An Electrophysiological Experimental Study on the Spontaneous Sympathetic Nerve Activity in the Rostral Ventrolateral Medulla Oblongata

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Introduction

The sympathetic postganglionic nerve fibers, which are controlled by preganglionic fibers originating from specific nuclei in the medulla oblongata, and the thoracic and upper lumbar segments of the spinal cord, together with the local autoregulatory mechanisms and circulating hormones, directly influence the cardiovascular function. Recently, the studies on the sympathetic preganglionic fibers have remarkably progressed, and the anatomical *(Strack et al., 1988)*, functional *(Janig, 1985)*, and chemical *(Krukoff, 1985)* characteristics of the synaptic input have been clarified. However, the peripheral sympathetic nerve activities vary depending on the organs concerned (including the skin, muscle, or internal organs) as they have their own physiological characteristics *(Janig and McLachlan, 1986)* including the response pattern to the peripheral receptor stimulation. Many areas, including the histological and functional roles of the peripheral part, nerve centers, and central pathway of the circulatory system, are still unknown.

The peripheral sympathetic nerve activities in humans consist of the skin sympathetic activity (SSA) that controls the sweat glands / skin vasomotion, and the muscle sympathetic activity (MSA) that controls the vascular smooth muscles in the skeletal muscles, and each activity has different characteristics. SSA involving regulation of the body temperature and MSA involving regulation of the blood pressure can be separately recorded *(Burke et al., 1977; Hagbarth et al., 1975; Vallbo et al., 1979; Wallin and Eckberg, 1982; Yatomi et al., 1989)* from the sympathetic postganglionic efferent fibers by microneurography *(Hagbarth et al., 1972)*.

By recording and comparing the action patterns and responses to stimulations of the premotor nuclei, along with other vital rhythms, we hoped to clarify the complex mechanism of the sympathetic nerve activities and to contribute in the treatment of disorders resulting from sympathetic dysfunction. We also report our findings on the premotor nuclei that produce sympathetic preganglionic fiber activities by using topographic mapping analysis of the changes in the central action potentials in the rostral ventrolateral medulla oblongata (RVLM) region to visually capture the complicated action patterns to compare the cross correlations with MSA and SSA using microneurography and ECG.

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Materials and Methods

Subjects

Thirty male Wistar rats weighing about 300g on average were used. This study was approved and conducted according to the ethical regulations for animal experiments in Kyoto Prefectural University of Medicine (Approval number: M101).

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Methods

a) Measurement of the action potentials of MSA and SSA

Each rat was immobilized on a stereotaxic table after inducing general anesthesia with pentobarbital sodium (PS) (25 mg/kg) while the room temperature was maintained at 26-28°C. The right sciatic nerve was exposed, and tungsten microelectrodes with an apical diameter of 1 μm and impedance of 3-5 MΩ (Unique Medical Co., UJ3002B, Tokyo, Japan) were inserted and fixed in the muscular branch to detect MSA and in the cutaneous branch to detect SSA using microneurography. The action potentials of the sympathetic postganglionic efferent fibers were measured using an amplifier of 500Hz-5 KHz (Nihon kohden Co., MEM 4104, Tokyo, Japan).

b) Measurement of the action potentials in the RVLM (rostral ventrolateral medulla oblongata) region

A longitudinal incision was made posteriorly from the upper cervical vertebrae, the paravertebral muscles were removed, and the interparietal bone was stripped off to expose the region from the pons / medulla oblongata to the caudal cerebellum. 20 channel tungsten electrodes (Unique Medical Co., PS-96006-20, Tokyo, Japan), made by clustering microelectrodes with an apical diameter of 1 μm and impedance of 3-5 MΩ that can detect (through the corresponding electrical leads) 20 potentials from the head and neck transition zone at a time, were inserted into the dorsal side 11.0 mm caudal to the bregma and fixed at a depth of 2.0 mm from the surface of the medulla oblongata. This was done using a universal electrode carrier that can move and fix an electrode three dimensionally to 1 mm (Fig.1). The action potentials of the RVLM region were detected using an amplifier (Nihon kohden Co., EEG4400, Tokyo, Japan) with a TC set at 0.3 second and the high-frequency cut-off at 3 kHz. An electrocardiogram was simultaneously recorded using a bedside monitor (Nihon kohden Co., BSM 8302, Tokyo, Japan).

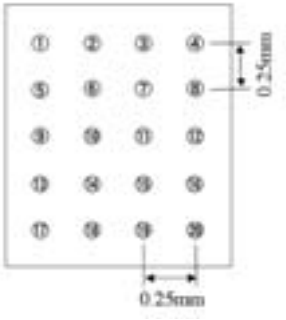

Figure 1. Muti-channel tungsten electrode
The 20 channel tungsten electrode (Unique Medical Co., PS-96006-20, Tokyo, Japan), made by elustering microelectrodes with apical diameter of 1 μm and impedance of 3-5 MΩ, that can derive 20 potentials at a time and which were placed into the RVLM region. The layout of each electrode is shown. The numbers from 1 to 20 represent the various electrical leads. Leads No. 1 to No. 4 were placed on the rostral side and leads No. 17 to No. 20 on the caudal side. Leads No. 1 and 2, No. 5 and 6, No. 9 and 10, No. 13 and 14, No. 17 and 18 were placed on the medial side, and leads No. 2 and 3, No. 7 and 8, No. 11 and 12, No. 15 and 16, No.19 and 20 were placed on the lateral side.
RVLM: rostral ventrolateral medulla oblongata.

c) Recording method of the action potentials and analysis method

After 5-6 hours, on average, when the animals awoke, MSA, SSA, action potentials in the RVLM region, and an ECG were recorded in a hard disk after A/D conversion at sampling frequency of 500 Hz using a 12-bit A/D conversion board (Keithley Instruments, KPCMCIA-16AI-C, Tokyo, Japan) to import the data into a personal computer (Sony Co., PCG-F37, Tokyo, Japan) following digital processing. After full-wave rectification of the MSA and SSA action potentials while off line, the time reset integration was calculated every 1 millisecond to record the action potentials at rest. The action potentials in the RVLM region were integrated

every 1 ms to make topographic mapping analysis with spline interpolation between the electrodes to record the changes in the distribution of action potentials in the RVLM region at rest.

The changes in action potentials in the RVLM region obtained from topographic mapping analysis were visualized, and the cross correlations with MSA, SSA and ECG were examined and compared, to clarify the action patterns in the RVLM region.

Results

a) Relationship between each nerve activity in the RVLM region and the changes in the distribution of the nerve activities

Figure 2 shows the action potentials at rest recorded from the RVLM region, MSA, SSA and ECG. Leads No.3-No.20 show each of the nerve activities obtained from the lateral side of the RVLM region. Regular spontaneous action potentials preceding the R wave of the ECG, and synchronizing with the heart beat, were found in MSA whereas no regular spontaneous action potentials synchronizing with the heart beat was found previously in SSA *(see Kira, 2005)*.

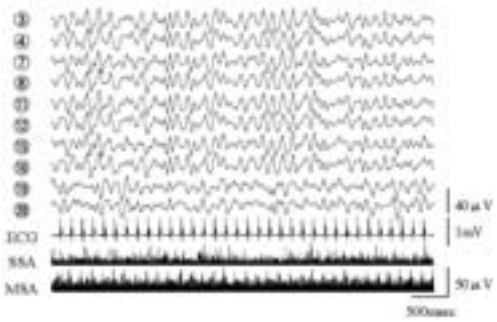

Figure 2. Action potentials in the RVLM region

Each of action potentials in the RVLM region showed waveform obtained from RVLM, integrated SSA, integrated MSA and ECG. The numbers from 3 to 20 represent the lead electrodes.

RVLM: rostral ventrolateral medulla oblongata, SSA: skin sympathetic nerve activity, MSA: muscle sympathetic nerve activity, ECG: electrocardiogram.

b) Relationship between each nerve activity in the RVLM region and MSA

Spontaneous action potentials appeared in leads No.3, No.4, No.7, No.8, No.11, No.12, No.15, and No.16 from the lateral side of the RVLM region about 61 ms before MSA. However, no spontaneous action potentials were found in leads No.19 and No.20 at the same timing. Spontaneous action potentials appeared in leads No.2, No.6, and No.14 led from the medial side of the RVLM region (Fig.3A) whereas no spontaneous action potentials were found in the other leads at the same timing.

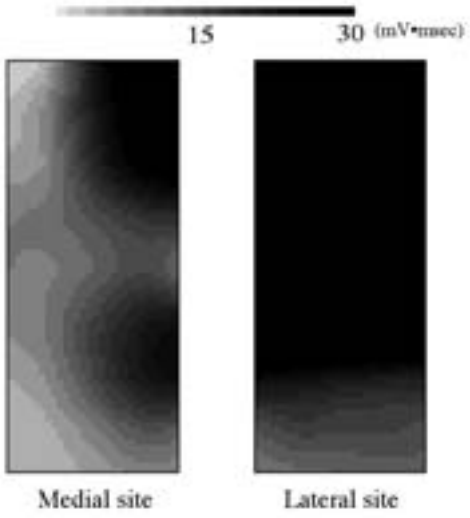

Figure 3 A.

Topographic mapping of the RVLM region before the action potentials were generated in MSA

Topographic mapping showed potentials when regular spontaneous action potentials were found in the RVLM region and topographic mapping of RVLM showed action potentials 61 ms before the action potentials were generated in MSA.

Regular spontaneous action potentials were recorded from leads No. 3, No. 4, No. 7, No. 8, No. 11, No. 12, No. 15, and No. 16 placed on the lateral side, and from leads No. 2, No. 6, and No. 14 placed on the medial side.

c) Relationship between each nerve activity in the RVLM region and SSA

Spontaneous action potentials appeared in leads No.2, No.6, No.10, No.13, and No.14 led from the medial side of the RVLM region about 61.0 ms before SSA. However, no spontaneous action potentials were found in the other leads at the same timing. Spontaneous action potentials appeared in leads No.11 and No.15 from the lateral side of the RVLM region (Fig.3B) whereas no spontaneous action potentials were found in the other leads at the same timing.

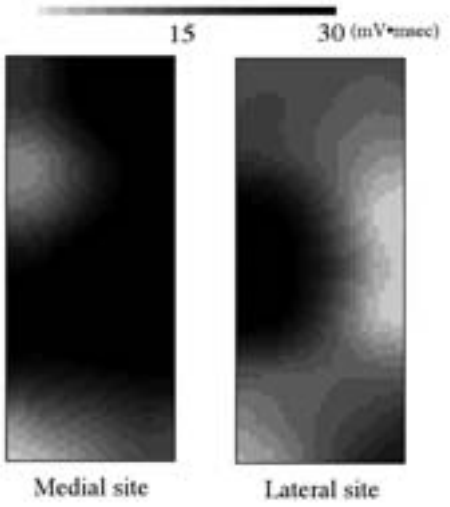

Figure 3 B.

Topographic mapping of the RVLM region before the action potentials were generated in SSA

Topographic mapping of RVLM showed action potentials 61 ms before the action potentials were generated in SSA. Regular spontaneous action potentials were recorded from leads No. 2, No. 6, No. 10, No. 13, and No. 14 placed on the medial side, and leads No. 11 and No. 15 placed on the lateral side.

RVLM: rostral ventrolateral medulla oblongata, MSA: muscle sympathetic nerve activity, SSA: skin sympathetic nerve activity.

d) Natural frequency of each nerve activity obtained from the medial and lateral sides of the RVLM region

The potentials obtained from the medial and lateral sides of the RVLM region fell into the frequency band of 3-3.5 Hz in all leads. No significant differences were found between the frequency bands after frequency analysis with Fourier transformation (Fig.4).

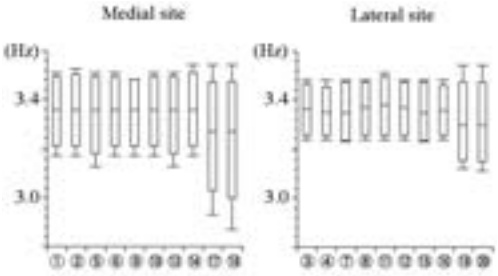

Figure 4. Comparison of the frequency bands between the lateral and medial sides of the RVLM region

Frequency bands were obtained from each action potential on the medial and lateral sides of the RVLM region. No significant differences in the frequency bands obtained within the RVLM region were found.

RVLM: rostral ventrolateral medulla oblongata.

Discussion

MSA, that plays a role in controlling the blood flow in the skeletal muscles and the blood pressure, has spontaneous action potentials synchronizing with the heart beat *(Kira, 2005)*. Topographic mapping analysis showed clear regular spontaneous action potentials synchronizing with MSA in the lateral side of the RVLM region. As these action potentials were generated preceding MSA, it is suggested that the regular spontaneous action potentials recorded from the RVLM region closely correlate with the peripheral vasomotor function.

Retrograde labeling by tracer injection *(Amendt et*

al., 1979) proved that the efferent fibers from the nuclei in the RVLM region specifically ended at the sympathetic preganglionic fiber nuclei in the intermediolateral cell column of the thoracic and lumbar spinal cord. These fibers are considered to regulate the sympathetic nerve activities via direct output to the major sympathetic ganglia. It was reported that a marked drop in the blood pressure is observed when the spinal cord is destroyed and similarly when the cells in the bilateral RVLM regions are destroyed or suppressed *(Feldberg and Guertzenstein, 1976; Guertzenstein and Silver, 1974)*. On the other hand, it was reported that the activities of the cutaneous and skeletal muscle vasomotor fibers increased when the cells in the RVLM region were stimulated by local microinjection of excitatory amino acids *(Dampney and McAllen, 1988)*. These findings suggest that there are neurons regulating the sympathetic nerve activities in the RVLM region as suppression or stimulation of the neurons in the RVLM region immediately produces major impacts on the sympathetic nerve activities.

It is believed that there are neurons regulating the vasomotor function of the skin and muscles *(Hancock, 1982)* and neurons regulating the non-vasomotor function *(Janig and MxLachlan, 1986)* of the visceral organs in the lateral and medial sides of the RVLM region, respectively. In this study, however, clear regular spontaneous action potentials synchronizing with MSA were found mainly in the lateral side of the RVLM region, and action potentials synchronizing with SSA were found mainly in the medial side of the RVLM region. MSA and SSA may not be disproportionately distributed but overlapped in the RVLM region.

No significant differences were found between the frequency bands of action potentials obtained from the RVLM region. Graphs obtained from leads No.3-No.16 showed dispersion of similar frequencies in the wavelet analysis *(Tsai et al., 2004)*, thus demonstrating the temporal dispersion of frequency for the action potentials obtained from the lateral side of the RVLM region. However, lead No.19 and No.20 showed dispersion of different frequency from leads No.3-No.16 (Fig.5). The cross-correlation analysis between the action potentials obtained from the lateral side of the RVLM region and MSA showed clear cross correlation in leads No.3-No.16 whereas no clear cross-correlation was found in leads No.19 and No.20 (Fig.6). These findings suggested that the rostral side had different action patterns in the RVLM region.

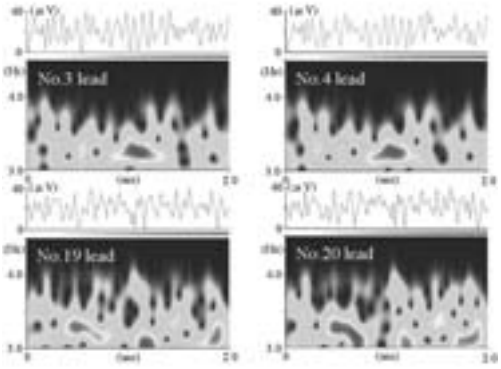

Figure 5. Wavelet analysis of the action potentials obtained from the lateral side of the RVLM region
The wavelet analysis was carried out for the action potentials obtained from the lateral side of the RVLM. The numbers 3, 4, 19, and 20 represent the lead electrodes. No differences were found in the analyzed potentials led from leads No. 3, No. 4, No. 7, No. 8, No. 11, No. 12, and from leads No. 15 and No. 16. However, clear differences were found between the analyzed potentials led from leads No. 3, No. 4, No. 7, No. 8, No. 11, No. 12, No. 15, No. 16 and from leads No. 19 and No. 20.
RVLM: rostral ventrolateral medulla oblongata.

The individual cells in the RVLM region are controlled by NTS (nucleus tractus solitarii), CVLM (caudal ventrolateral medulla), KF nucleus, PAG (preaqueductal grey substance), PVN corresponding to the hypothalamus (hypothalamus PVN), and superior center of LHA *(Carriver et al., 1988;*

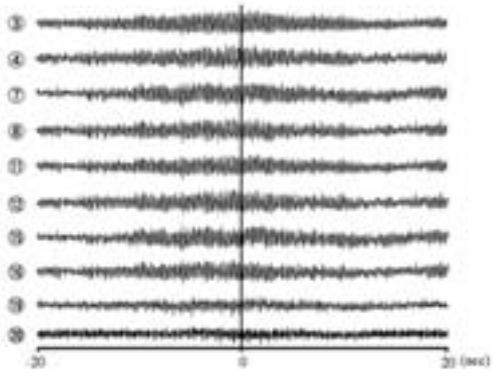

Figure 6. Cross correlation between the action potentials in the lateral side of the RVLM region and MSA
The numbers from 3 to 20 represent the lead electrodes. A clear cross correlation with MSA was found in the potentials led from leads No. 3, No. 4, No. 7, No. 8, No. 11, No. 12, No. 15, and No. 16 whereas no clear cross correlation was found in the potentials led from leads No. 19 and No. 20.
RVLM: rostral ventrolateral medulla oblongata, MSA: muscle sympathetic nerve activity.

Dampney, 1987). In this study, the different patterns of action potentials and the temporal dispersion of frequencies were considered to be shown according to the difference in distribution of these controlled areas. Similar to the findings in our present study, it was reported that different local vasomotion patterns depending on the stimulated part were induced by stimulation via microinjection of excitatory amino acids into various parts in the RVLM region *(Coote JH, 1988)*. Therefore, it is considered that the individual cells in the RVLM region have different action patterns and functional dispersion.
A hypothesis has been advocated that the electrophysiological properties some of the RVLM sympathetic premotor neurons, in which the activity of the neurons is governed entirely by their sympatric inputs, exhibit a pacemaker-like activity, i.e., a reguæar spontaneous activity, even in absence of sympatric input *(Sun et al., 1988)*. The cells in the RVLM region identified in this study are considered to control generation of MSA and SSA action potentials, thus electrophysiologically suggesting that there are premotor nuclei generating peripheral sympathetic nerve action potentials in the RVLM region.

References

Amendt K, J Czachurski & K Dembowsky K: Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J Auton Nerv Syst, 1979, *1*(1), 103-107.

Aramaki S, Y Kira & F Nakanishi: The experimental study on peripheral autonomic nerve potential of the spinal cord injury model by microneurogram method. 1st ISPRM Congress, 2001, *1*, 579-587.

Burke D, KE Hagbarth & BG Wallin: Reflex mechanism in Parkinsonian rigidity. Scand. J. Rehabil Med, 1977, *9*, 15-23.

Cabot JB, V Alessi & A Bushnell: Glycine-like immunoreactive input to sympathetic preganglionic neurons. Brain Res., 1992, *571*, 1-18.

Carriver P, R Bandler & RAL Dampney: Anatomical evidence that hypertension associated with the defence reaxtion in the cat is mediated by a direct projection from a restricted portion of the midbrain periaqueductal grey to the subretrofacial nucleus of the medulla. Brain Res., 1988, *460*, 339-345.

Coote JH: The organisation of cardiovascular neurons in the spinal cord. Rev. Physiol. Biochem. Pharmacol., 1988, *110*, 147-285.

Dampney RAL, J Czachurski & K Dembowsky: Afferent connections and spinal projections of the vasopressor region in the rostral ventrolateral medulla of the cat. J. Auton. Nerv. Sust., 1987, *20*, 73-86.

Dampney RAL & RM McAllen: Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. J. Physol., 1988, *395*, 41-56.

Feldberg W & PG Guertzenstein: Vasodepressor effects obtained by drugs acting on the ventral

surface of the brain stem. J. Physiol., 1976, *258*, 337-355.

Guertzenstein PG & A Silver: Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. J. Physiol., 1974, *242*, 489-503.

Hagbarth KE, RG Hallin & HE Hongell: General characteristics of sympathetic activity in human skin nerve. Acta. Physiologica. Scandinavica., 1972, 84, 164-176.

Hagbarth KE, G Wallin & L Lofstedt: Muscle spindle activity in alternating tremor of Parkinsonism and clonus. J. Neurol. Neurosurg. & Psychiatry, 1975, *38*, 636-641.

Hancock MB: Separate populations of lumbar preganglionic neurons identified with the retrograde transport of horseradish peroxidase (HRP) and 4,6-diamidino-2-phenylindole (DAPI). J. Auton. Nerv. Syst., 1982, *5*, 135-143.

Janig W: Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. Rev. Physiol. Biochem. Pharmacol., 1985, *102*, 119-213.

Janig W & EM McLachlan: Identification of distinct topographical distributions of lumbar sympathetic and sensory neurons projecting to end organs with different functions in the cat. J. Comp. Neurol. 1986, *246*, 104-112.

Kira Y, T Ogura, Y Mikami, S Aramaki, F Nakanishi & T Kubo: A neurophysiological study on the sympathetic premotor nuclei in the pons and medulla oblongata. Scand. J. Lab. Anim. Sci., 2005, *32*, 1-7.

Krukoff TL, J Ciriello & FR Calaresu: Segmental distribution of peptide-like immunoreactivity in cell bodies of the thoracolumbar sympathetic nuclei of the cat. J Comp. Neurol., 1985, *240*, 90-102.

Krukoff TL: Coexistence of neuropeptides in sympathetic preganglionic neurons of the cat. Peptides, 1987, *8*, 109-112.

Strack AM, WB Sawyer & M Marubio: Spinal origin of sympathetic preganglionic neurons in the rat.Brain Res. 1988, *455*, 187-191.

Sun MK, JT Hackett & PG Guyenet: Sympathoexcitatory neurons of rostral ventrolateral medulla exhibit pacemaker properties in the presence of a glutamate-receptor antagonist. Brain Res. 1988, *438*, 23-40.

Tsai ML, WC Shann, WR Luo & CT Yen: Wavelet-based analysis of low-frequency fluctuations of blood pressure and sympathetic nerve activity in rats. Neurosci. Lett., 2004, *358*, 165-168.

Vallbo ÅB, KE Hagbarth & HE Torebjörk: Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. Physiol. Rev., 1979, *59*, 919-957.

Wallin BG & DL Eckberg: Sympathetic transients caused by abrupt alterations of carotid baroreceptor activity in humans. Am. J. Physiol., 1982, *242*, 185-190.

Yatomi A, A Iguchi& K Uemura: A rare case of recurrent vasodepressive attacks of 2-hours duration, analysis of the mechanism by muscle sympathetic nerve activity recording. Clin. Cardiol., 1989, *12*, 164-168.