Structure-Activity Relationships of Phenylethylamine Analogs in Their Serotonergic Depressant Effects on the Spinal Monosynaptic Reflex in Rats

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The effects of 2-phenylethylamine (PEA) and related compounds on the spinal monosynaptic reflex (MSR) were examined using C1-spinalized rats. At low doses, PEA, *S*(+)-amphetamine, *S*(+)-methamphetamine and phentermine increased the amplitude of the MSR, whereas high doses of these drugs reduced it. *p*-Substituted PEA analogs (*p*-Cl-PEA, *p*-methoxy-PEA and (±)-*p*-Cl-amphetamine) only reduced the MSR. Low doses of PEA-related rigid compounds, *R*(+)-2-aminotetralin, (±)-*N*-methyl-2-aminotetralin and (±)-*N,N*-dimethyl-2-aminotetralin only reduced the MSR. *S*(-)-2-Aminotetralin did not affect the MSR. Depressions of MSR produced by PEA, *S*(+)-methamphetamine and *R*(+)-2-aminotetralin were antagonized by ketanserin and haloperidol which have 5-hydroxytryptamine (5-HT) antagonistic activity, and the MSR depression caused by *S*(+)-methamphetamine but not PEA and *R*(+)-2-aminotetralin was abolished by intracisternal 5,6-dihydroxytryptamine treatment or chronic spinal transection. These results suggest that PEA-related compounds cause MSR depression by direct and indirect 5-HT agonistic mechanisms, and support the proposal that the PEA moiety which exists in *R*(+)-2-aminotetralin is important for the direct 5-HT agonistic activity of some hallucinogens.

Keywords — phenylethylamine; amphetamine; 2-aminotetralin; spinal reflex; structure-activity relationship

Introduction

2-Phenylethylamine (PEA) is an endogenous amine that has been reported to be present in the mammalian central nervous system, especially the striatum and the spinal cord.¹⁾ PEA structurally resembles stimulants such as amphetamines, and possesses similar pharmacological properties, which include an increase in spontaneous motor activity,²⁾ the appearance of stereotypy³⁾ and decrease in food and water intake.⁴⁾ These actions may be due to the release of monoamines. On the other hand, PEA has been reported to induce direct 5-hydroxytryptamine (5-HT) agonistic activities,⁵⁾ and *p*-substituted PEAs possess strong indirect and direct serotonergic activities.^{6,7)} Clear comparison of structure-activity relationships using behavioral experiments is relatively difficult, since effects of drugs on behavior are mediated *via* complex neural networks. The spinal

monosynaptic reflex (MSR) of spinalized rats is a clear and simple tool for evaluating the activity of neurotransmitters or neuromodulators on the spinal cord, and we have found that α_1 -adrenoceptors and 5-HT receptors mediate the increase and decrease of the MSR, respectively.^{8,9)} In the present study, the conformation of the PEA moiety that is required for the induction of MSR depression was studied using spinalized rats, and we showed that PEA and its related compounds reduced the MSR *via* a serotonergic mechanism.

Materials and Methods

Male Wistar rats (300—400 g) were anesthetized with urethane (1 g/kg, i.p.) and α -chloralose (25 mg/kg, i.p.), and then artificially ventilated. The vagus nerves were severed bilaterally at the cervical region, and the spinal cord was transected at C1. Laminectomy was

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performed in the lumbo-sacral region. The ventral and dorsal roots of segments L4 and L5 were isolated. A skin pouch was formed at the site of the dissection so that the exposed tissues could be covered with liquid paraffin maintained at $36 \pm 0.5^\circ\text{C}$. Rectal temperature was maintained at $36 \pm 0.5^\circ\text{C}$.

The dorsal and ventral roots of segment L5 were placed on bipolar silver-silver chloride wire electrodes for stimulation (0.2 Hz, 0.05 ms, supramaximal) and recording, respectively. The spinal reflexes were amplified (Nihon Kohden AVB-10), displayed on an oscilloscope (Nihon Kohden VC-10) and averaged 8 times by an averaging computer (Nihon Kohden DAT-1100), the analog output of which was recorded by a recorder (Toa Electronics FBR-252A). The amplitude of the MSR was then measured.

In some experiments, rats were pretreated with ketanserin, haloperidol and 5,6-dihydroxytryptamine. Ketanserin and haloperidol were administered 10 min before the drug injection. 5,6-Dihydroxytryptamine creatinine sulfate ($75\text{ }\mu\text{g}$ as free base) was dissolved in 0.9% saline containing 0.2 mg/ml ascorbic acid and injected intracisternally 14 d beforehand in a volume of $20\text{ }\mu\text{l}$, using a Hamilton syringe. In some rats, chronic spinal transection was performed. Com-

plete spinal transection at the mid-thoracic level (T5, 6), was performed under pentobarbital Na anesthesia (30 mg/kg, i.p.). After the skin had been sutured, all animals received penicillin-G potassium (100000 I.U.) and kanamycin sulfate (10 mg) intramuscularly. Quinine HCl, which has a bitter taste, was dissolved in vaselinum album and then daubed over part of the hind limbs and the tail to prevent the rats from biting themselves. For the next 14 days, the urinary bladder was emptied manually and the skin was cleaned with 70% alcohol solution every day.

The drugs tested were 2-phenylethylamine (Tokyo Kasei), *p*-chlorophenylethylamine (Nakarai), *p*-methoxyphenylethylamine (Sigma), *S*(+)-methamphetamine HCl (Dainippon), *S*(+)-amphetamine sulfate (Dainippon), *R*(-)-amphetamine sulfate (Dainippon), phentermine (Tokyo Kasei), ($\pm$)-*p*-chloroamphetamine HCl (Sigma), benzylamine (Nakarai), 3-phenylpropylamine (Tokyo Kasei), *R*(+)-2-aminotetralin, *S*(-)-2-aminotetralin, ($\pm$)-*N*-methyl-2-aminotetralin and ($\pm$)-*N,N*-dimethyl-2-aminotetralin (Fig. 1). *R*(+)- and *S*(-)-2-aminotetralin were separated from ($\pm$)-2-aminotetralin (Tokyo Kasei) and their optical purities were more than 95%. ($\pm$)-*N*-Methyl-2-aminotetralin and ($\pm$)-*N,N*-dimethyl-2-aminotetralin were synthesized.

Fig. 1. Structures of PEA and PEA-Related Compounds

These drugs were dissolved in physiological saline or dissolved in equimolar HCl solution and injected into the femoral vein *via* a cannula.

Haloperidol (Serenace Injection, Dainippon),

ketanserin tartrate and 5,6-dihydroxytryptamine creatinine sulfate (Sigma) were used for the pretreatment.

Fig. 2. Effects of PEA and PEA-Related Compounds on the MSR in C1-Spinalized Rats

Ordinates: mean of MSR amplitudes calculated as a percentage of the value just prior to drug injection, with the S. E. M. indicated ($n = 4$). Abscissae: time in min after the injection of the drugs. The doses of the drugs are expressed as mol/kg, i.v. A, 2-Phenylethylamine; B, S(+)-amphetamine; C, S(+)-methamphetamine; D, p-chlorophenylethylamine; E, R(+)-2-aminotetralin; F, S(-)-2-aminotetralin.

Results

Effects of PEA, *p*-Chlorophenylethylamine and *p*-Methoxyphenylethylamine on MSR

PEA increased the MSR at low doses ($3 \times 10^{-6} - 1 \times 10^{-5}$ mol/kg, i.v.) and decreased the MSR at high doses ($3 \times 10^{-5} - 3 \times 10^{-4}$ mol/kg, i.v., Fig. 2A). *p*-Chlorophenylethylamine and *p*-methoxyphenylethylamine reduced the MSR at doses of $3 \times 10^{-6} - 3 \times 10^{-5}$ mol/kg, i.v., although lower doses of both drugs ($3 \times 10^{-7} - 1 \times 10^{-6}$ mol/kg, i.v.) did not affect the MSR (Figs. 2D and 3A).

Effects of Amphetamines on MSR

Low doses of *S*(+)-amphetamine (10^{-5} mol/kg, i.v.) increased the MSR, and high doses of *S*(+)-amphetamine ($3 \times 10^{-5} - 1 \times 10^{-4}$ mol/kg, i.v.) decreased the MSR (Fig. 2B). *S*(+)-Methamphetamine (3×10^{-6} mol/kg, i.v.) increased the MSR and *S*(+)-methamphetamine ($1 \times 10^{-5} - 3 \times 10^{-5}$ mol/kg, i.v.) decreased the MSR (Fig. 2C). *R*(-)-Amphetamine ($10^{-5} - 10^{-4}$ mol/kg, i.v.) increased the amplitude of the MSR (Fig. 3A). Phentermine ($1 \times 10^{-5} - 3 \times 10^{-5}$ mol/kg, i.v.) increased the MSR and at high dose (1×10^{-4} mol/kg, i.v.) decreased the MSR (Fig. 3A). ($\pm$)-*p*-Chloroamphetamine ($3 \times 10^{-6} - 3 \times 10^{-5}$ mol/kg, i.v.) decreased the MSR (Fig. 3B). The durations of MSR inhibition produced by amphetamines were longer than

those of the previously mentioned PEA analogs (Fig. 2A, B, C, D).

Effects of Benzylamine and 3-Phenylpropylamine on MSR

Benzylamine ($3 \times 10^{-5} - 3 \times 10^{-4}$ mol/kg, i.v.) and 3-phenylpropylamine (1×10^{-6} mol/kg, i.v.) did not affect the MSR, and 3-phenylpropylamine (1×10^{-5} and 1×10^{-4} mol/kg, i.v.) increased the MSR by 50% (Fig. 3B). These drugs did not reduce the amplitude of the MSR.

Effects of 2-Aminotetralins on MSR

R(+)-2-Aminotetralin ($1 \times 10^{-7} - 1 \times 10^{-6}$ mol/kg, i.v.) dose-dependently inhibited the MSR (Fig. 2E). A lower dose of less than 1×10^{-7} mol/kg, i.v. did not affect the MSR. *S*(-)-2-Aminotetralin slightly increased the MSR at the doses of 3×10^{-5} mol/kg, i.v. (Fig. 2F).

($\pm$)-*N*-Methyl-2-aminotetralin and ($\pm$)-*N,N*-dimethyl-2-aminotetralin ($1 \times 10^{-7} - 1 \times 10^{-6}$ mol/kg, i.v.) dose-dependently decreased the MSR (Fig. 3B).

Influences of Drug Treatments on the Depressant Effects of PEA Analogs on the MSR

Depressant effects on the MSR produced by PEA and *R*(+)-2-aminotetralin were reduced by the 5-HT₂ antagonist, ketanserin tartrate (1 mg/kg, i.v., Fig. 4A, C). The MSR decreases caused by PEA and *R*(+)-2-aminotetralin were also abolished by haloperidol (0.5 mg/kg,

Fig. 3. Dose-Response Relationships for the Effects of PEA-Related Compounds on the MSR in C1-Spinalized Rats

Ordinates: mean of maximal change in MSR amplitude within 10 min after injection, with the S. E. M. indicated ($n=4$).

Abscissae: log dose of drugs (mol/kg, i.v.). Cl, chloro; MeO, methoxy; MA, methamphetamine; AT, aminotetralin.

i.v., not shown). In the presence of ketanserin pressant effect of *S* (+)-methamphetamine (Fig. 4B) or haloperidol (not shown), the de- reversed to enhancement.

Fig. 4. Effects of PEA, *S* (+)-Methamphetamine and *R* (+)-2-Aminotetralin in Ketanserin-Treated (A, B, C) and Chronic Spinal-Transected Rats (D, E, F)

Ordinates: mean of MSR amplitudes calculated as a percentage of the value just prior to drug injection, with the S. E. M. indicated ($n=4$). Abscissae: time in min after the injection of PEA and its related drugs. KET: ketanserin tartrate 1 mg/kg, i.v. Open symbols: PEA, *S* (+)-methamphetamine or *R* (+)-2-aminotetralin alone. Closed symbols: ketanserin-treated or chronic spinal-transected.

A, PEA 10^{-4} mol/kg; B, *S* (+)-methamphetamine 10^{-5} mol/kg; C, *R* (+)-2-aminotetralin 10^{-6} mol/kg; D, PEA 10^{-4} mol/kg, chronic spinal; E, *S* (+)-methamphetamine 10^{-5} mol/kg, chronic spinal; F, *R* (+)-2-aminotetralin 10^{-6} mol/kg, chronic spinal.

Effects of Chronic Spinal Transection and 5,6-Dihydroxytryptamine Treatment on the Depressant Effects of PEA, *S* (+)-Methamphetamine and *R* (+)-2-Aminotetralin

The amplitudes of MSR and polysynaptic reflex in chronic spinal rats were about twice as large as those in acute spinal rats.¹⁰⁾ Chronic spinal transection 2 weeks beforehand increased the depressant action of PEA on the MSR (Fig. 4D). The depressant effect of *S* (+)-methamphetamine on the MSR was completely abolished by chronic spinal transection, and *S* (+)-methamphetamine increased the MSR in this preparation (Fig. 4E). The depressant effect of *R* (+)-2-aminotetralin on the MSR was not affected by chronic spinal transection (Fig. 4F). 5,6-Dihydroxytryptamine treatment produced quite similar results to those observed in chronically spinalized rats (not shown).

Discussion

There has been a possibility that changes in the reflex amplitude caused by PEA analogs, amphetamines and 2-aminotetralins are due to the peripheral changes in blood pressure. In the present study, PEA analogs and amphetamines elevated blood pressure, and high doses of these drugs decreased the MSR. 2-Aminotetralins, which did not change the blood pressure, strongly decreased the MSR. In chronic spinal rats, *S* (+)-methamphetamine elevated the blood pressure without decreasing the amplitude of MSR. Also, rats pretreated with intracisternal 6-hydroxydopamine, low doses of PEA and *S* (+)-methamphetamine elevated blood pressure without enhancing the MSR.¹¹⁾ These results suggest that the decrease or increase in MSR amplitude produced by these drugs is not caused by the change in blood pressure.

In the previous study, increasing effects on the MSR of low-dose PEA and *S* (+)-methamphetamine, nomifensine and mazindol were antagonized by a selective α_1 -blocker, prazosin¹²⁾ and abolished by intracisternal 6-hydroxydopamine treatment.¹¹⁾ These results suggested that the increase in the MSR was due to the release of noradrenaline from the descending noradrenergic terminals and that the conformation of

the PEA moiety, in which the nitrogen atom is close to the phenyl ring, can cause the enhancing effects of MSR. Low doses of other PEA-related compounds *S* (+)-amphetamine, *R* (–)-amphetamine and phentermine, also increased the amplitude of the MSR (Fig. 2).

High doses of PEA, *S* (+)-methamphetamine, *S* (+)-amphetamine and phentermine decreased the amplitude of the MSR. *p*-Chlorophenylethylamine, *p*-methoxyphenylethylamine and ($\pm$)-*p*-chloroamphetamine only decreased the amplitude of the MSR. Since benzylamine and 3-phenylpropylamine did not reduce the MSR, the presence of a PEA moiety in the molecule seems to be necessary for MSR reduction. *p*-Substituted analogs of PEA and amphetamine reduced the MSR without increasing the MSR. This may have been due to the strong serotonergic activities of *p*-substituted PEAs, *i.e.* 5-HT uptake inhibition, 5-HT-releasing and 5-HT agonistic actions.^{6,7)} 2-Aminotetralins are rigid analogs of PEA in which the conformation of the PEA moiety is fixed in such a form that the nitrogen is distant from the phenyl ring. 2-Aminotetralin, *N*-methyl-2-aminotetralin and *N,N*-dimethyl-2-aminotetralin reduced the amplitude of the MSR without enhancing its amplitude. *R* (+)-2-Aminotetralin but not *S* (–)-2-aminotetralin was effective on the MSR.

To examine the types of receptors or transmitters involved in the depressant action on the MSR, drug treatments and chronic spinal transection were conducted. In our previous experiments, 5-HT agonists, 5-methoxy-*N,N*-dimethyltryptamine and *d*-lysergic acid diethylamide (*d*-LSD), and a 5-HT precursor, 5-hydroxytryptophan, decreased the amplitude of the MSR.^{9,10,13,14)} The MSR decrease caused by 5-methoxy-*N,N*-dimethyltryptamine was antagonized by ketanserin¹³⁾ and haloperidol (unpublished observation), which have high and moderate affinity for 5-HT₂ receptors, respectively.¹⁵⁾ In the present study, ketanserin and haloperidol antagonized the depressant effects of PEA, *S* (+)-methamphetamine and *R* (+)-2-aminotetralin. Chronic spinal transection, which destroys all the descending fibers, and 5,6-dihydroxytryptamine treatment, which selectively destroys 5-HT fibers, did not reduce

the depressant effects of PEA and *R* (+)-2-aminotetralin. These results suggest that the depression of the MSR by PEA and *R* (+)-2-aminotetralin is due to the direct serotonergic activity of these drugs. On the other hand, chronic spinal transection and 5,6-dihydroxytryptamine treatment enhanced the depressant effects of PEA. These effects may have been due to the reduction of PEA uptake or metabolism. In contrast, the depressant effect of *S* (+)-methamphetamine was abolished by chronic spinal transection and 5,6-dihydroxytryptamine treatment. These results suggest that the depressant effect produced by *S* (+)-methamphetamine is due to 5-HT release from the serotonergic nerve terminals, and are compatible with the findings of Sloviter *et al.*,⁵⁾ who showed that *S* (+)-amphetamine and PEA caused 5-HT syndromes by releasing 5-HT and directly affecting 5-HT receptors, respectively.

The decreasing effects of *S* (+)-methamphetamine on the MSR reversed to enhancement in the presence of ketanserin or haloperidol, or in chronic spinal rats. These effects were not observed in the case of PEA. This difference may be due to a dose-effect relationship of *S* (+)-methamphetamine and PEA (Fig. 3). The stimulatory effect of a high dose of *S* (+)-methamphetamine on the MSR was considered to be unmasked by those treatments. A high dose of *S* (+)-methamphetamine may have direct noradrenergic actions.

It has been reported that *R*-isomers of phenylethylamines-hallucinogens and amphetamines have 5-HT₂ agonistic activity¹⁶⁾ and that *S*-isomers of amphetamines exert releasing effects on noradrenaline¹⁷⁾ and 5-HT.⁵⁾ Thus, the 5-HT-releasing action of *S* (+)-methamphetamine observed in the present study is in agreement with the above findings. The depressant effects of PEA and *R* (+)-2-aminotetralin on the MSR were antagonized by 5-HT antagonists but not reduced by the depletion of 5-HT in 5,6-dihydroxytryptamine-treated rats or chronic spinal rats. These results suggest that PEA and *R* (+)-2-aminotetralin possess direct 5-HT-agonistic effects on the MSR. It is noteworthy that *d*-LSD which depressed the MSR in our previous study¹⁴⁾ and an *S*-isomer of α -methyltrypt-

Fig. 5. Structures of PEA-Related Compounds, *d*-LSD, *S* (+)- α -Methyltryptamine and 5-HT

amine, which show 5-HT₂-agonistic and hallucinogenic actions,¹⁶⁾ have a similar spatial relationship between the phenyl ring and the nitrogen atom in the PEA moiety to that of *R* (+)-2-aminotetralin (Fig. 5). Thus, it may be proposed that the PEA moiety in *d*-LSD is involved in the serotonergic effects of *d*-LSD and PEA-related hallucinogens, and that the conformation of the PEA moiety in *R* (+)-2-aminotetralin is important for the direct 5-HT-agonistic activity of these drugs (Fig. 5).

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