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Characteristics of Sympathetic Nerve Activity in the Rat Sciatic Nerve in Response to Microstimulation in a Sympathetic Fascicle in the Contralateral Side

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Abstract— **Microneurography is used for the monitor of various peripheral nerve activities. We recently reported that the electrical stimulation of peripheral sympathetic nerve fascicle via the microelectrode, i.e., microstimulation, temporarily reduced the blood glucose level in rats in case that the stimulation intensity was set high enough to induce small muscle contraction. However, the nature of microstimulation has little been clarified yet. Therefore, in the present study, we first detected sympathetic nerve signal microneurographically in the bilateral sciatic nerves of rats, and one of the microelectrodes was used for the microstimulation (0.25 ms-width pulse train at a rate of 1 Hz) while sympathetic nerve activity (SNA) was recorded in the contralateral side as a parameter of systemic sympathetic effects. The SNA, expressed as action potential rate, was transiently increased 150 ms after each stimulation pulse in case that the stimulation intensity was set not less than -0.1 V from the contraction threshold (around 0.32 V). To confirm that the increase was not caused by the activation of low threshold, thick fibers such as motor nerves in the vicinity of the microelectrode tip, next, a bipolar hook electrode, instead of the microelectrode, was then used in the stimulation side. As a result, the above-mentioned, transient increase in SNA was not observed any more in the contralateral side. These results suggest that systemic SNA could be enhanced with lower stimulation intensity than that inducing muscle contraction, and that thicker fibers may little affect the increase in the contralateral SNA.**

I. Introduction

Microneurography allows us to monitor various peripheral nerve activities *in situ* [1], depending on the fascicle in which a fine microelectrode tip is located. The electrical stimulation via the microelectrode is called "microstimulation" of which nature has not been documented well.

It is known that central sympathetic nerve activation induces peripheral glucose uptake independently of insulin secretion [2]. Therefore, peripheral sympathetic activation might reduce blood glucose level in diabetic patients. Recently, we conducted the microstimulation of a sympathetic fascicle in the rat sciatic nerve, and reported that the microstimulation induces transient reduction in blood glucose level probably due to the enhancement of glucose uptake [3]. We speculated that the response in blood glucose may possibly be induced by some systemic response because the blood glucose reduction could be a too large event to be attributed to such small, local response.

On the other hand, because high sympathetic activity can cause small muscle contraction [4], enhancement of sympathetic nerve activity (SNA) was confirmed with minimal muscle contraction in our previous study shown above. Therefore, the effects of muscle contraction derived from SNA enhancement need to be separated from that caused by the motor nerve system. Furthermore, since muscle contraction itself enhances glucose uptake [5], we need to determine that the blood glucose reduction induced with the microstimulation may not be due to the effect.

In the present study, we again applied the microstimulation to a sympathetic fascicle of the sciatic nerve while the contralateral SNA was monitored as a parameter of systemic effect, and the effects of stimulation intensity on the contralateral SNA was evaluated. In addition, to clarify the effects of muscle contraction evoked via motor fibers in the sciatic nerve on the contralateral SNA, muscle contraction was induced with a bipolar hook electrode as well.

II. Materials and Methods

All of the surgical and experimental procedures described below were conducted with the approval of the Yamagata University Animal Research Committee (no. 23067). All animals were allowed free access to food and tap water, and were housed in a room illuminated daily from 0800 to 2000 hr (a 12:12-hr light/dark cycle) and maintained at 21 ± 1 °C.

Male Wistar rats (approximately 12 weeks of age) obtained from an in-house breeding colony were randomized into microstimulation (MS) and bipolar hook (BH) groups. The rats in both groups were anesthetized with intraperitoneal administration of sodium pentobarbital (50 mg/kg) after overnight fast, and placed in the supine position. Body temperature was concerned to keep 37 °C by continuous monitoring of rectal temperature. The right jugular vein was cannulated, and the catheter was filled with heparinized saline (20 U/mL). Additional doses of sodium pentobarbital were intermittently administered via the venous line to keep the animals in stable condition.

The bilateral sciatic nerves were exposed at the thigh level, and unilateral muscle sympathetic nervous signal was detected

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using the microneurographic technique [6]. In brief, the SNA was observed when a fine tungsten microelectrode (model 10-26-1 [approximately 5 MΩ]; FHC, ME, USA) with a 1 μm tip diameter and 250 μm shaft diameter was inserted into a sympathetic fascicle of the sciatic nerve. The signal was identified with spontaneous and intermittent burst signal; it was the general characteristics. The sympathetic nervous signal was recorded on a personal computer (PC) using LabVIEW Signal Express (National Instruments Japan, Tokyo, Japan) with a 16 bit A/D converter at a sampling rate of 16 kHz, for later analyses.

In the MS group, the sympathetic signal was also detected in the contralateral sciatic nerve in the same manner, and the microelectrode was used for microstimulation [3]. By contrast, a bipolar hook electrode was attached to the contralateral sciatic nerve in the BH group.

Electrical stimulation (Electronic Stimulator, SEN-3301; Nihon Kohden, Tokyo, Japan) was applied to the sympathetic nerve fibers with 0.25 ms-width pulse train via the microelectrode at a rate of 1 Hz. Its intensity was increased in a stepwise manner by 0.1 V (MS group) or 0.05 V (BH group), and the maximal intensities were set to 0.1 (MS group) or 0.05 V (BH group) above the voltage inducing eye-detectable muscle contraction. The duration of each intensity level was set to 1 min.

The implications of these stimulation intensities in MS and BH groups are different with each other because the target of each stimulation was different. Hence, it may be difficult to directly compare the data in MS and BH groups each other.

III. Effects of Unilateral Microstimulation on Contralateral SNA

A. Analyses Methods

After the animal experiments shown above, stored SNA data was analyzed on PC with MATLAB. The filtered signal (0.5-5 kHz band-pass filter) was full-wave rectified and integrated to obtain an integrated neurogram [6]. To emphasize the response in SNA caused by each stimulation pulse, sympathetic nerve signal obtained during last 30 s of each intensity was divided into 30 waveforms of 1-s length, and then the waveforms were averaged.

B. Results

Figs. 1-4 show examples of the averaged waveform of SNA obtained during contralateral stimulation in the sciatic nerve.

In MS group, when the muscle contraction was not observed, no visible change was detected after the artifact of microstimulation pulse (Fig. 1). In contrast, a large peak lasting about 100 ms was observed 150 ms after the artifact while the muscle contraction was evoked (Fig. 2).

In contrast in BH group, little change was observed regardless of existence of muscle contraction (Fig. 3 and 4).

That is, the response in SNA was observed only when the intensity of contralateral microstimulation was high enough to induce muscle contraction in MS group. These results indicate that the effects of the electrical stimulation to the sciatic nerve in MS and BH groups differ from each other. Furthermore, the sympathetic nerve activation induced by unilateral microstimulation can be identified via the observation of contralateral SNA.

IV. Relationship between Stimulation Intensity and SNA

A. Analyses Methods

Based on the results shown above, we calculated the action potential (AP) rate during the period while the peak was seen in Fig. 2, and compared it with that figured during the rest of the period. The ratio (relative AP rate) was made to evaluate the activation effect. In case that the relative AP rate exceeds 100%, it indicates that SNA during the "peak" period is high. APs were manually detected from the sympathetic signal after wavelet de-noising procedure proposed by Diedrich *et al* [7].

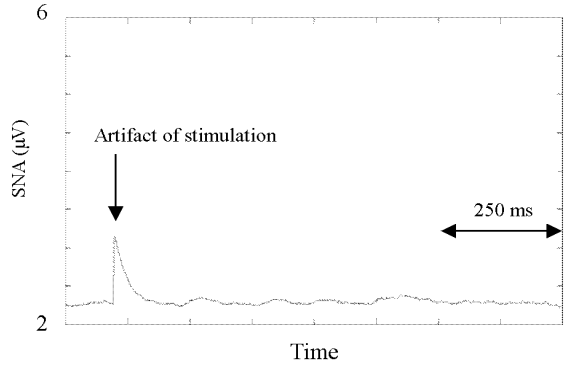

Figure 1. MS group: averaged SNA waveform in response to 0.2 V microstimulation in the contralateral side (muscle contraction was not observed).

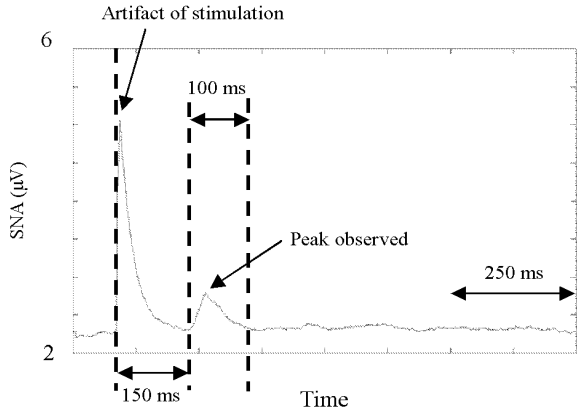

Figure 2. MS group: averaged SNA waveform in response to 0.5 V microstimulation in the contralateral side (muscle contraction was observed).

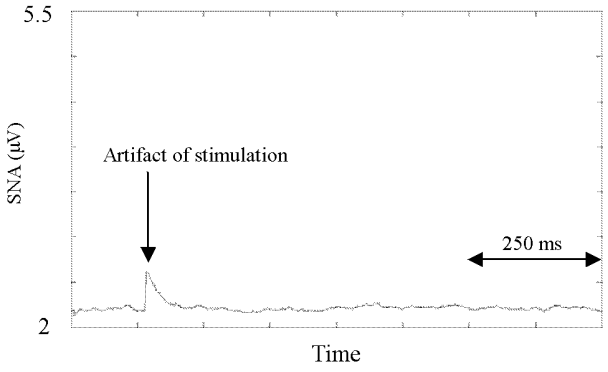

Figure 3. BH group: averaged SNA waveform in response to 0.27 V stimulation in the contralateral side (muscle contraction was not observed).

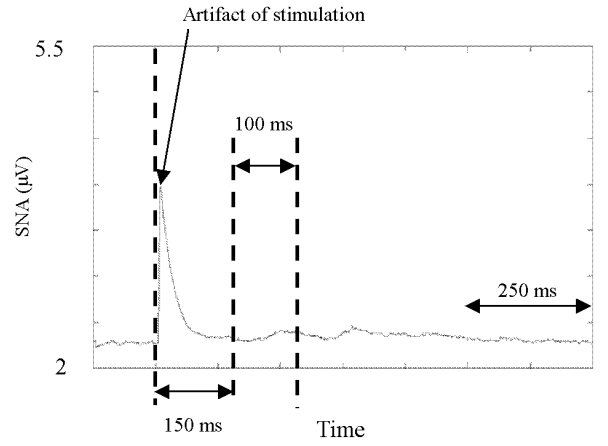

Figure 4. BH group: averaged SNA waveform in response to 0.32 V stimulation in the contralateral side (muscle contraction was observed).

B. Results

The threshold intensities of eye-detectable muscle contraction in MS and BH group were 0.32 ± 0.16 V (n=5, mean ± SD) and 0.38 ± 0.10 V (n=5), respectively. The relative AP rate in response to relative stimulation-intensity from each threshold was summarized in Figs. 5 and 6.

In MS group, the relative AP rate was not different from background AP rate when the relative stimulation-intensity was -0.2 V. However, when the intensity was increased to -0.1 V, the rate was higher than that observed at -0.2 V intensity in spite of no muscle contraction was observed yet. Thereafter, the rate was risen with increasing the intensity (Fig. 5). Each rate observed during the stimulation from -0.1 to +0.1 V was significantly higher than that obtained at -0.2 V stimulation ($p < 0.01$).

In contrast, the relative AP rate in BH group was maintained approximately 120% regardless of applied voltage (Fig. 6). The rate exceeded 100% in BH group even when the applied relative voltage was -0.1 V. All of the AP data in MS group observed at the stimulation levels equal to and more than -0.1 V tended to be higher than those in BH group, and significant difference were detected at 0 V stimulation by Student's t-test (MS: 148 ± 10% vs. BH: 125 ± 17%, $p < 0.05$).

V. Discussion

In the present study, we first examined the feasibility of other site evaluation of sympathetic activation caused by microstimulation. Microelectrodes were inserted into sympathetic nerve fascicles in the bilateral sciatic nerves, and SNA in response to contralateral microstimulation was evaluated (MS group). As a result, SNA in MS group was temporarily elevated 150 ms after the artifact of stimulation pulses when the intensity was set to a level inducing eye-detectable muscle contraction (Fig. 2). Although the mechanism of this phenomenon was unclear, it indicates that the local sympathetic microstimulation evoked a systemic response in SNA as expected.

In our previous work, the sympathetic microstimulation induced temporal reduction of blood glucose level [3]. This reduction may also be caused by the systemic sympathetic activation suggested in the present study.

In contrast, in case of the stimulation via the bipolar hook electrode (BH group), the transient elevation of SNA, seen in MS group, was not clear (Fig. 4). The results show that this stimulation may not affect the sympathetic system.

In the next step, to know the increased amount of SNA in response to the stimulation, we calculated the relative AP rate. The rate was significantly elevated ($p < 0.01$) even at the intensity 0.1 V lower than the muscle contraction threshold (Fig. 5). It suggests that the microstimulation could be able to enhance SNA without muscle contraction, a side effect. In the future, it should be clarified whether the blood glucose reduction, seen in our previous work [3], could be induced with lower intensity of the microstimulation.

On the other hand, the stimulation via bipolar hook electrode maintained the relative AP rate rather constant independently of the stimulation intensity (Fig. 6). The result suggests that the muscle contraction induced via the motor nerve does not affect SNA at least when the stimulation intensity was low enough.

However, in BH group, the relative AP rate was more than 100%, approximately 120%, although the extent in general was lower than that of sympathetic activation seen in MS group. This high SNA may be attributed to the effects of thicker fibers having lower threshold, such as afferent ones, although the mechanism is not known.

Since significant differences on the sympathetic activation were detected between MS and BH group ($p < 0.05$ at the muscle contraction threshold intensity, Fig. 6), the microstimulation may have little activated thicker sensory and/or motor nerves. Therefore, these results in this study suggest that systemic SNA could be enhanced with lower stimulation intensity than that inducing muscle contraction, and that thicker fibers may little affect the increase in the contralateral SNA.

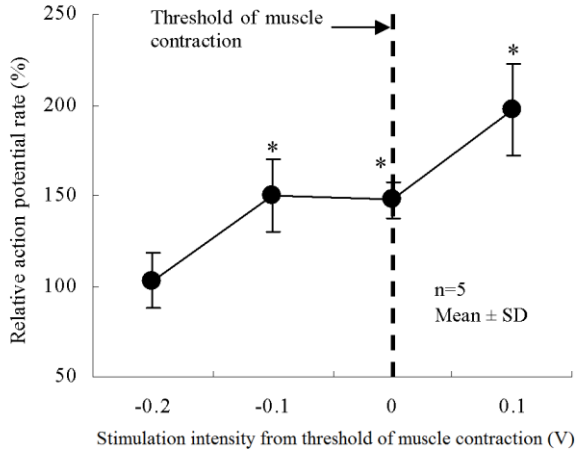

Figure 5. MS group: contralateral relative AP rate for 100 ms after 150 ms latency in response to unilateral microstimulation (*p < 0.01 vs. baseline (-0.2 V)). Significant difference was detected by ANOVA.

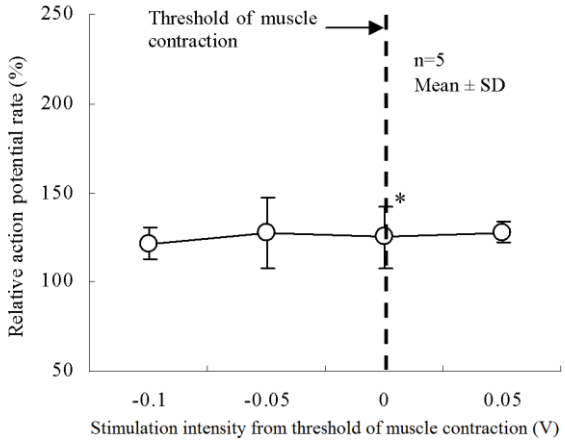

Figure 6. BH group: contralateral relative AP rate for 100 ms after 150 ms latency in response to unilateral stimulation. *p < 0.05 vs. 0 V-data in MS group (Student's t-test)

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