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Effects of NK433, a new centrally acting muscle relaxant, on masticatory muscle reflexes in rats

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Abstract

The effects of (−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride (NK433), a novel centrally acting muscle relaxant, on masticatory muscle reflexes were investigated in rats. NK433 inhibited the monosynaptic tonic vibration reflex of the masseter muscle and the polysynaptic tonic periodontal masseteric reflex. These reflexes are increased by γ-motor activity. NK433 had a weak inhibitory effect on the polysynaptic jaw opening reflex evoked by electrical stimulation of the tooth pulp, which is little related with γ-motor activity. Eperisone-HCl depressed the three types of masticatory muscle reflexes. When intravenously administered, eperisone-HCl was equipotent to NK433, but the effect of eperisone-HCl was shorter-lasting than that of NK433. The effect of intragastrically administered NK433 on the periodontal masseteric reflex was about three times stronger than that of eperisone-HCl. These results suggest that NK433 inhibits masticatory muscle reflexes controlled by the γ-motor system and thus may ameliorate the temporomandibular joint syndrome in man.

Keywords: NK433; Tonic vibration reflex; Tonic periodontal masseteric reflex; Jaw opening reflex

1. Introduction

The temporomandibular joint dysfunction syndrome, characterized by pain and clicking in the temporomandibular joint and limitation of function, involves a condition caused by hypertonia combined with parafunctional habits such as clenching or grinding of teeth and hyperreflexia (Laskin, 1969; Laskin and Block, 1986). Tension of the masseter muscle has been reported to increase in patients with occlusal disharmonies resulting from hyperactivity of the γ-motoneuron (Funakoshi et al., 1976; Funakoshi and Nagasawa, 1980). Centrally acting muscle relaxants, which reduce the excessive muscle tone and hyperreflexia in the extremity muscles in human spasticity, have been prescribed to release the hypertonia and hyperreflexia of the masseter muscle in the temporomandibular joint dysfunction syndrome, and ameliorating effects have been reported (Greene and Laskin, 1971; Jagger, 1973; Amir et al., 1978; Enomoto et al., 1991). We have shown that (−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride (NK433; Fig. 1A), an analog of 2-methyl-3-aminopropiophenones (Shiozawa et al., 1995), had more potent and longer-lasting inhibitory effects on the excessive muscle tone of decerebrate rigidity and on the spinal reflexes in the limb muscles than eperisone (Fig. 1B) and tolperisone, which also have a 2-methyl-3-aminopropiophenone moiety (Sakitama et al., 1990, 1995). NK433 also has inhibitory effects on γ-motor activity (Sakitama et al., 1995). This prompted us to investigate the effects of NK433 on the reflexes of the masticatory muscle in rats.

2. Materials and methods

2.1. Subjects and drugs

Male Wistar rats (228–534 g) were used. The reflexes were recorded by means of the electromyogram (EMG) evoked in the masseter or digastric muscles using paired enamel-coated wire electrodes.

The drugs used were NK433 (Nippon Kayaku, Japan), eperisone-HCl (synthesized by Nippon Kayaku), and pentobarbital-Na (Abott, USA). In the case of intravenous administration, NK433 and eperisone-HCl were dissolved

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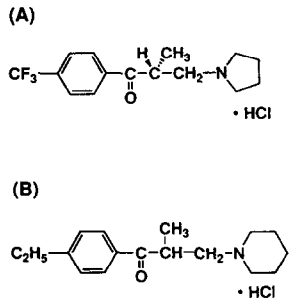

Fig. 1. Structure of NK433 ((−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride) (A) and eperisone-HCl (B).

in physiological saline (1 ml/kg body weight), and given through a catheter previously inserted into the jugular vein. In the study of the tonic periodontal masseteric reflex, NK433 and eperisone-HCl dissolved in distilled water (5 ml/kg body weight) were given intragastrically via a catheter previously inserted into the stomach.

2.2. Recording of the tonic vibration reflex of the masseter muscle

The animals were anesthetized with ether, intubated with a tracheal cannula and fixed onto a stereotaxic apparatus. Decerebration was performed by radiofrequency lesion of the midbrain (AP +3.5, V 0, +1.5, +3.0, +4.0, L ±1.5, Paxinos and Watson, 1982), using a Lesion Generator (Radionics, RFG-4, USA) and a lesioning electrode inserted into the midbrain. After lesioning, ether anesthesia was discontinued. The tonic vibration reflex of the masseter muscle, recorded as EMGs, was induced every 10–12 s by a sinusoidal vibration (100–500 Hz, 2 s in period) which was applied to the mandibula, and was delivered by a vibration generator (V101, Akashi, Japan) driven by a low frequency oscillator (VP-702B, National, Japan) and an amplifier (PA25E, Akashi). The evoked EMG was amplified by a biophysical amplifier (AB-621B, Nihon Kohden) and recorded on a thermal array recorder (RTA-1200, Nihon Kohden). The root mean square of the EMG was also recorded through an integrator (EI-601, Nihon Kohden).

2.3. Recording of the jaw opening reflex

The animals, anesthetized by intraperitoneal injection of pentobarbital-Na (50 mg/kg) initially, which was supplemented as required, were fixed into position on their back, and intubated with a tracheal cannula. Intrapulpal stimulation (0.5 Hz, 0.2 ms in pulse duration, supramaximal intensity) delivered by an electrical stimulator (SEN-7203, Nihon Kohden) was performed via a dental reamer inserted into the dental pulp of the mandibula. The jaw opening reflex recorded as the phasic component of the EMG evoked in the ipsilateral digastric muscle was amplified by a biophysical amplifier and recorded on a thermal array recorder.

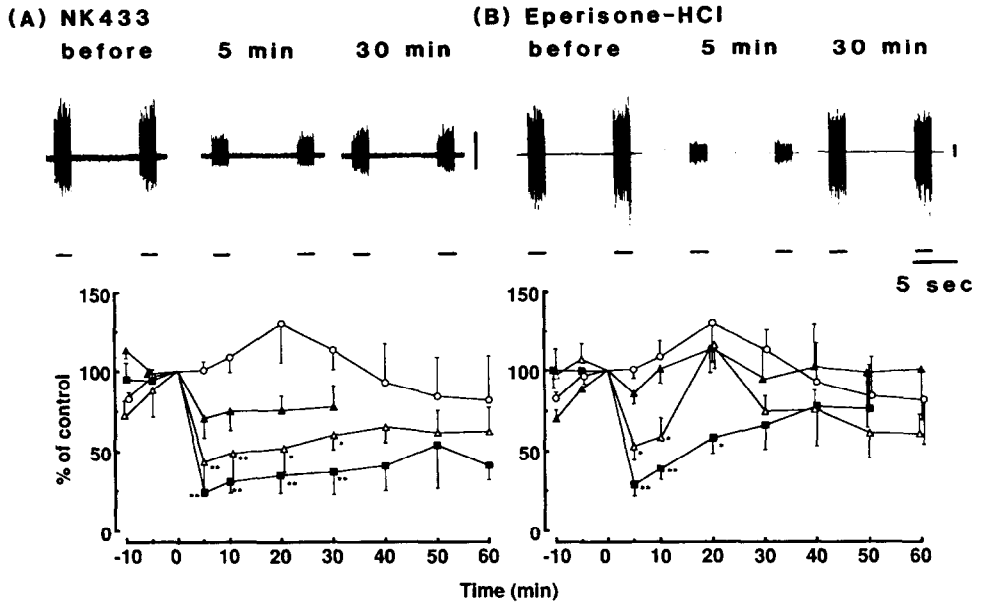

Fig. 2. Effects of intravenously administered NK433 (A) and eperisone-HCl (B) on electromyogram (EMG) of the masseter muscle evoked by the sinusoidal vibration applied to the submaxilla of decerebrate rats. The upper records of (A) and (B) show typical responses of the effects of NK433 and eperisone-HCl (10 mg/kg i.v.). Horizontal bars indicate time of the application of sinusoidal vibration to the mandibula. Vertical bars indicate a calibration of 0.1 mV. The lower graphs show the time course of the effects of NK433 and eperisone-HCl (○: control; ▲: 2.5 mg/kg; △: 5 mg/kg; ■: 10 mg/kg). Ordinates: mean area of the integrated EMG, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 3–5$). Abscissae: time in min after drug administration. * $P < 0.05$, ** $P < 0.01$: statistically significant difference from control group (Dunnett's test).

2.4. Recording of the tonic periodontal masseteric reflex

Recording of the reflex was performed according to the methods of Funakoshi and Amano (1974). Briefly, the animals were anesthetized by intraperitoneal injection of pentobarbital-Na (35 mg/kg), which was supplemented as required, intubated with a tracheal cannula and fixed onto a stereotaxic apparatus. The maxillary incisor was stimulated by pressing for 5 s every 5 min using a vibration generator driven by a trapezoid generator (DPS-204, Diamedical, Japan). The EMG responses to this stimulation were amplified by a biophysical amplifier and recorded on a thermal array recorder. The evoked EMG was transformed into square-wave pulses, fed into a staircase generator (EW-601, Nihon Kohden) and recorded on a thermal array recorder.

2.5. Statistics

The significance of differences between the control and the drug-treated groups was evaluated with Dunnett's test.

3. Results

3.1. Effects on the tonic vibration reflex in the masseter muscle

As shown in the upper records of Fig. 2, the sinusoidal vibration applied to the mandibula evoked the tonic vibration reflex of the masseter muscle recorded as the EMG. NK433 inhibited this reflex dose dependently. At a dose of 10 mg/kg, the inhibition reached its maximum of approximately 70% at 5 min after administration. Significant inhibition was observed at a dose of 5 mg/kg or more within 30 min, and complete recovery was not observed within 60 min (Fig. 2A). Eperisone-HCl also inhibited the tonic vibration reflex in the masseter muscle in a dose-related manner, and the inhibition reached its maximum 2 min after administration. Although the maximal inhibition of eperisone-HCl at a dose of 10 mg/kg was approximately the same as that of NK433, the effect of eperisone-HCl disappeared within 30 min (Fig. 2B).

3.2. Effects on the jaw opening reflex

Electrical stimulation of the tooth pulp evoked a phasic EMG component in the digastric muscle with a latency of approximately 5 ms (the upper records of Fig. 3), which was consistent with the latency of the jaw opening reflex reported by Vassel et al. (1986). EMG with a latency of more than 10 ms was not observed. Although NK433, at a dose of 10 mg/kg, significantly depressed the jaw opening reflex, the maximal inhibition was no more than 25%, thereafter the reflex was restored. Eperisone-HCl also inhibited the jaw opening reflex. The potency and time

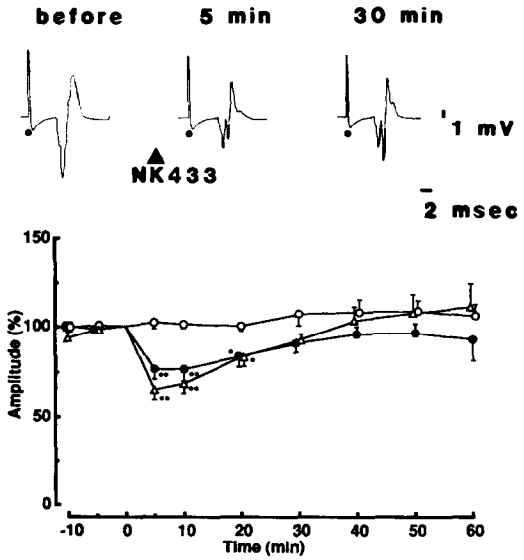

Fig. 3. Effects of intravenously administered NK433 and eperisone-HCl on electromyogram (EMG) of the digastric muscle evoked by electrical stimulation of the ipsilateral tooth-pulp in anesthetized rats. The upper records show typical responses of the effects of NK433 (10 mg/kg i.v.). The evoked EMG was averaged 30 times. Dots indicate stimulation. The lower graph shows the time course of the effects of NK433 and eperisone-HCl (○: control; ●: NK433 10 mg/kg; △: eperisone-HCl 10 mg/kg). Ordinate: mean amplitude of the evoked EMG, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 6$). Abscissa: time in min after drug administration. * $P < 0.05$, ** $P < 0.01$: statistically significant difference from control group (Dunnett's test).

course of eperisone-HCl were approximately the same as those of NK433 (Fig. 3).

3.3. Effects on the tonic periodontal masseteric reflex

When the maxillary incisor was pressed, the tonic periodontal masseteric reflex was recorded as a tonic increase in EMG activities of the masseter muscle (Fig. 4). Intravenously administered NK433 dose dependently inhibited this reflex. At a dose of 10 mg/kg, a maximal inhibition

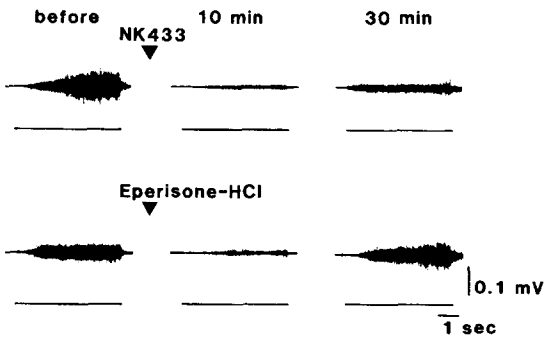

Fig. 4. Typical responses of the effects of intravenously administered (10 mg/kg) NK433 and eperisone-HCl on the masseter muscle electromyogram evoked by pressing on the maxillary incisor in anesthetized rats. Horizontal bars indicate time of pressing.

of about 90% was observed immediately after administration. Significant effects were observed within 20 min, and the reflex returned to its preadministration value within 60 min (Fig. 5, left graphs). When administered intragastrically, NK433 significantly suppressed the tonic periodontal reflex at doses of 25 mg/kg or more, but reached its maximum more slowly than the effects of intravenous administration. At an NK433 dose of 100 mg/kg, the suppression reached a peak of approximately 70% at 20 min after administration, and the reflex disappeared completely 20 min after administration of 200 mg/kg. Recovery was not observed within 60 min (Fig. 5, right graphs).

Eperisone-HCl administered by either route depressed the tonic periodontal masseter reflex significantly. When administered intravenously, 10 mg/kg of eperisone-HCl significantly inhibited the reflex within 10 min, and the maximal inhibition of the reflex was about 90%. The effects of eperisone-HCl were eliminated within 30 min (Figs. 4 and 5, left graphs). In the intragastric administration study, eperisone-HCl did not affect the tonic periodontal reflex at doses of 100 mg/kg or less. At a dose of 200 mg/kg, eperisone-HCl depressed the reflex significantly. The reflex almost disappeared at 20 min after administration and thereafter tended to be restored (Fig. 5, right graphs).

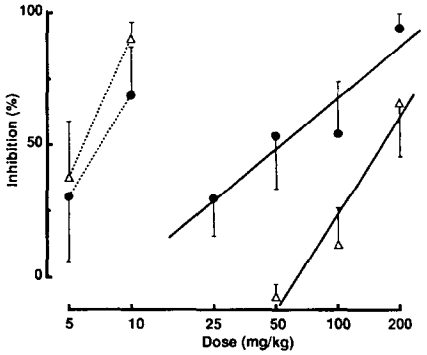

Fig. 6. Dose-response relationships for NK433 (●) and eperisone-HCl (△) administered intravenously (dotted lines) and intragastrically (solid lines) on the masseter muscle electromyogram (EMG) evoked by pressing on the maxillary incisor in anesthetized rats. Ordinate: maximal inhibition of the frequency of evoked EMG, as percentages of the value just prior to drug administration, with S.E.M. indicated. Abscissa: dose (mg/kg).

The dose-response relationships for NK433 and eperisone-HCl on the tonic periodontal masseteric reflex are shown in Fig. 6. The inhibitory effects of NK433 given intravenously were similar to those of eperisone-HCl. The intragastric doses producing 50% inhibition of the tonic periodontal masseteric reflex for NK433 and eperisone-HCl

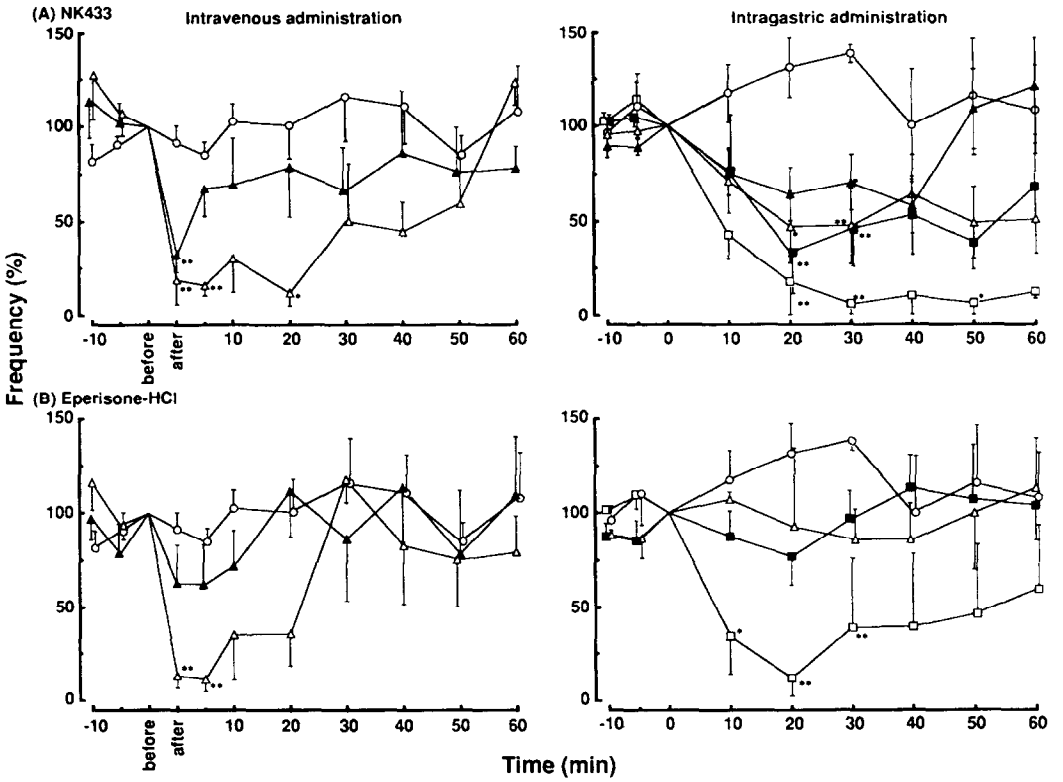

Fig. 5. Effects of NK433 (A) and eperisone-HCl (B) administered intravenously (left graphs; ○: control; ▲: 5 mg/kg; △: 10 mg/kg) or intragastrically (right graphs; ○: control; ▲: 25 mg/kg; △: 50 mg/kg; ■: 100 mg/kg; □: 200 mg/kg) on the masseter muscle electromyogram (EMG) evoked by pressing on the maxillary incisor in anesthetized rats. Ordinates: mean frequency of the evoked EMG, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 3$–6). Abscissae: time in min after drug administration. * $P < 0.05$, ** $P < 0.01$: statistically significant difference from control group (Dunnett's test).

were 54 and 168 mg/kg, respectively. Thus NK433 was approximately 3 times more potent than eperisone-HCl.

4. Discussion

In this series of experiments, NK433 inhibited both the tonic vibration reflex of the masseter muscle and the tonic periodontal masseteric reflex in rats. It is known that the tonic vibration reflex of the masseter muscle in patients with the acute stage of temporomandibular joint dysfunction is larger than that of normal subjects (Koizumi and Homma, 1984). An increase in muscle tension of the masseter muscle, resulting from continuous stimulation of the periodontal membrane caused by chronic oral habits, such as clenching and grinding, has been reported to be related to temporomandibular joint dysfunction (Laskin, 1969; Laskin and Block, 1986). Thus the tonic vibration reflex of the masseter muscle and the tonic periodontal masseteric reflex in rats are considered to be animal models of human temporomandibular joint dysfunction. Taken together, the inhibitory effects of NK433 on these reflexes suggest that NK433 may ameliorate human temporomandibular joint dysfunction.

Godaux and Desmedt (1975), by measuring the latency of the EMG spikes, have shown that the tonic vibration reflex of the masseter and temporalis muscles is a monosynaptic reflex. Funakoshi and Amano (1974) also measured the latency of the EMG evoked in the masseter muscle by stimulation of the maxillary incisor, and indicated that the tonic periodontal masseteric reflex is a polysynaptic reflex. We reported previously that NK433 has inhibitory effects on mono- and polysynaptic reflexes, such as the patellar reflex, the flexor reflex and the ventral root reflex potentials in the extremity muscles (Sakitama et al., 1990; Tomita et al., 1994). Our results showed that NK433 suppresses mono- and polysynaptic reflexes of the brainstem as well as of the spinal cord.

Although NK433 reduced the jaw opening reflex, another polysynaptic reflex of the brainstem, the effects were weaker than those on the tonic periodontal masseteric reflex. The activity of the γ-motor system may not be involved in the jaw opening reflex, because the digastric muscle has few muscle spindles (Shehata, 1971; Lennartsson, 1980). In contrast, the tonic periodontal masseteric reflex is considered to be facilitated by γ-motor activities (Funakoshi and Nagasawa, 1980; Ide, 1980). The inhibitory effect of NK433 on γ-motor activity seems to contribute to the preferential depressant activity on the tonic periodontal masseteric reflex in comparison to the jaw opening reflex. The threshold of the EMG component of the jaw opening reflex evoked by nociceptive stimulation of the tooth pulp is lower than that of the reflex evoked by non-nociceptive stimulation (Vassel et al., 1986). The jaw opening reflex is suppressed by stimulation of the raphe nuclei or the central gray matter (Sessle and Hu, 1981; Dostrovsky et al., 1982; Tanaka and Toda, 1982), which exerts central analgesia, and by administration of opiate agonists (Chan and Fung, 1976). These facts show that the EMG component evoked by nociceptive stimulation is important in producing the jaw opening reflex. As shown in a previous study, NK433 has little effect on the biting response caused by tail pinching, which is a nociceptive stimulus (Sakitama et al., 1995). These results suggest that the slight effects of NK433 on the reflex evoked by nociceptive stimulation might result in the weak inhibition by NK433 of the jaw opening reflex.

Eperisone-HCl given intravenously exerted inhibitory effects equipotent to those of NK433 on three types of masticatory muscle reflexes. However, the duration of the effects on the tonic vibration reflex of the masseter muscle and the tonic periodontal masseteric reflex was shorter than that of the NK433 effect. When administered intragastrically, eperisone-HCl had effects on the tonic periodontal masseteric reflex approximately one-third as potent as those of NK433.

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References

Amir, A., M. Sakuda and T. Miyazaki, 1978, Comparative effects of muscle relaxants and occlusal bite plate in treatment of temporomandibular joint dysfunction syndrome, J. Osaka Univ. Dent. Sch. 18, 1.

Chan, S.H.H. and S.J. Fung, 1976, The effects of morphine and nalorphine on the jaw-opening reflex in rabbits, Exp. Neurol. 53, 363.

Dostrovsky, J.O., J. Hu, B. Sessle and R. Suminor, 1982, Stimulation sites in periaqueductal gray, nucleus raphe magnus and adjacent regions effective in suppressing oral-facial reflexes, Brain Res. 252, 287.

Enomoto, S., S. Nagahata, K. Turuta, H. Sadamori, M. Miyake and K. Gouda, 1991, The effects of eperisone hydrochloride (Myonal) for patients with temporomandibular arthrosis, Oral Ther. Pharmacol. 10, 74.

Funakoshi, M. and N. Amano, 1974, Periodontal jaw muscle reflexes in the albino rat, J. Dent. Res. 53, 598.

Funakoshi, M. and S. Nagasawa, 1980, Effects of periodontal stimulation on the masseteric gamma motor fiber, J. Gifu Dent. Soc. 8, 216.

Funakoshi, M., N. Fujita and S. Takehana, 1976, Relations between occlusal interference and jaw muscle activities in response to changes in head position, J. Dent. Res. 55, 684.

Godaux, E. and J.E. Desmedt, 1975, Evidence for a monosynaptic mechanism in the tonic vibration reflex of the human masseter muscle, J. Neurol. Neurosurg. Psychiatry 38, 161.

Greene, C.S. and D.M. Laskin, 1971, Meprobamate therapy for the myofascial pain-dysfunction (MPD) syndrome: a double-blind evaluation, J. Am. Dent. Assoc. 82, 587.

Ide, R., 1980, Analysis of reflex patterns of the masseter induced by controlled periodontal stimulation in the rat, J. Gifu Dent. Soc. 8, 93.

Jagger, R.G., 1973, Diazepam in the treatment of temporomandibular joint dysfunction syndrome – a double blind study, J. Dent. 2, 37.

Koizumi, H. and I. Homma, 1984, Tonic vibration reflex of jaw closing muscles in normal man and the patient with temporomandibular joint dysfunction, Jikei-kai Med. J. 31, 471.

Laskin, D.M., 1969, Etiology of the pain-dysfunction syndrome, J. Am. Dent. Assoc. 79, 147.

Laskin, D.M. and S. Block, 1986, Diagnosis and treatment of myofascial pain-dysfunction (MPD) syndrome, J. Prosthet. Dent. 56, 75.

Lennartsson, B., 1980, Number and distribution of muscle spindle in the masticatory muscles of the rat, J. Anat. 130, 279.

Paxinos, G. and C. Watson, 1982, The Rat Brain in Stereotaxic Coordinates (Academic Press, Australia).

Sakitama, K., Y. Ozawa, N. Aoto, C. Komai, Y. Kojima, M. Ishikawa and A. Shiozawa, 1990, Pharmacological studies on (*R*)-(−)-2-methyl-3-pyrrolidino-1-(4-trifluoromethylphenyl)-1-propanone hydrochloride (NK433), a new centrally acting muscle relaxant, Eur. J. Pharmacol. 183, 1471p.

Sakitama, K., Y. Ozawa, N. Aoto, K. Nakamura and M. Ishikawa, 1995, Pharmacological properties of NK433, a new centrally acting muscle relaxant, Eur. J. Pharmacol. 273, 47.

Sessle, B.J. and J. Hu, 1981, Raphe-induced suppression of the jaw-opening reflex and single neurons in trigeminal subnucleus oralis, and influence of naloxone and subnucleus caudalis, Pain 10, 19.

Shehata, S.H., 1971, The pattern of innervation of the digastric muscle of the albino rat, Acta Anat. 78, 117.

Shiozawa, A., K. Narita, G. Izumi, S. Kurashige, K. Sakitama and M. Ishikawa, 1995, Synthesis and activity of 2-methyl-3-amino-propiophenones as centrally acting muscle relaxants, Eur. J. Med. Chem. 30, 85.

Tanaka, H. and K. Toda, 1982, Inhibition of the tooth pulp-evoked jaw-opening reflex by stimulation of raphe nuclei in the rat, Exp. Neurol. 77, 102.

Tomita, H., Y. Ozawa and K. Sakitama, 1994, Effects of NK433 and some centrally acting muscle relaxants (CAMRs) on spinal reflex potentials, Jpn. J. Pharmacol. 64, 209p.

Vassel, A., J. Pajot, L. Aigouy, J. Rajaona and A. Woda, 1986, Effects, in the rat, of various stressing procedures on the jaw-opening reflex induced by tooth-pulp stimulation, Arch. Oral Biol. 31, 159.