Characteristics of Antinociception Induced by Noncatecholic Phenylethylamine Derivatives: The Involvement of Alpha-2-Adrenoceptors

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ABSTRACT—Characteristics of the antinociceptive action of phenylethylamine derivatives, amphetamine, β-phenylethylamine (PEA) and β-hydroxyphenylethylamine (OHPEA), were examined. The antinociception induced by PEA derivatives was enhanced by intracisternal injection of norepinephrine or clonidine and attenuated by intracisternal injection of phentolamine or yohimbine, but was not affected by intracisternal injection of prazosin in the mouse hot plate method. PEA derivatives induced a contraction of the rat vas deferens, and this contraction by PEA derivatives was attenuated by the application of phentolamine. The contractions induced by PEA or OHPEA in the reserpinized vas deferens were much smaller than those in the normal one. PEA derivatives inhibited the electrical stimulation-evoked contractions of the vas deferens, and the inhibition by PEA derivatives was reversed by the application of yohimbine. These findings indicate that PEA derivatives may induce the antinociception as a result of stimulating the α2-adrenoceptors. The stimulation of α2-adrenoceptors by PEA derivatives may result from the release of endogenous norepinephrine and/or from direct action on the α2-adrenoceptors.

Keywords: Phenylethylamine, Amphetamine, Phenylethanolamine, Antinociception, α2-Adrenoceptor

The existence of β-phenylethylamine (PEA) has been demonstrated in the brain and body fluids of various species of animals including humans (1). Boulton (2) and Niddam et al. (3) claimed that PEA and other related trace amines could act as cotransmitters or neuromodulators in catecholaminergic neuro-transmission. Giardina (4) reported that PEA and β-hydroxyphenylethylamine (phenylethanolamine; OHPEA) induce an antinociceptive action in pargyline-pretreated mice. Amphetamine, one of the noncatecholic phenylethylamine derivatives, produces a slight but significant antinociceptive action (5). We (6, 7) have also reported that PEA derivatives such as PEA, OHPEA and amphetamine have antinociceptive action which may involve endogenous serotonin, norepinephrine and opioid peptides.

Braestrup and Randrup (8) reported that PEA appears to have a strong action in the release of norepinephrine in the central nervous system (CNS). We (7) have also reported that PEA derivatives may induce the release of norepinephrine in the CNS, and produce an antinociception mediated partly by norepinephrine via α-adrenoceptors.

α-Adrenoceptors have been classified into alpha-1 and alpha-2 subtypes (9, 10). The present study was performed to clarify the correlation of α-adrenoceptor subtypes to the antinociception induced by PEA derivatives through examining the effects of α-adrenergic agonists and antagonists on PEA analog-induced antinociception in mice and the effects of PEA derivatives on contractile responses of the rat vas deferens.

MATERIALS AND METHODS

Materials
Amphetamine sulfate (Zedrin; Takeda Chem., Inc., Osaka), dl-β-hydroxyphenylethylamine (OHPEA; Sigma, St. Louis, MO, USA) and β-phenylethylamine hydrochloride (PEA; Tokyo Kasei, Tokyo) were used as PEA derivatives. l-Norepinephrine bitartrate, yohimbine hydrochloride, clonidine hydrochloride and prazosin hydrochloride (Sigma); phentolamine mesylate (Ciba-Geigy, Basel, Switzerland); and reserpine (Tokyo Kasei) were...
Measurement of contractions of isolated rat vas deferens

Male ddY mice (Shizuoka Laboratory Animal Center, Hamamatsu), weighing 17 to 20 g, were used. Pain thresholds of mice were measured by the hot plate method of Woolfe and MacDonald (11). The hot plate was maintained at 55±0.5°C by a thermostat. To determine the pain thresholds of mice, the latency, the length of time which mice placed on the hot plate required to initiate any one of the following behaviors, jumping or shaking, licking, holding and lifting their paws, was measured in a room kept at 24±1°C and 55±5% relative humidity and protected from external noise. Mice were preliminarily tested twice for the latency, and those showing a latency ranging from 2 to 8 sec were used for the experiments. Ten microliters of an aqueous solution of phenolamine (4 μg/10 μl), yohimbine (2 μg/10 μl), clonidine (2 μg/10 μl) or prazosin (2 μg/10 μl) was injected into the cerebro-medullary cistern of a mouse by the method of Ueda et al. (12) 15 min before the i.p. administration of PEA derivatives, and norepinephrine (5 μg/10 μl) was intracisternally administered 5 min after the PEA derivatives. The pain thresholds of mice were measured at 15 min and immediately before and at 15, 30, 45, 60, 90 and 120 min after the i.p. administration of PEA derivatives.

Measurement of contractions of isolated rat vas deferens

Male Wistar rats weighing 300 to 400 g were stunned and bled, and the vasa deferentia were removed. The isolated vas deferens was suspended in an organ bath filled with 10 ml Locke-Ringer solution which was aerated continually and maintained 32°C. The tension of the isolated organ loaded with 0.3 g resting tension was recorded isotonically through an isotonic lever transducer (ME 4012; ME Commercial Co., Tokyo). The composition of Locke-Ringer solution was as follows: 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl2, 2.1 mM MgCl2, 5.9 mM NaHCO3 and 2.8 mM glucose. Dose-response curves for norepinephrine and PEA derivatives were obtained from the cumulative application of agonists to the isolated organ.

After relatively constant dose-response curves for norepinephrine were obtained, the dose-response curves for the PEA derivatives were obtained, and then another dose-response curve for norepinephrine was obtained to confirm whether the sensitivity of the tissue to norepinephrine remained unchanged. Contraction of the vas deferens by the PEA derivatives was expressed as the percent height of the maximum response induced by norepinephrine.

Measurement of antinociceptive effect

Male Wistar rats, weighing 300 to 400 g, were sacrificed by a blow on the head. The prostatic portions of the vasa deferentia (2- to 3-cm-long) of the rats were removed and suspended in a 10-ml organ bath containing Krebs solution which was kept at 32±0.5°C and gassed with 95% O2 and 5% CO2. The Krebs solution had the following composition: 118 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl2, 1.19 mM KH2PO4, 17.19 mM NaHCO3 and 11 mM glucose. The strip was loaded with a resting tension of 0.5 g. The intramural nerves were field-stimulated through two platinum ring electrodes with rectangular pulses that induced sub-maximal responses of the isolated vas deferens. The intramural nerves were field-stimulated through two platinum ring electrodes with rectangular pulses that induced sub-maximal responses of the isolated vas deferens. Stimulation parameters were 2-msec duration and 0.08 Hz (Electrostimulator: ME 6052; ME Commercial Co.). The electrically evoked contractions of the strip were recorded isometrically by means of a strain gauge transducer (ME 4021, ME Commercial Co.) and an ink-writing pen oscillograph (R-22; Rikadenki, Tokyo).

After the twitch responses of the vas deferens to the electrical stimulation became constant, the preparation was applied with PEA derivatives. Other experimental conditions are detailed in the description of the results.
Statistics
Results were expressed as the mean ± S.E. Statistical significance was assessed by Student’s t-test with two-tailed probability for unpaired data. The difference was considered significant at the P < 0.05 level.

RESULTS

Effect of central administration of alpha agonists and antagonists on PEA analog-induced antinociception
Intraperitoneal injection of 50 mg/kg of PEA, 50 mg/kg of OHPEA and 2 mg/kg of amphetamine induced a significant increment of the latency in the hot plate method (Figs. 1–4). Intracisternally injected norepinephrine enhanced the prolongation of latency induced by 50 mg/kg, i.p. of PEA and by 50 mg/kg, i.p. of OHPEA (P < 0.01 and P < 0.05, respectively), but did not affect the amphetamine (2 mg/kg, i.p.)-induced prolongation of latency (Fig. 1). On the other hand, phentolamine (4 μg/mouse, i.cist.) did not affect the latency by itself, but inhibited the prolongation of latency induced by amphetamine, PEA and OHPEA (P < 0.01, P < 0.05 and P < 0.01, respectively; data not shown).

Clonidine (2 μg/mouse) enhanced PEA- or OHPEA-induced prolongation of latency (P < 0.01 and P < 0.05, respectively) and tended to enhance the amphetamine-induced one (Fig. 2). Yohimbine (2 μg/mouse) did not affect the latency by itself, but inhibited amphetamine- or PEA-induced prolongation of latency (P < 0.05 and P < 0.01, respectively), and tended to inhibit the OHPEA-

Fig. 1. Effect of intracisternal (i.cist.) administration of norepinephrine (5 μg) on antinociception induced by amphetamine (A), PEA (B) and OHPEA (C) in mice. The time of the i.cist. injection of norepinephrine was indicated by white arrows (↑). Each point represents the mean value of latency from 10 mice, and the S.E. is indicated by vertical bars if it is larger than the size of the symbols. Symbols represent: ○, control; ●, norepinephrine; △, PEA derivatives; ▲, norepinephrine + PEA derivatives. Significant difference from the control by Student’s t-test: **P < 0.01, *P < 0.05 and significant difference from PEA derivatives: †P < 0.01, †P < 0.05.

Fig. 2. Effect of i.cist. administration of clonidine (2 μg) on the antinociception induced by amphetamine (A), PEA (B) and OHPEA (C) in mice. The time of the i.cist. injection of clonidine was at 15 min before the injection of 2 mg/kg of amphetamine, 50 mg/kg of PEA or 50 mg/kg of OHPEA, being indicated by white arrows (↑). Each point represents the mean value of latency from 8 mice. Vertical bars indicate S.E. Symbols represent: ○, control; ●, clonidine; △, PEA derivatives; ▲, clonidine + PEA derivatives. Significant difference from the control by Student’s t-test: **P < 0.01, *P < 0.05 and significant difference from PEA derivatives: †P < 0.01, †P < 0.05.
induced one (Fig. 3). Prazosin (2 pg/mouse), which had no effect on the latency per se, failed to affect the prolongation of latency induced by PEA derivatives (Fig. 4).

**Effects of phenylethylamine derivatives on the isolated vas deferens from rats**

Application of PEA, OHPEA and amphetamine to the isolated rat vas deferens produced a dose-dependent contraction. When their dose-response curves were obtained by cumulative applications, their EC₅₀s were approximately 2.4 × 10⁻⁵ M for PEA, 4.1 × 10⁻⁵ M for OHPEA and 2.5 × 10⁻⁶ M for amphetamine, and their maximal contractile responses were smaller than that induced by norepinephrine (Fig. 5A).

**Effects of alpha-adrenergic blocker on PEA analog-induced contraction of rat vas deferens**

Application of 10⁻⁷ M of phentolamine, an α-blocker, inhibited the contractile responses of the isolated vas deferens to PEA, OHPEA or amphetamine with statistical significance (P < 0.05, respectively) (Fig. 6).

**Effects of phenylethylamine derivatives on the isolated vas deferens from reserpinized rats**

In the isolated vas deferens from reserpinized rats, PEA and OHPEA produced a small but dose-dependent contraction, whose EC₅₀s were about 1.8 × 10⁻³ and 8.5 × 10⁻⁵ M, respectively (Fig. 5B), while amphetamine and tyramine produced no contraction even at a dose of 10⁻⁴ M. Maximal responsiveness and sensitivity of the vas deferens from reserpinized rats to PEA derivatives were lower than those of the one from the normal rats (Fig. 5).
Although the data are not shown, phentolamine ($10^{-7}$ or $3 \times 10^{-7}$ M) also inhibited the PEA- or OHPEA-induced contractions of the vas deferens from reserpinized rats with statistical significance ($P < 0.05$ and $P < 0.01$, respectively).

**Effects of phencytlylamine derivatives on the electrical stimulation-induced contraction of rat vas deferens**

Contractions of the rat vas deferens induced by electrical rectangular pulses, 2-msec duration, 1/12 Hz and 40–80 V, were inhibited completely by tetrodotoxin ($10^{-7}$ M) and significantly inhibited by clonidine ($10^{-7}$ M).
M), a selective α₂-agonist (data were omitted here). Application of PEA derivatives inhibited the electrical stimulation-evoked contractions in a biphasic manner (Fig. 7). The inhibition reached the first maximum in 1 to 2 min after the application of PEA derivatives, and then plateaued in 12 to 24 min after the application (Fig. 7 and Table 1). To observe the effects of yohimbine on the action of PEA derivatives which inhibited the electrical stimulation-evoked contraction of the vas deferens, \(10^{-7}\) M yohimbine was applied 24 min after the application of PEA derivatives. The post application of yohimbine significantly reversed the inhibitory effects of PEA derivatives on the electrical stimulation-evoked contractions (Table 2).

![Figure 7](image_url)

**Figure 7.** Representative recording of the inhibitory effects of PEA derivatives on the twitch responses induced by electrical stimulation (2 msec, 0.08 Hz, submaximal voltage) and the effects of the pre- and post-treatment with yohimbine on the inhibition in the isolated rat vas deferens. Drugs were added to the organ bath as indicated by arrows. Note that PEA derivatives inhibited the twitch-response and failed to contract the muscle as shown in the baseline of the responses. Interval of each vertical line indicates 6 min.

**Table 1.** Inhibitory effects of PEA derivatives on electrical stimulation evoked contraction and the effects of pretreatment with yohimbine on the inhibition in the rat vas deferens

| Treatment     | % Inhibition of electrical stimulation evoked contraction* | control | yohimbine 10⁻⁷ M |
|---------------|---------------------------------------------------------|---------|------------------|
|               | (1 to 2 min)                                            | plateau level (24 min) | first maximum |
| PEA 10⁻⁶ M    | 24.6 ± 7.4 (8)                                          | 12.3 ± 7.1 (8)          | 4.2 ± 1.7* (6) |
| OHPEA 10⁻⁶ M  | 14.7 ± 3.8 (7)                                          | 10.5 ± 3.9 (7)          | -0.1 ± 2.2** (7) |
| Amphetamine 10⁻⁷ M | 30.5 ± 6.1 (7)                                      | 29.5 ± 6.1 (7)          | 7.1 ± 5.2** (7) |

*Inhibition percent value was calculated from the equations as follows: \(\%\text{Inh} = (A - B)/A \times 100\%\) as first maximum and \(\%\text{Inh} = (A - C)/A \times 100\%\) as plateau level of the control, and \(\%\text{Inh} = (F - G)/F \times 100\%\) as first maximum of the yohimbine-treated group, with the parameters illustrated in Fig. 7(D). Numbers in parentheses indicate the number of experiments. Significantly different from the first maximum value of the corresponding control at *\(P<0.01\) or **\(P<0.05\) by Student's t-test.
When 10⁻⁷ M of yohimbine was applied to the vas deferens 12 min before the application of PEA derivatives, the pretreatment with yohimbine also attenuated the inhibitory effects of PEA derivatives on the electrical stimulation-evoked contractions (Table 1).

### Table 2. Reversal of the PEA analoge-induced inhibition of electrical stimulation-evoked contraction of the rat vas deferens by the post-treatment with yohimbine

| Treatment   | No. of experiments | % Enhancement by 10⁻⁷ M of yohimbine |
|-------------|--------------------|-------------------------------------|
| Control     | 19                 | 4.0 ± 0.58                          |
| PEA 10⁻⁴ M | 8                  | 41.1 ± 22.5                         |
| OHPEA 10⁻⁴ M| 7                  | 21.0 ± 5.6*                         |
| Amphetamine 10⁻⁷ M | 7               | 72.4 ± 17.4**                       |

Percent reverse was calculated by the equation: % enhancement = (D - C)/C × 100%, with the parameters illustrated in Fig. 7(D). Significantly different from the control at **P < 0.01 or *P < 0.05 by Student's t-test.

DISCUSSION

Tocco et al. (13) reported that i.p. injection of α-antagonists, such as phentolamine and yohimbine, did not affect the antinociception induced by amphetamine in the hot plate method in mice. We (7) have previously demonstrated that i.p. treatment with 5 mg/kg of phentolamine did not affect the antinociception induced by PEA derivatives. PEA is known to pass through the blood-brain barrier with ease (14) and to be rapidly accumulated in the brain (15). Therefore, there is a possibility that PEA derivatives may produce their antinociceptive action through direct acting on the brain. Furthermore, it is well-known that α-adrenergic agonists and antagonists produce peripheral effects such as cardiac effects, vasoconstriction or relaxation which could affect the latency in the hot plate method. Therefore, when we observed the effects of α-agonists and α-antagonists on PEA analog-induced antinociception, we administered the drugs intracisternally to mice in order to minimize the peripheral actions of α-agonists and α-antagonists.

Intraperitoneal injection of amphetamine, PEA or OH-PEA significantly increased the latency of mice in the hot plate method (Figs. 1–4), suggesting that PEA derivatives have antinociceptive action. Intracisternal injection of norepinephrine augmented the antinociceptive effect of PEA or OHPEA (Fig. 1), and intracisternal injection of phentolamine attenuated the antinociceptive effect of PEA derivatives. PEA derivatives induced a contraction of the vas deferens, which was inhibited by a pretreatment with phentolamine (Figs. 5 and 6). These results suggest that PEA derivatives have an α-agonistic action and may induce antinociception through α-adrenoceptors in the CNS.

α-Adrenoceptors have been classified into α₁ and α₂ subtypes. PEA analog-induced antinociception was enhanced by intracisternal injection of clonidine, an α₂ agonist, and attenuated by intracisternal injection of yohimbine, an α₂-antagonist, but failed to be affected by intracisternal injection of prazosin, an α₁-antagonist. Therefore, it seems that PEA analog-induced antinociception does not involve α₁-receptors but involves α₂-receptors in the CNS. The stimulation of post-synaptic α₂-adrenoceptors in the CNS is known to mediate analgesia (16–18). Therefore, PEA derivatives may act on central postsynaptic α₂-adrenoceptors and cause antinociception.

PEA- and OHPEA-induced contractions of the vas deferens from reserpinized rats were much smaller than those of normal rats (Fig. 5), while amphetamine did not induce any longer contraction of the vas deferens in reserpinized rats. Previously, we (7) have demonstrated that PEA derivatives induced the release of norepinephrine in the CNS. Lundberg et al. (19) reported that the endogenous norepinephrine released from the reserpine-sensitive pool by PEA injected intravenously or local microiontophoresically might act on α₂-adrenoceptors, resulting in the inhibition of the firing of locus coeruleus neurons. Therefore, PEA derivatives may induce the release of norepinephrine in the CNS and the released norepinephrine could produce an antinociception.

On the other hand, our present results of electrical stimulation using the vas deferens (Fig. 7 and Tables 1 and 2) suggest that PEA derivatives have an α₂-agonistic action and inhibit the twitch responses of the vas deferens through stimulating presynaptic α₂-receptors at concentrations lower than those causing smooth muscle contraction via action on α₁-receptors. Furthermore, norepinephrine (10⁻⁵ M) also inhibited the twitch induced by the electrical stimulation but concomitantly elevated the baseline of the tension in the rat vas deferens (data was not shown), while PEA derivatives failed to increase the baseline (Fig. 7). Taguchi et al. (20) suggested that PEA acts on α₂-receptors by itself. These findings strongly suggested that PEA derivatives may act on α₂-adrenoceptors per se. Hansen et al. (21) argued that the effects of PEA involve a direct action upon α-adrenergic receptors. Binding experiments using PEA and amphetamine (22, 23) suggested that specific binding sites for PEA and amphetamine exist in the central nervous system. It is unclear whether or not the binding sites of the PEA derivatives are similar to α₂-adrenoceptors, but it is likely that PEA derivatives act directly on the central α₂-adrenoceptors, resulting in the production of antinociception.

Ueda et al. (12) reported that the distribution of dye injected by intracisternal injection was limited mainly to the
cisterna magna and the ventral surface of the brain stem. Therefore, adrenoceptor agonists and antagonists in this study may act on the brain stem, and PEA derivatives could act on $\alpha_2$-adrenoceptors in the brain and induce antinociception. Ono et al. (24) claimed that PEA and methamphetamine act on the spinal cord, and, activation of $\alpha_2$-adrenoceptor in the spinal cord produces antinociception (25). Therefore, it is still unclear which portion of the CNS is the important part containing the central $\alpha_2$-adrenoceptors involved in PEA analog-induced antinociception.

From the above findings, it is concluded that PEA derivatives may induce antinociception as a result of stimulating $\alpha_2$-adrenoceptors in the CNS. The $\alpha_2$-adrenoceptors in the CNS may be stimulated directly by PEA derivatives and/or indirectly by endogenous noradrenaline released by the PEA derivatives.

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