatory response was demonstrated in 3 experiments by interrupting a vast contingent of cardiovascular sympathetic afferent fibers by dorsal root (C7 to T7) section. After this intervention, aortic constriction was no longer accompanied by sympathetic excitation (SNA from 16.3±11.9 to 9.2±7.7 spikes/s; $P=NS$).

Discussion

The following are the novel findings of our study: (1) In decerebrate-vagotomized animals, LF and HF components were present in the variability spectra of SNA, R-R interval, and SAP; (2) similar spectral profiles were observed after an additional cervical spinal section; (3) during a positive-feedback cardiovascular excitatory reflex, a marked incre-
Decerebration and Vagotomy

We have already described the presence of an LF component in the spectral profiles of SNA, R-R, and SAP variabilities in decerebrate unanesthetized cats. However, the additional observation reported in this study is the existence of a similar oscillatory component and in particular of LFR-R in the absence of cardiac vagal innervation, as already suggested by the finding in heart-transplanted patients of this spectral component attributed to sympathetic reinnervation. Thus, these observations strengthen the view that this rhythm can in some circumstances rely only on sympathetic modulation, independent of vagal activity.

The presence of HF SNA after vagotomy, interrupting not only efferent fibers but also the largest population of pulmonary afferents, can be explained by the arterial blood pressure (ABP) changes (HF SAP) induced by the positive-pressure artificial ventilation, sensed by baroreflex mechanisms modulating sympathetic efferent discharge. Obviously, the unphysiological positive-pressure ventilation, acting as a strong forcing input, represents a limitation of our experimental preparation and hence of the interpretation of the HF component. In this regard, the presence of HF_R-R after vagotomy may mainly reflect the mechanical influence exerted by ventilation on sinus node pacemaker activity, as suggested by the observation of an HF_R-R oscillation in heart-transplanted patients.

Spinal Section

The main purpose of our experiments was to investigate the characteristics of neural and cardiovascular oscillatory patterns in the presence of a spinal section, which interrupts the neural pathways linking the supraspinal structures to the sympathetic outflow.

The experiments by Fernandez de Molina and Perl were the first to suggest a spinal genesis for slow sympathetic oscillations corresponding to Mayer’s waves. However, these authors gave only a qualitative description of this neural oscillatory phenomenon and did not address the issue of the relation between this sympathetic oscillation and that present in heart rate variability. Our experiments, by using spectral methodology, quantified the occurrence in vagotomized spinal animals of an LF component in SNA, R-R, and SAP.
variabilities, thus providing further support to the hypothesis of a contribution of spinal structures.

A significant shift of LF component of SNA, R-R, and SAP variabilities toward lower central frequencies was also observed. This finding may depend on the separation of supraspinal oscillators from the spinal structures regulating the sympathetic outflow. In fact, it is likely that in normal conditions, LF and HF rhythms interact continuously, not only in terms of power but also of frequencies.

After spinal section, the detection of an HF SNA might be considered as unexpected because this intervention abolished the possibility that ventilatory blood pressure changes (HFSAP) could affect SNA through baroreflex mechanisms. However, the mechanical stimulus related to ventilation was also likely to activate somatic and visceral afferents projecting to the spinal cord and thus, also in this case, the rhythmic pattern of discharge related to respiration could modulate the sympathetic efferent discharge.

**Positive-Feedback Reflex**

The additional observation that we report is that LF SNA and HF SNA were markedly increased during an excitatory sympathetic spinal reflex induced by moderate aortic constriction. This stimulus has been found quite well suited to gradually activate the afferent sympathetic fibers with aortic endings.

The reflex sympathetic excitation was no longer elicitable after abolishing the afferent input from the cardiovascular system to the spinal cord, obtained by sectioning C7 to T7 dorsal roots. It is interesting to notice that, probably as a consequence of the unanesthetized state, a rise in the aortic pressure elicited an increase in SNA of a magnitude that had not been previously observed in spinal animals under anesthesia. Thus, the relevant finding is that a marked increase in average SNA is capable of drastically potentiating the two rhythmic components already present.

In this regard, we may hypothesize that the recovery with time of an LF component in R-R and SAP variabilities that has been observed in tetraplegic patients may be ascribed to the existence of a spinal rhythmicity enhanced by excitatory reflexes.

Unfortunately, the local mechanical disturbance associated to aortic constriction prevents a sound interpretation of the changes in SAP variability spectral components during the excitatory sympathetic reflex.

On the other hand, the weak changes observed in mean R-R and its variability during the excitatory reflex may be partly ascribed to the interruption of an important contingent of the left cardiac sympathetic innervation, caused by our recording procedures and by the acute abolition of the interaction with vagal modulation likely to potentiate cardiovascular oscillations. Nevertheless, during aortic constriction, LF SNA and LF R-R became coherent in all cases, suggesting an increased coupling within this frequency range.

It is relevant that LF and HF components of SNA and R-R variabilities when expressed in normalized units were unchanged during sympathetic excitation. The normalization procedure as well as the use of the LF/HF ratio have been proposed to underscore, independent of variance, the reciprocal changes of LF and HF that often characterize the spectral profiles of R-R interval and sympathetic activity in physiological conditions. These reciprocal changes have been interpreted as a reflection of sympathovagal balance. Thus, the fact that LF nu and HF nu during a sympathetic excitatory reflex did not undergo reciprocal
changes and, accordingly, LF/HF ratio remained unchanged, suggests that reciprocal pattern organization depends on a supraspinal integration because it was present in decerebrate animals but undetectable in spinal animals.

Conclusions

The occurrence of an LF oscillation in spinal animals and its potentiation during an excitatory reflex strongly suggest a contribution of spinal structures to its genesis. However, this concept is quite different from that of a physiological modulation. Indeed, in normal closed-loop conditions, peripheral vasomotion, autochthonous rhythmicity of the neural substratum, afferent, and efferent pathways, and the multitude of interacting cardiovascular reflexes are all likely to participate in the apparently simple event represented by an LF oscillation.

In this regard, it has been clearly demonstrated that the baroreflex circuitry can oscillate quite efficiently in the LF range, making it likely its important participation in the physiological modulation. However, this does not contrast the view that part of LF rhythmicity may be intrinsic in sympathetic excitation, whatever the mechanisms leading to it.

Acknowledgments

Dr Dias da Silva, a visiting research scientist from the Department of Biological Sciences, Medical School of the Triângulo Mineiro, Uberaba (MG), Brazil, was supported by a Postdoctoral Training Grant (PDE 200838/98-9) from CNPq–Brazil. This work was partly supported by MURST (60% 1999) and ASI (Agenzia Spaziale Italiana, Grant 97). We gratefully acknowledge the assistance of Isabella Ghirardelli in typing the manuscript.

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Hypertension. 2000;36:1029-1034
doi: 10.1161/01.HYP.36.6.1029

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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