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Endogenously released 5-hydroxytryptamine depresses the spinal monosynaptic reflex via 5-HT_{1D} receptors

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Abstract

In the spinal cord, various 5-hydroxytryptamine (5-HT) receptor subtypes are involved in the modulation of motor output. Previously, we have shown that 5-HT_{1B} receptors mediate the monosynaptic reflex depression induced by exogenously applied 5-HT that was formed from the precursor L-5-hydroxytryptophan in spinalized rats. In this study, we determined the effects of endogenous 5-HT, which was released from serotonergic terminals by DL-p-chloroamphetamine, on spinal reflexes. DL-p-Chloroamphetamine depressed the monosynaptic reflex and increased the polysynaptic reflex. The depletion of 5-HT abolished the monosynaptic reflex depression, but the increase in polysynaptic reflexes was maintained, suggesting that endogenous 5-HT released by DL-p-chloroamphetamine mediates depression of the monosynaptic reflex in the spinal cord. The depression of the monosynaptic reflex was antagonized by GR127935 (*N*-[methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide; 5-$HT_{1B/1D}$ receptor antagonist) and BRL15572 (3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol; 5-HT_{1D} receptor antagonist) but not by isamoltane (5-HT_{1B} receptor antagonist). These results suggest that 5-HT released from serotonergic terminals depresses monosynaptic reflex transmission via 5-HT_{1D} receptors.

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

1. Introduction

In the ventral horn of the spinal cord, 5-hydroxytryptamine (5-HT) and serotonergic agonistic modulate neuronal excitability or synaptic transmission through various subtypes of 5-HT receptors. 5-HT_2 receptors have been shown to mediate motoneuron excitability in adult and neonatal rats in both in vitro and in vivo studies (Yamazaki et al., 1992a,b; Elliott and Wallis, 1993). However, identity of receptor subtypes that mediate the inhibitory effects of 5-HT remains to be established. In our previous study, we showed that, in the presence of clorgyline, a monoamine oxidase-A inhibitor, L-5-hydroxytryptophan markedly reduces the amplitude of the monosynaptic reflex and increases that of the polysynaptic reflex in spinalized rats (Nagano et al., 1987), and that this reduction of the monosynaptic reflex is antagonized by the 5-HT_{1B} receptor antagonist isamoltane (Honda et al., 2003). Thus, 5-HT_{1B} receptors mediate the depression of monosynaptic transmission induced by 5-HT in the spinal cord. However, because exogenously applied, 5-HT might also activate extrasynaptic receptors, it is not likely that our observation revealed the true function of endogenously released 5-HT.

DL-p-Chloroamphetamine is a derivative of amphetamine that has the effect of releasing endogenous 5-HT from presynaptic terminals. It has been demonstrated that systemic administration of DL-p-chloroamphetamine increases the extracellular concentration of 5-HT in the central nervous system and alters the behavior of rats (Hutson and Curzon, 1989; Fuller, 1992; Saito et al., 1999). 5-HT depletion abolishes both the increase in extracellular 5-HT concentration in the central nervous system and the behavioral changes, suggesting that the effects of DL-p-chloroamphetamine are due to the acute release of 5-HT from the serotonergic system (Trulson and Jacobs, 1976;

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Kehne et al., 1992). In the study presented here, we determined whether the endogenous 5-HT that is released from the serotonergic system in response to the systemic administration of DL-p-chloroamphetamine alters the spinal reflex potentials of spinalized rats and established which 5-HT receptor subtypes are involved in this effect.

2. Materials and methods

2.1. Measurement of spinal reflexes

All of the experimental protocols used in this study were approved by the Animal Care and Use Committee of Nagoya City University and were performed 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 for drug administration, respectively. The vagus nerves were cut bilaterally in the cervical region to eliminate any parasympathomimetic effects on the heart. The spinal cord was transected at the first cervical segmental (C1) level under topical lidocaine anesthesia (4%, 50 μl). A dorsal laminectomy was performed in the lumbosacral region of each rat. Both of the ventral and dorsal roots below fourth lumber segment (L4) were cut distally at their point of exit from the vertebral column, and the entire exposed surgical area was covered with liquid paraffin that was maintained at a temperature of 36±0.5 °C by radiant heat. Bipolar Ag-AgCl wire electrodes were used for stimulation and recording. A fifth lumber segmental (L5) dorsal root was

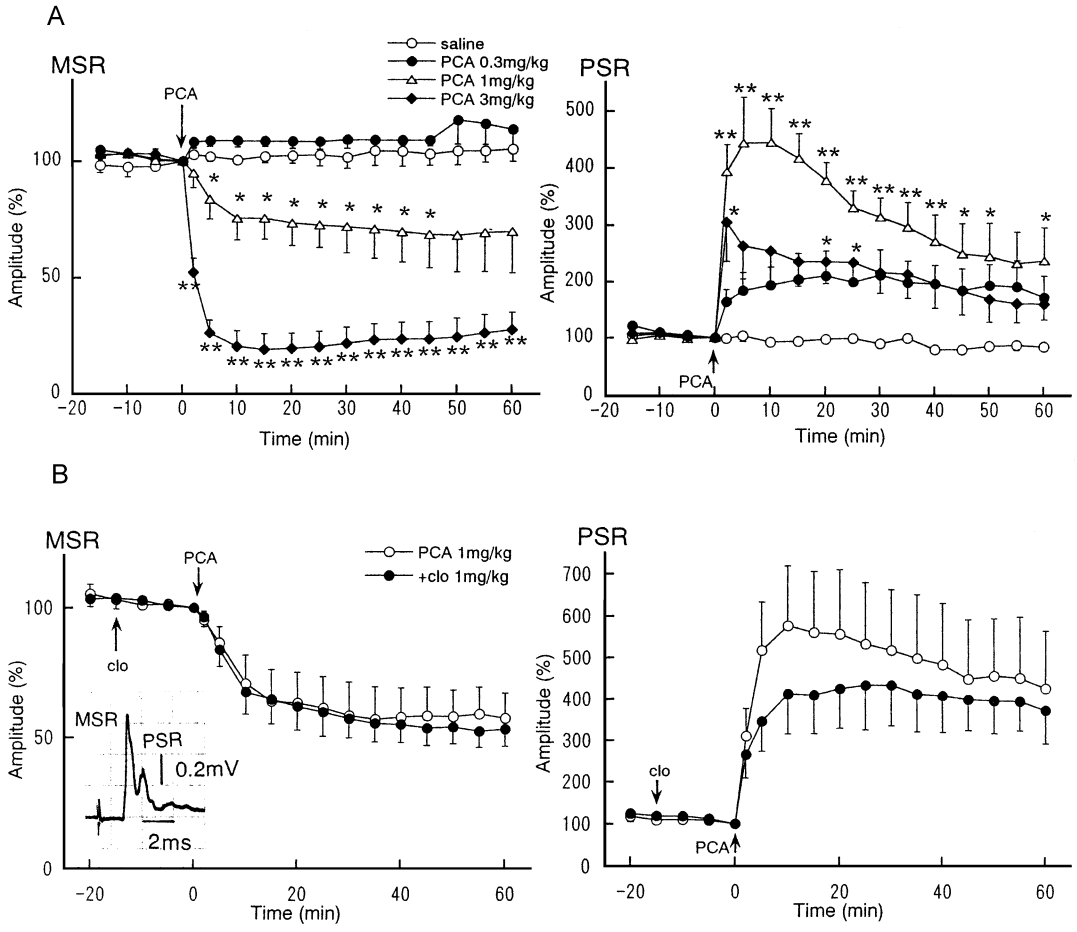

Fig. 1. Effects of DL-p-chloroamphetamine hydrochloride (PCA, 0.3, 1 and 3 mg/kg, i.v., [A]) on monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials in spinalized rats and the effects of pretreatment with clorgyline hydrochloride (clo, 1 mg/kg, i.v., [B]) on the response to DL-p-chloroamphetamine. Clorgyline hydrochloride was administered i.v. 15 min before the administration of DL-p-chloroamphetamine. Each point represents the mean±S.E.M. of four rats per group. Ordinates—mean reflex amplitude expressed as a percentage of the corresponding value at time 0. Abscissae—time in minutes after the injection of DL-p-chloroamphetamine. The significance of differences between test and control values was determined by the two-tailed multiple *t*-test with Bonferroni correction following one-way analysis of variance (ANOVA; three comparisons in four groups); $*P<0.05$ and $**P<0.01$. Inset—a sample record of the monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials.

stimulated with 0.2-Hz rectangular pulses, 0.05 ms in duration, at a supramaximal voltage of approximately twice that required to evoke a maximal reflex response. Monosynaptic and polysynaptic reflex potentials were recorded from the ipsilateral L5 ventral root. The recorded signal was digitized by the PowerLab system (ADInstruments) and analyzed using Scope software (ADInstruments). Twelve consecutive responses were averaged, and the amplitudes of the monosynaptic and polysynaptic reflex potentials were measured. The latency of the monosynaptic reflex potential was 1–1.5 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. Neurotoxic lesions

Depletion of 5-HT was effected by administering DL-p-chlorophenylalanine (300 mg/kg/day, i.p.) on days 1–3 before measuring the monosynaptic and polysynaptic reflex potentials. DL-p-Chlorophenylalanine was suspended in a 0.5% w/v carboxymethyl cellulose sodium solution, and 10 ml/kg was injected. Control animals received 0.5% w/v carboxymethyl cellulose sodium solution (i.e., vehicle alone).

2.3. Drugs

DL-p-Chloroamphetamine hydrochloride, clorgyline hydrochloride, prazosin hydrochloride, WAY-100635 (*N*-[2-(4-[2-methoxyphenyl]-1-piperazinyl)ethyl]-*N*-2-pyridinylcyclohexanecarboxamide) maleate and urethane were obtained from Sigma-Aldrich (St. Louis, MO, USA), BRL15572 (3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol) hydrochloride, GR127935 (*N*-[methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide) hydrochloride and isamoltane hemifumarate were from Tocris Cookson (Ballwin, MO, USA), and α-chloralose and DL-p-chlorophenylalanine were from Tokyo Kasei (Tokyo, Japan). Urethane and α-chloralose were dissolved in distilled water. All of the test compounds except BRL15572 and GR127935 were dissolved in 0.9% w/v physiological saline. BRL15572 and GR127935 were dissolved in 1% Tween 80 and distilled water, respectively. Drugs were administered intravenously (i.v.) at 1 ml/kg. Clorgyline or each antagonist was administered 15 min before injecting DL-p-chloroamphetamine hydrochloride. The dose of each drug used in these experiments represents the weight of the salt. Control rats received vehicle alone at 1 ml/kg. The drugs were administered at least 2 h after spinalization.

2.4. Statistical analysis

The amplitudes of the monosynaptic and polysynaptic reflexes recorded after drug administration are expressed as a percentage of the corresponding predrug (time 0) amplitude. 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 between control and treated groups (Wallenstein et al., 1980). Differences at $P<0.05$ (two tailed) were considered to be significant.

3. Results

3.1. DL-p-Chloroamphetamine inhibits the monosynaptic reflex in spinalized rats

DL-p-Chloroamphetamine hydrochloride (1–3 mg/kg, i.v.) reduced the amplitude of the monosynaptic reflex potential in a dose-dependent manner and increased that of the polysynaptic reflex potential (Fig. 1A). At a dose of 3 mg/kg, DL-p-chloroamphetamine significantly reduced the amplitude of the monosynaptic reflex to 18.6±6.6% of the pretreatment value, and this effect persisted for over 60 min (Fig. 1A, left, $n=4$). The amplitude of the polysynaptic reflex was enhanced to about 450% of the pretreatment level. This effect was not dose-dependent (Fig. 1A, right). Blockade of monoamine oxidase-A by clorgyline hydrochloride (1 mg/kg, i.v.) did not affect the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex or enhancement of the polysynaptic reflex (Fig. 1B).

3.2. Depletion of 5-HT abolished the monosynaptic reflex depression induced by DL-p-chloroamphetamine

In 5-HT-depleted rats, DL-p-chloroamphetamine hydrochloride (3 mg/kg, i.v.) significantly enhanced the amplitude of the monosynaptic reflex potential to 143.8±12.3% of the pretreatment value at 10 min after administration (Fig. 2A, left, $n=4$). On the other hand, that of the polysynaptic reflex potential was enhanced by DL-p-chloroamphetamine in both 5-HT-depleted and vehicle-treated rats (Fig. 2A, right). Thus, these results indicate that the 5-HT released in response to DL-p-chloroamphetamine administration inhibits the monosynaptic reflex, and mechanisms other than the 5-HT system are involved in the enhancement of the polysynaptic reflex. Pretreatment with prazosin hydrochloride (1 mg/kg, i.v.), a selective α_1-adrenoceptor antagonist, partly diminished the enhancement of the monosynaptic and polysynaptic reflexes induced by DL-p-chloroamphetamine in 5-HT-depleted rats (Fig. 2B). In the following experiments, we determined which receptor subtypes mediated the monosynaptic reflex depression induced by DL-p-chloroamphetamine.

3.3. 5-HT$_{1A}$ receptors are not involved in the depression of spinal reflexes by DL-p-chloroamphetamine

The selective 5-HT$_{1A}$ receptor antagonist WAY-100635 maleate (0.1 and 0.3 mg/kg, i.v.) had little effect on the DL-

p-chloroamphetamine-induced depression of monosynaptic reflex (Fig. 3A). Inasmuch as these doses of WAY-100635 maleate are thought to sufficiently block 5-HT_{1A} receptors (Forster et al., 1995), this finding suggests that it is unlikely that 5-HT_{1A} receptors are involved in the depression of the monosynaptic reflex induced by DL-p-chloroamphetamine.

3.4. 5-HT_{1D} receptors mediate the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex

Pretreatment with GR127935 hydrochloride (1 mg/kg, i.v.), a selective 5-$HT_{1B/1D}$ receptor antagonist, significantly reduced the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex (Fig. 3B). In the presence of GR127935, DL-p-chloroamphetamine hydrochloride (3 mg/kg, i.v.) depressed the amplitude of the monosynaptic reflex to about 50% of the pretreatment level. Although GR127935 alone markedly depressed the monosynaptic reflex, the depression induced by subsequent administration of DL-p-chloroamphetamine was less than in the control group. The effects of selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists were examined to determine which 5-HT receptor subtypes are involved in the DL-p-chloroamphetamine-induced depression. Isamoltane hemifumarate (1 and 3 mg/kg, i.v.), which is relatively selective for 5-HT_{1B} receptors, did not alter the monosynaptic reflex depression induced by DL-p-chloroamphetamine (Fig. 4A). On the other hand, BRL15572 hydrochloride (10 mg/kg, i.v.), a selective 5-HT_{1D} receptor antagonist, significantly antagonized (Fig. 4B).

4. Discussion

Monosynaptic reflex transmission is the synaptic excitation of motoneurons induced by the stimulation of group Ia afferent fibers that originate from the muscle spindles. This phenomenon is modulated by 5-HT receptors that are located in the presynaptic terminals and motoneuronal somata and dendrites (Marlier et al., 1991; Ridet et al., 1994). In the study presented here, an in vivo spinal cord preparation was

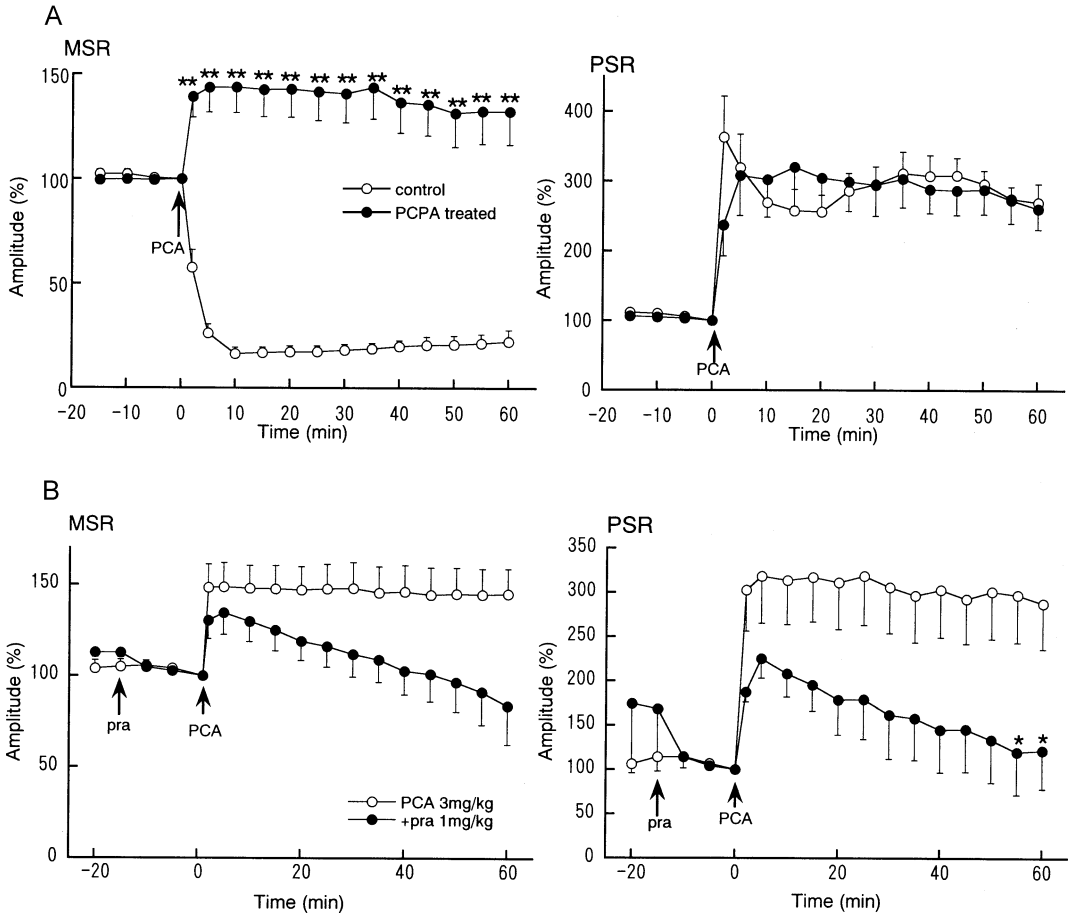

Fig. 2. Effects of DL-p-chloroamphetamine hydrochloride (PCA, 3 mg/kg, i.v., [A]) on monosynaptic reflex (MSR) and polysynaptic reflex (PSR) potentials in DL-p-chlorophenylalanine-treated rats and the effects of pretreatment with prazosin hydrochloride (pra, 1 mg/kg, i.v., [B]) on the response to DL-p-chloroamphetamine. Prazosin hydrochloride was administered i.v. 15 min before the administration of DL-p-chloroamphetamine. Each point represents the mean±S.E.M. of four rats per group. Ordinates—mean reflex amplitude expressed as a percentage of the corresponding value at time 0. Abscissae—time in minutes after the injection of DL-p-chloroamphetamine. 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.

used to examine monosynaptic reflex transmission, because the synaptic connections between dorsal roots and motoneurons are intact in this preparation, but not in slice and cell preparations. Inasmuch as we used spinalized rats, DL-p-chloroamphetamine affected the spinal reflexes via the terminals of descending serotonergic fibers at the spinal cord but not the supraspinal serotonergic terminals.

Consistent with results obtained from the isolated neonatal rat spinal cord (Ohno and Warnick, 1989; Wallis et al., 1993), DL-p-chloroamphetamine significantly depressed the monosynaptic reflex (Fig. 1A, left). In a previous study, we showed that, in the presence of clorgyline, L-5-hydroxytryptophan markedly reduced the amplitude of monosynaptic reflex and increased the amplitude of polysynaptic reflex in spinalized rats, although the same dose of L-5-hydroxytryptophan alone had no effect on the amplitude of either reflex (Nagano et al., 1987). It is suggested that inhibition of 5-HT inactivation is required when L-5-hydroxytryptophan exerts the effects. Although we expected that clorgyline could enhance the DL-p-chloroamphetamine-induced effects on the monosynaptic and polysynaptic reflex potentials, in fact, it had no effect (Fig. 1B). It has been suggested that DL-p-chloroamphetamine inhibits monoamine oxidase-A activity (Mantle et al., 1976; Benmansour and Brunswick, 1994; Sprague et al., 1996). It is, therefore, possible that when administered alone, DL-p-chloroamphetamine had already increased the level of monoamines after blockade of their degradation.

In 5-HT-depleted rats, the depression of the monosynaptic reflex potential induced by DL-p-chloroamphetamine was abolished, and the amplitude of the monosynaptic reflex rather increased to about 140% of the pretreatment

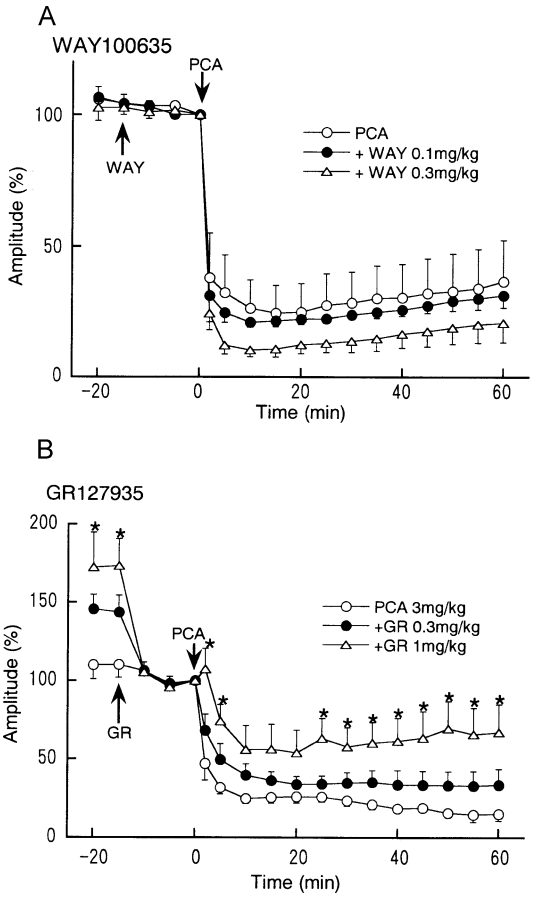

Fig. 3. Effects of WAY-100635 maleate (WAY, 0.1 and 0.3 mg/kg, i.v., [A]) and GR127935 hydrochloride (GR, 0.3 and 1 mg/kg, i.v., [B]) on DL-p-chloroamphetamine (PCA)-induced changes in the monosynaptic reflex potentials in spinalized rats. Each antagonist was administered i.v. 15 min before the administration of DL-p-chloroamphetamine. Each point represents the mean±S.E.M. of five rats per group. Ordinates—mean reflex amplitude expressed as a percentage of the corresponding value at time 0. Abscissae—time in minutes after the injection of DL-p-chloroamphetamine. 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 $**P<0.01$.

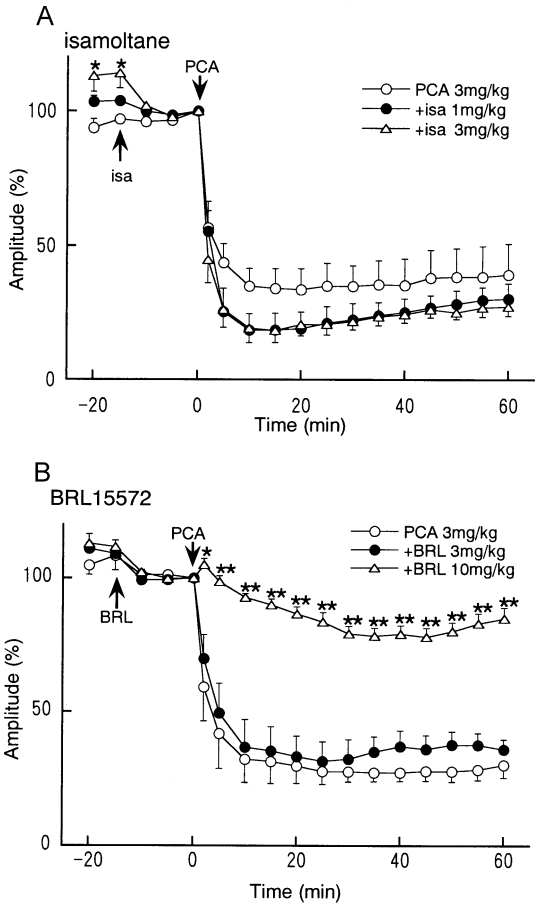

Fig. 4. Effects of isamoltane hemifumarate (isa, 1 and 3 mg/kg, i.v., [A]) and BRL15572 hydrochloride (BRL, 3 and 10 mg/kg, i.v., [B]) on DL-p-chloroamphetamine (PCA)-induced changes in the monosynaptic reflex potentials in spinalized rats. Each antagonist was administered i.v. 15 min before the administration of DL-p-chloroamphetamine. Each point represents the mean±S.E.M. of four rats per group. Ordinates—mean reflex amplitude expressed as a percentage of the corresponding value at time 0. Abscissae—time in minutes after the injection of DL-p-chloroamphetamine. 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 $**P<0.01$.

level (Fig. 2A, left). Thus, it appears that an increase in extracellular levels of 5-HT in the synaptic cleft leads to a depression of the spinal reflex, which is in agreement with our previous finding that 5-HT generated from L-5-hydroxytryptophan depresses the amplitude of the monosynaptic reflex (Nagano et al., 1987; Honda et al., 2003). However, the increase in polysynaptic reflex potential induced by DL-p-chloroamphetamine was not affected by depletion of 5-HT (Fig. 2A, right), suggesting that the endogenous serotonergic system does not play an important role in the facilitation of the polysynaptic reflex by DL-p-chloroamphetamine. Several studies have revealed that DL-p-chloroamphetamine also acts on the noradrenaline and dopamine transporters (Sugita et al., 1994; Wall et al., 1995; Crespi et al., 1997; Sugimoto et al., 2001). This may be reflected by our present observation that the α_1-adrenoceptor antagonist prazosin substantially reduced the DL-p-chloroamphetamine-induced increase in the spinal reflex amplitude in 5-HT-depleted rats (Fig. 2B). The descending noradrenergic systems are facilitatory to the motor system, and α_1-adrenoceptor antagonistic action at the spinal cord inhibits spinal motor activity via spinal α_1-adrenoceptors (Ono and Fukuda, 1995). Thus, DL-p-chloroamphetamine may employ other monoamine systems, including noradrenaline to enhance the amplitude of the spinal reflex.

In our previous study, we found that when levels of 5-HT are increased following systemic administration of L-5-hydroxytryptophan, the amplitude of the monosynaptic reflex is reduced, an effect that occurs via 5-HT_{1B} receptors (Honda et al., 2003). This is consistent with the finding that 5-HT_{1A} receptors are not involved in the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex (Fig. 3A). By contrast, the results obtained in the present study indicate clearly that endogenously released 5-HT stimulates 5-HT_{1D} but not 5-HT_{1B} receptors to depress the monosynaptic reflex (Fig. 4). We first used the mixed 5-HT_{1B} and 5-HT_{1D} receptor antagonist GR127935 (Skingle et al., 1996). GR127935 significantly attenuated the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex potential at a dose of 1 mg/kg (Fig. 3B). When administered alone, GR127935 markedly depressed the monosynaptic reflex, an effect that could be attributable to the inhibition of glutamate release (Maura et al., 1998) or an agonistic action at 5-HT_{1D} receptors (Pauwels et al., 1996). To examine further which subtypes are involved in the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex, isamoltane and BRL15572 were used as selective 5-HT_{1B} and 5-HT_{1D} receptor antagonists, respectively. Unlike our previous study, which demonstrated that isamoltane antagonizes the inhibitory effect of exogenous 5-HT on the monosynaptic reflex (Honda et al., 2003), in the present study, isamoltane did not affect the depression of monosynaptic reflex induced by DL-p-chloroamphetamine (Fig. 4A). The selective 5-HT_{1D} receptor antagonist BRL15572 completely antagonized the DL-p-chloroamphetamine-induced depression of the monosynaptic reflex (Fig. 4B). These results indicate that it is 5-HT_{1D} receptors that mediate the depression of monosynaptic reflex induced by endogenously released 5-HT. It has been proposed that distinct 5-HT receptor subtypes could be activated in response to both endogenous and exogenous 5-HT (Crick and Wallis, 1991; Yomono et al., 1992; Manuel et al., 1995). We therefore suggest that extrasynaptic 5-HT_{1B} receptors and subsynaptic 5-HT_{1D} receptors are involved in the depression of the monosynaptic reflex induced by exogenously applied and endogenously released 5-HT, respectively, in the spinal cord of rats. Mlinar and Corradetti (2003) demonstrated that endogenous 5-HT released by 3,4-methylenedioxy-methamphetamine, a 5-HT releaser, reduced excitatory synaptic transmission in rat hippocampal CA1 pyramidal cells by preferential activation of 5-HT_{1B} receptors. On the other hand, a study with synaptosomal preparations from fresh human neocortical samples has indicated that terminal 5-HT_{1D} heteroreceptors regulate glutamate release, and that presynaptic 5-HT_{1B} autoreceptors mediate the feedback inhibition of 5-HT release (Marcoli et al., 1999). Our present results, however, do not provide any evidence to allow us to establish whether endogenously released 5-HT acts on 5-HT_{1B} and 5-HT_{1D} receptors located postsynaptically on motoneurons or presynaptically on the terminals of primary afferent fibers; this remains to be determined. To clarify further the physiological function of 5-HT_{1B} and 5-HT_{1D} receptors, in vivo experiments investigating the effects of endogenous 5-HT released by electrical stimulation of descending serotonergic systems on the spinal reflexes are required.

In conclusion, endogenous 5-HT released by DL-p-chloroamphetamine depresses the amplitude of the monosynaptic reflex in spinalized rats, and this effect is mediated by 5-HT_{1D} receptors in the spinal cord.

Acknowledgements

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