Modulation of Morphine Action by Lauric Acid

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Intraperitoneal administration of lauric acid (C_{12}) at the high doses, 100—1000 μmol/kg, showed weak but dose-dependent antinociceptive effect in mice. Pretreatment of the animals with 0.1 μmol/kg of i.p. C_{12} tended to suppress the antinociceptive effect of 7 mg/kg of s.c. morphine and daily combination of this dose of C_{12} with 10 mg/kg of s.c. morphine blocked the development of antinociceptive tolerance to morphine. However, increasing or decreasing of the dose of C_{12} resulted in the loss of its modulatory effect on morphine. The strict dose-dependency of C_{12} in its action on morphine suggests that there is a regulatory role for C_{12}, a medium length straight chain fatty acid, in the endogenous pain inhibitory system.

Keywords lauric acid; antinociceptive effect; morphine; tolerance

In a series of experiments on the pharmacological effect of medium length straight chain fatty acids, C_6–C_{18}, we found that these acids exhibit anticonvulsive and antinociceptive effect in mice. The effects depend on chain length,[1,2] and, among the fatty acids tested, lauric acid (C_{12}) showed almost the maximal effect in both tests. In the present paper, we have carried out further studies on the central actions of C_{12}, especially its interaction with morphine. This was to investigate the possible participation of C_{12} in the activation of an endogenous pain inhibitory system.

Materials and Methods

Materials Sodium salt of lauric acid (C_{12}, Nacalai Tesque) and morphine–HCl (Takeda) were dissolved in saline so that the dose was contained in a volume of 0.1 ml/10 g of body weight. C_{12} was administered intraperitoneally (i.p.) and morphine was injected subcutaneously (s.c.).

Animals Male mice of the ddY strain, weighing 20 to 23 g (Ohtsubo Experimental Animals), were purchased and housed in a temperature-controled room with free access to food and water. After reaching 25 to 30 g, they were used for the experiments.

Evaluation of Antinociceptive Effect The antinociceptive effect was measured by a modification of Haffner's method,[3] using a cut-off time of 6 s to avoid tissue damage. Measurements were made at intervals of 15 min for 60 or 90 min after administration of C_{12} and morphine, respectively. The effect was calculated as an area under the curve (*AUC*) by plotting the increase of response time (s) on the ordinate and time intervals (min) on the abscissa.

Assessment of Tolerance The antinociceptive effect of morphine alone or in combination with C_{12} was determined daily for 5 d and the decrease in *AUC*, compared with that on the 1st day, was considered to indicate the development of tolerance.

Statistical Analysis The statistical significance of the data was evaluated by the analysis of variance followed by Dunnett's analysis for individual comparisons.

Results and Discussion

Antinociceptive Effect of C_{12} As reported in our previous paper,[2] C_{12} produced a dose-dependent antinociceptive effect with a peak at 15 min after administration (Fig. 1). Because of the solubility of C_{12} in saline the maximum dose was 1000 μmol/kg; this dose was maximum tolerable dose after a single injection and 3 out of 7 animals died within 24 h.

Effect on Morphine Antinociception The antinociceptive effect of 7 mg/kg of morphine was slightly, but not significantly, suppressed by pretreatment with 0.1 μmol/kg of C_{12}, 15 min before injection of morphine. However, any further decrease or increase in the dose of C_{12} failed to affect morphine antinociception (Fig. 2).

Effect on the Development of Antinociceptive Tolerance to Morphine Daily repeated treatment with 10 mg/kg of s.c. morphine resulted in gradual loss of the antinociceptive effect, indicating the development of tolerance to the effect. The development of tolerance was completely suppressed by daily combined treatment with 0.1 μmol/kg of C_{12}, given 5 min after morphine injection, which did not affect the antinociceptive effect of morphine. The lower dose, 0.01 μmol/kg, or the higher dose, 10 μmol/kg of C_{12} that did not show any apparent toxic effect by daily administration, was ineffective as far as the development of tolerance was concerned. The suppressive effect of C_{12} on the development of morphine tolerance was maintained as long as the combined treatment was continued for 10 d. However, when

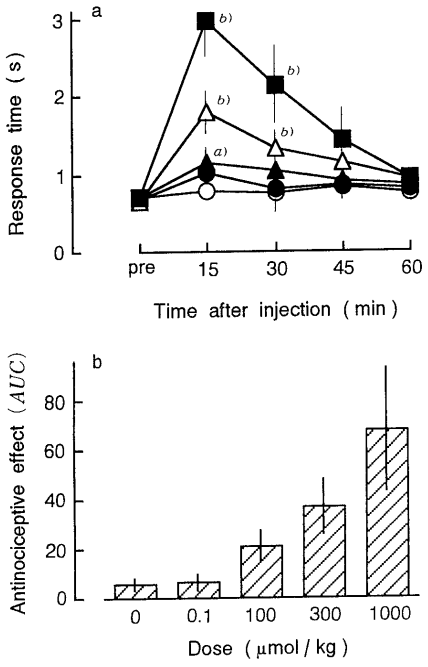

Fig. 1. Antinociceptive Effect of Lauric Acid

a) Dose–response curve. b) The antinociceptive effect was expressed as *AUC* calculated from a). Mice were given 0.1 (●), 100 (▲), 300 (△) and 1000 (■) μmol/kg of i.p. lauric acid. Control group (○) was treated with saline instead of lauric acid. The antinociceptive effect was measured by a modification of Haffner's method, every 15 min for 60 min after injection. Data are the mean ± S.E.M. of 6 animals. Significantly different from control, *a*) $p<0.05$, *b*) $p<0.01$.

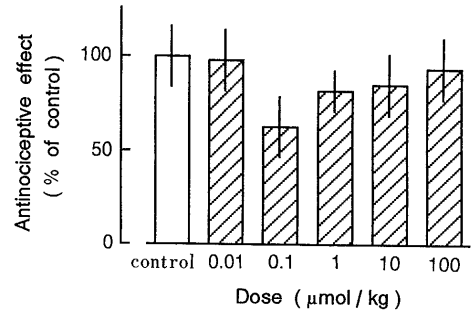

Fig. 2. Effect of Lauric Acid on Morphine Antinociception

Mice were treated with various doses of i.p. lauric acid 20 min before 7 mg/kg of s.c. morphine. Control group was given saline instead of lauric acid. Data are the mean ± S.E.M. of 6—7 animals.

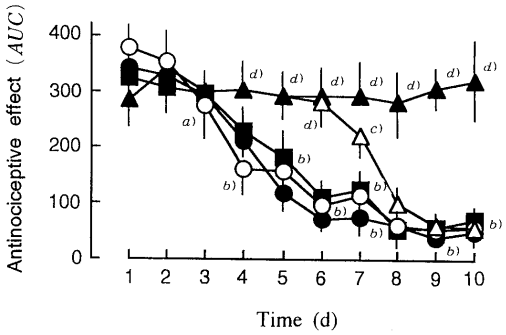

Fig. 3. Effect of Lauric Acid on the Development of Antinociceptive Tolerance to Morphine

Lauric acid, 0.01 (●), 0.1 (▲), and 10 (■) μmol/kg, i.p., was given daily 5 min after 10 mg/kg of s.c. morphine. Control group (○) received vehicle instead of lauric acid. Saline was given instead of lauric acid from the 6th day (△). Data are the mean ± S.E.M. of 6—7 animals. Significantly different from the control group on the 1st day, *a*) $p<0.05$, *b*) $p<0.01$. Significantly different from the control group on respective days, *c*) $p<0.05$, *d*) $p<0.01$.

the C_{12} was omitted, tolerance developed as rapidly as in control group (Fig. 3).

Thus, we have confirmed our previous finding that i.p. administration of C_{12} produces a dose-dependent antinociceptive effect in mice.[2] The C_{12}, at the dose of 0.1 μmol/kg, i.p. which is almost an ineffective dose on its own, was able to suppress the antinociceptive effect of morphine and also the development of tolerance to the effect.

It is widely accepted that various stressful stimuli induce an antinociceptive effect, known as stress-induced analgesia, SIA,[4] and we have demonstrated that the development of antinociceptive tolerance to daily morphine is suppressed by combined exposure of the animals to some kinds of stresses.[5] It is also well recognized that stressful stimuli activate the hypothalamo-pituitary-adrenocortical system and result in elevated plasma levels of adrenal cortical steroids and adrenaline,[6,7] accompanied by a marked increase in free fatty acids (FFA).[6] The changes occured even in the brain with raised levels of long chain and unsaturated fatty acids, such as palmitic acid (C_{16}), stearic acid (C_{18}), oleic acid ($C_{18:1}$) and arachidonic acid ($C_{20:4}$).[8] Plasma levels of the short chain fatty acids, including C_{12}, in naive animals are extremely low compared with those of long chain and unsaturated fatty acids and the change in plasma C_{12} levels after exposure to stress has not been reported. The fact that C_{12} tended to suppress the antinociceptive effect of morphine and blocked the development of tolerance to the effect only at a dose of 0.1 μmol/kg, while lower or higher doses were ineffective, suggests a characteristic importance of medium chain length fatty acids in central nervous system functions. The higher dose may cause some non-specific effects though no remarkable behavioral changes or toxic effects were observed. In a recent preliminary experiment, we found that the plasma level of C_{12} after a single i.p. injection of 0.1 μmol/kg, the effective dose for the suppression of morphine tolerance, declined quickly within 10 min but tended to induce a fairly sustained increase in plasma C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$ levels in mice (data not shown).

We concluded that C_{12}, presumably mediating by mobilization of long chain and unsaturated fatty acids, similar to the response subsequent to stress exposure, produced antinociception and suppression of tolerance development. This suggests an important role for medium length fatty acids in the endogenous pain inhibitory system.

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