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Involvement of Supraspinal Imidazoline Receptors and Descending Monoaminergic Pathways in Tizanidine-Induced Inhibition of Rat Spinal Reflexes

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Abstract. The neuronal pathways involved in the muscle relaxant effect of tizanidine were examined by measurement of spinal reflexes in rats. Tizanidine (i.v. and intra-4th ventricular injection) decreased the mono- and disynaptic (the fastest polysynaptic) reflexes (MSR and DSR, respectively) in non-spinalized rats. Depletion of central noradrenaline by 6-hydroxydopamine abolished the depressant effect of tizanidine on the MSR almost completely and attenuated the effect on the DSR. Co-depletion of serotonin by 5,6-dihydroxytryptamine and noradrenaline resulted in more prominent attenuation of tizanidine-induced inhibition of the DSR. Supraspinal receptors were then studied using yohimbine- and some imidazoline-receptor ligands containing an imidazoline moiety. Idazoxan (I_1, I_2, I_3, and α_2), efaroxan (I_1, I_3, and α_2), and RX821002 (I_3 and α_2), but not yohimbine, an α_2-adrenergic receptor antagonist with no affinity for I receptors, antagonized the inhibitory effects of tizanidine. Thus, supraspinal I receptors (most likely I_3) and descending monoaminergic influences are necessary for tizanidine-induced inhibition of spinal segmental reflexes.

Keywords: tizanidine, spinal reflex, 6-hydroxydopamine, 5,6-dihydroxytryptamine, imidazoline receptor

Introduction

Tizanidine is a centrally acting muscle relaxant used clinically for the treatment of spasticity. Its applications have also been extended to patients with migraine, tension-type headache and other related disorders (1).

Tizanidine, structurally similar to clonidine, has high affinity for α_2-adrenergic receptors and imidazoline (I) receptors (2), and the muscle relaxant activity of tizanidine, deduced by depression of the spinal reflex, is mediated by imidazoline receptors (3). Several studies have indicated that there are three subtypes of imidazoline binding sites. I_1 receptors are located in the rostral ventrolateral medulla area of the brain stem (4, 5) and regulate blood pressure (6) as well as food intake (7), while I_2 receptors are widely distributed in the brain. Non-I_1/I_2 (subtype I_3) imidazoline binding sites mediate the insulinotropic effect of some imidazoline ligands (8 – 10) by acting as blockers of ATP-sensitive K^+ channels (9, 10), and more recently, they have been suggested to regulate neuronal activity in the locus coeruleus (11). So far, there have been no attempts to evaluate imidazoline-receptor subtypes involved in the depression of spinal reflexes by tizanidine. Here, we used the term "ligands" for the imidazoline compounds when they were related to I_3 receptors, since it is still uncertain whether they work as the agonist or the antagonist at the I_3 receptors.

We have previously demonstrated that tizanidine reduces noradrenaline (NA) release from descending noradrenergic nerve terminals in the spinal cord by acting at the supraspinal level (12), and we have suggested that the descending noradrenergic system, which provides facilitatory influences on motor output from the spinal ventral horn (13 – 15), may be primarily affected by tizanidine (16).

In the study presented here, we investigated the

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neuronal pathways involved in the muscle relaxant effect of tizanidine, assessed by the depression of spinal reflexes in non-spinalized rats in which either central noradrenaline or serotonin (5-HT) was depleted, and evaluated its pharmacological characteristics using yohimbine- and some imidazoline-receptor ligands containing an imidazoline moiety.

Materials and Methods

Measurement of spinal reflexes

All experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University and were conducted according to the guidelines of the National Institutes of Health and The Japanese Pharmacological Society.

Male Wistar/ST rats (7 – 8-week-old; SLC, Shizuoka) 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 preparations, the spinal cord was transected at the level of the Th8 vertebra 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 recorded from the ipsilateral L5 ventral root. The spinal reflex employed in the present study consists of mono- and disynaptic (the fastest polysynaptic) reflex potentials (MSR and DSR, respectively) which are elicited by monosynaptic excitation and disynaptic excitation via an interneuron of the motoneurons of skeletal muscles, respectively. The MSR and DSR were displayed on an oscilloscope, and eight consecutive responses were averaged by an averager. The amplitudes of the averaged MSR and DSR were measured. The latency of the MSR was 1.5 – 2.0 ms, and the time between the MSR and DSR was 0.8 – 1.0 ms.

Neurotoxic lesions

Neurotoxic lesions were made as were described previously (17). Depletion of 5-HT was performed by intracisternal injection of 5,6-dihydroxytryptamine creatinine sulfate (5,6-DHT, 75 μg/animal), which was dissolved in 20 μl of 0.9% w/v physiological saline containing ascorbic acid (100 μg/ml). To avoid NA depletion, desipramine hydrochloride (25 mg/kg, i.p.) dissolved in distilled water was administered 1 h before 5,6-DHT injection. The noradrenergic neurons were lesioned by intracisternal injection of 6-hydroxydopamine hydrobromide (6-OHDA, 36.7 μg/animal), which was dissolved in 20 μl of 0.9% w/v physiological saline containing ascorbic acid (100 μg/ml). Co-depletion of NA and 5-HT was attained by intracisternal injection of 6-OHDA (36.7 μg/animal) and 5,6-DHT (75 μg/animal), which were dissolved in 20 μl of 0.9% w/v physiological saline containing ascorbic acid (100 μg/ml). Control animals received an injection of the vehicle alone. The spinal reflexes were measured 2 weeks after treatment with the neurotoxin(s). After assessment of the effect of tizanidine on the spinal reflex, the rats were sacrificed by an overdose injection of pentobarbital sodium, and the brain stem and spinal cord were dissected out. The contents of NA and 5-HT were measured using reverse-phase high-performance liquid chromatography with electrochemical detection.

Drugs

Tizanidine hydrochloride was obtained from Novartis (Tokyo). RX821002 was kindly donated by Pierre Fabre (Castres, France). 6-OHDA, desipramine hydrochloride, idazoxan hydrochloride, yohimbine hydrochloride, and efaroxan hydrochloride were purchased from Sigma (St. Louis, MO, USA), and 5,6-DHT was obtained from RBI (Natick, MA, USA). The drugs, except for the neurotoxins, were dissolved in 0.9% w/v physiological saline and administered at 1 ml/kg. Control rats received the vehicle only at 1 ml/kg. When rats were spinalized, drugs were administered at least 2 h after spinalization. Yohimbine- or imidazoline-receptor antagonists/ligands were administered 10 min before tizanidine injection.

Statistical analyses

The effects of tizanidine on the spinal reflex and the effects of either pharmacological or neurotoxic pretreatments on the tizanidine-induced inhibition of the spinal reflex were evaluated with respect to time; the time of administration of tizanidine was designated as time zero. The MSR and DSR amplitudes were calculated as percentages of the corresponding predrug (time zero) amplitudes. All data were expressed as the mean ± S.E.M. Two-tailed Bonferroni-type multiple *t*-test following one-way analysis of variance (ANOVA) was used for multiple comparisons between the control and the treated groups (18). Student's *t*-test was used to compare the data for two groups. Differences at $P<0.05$ (two-tailed) were considered significant.

Results

Effects of systemically and intra-4th ventricularly administered tizanidine on spinal reflexes

We first confirmed the systemic effect of tizanidine on the MSR and DSR in both non-spinalized and spinalized rats. Tizanidine hydrochloride (0.03 and 0.1 mg/kg, i.v.) reduced the MSR and DSR in a dose-dependent manner in non-spinalized rats (Fig. 1A). In contrast, in spinalized rats, the same dose of tizanidine hydrochloride (0.1 mg/kg, i.v.) transiently increased the MSR, which returned to preinjection levels within 10 min, with concomitantly less reduction of the DSR (Fig. 1C). These observations were consistent with our previous study (3). The difference in the results between the preparations (non-spinalized or spinalized) suggested that tizanidine requires intact neuronal connections between the supraspinal structures and spinal cord to generate inhibition of the spinal reflex. Indeed, injection of tizanidine hydrochloride into the 4th ventricle (10 and 30 μg) in non-spinalized rats reduced the MSR and DSR (Fig. 1B), thus largely resembling the systemic effect of tizanidine in non-spinalized rats. In the next series of experiments, we investigated descending monoaminergic fibers to explore their relevance in tizanidine-induced inhibition of the spinal reflex in non-spinalized rats.

Effects of depletion of descending monoaminergic fibers on tizanidine-induced inhibition of spinal reflexes

Figures 2 and 3 describe time-course graphs of tizanidine in animals treated with neurotoxins and the mean maximum percent inhibition by tizanidine calculated from the maximum effect in each animal, respectively. Treatment with 6-OHDA reduced the NA content of the spinal cord to $39.4 \pm 11.1\%$ of that in control animals treated with vehicle (ascorbic acid) alone (data obtained from 6 control and 6 6-OHDA-treated animals), as quantified after the spinal reflex experiments with tizanidine. In those rats pretreated with 6-OHDA, tizanidine hydrochloride (0.1 mg/kg, i.v.) elicited a significantly weaker reduction of the MSR and DSR (Figs. 2A and 3). In 4 rats in which NA and 5-HT were codepleted with 6-OHDA and 5,6-DHT, where the spinal NA and 5-HT contents were reduced to $24.6 \pm 9.4\%$ and $36.5 \pm 12.9\%$ of those in 4 vehicle-treated rats, respectively, the effect of tizanidine hydrochloride (0.1 mg/kg, i.v.) was much less marked (Figs. 2C and 3). In contrast, depletion of 5-HT alone in 5 rats did not have a significant influence on tizanidine-induced inhibition of the spinal reflex (Figs. 2B and 3), where the spinal 5-HT content was reduced to $24.0 \pm 21.9\%$ of that in 6 vehicle-treated rats. Taken together, these results suggest that the descending noradrenergic system originating in the brain stem is primarily required for generation of tizanidine-induced inhibition of the segmental spinal reflex.

Effects of yohimbine- or imidazoline-receptor antagonists/ligands on tizanidine-induced inhibition of spinal reflexes

Figures 4, 5, and 6 describe time-course graphs of tizanidine in animals treated with yohimbine or some imidazoline-receptor antagonists/ligands (Figs. 4 and 5) and the mean maximum percent inhibition by tizanidine calculated from the maximum effect in each animal (Fig. 6). As we revealed in our previous study (3), tizanidine reduces the spinal reflex via imidazoline receptors, and this was also confirmed in the present study. Indeed, inhibition of the spinal reflex by tizanidine hydrochloride (0.1 mg/kg, i.v.) was almost abolished by idazoxan hydrochloride (0.3 mg/kg, i.v., Figs. 4B and 6, $n = 6$), an $\alpha_2/I_{1,2}$-receptor antagonist with an affinity for I_3 receptors, but not by the α_2-receptor antagonist yohimbine hydrochloride (0.1 mg/kg, i.v., Figs. 4A and 6, $n = 5$). These doses of idazoxan and yohimbine have been demonstrated to be sufficient to antagonize the effect via α_2-adrenergic receptors (3). We then examined the effect of efaroxan, an α_2/I_1-receptor antagonist with an affinity for I_3 receptors, and RX821002, an α_2-receptor antagonist with an affinity for I_3 receptors, on tizanidine-induced inhibition of the spinal reflex. In the presence of efaroxan hydrochloride (0.3 mg/kg, i.v.), tizanidine hydrochloride (0.1 mg/kg, i.v.) did not influence the MSR and DSR significantly (Figs. 5A and 6). Moreover, RX821002 (0.3 mg/kg, i.v.) completely abolished the inhibitory effects of tizanidine on the MSR and DSR (Figs. 5B and 6). Although efaroxan alone increased the MSR and DSR (Fig. 5A), which may be partly attributed to its higher affinity to I_1 receptors, this is not further studied here.

Discussion

Interaction of tizanidine with descending monoaminergic systems

Tizanidine depresses muscle tonus by reducing the spinal reflex. The present study demonstrated that supraspinally applied tizanidine largely mimicked the systemic effect on the MSR and DSR and that impairment of descending monoaminergic function abolished tizanidine-induced inhibition of the spinal reflex. Pharmacological experiments with α_2- and I-receptor antagonists/ligands suggested that these supraspinal actions of tizanidine were mediated by I_3 receptors.

The descending noradrenergic and serotonergic fibers

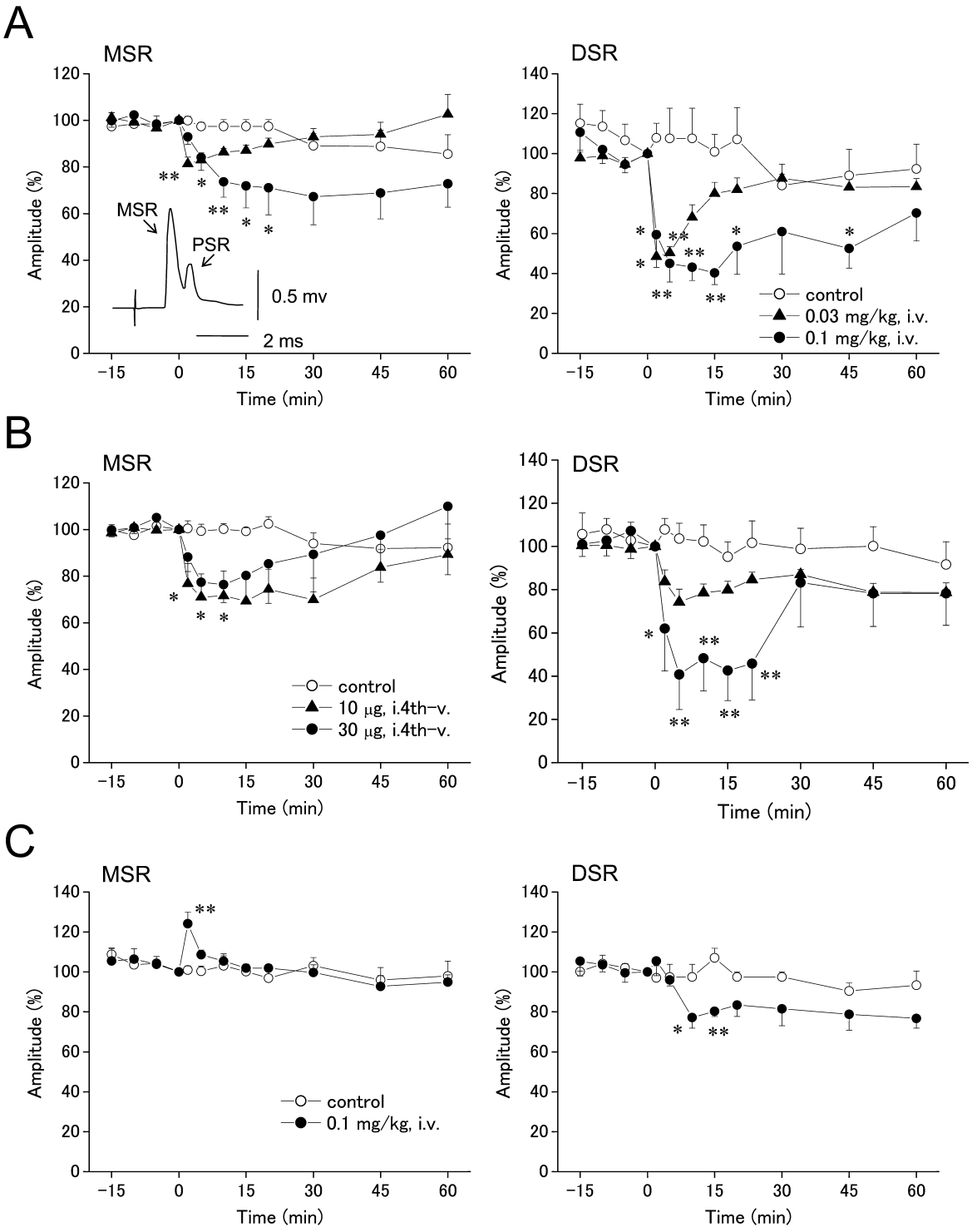

Fig. 1. Supraspinal mediation of tizanidine-induced inhibition of the spinal reflex. Intravenous (A, 0.03 and 0.1 mg/kg, i.v.) or intra-4th ventricular (B, 10 and 30 μg, i.c.v.) administration of tizanidine hydrochloride reduced both the mono- and disynaptic reflexes (MSR and DSR, respectively) in non-spinalized rats. In contrast, systemic tizanidine hydrochloride (0.1 mg/kg, i.v.) produced a slight increase in the MSR and smaller reduction of the DSR in spinalized rats (C). Tizanidine was administered at time zero. Each point represents the mean ± S.E.M. of 4 – 6 separate experiments. Ordinates: the MSR and DSR amplitudes expressed as percentages of the corresponding values at time zero. Abscissae: time in minutes after tizanidine administration. In A and B, the significance of differences between the values for the control and tizanidine-treated preparations was determined with the two-tailed Bonferroni-type multiple *t*-test following ANOVA (two comparisons in three groups). In C, Student's *t*-test was used to compare the data between the control and tizanidine-treated groups. **P*<0.05 and ***P*<0.01. Inset shows a sample record of the MSR and DSR.

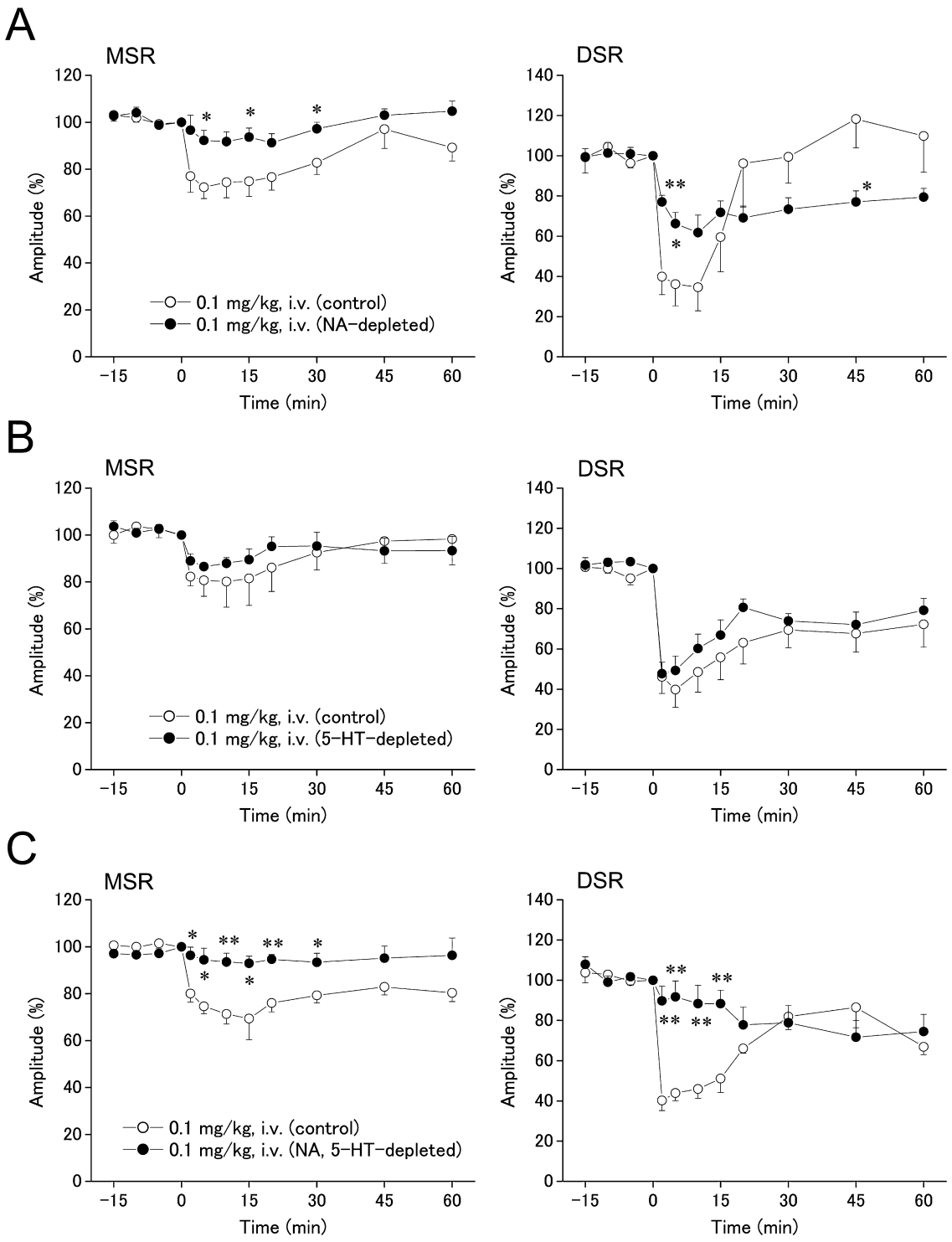

Fig. 2. Strong reduction of tizanidine-induced inhibition of the spinal reflex by either depletion of the descending noradrenaline (NA) level or co-depletion of the descending noradrenaline and serotonin (5-HT) levels. 6-Hydroxydopamine (6-OHDA) and 5,6-dihydroxytryptamine (5,6-DHT) were injected intracisternally to deplete NA and 5-HT, respectively. When both 6-OHDA and 5,6-DHT were injected, NA and 5-HT were co-depleted (for the detailed protocol, see Material and Methods). Tizanidine hydrochloride (0.1 mg/kg, i.v.)-induced inhibition of the spinal reflex was reduced largely in NA-depleted rats (A) and more pronouncedly in both NA and 5-HT-depleted rats (C). However, depletion of 5-HT alone did not have any influence on the effect of tizanidine (B). Tizanidine was administered at time zero. Each point represents the mean ± S.E.M. of 4 – 6 separate experiments. Ordinates: the MSR and DSR amplitudes expressed as percentages of the corresponding values at time zero. Abscissae: time in minutes after tizanidine administration. Student's *t*-test was used to compare the data between the control (ascorbic acid-treated) and depleted groups. **P*<0.05 and ***P*<0.01.

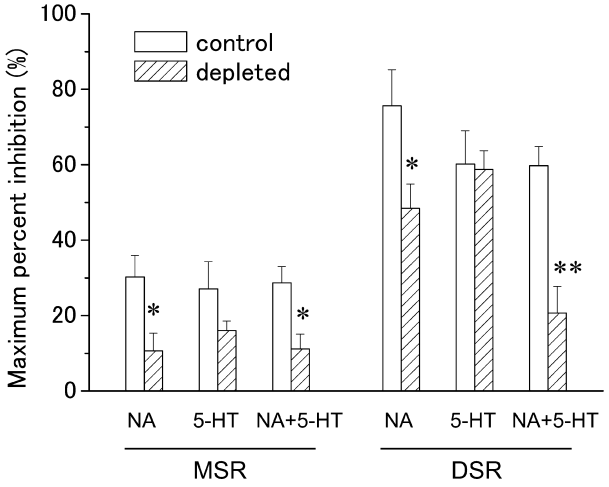

Fig. 3. Summary graph demonstrating the effects of depletion of central monoamines. Each column represents the mean ± S.E.M. of the maximum percentage inhibition of the MSR (left half) or DSR (right half) by tizanidine hydrochloride (0.1 mg/kg, i.v.) after vehicle (ascorbic acid, open column) treatment or either depletion of NA or 5-HT or co-depletion of NA and 5-HT (hatched column), obtained from 4 – 6 separate experiments. Student's *t*-test was used to compare the data between the control (ascorbic acid-treated) and depleted groups. **P*<0.05 and ***P*<0.01.

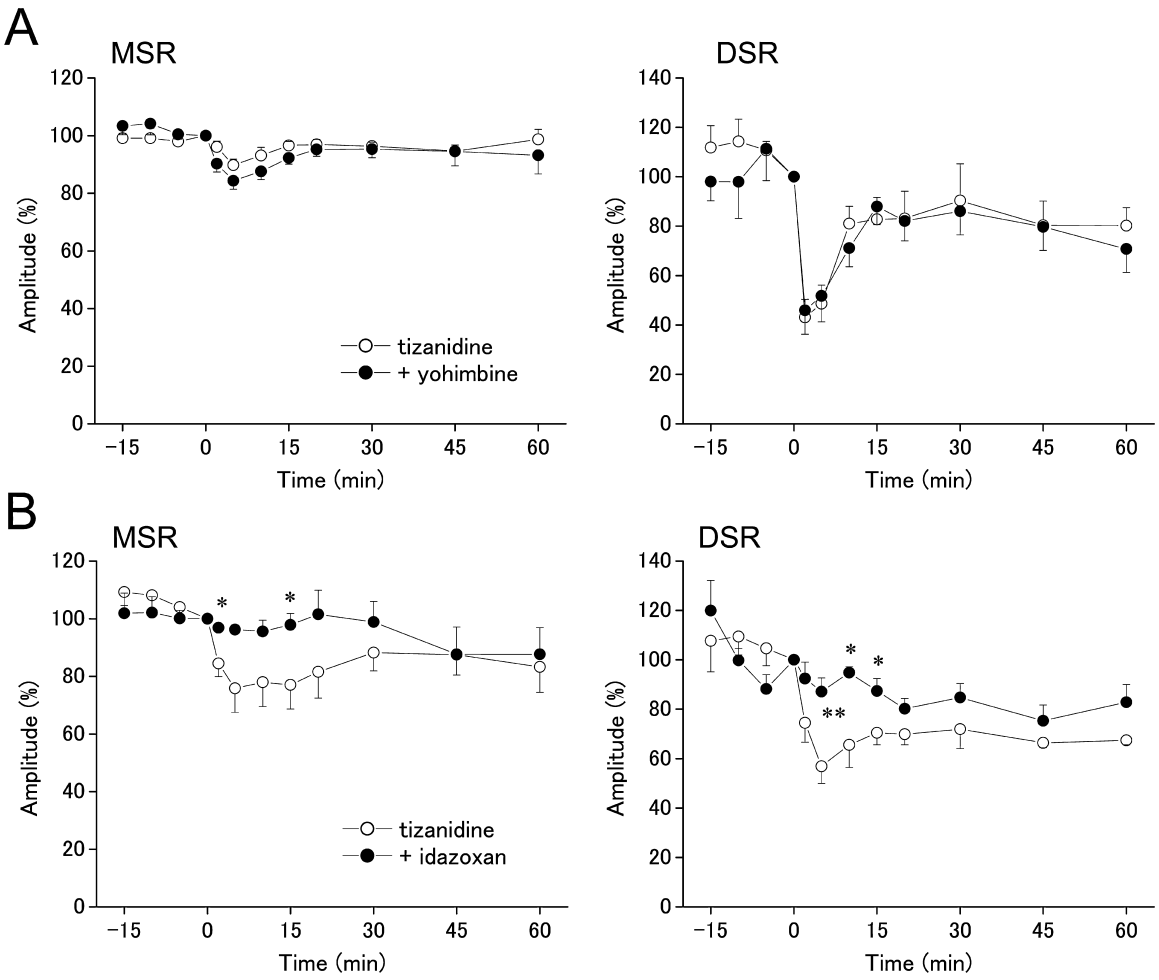

Fig. 4. Reduction of the spinal reflex by tizanidine via imidazoline receptors. Idazoxan hydrochloride (B, 0.3 mg/kg, i.v.), an $\alpha_2/I_{1,2}$-receptor antagonist with an affinity for I_3 receptors, but not the α_2-receptor antagonist yohimbine hydrochloride (A, 0.1 mg/kg, i.v.), abolished tizanidine-induced inhibition of the spinal reflex. Idazoxan or yohimbine was administered 10 min before tizanidine injection (0.1 mg/kg, i.v., injected at time zero). Each point represents the mean ± S.E.M. of 5 – 6 separate experiments. Ordinates: the MSR and DSR amplitudes expressed as percentages of the corresponding values at time zero. Abscissae: time in minutes after tizanidine administration. Student's *t*-test was used to compare the data between the control and antagonist/ligand-treated groups. **P*<0.05 and ***P*<0.01.

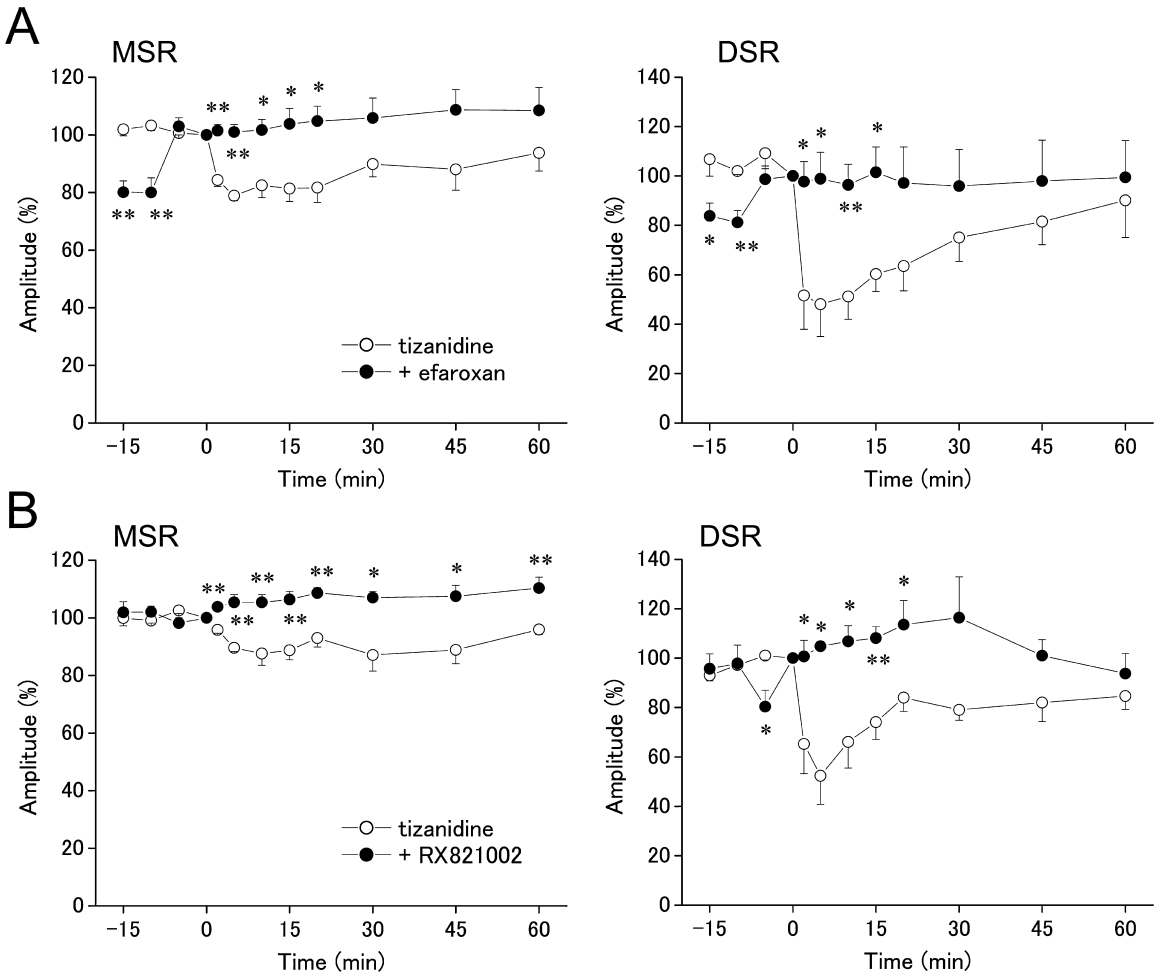

Fig. 5. Abolition of tizanidine-induced inhibition of the spinal reflex by efaroxan and RX821002. Efaroxan hydrochloride (A, 0.3 mg/kg, i.v.), an α_2/I_1-receptor antagonist with an affinity for I_3 receptors, and RX821002 (B, 0.3 mg/kg, i.v.), an α_2-receptor antagonist with an affinity for I_3 receptors, abolished tizanidine-induced inhibition of the spinal reflex. Efaroxan or RX821002 was administered 10 min before tizanidine injection (0.1 mg/kg, i.v., injected at time zero). Each point represents the mean ± S.E.M. of 4 – 5 separate experiments. Ordinates: the MSR and DSR amplitudes expressed as percentages of the corresponding values at time zero. Abscissae: time in minutes after tizanidine administration. Student's *t*-test was used to compare the data between the control and antagonist/ligand-treated groups. **P*<0.05 and ***P*<0.01.

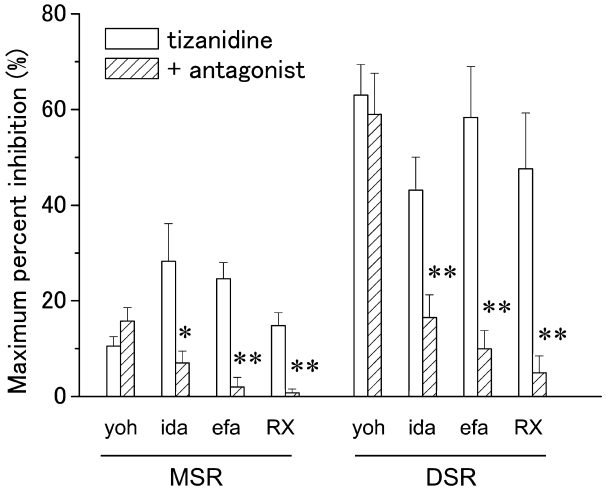

Fig. 6. Summary graph demonstrating the effects of yohimbine- or imidazoline-receptor antagonists/ligands. Each column represents the mean ± S.E.M. of the maximum percentage inhibition of the MSR (left half) or DSR (right half) by tizanidine hydrochloride (0.1 mg/kg, i.v.) in the absence (clear column) or presence (hatched column) of yohimbine, idazoxan, efaroxan, or RX821002, obtained from 4 – 6 separate experiments. Student's *t*-test was used to compare the data between the control and antagonist/ligand-treated groups. **P*<0.05 and ***P*<0.01.

that originate in the locus coeruleus (12, 13) and the medullary raphe (19), respectively, are the major descending neuronal systems that have tonic influences on lumbar α-motoneuronal activity. Although pharmacological modulation of these descending systems may have large influences upon spinal motor output, the relevance of these descending pathways in tizanidine-induced inhibition of the spinal reflex has not been addressed. Under reduced noradrenergic influence after treatment with 6-OHDA, tizanidine exhibited little inhibition of spinal reflexes (Figs. 2A and 3). Together with the established facilitatory roles of the descending noradrenergic system in the regulation of spinal motoneuronal activity (13 – 15), the supraspinal action of tizanidine seems to remove this facilitation, resulting in reduced segmental motor output. Furthermore, removal of both noradrenergic and serotonergic influences by simultaneous treatment with 6-OHDA and 5,6-DHT abolished the inhibitory effect of tizanidine on the spinal reflex almost completely (Figs. 2C and 3). In contrast, depletion of the descending 5-HT alone did not hamper the effect of tizanidine (Figs. 2B and 3). We may conclude that the primary target of tizanidine is the descending noradrenergic system, bearing in mind that there is interaction between the noradrenergic and serotonergic systems in the brain stem that mutually modifies the spontaneous firing activity of noradrenergic and serotonergic neurons. Serotonergic influences on noradrenergic neuronal activity are either inhibitory (20 – 22) or negligible (23 – 25), while noradrenergic influences on serotonergic neuronal activity are slightly excitatory (25 – 27). The stronger impact of removal of both noradrenergic and serotonergic influences than that of noradrenergic depletion alone on tizanidine-induced inhibition of the spinal reflex may reflect the interaction between these monoaminergic neurons.

Imidazoline-receptor subtypes involved in tizanidine-induced inhibition of spinal reflexes

In line with our previous study (3), the present result indicates that imidazoline receptors, but not α_2-adrenergic receptors, mediate tizanidine-induced inhibition of spinal reflexes.

It is widely accepted that I_1 and I_2 receptors are distributed in the central nervous system (4, 5, 28). Although their physiological functions are not fully determined, it has been demonstrated that I_1 receptors, found predominantly in the brain stem (5), play a role in the regulation of arterial blood pressure (6) and food intake (7). It is known that I_2 receptors mediate antinociceptive effects (29) and that some populations of I_2 receptors are located intracellularly in the outer membrane of the mitochondrion and inhibit monoamine oxidase activity (30). Non-I_1/I_2 (= I_3) receptors have been shown to act peripherally in the regulation of insulin secretion (8 – 10) and centrally in the modulation of firing activity of locus coeruleus neurons (11). Given that all subtypes of I receptors are present in the brain stem, which I-receptor subtypes mediate tizanidine-induced inhibition of the spinal reflex? The I-receptor antagonists/ligands employed in this study (idazoxan, eferoxan, and RX821002) effectively abolished the effect of tizanidine on spinal reflexes (Figs 4 and 5). Although all these I-receptor antagonists/ligands block α_2-adrenergic receptors, each exhibits a distinct affinity spectrum for I-receptor subtypes. Idazoxan is relatively I_2-selective, efaroxan is relatively I_1-selective (31), and RX821002 has affinity for I_3 receptors with negligible affinity for I_1 and I_2 receptors (32). Moreover, idazoxan and efaroxan also induce insulin release via imidazoline binding sites that are distinct from I_1/I_2 receptors (presumably I_3 receptors), coupled with ATP-sensitive K^+ channels (33, 34). Taken together, the results of the experiments using these three I-receptor antagonists/ligands suggest that I_3 receptors are the most likely receptor subtype on which tizanidine acts to inhibit the spinal reflex.

In summary, our present results suggest that the supraspinal inhibitory effects of tizanidine on spinal reflexes involve I receptors (most likely I_3) and the descending monoaminergic systems. However, the molecular basis responsible for the function of central I_3 receptors remains poorly understood, and potent ligands that bind selectively to I_3 receptors will be needed to further explore the relationship between supraspinal I_3 receptors and the descending monoaminergic system that regulates spinal motor control.

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