Implantable electrode for recording nerve signals in awake animals

ISHIO NINOMIYA, YOSHIHARU YONEZAWA, AND MICHAEL F. WILSON
Department of Physiology, School of Medicine, Hiroshima University, Kasumicho, Hiroshima, Japan;
Department of Electrical Engineering, Hiroshima Institute of Technology, Itsukaichi, Hiroshima, Japan, and
Department of Physiology and Biophysics, West Virginia University Medical Center,
Morgantown, West Virginia 26506

Ninomiya, Ishio, Yoshiharu Yonezawa, and Michael F. Wilson. Implantable electrode for recording nerve signals in awake animals. J. Appl. Physiol. 41(1): 111-114. 1976. An implantable electrode assembly consisting of collagen and metallic electrodes was constructed to measure simultaneously neural signals from the intact nerve and bioelectrical noises in awake animals. Mechanical artifacts, due to bodily movement, were negligibly small. The impedance of the collagen electrodes, measured in awake cats 6-7 days after implantation surgery, ranged from 39.8-11.5 kΩ at a frequency range of 20-5 kHz. Aortic nerve activity and renal nerve activity, measured in awake conditions using the collagen electrode, showed grouped activity synchronous with the cardiac cycle. Results indicate that most of the renal nerve activity was from postganglionic sympathetic fibers and was inhibited by the baroceptor reflex in the same cardiac cycle.

Implantable cowskin collagen electrode, aortic and renal nerve activity in awake cats; grouped activity synchronous with cardiac cycle; baroceptor reflex

THE IMPLANTABLE ELECTRODE was developed to fill the need for continuous recording of neural signals from multifiber preparations in both anesthetized and unanesthetized animals. In most previous studies of neural signals controlling the cardiovascular system, conventional metallic electrodes were used in a liquid paraffin pool under anesthetized conditions (2, 3, 5). When various kinds of stimuli were applied during an experiment, continuous recording of neural signals was often interrupted by unsatisfactory connection between the nerve and electrodes with passive or spontaneous movement of the body. Moreover, neural signals were recorded from the cut end of the nerve fibers, placing the biological system in an open loop condition. The implantable electrode is designed to record neural signals from the intact nerve which preserves a closed loop configuration. Efferent neural signals have been recorded by metallic electrodes in unanesthetized conditions (1, 4, 6, 7). In these studies, the influence of anesthesia on neural signals could be neglected, but technical difficulties existed in implanting the electrodes, and in eliminating signal artifacts and nerve fiber injury. In this study, to minimize noise and injury, a sheet of collagen fibers was placed between the nerve fibers and the metal of the recording electrode.

PREPARATION AND FABRICATION OF ELECTRODE

The details of the size and configuration of the implantable electrode assembly are shown in Fig. 1. A sheet of collagen fibers was made as follows: a raw cowskin (about 10 x 5 cm) was immersed in a saturated NaCl solution for a period of 10 days and in a solution of Ca(OH)₂ (450% of Ca(OH)₂ per unit weight of a wet salted hide) for a period of 4 days. After removing the hair, the limed hide was immersed in borac acid (pH 9.0, temperature 30°C) for 2.5 days, and then tanning was performed sequentially in a tannic acid solution with a pH of 5.5, 5.0, 4.5, 4.0, and 3.5 for 2 days at each pH, respectively. Finally, the collagen fibers washed by distilled water (pH 7.0) were exsiccated gradually and cut in a sheet of 1.5 × 1.0 × 0.3 mm under a dissecting microscope (Fig. 1A). One end of the collagen fiber sheet was connected to a silver wire (Teflon-coated, 9999 Ag, 0.005 ft in diameter), and a hole (0.1-0.3 mm in diameter) was made in the center of the collagen fiber sheet. As shown in Fig. 1B, two collagen fiber sheets with attached wires were arranged in parallel configuration at a distance of 3 mm by inserting a stainless steel rod and were coated completely by silicone rubber. After the process cured for 1 day a slit was cut into the silicone rubber and collagen fiber sheets opposite the lead wires (SL in Fig. 1, A and C), and then the stainless steel rod was removed through the slit for making a channel. The size of the channel for the nerve fibers ranged from 0.1 to 0.3 mm in diameter and from 6 to 8 mm in length. Clear silicone rubber was used for structural support and insulation so that the relative position of the nerve fibers in the channel of the electrodes could be seen at the time of implantation.

A pair of wires (Ag or Pt, Teflon-coated, 0.005 ft in diameter) was placed on the outside of the silicone rubber parallel to the sheet of recording electrodes and used as reference electrodes (RE in Fig. 1, B and C) for monitoring bioelectrical noises around the nerve signals. The four lead wires (10 cm in length) from the recording and reference electrodes were covered by a silicone rubber tube (ID 0.5 mm) and were connected to a socket (Fig. 1C). An implantable electrode assembly of small size and light weight with adequate electrical insulation is preferred. In this study, a size of 2 × 3 × 6 mm was usually selected for recording renal nerve activity (RNA) and aortic nerve activity (ANA) in unanesthetized cats.

MECHANICAL NOISE

Mechanical noise of the electrode assembly was examined by measuring the induced potentials between two recording electrodes and between two reference electrodes. The implantable electrode was immersed in 0.9% NaCl solution for at least 24 h, and then displacement sine wave signals were applied. When the implantable electrode was moved sinusoidally with a displacement of 1 cm at various frequencies in 0.9% NaCl solution at 37.0°C, the induced potentials in the recording electrodes were 14 µV (peak to peak) at all frequencies tested. On the other hand, the induced potentials in the reference electrodes varied from 4,000, 2,600, 2,300, 1,670, 933 to 867 µV with Ag and from 800, 667, 600, 533, 467, to 400 µV with Pt at 0.15, 0.24, 0.42, 0.57, 0.80 and 1.00 Hz, respectively. Thus, the mechanical noise was much smaller in the collagen-covered recording electrodes compared to conventional metallic
FIG. 1 Details of a cowskin collagen fiber electrode (SkE) are shown in A. LW = lead wire (Teflon-coated), Ag = silver wire, H = hole, SL = slit. Numerals indicate size in mm. Relative position of two SkE and reference electrodes (RE) in an implantable electrode assembly is shown in B. At the time of implantation, the nerve was placed in the position denoted by N through the slit (SL). The size and configuration of the implantable electrode is shown in C. SR = silicone rubber material, S = silk suture. Recording and reference electrodes were connected to a preamplifier through the socket for recording neural signals (NS) and for monitoring bioelectrical noises (BEN).

Electrical properties were tested by measuring the impedance in NaCl solution and in vivo. For small voltage (of the order of 5 mV) applied to a pair of recording electrodes, the impedance varied dependent on the frequency, time after immersion, and concentration and temperature of the test solution.

In Fig. 2A, the impedance measured 4 days after immersion in 0.9% NaCl solution at 37°C is shown plotted against the frequency by the lower curve of open circles. The impedance decreased from 9.5 kΩ to 8.0 kΩ as the frequency was increased from 20 Hz to 5 kHz. An inverse relationship between the frequency and the impedance obtained 4 days after immersion is shown in Fig. 2B by the upper curve of solid circles. The impedance was higher at all frequencies tested, in particular at high frequency range, as compared to that obtained in 0.9% NaCl solution. The effects of time on the impedance is shown in Fig. 2C by the lower curve of open circles. The impedance was 12.9 kΩ at a frequency of 1 kHz on the day of operation, but it increased after operation and reached a maximum value (mean, 21.3 kΩ) on the 3rd postoperative day. Mean values measured from three cats are summarized in Table 1. The increase in impedance may be caused partly by a decrease in concentration of fluids in the collagen fiber sheet. In addition, scar formation between the nerve fibers and recording electrodes may increase the impedance. To minimize the influence of electrode impedance, a preamplifier (band pass, 50-2 kHz) was employed. The method of analysis of the neural signal has been described previously.

In three cats, the impedance of the recording electrode was measured over a period of 1 wk following operation while recording the neural signals. Relationship between the frequency and the impedance obtained 4 days after operation is shown in Fig. 2A by the upper curve of solid circles. The impedance was higher at all frequencies tested, in particular at high frequency range, as compared to that obtained in 0.9% NaCl solution. The effects of time on the impedance is shown in Fig. 2B by the upper curve of solid circles. The impedance was 12.9 kΩ at a frequency of 1 kHz on the day of operation, but it increased after operation and reached a maximum value (mean, 21.3 kΩ) on the 3rd postoperative day. Mean values measured from three cats are summarized in Table 1. The increase in impedance may be caused partly by a decrease in concentration of fluids in the collagen fiber sheet. In addition, scar formation between the nerve fibers and recording electrodes may increase the impedance. To minimize the influence of electrode impedance a preamplifier (band pass, 50-2 kHz) with an input impedance of 5 MΩ was employed. The method of analysis of the neural signal has been described previously.

Implantation of Electrode

Under sodium pentobarbital anesthesia (35 mg/kg, ip), a bundle of nerve fibers from either the renal or aortic nerve (1.2-2 cm in length) was isolated from the surrounding connective tissue and the nerve bundle (about 1 cm in length) was desheathed carefully at the site of electrode implantation. After the nerve bundle was placed in the channel through the slit of the electrode assembly (Fig. 1C), the slit was closed completely by suture (S). Then the electrode assembly was sutured on the surrounding connective tissue. The lead wires from the electrodes were brought out to the body surface and the socket was firmly attached to the skin of the back. Connections between this socket and the recording apparatus were made by lightweight cable.

In three cats, the impedance of the recording electrode was measured over a period of 1 wk following operation while recording the neural signals. Relationship between the frequency and the impedance obtained 4 days after operation is shown in Fig. 2A by the upper curve of solid circles. The impedance was higher at all frequencies tested, in particular at high frequency range, as compared to that obtained in 0.9% NaCl solution. The effects of time on the impedance is shown in Fig. 2B by the upper curve of solid circles. The impedance was 12.9 kΩ at a frequency of 1 kHz on the day of operation, but it increased after operation and reached a maximum value (mean, 21.3 kΩ) on the 3rd postoperative day. Mean values measured from three cats are summarized in Table 1. The increase in impedance may be caused partly by a decrease in concentration of fluids in the collagen fiber sheet. In addition, scar formation between the nerve fibers and recording electrodes may increase the impedance. To minimize the influence of electrode impedance a preamplifier (band pass, 50-2 kHz) with an input impedance of 5 MΩ was employed. The method of analysis of the neural signal has been described previously.

FIG. 2 Impedance of collagen-covered recording electrodes was plotted against frequency (A), time after immersion or operation (B), concentration of NaCl solution at 37°C (C), and temperature of 0.9% NaCl solution (D). Open circles (lower curves in A and B) indicate impedance obtained in NaCl solution (0.9% in A, B, and D); solid circles indicate impedance obtained in 3 cats.
Using the implantable electrodes, complex action potentials were recorded from the aortic nerve in 6 of 7 cats and from the renal nerve in 10 of 12 cats. As shown in Fig. 3A, in the anesthetized condition the original neurograms were recorded simultaneously from the peripheral cut end of the aortic nerve using the conventional metallic electrode in a paraffin pool and from the intact portion of the same nerve using the collagen-covered recording electrode. In three cats tested, the amplitude of the original neurograms was smaller in the collagen-covered recording electrode than in the conventional metallic electrode. However, the discharge patterns of the original neurograms resembled each other.

In the awake condition, using the implantable cown collagen electrode, it is possible to record the neural signals continuously from the intact nerve. In Fig. 3B, although the electromyogram (EMG) was observed in the electrocardiogram (ECG) tracing, neither the EMG nor the ECG was detected in the neurogram. Since skeletal muscle potentials during spontaneous movement may cause interpretation difficulties (6, 7), in this study the EMG around the implantable electrode was monitored during experiments. By comparing the EMG in the reference electrodes and the neural signals in the recording electrodes, if the time courses of EMG and neural signals resemble each other, it can be assumed that the neural signals are marked by the EMG. The aortic and renal nerve activity were recorded simultaneously under closed baroceptor loop conditions using the implantable electrode (Fig. 3C). The shape and magnitude of spikes differed between nerves. The peak-to-peak amplitude of the spikes ranged from 5 to 100 μV, mostly 10 to 20 μV, while the peak-to-peak amplitude of the noise signal in the recording system including the implantable electrode was approximately 5 μV at rest in the awake condition and under anesthesia. In many cats, the signal-to-noise ratio tends to decrease in the awake condition, because of the decrease in amplitude of the neurogram and/or the increase of the noise signals due to the EMG. However, the signal-to-noise ratio was larger in the collagen-covered electrodes than in the metallic electrodes, since the mechanical artifacts were negligibly small.

In the awake conditions grouped discharges synchronous with the cardiac cycle were observed in all renal nerves examined. To enhance the signal-to-noise ratio and to reduce the cycle variation of the grouping, the integrated neural signals were averaged over 50 cardiac cycles using the R spike of the ECG as a trigger signal. Examples of the averaged renal nerve activities (RNA) synchronous with the cardiac cycle are shown in Fig. 4, A1 and B1. During resting, sitting, standing, eating of food, drinking of milk, or walking conditions from 0 to 8 days following operation, the averaged renal nerve activity was sampled randomly at 237 periods. In 144 of 237 averaged data obtained from eight cats, the grouped activity synchronous with the cardiac cycle was clearly detected in the renal nerve activity. Both the grouped and tonic activity in the renal nerve were inhibited by the administration of norepinephrine (Fig. 4, A2) or by the injection of hexamethonium bromide, i.e., ganglion blockade. (1 mg/kg, iv), the major portion of RNA was inhibited.

**TABLE 1. Influence of time after operation and frequency on impedance of collagen-covered recording electrodes**

<table>
<thead>
<tr>
<th>Postoperative Day</th>
<th>Frequency, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>0</td>
<td>38.0</td>
</tr>
<tr>
<td>1</td>
<td>37.6</td>
</tr>
<tr>
<td>2</td>
<td>37.7</td>
</tr>
<tr>
<td>3</td>
<td>39.8</td>
</tr>
<tr>
<td>4</td>
<td>36.9</td>
</tr>
<tr>
<td>5</td>
<td>35.9</td>
</tr>
<tr>
<td>6</td>
<td>34.9</td>
</tr>
<tr>
<td>7</td>
<td>36.2</td>
</tr>
</tbody>
</table>

Data are mean values of the impedance obtained from 3 cats, calibration = M1.

**FIG. 3. Original neural signals recorded in anesthetized and in awake conditions.** In A, discharge patterns of original neural signals in the aortic nerve recorded simultaneously by collagen-covered implantable electrode (2) and by conventional metallic electrode (3) are compared. The aortic pressure (1) is 148/106 mmHg. In B, the original neural signals (1) in the intact aortic nerve were recorded simultaneously with the electrocardiogram (2) in the awake condition. In the electrocardiogram tracing bioelectrical noise due to the electromyogram can be seen while no detectable bioelectrical noise due to ECG and EMG is observed in the neural signals. In C, neural signals from the left aortic nerve (2) and left renal nerve (3) are illustrated. Both tracings show grouped discharges synchronous with the cardiac cycle. In D, the original renal activity (1) and its integrated activity (2) are shown. Time calibration = 200 ms, vertical bar = 25 μV. Parts of the original neurograms are retouched.

**FIG. 4. Integrated neural signals were averaged over 50 cardiac cycles.** Averaged neural signals (RNA) were obtained 2 days after operation from the left renal nerve in the cat in the prone position. R spikes in the electrocardiogram (ECG) were used as the trigger signal for the averaging device. At control (A1 and B1) grouping of RNA synchronous with the cardiac cycle was dominantly observed. In A2, with administration of norepinephrine (5 μg/kg, iv), both grouped and tonic RNA were inhibited by an increase in baroceptor inputs. In B2, with administration of hexamethonium bromide, i.e., ganglion blockade, (1 mg/kg, iv), the major portion of RNA was inhibited.
cats examined. These studies indicate that the major portion of renal nerve activity, recorded by the implanted cowskin collagen electrode, originated from efferent postganglionic sympathetic fibers and was inhibited reflexly by baroceptor inputs in the same cardiac cycle.

This study was supported in part by Japanese Ministry of Education Grant 021514 and National Aeronautics and Space Administration Grant NGR 49-001-048.

Received for publication 24 September 1975.

REFERENCES