EFFECTS OF DIAZEPAM (CERCINE) ON THE SOMATOSENSORY EVOKED RESPONSES FOLLOWING TOOTH PULP STIMULATION IN RAT

BY

Kazuho Toda,*1 Hiroyoshi Tanaka*2 and Atsushi Iriki*1

ABSTRACT

Effect of diazepam on the somatosensory evoked responses (SER) following tooth pulp stimulation was investigated in Wistar albino rats. The SERs were recorded from the contralateral surface of the skull with a silver ball electrode and 200 responses were averaged with a medical computer. Generally, SERs were found to be composed of a sequence of four components named P1 (first positive wave), N1 (first negative wave), P2 (second positive wave), and N2 (second negative wave) in a 100 msec analysis time. Diazepam enhanced only the amplitude of the P1 component to about 500% of the control, while it suppressed other N1, P2, and N2 components to about 30, 40, and 20% of the control, respectively. The maximum suppressed effect appeared about 30 min after the diazepam injection and the effect was maintained for about 150 min. One possible explanation for the present result is that the activities of the synapses mostly in cortical layer IV evoked by tooth pulp stimulation may be enhanced but the activities of the cortical cells may be suppressed by diazepam injection.

INTRODUCTION

It has been reported that diazepam injection produces psychosedative effect on man in clinical treatment.4,7) On the other hand, weak analgesic effect of diazepam has been shown by some investigators.2,3) It has been accepted that pain is the only sensation which can be produced by stimulating dentine or pulp1) and that the somatosensory evoked response (SER) elicited by tooth pulp stimulation is an indicator of pain sensation in man.5) Therefore, in the present study, the effect of diazepam (Cercine) on the rat SERs elicited by tooth pulp stimulation was investigated by recording them from the surface of the skull.

MATERIALS AND METHODS

Experiments were carried out on 7 female Wistar albino rats weighing about 400 g under the anesthesia as reported previously.10–14) A bipolar stimulating electrode (interpolar distance, 1.5 mm) of stainless steel wire, 0.1 mm in diameter, insulated except for the tips, was inserted into the tooth pulp of a lower incisor. The whole tooth was covered with dental cement or wax to prevent short circuiting by saliva. The pulp nerve was stimulated electrically by rectangular pulses of 0.1 msec duration at 1 Hz. The stimulus intensity employed was about 1.5 times stronger than the SER

*1 岩田一雄, 医学博士: Department of Physiology (Chief: Prof. M. Ichikawa), Faculty of Dentistry, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
*2 田中晃彦: Department of Anesthesiology (Chief: Prof. E. Ikezono), Faculty of Medicine, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).

Received for publication, March 14, 1979.
SERs were recorded from the contralateral surface of the skull with a silver ball electrode, 0.8 mm in diameter, at the point of 8 mm at the frontal plane and 3 mm at the horizontal plane, according to König and Klippel’s brain atlas. An indifferent electrode was inserted into the neck muscle.

A medical computer (ATAC 501-10, Nihon Kohden, Tokyo) was used to average SERs. The computer, set to a 100 msec analysis time, was externally triggered at 1 Hz intervals. The pulse counter was used to stop the triggering automatically after 200 responses. The amplitude of each component in the SER was measured between the baseline as indicated by a horizontal line and the peak of each component (Fig. 1).

Diazepam (Cercine, Takeda Chem. Ind. Ltd., Osaka) was administered once intraperitoneally in a dose of 5 mg/kg b.wt. As a control experiment, the same amount of physiological saline was injected intraperitoneally. Changes of the SERs were measured 30, 90, and 150 min after diazepam injection.

The animal was immobilized with gallamine triethiodide (10 mg/kg) under artificial respiration. The body temperature of the animal was kept between 36 and 37°C.

**Results**

Figure 1 shows typical examples of the SERs elicited by tooth pulp stimulation in normal rats. In all but two rats, SERs were composed of four components in a 100-msec analysis time; these components were named P1 (first positive wave), N1 (first negative wave), P2 (second positive wave), and N2 (second negative wave) as shown in Fig. 1A. In two exceptional animals, two new components, named P0 and N0, preceded before appearance of P1 component (B). The latencies of all components in the

---

**Fig. 1.** Typical examples of contralateral SERs elicited by tooth pulp stimulation in normal rats and recorded from (8.3) according to König and Klippel’s brain atlas. P1, N1, P2, and N2 components were found in all the animals (A,B). In two of seven animals, P0 and N0 components appeared before P1 component (B).

**Table 1.** Latencies in msec of the peaks of P0, N0, P1, N1, P2, and N2 waves in the contralateral SER elicited by tooth pulp stimulation in normal rats

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>N0</th>
<th>P1</th>
<th>N1</th>
<th>P2</th>
<th>N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>4.2</td>
<td>6.1</td>
<td>7.6</td>
<td>16.3</td>
<td>29.5</td>
<td>58.0</td>
</tr>
<tr>
<td>SE</td>
<td>—</td>
<td>—</td>
<td>1.8</td>
<td>3.5</td>
<td>4.9</td>
<td>10.6</td>
</tr>
<tr>
<td>n</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

SER in the normal rat are shown in Table 1.

An example of the effect of 5 mg/kg of diazepam injection is shown in Fig. 2. The amplitude of P1 component 30 min after the diazepam injection (B) was significantly enhanced relative to the control (A), whereas the amplitudes of the N1, P2, and N2 components were reduced to about 20–40% of the controls. The latency of each component after diazepam injection was not changed significantly as compared with the
EFFECTS OF DIAZEPAM

Fig. 2. A typical example of the effect of diazepam injection on the contralateral SER elicited by tooth pulp stimulation. A: control (just before diazepam injection). B: 30 min after diazepam injection. C: 90 min later. D: 150 min later.

In Fig. 3, the effect of diazepam on the P1 (○), N1 (◇), P2 (△), and N2 (□) components is summarized from seven experiments. Control represents the changes in each component amplitude relative to the amplitude at time 0 after the same amount of saline was injected. Diazepam or saline was injected immediately after recording SERs at 0 min. The amplitude of the P1 component was enhanced to 520% (mean value) of the control after the diazepam injection, but the amplitudes of N1, P2, and N2 components were suppressed to 28, 35, and 18% of the controls, respectively. These effective degrees were statistically significant as compared with the control (p<0.05). At

changes of P1 (○), N1 (◇), P2 (△), and N2 (□) components after the injection of the same amount of physiological saline. Diazepam or saline was injected immediately after recordings of normal SER at 0 min. Ordinate: Relative amplitude of each component. Initial amplitude at 0 min is taken as 100%. Abscissa: Time in min after diazepam or saline injection. Vertical bars indicate SE (n=7).

Fig. 3. Changes of the amplitudes of P1 (○), N1 (◇), P2 (△), and N2 (□) components after diazepam injection. Control represents the
about 150 min after diazepam injection, the amplitude of the SER recovered to the control level.

Figure 4 shows the relative latencies between the stimulus onset and the peaks of P1, N1, P2, and N2 components after diazepam or saline had been injected. No significant difference in the latencies was found between diazepam and saline group ($p>0.05$).

**Discussion**

In the present study, the SERs were recorded from the surface of the skull without removing the calvarium and the dura. This method was very convenient for estimating the effect of diazepam, because the surface of the cortex remained intact without the fear of damaging it.$^{14}$

Eccles$^3$ postulated the neural mechanisms of generating initial positive and negative waves in the SER. He suggested that afferent cortical volleys from the thalamus ending mostly in layer IV may generate the initial positive wave (P1 in the present study) and that the negative wave (N1) may be evoked when the cortical cells are activated. Thus, enhancement of the amplitude of the P1 component in the present study suggests the possibility that the activities of the synapses mostly in cortical layer IV evoked by tooth pulp stimulation are enhanced by diazepam injection. Further, it can be said that the depression of N1 component may be due to the diazepam-induced inactivation of cortical cells.
Shigenaga\textsuperscript{8}) reported that morphine injection (2.5–10 mg/kg) suppressed the amplitude of positive (latency \(8.1\pm1.0\) msec) and negative (13.1\(\pm1.6\) msec) waves in rat SER elicited by tooth pulp stimulation. Comparison between the latencies, the P1 and N1 components recorded in the present study are through to correspond to the positive and negative waves reported by Shigenaga. These observations suggest that pain sensation can be suppressed by diazepam injection to some degree. However, the present authors suspect that diazepam must not be applied for relieving pain sensation in clinical fields, because the pain inhibitory action of diazepam appears only when a large dose of the drug as used in the present study is injected.

Acknowledgement

The authors wish to express thanks to Prof. M. Ichioka for instruction and encouragement throughout the present study and also to Prof. E. Ikezono and Dr. T. Sato for valuable discussions. Mr. A. Iriki is a first year student at the Faculty of Dentistry, Tokyo Medical and Dental University.

References


