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M_1 receptor activation is a requirement for arecoline analgesia☆

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Abstract

Arecoline, a drug obtained from the *Areca Catechu L.*, induced a dose-dependent antinociception (0.3–1 mg kg^{-1} i.p.) which was prevented by the muscarinic antagonists pirenzepine (0.1 μg per mouse i.c.v.) and *S*-(−)-ET-126 (0.01 μg per mouse i.c.v.). A dose-dependent inhibition of the antinociception induced by arecoline was observed after inactivation of the M_1 gene by an antisense oligodeoxyribonucleotide (aODN). This effect was detected 24 h after the last i.c.v. injection of aODN. These results indicate that arecoline antinociception is mediated by the activation of central M_1 muscarinic receptors. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: Arecoline; Analgesia; Central cholinergic system; M1 receptor; aODN

1. Introduction

Arecoline is a component, together with arecaine, guvacine and guvacholine, in the areca walnut that is the seed of *Areca Catechu L.* endowed with antinociceptive properties through a cholinergic mechanism [1,2].

M_1 selective agonists McN-A-343 and AF-102B are able to produce a significant enhancement of the pain threshold antagonised by the M_1 antagonists dicyclomine and pirenzepine [3]. Since it has been demonstrated that muscarinic analgesia in mice and rats is mediated by post-synaptic M_1 receptors, we thought it worthwhile to identify the muscarinic receptor subtype responsible for arecoline antinociception.

2. Experimental

2.1. Animals

Male Swiss albino mice (23–30 g) from Morini (San Polo d'Enza, Italy) were used. All experiments were carried out according to the guidelines of the European Community Council for experimental animal care.

2.2. Antisense oligonucleotides

The 18-mer phosphorothioate antisense ODN (aODN) 5′-CAC TGA GGT GTT CAT TGC-3′ complementary to the residues 112–129 of the published mouse M_1 cDNA sequence [4] and the 18-mer phosphorothioate fully degenerate ODN (dODN) 5′-NNN NNN NNN NNN NNN NNN-3′ (where N is G, C, A, or T) were vehiculated intracellularly by an artificial cationic lipid (DOTAP 13 μM). The i.c.v. administration of ODNs was performed under ether anaesthesia according to the method described by Haley and McCormick [5].

2.3. Hot-plate test

The method adopted was described by O'Callaghan and Holtzman [6].

2.4. Statistical analysis

All experimental results are given as the means ± SEM. An analysis of variance (ANOVA), followed by Fisher's protected least significant difference (PLSD) procedure for post-hoc comparison, was used to verify

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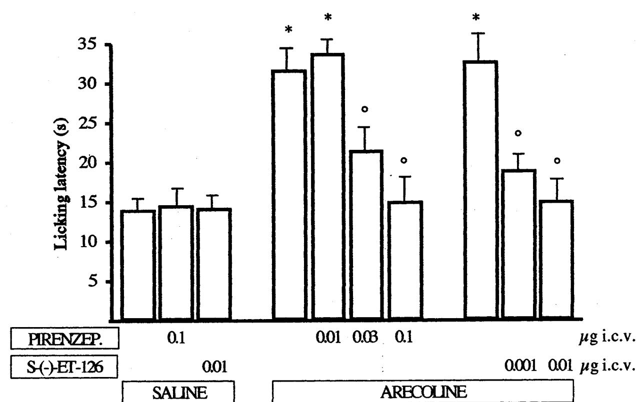

Fig. 1. Effect of pirenzepine and S-(−)-ET-126 on arecoline-induced antinociception in mouse hot-plate test. Pirenzepine and S-(−)-ET-126 were administered 10 min before arecoline. Nociceptive response was recorded 15 min after arecoline (1 mg kg^{-1} i.p.) injection. Number of mice ranged between 8 and 18. Vertical lines show SEM. $^{*}P<0.01$ vs saline-treated mice. $^{\circ}P<0.01$ vs arecoline-treated mice.

the significance of differences between two means. Data were analysed with the StatView software for the Macintosh (1992).

3. Results and discussion

The selective M_1 antagonists pirenzepine (0.03–0.1 μg per mouse i.c.v.), and S-(−)-ET-126 (0.001–0.01 μg per mouse i.c.v.) prevented arecoline (1 mg kg^{-1} i.p.) antinociception (Fig. 1). Pirenzepine and S-(−)-ET-126, at the same concentration, did not prevent the analgesia induced by morphine (7 mg kg^{-1} i.p.) and amitriptyline (15 mg kg^{-1} i.p.) (data not shown). aODN, at the dose of 0.3 nmol per i.c.v. injection, did not significantly affect arecoline (Fig. 2, panel A) analgesia whereas at the dose of 1.0 and 2.0 nmol per i.c.v. injection, aODN dose-dependently prevented arecoline (Fig. 2, panels B and C) antinociception. The regression line which illustrates the dose-dependent reduction of arecoline antinociception produced by increasing concentrations of aODN is shown in Fig. 2, panel D. The prevention by the i.c.v. injection of the aODN also indicates that the antinociception induced by arecoline is centrally mediated.

The aODN pretreatment (2.0 nmol per i.c.v. injection) did not reduce the pain threshold in mice showing lack of any hyperalgesic effect (Fig. 2), excluding the

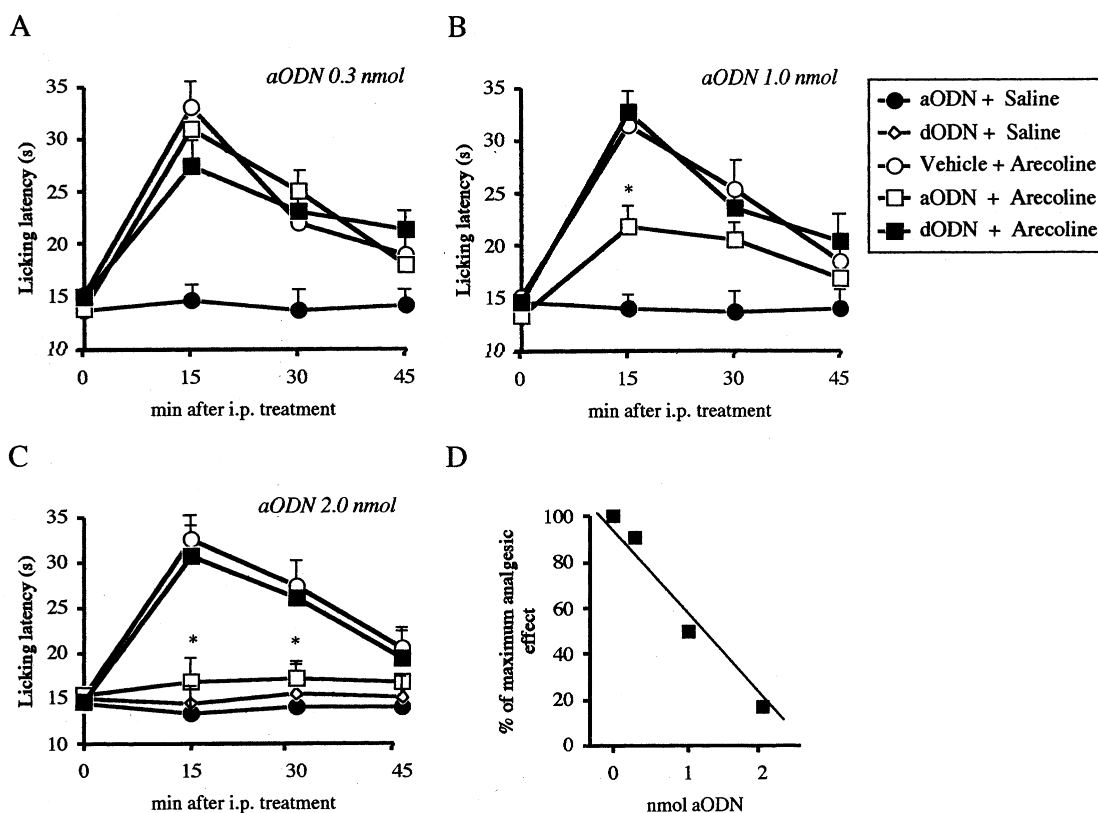

Fig. 2. Prevention of arecoline (1 mg kg^{-1} i.p.)-induced antinociception by pretreatment with an antisense ODN (aODN) to M_1 gene in the mouse hot-plate test (panels A–C) and effect of increasing concentrations of aODN to M_1 gene on arecoline (1 mg kg^{-1} i.p.)-induced antinociception in the same test (panel D). Mice were i.c.v. injected with vehicle, aODN or degenerated ODN (dODN) at the dose of 0.3 (panel A), 1.0 (panel B) and 2.0 nmol (panel C) per single i.c.v. injection on days 1, 4 and 7. The hot-plate test was performed 24 h after the last i.c.v. injection. The evaluation of the analgesic effect was carried out 15 min after arecoline administration. Vertical lines give SEM. Each point represents the mean of 10–14 mice. $^{*}P<0.01$ in comparison with dODN + arecoline-treated mice.

possibility that the prevention of arecoline antinociception is due to a hyperalgesic effect of the treatment used.

In summary, our results have shown that arecoline is able to produce antinociception in mice by activating M_1 muscarinic receptor subtype.

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