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DIAZEPAM-INDUCED SUPPRESSION OF JAW OPENING REFLEX IN RATS

BY

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ABSTRACT

Effect of an i.p. injection of diazepam on the jaw opening reflex evoked by electrical stimuli applied to the tooth pulp innervated by mainly high threshold afferents and the lower lip innervated by low threshold afferents was investigated using Wistar albino rats. Small doses of diazepam, less than 2.5 mg/kg, suppressed to the same degree the jaw opening reflexes evoked by stimulation of tooth pulp or lower lip. However, when larger doses than 5.0 mg/kg were administered, the suppression of tooth pulp-evoked jaw opening reflex was greater than that of lower lip-evoked one. In this case, the period of suppression of tooth pulp-evoked jaw opening reflex was longer than that of lower lip-evoked one. It is concluded that (1) the pain relieving effect of diazepam appears only when larger doses than 5.0 mg/kg are administered and that (2) the pain-relieving effect lasts longer than muscle-relaxative effect of diazepam.

INTRODUCTION

Weak analgesic or muscle relaxing effects induced by diazepam application have been reported in clinical treatment [1]. Our previous study [2] showed the suppressive effect of diazepam on somatosensory evoked responses elicited by stimulation of tooth pulpal nerve, mostly consisting of pain fibers [3–7].

The jaw opening reflex elicited by high threshold tooth pulpal afferents has been considered as a possible model of reflexes evoked by stimuli and pharmacological agents relative to pain sensation [8]. On the other hand, jaw opening reflex can also be evoked by stimulating low threshold afferents [9] innervating in lips, oral mucosa, or tongue, which are not related to pain sensation.

In the present study, we investigated the effects of diazepam on the jaw opening reflex elicited by stimulating tooth pulp or lower lip to obtain a neuropharmacological action of the drug.

MATERIALS AND METHODS

Experiments were carried out on 22 Wistar albino rats weighing about 400 g. Each animal was initially anesthetized with thiamylal sodium as described in our previous reports [10–13].

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 the lower incisor. The whole tooth was covered with dental cement to prevent

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short circuiting by saliva. A bipolar hook electrode (interpolar distance, 2 mm) of silver wire, 0.5 mm in diameter, was used for stimulation of the lower lip. The pulp nerve and lower lip were stimulated at 2 Hz with rectangular constant current pulses of 0.1-ms duration. Stimulus intensity used for stimulating tooth pulp and lower lip were 68±13 μA (mean±SE, N=22) and 32±9 μA (N=22), respectively, which were about 1.2 times the threshold for evoking jaw opening reflex.

The electromyogram of the digastric muscle (dEMG) was used as an index of the magnitude of the jaw opening reflex [10]. The dEMG was recorded with a bipolar

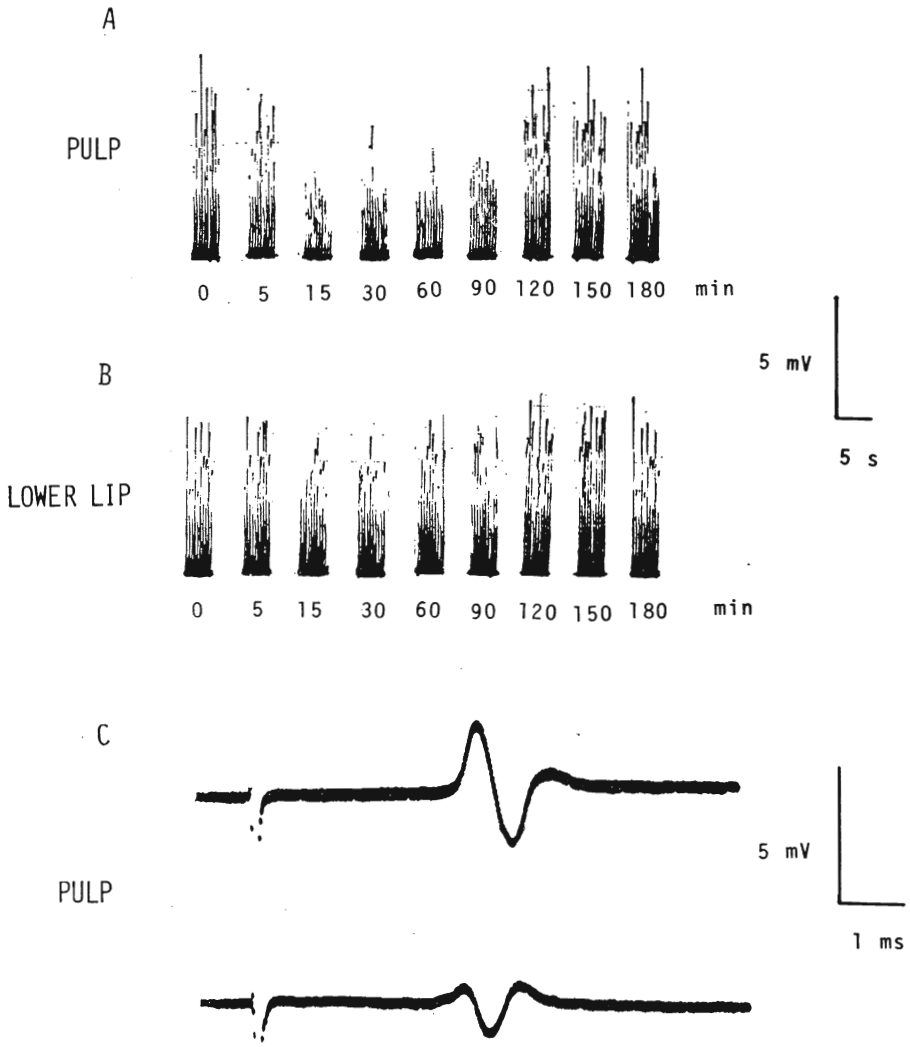

Fig. 1. A, B; Effect of diazepam injection (10.0 mg/kg, i.p.) on the integrated dEMG responses elicited by electrical stimulation of tooth pulp (A) and lower lip (B). C: Oscillograph records of the dEMG by tooth pulp stimualtion. The upper trace represents the control dEMG (0 min) and the lower trace the dEMG 30 min after diazepam injection (10.0 mg/kg, i.p.). Each trace was obtained by averaging 10 summated responses by a medical computer.

needle electrode (interpolar distance, 4 mm) inserted into the anterior belly of ipsilateral digastric muscle [14] and was integrated with an integrator of 20 ms time constant [10, 15]. The mean amplitude of the dEMG activities in each experiment was obtained by averaging 10 integrated dEMG responses. In some experiments, a medical computer (ATAC 501-10, Nihon Kohden, Tokyo), set to a 10-ms analysis time and triggered externally at 0.5-s intervals, was employed to average dEMG responses. The pulse counter was used to stop the triggering automatically after 10 responses.

Diazepam (CERCINE, Takeda Chem. Ind. Ltd., Osaka) was administered once intraperitoneally in various doses of 2.5, 5.0, 7.5, 10.0, and 15.0 mg/kg. In each injection, the drug was adjusted to 4.0 ml with physiological saline. As a control experiment, the

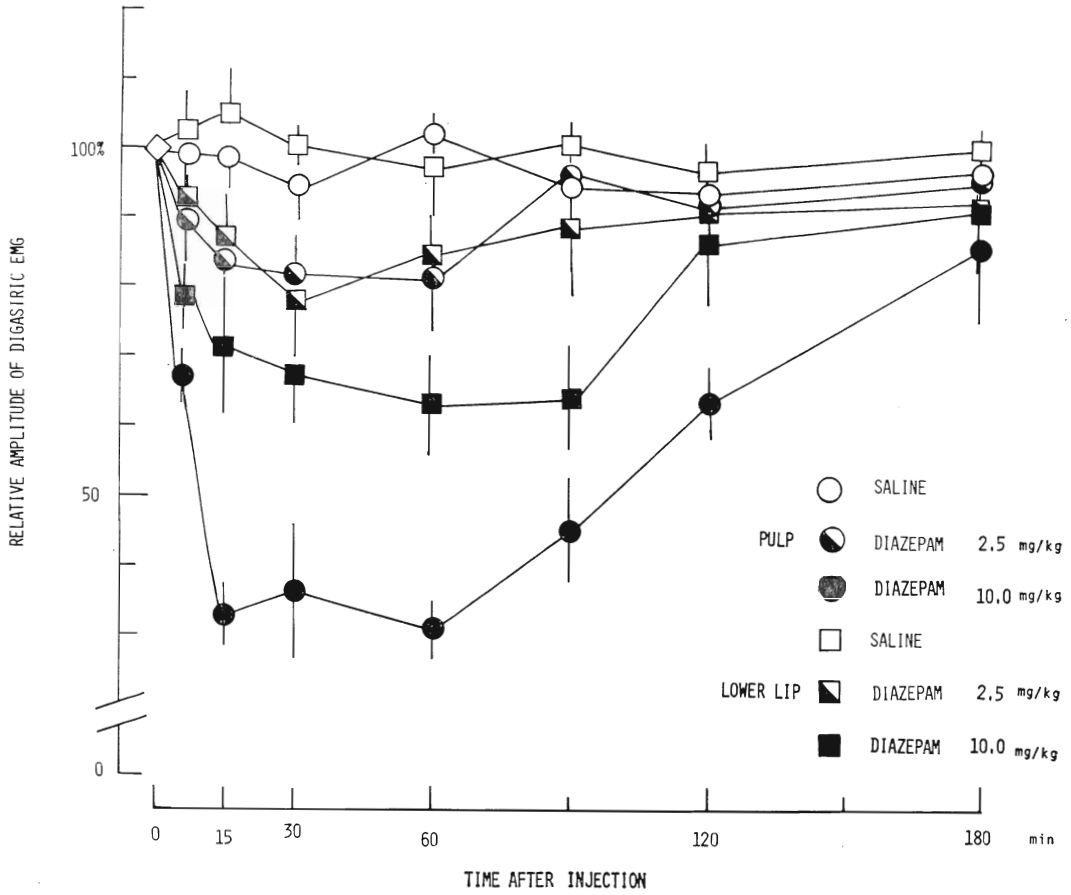

Fig. 2. Changes of the amplitude of dEMGs after an i.p. injection of physiological saline or diazepam. ○ Changes of tooth pulp-evoked dEMGs after saline injection. ◔ Changes of those 2.5 mg/kg i.p. injection of diazepam. ● Changes of those after 10.0 mg/kg i.p. injection of diazepam. □ Changes if lower lip-evoked dEMGs after saline injection. ◧ Changes of those after 2.5 mg/kg i.p. injection of ciazepam. ■ Changes of those after 10.0 mg/kg i.p. injection of diazepam.
Ordinate: Relative amplitnde of dEMGs; the initial amplitude at 0 min is taken as 100%. Abscissa: Time after injection. Vertical bars indicate SE (N=11).

same volume of saline was injected in the same way. Changes of the dEMGs were observed during 180 min after the injection of diazepam.

Results

Typical examples of the effect of a single diazepam injection (10.0 mg/kg, i.p.) on the integrated dEMG evoked by stimulation of tooth pulp or lower lip are shown in Fig. 1, A and B. In both cases, the dEMG responses were gradually suppressed by diazepam injection. The maximal suppression appearing about 15 min after injection was maintained for about 45 min and then slowly recovered to the control level. As can be seen in the figure, the degree of suppression of dEMG after stimulation of tooth pulp was much greater than that after stimulation of lower lip.

In Fig. 1C are shown the oscillograph records of the tooth pulp-evoked dEMG responses before (upper) and 30 min after (lower) diazepam injection of 10.0 mg/kg. Each trace was obtained by averaging 10 summated responses with a medical computer. The amplitude of the dEMG was reduced to about 30% of the control. The latency of the tooth pulp-evoked dEMG before diazepam injection was 6.3±0.2 ms (mean±SE, N=22), and it did not statistically change ($p>0.05$) after diazepam injection of any doses used in the present study. The latency of the lower lip-evoked dEMG before injection was 7.4±0.5 ms (N=22), which did not statistically change ($p>0.05$) after diazepam injection.

Figure 2 summarises the effect of an i.p. administration of diazepam in doses of 2.5 and 10.0 mg/kg. Solid circle and solid square represent the effect of 10.0 mg/kg of diazepam injection on tooth pulp and lower lip-evoked dEMGs, respectively, and semisolid circle and semisolid square, the effect of 2.5 mg/kg of diazepam injection on the tooth- or lip-evoked dEMGs, respectively. Open circle and open square show the changes in the dEMGs after the same amount of a physiological saline was injected. Diazepam or saline was injected once immediately after recording control dEMGs at 0 min. When 10.0 mg/kg of the drug was injected, the tooth pulp-evoked dEMG was suppressed to as much as 30% of the control 15–60 min after injection, whereas the lower lip-evoked dEMG was suppressed to about 65% of the control 15–60 min after injection. The suppressed effect on dEMG evoked by stimulation of lower lip returned to the control by 120 min after injection, while that on tooth pulp-evoked dEMG returned to the control by 180 min. In both cases, the amplitudes of suppressed dEMGs were statistically smaller ($p<0.05$) than that of the controls. The suppression of tooth pulp-evoked dEMG was statistically larger than that of lower lip-evoked dEMG ($p<0.05$) during 3 h after diazepam injection. In contrast, when 2.5 mg/kg of diazepam was injected, either of dEMGs was suppressed only to about 80% of the control 15–60 min later; there was no significant differences between the amplitudes of dEMGs evoked by stimulating tooth pulp and lower lip ($p>0.05$).

Figure 3 illustrates different suppressive effects of various doses of diazepam on tooth pulp-evoked (circle) and lower lip-evoked dEMGs (square). Each point was obtained 30 min after injection. With doses smaller than 2.5 mg/kg, there was no significant difference ($p>0.05$) between the diazepam effects on both dEMGs. In the case of lower lip-evoked dEMG, the dose more than 5.0 mg/kg scarcely changed the degree of suppression of dEMG. In contrast, in the case of tooth pulp-evoked dEMG, the degree of suppression increased with increasing dose

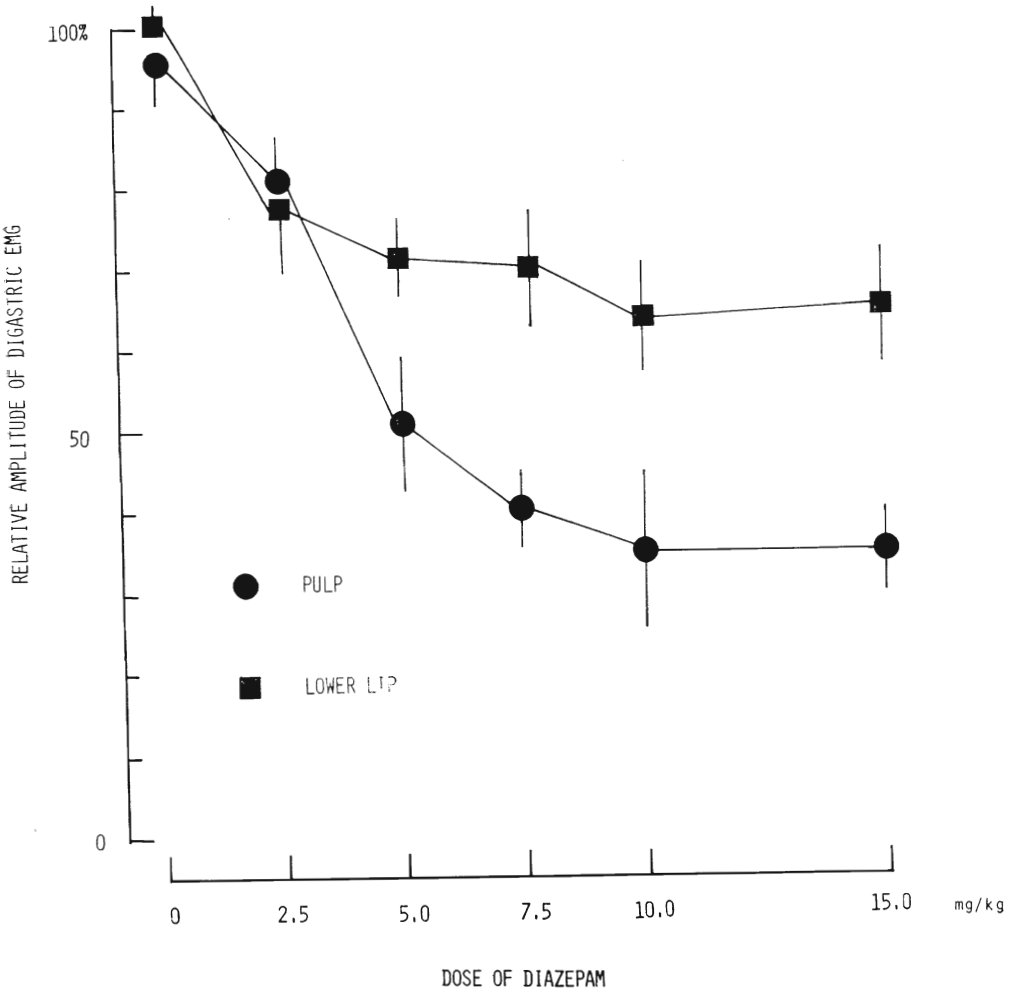

Fig. 3. Relation between doses of i.p. administered diazepam and dEMG responses evoked by electrical stimulation of tooth pulp (●) or lower lip (■). Each point was obtained 30 min after injection. Ordinate: Relative amplitude of dEMGs. Initial amplitude at 0 min is taken as 100%. Abscissa: Doses of diazepam injection. Vertical bars indicate SE (N=4).

up to a dose of 10.0 mg/kg. With dose more than 5.0 mg/kg, relative amplitude of dEMGs evoked by stimulation of tooth pulp and lower lip induced by diazepam injection were statistically different ($p<0.05$).

Discussion

It has been reported that the jaw opening reflex evoked by tooth pulp stimulation can be used as an index of the pain sensation [8]. Also, the jaw opening reflex is reported [9] to be elicited by stimulation of lower threshold afferents innervating the lip or tongue. Stimulation of the lower lip, in this study, is considered to induce an excitation of mainly lower threshold afferents such as $A\beta$ fibers of the cutaneous nerve. However, the latency of dEMG elicited by stimulation of the lower lip was longer than that by tooth pulp stimulation. We suspect that this might be possibly explained by the difference of conduction distance; the dis-

tance between the apical foramen of lower incisor and the semilunar ganglion is 12–15 mm, the distance between the lower lip and the ganglion being 21–27 mm (unpublished data).

It has been reported [1] that the centrally evoked muscle relaxing effect is mainly produced by small doses of diazepam application, and that, by larger doses, not only muscle relaxing effect but sedative or pain relieving effect can be produced by diazepam. In the present study, suppression of jaw opening reflex elicited by stimulation of lower lip was 30% when a sufficient dose was administered. This suppression is assumed to be mainly induced by the muscle relaxing effect of diazepam. Consequently, the difference in the suppression degree between tooth pulp-evoked dEMG and lower lip-evoked dEMG may be due to the presence and absence of pain relieving effect. There was no difference in the suppression degree between the tooth pulp-evoked dEMG and lower lip-evoked dEMG, when the dose less than 2.5 mg/kg was injected. However, the injection of larger doses than 5.0 mg/kg produced a great suppression of the tooth pulp-evoked dEMG alone. This might be due to the effect of diazepam on relieving pain sensation. This suggestion is quite coincident with our previous study [2] that diazepam injection (5.0 mg/kg, i.p.) produced suppression of somatosensory evoked responses elicited by tooth pulp stimulation.

On the other hand, the suppression of dEMG evoked by tooth pulp stimulation lasted longer than the suppression of dEMG elicited by lower lip stimulation, when a sufficient dose of diazepam was injected. This suggests that the duration of pain relief induced by diazepam injection is longer than that of diazepam-produced muscle relaxing effect.

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