

# An experimental study on the peripheral autonomic nerve potential in the spinal cord injury model by microneurography

by Taku Ogura<sup>1</sup>, Yasuhiko Kira<sup>2\*</sup>, Shunzo Aramaki<sup>2</sup>, Fumihiko Nakanishi<sup>2</sup>, Kookho Lee<sup>1</sup>, Toshikazu Kubo<sup>1</sup>

<sup>1</sup> Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

<sup>2</sup> Department of Orthopedic Surgery, Kyoto Prefectural Rehabilitation Hospital for the Mentally and Physically Disabled, Kyoto, Japan

## Summary

The purpose of the present investigation was to analyze the effects of spinal cord injury (SCI) on the peripheral sympathetic nerve, skin sympathetic activity (SSA), and muscle sympathetic activity (MSA). To do this sixteen male Japanese white rabbits weighing 1.0 kg-1.5 kg were used. The exposed spinal cord was transected at various levels. Microelectrodes were placed on the muscular branch and on the cutaneous branch, and they were separately induced using a 0.5 -5 kHz amplifier. The data were calculated with the time reset integration value at 60 seconds.

Results: It is usually satisfactory to considered that the peripheral sympathetic fibers from T1-3 generally pass up through the sympathetic chain to the upper extremities, and that fibers from T9-11 pass up through the sympathetic chain to the lower limbs. In the electrophysiological properties studied, the SSA could not be recognized as a spontaneous activity. On the other hand, the MSA could be recognized as a spontaneous regular activity which synchronizes with the R wave of the electrocardiogram.

Conclusions: The MSA potentials synchronized with the heart rate, and they seem to correlate with the body homeostasis. The existence of a central regulatory mechanism is suggested from those findings not only in vital rhythms, such as the heart rate variability but also in the MSA.

## Introduction

As one of the modalities for assessment of the autonomic nervous system function, Hagbarth et al. (Hagbarth & Vallbo, 1967; Hagbarth & Vallbo, 1967) have recently induced the skin sympathetic nerve activity (SSA) in man directly by means of microneurography, and recorded (Sugenoya and Ogawa, 1985; Hagbarth et al., 1972) the burst activities as joint responses from the sudomotor and vasomotor nerves that supply the sweat glands and the cutaneous blood vessels, respectively. By using metal microelectrodes, this microneurography technique made identification of the types of

nerve fibers feasible, and allowed recording of the action potentials of these nerve fibers. Then, it became possible to induce and record the action potentials of each and every individual nerve fiber in most of the peripheral nerve trunks. Moreover, microneurography enabled one to induce and record the action potentials of the sympathetic postganglionic efferent nerve fibers in the peripheral nerves of man, that could not be done by any of the conventional techniques. Therefore, microneurography is currently used as a direct method for analysis of the autonomic nerve activities (Hagbarth & Vallbo, 1968; Vallbo, 1971; Vallbo et al., 1979; Wallin & Eckberg, 1982).

In man, the peripheral sympathetic nerve activities consist of SSA, that controls the sweat glands and the cutaneous blood vessels, and muscle sympathetic activity (MSA) that controls the smooth muscles of the blood vessels in the skeletal muscles

\* Correspondence: Department of Orthopedic Surgery, Kyoto Prefectural Rehabilitation Hospital for the Mentally and Physically Disabled, Naka Ashihara, Johyo City, Kyoto 610-0113, Japan. Tel: +81-0774-54-1400, Fax: +81-0774-54-3616, E-mail: kiray@mb.neweb.ne.jp

(Hagbarth *et al.*, 1975; Burke *et al.*, 1977; Yatomi *et al.*, 1989). Each of those activities reportedly has its own peculiar characteristics. By means of microneurography, SSA (that is involved in the body temperature control) and MSA (that is involved in the blood pressure control) of the sympathetic postganglionic efferent nerve fibers can be recorded separately.

Lesions of the peripheral autonomic nerves due to spinal cord injury result in many clinical problems, such as failure of body temperature control due to perspiration disorders and impairment of the peripheral blood flow; orthostatic hypotension due to failure of blood pressure control; autonomic nerve hyper-reflexia, and so on. Valsalva's technique can reportedly demonstrate the differences in the reflex potentials between SSA and MSA (Wallin and Elam, 1993), suggesting that each activity has its characteristic reflex features in response to stimulation.

In the present study, to clarify the mechanism of the peripheral sympathetic nerve functions and to investigate the effects of spinal cord injury on the sympathetic nerves, we made a model of spinal cord injury by transecting the spinal cord, recorded the sympathetic nerve action potentials of SSA and MSA from the peripheral nerves by microneurography, and compared the differences in the action potentials. Herein, we report the results.

## **Materials and Methods**

### *Subjects*

Sixteen male Japanese white rabbits (Shimizu experimental material Co., Ltd., Kyoto, Japan) weighing 1.0 kg-1.5 kg were used. The rabbits were fed a commercial diet (CRF-1, Charles River Japan, Kanagawa, Japan). They were individually housed in standard rabbit cages (Natsume Co., Ltd., Tokyo, Japan).

### *Method of inducing the sympathetic nerve action potentials from the upper limbs*

The room temperature was kept at 26-28°C and the relative humidity varied between 50% and 60%, and

each rabbit was fixed onto an operating table after intravenous injection of pentobarbital sodium (25 mg/kg), and the spinal cord from the level of C 6/7 to T 2/3 was exposed. The right median nerve was exposed to record the efferent sympathetic nerve potentials of the postganglionic nerve fibers. Tungsten  $\mu$  microelectrodes, with tip diameter of 1 $\mu$ m, and impedance of 3-5 M  $\Omega$  (Unique  $\Omega$  Medical Co., UJ3002B, Japan), were placed on the muscular branch of the median nerve for MSA recording and on the cutaneous branch for SSA recording, and they were separately induced using a 500 Hz-5 kHz amplifier (Nihon Kohden, MEM4104, Japan). For electrocardiogram (ECG) monitoring, a bedside monitor (Nihon Kohden, BSM8302, Japan) was used. A disposable needle electrode (Medelec Co., DMC25, Japan) was also used for inducing an electromyogram (EMG) response from the right flexor carpi radialis muscle by the apparatus (Nihon Kohden, MEM4104, Japan) and the waves taken from the muscle were amplified by a 10 Hz-3 KHz amplifier (Fig. 1A). The whole procedure lasted about 90 minutes. The animals were killed during anesthesia with an overdose of pentobarbital sodium.

### *Method of inducing the sympathetic nerve action potentials from the lower limbs*

The procedure was almost the same as that for the upper limbs. The spine from T 8/9 to T 11/12 level and the right sciatic nerve for recording the action potentials were exposed. As in the upper limbs, tungsten microelectrodes were placed on both the muscular and cutaneous branches for MSA and SSA inductions, respectively, and a 500 Hz-5 KHz amplifier was used for recording. ECG and EMG from the right gastrocnemius muscle were also recorded (Fig. 1B). The whole procedure lasted about 90 minutes. The animals were killed during anesthesia with an overdose of pentobarbital sodium.

### *Method of recording and analyzing the potentials*

The rabbits were anesthetized with pentobarbital sodium (dose: 25 mg/kg body weight). After five to six hours, the spinal cord was exposed at the thoracic

and lumbar regions, and then completely transected at various peripheral levels using a disposable scalpel (Feather Co., No. 23, Japan). Furthermore, at each level of transaction, right-angled wave electric stimulation (strength: 15 mA, duration: 0.25 msec) was applied using an electric stimulator (Unique Medical Inc., KS-101, Japan) through stimulation electrodes placed on the rabbit's forehead. Stimulation-induced SSA and SMA as well as electrocardiography and electromyography were continually recorded using a data recorder (RD

130T, PCM DATA RECORDER TEAK Inc., Japan). All raw data recorded by the data recorder off-line were input into a personal computer using a 12-bit conversion board at a sampling frequency of 1 kHz, and then digital processing was carried out. BIMUTAS II (Kissei Co., Japan) was utilized for recording and analysis. After full-wave rectification, the integral values of SSA, MSA, and electromyography were calculated separately at a time reset of 60 seconds. Regarding the calculated integral values, we compared the integral values

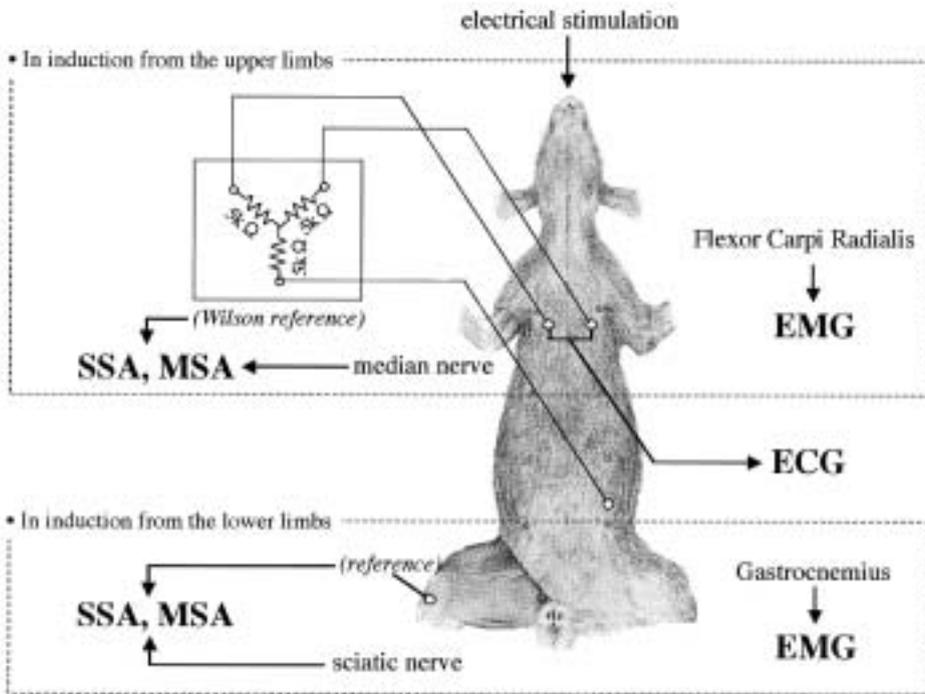


Figure 1: Methods of induction of the sympathetic nerve action potentials.

In induction from the upper limbs, the SSA and MSA were recorded from the cutaneous branch and the muscular branch of the median nerve, respectively. The Wilson electrode was used as a reference electrode. For ECG monitoring, a bedside monitor was used, and EMG was recorded from the right flexor carpi radialis muscle. In induction from the lower limbs, the MSA and SSA were recorded from the muscular and cutaneous branches of the sciatic nerve, respectively. The ipsilateral electrode was used as the reference electrode. ECG and EMG from the right gastrocnemius muscle were recorded. SSA = skin sympathetic nerve activity, MSA = muscle sympathetic nerve activity, ECG= electrocardiogram, EMG = electromyogram.

obtained on applying electric stimulation with the reduced integral values taken at rest. The differences were considered significant when the level of significance (p value) was less than 5%. In addition, a statistical analysis was conducted using one way analysis of variance (ANOVA) for comparison among the individual groups, and the Scheffe F-test was applied when the differences were significant.

## Results

### *Nerve action potentials before SCI*

#### *Action potentials at rest*

In MSA, the regular spontaneous action potentials preceded and synchronized with the R wave of ECG as shown in Fig. 2. The spontaneous action potentials in SSA and EMG were not as in MSA.

#### *Action potentials by applying electrical stimulation on the rabbit's forehead*

MSA, SSA, and EMG showed large action potentials in response to the electrical stimulations. Moreover, regular spontaneous potentials synchronizing with ECG were seen similar to those at rest (Fig 3.).

### *Nerve action potentials of in the sympathetic nerves of the upper limbs in the cervical SCI model*

#### *Action potentials at rest*

In MSA, regular spontaneous action potentials that synchronized with ECG as in the pre-SCI model were noticed at the levels from T3/T4 to C7/T1, but not in SSA. All the potentials in both MSA and SSA disappeared following transection at the level of C6/C7.

#### *Action potentials by electrical stimulation*

The mean integration values (mV, sec) of SSA at each level were: in pre-SCI:  $1.95 \pm 0.53$ , T3/T4:  $1.85 \pm 0.53$ , T2/T3:  $0.92 \pm 0.22$ , T1/T2:  $0.48 \pm 0.06$ , and C7/T1:  $0.12 \pm 0.02$ . Moreover, the mean integration values of MSA at each level were: pre-SCI:  $2.91 \pm 0.27$ , T3/T4:  $2.51 \pm 0.33$ , T2/T3:  $1.22 \pm 0.24$ , T1/T2:  $0.71 \pm 0.26$ , and C7/T1:  $0.11 \pm 0.07$ . The action potentials in both MSA and SSA decreased

significantly as the transection levels were shifted from caudal to rostral (Fig. 4) and finally, at the level of C6/C7, all the potentials disappeared.

### *Nerve action potentials in the sympathetic nerves of the lower limbs of the lumbar SCI models*

#### *Action potentials at rest*

In MSA, regular spontaneous action potentials that synchronized with ECG as in the pre-SCI models were noticed at the levels from T12/L1 to T9/T10, but not in SSA. All the potentials in both MSA and SSA disappeared following transection at the level of T8/T9.

#### *Action potentials by electrical stimulation*

The mean integration values (mV, sec) of SSA at each level were: pre-SCI:  $2.36 \pm 0.16$ , T12/L1:  $2.23 \pm 0.32$ , T11/12:  $1.51 \pm 0.52$ , T10/11:  $0.91 \pm 0.40$ , and T9/10:  $0.09 \pm 0.02$ . Moreover, the mean integration values of MSA at each level were: pre-SCI:  $3.15 \pm 0.23$ , T12/L1:  $2.91 \pm 0.22$ , T11/T12:  $2.18 \pm 0.25$ , T10/T11:  $0.88 \pm 0.24$  and T9/T10:  $0.09 \pm 0.18$ . The action potentials in both MSA and SSA tended to decrease significantly as the transection levels were shifted from caudal to rostral (Fig. 5) and finally, at the level of T7/T8, all the potentials disappeared.

## Discussion

### *Anatomical features of the sympathetic nerves*

The postganglionic sympathetic nerves receive impulses from the preganglionic neurons which originate from the nuclei located in the thoracic and lumbar spinal cord. They have a direct influence on the circulatory functions, etc., so the sympathetic nerve disturbances in SCI depend mainly on the anatomical characteristics. Clinically, body temperature regulation disturbance, due to perspiration and blood flow failures, autonomic nerve dysreflexia and orthostatic hypotension, etc., are often experienced. These phenomena are considered to reflect the peripheral sympathetic nerve dysfunctions including MSA and SSA, suggesting

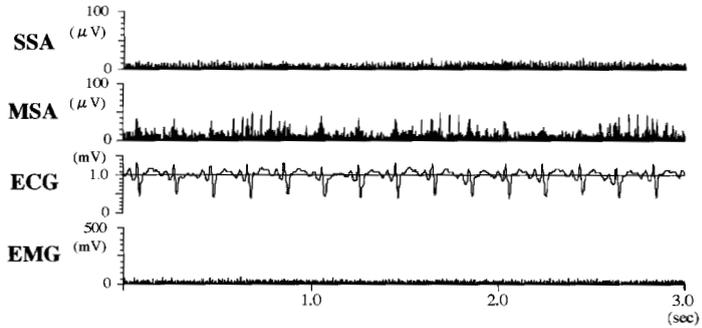


Figure 2: In the pre-SCI model, the integrated nerve action potentials for 3.0 seconds at rest. No spontaneous activities were recognized for SSA and EMG. On the other hand, MSA showed spontaneous regular activities which synchronized with the R wave of the ECG. SCI = spinal cord injury, SSA = skin sympathetic nerve activity, MSA = muscle sympathetic nerve activity, ECG= electrocardiogram, EMG = electromyogram.

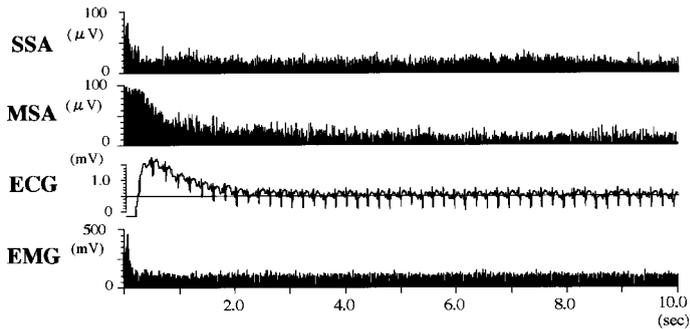


Figure 3: In the pre-SCI model, the integrated nerve action potentials for 10.0 seconds by electrical stimulation. SSA, MSA and EMG showed clear action potentials by electrical stimulation. And MSA showed spontaneous regular activities which synchronized with the R wave of the ECG. Electrical stimulation was applied at the beginning of each sweep. SCI = spinal cord injury, SSA = skin sympathetic nerve activity, MSA = muscle sympathetic nerve activity, ECG= electrocardiogram, EMG = electromyogram.

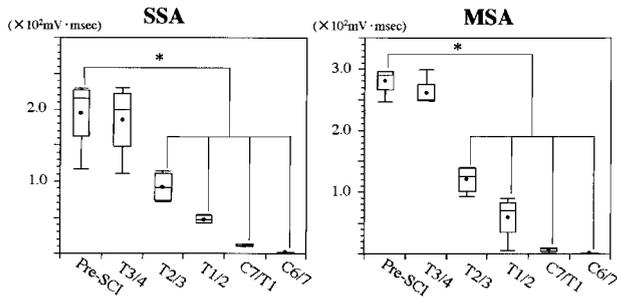


Figure 4: Comparison between the integral values for the upper limbs.

In both SSA and MSA, the integral values were significantly lower in the SCI model at C6/7 to T2/3 level than those in the pre-SCI model. SSA = skin sympathetic nerve activity, MSA = muscle sympathetic nerve activity.

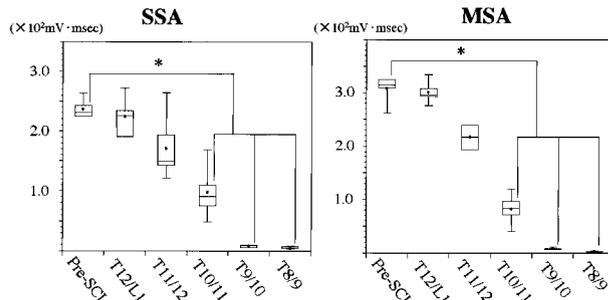


Figure 5: Comparison between the integral value for the lower limbs.

Similarly, in both SSA and MSA, the integral values were significantly lower in the SCI model at T8/9 to T10/11 level than those in the pre-SCI model. SSA = skin sympathetic nerve activity, MSA = muscle sympathetic nerve activity.

a singularity of function of the sympathetic nerves in effectors, such as the sweat gland and baroreceptors.

Moreover, the sympathetic nervous system descends in the spinal cord and reaches the intermediate lateral nuclei located between T1 and L3 (Coote, 1988; Cabot, 1990). The myelinated preganglionic fibers start from these nuclei and go into the sympathetic chain through the white rami. The trunk consists of almost 20 nerve ganglia from the neck to the sacrum and has communications upwards and downwards. The non-myelinated postganglionic fibers join with the somatic nerves through the gray white rami and return to the spinal nerve. The centers of the sym-

pathetic nerves are in the thoracic and lumbar spinal cord and mix with the somatic nerves through the sympathetic chain, so the distribution of the sympathetic nerve differs from that of the somatic nerve. The action potentials of SSA and MSA disappeared following C6/7 level transection in the upper limbs in this study, but not in EMG. And by T9/T10 level transection, the action potentials of EMG disappeared in the lower limbs, but SSA and MSA still showed such potentials. These findings suggest that the sympathetic nerves and somatic nerves definitely take different pathways.

Moreover, the comparison of the integrated values suggested that the main center of the sympathetic

nerve lies probably between T2/T3-C7/T1 for the upper limbs and between T10/T11-T8/T9 for the lower limbs.

*Electrophysiological features of SSA and MSA*

SSA regulates the body temperature by controlling the sweat glands and cutaneous blood vessels. At a particular temperature, the vessels' motor nerves could be suppressed and only the sweat gland function appear. Therefore SSA did not show clear action potentials in this study.

On the other hand, MSA controls the blood volume in the muscles in relation to the blood pressure control, and it showed spontaneous action potentials that synchronized with the R wave of ECG, but not in SSA at rest (Fig. 6). There was an apparent relationship between the action potentials of MSA and the R wave of ECG (almost 1 : 1). Moreover, the action potentials of MSA were seen not only on transection at C7/T1, but also even when the R wave disappeared (Fig. 7). It has been considered long ago that the autonomic nervous system main-

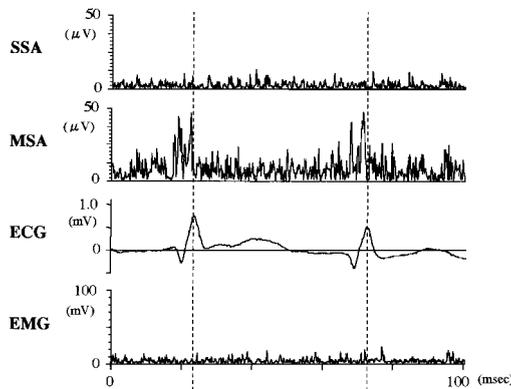


Figure 6: The integrated nerve action potentials for 0.1 second at rest.

The waveforms obtained at rest were integrated. MSA showed spontaneous regular activities which synchronized with the R wave of the ECG. The dotted line represents the R wave of the ECG. MSA = muscle sympathetic nerve activity, ECG = electrocardiogram.

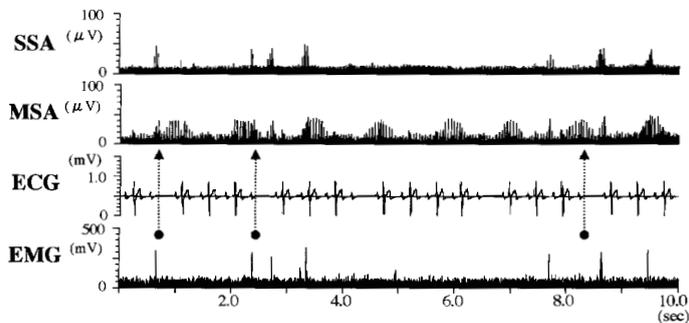


Figure 7: In the SCI model at C7/T1 level, the integrated nerve action potentials for 10.0 seconds at rest. The QRS waves that disappeared on the ECG are indicated by arrows with the dotted line representing MSA that remained intact. SCI = spinal cord injury, MSA = muscle sympathetic nerve activity, ECG= electrocardiogram.

tains homeostasis of the body. MSA could be involved in the homeostasis as well.

### Conclusions

(1) This study was conducted on 16 (male) Japanese white domestic rabbits to investigate the effects of spinal cord injury on the peripheral sympathetic nerve activities.

a) In the study on the peripheral sympathetic nerve activities in the upper limbs, transaction of the spinal cord was done at intervertebral levels starting from C6/7 till T2/3, SSA and MSA were recorded from the median nerve, and finally ECG and EMG were simultaneously recorded and compared with SSA and MSA.

b) In the study on the peripheral sympathetic nerve activities in the lower limbs, transaction of the spinal cord was done at intervertebral levels starting from T8/9 till T12/L1, SSA and MSA were recorded from the sciatic nerve, and finally ECG and EMG were simultaneously recorded and compared with SSA and MSA.

(2) Regarding the integral values of SSA and MSA:

a) In the study on the peripheral sympathetic nerves in the upper limbs, the potentials showed a significant reduction with transection at the level of T2/3 as compared with the corresponding pre-SCI values, and complete disappearance of the potentials was noticed with transection at the level of C6/7.

b) In the study on the peripheral sympathetic nerves in the lower limbs, the potentials similarly showed a significant reduction with transection at the level of T10/11 as compared with the corresponding pre-SCI values, and complete disappearance of the potentials was noticed with transection at the level of T8/9.

(3) In the Japanese white domestic rabbits, it is suggested that the principal center supplying the peripheral sympathetic nerves in the upper limbs extends from T2/3 till C7/T1, and that the principal center supplying the peripheral sympathetic nerves in the lower limbs extends across 3 intervertebral segments from T10/11 till T8/9.

(4) The autonomic nervous system activities synchronize with the vital rhythm, such as the heart rate, and strongly correlate with the body homeostasis, indicating the presence of an independent central regulatory mechanism for each activity.

### References

- Burke D, KE Hagbarth & BG Wallin*: Reflex mechanism in Parkinsonian rigidity. *Scand. J. Rehabil. Med.* 1977, 9, 15-23.
- Cabot JB*: Sympathetic preganglionic neurons: cytoarchitecture, ultrastructure, and biophysical properties. In: Loewy AD and Spyer KM eds. *Central Regulation of Autonomic Functions* Oxford Univ. Press. New York. 1990, pp22-34.
- Coote JH*: The organization of cardiovascular neurons in the spinal cord. *Rev. Physiol. Biochem. Pharmacol.* 1988, 110, 147-285.
- Hagbarth KE & ÅB Vallbo*: Mechanoreceptor activity recorded percutaneously with semi-microelectrodes in human peripheral nerve. *Acta. Physiol. Scand.* 1967, 69, 121-122.
- Hagbarth KE & ÅB Vallbo*: Afferent response to mechanical stimulation of muscle receptors in man. *Acta. Soc. Med. Upsal.* 1967, 72, 102-104.
- Hagbarth KE & ÅB Vallbo*: Pulse and respiratory grouping of sympathetic impulses in human muscle nerve. *Acta. Physiol. Scand.* 1968, 74, 96-108.
- Hagbarth KE, RG Hallin, A Hongell, HE Torebjörk & BG Wallin*: General characteristics of sympathetic activity in human skin nerve. *Acta. Physiol. Scand.* 1972, 84, 164-176.
- Hagbarth KE, G Wallin & L Lofstedt et al*: Muscle spindle activity in alternating tremor of Parkinsonism and clonus. *J. Neurol. Neurosurg. Psychiat.* 1975, 38, 636-641.
- Sugenoya J & T Ogawa*: Characteristics of central sudomotor mechanism estimated by frequency of sweat expulsions. *Jpn. J. Physiol.* 1985, 35, 783-794.
- Vallbo ÅB, KE Hagbarth, HE Torebjörk & BG Wallin*: Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol. Rev.* 1979, 59, 919-957.

*Vallbo ÅB*: Muscle spindle response at the onset of isometric voluntary contractions in man. Time difference between fusimotor and skeletomotor effects. *J. Physiol.* 1971, *318*, 405-431.

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

*Wallin BG & M Elam*: Microneurography and autonomic dysfunction, Low PA (ed). *Clinical Autonomic Disorders*, Little Brown, 1993, pp 243-251.

*Yatomi A, A Iguchi A & K Uemura et al*: 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.