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Short communication

No involvement of 5-HT_7 or 5-HT_{1D} receptors in the (*R*)-8-OH-DPAT-induced depression of the monosynaptic reflex in spinalized rats

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Abstract

(*R*)-8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) depressed the monosynaptic reflex. This effect was not antagonized by 5-HT_{1A} receptor antagonists. We examined whether 5-HT_{1D} and 5-HT_7 receptors are involved in (*R*)-8-OH-DPAT-induced inhibition of the monosynaptic reflex in spinalized rats. Pretreatment with methiothepin and mesulergine, but not clozapine, inhibited (*R*)-8-OH-DPAT-induced monosynaptic reflex depression. Pretreatment with 2*a*-(4-phenyl-1,2,3,6-tetrahydropyridal)butyl)-2*a*,3,4,5-tetrahydrobenzo[*c*,*d*]indol-2(1*H*)-one (DR4004) and (*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrolidine (SB-269970), new selective 5-HT_7 receptors antagonists, and *N*-[methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide (GR127935), a selective 5-HT_{1D} receptor antagonist, had no effect on (*R*)-8-OH-DPAT-induced depression. These results suggested that 5-HT_7 and 5-HT_{1D} receptors are not involved in (*R*)-8-OH-DPAT-induced monosynaptic reflex depression.

Keywords: Spinal reflex; 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin); 5-HT_7 receptor; 5-HT_{1D} receptor

1. Introduction

8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) has been used as a selective ligand to study the function of the 5-HT_{1A} receptor. In a previous study, we found that (*R*)- and (*S*)-8-OH-DPAT have different pharmacological effects on the monosynaptic spinal reflex in rats (Honda and Ono, 1999). In intact rats, the supraspinal 5-HT_{1A} receptor and the descending serotonergic system are involved in the stimulatory action of (*R*)-8-OH-DPAT on the monosynaptic reflex, whereas the descending noradrenergic system and α_1-adrenoceptor are involved in the stimulatory action of (*S*)-8-OH-DPAT on this reflex. A higher dose of (*R*)-8-OH-DPAT (30 μg/kg, i.v.) inhibited the monosynaptic reflex in intact and spinalized rats, suggesting that, at this dose, (*R*)-8-OH-DPAT inhibits the monosynaptic reflex at the spinal cord level. However, the depressant effects of (*R*)-8-OH-DPAT on the monosynaptic reflex were not antagonized by the 5-HT_{1A} receptor antagonists 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]-piperazine (NAN-190) and spiroxatrine. Racemic 8-OH-DPAT inhibited the monosynaptic reflex recorded in vitro from the neonatal rat spinal cord and this inhibitory effect was blocked by spiperone, a selective 5-HT_{1A} receptor antagonist (Crick et al., 1994). Manuel et al. (1995) showed that 8-OH-DPAT depressed the monosynaptic reflex of the neonatal rat spinal cord via ketanserin-sensitive receptors, but this depressant effect was not affected by spiroxatrine. 8-OH-DPAT increased spinal reflexes evoked by moderate-to-high stimulus intensities and depressed responses to low-intensity stimuli in the decerebrated and spinalized rabbit. Although the inhibitory effects of 8-OH-DPAT were abolished by the selective 5-HT_{1A} receptor antagonist *N*-*tert*-butyl-3-(4-(2-methoxyphenyl)piperazin-l-yl)-2-phenylpropionamide dihydrochloride ((*S*)-WAY-100135), the facilitatory effects could not be blocked by a 5-HT_{1A} receptor antagonist (Clarke et al., 1997). From these observations, it is not clear which receptor subtypes mediate the 8-OH-DPAT-induced depressant effect on the monosynaptic reflex. 8-OH-DPAT has been suggested to be a ligand of 5-HT_7 (Lovenberg et al., 1993; Eglen et al., 1997) and 5-HT_{1D} (Pauwels and Colpaert, 1996) receptors. Therefore, it is

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possible that these receptors are involved in the inhibitory effect of (*R*)-8-OH-DPAT on the monosynaptic reflex. In the present study, we examined whether the 5-HT_{1D} or the 5-HT_7 receptor is involved in the inhibitory effect of (*R*)-8-OH-DPAT on the monosynaptic reflex, using several drugs displaying a high affinity for either the 5-HT_{1D} or the 5-HT_7 receptor.

2. Materials and methods

2.1. Measurement of monosynaptic reflexes

All 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 rats (8–9 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 for respiration and the femoral vein for drug administration. 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 lidocaine anesthesia (4%, 50 μl). A dorsal laminectomy was performed in the lumbo-sacral 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. Monosynaptic 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. Drugs

(*R*)-8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide, methiothepin mesylate, clozapine and mesulergine hydrochloride were obtained from Research Biochemicals International (Natick, MA, USA). (*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrolidine (SB-269970) hydrochloride was a gift from SmithKline Beecham Pharmaceuticals (Harlow, UK). 2*a*-(4-Phenyl-1,2,3,6-tetrahydropyridal)butyl)-2a,3,4,5-tetrahydrobenzo[*c*,*d*]indol-2(1*H*)-one (DR4004) was a gift from the Pharmaceutical Research Center of Meiji Seika Kaisha, Ltd. (Yokohama, Japan). *N*-[Methoxy-3-(4-methyl-l-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide (GR127935) hydrochloride monohydrate was a gift from Glaxo Wellcome Research and Development (Stevenage, UK). Urethane and α-chloralose were obtained from Aldrich Chemical (Milwaukee, WI, USA) and Tokyo Kasei (Tokyo, Japan), respectively. Both were dissolved in distilled water. All the test compounds, except DR4004 and GR127935 hydrochloride monohydrate, which were dissolved in 1% Tween 80 and distilled water, respectively, were dissolved in 0.9% w/v physiological saline and administered i.v. at 1 ml/kg. Each antagonist was administered 10 min before injection of (*R*)-8-OH-DPAT, except DR4004, which was administered 20 min before. The dose of each drug used in these experiments represents the weight of the salt. Control rats received 1 ml/kg vehicle. Drugs were administered at least 2 h after spinalization.

2.3. Statistical analysis

The monosynaptic reflex amplitudes after drug administration were calculated as percentages of the corresponding pre-drug (time 0) amplitudes. All data are expressed as means ± S.E.M. The 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.

3. Results

3.1. Effects of several 5-HT_7 receptor ligands on (R)-8-OH-DPAT-induced monosynaptic reflex depression

We have reported that (*R*)-8-OH-DPAT depressed the monosynaptic reflex of spinalized rats in a dose-dependent manner (Honda and Ono, 1999). In the present study, a lower dose of (*R*)-8-OH-DPAT (30 μg/kg) was used so that the antagonistic effects of serotonergic antagonists against (*R*)-8-OH-DPAT would be clearly visible.

Although methiothepin mesylate (0.1 mg/kg, i.v.) had no effect on (*R*)-8-OH-DPAT (30 μg/kg, i.v.)-induced monosynaptic reflex depression, a high dose of methiothepin mesylate (0.5 mg/kg) significantly inhibited this depression (Fig. 1A). Mesulergine hydrochloride (0.5 and 1 mg/kg, i.v.) significantly reduced (R)-8-OH-DPAT-induced monosynaptic reflex depression (Fig. 1B). The higher dose of mesulergine hydrochloride (1 mg/kg) alone significantly reduced the amplitude of the monosynaptic reflex. Although clozapine (0.5 and 1 mg/kg, i.v.) at the higher dose reduced the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT, complete antagonism was not observed (Fig. 1C). SB-269970 hydrochloride (1, 5 and 10 mg/kg, i.v.), a selective 5-HT_7 receptor antagonist, had little effect on the monosynaptic reflex depression produced by (R)-8-OH-DPAT (Fig. 1D).

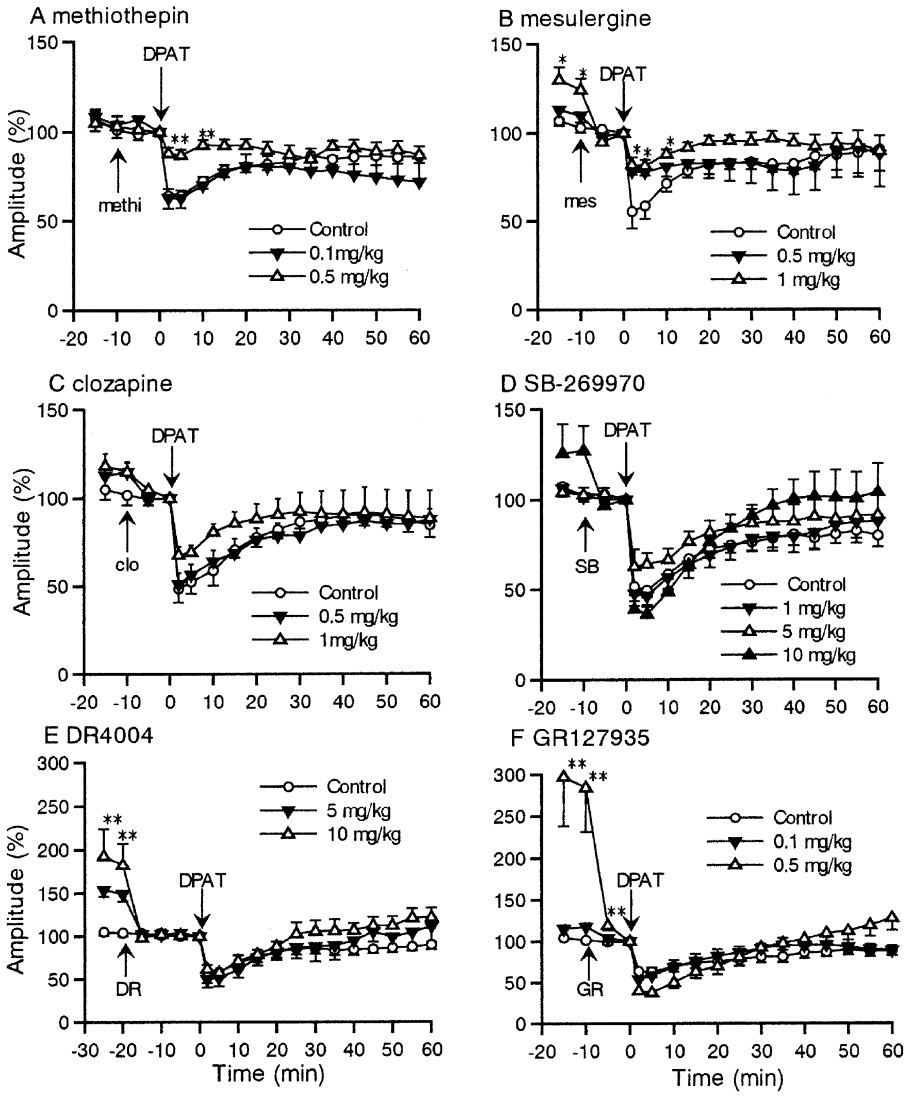

Fig. 1. Influence of pretreatment with methiothepin mesylate (0.1 and 0.5 mg/kg, i.v. (A)), mesulergine hydrochloride (0.5 and 1 mg/kg, i.v. (B)), clozapine (0.5 and 1 mg/kg, i.v. (C)), SB-269970 hydrochloride (1, 5 and 10 mg/kg, i.v. (D)), DR4004 (5 and 10 mg/kg, i.v. (E)) and GR127935 hydrochloride monohydrate (0.1 and 0.5 mg/kg, i.v. (F)) on the effect of (*R*)-8-OH-DPAT (30 μg/kg, i.v.) on the monosynaptic reflex in spinalized rats. Each point represents the mean ± S.E.M. for five 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 (*R*)-8-OH-DPAT. The significance of the differences between the control and test values was determined with the two-tailed Bonferroni-type multiple *t*-test following ANOVA: $^{*}P < 0.05$ and $^{**}P < 0.01$ (two comparisons in three groups (A, B, C, E and F), three comparisons in four groups (D)).

Similarly, DR4004 (5 and 10 mg/kg, i.v.), a selective 5-HT_7 receptor antagonist, had no effect on the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT (Fig. 1E). DR4004 (10 mg/kg, i.v.) significantly inhibited the monosynaptic reflex by itself.

3.2. Effect of 5-HT_{1D} receptor antagonist on (R)-8-OH-DPAT-induced monosynaptic reflex depression

GR127935 hydrochloride monohydrate (0.1 mg/kg, i.v.), a selective 5-HT_{1D} receptor antagonist, had no effect on the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT (Fig. 1F). The depressant effect of (*R*)-8-OH-DPAT was not reduced by a high dose of GR127935 hydrochloride monohydrate (0.5 mg/kg). At this dosage the compound strongly inhibited the monosynaptic reflex by itself.

4. Discussion

In this study, there was a possibility that changes in the amplitude of the monosynaptic reflex could be due to changes in blood pressure caused by the i.v. injections of 8-OH-DPAT. In a previous study (Honda and Ono, 1999), (*R*)-8-OH-DPAT did not alter the carotid arterial blood

pressure significantly within 10 min of its administration to spinalized rats. Furthermore, we showed that changes in reflex amplitudes were not affected by large changes in blood pressure (Ono et al., 1991, 1993). These results suggest that the effect of (*R*)-8-OH-DPAT on the spinal reflex was not due to changes in blood pressure.

In a previous study (Honda and Ono, 1999), we found that the depressant effects of (*R*)-8-OH-DPAT on the monosynaptic reflex in spinalized rats were not antagonized by the 5-HT_{1A} receptor antagonists NAN-190 and spiroxatrine. 8-OH-DPAT has been suggested to be a ligand at 5-HT_{1D} (Pauwels and Colpaert, 1996) or 5-HT_7 (Lovenberg et al., 1993, Eglen et al., 1997) receptors. Therefore, we examined whether either of these receptors is involved in the depressant effect of (*R*)-8-OH-DPAT on the monosynaptic reflex. We found that the monosynaptic reflex depression by (*R*)-8-OH-DPAT was reduced by both methiothepin and mesulergine, but less so by clozapine (Fig. 1A, B and C). The rank order of potency in this study was methiothepin = mesulergine > clozapine. These drugs have high affinity for the cloned 5-HT_7 receptor, and the reported rank order of affinity in vitro is methiothepin > mesulergine > clozapine (Hoyer et al., 1994). However, these drugs have affinity for other 5-HT receptor subtypes, and their selectivity for 5-HT_7 receptors is low (Hoyer et al., 1994).

For this reason, the effects of SB-269970 and DR4004, which are new selective 5-HT_7 receptor antagonists, were studied on the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT. SB-258719 ((*R*)-3, *N*-dimethyl-*N*-[1-methyl-3-(4-methylpiperiden-1-yl)propyl]benzenesulfonamide) has high affinity for the human cloned 5-HT_7 receptor and antagonizes 5-CT-stimulated adenylyl cyclase activity in membranes from human embryonic kidney (HEK293) cells expressing recombinant human cloned 5-HT_7 receptor (Thomas et al., 1998). SB-269970 displays a similar selectivity for the 5-HT_7 receptor to that of SB-258719, but has a higher affinity. DR4004 has a high affinity and a high selectivity for the 5-HT_7 receptor and inhibits the 5-HT-induced stimulation of cAMP accumulation in a mammalian cell line (COS-7 cells) expressing the 5-HT_7 receptor (Kikuchi et al., 1999). Pretreatment with SB-269970 and DR4004 had no effect on the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT (Fig. 1D and E). Therefore, these results suggested that the 5-HT_7 receptor is not involved in the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT.

(*R*)-8-OH-DPAT has affinity for the 5-HT_{1D} receptor (Hoyer et al., 1994) Pretreatment with GR127935, a selective 5-HT_{1D} receptor antagonist (Skingle et al., 1996; DeVries et al., 1997), had little effect on the monosynaptic reflex depression produced by (*R*)-8-OH-DPAT (Fig. 1F). This result was consistent with the observation that (*R*)-8-OH-DPAT depresses the monosynaptic reflex in the neonatal isolated rat spinal cord via receptors that are not blocked by GR127935 (Manuel et al., 1995). Thus, it is suggested that 5-HT_{1D} receptors are not involved in the inhibitory effect of (*R*)-8-OH-DPAT on the monosynaptic reflex.

In the present study, DR4004 and GR127935 significantly inhibited the amplitude of the monosynaptic reflex by themselves, suggesting that these antagonists penetrate the blood–brain barrier and act on the spinal cord. These inhibitory effects suggest that 5-HT_7 and 5-HT_{1D} receptors may be involved in the excitability of motoneurons or in the release of excitatory amino acids from the primary afferent terminal.

The possibility that the depressant effect of (*R*)-8-OH-DPAT on the monosynaptic reflex is due to an effect on the 5-HT_2 receptor is inconsistent with the following evidences. In our previous studies, 5-HT_2 receptors on the monosynaptic reflex pathway were considered to mediate enhancement of the monosynaptic reflex (Yamazaki et al., 1992a,b). Ketanserin, a selective 5-HT_2 receptor antagonist, did not antagonize the depressant effect of (*R*)-8-OH-DPAT on the monosynaptic reflex (Honda and Ono, 1999), although this is reported to occur in the isolated neonatal rat spinal cord (Manuel et al., 1995).

From our present and previous data (Honda and Ono, 1999), it is suggested that 5-HT_{1A}, 5-HT_{1D}, 5-HT_2 or 5-HT_7 receptors are not involved in the depressant effect of (*R*)-8-OH-DPAT on the monosynaptic reflex. It is possible that (*R*)-8-OH-DPAT inhibits the monosynaptic reflex via a mechanism other than one involving 5-HT receptors. (*R*)-8-OH-DPAT should not be regarded as a selective 5-HT receptor agonist.

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