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Serotonergic depression of spinal monosynaptic transmission is mediated by 5-HT_{1B} receptors

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Abstract

In the spinal cord, various subtypes of serotonin (5-hydroxytryptamine; 5-HT) receptors are involved in the modulation of motor output. Although the excitatory role of 5-HT_2 receptors is known, the receptor subtypes mediating the inhibitory effect of 5-HT on monosynaptic reflex transmission remain unclear. In this study, segmental spinal reflexes were recorded to examine the receptor subtypes underlying 5-HT-mediated inhibition of monosynaptic reflex transmission in spinalized rats. Under conditions of monoamine oxidase blockade with clorgyline, the 5-HT precursor L-5-hydroxytryptophan depressed the monosynaptic reflex. 3-Hydroxybenzylhydrazine dihydrochloride (NSD-1015), a centrally active decarboxylase inhibitor, abolished this inhibition, confirming that the depression of the monosynaptic reflex by L-5-hydroxytryptophan was due to 5-HT. In the presence of GR127935 or isamoltane, which show high affinity for 5-HT_{1B} receptors, L-5-hydroxytryptophan did not suppress the monosynaptic reflex, whereas 5-HT_{1A}, 5-HT_{1D}, 5-HT_2 and 5-HT_7 receptor antagonists did not alter the inhibitory effect of L-5-hydroxytryptophan. These results suggest that serotonergic depression of monosynaptic reflex transmission is mediated by 5-HT_{1B} receptors.

Keywords: 5-Hydroxytryptophan; Spinal reflex; 5-HT receptor subtype

1. Introduction

Autoradiographic and immunocytochemical studies have demonstrated the presence of 5-hydroxytryptamine (5-HT)$_{1A}$, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_3 receptor subtypes in the spinal cord (Marlier et al., 1991; Ridet et al., 1994). Although evidence for the physiological importance of 5-HT in the modulation of pain transmission (Nadeson and Goodchild, 2002) and motor output (Roberts et al., 1988; White and Fung, 1989; Wang and Dun, 1990; Takahashi and Berger, 1990; Wallis et al., 1991; Wu et al., 1991) in the spinal cord is accumulating, the functional relationships of 5-HT with specific receptor subtypes have not been fully elucidated.

In the ventral horn of the spinal cord, 5-HT and serotonergic agonists affect neuronal excitability or synaptic transmission through various subtypes of receptors. In neonatal rat spinal motoneurons, 5-HT exerts excitation, presumably via 5-HT_{1A} receptors (Takahashi and Berger, 1990). The slow excitatory synaptic potential elicited in motoneurons of the neonatal rat spinal cord is mediated by 5-HT_2 receptors (Elliott and Wallis, 1993). Moreover, in previous in vitro and in vivo studies (Yamazaki et al., 1992a,b), we showed that 5-HT_2 receptors mediate motoneuronal excitatory activities in adult rats. However, we know relatively little about the inhibitory effects of 5-HT. Indeed, 5-hydroxytryptophan reduces the amplitude of the spinal monosynaptic reflex (Nagano et al., 1987), but the receptor subtypes underlying this inhibition remain unknown. In our previous study attempting to characterize this inhibitory effect of 5-HT, we used (*R*)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). 8-OH-DPAT inhibited monosynaptic reflex transmission in spinalized rats, but this effect was not antagonized by 5-HT_{1A}, 5-$HT_{1B/1D}$, 5-HT_2 or 5-HT_7 receptor antagonists (Honda and Ono, 2001). These results suggest that non-serotonergic mechanisms are involved in the inhibitory effect of (*R*)-8-OH-DPAT at the spinal level. Therefore, it was necessary to use 5-HT itself as an agonist in order to elucidate the inhibitory mechanism(s) of 5-HT. The goal of this study

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was to characterize the receptor subtypes responsible for the inhibitory effects of L-5-hydroxytryptophan, a precursor of 5-HT, on spinal reflexes of spinalized rats.

2. Materials and methods

2.1. Measurement of spinal reflexes

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

Male Wistar/ST rats (7–9 weeks old) were anesthetized with α-chloralose (25 mg/kg, intraperitoneally, i.p.) and urethane (1000 mg/kg, i.p.). Cannulae were inserted into the trachea and the femoral vein for respiration and drug administration, respectively. The vagus nerves were cut bilaterally in the cervical region to eliminate parasympathomimetic effects on the heart. The spinal cord was transected at the C1 level under topical lidocaine anesthesia (4%, 50 μl). A dorsal laminectomy was performed in the lumbosacral region of each rat. Both 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

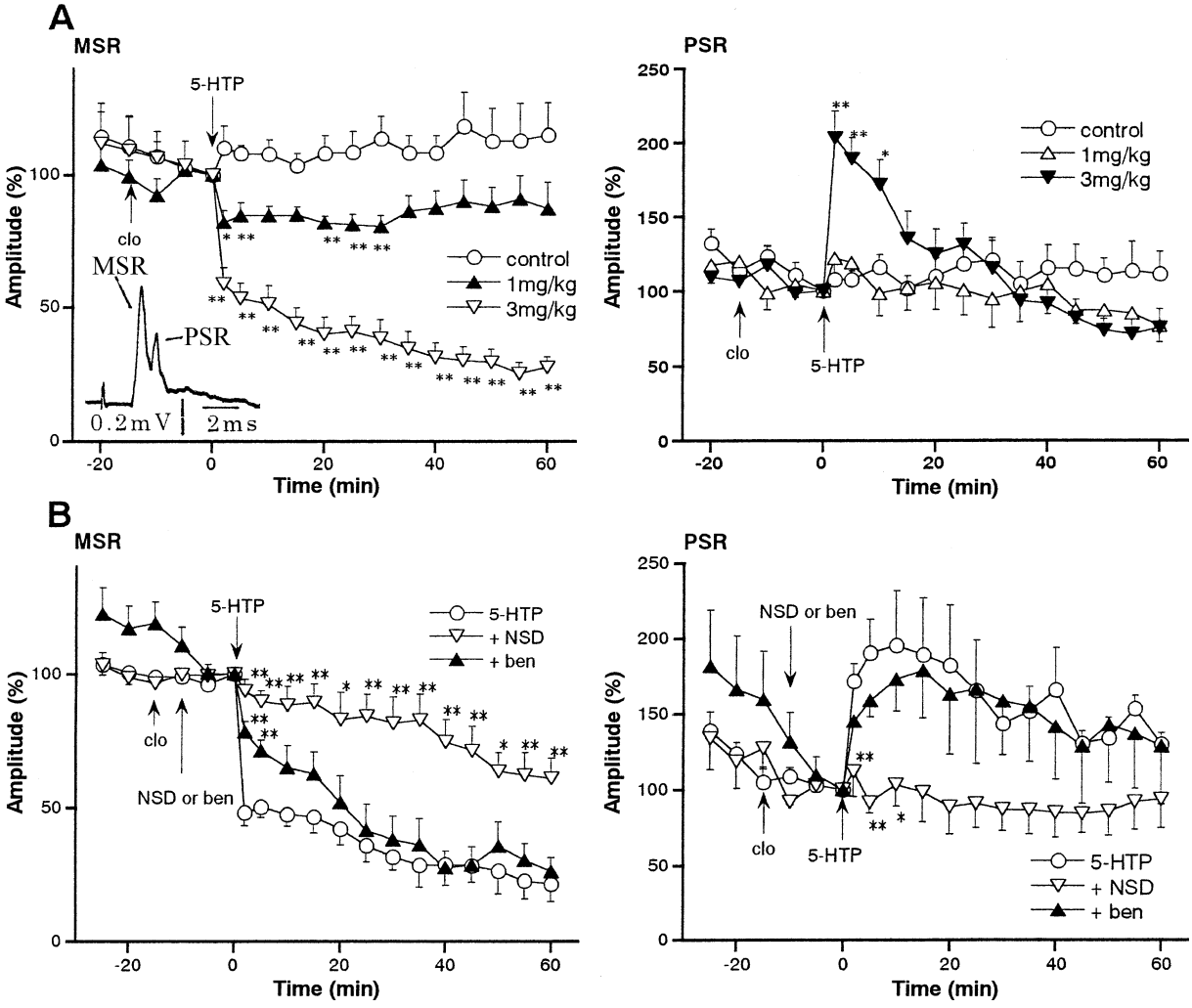

Fig.1. Effects of L-5-hydroxytryptophan (5-HTP, 1 and 3 mg/kg, i.v., A) on monosynaptic (MSR) and polysynaptic (PSR) reflex potentials in clorgyline-treated spinalized rats and the effects of pretreatment with aromatic L-amino acid decarboxylase inhibitors (B) on the response to L-5-hydroxytryptophan. Clorgyline hydrochloride (clo,1 mg/kg) and NSD-1015 (NSD,1 mg/kg) or benserazide hydrochloride (ben,1 mg/kg) were administered i.v. 15 and 10 min, respectively, before the administration of L-5-hydroxytryptophan. Each point represents the mean ± S.E.M. of five rats per group. Ordinates: means of amplitudes of reflexes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of L-5-hydroxytryptophan. The significance of differences between test and control values was determined by the two-tailed multiple *t*-test with Bonferroni correction following ANOVA (two comparisons in three groups); $*P<0.05$ and $**P<0.01$. Insert: a sample record of monosynaptic (MSR) and polysynaptic (PSR) reflex potentials.

reflex potentials were recorded from the ipsilateral L5 ventral root, displayed on an oscilloscope and eight consecutive responses were averaged by an averager. Then, the amplitudes of the monosynaptic and polysynaptic reflex potentials were measured. The latency of the monosynaptic reflex potential was 2–3 ms and the delay of the polysynaptic reflex peak after the monosynaptic reflex peak was 0.8–1 ms, corresponding to the disynaptic reflex (Fig. 1).

2.2. Drugs

WAY-100635 (*N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexanecarboxamide) maleate, ketanserin tartrate, clorgyline hydrochloride and 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015) were obtained from Research Biochemicals International (Natick, MA, USA). Benserazide (DL-serine 2-[2,3,4-trihydroxybenzyl]-hydrazine) hydrochloride and urethane were from Sigma-Aldrich (St. Louis, MO, USA), BRL15572 (3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol) hydrochloride and isamoltane hemifumarate were from Tocris Cookson (Ballwin, MO, USA) and L-5-hydroxytryptophan and α-chloralose were from Tokyo Kasei (Tokyo, Japan). SB-269970 ((*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrolidine) hydrochloride was a gift from SmithKline Beecham Pharmaceuticals (Harlow, UK). DR4004 (2a-(4-phenyl-1,2,3,6-tetrahydropyridal)butyl)-2a,3,4,5-tetrahydrobenzo[cd]indol-2(1H)-one) was from the Pharmaceutical Research Center of Meiji Seika Kaisha Ltd., (Yokohama, Japan) and GR127935 (*N*-[methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxa-

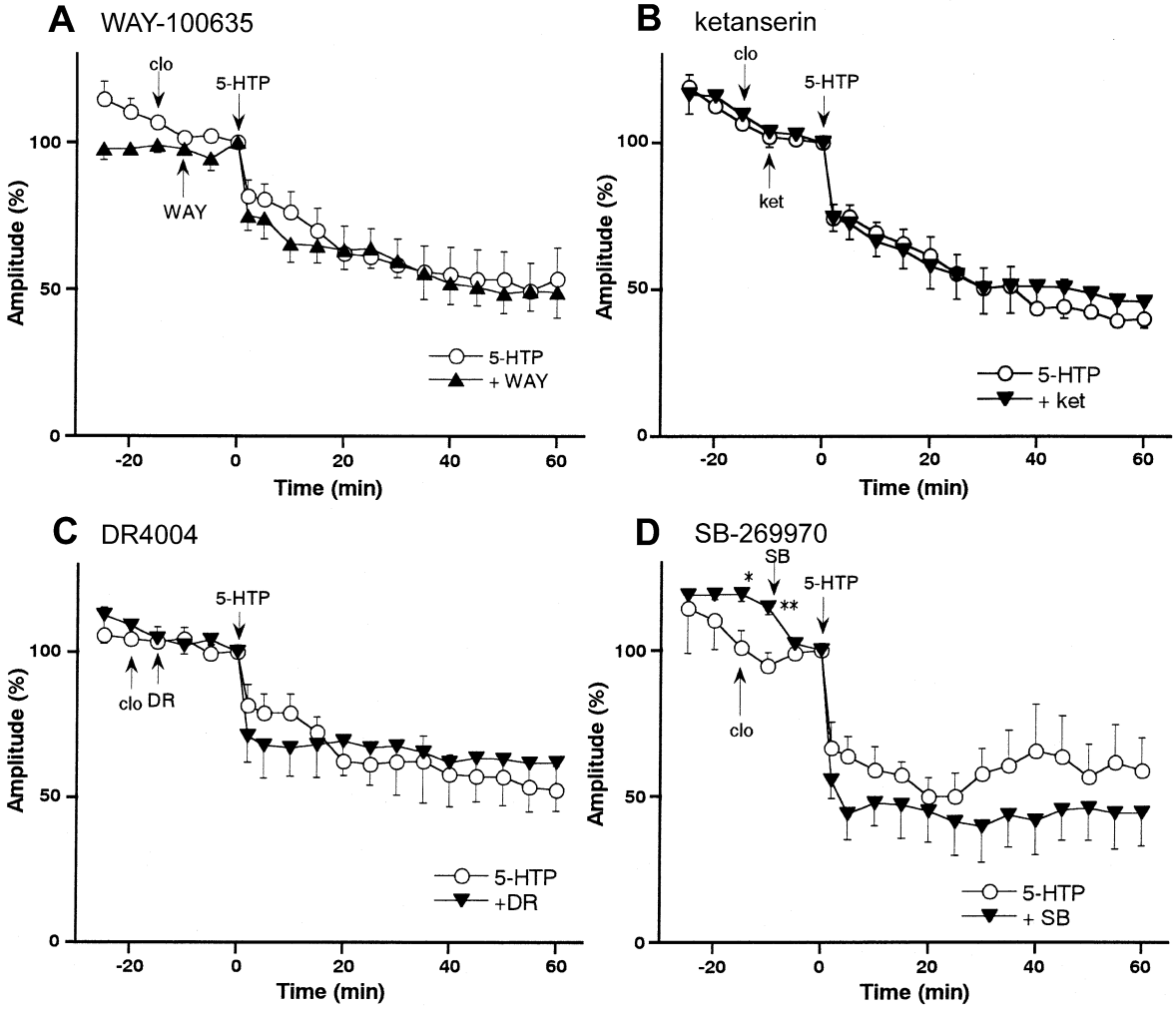

Fig. 2. Effects of WAY-100635 maleate (WAY, 0.1 mg/kg, i.v., A), ketanserin tartrate (ket,1 mg/kg, i.v., B), DR4004 (DR,1 mg/kg, i.v., C) and SB-269970 (SB, 10 mg/kg, i.v., D) on L-5-hydroxytryptophan (5-HTP)-induced changes in the monosynaptic reflex potentials in clorgyline-treated spinalized rats. Clorgyline hydrochloride (clo, 1 mg/kg) and each antagonist were administered i.v. 15 and 10 min, respectively, before the administration of L-5-hydroxytryptophan. Each point represents the mean ± S.E.M. of five to seven rats per group. Ordinates: means of amplitudes of reflexes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of L-5-hydroxytryptophan. The significance of differences between test and control values was determined by the two-tailed Student's *t*-test; $*P<0.05$ and $**P<0.01$.

Table 1
Effects of 5-HT receptor antagonists on 5-hydroxytrytophan-induced enhancement of polysynaptic reflex potentials

Antagonists (salt)	Vehicle + 5-HTP	Antagonist + 5HTP
WAY10635 (0.1 mg/kg)	1462.3 ± 814.6	1019.0 ± 301.1
Ketanserin (1 mg/kg)	1319.0 ± 509.7	− 89.8 ± 247.2**
DR4004 (1 mg/kg)	753.9 ± 293.0	1336.1 ± 566.4
SB-26990 (10 mg/kg)	1026.1 ± 341.2	682.4 ± 402.5
GR127935 (0.3 mg/kg)	1133.4 ± 311.0	1242.8 ± 701.8
Isamoltane (1 mg/kg)	1062.6 ± 246.1	1652.1 ± 310.2
BRL15572 (3 mg/kg)	2145.8 ± 667.8	748.6 ± 581.9

Each value represents area under the time-course curve (AUC, amplitude (%) × time (min)) over 100% line for 30 min after administration of 5-hydroxytrytophan in clorgyline-treated spinalized rats. The significance of the differences between each control (vehicle + 5-HTP) and test group (antagonist + 5-HTP) was determined by the two-tailed Student's *t*-test.
* $P < 0.05$.

mide) hydrochloride monohydrate was from Glaxo Wellcome Research and Development (Stevenage, UK). Urethane and α-chloralose were dissolved in distilled water. All the test compounds were dissolved in 0.9% w/v physiological saline, except that DR4004 and BRL15572 were dissolved in 1% Tween 80 and GR127935 hydrochloride monohydrate was dissolved in distilled water, and administered intravenously (i.v.) at 1 ml/kg. Clorgyline, a monoamine oxidase A inhibitor, was administered 15 or 20 min before injecting L-5-hydroxytryptophan. Each antagonist was administered 10 min before injecting L-5-hydroxytryptophan, except that DR4004 was administered 15 min before. The dose of each drug used in these experiments represents the weight of the salt. Control rats received vehicle at 1 ml/kg. Drugs were administered at least 2 h after spinalization.

2.3. Statistical analysis

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 data for two groups, and the multiple *t*-test with Bonferroni correction following one-way analysis of variance (ANOVA) was used for multiple comparisons of

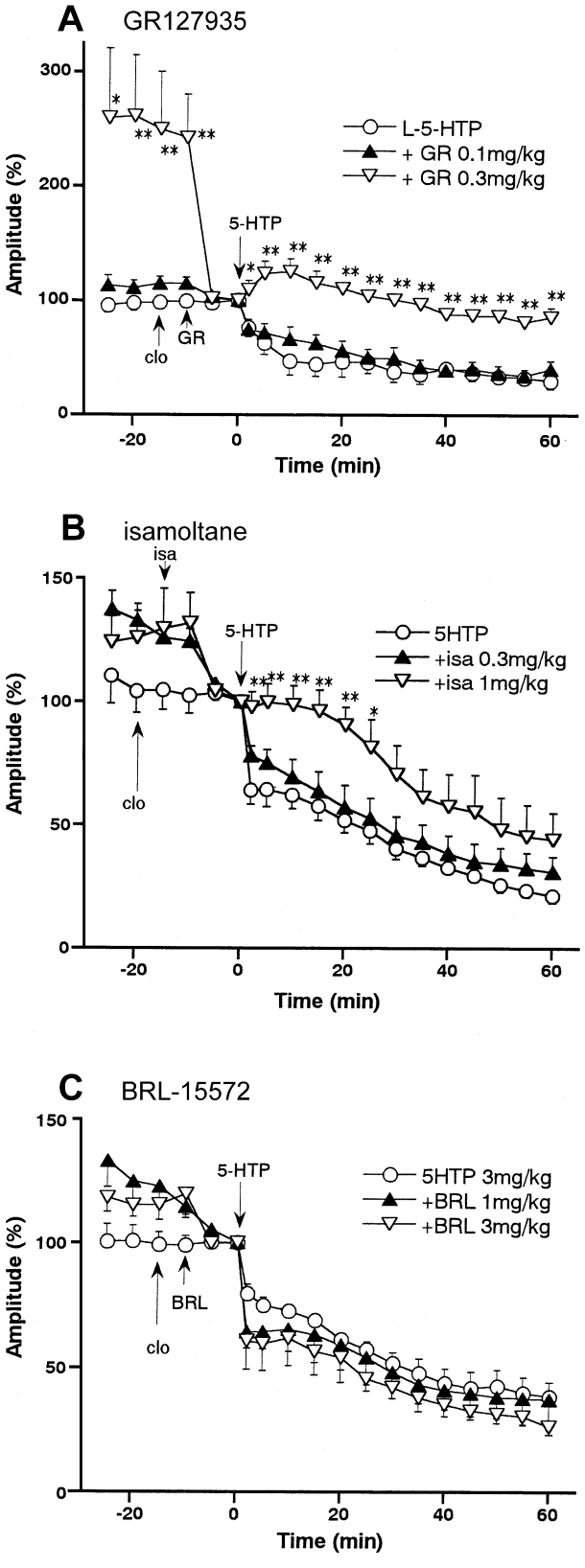

Fig. 3. Effects of GR127935 hydrochloride monohydrate (GR, 0.1 and 0.3 mg/kg, i.v., A), isamoltane hemifumarate (isa, 0.3 and 1 mg/kg, i.v., B) and BRL-15572 hydrochloride (BRL, 1 and 3 mg/kg, i.v., C) on L-5-hydroxytryptophan (5-HTP)-induced changes in the monosynaptic reflex potentials in clorgyline-treated spinalized rats. Clorgyline hydrochloride (clo, 1 mg/kg) and each antagonist were administered i.v. 15 and 10 min, respectively, before the administration of L-5-hydroxytryptophane. Each point represents the mean ± S.E.M. of five to six rats per group. Ordinates: means of amplitudes of reflexes expressed as percentages of the corresponding values at time 0. Abscissae: time in minutes after the injection of L-5-hydroxytryptophan. The significance of differences between test and control values was determined by the two-tailed multiple *t*-test with Bonferroni correction following ANOVA (two comparisons in three groups); * $P < 0.05$ and ** $P < 0.01$.

control and treated groups (Wallenstein et al., 1980). Differences at $P < 0.05$ (two-tailed) were considered to be significant.

3. Results

3.1. 5-HT generated from L-5-hydroxytryptophan inhibits the monosynaptic reflex in spinalized rats

In clorgyline-treated rats, L-5-hydroxytryptophan (1 and 3 mg/kg, i.v.) reduced monosynaptic reflex potentials in a dose-dependent manner and increased polysynaptic reflex potentials (Fig. 1A). L-5-Hydroxytryptophan, at a dose of 3 mg/kg, significantly reduced the amplitude of the monosynaptic reflex to about 30–40% of the pretreatment value and this effect persisted for over 60 min (Fig. 1A, MSR). The amplitude of the polysynaptic reflex was enhanced to about 200% of the pretreatment level (Fig. 1A, PSR). NSD-1015, a centrally active aromatic L-amino acid decarboxylase inhibitor, significantly inhibited the L-5-hydroxytryptophan-induced effects on the mono- and polysynaptic reflexes. In the presence of benserazide hydrochloride (1 mg/kg, i.v.), a peripherally active aromatic L-amino acid decarboxylase inhibitor, the depression was attenuated for the initial 15 min after the administration of L-5-hydroxytryptophan, but L-5-hydroxytryptophan then progressively and strongly depressed the monosynaptic reflex (Fig. 1B). Thus, these results indicate that 5-HT, generated from L-5-hydroxytryptophan in the spinal cord, inhibits the monosynaptic reflex and potentiates the polysynaptic reflex.

3.2. 5-HT_{1A}, 5-HT_2, or 5-HT_7 receptors are not involved in the inhibition of spinal reflexes by 5-HT

The selective 5-HT_{1A} receptor antagonist WAY-100635 maleate (0.1 mg/kg, i.v.), the selective 5-HT_2 receptor antagonist ketanserin tartrate (1 mg/kg, i.v.) and the selective 5-HT_7 receptor antagonists DR4004 (1 mg/kg, i.v.) and SB-269970 hydrochloride (10 mg/kg, i.v.) had little effect on the monosynaptic reflex depression induced by L-5-hydroxytryptophan (Fig. 2). We consider that the doses of these antagonists were adequate to block their target receptors, because each dose has previously been found to be effective in studies of the specific functions of each receptor subtype (Yamazaki et al., 1992b; Forster et al., 1995; Kogan et al., 2002). Ketanserin pretreatment inhibited the enhancement of the polysynaptic reflex (Table 1), which is consistent with our previous observation (Yamazaki et al., 1992b). WAY-100635, DR4004 and SB-269970 did not affect this L-5-hydroxytryptophan-induced enhancement of the polysynaptic reflex. None of these antagonists alone, except SB269970, changed the spinal reflexes. These results suggest that 5-HT_{1A}, 5-HT_2, or 5-HT_7 receptors are not involved in the inhibition of the monosynaptic reflex induced by 5-HT.

3.3. 5-HT_{1B} receptors mediate the inhibition of monosynaptic reflexes by 5-HT

Pretreatment with GR127935 hydrochloride monohydrate (0.3 mg/kg, i.v.), a selective 5-$HT_{1B/1D}$ receptor antagonist, significantly reduced the monosynaptic reflex depression induced by L-5-hydroxytryptophan (Fig. 3A). Although this dose of GR127935 alone resulted in a considerable decrease in the amplitude of the monosynaptic reflex, subsequent administration of L-5-hydroxytryptophan did not further attenuate the monosynaptic reflex, which was increased to $124.7 \pm 11.4\%$ of the pre-L-5-hydroxytryptophan level. Then, the effects of selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists were examined to determine which 5-HT receptor subtypes were involved in the L-5-hydroxytryptophan-induced inhibitory effect. Isamoltane hemifumarate (0.3 and 1 mg/kg, i.v.), which has relative selectivity for 5-HT_{1B} receptors, antagonized the monosynaptic reflex depression induced by L-5-hydroxytryptophan in a dose-dependent manner, and the effect was significant at a dose of 1 mg/kg (Fig. 3B). On the other hand, BRL15572 hydrochloride (1 and 3 mg/kg, i.v.), a selective 5-HT_{1D} receptor antagonist, did not impair the monosynaptic reflex depression induced by L-5-hydroxytryptophan (Fig. 3C). GR127935, isamoltane and BRL15572 had no effect on the polysynaptic reflex enhancement induced by L-5-hydroxytryptophan (Table 1).

4. Discussion

Monosynaptic reflex transmission is the synaptic excitation of motoneurons induced by stimulation of group Ia afferent fibers originating from the muscle spindles and 5-HT receptors are located in the presynaptic terminals and motoneuronal somata and dendrites. In the present study, an in vivo spinal cord preparation was used to study monosynaptic reflex transmission, because synaptic connections between dorsal roots and motoneurons are intact in this preparation, but not in slice and cell preparations.

The somata of 5-HT neurons in the raphe regions of the pons and medulla project to the ventral horn of the spinal cord, where they are thought to regulate motor function (Björklund and Skagerberg, 1982). Although 5-HT_2 receptor subtypes are involved in the excitatory effects of 5-HT and serotonergic agonists (Yamazaki et al., 1992a,b; Elliott and Wallis, 1993), it is not known which receptor subtypes are involved in the inhibitory effect of 5-HT on monosynaptic reflex transmission.

In a previous study, we showed that L-5-hydroxytryptophan in the presence of clorgyline markedly reduced the amplitude of the monosynaptic reflex and increased the amplitude of the polysynaptic reflex in spinalized rats, although the same dose of L-5-hydroxytryptophan alone had no effect on either mono- or polysynaptic reflex amplitudes (Nagano et al., 1987). Thus, in all the experi-

ments in the present study, clorgyline was administered before injecting L-5-hydroxytryptophan. As expected, L-5-hydroxytryptophan reduced the amplitude of the monosynaptic reflex and enhanced the amplitude of polysynaptic reflex of clorgyline-pretreated spinalized rats (Fig. 1A). These effects were abolished by pretreatment with NSD-1015, a centrally active aromatic L-amino acid decarboxylase inhibitor, but not with benserazide, a peripherally active aromatic L-amino acid decarboxylase inhibitor (Fig. 1B). The effects of L-5-hydroxytryptophan were attenuated during the initial 15 min after administration of benserazide. This may partly be due to the central effect of benserazide, since the i.p. administration of benserazide at high doses can penetrate the blood–brain barrier (Silva et al., 1997; Jonkers et al., 2001). These results suggest that the effects observed with L-5-hydroxytryptophan were mediated by 5-HT generated from L-5-hydroxytryptophan within the spinal cord.

Our findings indicate that 5-HT_{1A}, 5-HT_2 or 5-HT_7 receptors are not involved in 5-HT-elicited inhibition of the monosynaptic reflex (Fig. 2). We consider that the doses of these antagonists were adequate to block each receptor. The 5-HT_{1A} receptor antagonist WAY-100635 at a dose of 0.1 mg/kg, i.v. attenuated 8-OH-DPAT-induced inhibition of firing of dorsal raphe neurons in the anesthetized rat (Forster et al., 1995). In our previous study, the 5-HT_2 receptor antagonist ketanserin tartrate at a dose of 1 mg/kg, i.v. antagonized the increase in spinal motoneuronal excitability elicited by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a selective 5-HT_2 receptor agonist, in vivo (Yamazaki et al., 1992b). Furthermore, the selective 5-HT_7 receptor antagonists DR4004 (1, 5 and 10 mg/kg, i.p.) and SB-269970 (20 mg/kg, i.p.) induced hypothermia via 5-HT_7 receptors in conscious rats (Kogan et al., 2002). Our findings that L-5-hydroxytryptophan strongly reduced the monosynaptic reflex in the presence of these antagonists suggest that other subtypes of 5-HT receptors mediate this effect. Indeed, in the isolated neonatal rat spinal cord, 5-HT significantly depressed the monosynaptic reflex (Wallis et al., 1993) and this depression was not blocked by ketanserin (Crick and Wallis, 1991; Manuel et al., 1995). Manuel et al. (1995) suggested that 5-HT applied to the spinal cord acts via a different receptor, possibly a novel 5-HT receptor, to depress the monosynaptic reflex.

In the presence of GR127935, a potent and selective 5-$HT_{1B/1D}$ receptor antagonist (Skingle et al., 1996), L-5-hydroxytryptophan did not depress the monosynaptic reflex (Fig. 3A). At a high dose (0.3 mg/kg), GR127935 alone markedly depressed the monosynaptic reflex. GR127935 has potent but partial 5-HT_{1B} receptor agonistic activities in some functional studies, such as [^{35}S]-GTPγS binding and cAMP accumulation with recombinant receptors (Pauwels and Colpaert, 1996; Pauwels et al., 1996, 1997), which could contribute to the inhibitory effect on the monosynaptic reflex exerted by GR127935 itself in the present study. To examine further which subtypes are involved in the monosynaptic reflex depression induced by L-5-hydroxytryptophan, isamoltane and BRL15572 were used as selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists, respectively. Pretreatment with isamoltane hemifumarate (1 mg/kg) significantly antagonized L-5-hydroxytryptophan-induced monosynaptic reflex depression (Fig. 3B), whereas BRL15572 hydrochloride (1 and 3 mg/kg) had no effect (Fig. 3C). Although isamoltane shows affinities for both 5-HT_{1A} and 5-HT_{1B} receptors, selectivity for 5-HT_{1B} receptors is higher than for 5-HT_{1A} receptors (Waldmeier et al., 1988). Furthermore, the selective 5-HT_{1A} receptor antagonist WAY-100635 did not change the effect of L-5-hydroxytryptophan (Fig. 2A). These observations suggest that 5-HT_{1B} receptors are involved in the blockade of L-5-hydroxytryptophan-induced monosynaptic reflex depression. In the present study, SB-269970, isamoltane or BRL15572 alone tended to depress the monosynaptic reflex by about 25% (Figs. 2D, 3B and C, − 5 min). Although SB-269970 or BRL15572 alone depressed the monosynaptic reflex and the subsequent administration of L-5-hydroxytryptophan induced further depression of monosynaptic reflex amplitude; conversely, in the presence of isamoltane, no further depression of monosynaptic reflex amplitude was induced by L-5-hydroxytryptophan. We interpret these results to mean that 5-HT was able to depress, monosynaptic reflex amplitude beyond that caused by antagonists alone, and that isamoltane-induced blockade of monosynaptic reflex depression produced by L-5-hydroxytryptophan was genuine rather than apparent antagonism by isamoltane.

In conclusion, 5-HT depressed the monosynaptic reflex in the spinalized rats, an effect that appears to be mediated by 5-HT_{1B} receptors in the spinal cord. Further studies with selective 5-HT_{1B} receptor agonists will be needed to confirm this conclusion.

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