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An α-adrenoceptor-mediated mechanism of hypoactivity induced by β-amyrin palmitate

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Abstract—Inhibitory effects of β-amyrin palmitate in locomotor activity of mice were studied by combining this compound with α-adrenergic agonists or antagonists and a dopaminergic agonist. β-Amyrin palmitate (2·5, 5·0 and 10·0 mg kg^{-1}, i.p.) decreased locomotor activity of mice in a dose-dependent manner. It enhanced hypoactivity of mice treated with clonidine (0·025 mg kg^{-1}, i.p.) and antagonized hyperactivity produced by phenylephrine (40 μg, i.c.v.). The inhibitory action of β-amyrin palmitate was not affected by yohimbine (1·5 mg kg^{-1}, i.p.), but was potentiated by prazosin (0·75 mg kg^{-1}, i.p.). When combined with a dopaminergic agonist, apomorphine (2·0 mg kg^{-1}, i.p.), β-amyrin palmitate (5·0 and 10·0 mg kg^{-1}, i.p.) did not affect locomotor stimulation produced by apomorphine. These results suggest that β-amyrin palmitate might inhibit α_1-adrenoceptors.

In our recent studies on natural compounds possessing antidepressant activity, we have isolated β-amyrin palmitate as an active compound from the leaves of *Lobelia inflata*. This compound shows antidepressant activity in the forced swimming test in mice (Subarnas et al 1992). In the experiment to evaluate the effects of β-amyrin palmitate on the central nervous system, this compound decreased locomotor activity and antagonized methamphetamine-induced hyperactivity of mice (Subarnas et al 1993). The antagonistic effect of β-amyrin palmitate on the methamphetamine-induced locomotor stimulation led us to assume that this compound might have a blocking action on dopamine receptors or α_1-adrenoceptors. This assumption is based on the previous findings that the effect of amphetamine on locomotor activity is antagonized either by the non-selective dopamine receptor antagonist, pimozide, or the selective α_1-adrenoceptor antagonist, prazosin (Przegalinski & Jurkowska 1990).

In this experiment, we attempted to study the effect of β-amyrin palmitate on α-adrenergic receptors and dopamine receptors. For this purpose, we investigated the effect of β-amyrin palmitate in combination with α-agonists or antagonists and with a dopamine receptor agonist on locomotor activity of mice.

Materials and methods

Animals. Male ddy strain mice, 23–26 g, were placed in plastic cages with free access to food and water, and housed under standard conditions (room temperature: 23 ± 1°C; constant humidity). The room light was automatically controlled on a 12 h light/dark cycle.

Drugs. Drugs or compounds used in this study were β-amyrin palmitate, phenylephrine hydrochloride (Sigma Chemical Company, USA), clonidine hydrochloride (Tokyo Chemical Industry, Japan), yohimbine (Tokyo Chemical Industry), prazosin hydrochloride (Tokyo Chemical Industry), and apomorphine (Tokyo Chemical Industry).

Measurement of locomotor activity. Locomotor activity was measured by an Animex Auto MK-110 (Muromachi Kikai Co. Ltd, Japan). The mice were allowed 15 min to adapt to the activity cages. In the experiments in which β-amyrin palmitate was combined with α_1- or α_2-agonists (phenylephrine or clonidine) or a dopamine receptor agonist (apomorphine), the mice were first given β-amyrin palmitate, and 30 min later phenylephrine (40 μg), clonidine (0·025 mg kg^{-1}) or apomorphine (2·0 mg kg^{-1}) was administered. The activity was measured immediately after the administration of phenylephrine or clonidine. In the experiments in which β-amyrin palmitate was combined with α_1- or α_2-antagonists (prazosin or yohimbine), β-amyrin palmitate was given to mice 30 min after the administration of prazosin (0·75 mg kg^{-1}) or yohimbine (1·5 mg kg^{-1}). The activity was measured immediately after the administration of β-amyrin palmitate. In all experiments, the number of activity counts was recorded every 15 min in a 90-min period. The experiments were consistently carried out from 0900 to 1600 h. The doses of β-amyrin palmitate used were 2·5 and 5·0 mg kg^{-1} for the experiments with α-agonists or antagonists, and 5·0 and 10·0 mg kg^{-1} for the experiment with a dopamine receptor agonist. β-Amyrin palmitate, clonidine, prazosin, yohimbine and apomorphine were given to mice intraperitoneally, and phenylephrine was administered intracerebroventricularly. Nine to twelve mice were used for each dose.

Statistical analysis. Data were analysed by analysis of variance, and the significance of difference was calculated according to Dunnett's test.

Results

Effects of β-amyrin palmitate on clonidine-induced locomotor activity. Fig. 1A shows that clonidine (0·025 mg kg^{-1}) significantly reduced locomotor activity of mice 15–45 min after its administration. β-Amyrin palmitate at a dose of 5·0 mg kg^{-1} significantly decreased the clonidine-induced locomotor activity 60–75 min after the administration of clonidine. β-Amyrin palmitate itself at these two doses given to mice 30 min before treatment with 0·9% NaCl (saline) decreased locomotor activity significantly 15 min and 15–45 min, respectively, after the administration of saline.

Effects of β-amyrin palmitate on phenylephrine-induced locomotor activity. Fig. 1B shows that phenylephrine (40 μg) given to mice 30 min after pretreatment with vehicle increased locomotor activity significantly, as compared with that induced by control (Tween 80 + cerebrospinal fluid). The significant increase in locomotor activity was observed 60–90 min after the administration of phenylephrine. β-Amyrin palmitate at doses of 2·5 and 5·0 mg kg^{-1} significantly decreased hyperactivity of mice treated with phenylephrine 45–90 min after the injection of phenylephrine.

Effects of yohimbine on β-amyrin palmitate-induced locomotor activity. β-Amyrin palmitate at doses of 2·5 and 5·0 mg kg^{-1} administered to mice 30 min after the mice were treated with saline reduced locomotor activity (Fig. 2A). This decrease in locomotor activity was not affected by yohimbine (1·5 mg kg^{-1}). Yohimbine significantly decreased the activity of mice 30 min

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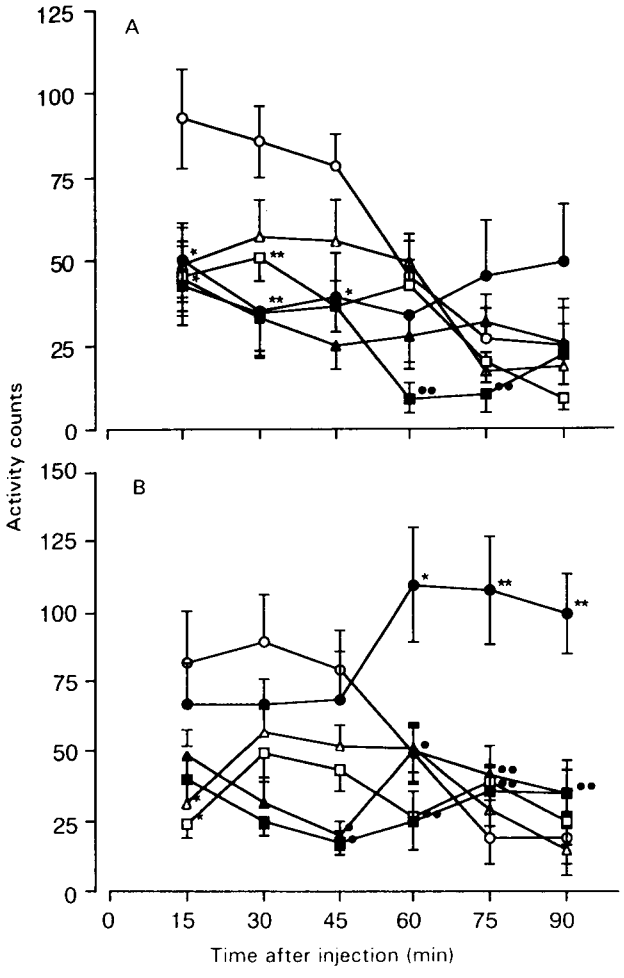

FIG. 1. Effects of β-amyrin palmitate on locomotor activity induced by clonidine (A) and phenylephrine (B). Each point indicates the mean value of activity. A. ○ Tween 80 + saline, ● Tween 80 + clonidine, △ β-amyrin palmitate (2·5 mg kg^{-1}) + saline, ▲ β-amyrin palmitate (2·5 mg kg^{-1}) + clonidine, □ β-amyrin palmitate (5·0 mg kg^{-1}) + saline, ■ β-amyrin palmitate (5·0 mg kg^{-1}) + clonidine. B. ○ Tween 80 + cerebrospinal fluid, ● Tween 80 + phenylephrine, △ β-amyrin palmitate (2·5 mg kg^{-1}) + cerebrospinal fluid, ▲ β-amyrin palmitate (2·5 mg kg^{-1}) + phenylephrine, □ β-amyrin palmitate (5·0 mg kg^{-1}) + cerebrospinal fluid, ■ β-amyrin palmitate (5·0 mg kg^{-1}) + phenylephrine. Vertical bars represent standard errors of means (s.e.m.). * $P < 0.05$, ** $P < 0.01$ compared with the Tween 80 + saline or cerebrospinal fluid values according to Dunnett's test. • $P < 0.05$, •• $P < 0.01$ compared with the Tween 80 + clonidine or phenylephrine values according to Dunnett's test.

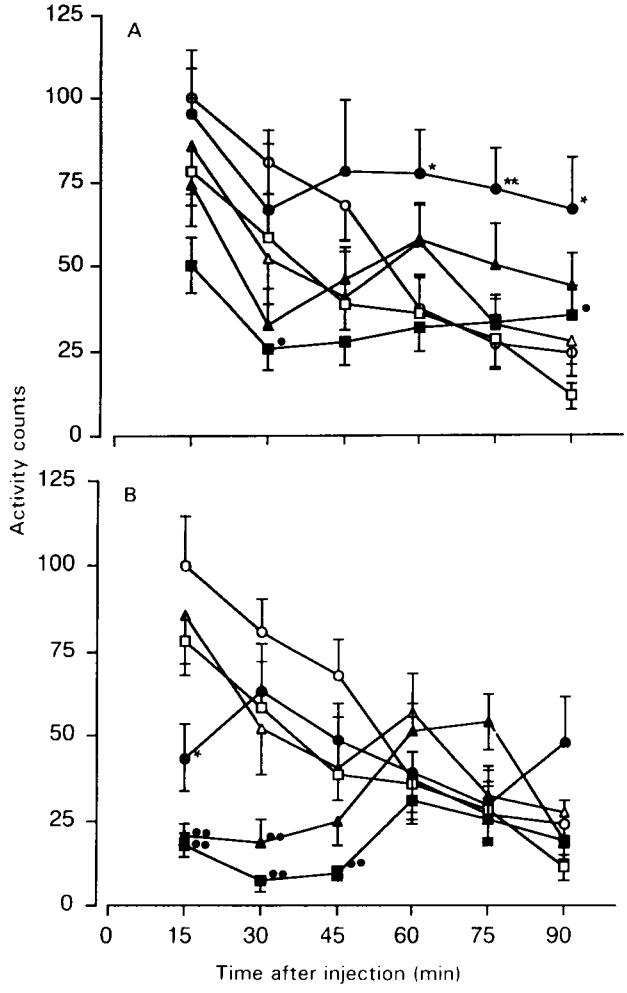

FIG. 2. Effects of yohimbine (A) and prazosin (B) on β-amyrin palmitate-induced locomotor activity. Each point indicates the mean value of activity counts. A. ○ Saline + Tween 80, ● yohimbine + Tween 80, △ saline + β-amyrin palmitate (2·5 mg kg^{-1}), ▲ yohimbine + β-amyrin palmitate (2·5 mg kg^{-1}), □ saline + β-amyrin palmitate (5·0 mg kg^{-1}), ■ yohimbine + β-amyrin palmitate (5·0 mg kg^{-1}). B. ○ Saline + Tween 80, ● prazosin + Tween 80, △ saline + β-amyrin palmitate (2·5 mg kg^{-1}), ▲ prazosin + β-amyrin palmitate (2·5 mg kg^{-1}), □ saline + β-amyrin palmitate (5·0 mg kg^{-1}), ■ prazosin + β-amyrin palmitate (5·0 mg kg^{-1}). Vertical bars represent standard errors of means (s.e.m.). * $P < 0.05$, ** $P < 0.01$ compared with the saline + Tween 80 values according to Dunnett's test. • $P < 0.05$, •• $P < 0.01$ compared with the saline + β-amyrin palmitate (2·5 or 5·0 mg kg^{-1}) values according to Dunnett's test.

after the administration of β-amyrin palmitate at a dose of 5·0 mg kg^{-1}, but significantly increased the activity 90 min after. Yohimbine given to mice 30 min before the administration of Tween 80 caused a significant increase in locomotor activity 60–90 min after the vehicle administration in comparison with that induced by control (saline + Tween 80).

Effects of prazosin on β-amyrin palmitate-induced locomotor activity. β-Amyrin palmitate at doses of 2·5 and 5·0 mg kg^{-1} decreased locomotor activity of mice. This inhibitory effect of β-amyrin palmitate was potentiated by prazosin (0·75 mg kg^{-1}) given to mice 30 min before treatment with β-amyrin palmitate (Fig. 2B). The significant potentiation was observed 15–30 and 15–45 min after the administration of β-amyrin palmitate at doses of 2·5 and 5·0 mg kg^{-1}, respectively, as compared with the activity counts of saline-pretreated mice treated with the related doses of β-amyrin palmitate. Prazosin itself decreased locomotor activity of mice and showed a significant difference from the control (saline + Tween 80) values 15 min after the administration of vehicle.

Effects of β-amyrin palmitate on apomorphine-stimulated locomotor activity. Apomorphine (2·0 mg kg^{-1}) increased locomotor activity of mice significantly 15–30 min after the administration of apomorphine and decreased it significantly 45–60 min after in comparison with that produced by control (Tween 80 + saline) (Fig. 3). β-Amyrin palmitate at doses of 5·0 and 10·0 mg kg^{-1} did not affect the increase in locomotor activity of mice treated with apomorphine.

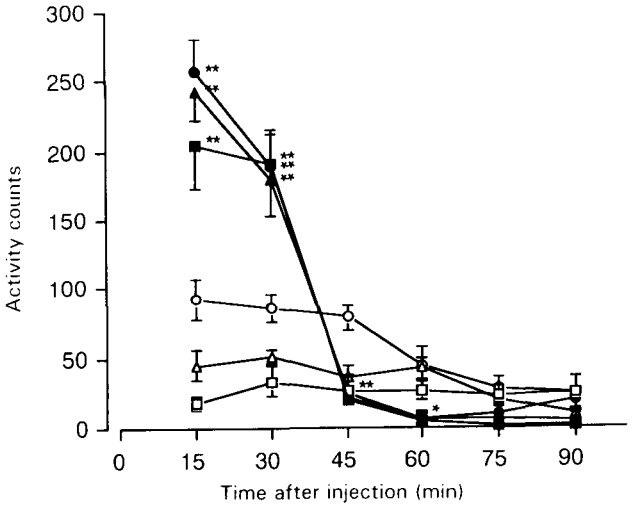

FIG. 3. Effects of β-amyrin palmitate on apomorphine-induced locomotor stimulation. Each point indicates the mean value of activity counts. ○ Tween 80+saline, ● Tween 80+apomorphine, △ β-amyrin palmitate (5·0 mg kg^{-1})+saline, ▲ β-amyrin palmitate (5·0 mg kg^{-1})+apomorphine, □ β-amyrin palmitate (10·0 mg kg^{-1})+saline, ■ β-amyrin palmitate (10·0 mg kg^{-1})+apomorphine. Vertical bars represent standard errors of means (s.e.m.). * $P<0.05$, ** $P<0.01$ compared with the Tween 80+saline values according to Dunnett's test.

Discussion

Clonidine decreased locomotor activity of mice. This inhibitory action of clonidine was not significantly potentiated by β-amyrin palmitate, a finding suggesting that β-amyrin palmitate might not stimulate α_2-receptors. This assumption is based on the evidence that the reduction in locomotor activity elicited by clonidine is due to the stimulation of central α_2-receptors (Clineschmidt et al 1979; Heal et al 1983). Moreover, yohimbine known as a selective α_2-antagonist did not affect the decrease in the activity elicited by β-amyrin palmitate, indicating that the inhibitory action of β-amyrin palmitate might not be due to the stimulation of α_2-receptors.

In the experiment with phenylephrine, β-amyrin palmitate significantly antagonized the effect of phenylephrine. This suggests that β-amyrin palmitate might block α_1-receptors, since the phenylephrine-induced locomotor stimulation is caused by the stimulation of α_1-receptors (Clineschmidt et al 1979; Heal 1984). Moreover, the potentiation of the β-amyrin palmitate-induced hypoactivity by prazosin also indicates that the inhibitory action of β-amyrin palmitate might be connected with α_1-receptors.

In the experiment with a dopamine receptor agonist, β-amyrin palmitate did not affect the locomotor stimulation produced by apomorphine. This indicates that β-amyrin palmitate might not have an effect on D_1 and D_2 dopamine receptors, since the behavioural effects of apomorphine are due to the simultaneous stimulation of D_1 and D_2 dopamine receptors (Braun & Chase 1986; Vasse et al 1988).

In conclusion, the inhibitory effect of β-amyrin palmitate on locomotor activity of mice might be due to its inhibitory action on α_1-receptors.

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