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Effects of a new centrally acting muscle relaxant, NK433 (lanperisone hydrochloride) on spinal reflexes

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Abstract

(−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride, lanperisone hydrochloride (NK433) administered intravenously or orally depressed the mono- and polysynaptic reflex potential, dorsal root reflex potential, flexor reflex mediated by group II afferent fibers, patellar and flexor reflexes. These effects were reduced by spinal transection. NK433 inhibited the facilitation of the flexor reflex mediated by group II afferent fibers that was induced by intrathecal administration of noradrenaline–HCl. (+)-(1*R*,2*R*)-2-methyl-3-(1-pyrrolidinyl)-1-(4-trifluoromethylphenyl)-1-propanol (LPS-9)–HCl, a metabolite of NK433, also inhibited the spinal reflexes. Given orally, NK433 had effects more than three times stronger and tending to be longer-lasting than those of eperisone–HCl. These results suggest that NK433 exerts a non-selective inhibition on spinal reflexes and that inhibition of the descending noradrenergic tonic facilitation within the spinal cord is involved in the mechanism of spinal reflex depression by NK433. LPS-9 could contribute to the potent activity of NK433 after oral administration. © 1997 Elsevier Science B.V.

Keywords: NK433; Muscle relaxant; Spinal reflex; Active metabolite

1. Introduction

Positive symptoms of spasticity are characterized not only by a tonic increase in muscle tone but also by exaggerated spinal reflexes such as flexor spasms and the appearance of abnormal reflexes (Young, 1987; Noth, 1991). In electrophysiological studies of spastic patients, increases in the ratio of the maximal amplitude of the H reflex to the M-response amplitude (H/M ratio) and increases in the amplitude and duration of the flexor reflex recorded by desynchronized electromyograms (EMGs) with long latency have been reported (Garcia-Mullin and Mayer, 1972; Delwaide, 1985; Meinck et al., 1985; Katz and Rymer, 1989). Centrally acting muscle relaxants have been shown to have depressant effects on spinal reflexes as well as on the excessive muscle tone of decerebrate rigidity seen in animal studies (Polc et al., 1974; Sayers et al., 1980; Tanaka et al., 1981; Farkas et al., 1989; Schwarz et al., 1995). They also suppress spinal reflexes, such as the H reflex or the flexor reflex in clinical studies (Delwaide, 1985; Meinck et al., 1985).

We previously reported that NK433, ((−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride; lanperisone hydrochloride) (Fig. 1A), has central muscle relaxant activity and selectively depresses the excessive muscle tone induced by decerebrate rigidity and not the muscle tone of normal animals (Sakitama et al., 1995; Shiozawa et al., 1995). One of the metabolites of NK433, (+)-(1*R*,2*R*)-2-methyl-3-(1-pyrrolidinyl)-1-(4-trifluoromethylphenyl)-1-propanol (LPS-9)–HCl (Fig. 1B), also has centrally acting muscle relaxant activity (Shiozawa et al., 1992).

We have now investigated the effects of NK433 on spinal reflexes, using rats and cats and compared the effects with those of eperisone and tolperisone (Fig. 1C and D) which have chemical structures similar to that of NK433. We used spinalized preparations as well as intact preparations and investigated the effects of NK433 on the intrathecal noradrenaline-induced facilitation of the flexor reflex mediated by group II afferent fibers (group II flexor reflex) (Sakitama and Ishikawa, 1992; Sakitama, 1993a) to identify the site of action. We also investigated the effects of LPS-9 on the ventral and dorsal root reflex potentials.

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2. Materials and methods

2.1. Animals and drugs

Mongrel cats of both sexes (2.4–5.3 kg) and male Wistar rats (244–630 g) were used. The drugs used were urethane, α-chloralose (Tokyo Kasei or Wako, Japan), NK433, LPS-9–HCl, tolperisone–HCl (Nippon Kayaku, Japan), eperisone–HCl (synthesized by Nippon Kayaku), noradrenaline–HCl (Sigma, USA) and yohimbine–HCl (Tokyo Kasei). In the parenteral study, drugs were dissolved in physiological saline and administered via a cannula previously inserted into the jugular, cephalic or femoral vein. In the oral administration study, NK433, eperisone–HCl and tolperisone–HCl dissolved in distilled water were administered via a cannula previously inserted into the stomach.

2.2. Recording of the ventral root reflex potential (rats)

The animals were anesthetized by intraperitoneal administration of urethane (1 g/kg) and α-chloralose (25 mg/kg), intubated with a tracheal cannula and fixed on their bellies. A laminectomy was performed in the lumbosacral region. After the dorsal and ventral roots were cut below L6, the dorsal root of L3, L4 or L5 was placed on paired platinum electrodes for electrical stimulation (0.2 Hz, 0.05 ms pulse duration, supramaximal intensity) delivered by an electrical stimulator (MSE-3, Nihon Kohden, Japan). The ventral root potential recorded through paired platinum electrodes was displayed on a memory-oscilloscope (VC-10, Nihon Kohden), averaged 6 times by an averaging computer (DAT-1100, Nihon Kohden) and recorded on an X–Y recorder (WX-2400, Graphtec, Japan). A skin pouch was formed and exposed tissues were covered with warm liquid paraffin maintained at 37°C. The amplitude of the averaged monosynaptic reflex potential and the areas of the averaged polysynaptic reflex potential were measured.

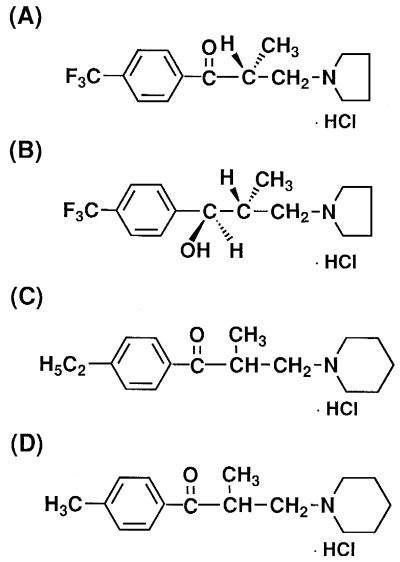

Fig. 1. Structure of NK433 ((−)-(*R*)-2-methyl-3-(1-pyrrolidinyl)-4′-trifluoromethylpropiophenone monohydrochloride) (A), LPS-9((+)-(1*R*,2*R*)-2-methyl-3-(1-pyrrolidinyl)-1-(4-trifluoromethylphenyl)-1-propanol)-HCl (B), eperisone–HCl (C) and tolperisone–HCl (D).

2.3. Recording of the ventral and dorsal root reflex potentials (intact and spinal cats)

The animals were anesthetized by intraperitoneal administration of urethane (400 mg/kg) and α-chloralose (50 mg/kg), intubated with a tracheal cannula and fixed on their bellies. A preparation similar to that outlined for recording the ventral root reflex potential in rats (Section 2.2) was adopted. The dorsal root reflex potential was recorded from the dorsal rootlet adjacent to the dorsal rootlet stimulated (L3, L4 or L5). The dorsal root reflex potential was displayed on a memory-oscilloscope, averaged 6 times and recorded on the recorder. The areas of the averaged dorsal root reflex potential were measured. A skin pouch was formed and exposed tissues were covered with warm liquid paraffin which was maintained at 37°C. In anesthetized spinal preparations, the spinal cord was transected at the level of Th8 more than 2 h prior to drug administration. Blood pressure was monitored and maintained at physiological levels.

2.4. Recording of the patellar and flexor reflexes (cats)

The animals were anesthetized by intraperitoneal administration of urethane (480 mg/kg) and α-chloralose (60 mg/kg), intubated with a tracheal cannula and fixed on their bellies. The patellar reflex was elicited by tapping the left patellar tendon using a Vibration Generator (V-101, Akashi, Japan) driven by a rectangular pulse (3–5 ms pulse duration). Reflex contraction of the ipsilateral quadriceps muscle of the thigh was recorded through a force transducer (SB-1T, Nihon Kohden) and a carrier amplifier (AP-5, Nihon Kohden). The flexor reflex was recorded as a contraction of the anterior tibial muscle of the right limb evoked by electrical stimulation (0.1 ms pulse duration, supramaximal intensity) of the ipsilateral tibial nerve through paired platinum electrodes. Tapping of the patellar tendon and electrical stimulation of the tibial nerve were performed alternately at intervals of 5 s.

2.5. Recording of the group II flexor reflex and intrathecal noradrenaline–HCl administration (rats)

2.5.1. Recording of the group II flexor reflex (intact rats)

The group II flexor reflex was recorded using the phasic EMG component in the anterior tibial muscle with a

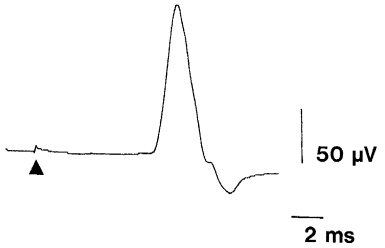

Fig. 2. Typical record of the averaged flexor reflex mediated by group II afferent fibres, showing the phasic electromyogram (EMG) component evoked in the anterior tibial muscle by stimulation of the ipsilateral tibial nerve in anesthetized rats. Stimulation is indicated by the arrowhead.

latency of less than 10 ms that was evoked by electrical stimulation of the ipsilateral tibial nerve (Fig. 2) according to a previous report (Sakitama and Ishikawa, 1992). Briefly, the animals were anesthetized by intraperitoneal injection of urethane (400 mg/kg) and α-chloralose (50 mg/kg) which were supplemented as required. The sciatic nerve was exposed and all branches were cut except the common peroneal nerve. The tibial nerve was placed on paired platinum electrodes for stimulation (0.1–0.2 Hz, 0.05 ms pulse duration, supramaximal intensity, Nihon Kohden MSE-3 or SEN-7103). A silver-ball tipped electrode was placed on the ipsilateral anterior tibial muscle to record the group II flexor reflex. An indifferent needle electrode was inserted in the skin. The group II flexor reflex was displayed on a memory-oscilloscope, averaged 6 times by an averaging computer and recorded on a recorder (RJG-4124, Nihon Kohden). The height of the initial negative wave of the group II flexor reflex was measured. A skin pouch was formed and the exposed tissues were covered with warm

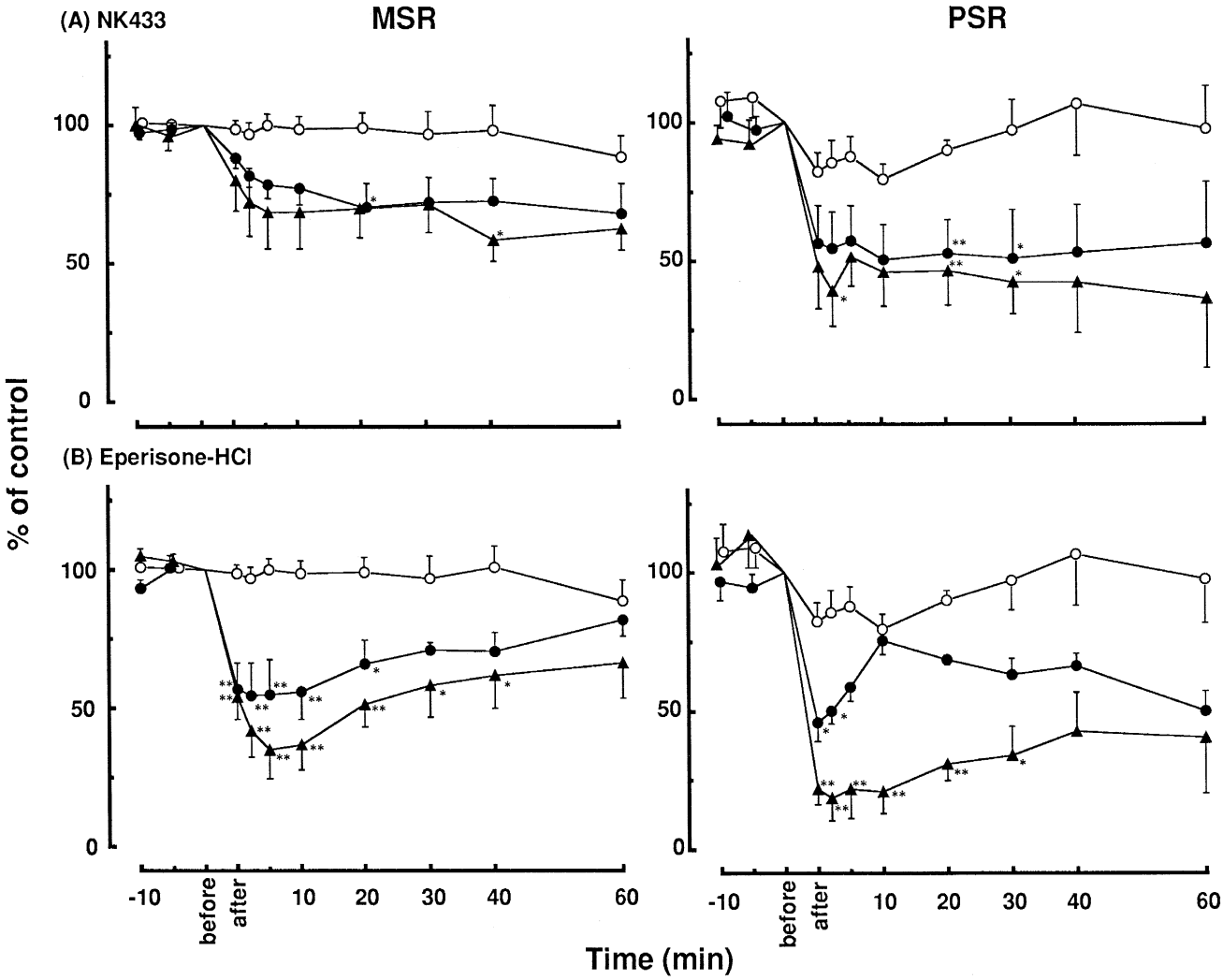

Fig. 3. The effects of NK433 (A) and eperisone–HCl (B) given intravenously (○: control; ●: 5 mg/kg; ▲: 10 mg/kg) on the monosynaptic reflex potential (MSR; left graphs) and polysynaptic reflex potential (PSR; right graphs) in anesthetized rats. Ordinates: mean amplitude of MSR or area of PSR, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 4–8$). Abscissae: time in min after drug administration. $^{*}P < 0.05$, $^{**}P < 0.01$: statistical significance of difference from control group (Dunnett's test).

liquid paraffin. The rectal temperature was monitored and maintained at 37 ± 1°C with a heat lamp.

2.5.2. Effects of NK433 on the intrathecal noradrenaline–HCl-induced facilitation of the group II flexor reflex (spinal rats)

Intrathecal drug administration was performed according to the method of Sakitama (1993a). The spinal cord was transected at the level of Th8-9 more than 2 h prior to recording of the group II flexor reflex. Laminectomy was performed from Th13 to L2. An intrathecal (i.t.) catheter (PE10) was inserted through a small slit in the dura at L1 and its tip was positioned on the left ventral aspect of the spinal cord at L2. As previously described (Sakitama, 1993a), noradrenaline–HCl exerts an α_2-adrenoceptor-mediated inhibition as well as an α_1-adrenoceptor-mediated facilitation of the group II flexor reflex, so yohimbine–HCl, an α_2-adrenoceptor antagonist, was given before treatment with noradrenaline–HCl to exclude an α_2-adrenoceptor-mediated effect. Noradrenaline–HCl and yohimbine–HCl were administered via the intrathecal catheter in a volume of 10 μl followed by 5 μl of saline. The animals were pretreated with NK433 30 min earlier to allow maximal effects on the group II flexor reflex in anesthetized intact rats coincident with the maximum of the intrathecal noradrenaline–HCl-induced facilitation. Yohimbine–HCl was administered 5 min before treatment with noradrenaline–HCl.

2.6. Statistics

The data are expressed as means ± S.E.M. Comparison for significance of differences between the control and drug-treated groups was performed with Student's *t*-test or one-way analysis of variance (ANOVA) with Dunnett's test. In the experiment on spinal reflex in cats, comparison for significance of differences between the effects observed in anesthetized intact preparations and those in spinal preparations was performed with a two-way ANOVA with the Tukey–Kramer test.

3. Results

3.1. Effects on the ventral root reflex potential (rats)

The effects of NK433 and eperisone-HCl on the ventral root reflex potential are shown in Fig. 3.

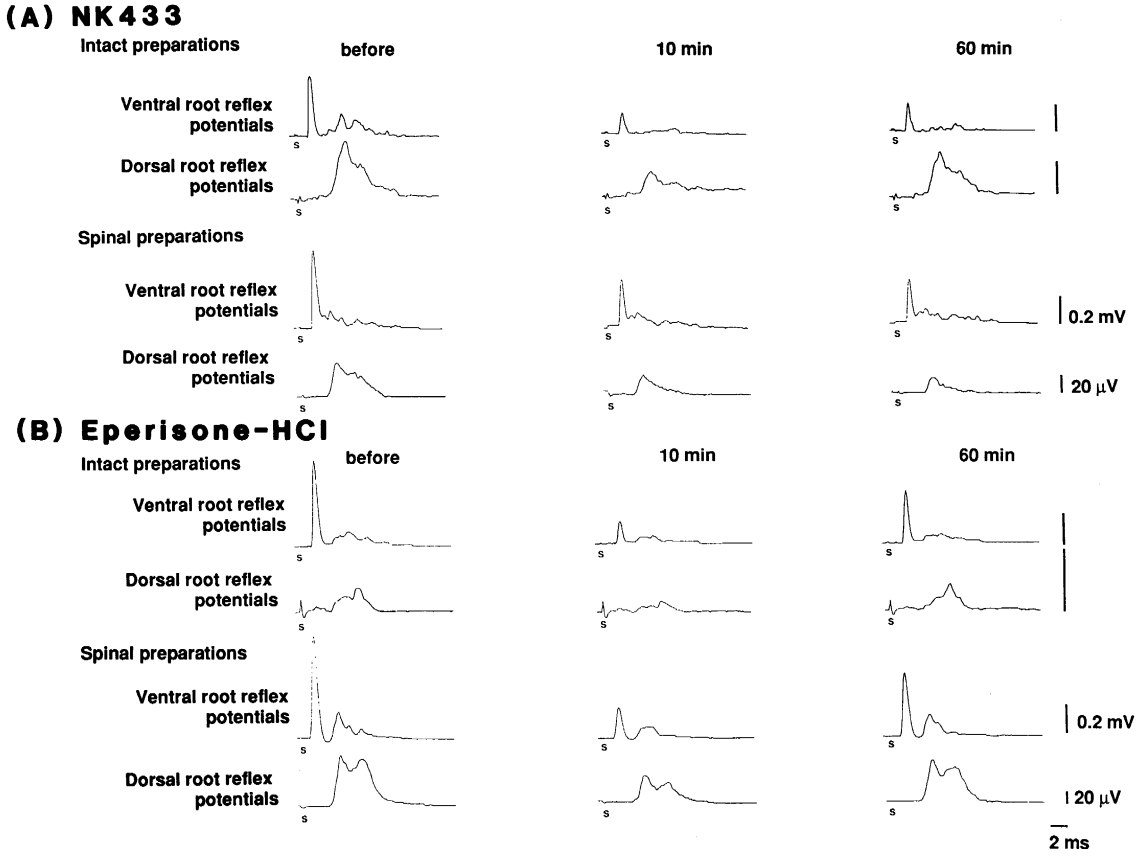

Fig. 4. Typical effects of NK433 (A) and eperisone–HCl (B) given intravenously at a dose of 10 mg/kg on the ventral root reflex potentials and dorsal root reflex potentials in anesthetized intact and spinal cats. Stimulation is indicated by the letter 's'.

NK433 reduced the mono- and polysynaptic reflex potential at doses of 5 and 10 mg/kg, i.v. The effect was observed immediately after administration; maximal suppression at a dose of 10 mg/kg, i.v. was about 40 and 60% for the mono- and polysynaptic reflex potential, respectively. Neither of the reflexes had recovered within 60 min (Fig. 3A).

Eperisone–HCl showed significant and dose-related in-

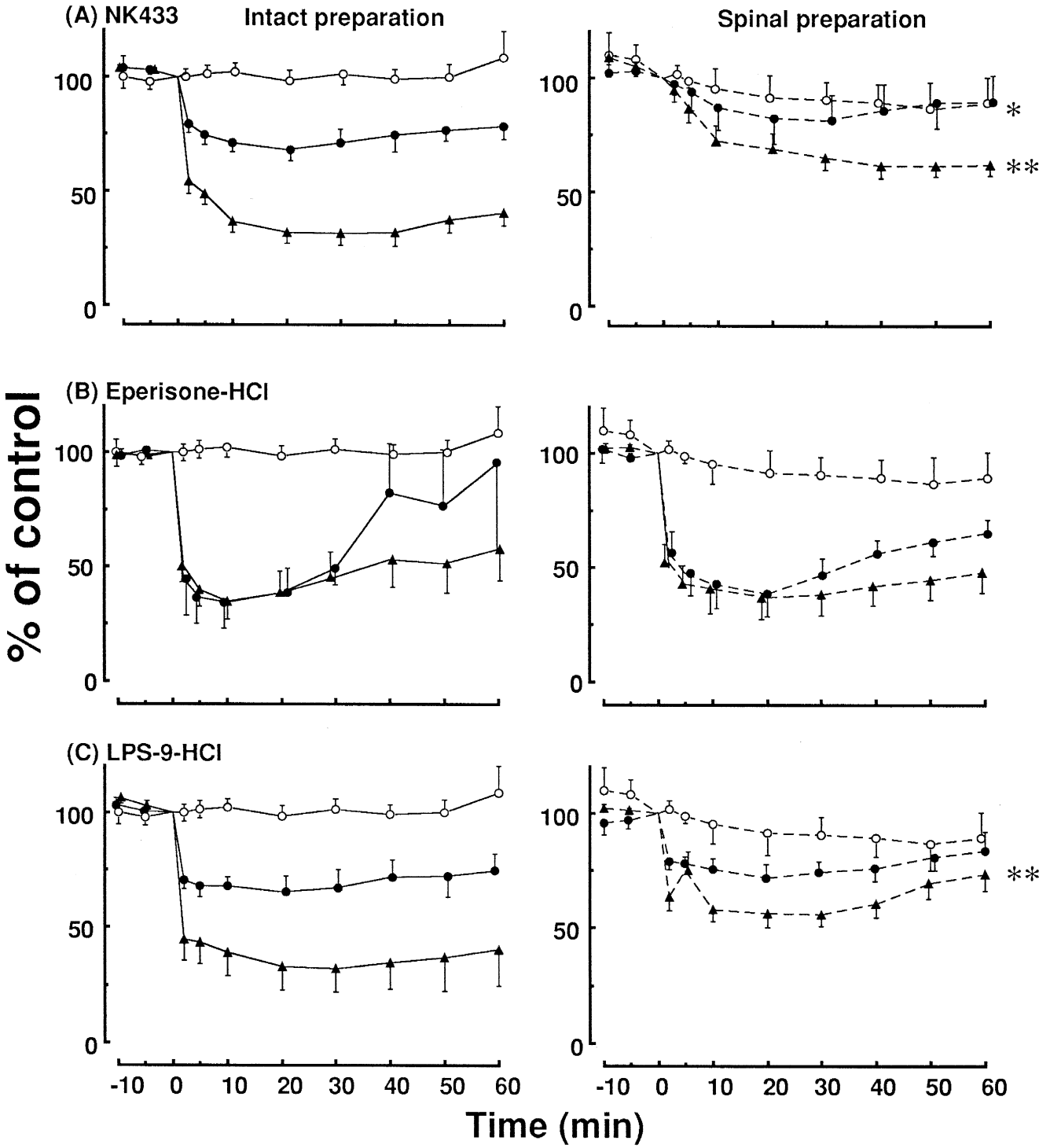

Fig. 5. The effects of NK433 (A), eperisone–HCl (B) and LPS-9–HCl (C) given intravenously (○: control; ●: 5 mg/kg; ▲: 10 mg/kg) on the monosynaptic reflex potential in anesthetized intact (left graphs) and spinal (right graphs) cats. Ordinates: mean amplitude of monosynaptic reflex potential, as percentages of the value just prior to drug administration, with S.E.M. indicated (n = 4–8). Abscissae: time in min after drug administration. $^{*}P < 0.05$, $^{**}P < 0.01$: significantly different from the effects of the corresponding dose of the same drug observed in anesthetized intact cats (Tukey–Kramer).

hibitory effects on the mono- and polysynaptic reflex potential. At a dose of 10 mg/kg, i.v., the inhibition reached a maximum of approximately 60 and 80% for the mono- and polysynaptic reflex potential, respectively, and thereafter the monosynaptic reflex potential showed some tendency to recover (Fig. 3B).

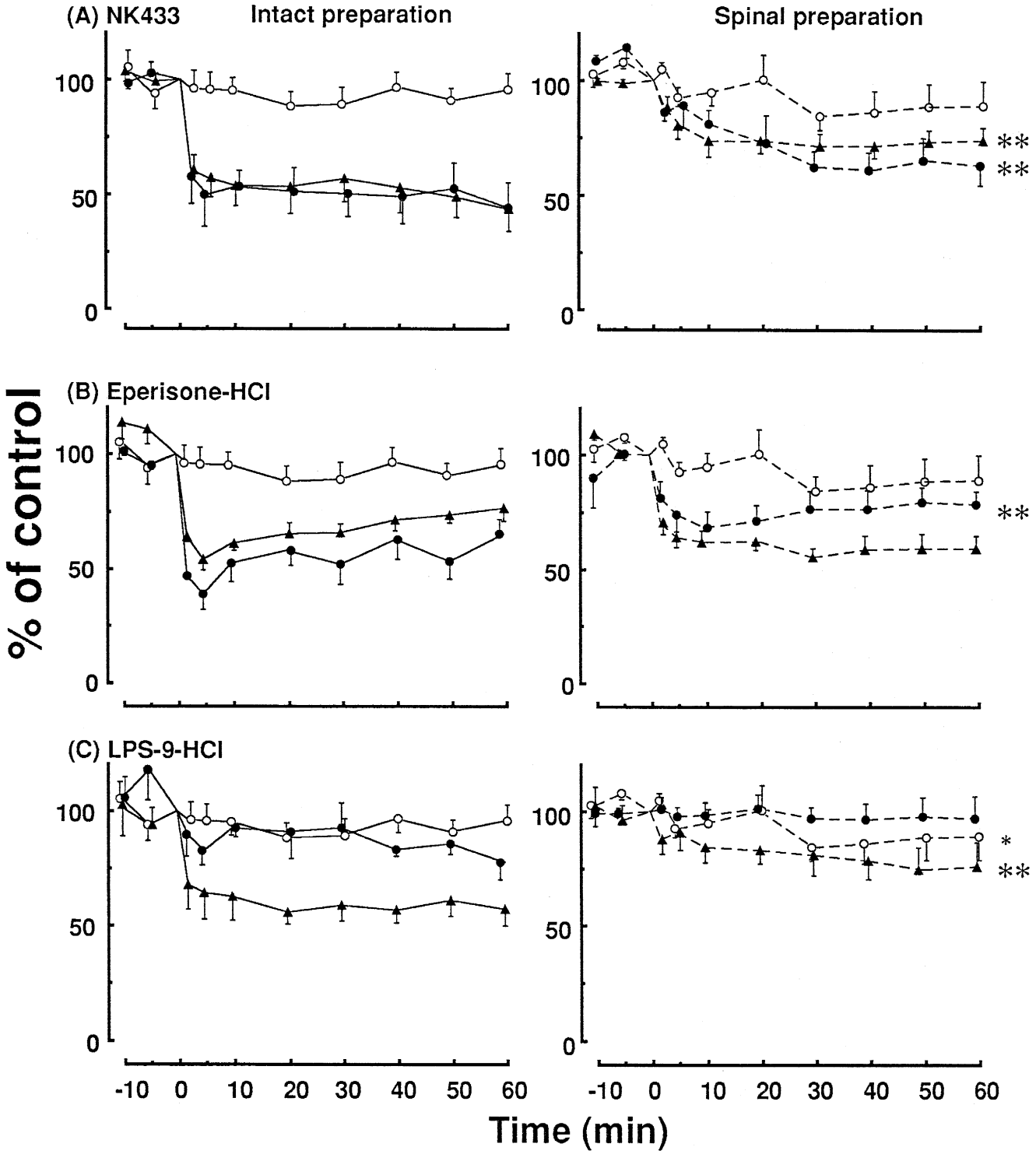

Fig. 6. The effects of NK433 (A), eperisone–HCl (B) and LPS-9–HCl (C) given intravenously (○: control; ●: 5 mg/kg; ▲: 10 mg/kg) on the polysynaptic reflex potential in anesthetized intact (left graphs) and spinal (right graphs) cats. Ordinates: mean area of polysynaptic reflex potential, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 4–8$). Abscissae: time in min after drug administration. $^{*}P < 0.05$, $^{**}P < 0.01$: significantly different from the effects of the corresponding dose of the same drug observed in anesthetized intact cats (Tukey–Kramer).

3.2. Effects on the ventral and dorsal root reflex potential (anesthetized intact and spinal cats)

3.2.1. Effects on the ventral and dorsal root reflex potential

Fig. 4 shows typical effects on spinal reflexes. The effects on the mono- and polysynaptic reflex potential are shown in Figs. 5 and 6, respectively.

Although spinalization caused hypotension in anesthetized spinal cats, blood pressure was restored gradually. The systolic blood pressure was 131 ± 4 mmHg before drug administration, i.e., 2 h or more after spinalization and thus was 22 ± 5 mmHg lower than before spinalization. Physiological conditions were taken to have been maintained.

In anesthetized intact preparations, NK433 depressed the monosynaptic reflex potential dose dependently. At a dose of 10 mg/kg, i.v., a maximal depression of approximately 70% was observed from 10 to 40 min after administration and no recovery was observed within 60 min (Fig. 5A, left graph). NK433 also exerted an inhibitory effect of about 50% on the polysynaptic reflex potential from 2 min after administration and the inhibition did not disappear within 60 min (Fig. 6A, left graph). In anesthetized spinal preparations, the effects of NK433 on the mono-and polysynaptic reflex potentials were significantly reduced in comparison with those observed in anesthetized intact preparations (Fig. 5A and Fig. 6A, right graphs).

In anesthetized intact preparations, eperisone–HCl inhibited the ventral root reflex potential at doses of 5 and 10 mg/kg, i.v.. The inhibition of mono- and polysynaptic reflex potentials reached its maximum of approximately 70 and 60%, respectively. These effects were saturated at a dose of 5 mg/kg, i.v. and no recovery was observed within 60 min (Fig. 5B and Fig. 6B, left graphs).

In anesthetized spinal preparations, the potency and duration of the effects on the monosynaptic reflex potential were similar to those observed in anesthetized intact preparations. At a dose of 5 mg/kg, i.v., the effects on the polysynaptic reflex potential were significantly reduced in comparison with those observed in anesthetized intact preparations (Fig. 5B and Fig. 6B, right graphs).

LPS-9–HCl dose dependently depressed the mono- and polysynaptic reflex potential in anesthetized intact preparations. The inihibitory effects on both reflexes were observed 2 min after administration and reached their maximum 20–30 min after administration. The maximal inhibition was approximately 70 and 40–50% for the mono- and polysynaptic reflex potential, respectively. Neither of the reflexes was restored within 60 min (Fig. 5C and Fig. 6C, left graphs) In anesthetized spinal preparations, LPS-9–HCl inhibited the monosynaptic reflex potential. The effect was observed from 2 min after administration and reached its maximum of about 50% 20–30 min after administration at a dose of 10 mg/kg, i.v.. The monosynaptic reflex potential did not recover within 60 min. The inhibitory effects on the monosynaptic reflex potential were significantly less than the effects in anesthetized intact preparations. The polysynaptic reflex potential was not affected by LPS-9–HCl even at a dose of 10 mg/kg, i.v. in anesthetized spinal preparations and the differences between the effects observed in anesthetized intact preparations and those in spinal preparations were statistically significant. (Fig. 5C and Fig. 6C, right graphs).

The drugs used in this study induced a transient hypotension, whose time course was much shorter than that of the inhibition of the spinal reflexes. Thus, it is unlikely that the alteration of blood pressure had affected the spinal reflexes. It is also possible that the drug distribution in the spinal cord may have been interrupted by the spinalization procedure, but the effect was considered slight because eperisone–HCl suppressed the spinal reflexes even in spinal preparations.

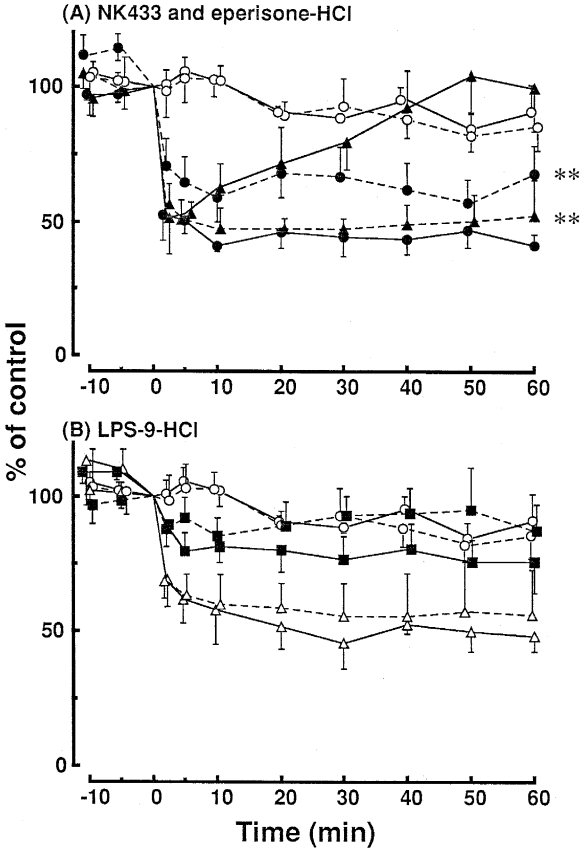

Fig. 7. Effects of NK433, eperisone–HCl and LPS-9–HCl administered intravenously on the dorsal root reflex potential in anesthetized intact (solid lines) and spinal (dotted lines) cats. Ordinates: mean area of dorsal root reflex potential, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 4$–6). Abscissae: time in min after drug administration. ○: control; (A) ●: NK433 10 mg/kg; ▲: eperisone–HCl 10 mg/kg; (B) ■: LPS-9–HCl 5 mg/kg; △: LPS-9–HCl 10 mg/kg. $^{*}P < 0.05$, $^{**}P < 0.01$: significantly different from the effects of the corresponding dose of the same drug observed in anesthetized intact cats (Tukey–Kramer).

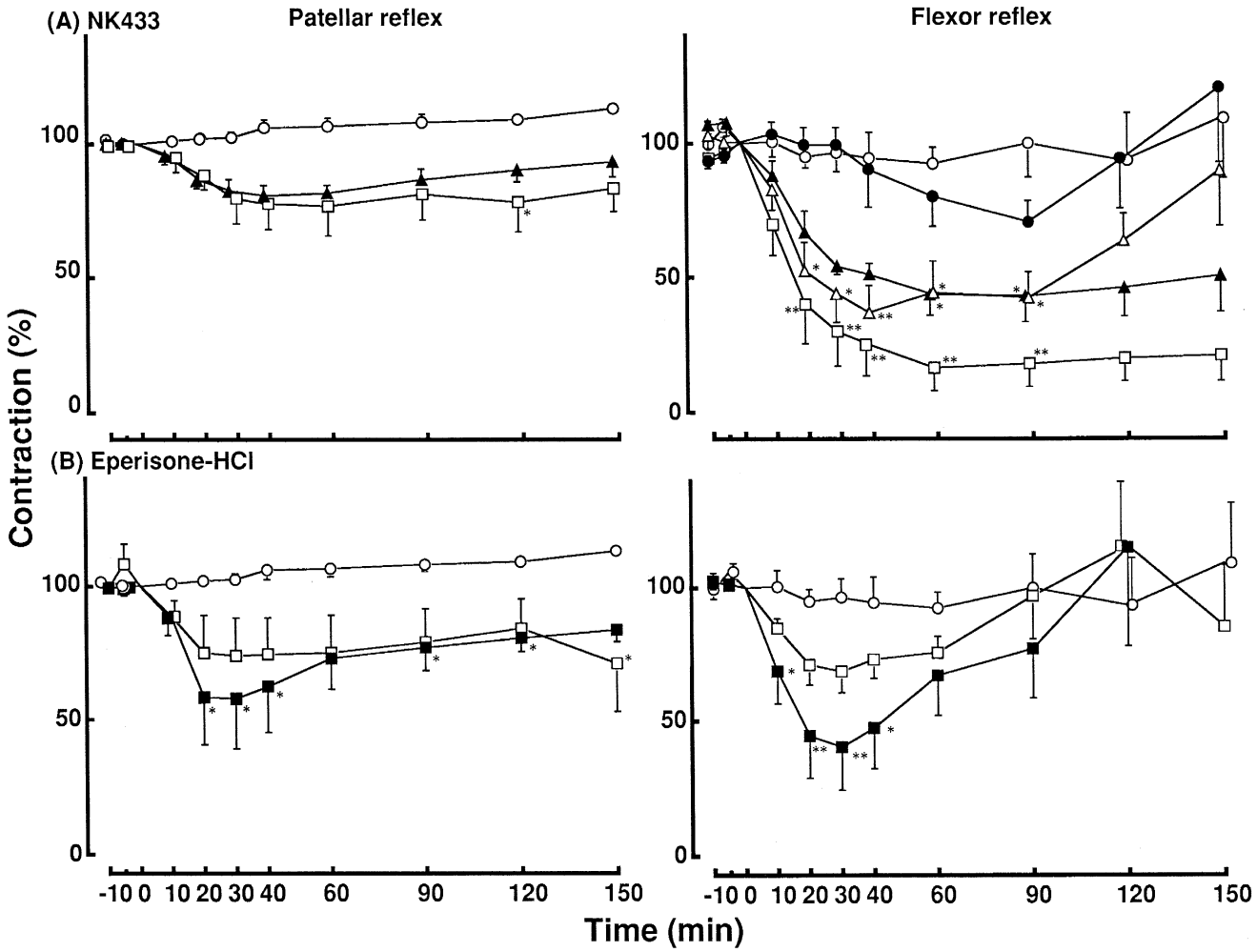

Fig. 8. Effects of NK433 (A) and eperisone–HCl (B) administered orally (○: control; ●: 3.1 mg/kg; △: 6.3 mg/kg; ▲: 12.5 mg/kg; □: 25 mg/kg; ■: 50 mg/kg) on the patellar (left graphs) and flexor (right graphs) reflexes in anesthetized cats. Ordinates: mean reflex contractions of the patellar and flexor reflexes, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 4–6$). Abscissae: time in min after drug administration. $^{*}P < 0.05$, $^{**}P < 0.01$: statistical significance. (Dunnett's test).

3.2.2. Effects on the dorsal root reflex potential

The effects of NK433 and eperisone–HCl are shown in Figs. 4 and 7A. NK433 at a dose of 10 mg/kg, i.v. inhibited the dorsal root reflex potential in both anesthetized intact and spinal preparations. The maximal inhibition was about 60 and 40% in anesthetized intact and spinal preparations, respectively. The effects were significantly reduced in anesthetized spinal preparations as compared to anesthetized intact preparations.

Although eperisone–HCl at a dose of 10 mg/kg, i.v. depressed the dorsal root reflex potential by approximately 50% of the pre-administration value in both anesthetized intact and spinal preparation, the effects in anesthetized spinal preparations were longer-lasting than those in anesthetized intact preparations and the difference reached statistical significance.

As shown in Fig. 7B, LPS-9–HCl inhibited the dorsal root reflex potential dose dependently. The inhibition was

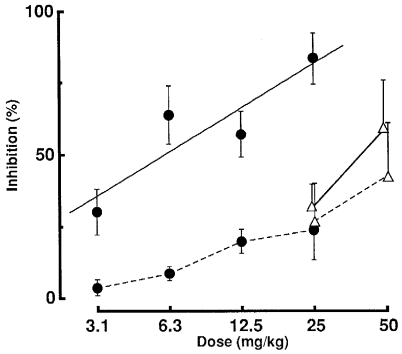

Fig. 9. Dose–response relationships for NK433 (●) and eperisone–HCl (△) administered orally on the patellar (dotted lines) and flexor (solid lines) reflexes in anesthetized cats. Ordinate: maximal inhibition of the patellar and flexor reflexes in each preparation, as percentages of the value just prior to drug administration, with S.E.M. indicated. Abscissa: dose (mg/kg).

observed from 2 min after administration and reached its maximum of approximately 50% within 30 min after administration at a dose of 10 mg/kg, i.v. in both preparations.

3.3. Effects on the patellar and flexor reflexes (cats)

Fig. 8 shows the effects on the patellar and flexor reflexes.

NK433 had no effect on the patellar reflex at doses of 12.5 mg/kg p.o. or less. At a dose of 25 mg/kg p.o., a slight inhibition was observed (Fig. 8A, left graph). The flexor reflex was significantly and dose dependently suppressed by NK433 at doses of 6.3 mg/kg p.o. or more. Suppression reached its maximum of approximately 60 and 80% or more for 12.5 and 25 mg/kg p.o., respectively.

With 12.5 and 25 mg/kg p.o., recovery was not observed within 150 min (Fig. 8A, right graph).

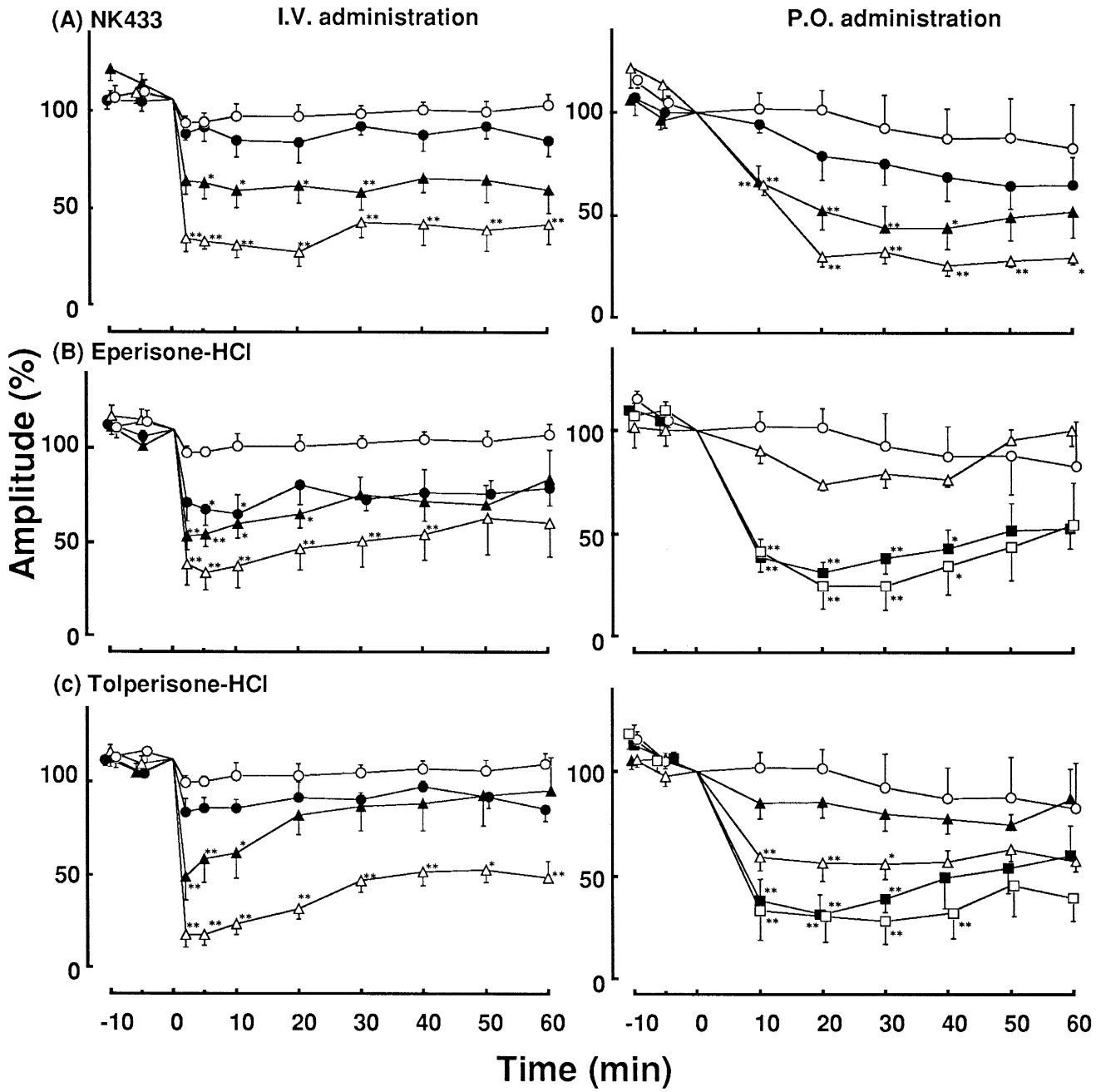

Fig. 10. Effects of NK433 (A), eperisone–HCl (B) and tolperisone–HCl (C) administered intravenously (left graphs; ○: control; ●: 2.5 mg/kg; ▲: 5 mg/kg; △: 10 mg/kg) or orally (right graphs; ○: control; ●: 12.5 mg/kg; ▲: 25 mg/kg; △: 50 mg/kg; ■: 100 mg/kg; □: 200 mg/kg) on the flexor reflex mediated by group II afferent fibers (group II flexor reflex) in anesthetized rats. Ordinates: mean amplitudes of the initial negative waves of the group II flexor reflex, as percentages of the value just prior to drug administration, with S.E.M. indicated ($n = 4–7$). Abscissae: time in min after drug administration. $^{*}P < 0.05$, $^{**}P < 0.01$: statistical significance from control group (Dunnett's test).

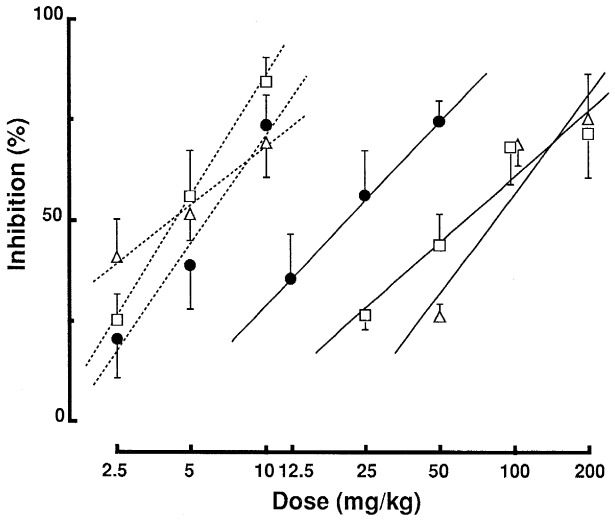

Fig. 11. Dose–response relationships for NK433 (●), eperisone–HCl (△) and tolperisone–HCl (□) administered intravenously (dotted lines) or orally (solid lines) on the flexor reflex mediated by group II afferent fibers (group II flexor reflex) in anesthetized rats. Ordinate: maximal inhibition of the amplitudes of the initial negative waves of the groups flexor reflex, as percentages of the value just prior to drug administration, with S.E.M. indicated. Abscissa: dose (mg/kg).

Eperisone–HCl dose dependently inhibited the patellar reflex. The inhibition at a dose of 50 mg/kg p.o. reached its maximum of approximately 40% 20–30 min after administration and thereafter the reflex was partially restored, but complete recovery was not observed within 150 min (Fig. 8B, left graph). The flexor reflex was depressed dose dependently. Significant inhibition was observed at a dose of 50 mg/kg p.o. and the peak of the effect was about 60% (Fig. 8B, right graph). The dose–response relationships of the effects on the patellar and flexor reflexes are shown in Fig. 9. As NK433 at doses of 6.3 and 12.5 mg/kg p.o. was equipotent to eperisone–HCl at a dose of 50 mg/kg p.o. to inhibit the flexor reflex, NK433 was more than 4 times more potent than eperisone–HCl.

3.4. Effects on the group II flexor reflex and intrathecal noradrenaline–HCl induced facilitation (rats)

3.4.1. Effects on the group II flexor reflex

The effects on the group II flexor reflex are shown in Fig. 10.

NK433 administered intravenously or orally depressed the group II flexor reflex. In the i.v. study, the depressant effects at a dose of 10 mg/kg, i.v. reached a maximum of approximately 70% from 2 to 20 min after administration and the reflex did not recover within 60 min. When given orally, NK433 exerted a significant inhibition within 40 and 60 min for doses of 25 and 50 mg/kg, p.o., respectively. The maximal inhibition of approximately 70% was observed 30 min after administration of NK433 at a dose

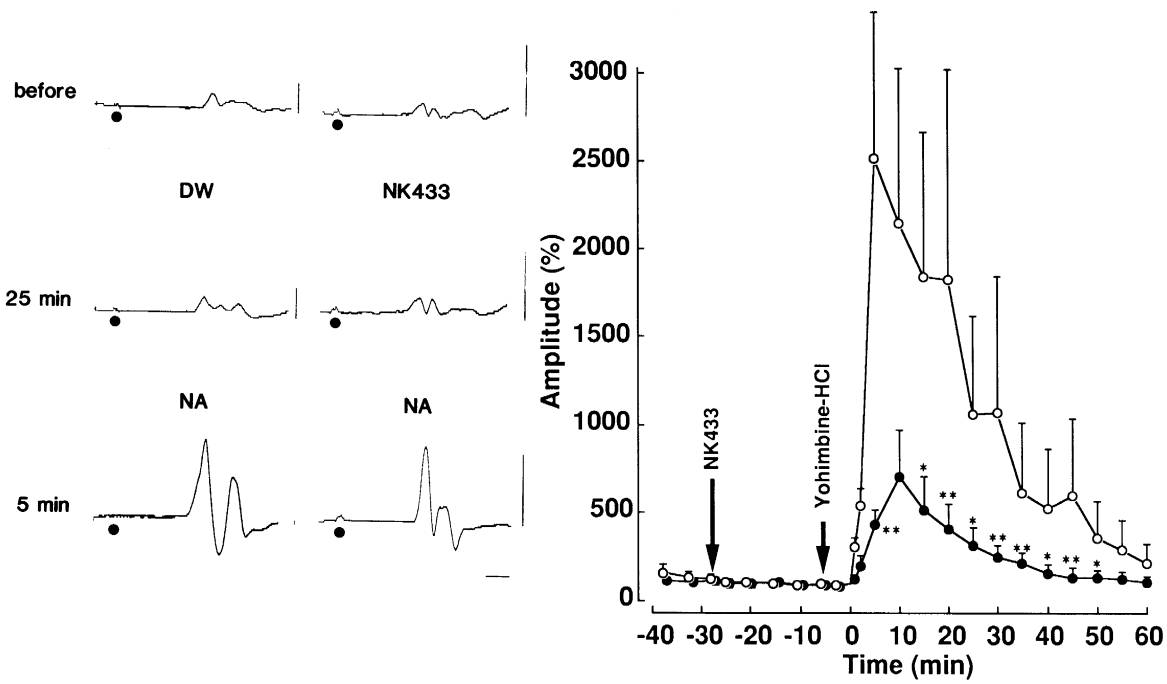

Fig. 12. Effect of NK433 on the intrathecal noradrenaline (NA)–HCl-induced facilitation of the flexor reflex mediated by group II afferent fibers (group II flexor reflex) in anesthetized spinal rats. The records on the left show typical control responses and the effects of distilled water (DW) and NK433 (50 mg/kg, p.o.). Calibration; 2 ms, 50 μV. Ordinate: mean amplitudes of the initial negative waves of the group II flexor reflex, as percentages of the value just prior to administration of NA–HCl, with S.E.M. indicated ($n = 4$). Abscissa: time in min after administration of NA–HCl. ○: Distilled water, p.o. (30 min before) + Yohimbine–HCl 0.1 μmol, i.t. (5 min before) + NA–HCl 0.1 μmol, i.t.; ●: NK433 50 mg/kg, p.o. + Yohimbine–HCl 0.1 μmol + NA–HCl 0.1 μmol. $^{*}P < 0.05$, $^{**}P < 0.01$: statistical significance of difference from distilled water group (Student's *t*-test).

of 50 mg/kg, p.o. and the reflex did not recover within 60 min (Fig. 10A).

Eperisone–HCl and tolperisone–HCl also inhibited the group II flexor reflex significantly and dose dependently. In i.v. experiments, the effects of eperisone–HCl and tolperisone–HCl at a dose of 10 mg/kg, i.v. reached their maximum of approximately 70–80% within 10 min after administration and the reflex tended to be restored, but recovery was not complete within 60 min. In p.o. experiments, the maximal inhibition of approximately 70% was observed 20–30 min after the administration of eperisone–HCl and tolperisone–HCl at a dose of 200 mg/kg, p.o.. Although the effects tended to disappear from 40 min after administration, recovery was not complete within 60 min (Fig. 10B and C).

The dose–response relationships for effects of the three drugs on the group II flexor reflex are shown in Fig. 11. Intravenous doses producing 50% inhibition of the group II flexor reflex for NK433, eperisone–HCl and tolperisone–HCl were 6, 4 and 4 mg/kg, respectively, whereas oral doses producing 50% inhibition of the group II flexor reflex for NK433, eperisone–HCl and tolperisone–HCl were 20, 78 and 63 mg/kg, respectively. Therefore orally administered NK433 was more than 3 times more potent than eperisone–HCl and tolperisone–HCl for these effects.

3.4.2. Effects on the intrathecal noradrenaline–HCl induced facilitation of the group II flexor reflex

As shown in Fig. 12, the group II flexor reflex was markedly facilitated after the intrathecal administration of noradrenaline–HCl at a dose of 0.1 μmol in the presence of yohimbine–HCl (0.1 μmol i.t.), which was consistent with previous reports (Sakitama, 1993a,b). The maximal amplitude of the group II flexor reflex observed 5 min after the intrathecal administration of noradrenaline–HCl was more than 25 times greater than that of the pre-administration value. The facilitatory effect tended to decline subsequently, but the amplitude did not recover to its pre-administration value. NK433 at a dose of 50 mg/kg p.o. failed to affect the group II flexor reflex within 25 min after administration (Fig. 12, left records), whereas the intrathecal noradrenaline–HCl-induced facilitation of the group II flexor reflex was significantly suppressed from 5 min after administration of noradrenaline–HCl. Although the maximal facilitation of the group II flexor reflex was observed 10 min after administration of noradrenaline–HCl, facilitation was less than one-third of that observed in rats treated with distilled water (Fig. 12).

4. Discussion

NK433 suppressed the mono- and polysynaptic reflex potential evoked by electrical stimulation of the ipsilateral dorsal rootlet and also inhibited the monosynaptic patellar reflex and polysynaptic flexor reflex recorded as contraction of the extensor or flexor muscle. In anesthetized intact cats, the inhibitory effects were slightly more potent for the monosynaptic reflex potential than for the polysynaptic reflex potential, but the effect on the patellar reflex was weaker than that on the flexor reflex. Crankshaw and Raper (1970) suggested that asynchronous discharges of the muscle spindle evoked in the patellar reflex counteract the inhibitory effects of drugs, which are unmasked in the monosynaptic reflex potential evoked by an electrical synchronous stimulus. In the patellar reflex experiments, the effect of NK433 on the monosynaptic reflex seems to be masked by the asynchronized discharges of the muscle spindle. Thus, our results indicate that NK433 inhibits spinal reflexes regardless of the presence or absence of an interneuron in the reflex arc.

The group II flexor reflex with a latency of 6–8 ms involves one or two interneurons in the reflex pathway (Eccles and Lundberg, 1959). The latency of the M-wave evoked in the anterior tibial muscle by stimulation of the ipsilateral common peroneal nerve is 1.5 ms (Sakitama and Ishikawa, 1992), therefore the difference between the latency of the groups flexor reflex and the M-wave is 4.5–6.5 ms, which is considered to be the net latency in the spinal cord. As shown in Fig. 4, the polysynaptic reflex potential recorded in the ventral rootlet that was evoked by stimulation of the ipsilateral dorsal rootlet had a latency in the range of 3–15 ms. Thus the polysynaptic reflex potential involves reflex components mediated by afferent fibers other than group II afferents and a component with more than two interneurons in the reflex arc, as well as the component of the groups flexor reflex. The similarity of the potencies of NK433 to inhibit the groups flexor reflex and the polysynaptic reflex potential suggests that NK433 suppresses polysynaptic reflexes independently of the types of afferent fibers and the number of interneurons within the reflex arc.

The dorsal root reflex potential is the primary afferent depolarization evoked by excitation of the adjacent group Ia fibers via a polysynaptic pathway. The primary afferent depolarization, which results in presynaptic inhibition, has been reported to involve γ-aminobutyric acid. As diazepam and tizanidine, centrally acting muscle relaxants, facilitate the dorsal root reflex potential, the facilitation of presynaptic inhibition may be involved in their inhibitory effects on the spinal reflexes (Polc et al., 1974; Ono et al., 1986). NK433 inhibited the dorsal root reflex potential with a potency that was approximately the same as that on the ventral root reflex potential. These results indicate that NK433 exerts a non-selective inhibition on the dorsal root reflex potential pathway as well as the ventral root reflex potential pathway.

In spinal cats, NK433 had little effect on the mono- and polysynaptic reflex potential and the effects on the ventral and dorsal root reflex potential were significantly reduced in comparison with those observed in intact cats. In spinal

rats, NK433 at a dose of 50 mg/kg p.o., which suppressed the group II flexor reflex by about 70% in intact rats, failed to affect the group II flexor reflex. These results suggest that a supraspinal connection is necessary to show the full depression of the spinal reflexes.

Several groups of supraspinal neurons, such as those in the vestibular nuclei and the reticular formation, project to the spinal cord and carry information for the control of spinal reflexes and posture. The noradrenergic descending system arising from the locus coeruleus and subjacent subcoeruleus/medial parabrachial nuclear complex exerts tonic facilitatory effects on the motor mechanisms via α_1-adrenoceptors (Fukuda and Ono, 1990). Some centrally acting muscle relaxants have been reported to block these tonic facilitatory effects (Kehne et al., 1985; Chen et al., 1987; Sakitama, 1993b). As supraspinal actions may be involved in the inhibitory effects of NK433 on the spinal reflexes, we studied its effects on the descending noradrenergic tonic facilitation of the group II flexor reflex induced by intrathecal administration of noradrenaline. Pre-administration of NK433 inhibited the noradrenaline-induced facilitation of the group II flexor reflex without affecting the group II flexor reflex itself. These results suggest that inhibitory effects on the descending noradrenergic tonic facilitation within the spinal cord are involved, at least in part, in the mechanism of spinal reflex depression of NK433. As mentioned in Materials and methods (Section 2.5.2), the noradrenaline-induced facilitation of the group II flexor reflex is thought to be evoked mainly via an α_1-adrenoceptor, thus NK433 might exert an α_1-adrenoceptor blocking action at the spinal level.

Eperisone, also an analog of 2-methyl-3-aminopropiophenone, inhibited the monosynaptic reflex potential, polysynaptic reflex potential, group II flexor reflex and dorsal root reflex potential in rats and cats. These results suggest that eperisone exerts non-selective inhibitory effects on the spinal reflexes, regardless of the presence of an interneuron in the reflex pathway or of the types of afferent fibers, which is similar to the profile of NK433.

In spinal cats, the effects of LPS-9 on the mono-and polysynaptic reflex potential were reduced in comparison with that observed in intact cats, as for the effects of NK433. The reduction of the effects of eperisone caused by spinal transection was smaller than the reduction observed with NK433 and LPS-9. Spinalization prolonged the duration of the dorsal root reflex potential inhibition by eperisone. Although the mechanism remains obscure, a direct membrane-stabilizing action may be involved in these prolonged inhibitory effects on dorsal root reflex potential, as Farkas et al. (1989) suggested. Collectively, there seem to be differences between NK433, LPS-9 and eperisone regarding the site of action for suppression of the spinal reflexes.

Eperisone given intravenously was slightly more potent on spinal reflexes than NK433. Eperisone, given orally, however, had about one-quarter and one-third the potency of NK433 on the flexor reflex of cats and on the group II flexor reflex of rats, respectively. In addition, the effect of NK433 tended to be more longlasting than that of eperisone at the dose producing equipotent inhibition of the flexor reflex in cats, 12.5 mg/kg for NK433 and 50 mg/kg for eperisone (Fig. 8, right graphs). These results are consistent with our previous findings for decerebrate rigidity in rats (Sakitama et al., 1995). In the present study, we showed the inhibitory effects of LPS-9 on spinal reflexes, which suggests a contribution of LPS-9 to the potent activity of NK433 in the p.o. experiments. As the maximal plasma concentration of LPS-9 in humans was 7.5–245 ng/ml after the administration of NK433 at a dose of 75 mg (Ishigami et al., 1995), in clinical use, the effects of LPS-9 could override those of NK433. Based on the above mentioned studies, it is possible that NK433 will have an ameliorating effect on spastic patients by reducing the exaggerated monosynaptic H reflex or the polysynaptic flexor reflex and on the radiculopathy of low back pain in which the H/M ratio has been reported to be increased (Date, 1995).

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