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 α₁-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 lumbo-sacral 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 ± 0.5°C. Rectal temperature was maintained at 36 ± 0.5°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 (Apoplon Injection, Daichi), 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 α1-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 α1-blocker
Effects of the dopamine D₁ antagonist SK&F 83566-HBr and the D₂ antagonist YM-09151-2

Pretreatment with the D₁ antagonist SK&F 83566-HBr (0.01 mg/kg, i.v.) or the D₂ 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 D₁ agonist SK&F 38393-HBr (1 mg/kg, i.v.) and the decreasing effect of the D₂ 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 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 D₁ antagonist SK&F 83566-HBr and the D₂ antagonist YM-09151-2

Pretreatment with the D₁ antagonist SK&F 83566-HBr (0.01 mg/kg, i.v.) or the D₂ 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 D₁ agonist SK&F 38393-HBr (1 mg/kg, i.v.) and the decreasing effect of 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.

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 ± S.E.M. for 4 experiments) calculated as a percentage of the value just prior to injection of PEA or MAP. (A) ●: PEA-HCl 1 mg/kg, i.v.; ○: PEA-HCl 0.3 mg/kg, i.v. (B) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: MAP-HCl 0.1 mg/kg, i.v. (C) ●: PEA-HCl 1 mg/kg, i.v.; ○: prazosin-HCl 1 mg/kg, i.v. + PEA-HCl 1 mg/kg, i.v. (D) ●: MAP-HCl 0.3 mg/kg, i.v.; ○: 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.
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 μg, 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.

Amphetamines on Spinal Reflex

The increase in the amplitude of the MSR induced by PEA and MAP was antagonized by the α₁-blocker prazosin, but not by the D₁ antagonist Sk&F 83566-HBr (20) and the D₂ antagonist YM-09151-2 (21) (Figs. 1 and 2). These results suggest that PEA and MAP increase the MSR via direct or indirect 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-dipropylaminotetralin, 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.
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 $\alpha_2$-receptors, and that the mechanism of the enhancing effect of PEA on the MSR is similar to that of MAP.

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REFERENCES
1 Inwang, E.E., Mosnaim, A.D. and Sabellig, H.C.: Isolation and characterization of phenylethylamine and phenylethanolamine from human brain. J. Neurochem. 20, 1469–1473 (1973)
2 Philips, S.R., Rozdilsky, B. and Boulton, A.A.: Evidence for the presence of m-tyramine, p-tyramine, and phenylethylamine in the rat brain and several areas of the human brain. Biol. Psychiatry 13, 51–57 (1978)
3 Borison, R.L., Havdala, H.S. and Diamond, B.I.: Chronic phenylethylamine stereotypy in rats: A new animal model for schizophrenia? Life Sci. 21, 117–122 (1977)
4 Cooper, S.J. and Dourish, C.T.: Hypodipsia, stereotypy and hyperactivity induced by $\beta$-phenylethylamine in the water-deprived rat. Pharmacol. Biochem. Behav. 20, 1–7 (1984)
5 Dourish, C.T. and Cooper, S.J.: Environmental experience produces qualitative changes in the stimulant effects of $\beta$-phenylethylamine in rats. Psychopharmacology (Berlin) 84, 132–135 (1984)
6 Karoum, F., Chuang, L.-W. and Wyatt, R.J.: Presence and distribution of phenylethylamine in the rat spinal cord. Brain Res. 225, 442–445 (1981)
7 Björklund, A. and Skagerberg, G.: Descending monoaminergic projections to the spinal cord. In Brain Stem Control of Spinal Mechanisms, Edited by Sjöland, B. and Björklund, A., p. 55–88, Elsevier Biomedical Press, Amsterdam (1982)
8 Tanabe, M., Ono, H. and Fukuda, H.: Spinal $\alpha_1$- and $\alpha_2$-adrenoceptors mediate facilitation and inhibition of spinal motor transmission, respectively. Japan. J. Pharmacol. 54, 77–85 (1990)
9 Nagano, N., Ono, H., Ozawa, M. and Fukuda, H.: Sensitivity of spinal reflexes to TRH and 5-HT in 5,6-dihydroxytryptamine-treated rats. Eur. J. Pharmacol. 139, 315–321 (1987)
10 Nagano, N., Ono, H., Ozawa, M. and Fukuda, H.: The spinal reflex of chronic spinal rats is supersensitive to 5-HTP but not to TRH or 5-HT agonists. Eur. J. Pharmacol. 149, 337–344 (1988)
11 Nagano, N., Ono, H. and Fukuda, H.: Functional significance of subtypes of 5-HT receptors in the rat spinal reflex pathway. Gen. Pharmacol. 19, 789–793 (1988)
12 Hasebe, Y., Ono, H., Fukuda, H., Ohta, S. and Hirobe, M.: The most desirable conformation of phenylethylamine (PEA) moiety stimulating noradrenergic neurons: Effects of PEA, methamphetamine, phentolamine, methylphenidate, nomifensine and mazindol on rat spinal reflexes. Gen. Pharmacol. 20, 375–379 (1989)
13 Hasebe, Y., Ono, H., Fukuda, H., Ohta, S. and Hirobe, M.: Enhancement of spinal monosynaptic reflexes with phenylethylamine and related drugs through descending noradrenergic neurons. J. Pharmacobiodyn. 12, 241–245 (1989)
14 Ono, H., Hasebe, Y., Mori, T., Fukuda, H., Kohno, M., Ohta, S. and Hirobe, M.: Structure-activity relationships of phenylethylamine analogs in their serotonergic depressant effects on the spinal monosynaptic reflex in rats. J. Pharmacobiodyn. 12, 384–391 (1989)
15 Welch, B.L.: A significance of the difference between two means when the population variances are unequal. Biometrika 29, 350–362 (1937)
16 Jacks, B.R., De Champlain, J. and Cordeau, P.: Effects of 6-hydroxydopamine on putative transmitter substances in the central nervous system. Eur. J. Pharmacol. 18, 353–360 (1972)
17 Hirayama, T., Ono, H. and Fukuda, H.: Effects of adrenergic agents on ventral horn cells in rat spinal cord slices. Biomed. Res. 9, 343–351 (1988)
18 Hino, M., Ono, H. and Fukuda, H.: Brain stem involvement in the effects of chlorpromazine on the monosynaptic reflex of the rat lumbar spinal cord. Gen. Pharmacol. 17, 379–383 (1986)
19 Hino, M., Ono, H. and Fukuda, H.: Involvement of noradrenergic systems in the effects of con-
ditioning stimulation of the lower brain stem on the lumbar spinal reflex in rats. Gen. Pharmacol. 18, 41–45 (1987)

20 O’Boyle, K.M. and Waddington, J.L.: SKF 83566 and 83692: Benzazepines with selective and stereospecific actions at the D₁ dopamine receptor. Br. J. Pharmacol. 83, Supp. 371P (1984)

21 Terai, M., Usuda, S., Kuroiwa, I., Noshiro, O. and Maeno, H.: Selective binding of YM-09152-2, a new potent neuroleptic, to D₂-dopaminergic receptors. Japan. J. Pharmacol. 33, 749–755 (1983)

22 Schlosser, W., Horst, W.D., Spiegel, H.E. and Sigg, E.B.: Apomorphine and its effects on the spinal cord. Neuropharmacology 11, 417–426 (1972)

23 Carp, J.S. and Anderson, R.J.: Dopamine receptor-mediated depression of spinal monosynaptic transmission. Brain Res. 242, 247–254 (1982)

24 Sloviter, R.S., Connor, J.D. and Drust, E.G.: Serotonergic properties of β-phenylethylamine in rats. Neuropharmacology 19, 1071–1074 (1980)

25 Dourish, C.T.: Behavioural effects of acute and chronic β-phenylethylamine administration in the rat: Evidence for the involvement of 5-hydroxytryptamine. Neuropharmacology 20, 1067–1072 (1981)