2-Phenylethylamine and Methamphetamine Enhance the Spinal Monosynaptic Reflex by Releasing Noradrenaline from the Terminals of Descending Fibers

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ABSTRACT—Experiments were performed on spinalized rats transected at C1. Intravenous administration of 2-phenylethylamine-HCl (PEA-HCl) (0.3 and 1 mg/kg, i.v.) and methamphetamine-HCl (MAP-HCl) (0.1 and 0.3 mg/kg, i.v.) increased the amplitude of the monosynaptic reflex (MSR). The increase of the MSR caused by PEA and MAP was antagonized by prazosin-HCl and abolished by the pretreatment with reserpine (i.p.) and 6-hydroxydopamine (intracisternally, 14 days previously). A dopamine D₁ antagonist, SK&F 83566-HBr (0.01 mg/kg, i.v.), and a D₂ antagonist, YM-09151-2 (0.3 mg/kg, i.v.), did not antagonize the increasing effects produced by PEA and MAP. An inhibitor of type-B monoamine oxidase, (–)deprenyl-HCl (1 mg/kg, i.v.), prolonged the effect of PEA but not that of MAP, suggesting that PEA alone, and not its metabolites, enhanced the MSR. These results suggest that PEA and MAP increase the amplitude of the MSR by releasing noradrenaline from the terminals of descending noradrenergic fibers, and that PEA, an endogenous trace amine, has a mechanism of action similar to that of MAP.

2-Phenylethylamine (PEA) is one of the endogenous trace amines present in mammals (1, 2). PEA is similar to amphetamine in its chemical structure and some pharmacological actions. Like amphetamine, PEA produces stereotyped behavior, increases locomotor activity and decreases feeding behavior (3–5). Thus it could be considered that PEA is an endogenous amphetamine-like substance. However, the mechanism of the synaptic action of PEA has not yet been clarified.

PEA is abundant in the caudate nucleus, putamen, globus pallidus and cerebellum in man (2) and relatively abundant in the rat

spinal cord (6). The spinal monosynaptic reflex (MSR) of spinalized animals can be used as a clear and simple tool for elucidating the mechanisms of drug action. Noradrenergic and serotonergic descending neurons innervate the spinal ventral horn (7), and α_1 -agonistic action and serotonergic actions enhance (8) and decrease the MSR (9–11), respectively. Thus, the MSR is a useful tool for evaluating amphetamines which affect the monoaminergic terminals. We have shown previously that low doses of PEA and MAP increase the MSR and that high doses decrease it (12–14). In the present study, the mechanisms of the

MSR-enhancing action of PEA and MAP were compared in detail using spinalized rats.

MATERIALS AND METHODS

Recording of MSR

Male Wistar rats, weighing 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 bilateral vagus nerves were severed at the cervical region, and the spinal cord was transected at the C1 level. Laminectomy was performed in the lumbosacral region. The ventral and dorsal roots below L4 were cut bilaterally, and the dorsal and ventral 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 kept at $36 \pm 0.5^\circ\text{C}$. Rectal temperature was maintained at $36 \pm 0.5^\circ\text{C}$ by a heating pad. The dorsal and ventral roots of segment L5 were placed on bipolar silver-silver chloride wire electrodes for stimulation (0.2 Hz, 0.05 msec, supramaximal) and recording, respectively. The reflex potentials 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 FBR-252A). The amplitude of the MSR was then measured.

Blood pressure in the femoral artery was monitored with a transducer (Bentley Trantec model 800) and recorded on a polygraph (Nihon Kohden RM-25).

Drug treatment

Rats were pretreated with several drugs. Prazosin-HCl (1 mg/kg), SK&F 83566-HBr (0.01 mg/kg), YM-09151-2 (0.3 mg/kg), (–)-deprenyl-HCl (1 mg/kg, i.v.) and phentolamine mesylate (2 mg/kg) were administered intravenously 10 min before injection of PEA or MAP. Reserpine (5 mg/kg, i.p.) was administered 24 hr before PEA or MAP injection.

Pretreatment with 6-hydroxydopamine was

performed 14 days before recording of the MSR. Rats were anesthetized with ether and given an intracisternal injection of 6-hydroxydopamine (200 μg free base in 10 μl saline with 0.1% ascorbic acid), whereas controls were administered 10 μl ascorbic acid. After the skin had been sutured all animals received penicillin-G potassium (100,000 I.U.) and kanamycin sulfate (10 mg) intramuscularly.

Drugs

Drugs used were 2-phenylethylamine-HCl (Tokyo Kasei), S(+)-methamphetamine-HCl (Dainippon), prazosin-HCl (Pfizer), phentolamine mesylate (Regitin Injection, Ciba Geigy), (–)-deprenyl-HCl (Research Biochemical Inc.), reserpine (Apoplone Injection, Daiichi), 6-hydroxydopamine-HCl (Sigma), SK&F 83566-HBr (Smith Kline & French Labs.) and YM-09151-2 (Yamanouchi). Prazosin-HCl was dissolved in HCl solution and prepared at pH 3, and 6-hydroxydopamine-HCl was dissolved in saline with 0.1% ascorbic acid. Other drugs were dissolved in physiological saline.

Statistics

Student's *t*-test (two-tailed) was employed to compare control values with treated values. When the population variances were unequal, Welch's procedure (15) was employed. Control and drug-treated data were obtained alternately in each graph of figures. One animal received only one injection of PEA or MAP, because PEA and MAP caused tachyphylaxis.

RESULTS

Enhancements of MSR by PEA and MAP and antagonism by the α_1 -antagonist prazosin

PEA-HCl (0.3 and 1 mg/kg, i.v.) and MAP-HCl (0.1 and 0.3 mg/kg, i.v.) increased the amplitude of the MSR (Fig. 1, A, B). The time courses of the effects of PEA and MAP were short and long, respectively; the effects of PEA disappeared within 10 min, whereas those of MAP continued for more than 30 min. In the presence of the α_1 -blocker

Fig. 1. Effect of PEA-HCl and MAP-HCl on the MSR (A and B) and antagonism by prazosin-HCl (C and D). Abscissae: time after the injection of PEA or MAP. Ordinates: reflex amplitude (mean $\pm$ S.E.M. for 4 experiments) calculated as a percentage of the value just prior to injection of PEA or MAP. (A) $\bullet$: PEA-HCl 1 mg/kg, i.v.; $\circ$: PEA-HCl 0.3 mg/kg, i.v. (B) $\bullet$: MAP-HCl 0.3 mg/kg, i.v.; $\circ$: MAP-HCl 0.1 mg/kg, i.v. (C) $\bullet$: PEA-HCl 1 mg/kg, i.v.; $\circ$: prazosin-HCl 1 mg/kg, i.v. + PEA-HCl 1 mg/kg, i.v. (D) $\bullet$: MAP-HCl 0.3 mg/kg, i.v.; $\circ$: prazosin-HCl 1 mg/kg, i.v. + MAP-HCl 0.3 mg/kg, i.v. Prazosin was administered at the arrow. Where S.E.M. bars are not shown, they lie within the dimension of the symbols. * $P < 0.05$ (t -test), as compared with the effect of PEA or MAP alone.

prazosin-HCl (1 mg/kg, i.v.), the increasing effects of PEA were reversed to a decrease (Fig. 1C). The enhancement of MSR caused by MAP was completely abolished by prazosin (Fig. 1D). Prazosin alone did not affect the MSR in the spinalized rats that were used in the present study.

Effects of the dopamine D_1 antagonist SK&F 83566-HBr and the D_2 antagonist YM-09151-2

Pretreatment with the D_1 antagonist SK&F 83566-HBr (0.01 mg/kg, i.v.) or the D_2 antagonist YM-09151-2 (0.3 mg/kg, i.v.) did not alter the effects of PEA and MAP on the MSR (Fig. 2). These doses of SK&F 83566-HBr and YM-09151-2 antagonized the increasing effect of the D_1 agonist SK&F 38393-HBr (1 mg/kg, i.v.) and the decreasing effect of

the D_2 agonist apomorphine-HCl (3 mg/kg, i.v.) on the MSR, respectively (not shown). SK&F 83566-HBr and YM-09151-2 tended to increase and decrease the MSR, respectively.

Influences of reserpine and 6-hydroxydopamine treatments

Reserpine (5 mg/kg, i.p.), which depletes monoamines in the monoaminergic terminals, administered 24 hr before PEA and MAP, abolished the increase of the MSR produced by the latter agents (Fig. 3, A, B). PEA decreased the MSR in reserpinized rats.

Pretreatment (14 days, previously) with intracisternal 6-hydroxydopamine, which destroys monoaminergic terminals, reduced the enhancing effects of PEA and MAP on the MSR without changing the pressor effects of

Fig. 2. Influence of SK&F 83566-HBr (A and B) and YM-09151-2 (C and D) on the stimulatory effects of PEA and MAP on the MSR. (A) ●: PEA-HCl 1 mg/kg, i.v.; ○: SK&F 83566-HBr 0.01 mg/kg, i.v. + PEA-HCl 1 mg/kg, i.v. (B) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: SK&F 83566-HBr 0.01 mg/kg, i.v. + MAP-HCl 0.3 mg/kg, i.v. (C) ●: PEA-HCl 1 mg/kg, i.v.; ○: YM-09151-2 0.3 mg/kg, i.v. + PEA-HCl 1 mg/kg, i.v. (D) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: YM-09151-2 0.3 mg/kg, i.v. + MAP-HCl 0.3 mg/kg, i.v. SK&F 83566-HBr and YM-09151-2 were administered at the arrow. $n = 4$.

PEA and MAP (Fig. 3, C, D). The treatment with reserpine or 6-hydroxydopamine did not change the amplitude and latency of MSR.

Influences of a monoamine oxidase inhibitor and blood pressure

An inhibitor of type-B monoamine oxidase (MAO), (–)deprenyl-HCl (1 mg/kg, i.v.), did not reduce, but increased and prolonged the enhancing effects of PEA (0.3 mg/kg, i.v.) on the MSR (Fig. 4A). The time course of the effect of MAP (0.3 mg/kg, i.v.) was not affected by (–)deprenyl (Fig. 4B). These results suggested that the short action of PEA is due to its rapid metabolism.

The possibility was also tested that changes in the MSR caused by PEA and MAP are due to peripheral changes in blood pressure. Phentolamine mesylate (2 mg/kg, i.v.) antagonized the pressor effects of PEA and MAP without

changing their enhancing effects on the MSR (Fig. 4, C, D).

DISCUSSION

Effects of drugs on the polysynaptic reflex (PSR) were not described in this study, since excitatory effects of PEA and MAP on the PSR were variable and the influences of drug-treatment on the PSR were not significant.

It has been considered that changes in the MSR caused by PEA and MAP may be due to peripheral changes in blood pressure. Phentolamine, which seems to have difficulty in penetrating the blood-brain barrier, antagonized the pressor effects but not the enhancing effects of PEA and MAP on the MSR (Fig. 4). In addition, pretreatment with intracisternal 6-hydroxydopamine, which destroys monoaminergic terminals in the CNS (16), antago-

Fig. 3. Influence of depletion of monoamines by reserpine (A and B) and 6-hydroxydopamine (C and D) on the stimulatory effects of PEA and MAP on the MSR. (A) ●: PEA-HCl 1 mg/kg, i.v.; ○: reserpine + PEA-HCl 1 mg/kg, i.v. (B) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: reserpine + MAP-HCl 0.3 mg/kg, i.v. (C) ●: PEA-HCl 1 mg/kg, i.v.; ○: 6-hydroxydopamine + PEA-HCl 1 mg/kg, i.v. (D) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: 6-hydroxydopamine + MAP-HCl 0.3 mg/kg, i.v. Reserpine (5 mg/kg, 24 hr before, i.p.) and 6-hydroxydopamine (200 μ g, 14 days before, intracisternally) were preadministered. Inset figures in C and D show the effects of drugs on blood pressure. Each point represents the change in the mean blood pressure (Δ mmHg). * $P < 0.05$ (*t*-test), as compared with the effect of PEA or MAP alone. $n = 4$.

nized the enhancing effects of PEA and MAP on the MSR but not their pressor effects (Fig. 3). These results suggest that the increase of the MSR produced by PEA or MAP is not due to a change in blood pressure.

It was also possible that the MSR enhancement caused by PEA was due to metabolites produced by conversion with type-B MAO. However, an inhibitor of type-B MAO, (–)deprenyl, did not reduce, but rather increased and prolonged the effect of PEA (Fig. 4). These results suggest that PEA itself increased the MSR.

There are abundant noradrenergic and serotonergic descending neurons innervating the spinal ventral horn, although dopaminergic innervation is very scarce (7). In our previous study, the serotonergic agonists, 5-methoxy-N,N-dimethyltryptamine and 8-hydroxy-di-

propylaminotetralin, and also 5-hydroxytryptophan, decreased the amplitude of the MSR (9–11). We have shown previously that noradrenergic systems mediate facilitation of the spinal motoneurons (8, 17) and that tonic noradrenergic facilitation of the spinal reflexes occurs (18, 19). In the present preparation, although tonic activities from the brain to the spinal cord were lost because of spinalization at the C1 level, the terminals of noradrenergic fibers descending from the brainstem were still viable.

The increase in the amplitude of the MSR induced by PEA and MAP was antagonized by the α_1 -blocker prazosin, but not by the D_1 antagonist SK&F 83566-HBr (20) and the D_2 antagonist YM-09151-2 (21) (Figs. 1 and 2). These results suggest that PEA and MAP increase the MSR via direct or indirect norad-

Fig. 4. Influence of (—)deprenyl-HCl (A and B) and phentolamine mesylate (C and D) on the stimulatory effects of PEA and MAP on the MSR. (A) ●: PEA-HCl 0.3 mg/kg, i.v.; ○: (—)deprenyl-HCl 1 mg/kg, i.v. + PEA-HCl 0.3 mg/kg, i.v. (B) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: (—)deprenyl-HCl 1 mg/kg, i.v. + MAP-HCl 0.3 mg/kg, i.v. (C) ●: PEA-HCl 1 mg/kg, i.v.; ○: phentolamine mesylate 2 mg/kg, i.v. + PEA-HCl 1 mg/kg, i.v. (D) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: phentolamine mesylate 2 mg/kg, i.v. + MAP-HCl 0.3 mg/kg, i.v. Deprenyl and phentolamine were administered at the arrow. Inset figures in C and D show the effects of drugs on blood pressure. Each point represents the change in the mean blood pressure (Δ mmHg). * $P < 0.05$ (*t*-test), as compared with the effect of PEA or MAP alone. $n = 4$.

renergic mechanisms and exclude the possibility that PEA and MAP increase the amplitude of the MSR by releasing dopamine or by direct stimulation of dopamine receptors. Although dopaminergic agents have been reported to affect the MSR (22, 23), the involvement of dopamine release in the effects of PEA and MAP was not suggested in the present study. This may be due to the existence of little dopaminergic innervation in the ventral horn (7). Reserpine, which depletes monoamines in the monoaminergic terminals, and 6-hydroxydopamine, which destroys noradrenergic terminals more rapidly than dopaminergic terminals (16), antagonized the enhancing effects of PEA and MAP on the MSR. These findings suggest that PEA and MAP increase the amplitude of the MSR by

releasing noradrenaline from the terminals of descending noradrenergic fibers.

In rats pretreated with prazosin or reserpine, PEA decreased the amplitude of the MSR. This may be due to the serotonergic agonistic action of PEA, which usually appears at high doses (14). The decrease of the MSR caused by PEA has been shown to be antagonized and reversed to enhancement by the serotonergic antagonist ketanserin (14). However, 6-hydroxydopamine treatment did not reverse the effect of PEA. This result was not consistent with the previous study (13) which showed the reversal after 6-hydroxydopamine treatment. The inconsistency may be due to a balance between the direct noradrenergic agonistic effect which is amplified after 6-hydroxydopamine treatment and the

serotonergic agonistic action of PEA. In the present study, a low dose of PEA had noradrenergic stimulatory effects on the MSR similar to those of MAP, and a high dose of PEA produced a serotonergic depressant action on the MSR (14). Serotonergic effects of PEA have also been reported in behavioral experiments (24, 25).

From the present results, it is concluded that PEA and MAP increase the amplitude of the MSR by releasing noradrenaline from the terminals of descending noradrenergic nerve fibers and through α_1 -receptors, and that the mechanism of the enhancing effect of PEA on the MSR is similar to that of MAP.

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