Depiction of perivascular micro-action potentials by microneurography

Hiroshi Miyauchi, Kenji Yamada, Hideaki Goto, Takehiko Tarui, Takeaki Matsuda, Shuji Shimazaki, Yoshihiro Yamaguchi

1Department of Traumatology and Critical Care Medicine, Kyorin University School of Medicine
2Emergency Life Saving Technique Academy, Tokyo
3Department of Emergency Medicine, Tokyo Metropolitan Hiroo General Hospital
4Trauma, Resuscitation and Prehospital Medicine, Graduate School, Kokushikan University

(Received: March 12, 2012; Accepted: September 10, 2012)

Summary

Objective: Circulatory system complications such as blood pressure fluctuations and arrhythmias often affect the prognosis of patients admitted to critical care medical centers and those requiring intensive care. Elucidating the influence of disease on the autonomic nervous system (ANS) is clinically important. We have conducted research with the aim of devising a novel method to monitor ANS dysfunction caused by disease.

Patients and methods: Subjects comprised 12 healthy volunteers (10 men, 2 women; mean age, 35.9 years; range, 26-50 years). After obtaining informed consent from each subject, Allen’s test was performed to ensure arterial patency. Absence of problems such as peripheral blood flow disturbance or bleeding diathesis was then confirmed. After subcutaneous infiltration anesthesia of the palmar aspect of the wrist, a 24-gauge micro-electrode was inserted using a technique for invasive indwelling monitoring of arterial pressure. The investigator held and touched the electrode tip against the radial artery wall, detecting micro-action potentials. Each subject underwent a Valsalva maneuver and tilt test, and the induced potential changes were recorded.

Results: Testing showed micro-action potentials in 8 of the 12 subjects at rest. On Fast-Fourier Transform (FFT) waveforms, waveform patterns showed peaks at low frequencies, gradually decreasing at integral multiples. Amplitudes of action potentials were clearly increased in 6 subjects with the Valsalva maneuver; and in 7 subjects with the tilt test. Detected micro-action potentials likely represented perivascular sympathetic nerve activity (SNA). Moreover, no complications occurred after testing.

Conclusion: The radial artery is often used clinically for invasive catheter insertion for arterial pressure monitoring. In the periphery, we were able to detect micro-potentials thought to represent sympathetic nerve activity. The subjects of this qualitative study were healthy volunteers. In future, adding evaluation of integrated peripheral blood flow and quantifying both integrated waveform (IW) and micro-action potentials (MP), we will be able to verify the significance of this achievement by examining the disease associated with ANS, such as sepsis and spinal cord injury.

Key words: sympathetic nerve activity; perivascular action potentials; Fast-Fourier Transform; a micro-electrode needle.
Introduction

Among patients admitted to high-level critical care medical centers who require intensive care, circulatory system complications such as blood pressure fluctuations and arrhythmia often affect the prognosis, and elucidating the influence of disease on the autonomic nervous system (ANS) is a clinically important issue. For example, in patients infected with tetanus, symptoms characterized by increased sympathetic nerve activity (SNA) often develop, including rises in blood pressure, increased heart rate, increased sweating, and elevated body temperature. However, a report by Shindo et al. in 1993, which a micro-electrode was inserted percutaneously in the peroneal nerve to record and analyze muscle SNA by microneurography, tetanus toxoid was also shown to have inhibitory effects on sympathetic nerves. In patients with tetanus, even when sympathetic inhibitors like β-blockers are used, control of increased heart rate and blood pressure can be difficult. Occasionally, severe hypotension and unstable hemodynamics can occur. Through analysis with microneurography, the causes have been clarified. Microneurography began in 1968, when Hagbarth and Vallbo in Sweden first recorded human SNA from peripheral nerves using tungsten micro-electrodes. Use of microneurography has gradually increased in various pathophysiologic studies, and Yamaguchi et al. reported the results of research on SNA during the course of sepsis or septic shock in 1994.

Recently, it has become widely known that overreactions in the body such as a "cytokine storm" worsen disease symptoms and affect vital prognosis. We have investigated methods of monitoring ANS overreaction and dysfunction caused and produced by diseases. With the aim of elucidating the effects of various diseases on the ANS and reflecting this in the development of therapeutic methods, we planned to continue studies on microneurography. However, previously reported methods of recording by direct nerve puncture in a shielded room are now difficult to accept in clinical practice. Instead, research has been initiated to identify safer methods to obtain this information.

For intensive care treatment in patients with unstable hemodynamic status, an arterial line is often placed in the radial artery, at a level about 2 cm proximal from the crease on the palmar aspect of the wrist to monitor 24-h changes in blood pressure. To date, attention has focused on the distribution of autonomic nerves to capillaries and arterioles, as depicted by electron micrography. We have devised a method of depicting action potentials, not of peripheral nerve trunks, but of sympathetic nerves from small perivascular sympathetic nerve networks. Compared to direct nerve puncture, which is associated with a high risk of complex regional pain syndrome (CRPS), our method can be performed more safely.

Materials and Methods

The total subject population comprised 10 male and 2 female healthy volunteers (mean age, 35.9 years; range, 26-50 years) under the ethics committee approval.

The protocol is described below. All tests were performed in an unshielded laboratory in the high-level critical care center at Kyorin University Hospital, because the test is intended to be performed in the intensive care unit in the future. In a laboratory that had been used as an operating theater until 5 years earlier, temperature was 26 degree in ceulcius, a temperature that would not be too cold or hot when wearing clothes, and the study was started. On the day of the study, subjects were called to the laboratory, the purpose of the study, details, and possible health hazards were explained.

Each subject adjusted their clothing so that the distal aspect of the arm to be tested was exposed from the elbow. After removing any metal objects that they were wearing, the subject lay in a supine position on the operating table in the center of the laboratory (Fig. 1A). A 3-body lead electrocardiogram (ECG) was monitored, and a fingertip plethysmograph (Finger Probe TL-201T; Nihon Kohden Corp., Tokyo, Japan) was placed on the contralateral index finger (Fig. 1B). The fingertip plethysmograph used in this study displays integrated waveforms, and so was mainly used to monitor changes in blood flow to the fingertip.

In all subjects, testing was performed on the left side, contralateral to the dominant hand. On the side tested, the hand was in supination with the palm upwards, and a towel was spread under the back of the hand to ensure a relaxed position. First, the radial artery was palpated at a site about 3 cm from the crease of the palmar aspect of the wrist, and this was marked with a felt-tip pen (Fig.
Allen’s test was performed and the absence of abnormal blood vessel distribution to the hand was confirmed, and then subjects rested in a supine position for 10 min. Next, at the planned puncture site, about 2 ml of 1% lidocaine was injected in the skin surface to raise a small wheal as local infiltration anesthesia. For another 5 min at rest, stable respiration, blood pressure, and heart rate were confirmed. The investigator inserted a 24-gauge micro-electrode needle (Stimuplex®, A25, 24G×25 mm; B. Braun Aesculap Japan, Tokyo, Japan), from proximally to distally, at an angle of about 60°. The investigator touched and held the needle tip to the arterial wall (Fig. 2).

After confirming detection of action potential waveforms on the monitor, resting potentials were recorded for a maximum of 5 min, using the Biomedical Research System LEG-1000® (Nihon Kohden, Tokyo, Japan). This system includes an ECG coupler (PC−101H; Nihon Kohden), ECG amplifier (AC−100H; Nihon Kohden), bioelectric coupler (PC−101H; Nihon Kohden), bioelectric amplifier (AB−100H; Nihon Kohden), sensor coupler (PP−101H; Nihon Kohden), and sensor amplifier (AP−100H; Nihon Kohden). Recording was continued, and subjects held their breath as long as possible up to 30 s (Valsalva maneuver). After resting for 5 min, a tilt test (Fig. 3) was performed by raising the head of the bed up 30° for 5 min. Next, the bed was returned to a flat position for 5 min of rest, and recording and testing were completed.

A total of 12 study participants were examined. The study was conducted over 8 days between January 21 and April 28, 2008. Testing in each subject took a mean of 13.8 min (range, 11–23 min).

Results

Figure 4a shows a representative monitoring image obtained from Subject d in Table 1.

On testing of the 12 healthy volunteers, micro-action
Figure 2. Image demonstrating the insertion of micro-electrode. A 24-G micro-electrode was inserted, from proximally to distally, at an angle of about 60°. The investigator touched and held the tip of the needle electrode (tip only is uncoated) to the artery wall (*) and micro-action potentials were detected within 15 min.

Figure 3. Tilt test. In the tilt test, the head of the bed is raised 30° for 5 min, then the bed is returned to a flat position.

Table 1. Detection results.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Time (min)</th>
<th>SNA potential pattern on FFT waveform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rest</td>
</tr>
<tr>
<td>a</td>
<td>45</td>
<td>M</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>b</td>
<td>50</td>
<td>M</td>
<td>11</td>
<td>+</td>
</tr>
<tr>
<td>c</td>
<td>39</td>
<td>M</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>d</td>
<td>30</td>
<td>M</td>
<td>23</td>
<td>+</td>
</tr>
<tr>
<td>e</td>
<td>29</td>
<td>F</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>f</td>
<td>45</td>
<td>M</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>g</td>
<td>35</td>
<td>M</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>h</td>
<td>34</td>
<td>M</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td>j</td>
<td>29</td>
<td>M</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td>k</td>
<td>42</td>
<td>F</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>l</td>
<td>26</td>
<td>M</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>m</td>
<td>27</td>
<td>M</td>
<td>18</td>
<td>-</td>
</tr>
</tbody>
</table>

Micro-action potentials at rest were detected in 8 subjects. Of these, changes in amplitude with the Valsalva maneuver or tilt test were observed in 7 subjects.

Three other subjects (Subjects f, g, and m) showed a similar amount of noise, and micro-action potentials could not be distinguished (Fig. 4B). Among the 8 subjects with detectable micro-action potentials at rest, changes in amplitude with the Valsalva maneuver or tilt test were observed in 7 subjects (Table 1).

Figure 5a shows a representative FFT waveform obtained from Subject k. At the center of the horizontal axis is a 50-Hz alternating current (AC) waveform, and smaller frequency waveforms are observed. The 50-Hz AC waveform is considered an artifact. A heart rate of 60 beats/min represents a frequency of 1 Hz. If complex potentials of SNA synchronous with the heart rate are detected, a waveform pattern like this develops, with peaks at low frequencies, and gradually decreasing at
Figure 4. Explanation of the waveform on the monitor screen

A: Expanded waveform of the monitor screen with Biomedical Research System LEG-1000® (Subject d).

The vertical axis shows the waveform (amplitude), and the horizontal axis shows the time (sec). The top waveform (ECG) is the ECG waveform. The middle upper waveform (FP) is the fingertip pulse wave. The middle lower waveform (IW) is the integration of the micro-action potentials = full-wave rectification integrated waveform. The bottom waveform (MP) is the micro-action potentials.

The lower right (*) area shows the waveform after analysis by real-time Fast Fourier Transform (FFT). The vertical axis is amplitude, and the horizontal axis is frequency. This shows typical sympathetic nerve action potentials, with a gradual decrease at integral multiples in the low-frequency range (white arrow). The waveform at 50 Hz is an AC power-supply artifact (black arrow).

B: Compressed waveform of micro-action potentials obtained from LEG-1000® (Subject e).

This shows a compressed waveform of the horizontal axis (time) of the monitor screen depicted in a). Changes due to waveform stimuli are easy to recognize. The micro-action potentials (MP) and full-wave rectification integrated waveform (IW) are distorted. Increases related to stimulation are also recognizable (white arrows), but are not synchronous with changes in the fingertip pulse wave (FP). On FFT (*), the AC noise at 50 Hz is very noticeable (black arrow), but amplification at low frequencies, typically seen with sympathetic nerve action potentials, is not observed.
decreased the fingertip rate or completing the bed-up position. The waveform at 50 Hz is the AC power supply artifact (black arrow).

B: Compressed waveform of micro-action potentials (Subject k).

With the Valsalva maneuver (start=S, end=E), from immediately after the head of the bed was raised to over 1 min, an increase in micro-action potentials was observed (MP), and the full-wave rectification integrated waveform (IW) increased. During the Valsalva maneuver, in the fingertip pulse wave (FP), a slight decrease was seen in amplitude. After the end of maneuver, heart rate increased (ECG).

C: The data analysis for the waveform of the tilt test (Subject l).

With the tilt test (start=S, end=E), from immediately after the head of bed was elevated with the tilt test, the full-wave rectification integrated waveform (IW in Fig. 5b), the waveform peak increases from just before completion of the Valsalva maneuver, and the amplitude of micro-action potentials increases markedly. In addition, in Subject l, as the head of bed was elevated with the tilt test, the full-wave rectification integrated waveform (IW in Fig. 5b) increased, and the amplitude of micro-action potentials increased. Moreover, the increase in waveforms decreased abruptly after completing the bed-up position.

Thus, for the micro-action potentials that we depicted, amplitudes clearly increased after the Valsalva maneuver or with the tilt test, which activated the SNA. Heart rate was also increased at the same time. Conversely, fingertip pulse waves represent integrated waveforms, and the amplitude reflects blood flow in the fingertips. Irrespective of the detection of micro-action potentials, the amplitude of fingertip pulse waves reproducibly decreased with the Valsalva maneuver or tilt test, when SNA was activated. When the amplitude of the micro-action potentials increased, the amplitude of the fingertip pulse wave decreased. The amplitude of micro-action potentials thus increased synchronously with SNA activation.

In all subjects in whom micro-action potentials could be detected during the above experiments, no action potentials were detected with the subcutaneous electrode. In addition, touching the electrode tip to the radial artery wall was essential to detect action potentials. Overall, our data support the hypothesis that the micro-action potentials detected by touching the electrode to the radial artery wall are consistent with perivascular wall SNA, considering that: 1) amplitudes increased with stimuli that activate SNA such as the Valsalva maneuver and tilt test; 2) sympathetic nerve-type complex action potential patterns were observed on FFT; and 3) the detection of action potentials was reproducible among subjects.  

integral multiples. Next, Figure 5b shows a waveform where the horizontal axis (the time axis) is compressed. If we observe the full-wave rectification integrated waveform (IW in Fig. 5b), the waveform peak increases from just before completion of the Valsalva maneuver, and the amplitude of micro-action potentials increases markedly. In addition, in Subject l, as the head of bed was elevated with the tilt test, the full-wave rectification integrated waveform (IW in Fig. 5b) increased, and the amplitude of micro-action potentials increased. Moreover, the increase in waveforms decreased abruptly after completing the bed-up position.

Thus, for the micro-action potentials that we depicted, amplitudes clearly increased after the Valsalva maneuver or with the tilt test, which activated the SNA. Heart rate was also increased at the same time. Conversely, fingertip pulse waves represent integrated waveforms, and the amplitude reflects blood flow in the fingertips. Irrespective of the detection of micro-action potentials, the amplitude of fingertip pulse waves reproducibly decreased with the Valsalva maneuver or tilt test, when SNA was activated. When the amplitude of the micro-action potentials increased, the amplitude of the fingertip pulse wave decreased. The amplitude of micro-action potentials thus increased synchronously with SNA activation.

In all subjects in whom micro-action potentials could be detected during the above experiments, no action potentials were detected with the subcutaneous electrode. In addition, touching the electrode tip to the radial artery wall was essential to detect action potentials. Overall, our data support the hypothesis that the micro-action potentials detected by touching the electrode to the radial artery wall are consistent with perivascular wall SNA, considering that: 1) amplitudes increased with stimuli that activate SNA such as the Valsalva maneuver and tilt test; 2) sympathetic nerve-type complex action potential patterns were observed on FFT; and 3) the detection of action potentials was reproducible among subjects. 
Discussion

In 1993, Mano et al. described a method of identifying SNA using microneurography. SNA is a spontaneous burst activity, and activity recorded by a micro-electrode is characterized by pulse-synchronous spontaneous and rhythmic activity. This activity is reportedly stimulated when blood pressure decreases with the tilt test, and is inhibited when increases occur. Likewise, this activity is markedly increased in the hypotensive phase of the Valsalva maneuver. This is in agreement with our study findings of micro-action potentials recorded from the periphery of the radial artery wall.

To date, although autonomic nerve distribution to capillaries and arterioles has been shown by electron micrography, for autonomic nerve distribution to medium-sized arteries such as the radial and ulnar arteries, the detection of those action potentials by electrodes as used in our study has been regarded as very difficult unless at a level that can be confirmed by gross anatomy. Therefore, following a report of Sihler’s staining of a cadaveric peripheral nerve by Sekiya et al. in 2005, we conducted a collaborative study with the Department of Anatomy at our university aimed at macroscopic investigation of how peripheral nerve branches are distributed in the arterial surface of the ulnar and radial arteries. The results using this staining method showed that nerves stained blue and other tissues were transparent. On observation using a stereomicroscope, small branches from the radial nerve cutaneous branch were distributed to the radial artery, and small nerves were distributed like a network around the radial artery. At a level 1–5 cm proximal from the crease of the palmar aspect of the wrist, distribution of many autonomic nerve fibers on the radial artery surface was predicted. The results supported the possibility of using this method to detect sympathetic nerve action potentials from small sympathetic nerve networks on the arterial surface.

Recently, microneurography studies have been performed by direct nerve puncture with a tungsten needle in a shielded room. The high levels of noise on the ground have led to recent experiments being conducted in a space environment. The consistent results obtained in the present study are due in large part to the detection electrode. Irrespective of the detection environment, the low level of noise was due to a large cross-sectional area of the needle tip and shielding of areas other than the needle tip. In addition, selection of the periphery of the radial artery as a detection site contributed to successful detection. Even if we evaluated the qualitative in this study, not to the quantitative, it may be a valuable method that we have never developed.

Although the problematic issues include the detection rate and dependence on investigator technique, but we will continue to research by the quantitative evaluation of micro-potentials, our method may be useful in future applied research for clinical monitoring of the sympathetic nervous system.

Conclusion

Use of this novel technique allowed us to detect micro-action potentials from the periphery of the radial artery. From analysis of the action potentials and histological studies, we have shown that sympathetic nerve complex potentials were detected. The subjects of this qualitative study were healthy volunteers. In future, adding evaluation of integrated peripheral blood flow and quantifying both IW and MP, we will be able to verify the significance of this achievement by examining the disease associated with the autonomic nervous system, such as sepsis and spinal cord injury.

Acknowledgments

We wish to thank Mr. Ohara, Mr. Yoshinori, Mr. Matsuo, and Mr. Higashigaki at Nihon Kohden Corp. for their cooperation in this study.

References


