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Involvement of imidazoline receptors in the centrally acting muscle-relaxant effects of tizanidine

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Abstract

The centrally acting muscle relaxant tizanidine has an imidazoline structure and binds not only to α_2-adrenoceptors but also to imidazoline receptors. The role of imidazoline receptors in the muscle-relaxant effect of tizanidine was studied using the α_2-adrenoceptor/imidazoline receptor antagonist idazoxan and the α_2-adrenoceptor antagonist yohimbine. Tizanidine decreased the spinal mono- and polysynaptic reflexes in intact rats, and the inhibitory effects were antagonized by idazoxan but not by yohimbine. After pretreatment with prazosin, tizanidine decreased the mono- and polysynaptic reflexes in spinalized rats. While yohimbine partly inhibited tizanidine-induced depression of the polysynaptic reflex, idazoxan completely abolished tizanidine-induced depression of spinal reflexes. Furthermore, tizanidine-induced muscle relaxation in the traction test was significantly inhibited by idazoxan but not by yohimbine. From these results, it is suggested that imidazoline receptors, but not α_2-adrenoceptors, are involved in the supraspinal inhibitory effects of tizanidine on spinal reflexes, and at the spinal level, α_2-adrenoceptors and imidazoline receptors are involved in the inhibitory effects of tizanidine.

Keywords: Spinal reflex; Imidazoline receptor; Tizanidine; Muscle relaxation

1. Introduction

Tizanidine is a centrally acting muscle relaxant used in spastic patients. Tizanidine and clonidine reduce the release of noradrenaline from descending noradrenergic fiber terminals when injected intravenously or into the fourth ventricle (Ono et al., 1988), and tizanidine inhibits spinal mono- and polysynaptic reflexes in rats which have an intact connection between the brain and the spinal cord (Ono et al., 1986). In spinalized rats, tizanidine and clonidine inhibit polysynaptic reflexes in the presence of prazosin, an α_1-adrenoceptor antagonist (Tanabe et al., 1990; Ono et al., 1993). Noradrenaline and clonidine reduce the excitability of motoneurons in spinal cord slices from adult rats in the presence of prazosin, and the reduction is antagonized by the α_2-adrenoceptor antagonist yohimbine (Hirayama et al., 1988). In the absence of prazosin, these adrenergic agents increased the excitability of spinal neurons via α_1-adrenoceptors in the spinal rat in vivo or spinal cord slices in vitro (Ono and Fukuda, 1995). These results suggest that the inhibitory effects of tizanidine on spinal reflexes are due to supraspinal and spinal effects on noradrenergic systems.

Tizanidine has an imidazoline structure and shows affinity for imidazoline receptors (Muramatsu and Kigoshi, 1992). Several studies on imidazoline binding sites have indicated that there are two subtypes: imidazoline I_1 and I_2 receptors. It is reported that imidazoline I_1 receptors are located in the rostral ventrolateral medulla area of the brain stem (Bricca et al., 1989), while imidazoline I_2 receptors are located extensively in the brain (Lione et al., 1998). Furthermore, it has been shown that imidazoline receptor protein exists in the spinal cord (Ruggiero et al., 1998). Therefore, we investigated the role of imidazoline receptors in the muscle-relaxant effects of tizanidine by measurement of spinal reflexes and traction tests in rats. Brief preliminary results have been published elsewhere (Sekiguchi et al., 2000).

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2. Materials and methods

2.1. Measurement of spinal reflexes

All experimental protocols were approved by the Animal Care and Use Committee of Tokyo University of Science and Nagoya City University, and were conducted in accordance with the guidelines of the National Institutes of Health and the Japanese Pharmacological Society.

Male Wistar rats (8–9 weeks old, NRC Haruna, Saitama, Japan) were anesthetized with α-chloralose (25 mg/kg, intraperitoneally, i.p.) and urethane (1 g/kg, i.p.). Cannulae were inserted into the trachea for ventilation and into the femoral vein for drug administration. In spinalized rats, the vagus nerves were cut bilaterally in the cervical region to eliminate any parasympathomimetic effects on the heart and the spinal cord was transected at the C1 level under lidocaine anesthesia (4%, 50 μl). A dorsal laminectomy was performed in the lumbo-sacral region of each rat. The ventral and dorsal roots below L4 were cut distally at their points of exit from the vertebral column, and the entire exposed surgical area was covered with liquid paraffin kept at 36 ± 0.5 °C by radiant heat. Bipolar Ag–AgCl wire electrodes were used for stimulation and recording. An L5 dorsal root was stimulated with 0.2-Hz rectangular pulses, 0.05 ms in duration, at a supramaximal voltage approximately twice that required to evoke a maximal reflex response. Mono- and polysynaptic reflex potentials were recorded from the ipsilateral L5 ventral root displayed on an oscilloscope, and eight consecutive responses were averaged by an averager.

2.2. Traction test

Male Wistar/ST rats (5 weeks old, SLC, Shizuoka, Japan) were used for a traction test (Kuribayashi et al., 1977). The animals were given free access to food and water, and housed under a 12-h light–dark cycle and a constant temperature of 23 ± 2 °C and 50 ± 10% humidity.

A stainless steel bar (8 mm in diameter) was set horizontally at a height of 40 cm. Each rat was first forced to grasp the bar with the forepaws, and the number of rats capable of climbing up the bar was counted. The trial was performed twice for each rat, and the effects of drugs were observed at 15-min intervals from −30 to 90 min (at time 0, tizanidine was administered subcutaneously, s.c.). Antagonists were administered i.p. at 20 min before the administration of tizanidine.

2.3. Drugs

Tizanidine hydrochloride and idazoxan hydrochloride were obtained from Sandoz (Tokyo, Japan) and Reckitt & Colman Pharmaceutical Division (Kingston-upon-Hull, UK), respectively. Yohimbine hydrochloride and clorgyline hydrochloride were obtained from Research Biochemicals International (Natick, MA, USA). L-3,4-Dihydroxyphenylalanine (L-dopa) and α-chloralose were obtained from Tokyo Kasei (Tokyo, Japan). Prazosin hydrochloride and urethane were obtained from Sigma (St. Louis, MO, USA) and Aldrich Chemical (Milwaukee, WI, USA), respectively. Urethane and α-chloralose were dissolved in distilled water. All the test compounds except prazosin hydrochloride, which was dissolved in distilled water, were dissolved in 0.9% w/v physiological saline and administered at 1 ml/kg. The dose of each drug used in these experiments is expressed as the weight of the salt. Control rats received the vehicle at 1 ml/kg. Drugs were administered to the spinalized rats at least 2 h after spinalization.

2.4. Statistical analysis

For studies on spinal reflexes, the mono- and polysynaptic reflex amplitudes after drug administration were calculated as percentages of the corresponding predrug (time 0) amplitudes. All data are expressed as means ± S.E.M. Student's *t*-test was used to compare the data for two groups, while two-tailed Bonferroni-type multiple *t*-test following one-way analysis of variance (ANOVA) was used for multiple comparisons of control and treated groups (Wallenstein et al., 1980). Differences at $P < 0.05$ (two-tailed) were considered to be significant. For the traction test, the data are expressed as the number of animals that were able to climb up the bar. Fisher's exact test was used to compare the data between control and treated groups. Differences at $P < 0.05$ (one-tailed) were considered to be significant.

3. Results

3.1. Effects of yohimbine and idazoxan on L-dopa-induced polysynaptic reflex depression in spinalized rats

To determine the effective α_2-adrenoceptor antagonistic doses of yohimbine and idazoxan, we studied the effect of both drugs on L-dopa-induced polysynaptic reflex depression in the presence of prazosin hydrochloride (500 μg/kg, i.v.), a selective α_1-adrenoceptor antagonist, and clorgyline hydrochloride (1 mg/kg, i.v.), a monoamine oxidase-A inhibitor, in spinalized rats. Our previous study (Tanabe et al., 1990) had shown that the inhibitory effect of L-dopa on the polysynaptic reflex was mediated via α_2-adrenoceptors. L-Dopa (5 mg/kg, i.v.) did not change the monosynaptic reflex but decreased the polysynaptic reflex (Fig. 1). The inhibitory effect of L-dopa on the polysynaptic reflex was significantly blocked by yohimbine hydrochloride (50 μg/kg, i.v.) or idazoxan hydrochloride (300 μg/kg, i.v.), suggesting that these doses are sufficient to antagonize the effect via α_2-adrenoceptors.

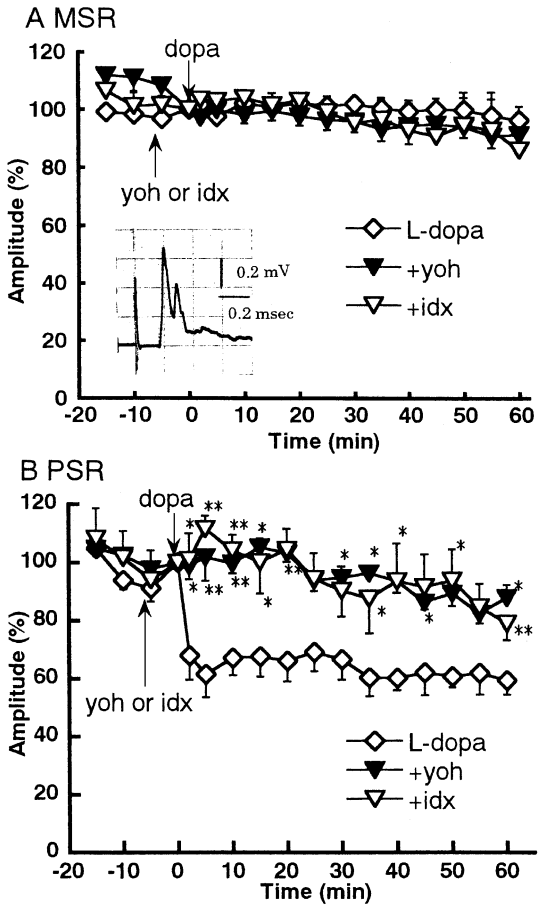

Fig. 1. Effects of L-dopa (5 mg/kg, i.v.) and influence of pretreatment with yohimbine hydrochloride (50 μg/kg, i.v.) and idazoxan hydrochloride (300 μg/kg, i.v.) on the effect of L-dopa on the mono- (A) and polysynaptic (B) reflexes in prazosin-treated spinalized rats. Prazosin hydrochloride (500 μg/kg) was administered i.v. 10 min before the administration of L-dopa. Each point represents the mean ± S.E.M. of four rats per group. Ordinates: mono- and polysynaptic reflex amplitudes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of L-dopa. The significance of the differences between the control and test values was determined with the two-tailed Bonferroni-type multiple *t*-test following ANOVA (two comparisons in three groups); * $P<0.05$ and ** $P<0.01$. Insert shows a sample of record of reflex potentials.

3.2. Effects of tizanidine and antagonists on spinal reflexes in intact rats

In intact (nonspinalized) rats, tizanidine hydrochloride (100 μg/kg, i.v.) decreased the amplitude of mono- and polysynaptic reflexes. Tizanidine depressed the amplitude of the monosynaptic reflex to 85.6 ± 1.1% of the preadministration level at 5 min after administration. The amplitude of the monosynaptic reflex returned to the control level within 20 min (Fig. 2A). The polysynaptic reflex was inhibited to 58.3 ± 5.6% at 2 min after tizanidine administration, and the amplitude returned to the control level within 60 min (Fig. 3A).

Pretreatment with yohimbine hydrochloride (100 μg/kg, i.v.) did not change the inhibitory effects of tizanidine for the period in which tizanidine significantly decreased the mono- and polysynaptic reflexes (Figs. 2B and 3B). In the yohimbine-treated rats, the amplitude of the monosynaptic reflex did not return to the preadministration level, and there was a significant difference between the control and the yohimbine-pretreated group (Fig. 2B). Idazoxan hydrochloride (300 μg/kg, i.v.) completely abolished the inhibitory effects of tizanidine on the mono- and polysynaptic reflexes (Figs. 2C and 3C).

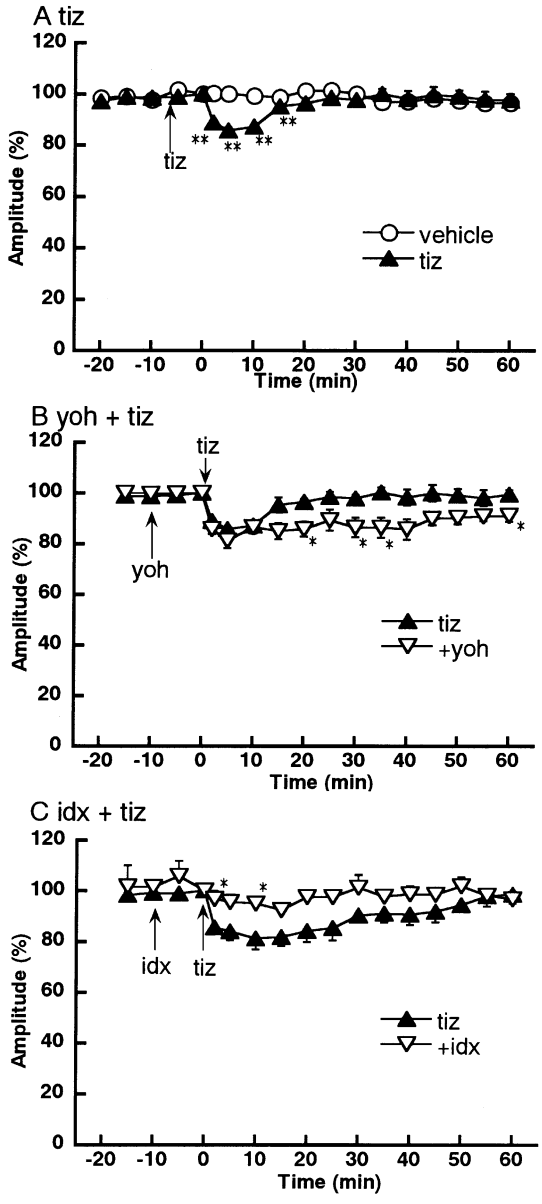

Fig. 2. (A) Effects of tizanidine hydrochloride (100 μg/kg, i.v.) and (B) influence of pretreatment with yohimbine hydrochloride (100 μg/kg, i.v.) and (C) idazoxan hydrochloride (300 μg/kg, i.v.) on the effect of tizanidine on the monosynaptic reflex in intact rats. Each point represents the mean ± S.E.M. of four rats per group. Ordinates: monosynaptic reflex amplitudes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of tizanidine. The significance of the differences between the control and test values was determined with the two-tailed Student's *t*-test; * $P<0.05$ and ** $P<0.01$.

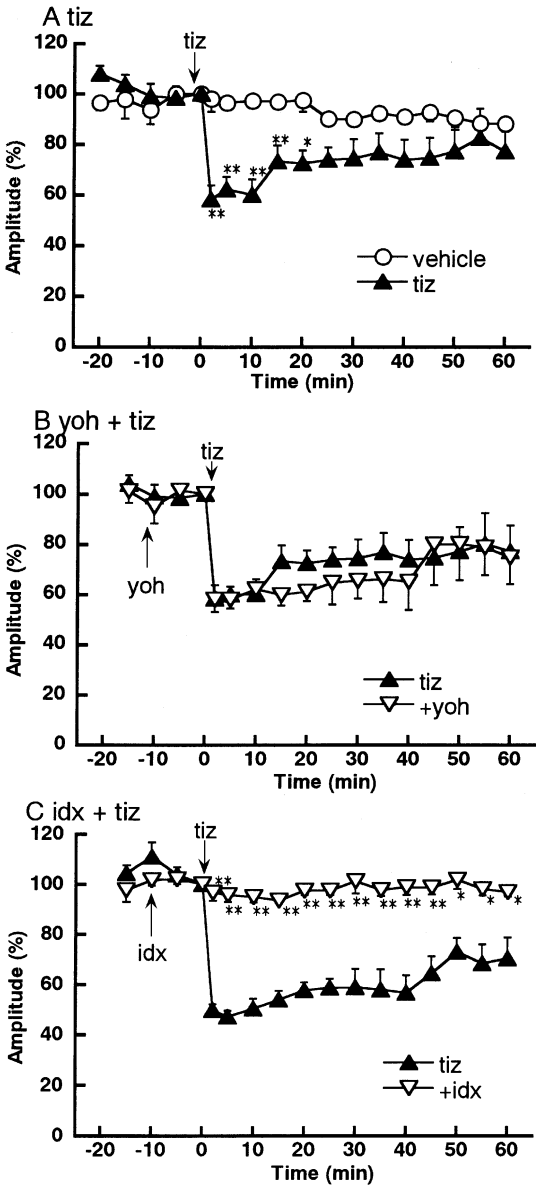

Fig. 3. (A) Effects of tizanidine hydrochloride (100 μg/kg, i.v.) and (B) influence of pretreatment with yohimbine hydrochloride (100 μg/kg, i.v.) and (C) idazoxan hydrochloride (300 μg/kg, i.v.) on the effect of tizanidine on the polysynaptic reflex in intact rats. Each point represents the mean ± S.E.M. of four rats per group. Ordinates: polysynaptic reflex amplitudes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of tizanidine. The significance of the differences between the control and test values was determined with the two-tailed Student's *t*-test; $^{*}P<0.05$ and $^{**}P<0.01$.

3.3. Effects of tizanidine and antagonists on spinal reflexes in spinalized rats

In spinalized rats, tizanidine hydrochloride (100 μg/kg, i.v.) produced slight and transient facilitation of the monosynaptic reflex (Fig. 4A) but significantly decreased the polysynaptic reflex to 73.3 ± 4.9% at 20 min after administration (Fig. 5A). After pretreatment with prazosin hydrochloride (500 μg/kg, i.v.), tizanidine hydrochloride (100 μg/kg, i.v.) decreased the mono- and polysynaptic reflexes to about 90% and 50%, respectively (Figs. 4B and 5B, tiz). The inhibitory effect on the polysynaptic reflex peaked at 2 min after tizanidine administration. While pretreatment with yohimbine hydrochloride (50 and 100 μg/kg, i.v.) partly

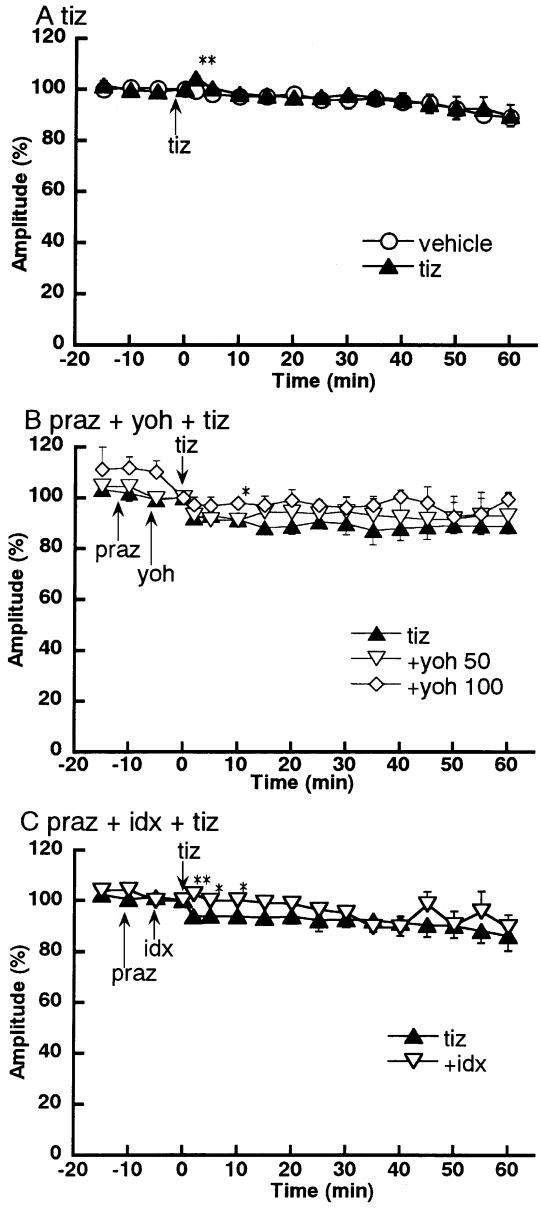

Fig. 4. (A) Effects of tizanidine hydrochloride (100 μg/kg, i.v.) and (B) influence of pretreatment with yohimbine hydrochloride (100 μg/kg, i.v.) and (C) idazoxan hydrochloride (300 μg/kg, i.v.) on the effect of tizanidine on the monosynaptic reflex in spinalized (A) and prazosin-treated spinalized rats (B, C). Prazosin hydrochloride (500 μg/kg) was administered i.v. 10 min before the administration of tizanidine. Each point represents the mean ± S.E.M. of four rats per group. Ordinates: monosynaptic reflex amplitudes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of tizanidine. The significance of the differences between the control and test values was determined with the two-tailed Bonferroni-type multiple *t*-test following ANOVA (two comparisons in three groups, B) or two-tailed Student's *t*-test (A and C); $^{*}P<0.05$ and $^{**}P<0.01$.

inhibited the tizanidine-induced depression of the polysynaptic reflex in the presence of prazosin (Fig. 5B), idazoxan hydrochloride (300 μg/kg, i.v.) completely abolished the tizanidine-induced mono- and polysynaptic reflex depressions (Figs. 4C and 5C).

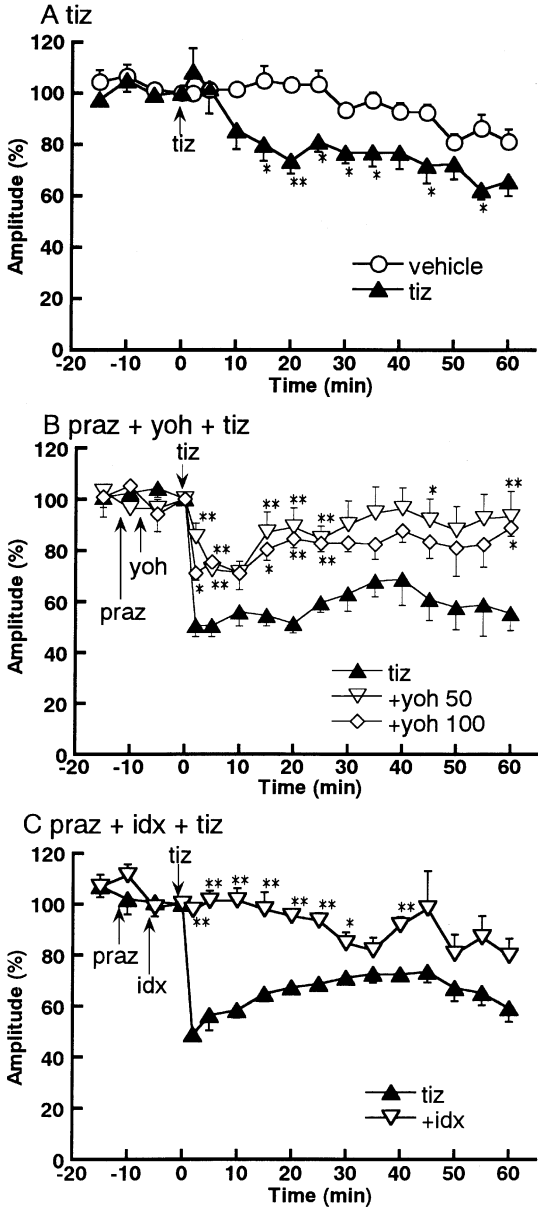

Fig. 5. (A) Effects of tizanidine hydrochloride (100 μg/kg, i.v.) and (B) influence of pretreatment with yohimbine hydrochloride (100 μg/kg, i.v.) and (C) idazoxan hydrochloride (300 μg/kg, i.v.) on the effect of tizanidine on the polysynaptic reflex in spinalized (A) and prazosin-treated spinalized rats (B, C). Prazosin hydrochloride (500 μg/kg) was administered i.v. 10 min before the administration of tizanidine. Each point represents the mean ± S.E.M. of four rats per group. Ordinates: polysynaptic reflex amplitudes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of tizanidine. The significance of the differences between the control and test values was determined with the two-tailed Bonferroni-type multiple *t*-test following ANOVA (two comparisons in three groups, B) or two-tailed Student's *t*-test (A and C); * $P<0.05$ and ** $P<0.01$.

Table 1
Effects of drugs on the traction test of rats

Vehicle	6/6
Tizanidine (1 mg/kg)	3/6
Tizanidine (2 mg/kg)	1/6[a]
Vehicle + tizanidine (2 mg/kg)	4/10
Yohimbine (1 mg/kg) + tizanidine (2 mg/kg)	3/10
Yohimbine (2 mg/kg) + tizanidine (2 mg/kg)	5/10
Vehicle + tizanidine (2 mg/kg)	4/10
Idazoxan (0.3 mg/kg) + tizanidine (2 mg/kg)	9/10[b]
Idazoxan (1 mg/kg) + tizanidine (2 mg/kg)	9/10[b]

Effects of tizanidine hydrochloride (1 and 2 mg/kg, s.c.) and influence of pretreatment with yohimbine hydrochloride (1 and 2 mg/kg, i.p.) and idazoxan hydrochloride (0.3 and 1 mg/kg, i.p.) on the traction test. Each value represents the number of rats which climbed the bar/number of rats used at 30 min after the administration of tizanidine. The significance of the differences between each control and test values was determined with Fisher's exact test.

[a] $P<0.01$ vs. vehicle.

[b] $P<0.05$ vs. vehicle + tizanidine (2 mg/kg).

3.4. Effects of tizanidine and antagonists in the traction test

Table 1 summarizes the result of the traction test. Tizanidine hydrochloride (1 and 2 mg/kg, s.c.) disrupted traction performance in a dose-dependent manner. At 2 mg/kg, the maximal effect was observed at 30 min after tizanidine administration and the effect was significant. Although i.p. administration of yohimbine hydrochloride (1 and 2 mg/kg) did not alter the effect of tizanidine hydrochloride (2 mg/kg, s.c.), i.p. administration of idazoxan hydrochloride (0.3 and 1 mg/kg) significantly inhibited the effect of tizanidine hydrochloride (2 mg/kg, s.c.).

4. Discussion

In the present study, there was a possibility that changes in the amplitude of the spinal reflexes could have been due to changes in blood pressure caused by the i.v. injection of tizanidine. It has been shown that changes in reflex amplitude are not affected by large changes in blood pressure (Ono et al., 1993). Thus, it is suggested that the effect of tizanidine on the spinal reflex in intact rats was not due to changes in blood pressure. It is considered that effects of tizanidine on spinal reflexes in spinalized rats have no relation to blood pressure, since effects of tizanidine were observed in spinalized rats after pretreatment with prazosin (Figs. 4 and 5), which eliminates the contribution of sympathetic tone to blood pressure.

The spinal reflex employed in the present study consists of the mono- and polysynaptic reflexes. Primary afferent fibers originating from skeletal muscles were stimulated and the synaptic excitation of the motoneurons of this muscle was recorded. Monosynaptic excitation and polysynaptic

excitation via interneurons were the mono- and polysynaptic reflexes, respectively.

The doses of yohimbine and idazoxan, which can block α_2-adrenoceptors, were determined using spinalized rats. L-Dopa depresses the polysynaptic reflex but not the monosynaptic reflex via the α_2-adrenoceptors in the presence of prazosin and clorgyline (Tanabe et al., 1990). The inhibitory effect of L-dopa was antagonized by yohimbine and idazoxan (Fig. 1). These results indicate that the doses of yohimbine hydrochloride (50 μg/kg, i.v.) and idazoxan hydrochloride (300 μg/kg, i.v.) were sufficient to block α_2-adrenoceptors, and therefore, these doses of drugs were employed in subsequent experiments.

In previous studies, we found that tizanidine depressed the mono- and polysynaptic reflexes through supraspinal structures (Ono et al., 1986, 1993), and that tizanidine and clonidine reduced the release of noradrenaline from the terminals of descending noradrenergic fibers (Ono et al., 1988). Chen et al. (1987) showed that tizanidine inhibited the flexor reflex of intact rats and enhanced that of spinalized rats. The inhibition of the flexor reflex in intact rats by tizanidine was antagonized by the α_2-adrenoceptor antagonist yohimbine, and tizanidine-induced enhancement of the flexor reflex in spinalized rats was antagonized by the α_1-adrenoceptor antagonist prazosin. These results suggested that tizanidine activated α_2-adrenoceptors in the supraspinal structures and removed the tonic facilitation of spinal neurons by descending noradrenergic fibers.

In the present study, the inhibitory effects of tizanidine on the mono- and polysynaptic reflexes in intact (nonspinalized) rats were antagonized by idazoxan but not by yohimbine (Figs. 2 and 3). Tizanidine has higher affinity for imidazoline receptors than for α_2-adrenoceptors (Muramatsu and Kigoshi, 1992), and idazoxan, but not yohimbine, interacts with imidazoline receptors (Mallard et al., 1992; Monroe et al., 1995). Therefore, it is suggested that the inhibitory effects of tizanidine on mono- and polysynaptic reflexes in intact rats is mediated via imidazoline receptors, but not by α_2-adrenoceptors.

Tizanidine hydrochloride (100 μg/kg, i.v.) slightly facilitated the monosynaptic reflex but depressed the polysynaptic reflex in spinalized rats (Figs. 4A and 5A). In the presence of prazosin, tizanidine hydrochloride (100 μg/kg, i.v.) depressed the mono- and polysynaptic reflexes in the spinalized rats (Figs. 4B and 5B, tiz). These results suggested that α_1-adrenoceptors were dominant in the spinal motor system, and thus supporting our previous reports (Tanabe et al., 1990; Ono et al., 1993). Pretreatment with yohimbine hydrochloride (50 and 100 μg/kg, i.v.) had little effect on tizanidine-induced monosynaptic reflex depression (Fig. 4B) and partly reduced the tizanidine-induced inhibitory effect on the polysynaptic reflex (Fig. 5B). However, pretreatment with idazoxan hydrochloride (300 μg/kg, i.v.) significantly antagonized the tizanidine-induced depression of the mono- and polysynaptic reflexes (Figs. 4C and 5C). These results using intact and spinalized rats suggest that supraspinal imidazoline receptors are involved in tizanidine-induced depression of the mono- and polysynaptic reflexes and that spinal imidazoline receptors and α_2-adrenoceptors are involved in tizanidine-induced polysynaptic reflex depression.

In the traction tests (Table 1), tizanidine hydrochloride (2 mg/kg, s.c.) disrupted traction performance in a dose-dependent manner and idazoxan hydrochloride (0.3 and 1 mg/kg, i.p.), but not yohimbine hydrochloride (1 and 2 mg/kg, i.p.), reduced the tizanidine-induced effect significantly. Since 50 μg/kg (i.v.) yohimbine hydrochloride antagonized L-dopa-induced depression of the polysynaptic reflex in spinalized rats (Fig. 1), it is considered that these doses of yohimbine penetrate the blood–brain barrier. However, yohimbine hydrochloride at 2 mg/kg (i.p.) did not inhibit the effect of tizanidine in the traction test. These results suggest that imidazoline receptors, but not α_2-adrenoceptors, are involved in the muscle-relaxant effects of tizanidine.

It is reported that imidazoline I_1 receptors have a limited distribution in the brain, being present mainly in the brain stem (Bricca et al., 1989; Ernsberger et al., 1987), while imidazoline I_2 receptors are widely distributed in the central nervous system (Lione et al., 1998). In the present study, the supraspinally mediated inhibitory effects of tizanidine were antagonized by idazoxan, which has higher affinity for imidazoline I_2 receptors (Eglen et al., 1998), and in spinalized rats, the inhibitory effect of tizanidine on the polysynaptic reflex was antagonized by idazoxan, suggesting that imidazoline I_2 receptors mediate the inhibitory effects. However, clonidine, which has higher affinity for imidazoline I_1 receptors (Eglen et al., 1998), depressed the spinal polysynaptic reflexes in spinalized rats and the effect was abolished by piperoxane, the nonspecific adrenergic antagonist (Tanabe et al., 1990). From these conflicting results, it cannot be concluded which subtypes of imidazoline receptors are involved in the effects of tizanidine on spinal reflexes and muscle relaxation. Furthermore, it has been reported that some imidazoline drugs can stimulate insulin secretion from pancreatic β-cells, and this effect is mediated via imidazoline I_3 site that is different from imidazoline I_1 and I_2 sites (Eglen et al., 1998; Zaitsev et al., 1999). Although the presence of imidazoline I_3 site was suggested only at pancreatic β-cells, we cannot exclude the possibility of imidazoline I_3 site. Further studies using highly selective antagonists are needed to elucidate the involvement and subtype of the imidazoline receptors in muscle-relaxant effects of tizanidine.

In conclusion, the present findings suggest that imidazoline receptors are involved in the supraspinal inhibitory effects of tizanidine on mono- and polysynaptic reflexes, and that at the spinal level, not only α_2-adrenoceptors but also imidazoline receptors are involved in the inhibitory effect of tizanidine on the polysynaptic reflex. The results of the traction test suggest that the muscle-relaxant effects of tizanidine are mediated by imidazoline receptors and not by α_2-adrenoceptors.

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