Evoked Electrospinogram from the Epidural Space by the Stimulation of Saphenous Nerve in Dog

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ABSTRACT. Using a signal-averaging technique, the evoked electrospinogram (EESG) by stimulating saphenous nerve was recorded from epidural space in order to clarify the characteristics of canine EESG. When saphenous nerve in neurologically normal dogs was stimulated, the segmental EESG was obtained from the epidural space adjacent to L3 to L6 segments (L2 to L5 vertebrae). The segmental EESG consisted of one positive (P1) and three negative peaks (N1, N2 and N3). A slow positive phase occasionally followed N3. When the stimulus intensity was changed, the N3 latency varied whereas the P1 and the N1 latencies were almost constant. The N2 and the N3 amplitudes were variable even in the same dog at the stimulus intensity strong enough to make the P1-to-N1 amplitude maximum. Spinal transection at a lower thoracic level had no influence upon the segmental EESG. After a temporal spinal ischemia, P1 and N1 did not change. On the contrary, N2 was diminished in the rigid as well as in the flaccid dogs and N3 was greatly decreased or almost disappeared in the rigid dogs and completely abolished in the flaccid dogs. Mephenesin did not alter the segmental EESG. On the other hand, baclofen decreased N2 and, more preferentially, N3. Based upon these results, it was suggested that P1 and N1 might be related to the arrival of the afferent volley at the spinal cord and N3 might be associated with the interneuronal activity.

The evoked electrospinogram (EESG) has been frequently used to diagnose the disease in spinal cord and to monitor the activity of spinal cord during its surgery in man [10–13]. EESG has also been introduced in canine neurology expecting that a better understanding of function and disorder of spinal cord can be obtained. Thus, Griffiths [7] has examined the evoked dorsal column potential in the experimental spinal cord compression of dogs, and Holliday et al. [8] have reported percutaneous recordings of the EESG in dogs. However, the systematic knowledge of canine EESG is not sufficient as yet and much is left to be studied.

Our preliminary experiment revealed that the EESGs were distorted to varying degree due to the artifact due to muscuar contraction when a mixed nerve such as tibial nerve was stimulated without a muscle relaxant. The purpose of the present study is to examine the EESGs recorded from the epidural space by the stimulation of saphenous nerve which is a purely sensory and an easily accessible nerve in dogs.

MATERIALS AND METHODS

Materials: Total of 27 neurologically normal mongrel dogs of either sexes, 6 months of age or older and weighing 5.5 to 10.0 kg, were used. Twelve out of 27 dogs, 9 intact and 3 spinal dogs, were used to determine the distribution and characteristics of the EESG. Other 8 dogs were subjected to the temporal spinal ischemia.Approximately 24 h after the release from the ischemic procedure, 5 dogs were found to be rigid whereas 3

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dogs were flaccid. The EESG was recorded in these dogs. In 3 of the former and 2 of the latter dogs, spinal cord was transected during the course of the EESG recording. The remaining 7 dogs were used for an experiment with drugs, 3 for mephenesin and 4 for baclofen.

Anesthesia: All dogs were anesthetized with a halothane-oxygen mixture, usually at a concentration of 2 to 2.5% of halothane. In the experiments with mephenesin and baclofen, lower concentration of halothane (1 to 1.5%) was used in order to keep the flexor reflex evocable and the breathing of the dogs were artificially maintained by manual deflation of the rebreathing bag of anesthesia apparatus when administered with the drugs. A decrease in the body temperature during the experiment was prevented by hot-water bottles.

Nerve stimulation: Saphenous nerve was exposed on the medial aspect of right thigh. A bipolar electrode made of two silver wires with an interelectrode distance of 5 mm was applied to the moistened saphenous nerve at the point where it emerged from the caudal part of sartorius muscle. Rectangular pulses with a duration of 0.3 msec and a frequency of 1 Hz were delivered by an electrostimulator (San-ei 3F-31 type). The stimulus intensity was changed stepwise to 0.3, 0.5, 1, 2 and 4 V. Intensities of 6 and 8 V were applied when necessary, particularly in the ischemic dogs.

In the experiment with mephenesin and baclofen, an incision was made in the right popliteal region and tibial nerve was separated and severed. Its proximal end was placed on a bipolar electrode in a warm paraffin pool and stimulated with a rectangular pulse with a duration of 0.3 msec and an intensity of 20 V to induce a response originated to a flexor reflex in the ipsilateral anterior tibial muscle.

The response of the muscle was monitored by recording electromyograms before and after the administration of these drugs.

EESG recording: The exploring electrode was a silver needle (0.34 mm in diameter) which was insulated with cashew except for a length of 4 mm from the tip. Hypodermic needles were used as reference and ground electrodes.

A skin incision was made on the dorsal midline from the lower thoracic to sacral regions and the paraspinal musculature was turned over. The exploring electrodes were inserted into the epidural space through the individual intervertebral foramina from T12 to L6 vertebrae on the right side. The reference electrode was positioned subcutaneously near the skin incision at the same spinal level as the exploring electrode. The ground electrode was placed subcutaneously usually at the left stifle joint. When the electrocardiographic artifact interfered with the EESG recording, the ground electrode was moved to an appropriate site to eliminate the artifact.

The exploring and the reference electrodes were connected to G1 and G2 of an amplifier (San-ei 130-system biophysiograph), respectively, and the electrical response was amplified at a time constant of 0.3 sec. The output of the amplifier was fed into a signal processor (San-ei 7TO7A type) at an amplification of 50 to 250 µV/cm. Two hundred responses were averaged at a sampling time of 20 µsec and an analyzing time of 20.48 msec. The averaged response was photographed.

Spinal ischemia: Dogs were anesthetized by an i. v. of sodium pentobarbital at a dose of 25 mg/kg. Thoracotomy was made at the left 4th or the 5th intercostal space under artificial respiration. According to the method of Tureen [14],
the thoracic aorta was clamped for a period of 45 to 100 min with rubbersheathed Péan's forceps at the level between the 4th and the 5th costovertebral junctions to make the spinal cord below the thoracic segments temporally ischemic.

**Drugs:** Mephenesin was given intravenously at a dose of 20 mg/kg and baclofen at a dose of 5 mg/kg. Physiological saline of the same volume as the drug solution served as control and was given 15 to 30 min prior to the administration of the drugs.

**Spinal transection:** After dorsal laminectomy, the spinal cord including the dura mater, was severed with scissors at a level between T11 and T12 or T12 and T13 segments. Hemorrhage was prevented by using cotton plugs.

The recording sites in relation to the spinal cord segment in the vertebral canal were examined in all dogs after fixation with formalin.

**RESULTS**

1. **Distribution and characteristics of electrical potential changes in neurologically normal dogs**

Although the changes in electrical potential were observed at levels ranging from T12 segment to cauda equina, large changes were obtained only at 1 to 2 recording sites adjacent to L3 to L6 segments (L2 to L5 vertebrae) (Table 1 and Fig. 1).

The typical electrical changes consisted of 4 components, i.e., one positive and 3 negative peaks. They were tentatively designated as P1, N1, N2 and N3 according to their polarity and the order of occurrence (Fig. 2). A slow positive

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**Fig. 1.** Changes in electrical potential in a neurologically normal dog. Obtained from dog 6. Stimulus intensity: 2 V. Calibration: 31 µV.
phase occasionally followed N3. P1 and N1 were sharp. N2 often looked like a hump on N3. In a few dogs, N1 and/or N2 were notched. N3 rised slowly and had a long descending phase returning to the zero line. These four components were clearly distinguishable at a stimulus intensity of more than 0.5 or 1 V at the recording sites adjacent to L3 to L6 segments. While all the components became small and ambiguous at the sites cranial to L2 to L3 segments, only P1 and N1 were clearly recorded at the sites caudal to L6 to L7 segments.

To characterize the individual components, measurements were performed on the changes recorded at the site where the maximum response was obtained at various stimulus intensities. Peak latencies from the stimulus artifact to P1 (P1 latency), to N1 (N1 latency), to N2 (N2 latency) and to N3 (N3 latency) were read by the signal processor (Table 2). The amplitude from P1 to N1 (P1-to-N1 amplitude), the amplitude from the zero line to N2 (N2 amplitude) and the amplitude from the zero line to N3 (N3 amplitude) were measured on enlarged prints (Table 3).

Both the P1 and the N1 latencies were

![Fig. 2. Typical segmental EESG. Obtained at the epidural space adjacent to the L4 segment (between L3 and L4 vertebrae) in dog 6. Stimulus intensity: 2 V. Calibration: 31 μV.](image-url)

| Table 1. Distribution of electrical potentials in neurologically normal dogs |
|------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     |
| T12                   | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| T13                   | +     | +     | +     | ±     | +     | +     | +     | +     | +     |
| L1                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| L2                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| L3                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| L4                    | +*    | +*    | +*    | +*    | +*    | +     | +     | +     | +     |
| L5                    | +     | +     | +*    | +*    | +*    | +*    | +*    | +*    | +*    |
| L6                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| L7                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| S1                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| S2                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| S3                    | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| C.E.                  | -     | -     | +     | ±     | ±     | ±     | ±     | ±     | ±     |


| Table 2. P1, N1, N2 and N3 latencies in neurologically normal dogs |
|------------------------|--------|--------|--------|--------|--------|
| Stimulus intensity    | P1 latency | N1 latency | N2 latency | N3 latency |
| 0.3                    | 3.6(3.0–4.7) | 4.3(3.5–5.9) | 5.7(4.6–7.3) | 7.5(6.4–9.2) |
| 0.5                    | 3.5(3.1–4.6) | 4.2(3.6–5.9) | 5.5(4.5–7.1) | 7.3(6.1–9.2) |
| 1                      | 3.5(3.1–4.6) | 4.3(3.5–5.8) | 5.4(4.4–7.0) | 7.5(6.0–9.5) |
| 2                      | 3.4(3.0–4.5) | 4.1(3.5–5.7) | 5.3(4.4–7.0) | 7.5(6.5–9.4) |
| 4                      | 3.5(3.1–4.5) | 4.0(3.5–5.7) | 5.3(4.4–6.9) | 7.3(6.0–9.5) |

Stimulus intensity in V. Latency: Mean (min.-max.) in msec (N=7 at 0.3 V and N=9 at 0.5 to 4 V). Measurements were performed on records obtained at the maximal recording site in 9 dogs (in one dog at L3, in 6 dogs at L4 and in 2 dogs at L5, as shown in Table 1). No response was manifested at 0.3 V in 2 dogs.
constant in individual dogs except in one dog regardless of the changes in the stimulus intensity. The N2 latency was also nearly stable in 6 out of 9 dogs irrespective of the stimulus intensity. However, the N2 latency decreased by 0.4 to 0.8 msec in other 3 dogs as the stimulus intensity was increased from 0.3 to 4 V. The N3 latency fluctuated considerably and irregularly in some of the dogs at various stimulus intensities.

In general, the individual variation in the amplitude of the peaks at a stimulus intensity was wide and a 3- to 13-fold change in the amplitude was observed among the dogs. The P1-to-N1 amplitude attained the maximum in all the dogs except one at a stimulus intensity of 0.5 to 2 V. The N2 and the N3 amplitudes were variable even in the same dog at the stimulus intensity strong enough to make the P1-to-N1 amplitude maximum.

Spinal transection at a lower thoracic level had no significant influence upon the distribution, latency and amplitude of the electrical changes.

II. Influences of the temporal spinal ischemia

About 24 h after the ischemia, a knee jerk could be manifested in the 5 rigid dogs but not in the 3 flaccid dogs. The flexor reflex of the hindlimbs was not observed in all of the 8 dogs. Only one rigid dog showed pain when pinched by a dressing forceps, and the other 7 dogs, 4 rigid and 3 flaccid, did not manifest pain even when pinched by Péan's forceps. The ischemic rigidity of the hindlimbs disappeared in all of the 5 rigid dogs after anesthesia with halothane.

In the rigid dogs, P1 and N1 were similar to those in neurologically normal dogs in their latency and amplitude. N2 occurred at a similar latency to that in neurologically normal dogs but its amplitude was small. N3 underwent a marked alteration and its amplitude decreased markedly or almost disappeared. However, N3 with a small amplitude was clearly noted in the rigid dog showing pain in response to pinching.

In the flaccid dogs, P1 and N1 always persisted and N2 decreased as in the case with the rigid dogs. N3 disappeared completely in all of the flaccid dogs (Fig. 3).

Spinal transection at a lower thoracic level did not alter these characteristics in both the rigid and the flaccid dogs.

III. Influences of mephensin and baclofen

The response of the anterior tibial muscle originated to a flexor reflex was completely depressed after the administration of mephensin and the evoked electromyograms were not demonstrated in the anterior tibial muscle. However,

<table>
<thead>
<tr>
<th>Stimulus intensity</th>
<th>P1-to-N1 amplitude</th>
<th>N2 amplitude</th>
<th>N3 amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>7.6(3.1–11.9)</td>
<td>14.2(7.1–25.0)</td>
<td>15.4(8.0–22.7)</td>
</tr>
<tr>
<td>0.5</td>
<td>11.6(4.6–17.6)</td>
<td>18.0(4.6–38.4)</td>
<td>21.6(4.6–49.1)</td>
</tr>
<tr>
<td>1</td>
<td>16.0(6.7–30.6)</td>
<td>20.5(3.8–45.4)</td>
<td>26.1(8.7–69.9)</td>
</tr>
<tr>
<td>2</td>
<td>16.0(6.6–33.6)</td>
<td>21.2(3.9–51.1)</td>
<td>28.0(10.3–67.9)</td>
</tr>
<tr>
<td>4</td>
<td>17.2(6.7–35.0)</td>
<td>21.1(6.5–55.6)</td>
<td>28.2(13.2–69.1)</td>
</tr>
</tbody>
</table>

Amplitude: Mean (min.-max.) in μV (N=7 at 0.3 V and N=9 at 0.5 to 4 V). See the foot-notes on Table 2.
this drug had no significant influence upon all the 4 components of the electrical changes (Fig. 4).

Baclofen also completely suppressed the response due to the flexor reflex. Although P1 and N1 were not significantly changed, N2 and N3 were gradually decreased in their amplitude following the administration of baclofen. The decrease in the amplitude of N3 was greater than that in N2 (Fig. 4).

**DISCUSSION**

Changes in electrical potential that occurred in the spinal cord by stimulation of a peripheral nerve are called the EESG. Generally, the EESG is classified into two types; the segmental and conductive EESGs. The former is an EESG obtained at or near the spinal cord segments from which the stimulated peripheral nerve arises whereas the latter is an EESG obtained at the remote segments. When the saphenous nerve was stimulated, changes in electrical potential were recorded at a wide range of the epidural space caudal to T12 segment. However, larger changes were observed only in a narrow range, that is, the epidural space adjacent to L3 to L6 segments. Saphenous nerve is a branch of femoral nerve and the latter arises from L4 to L6 segments [3]. Thus, the larger changes were manifested at the recording sites adjacent to these segments. They are indisputably the segmental EESG. At sites cranial to L2 to L3 segments, electrical potential changes were diminished and distorted. Therefore, these changes seem to be electrotonic propagation of the segmental EESG in the volume conductor but not the true conductive EESG. At the sites caudal to L6 to L7 segments, small changes in electrical po-
potential were persistently recorded, as discussed later.

The typical segmental EESG in the present study consisted of 4 peaks, that is, first positive peak P1, second negative peak N1, third negative peak N2 and fourth negative peak N3. Holliday et al. [8] recorded the EESG of dogs through monopolar needle electrodes placed in contact with the dorsal lamina of the vertebra or near the interarcuate space. The EESG consisted of the first positive peak of a considerable amplitude and the second negative wave of a large amplitude followed by a slow positive wave when the EESG was recorded at the site between L4 and L5 vertebrae on the stimulation of saphenous nerve. Comparing the order of occurrence, polarity and magnitude of deflections, their positive and negative peaks seem to correspond to P1 and N3, respectively, in the present study. Gelfan and Tarlov [4] distinguished 4 main deflections of the cord dorsum potential (CDP) of dogs; the initial positivity, the A spike (negative), the N wave (negative) and the P wave (positive). There are similarities between the CDP described by them and the segmental EESG observed in the present study; the initial positivity might correspond to P1 in our experiment, the A spike to N1 and the N wave to N3. According to them, the P wave was the
most vulnerable component. This wave might correspond to the slow positive phase which occasionally followed N3 in our experiment. As has been stated by them, the last component would be greatly dependent upon the physiological state of the spinal cord.

P1 and N1 were not influenced by the temporal spinal ischemia which brought about an extensive alteration of lumbar-sacral [5, 6] or thoracolumbar [9] neurons. Furthermore, clear P1 and N1 were recorded even at the sites caudal to the segments where saphenous nerve arose. These results indicate that P1 and N1 might be related to the arrival of the afferent volley at the spinal cord. In fact, the roots of the L5 and the L6 spinal nerves run long in the vertebral canal.

N2 and N3 were conspicuous at sites adjacent to the segments where saphenous nerve entered the spinal cord. Moreover, N2 and, in particular, N3 were reduced after a temporal spinal ischemia. These observations suggest that N2 and N3 are concerned with the intrinsic function of the spinal cord. According to Gelfan and Tarlov [5], the N wave of the CDP was suppressed almost completely by a temporal spinal ischemia. They concluded that the greatly reduced N wave was due to the destruction of interneurons. In the present study, N3 was extremely or completely depressed after the spinal ischemia. This finding suggests that N3 might be closely associated with the interneuronal activity. It is interesting that N3 of a small amplitude was clearly noted in the dog showing pain in response to forceps pinching whereas this peak was completely abolished in the flaccid dogs. The spinal cord lesion in the former dog would be less severe than the flaccid dogs.

Both mephenesin and baclofen abolished the response due to the flexor reflex but their influences on the segmental EESG were quite different. They are placed in the same pharmacological category called centrally acting muscle relaxants. However, mephenesin affords protection against the convulsions induced by electric shock and particularly by strychnine [1] whereas baclofen has no effect on the convulsions due to electric shock, strychnine or picrotoxin [2]. These results indicate that their pharmacological mechanisms differ and this difference may be responsible for their inconsistent influences upon the segmental EESG.

References


要約

犬の伏臥神経刺激による硬膜上腔誘導誘発脊髄電位: 河合正治・木下寛之・森田七郎（鹿児島大学農学部獣医学科畜生理学教室）——犬における誘発脊髄電位の特徴の一端を明らかにする目的で、神経学的に正常な犬の胸・腰・仙腸の硬膜上腔に椎間孔を経由して単極針電極を刺入し、伏臥神経刺激によって誘発される脊髄電位を同期加算、分析した。また、脊髄切断、胸大動脈の一時的閉塞による脊髄虚血、および mephenesin, baclofen による誘発脊髄電位の変化もあわせて観察した。神経学的に正常な犬では、最初に出現する陽性ピーク (P1), それに続く 2つの陰性ピーク (N1, N2) と、長い下降相をもつ陰性ピーク (N3) から構成される分節性誘導脊髄電位が、L3-L6 分節に誘導する硬膜上腔で記録され、ときに緩徐な陽性波が N3 に続いた。分節性誘発脊髄電位の特徴は、尾側胸髄レベルで脊髄を切断しても変化しなかった。脊髄虚血は P1, N1 に変化をもたらさなかったが、N2 の振幅を減少した。N3 は rigidity を示した犬では顕著に減少し、もしくはほとんど消失し、flaccidity を示したものでは完全に消失した。mephenesin は分節性誘発脊髄電位に有効な変化を示すことができなかったが、baclofen は P1, N1 に変化をもたらさなかったが、N2 および N3。特に後者の振幅を著明に減少した。以上の結果から、P1, N1 は求心性インパルスの脊髄への到達と関連し、N3 は介在神経細胞活動と関連すると推測された。