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European Journal of Pharmacology 485 (2004) 193–196
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Short communication

Loss of amitriptyline analgesia in α_{2A}-adrenoceptor deficient mice

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Received 25 August 2003; received in revised form 12 November 2003; accepted 18 November 2003

Abstract

Tricyclic antidepressants have analgesic and sedative effects in addition to their antidepressive properties. We tested the acute analgesic and locomotor inhibitory effects of the tricyclic antidepressant amitriptyline and the α_2-adrenoceptor agonist clonidine in wild-type control and in α_{2A}-adrenoceptor knockout mice in hot-plate and tail-flick tests. Amitriptyline-induced analgesia was lost in α_{2A}-adrenoceptor knockout mice. The locomotor inhibitory effect of amitriptyline was reduced, but not fully abolished in α_{2A}-adrenoceptor knockout mice. Similar results were obtained with clonidine. We conclude that α_{2A}-adrenoceptors appear to have a significant role in amitriptyline-induced acute analgesia in mice, and that α_{2A}-adrenoceptors also participate in the sedative effects of amitriptyline.

Keywords: Locomotor activity; Analgesia; Sedation; Amitriptyline; α_{2A}-Adrenoceptor; Clonidine; (Mouse)

1. Introduction

Tricyclic antidepressants are efficacious and commonly used in the management of chronic pain states, such as diabetic neuropathy (Max et al., 1987, 1991) and postherpetic neuralgia (Kishore-Kumar et al., 1990; Watson et al., 1992). In rodents, the analgesic efficacy of tricyclic antidepressants has been shown in both acute and chronic pain models (Sawynok et al., 2001). Tricyclic antidepressants block the neuronal reuptake of the monoamine transmitters noradrenaline and 5-hydroxytryptamine (5-HT), and interact with numerous neurotransmitter receptors including muscarinic, histamine, 5-HT and dopamine receptors, and α_1- and α_2-adrenoceptors. Amitriptyline is a typical tricyclic antidepressant that has been used as an analgesic for decades.

Both noradrenergic and serotonergic neuronal systems modulate pain transmission and may thus mediate the analgesia induced by tricyclic antidepressants. The exact analgesic mechanisms of these agents are not fully understood, and several routes have been proposed. α_2-Adrenoceptor antagonists have been shown to block amitriptyline-induced acute analgesia in mice (Ghelardini et al., 2000; Gray et al., 1999), and experiments with a limited set of subtype-preferring antagonists have suggested the involvement of the α_{2A}-adrenoceptor subtype (Ghelardini et al., 2000). Beyond the receptor level, activation of pertussis toxin-sensitive $G_{i/o}$-proteins has been shown to be critical for the acute analgesic effects of amitriptyline (Ghelardini et al., 2001).

Sedation is a well-established side effect of tricyclic antidepressants. In animals, amitriptyline appears to be among the most sedative tricyclic drugs (Ögren et al., 1981). The mechanism of the sedation induced by tricyclic antidepressants is usually thought to be related to blockade of histamine H_1 receptors. Moreover, antagonism of muscarinic and 5-HT receptors and α_1-adrenoceptors has been suggested to be important for the sedative effects of amitriptyline (Ögren et al., 1981).

α_2-Adrenoceptor agonists, such as clonidine and dexmedetomidine, induce analgesia and sedation in experimental animals and in man. Studies with transgenic mice have shown that both α_2-adrenergic analgesia and sedation are mainly mediated by the α_{2A}-adrenoceptor subtype (Lähdesmäki et al., 2003a,b; Lakhlani et al., 1997; Stone et al., 1997). The present study was aimed to explore the involvement of the α_{2A}-adrenoceptor subtype in mediation of the acute analgesic and sedative effects of the classical tricyclic antidepressant, amitriptyline, in mice.

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doi:10.1016/j.ejphar.2003.11.047

2. Materials and methods

2.1. Animals

Male C57 Bl/6J wild-type control mice and mice with targeted disruption of the α_{2A}-adrenoceptor gene (α_{2A}-knockout) weighing 20–35 g were used. The generation of an α_{2A}-adrenoceptor knockout mouse strain has been described previously (Altman et al., 1999). The α_{2A}-adrenoceptor knockout mice were backcrossed to C57Bl/6J mice for a minimum of five generations to produce a congenic strain. Animals had free access to food and water and were maintained under controlled conditions. All experiments were approved by the local committee for animal welfare and were in accordance with the European Communities Council Directive of 24 November 1986 (86/906/EEC). Each mouse was tested only once. The group *n* was 8–10.

2.2. Drugs

Amitriptyline HCl, morphine HCl, and clonidine HCl were from Sigma (St. Louis, MO), were dissolved in physiological saline, and were administered in a volume of 10 ml/kg i.p.

2.3. Antinociceptive assays

The tail-flick method with a cutoff time of 10 s and the hot-plate method with 55 °C temperature and a cutoff time of 15 s were used. For analgesic test validation, 5 mg/kg morphine was administered to separate groups of control and α_{2A}-adrenoceptor knockout mice [60% and 86% of maximal possible effect (MPE) for tail-flick and 44% and 76% of MPE for hot-plate] (not shown). Basal tail-flick and hot-plate scores were first measured, followed by a second tail-flick and hot-plate measurement 30 min after i.p. administration of the test drugs (amitriptyline 5, 10, and 20 mg/kg, clonidine 5 mg/kg) or saline.

2.4. Spontaneous locomotor activity test

Following analgesia tests and 1 h after drug administration, the mice were placed individually into transparent polypropylene locomotor activity cages housed in a photobeam recording system (San Diego Instruments, San Diego, CA). Three different types of activity were recorded: ambulations, fine movements, and rearings (Lähdesmäki et al., 2002). The locomotor activity was measured over 15 min, with no habituation.

2.5. Statistical analysis

The effects of each drug on analgesic scores and locomotor activity were tested using two-way analysis of variance (ANOVA) followed by Scheffé post-hoc tests using SPSS programs (SPSS 8.0 for Windows, SPSS, Chicago, IL). Differences between genotypes were compared using independent samples *t*-tests. All results are presented as mean ± S.E.M. The level of significance was set at $P < 0.05$.

3. Results

3.1. Amitriptyline and clonidine analgesia are abolished in mice lacking α_{2A}-adrenoceptors

Amitriptyline had dose-dependent but weak analgesic effects in the tail-flick test in control mice but did not show any analgesic effect in α_{2A}-adrenoceptor knockout mice ($P < 0.001$; genotype × amitriptyline interaction) (Fig. 1A). Amitriptyline produced dose-dependent analgesia in the hot-plate test in wild-type mice (10%, 34%, and 80% of MPE), whereas amitriptyline had no analgesic effect in α_{2A}-adrenoceptor knockout mice ($P < 0.001$; genotype × amitriptyline interaction) (Fig. 1B). Lack of α_{2A}-adrenoceptor expression also almost totally abolished clonidine-induced analgesia. The analgesic efficacy of 5 mg/kg clonidine was 81% and 18% of MPE in the tail-flick test, and 79% and 17%

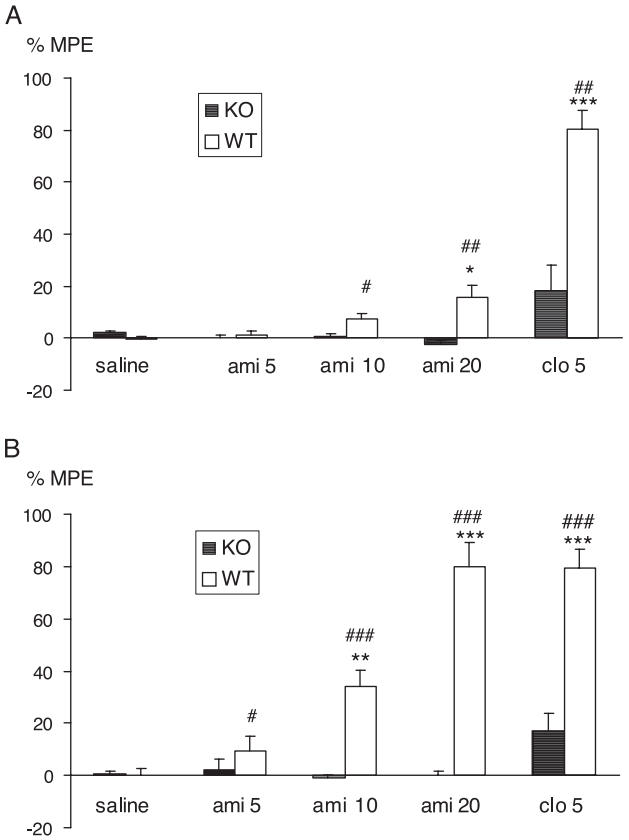

Fig. 1. The effects of amitriptyline (ami, mg/kg) and clonidine (clo, mg/kg) in analgesic tests in α_{2A}-adrenoceptor knockout and C57 wild-type control mice. Latencies were converted to mean ± S.E.M. percentage of maximal possible effect (% MPE). Asterisks (*) denote significant differences compared to saline groups (Scheffé tests) and # shows significant differences between mouse strains (*t*-tests; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$). (A) Tail-flick test; (B) hot-plate test.

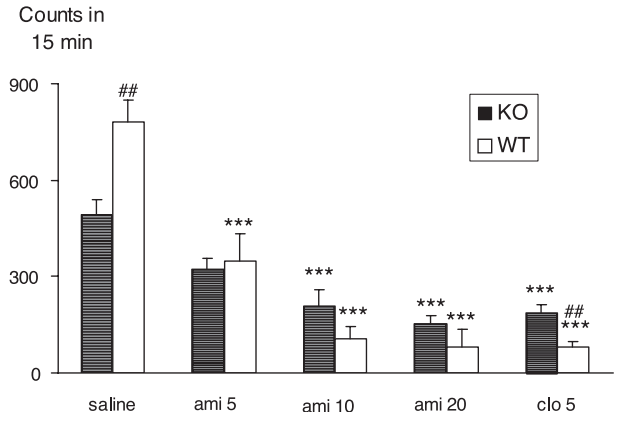

Fig. 2. The effects of amitriptyline and clonidine on locomotor activity in α_{2A}-adrenoceptor knockout and C57 wild-type mice. Total activity counts (ambulations + fine movements + rearings) are shown. Statistical significance and drug doses as indicated in Fig. 1.

in the hot-plate test in wild-type and α_{2A}-adrenoceptor knockout mice, respectively ($P<0.001$; genotype × clonidine interaction) (Fig. 1A and B).

3.2. Locomotor inhibition by amitriptyline and clonidine are attenuated in mice lacking α_{2A}-adrenoceptors

Amitriptyline dose-dependently decreased all modalities of locomotor activity (ambulations, fine movements, and rearings) in both genotypes, but the inhibitory effects were more pronounced in wild-type control mice ($P<0.001$, genotype × amitriptyline interaction, e.g. in total activity) (Fig. 2). In control mice, a highly significant decrease in activity was already noted with the lowest amitriptyline dose (66% reduction in total activity). Also in α_{2A}-adrenoceptor knockout mice, significant inhibition of locomotor activity was observed after amitriptyline 10 and 20 mg/kg ($P<0.001$, Scheffé test). Clonidine decreased all modalities of locomotor activity in both genotypes, but the sedative effect of clonidine was smaller in α_{2A}-adrenoceptor knockout than in wild-type control mice (62% decrease of total activity counts in α_{2A}-adrenoceptor knockout and 90% decrease in control mice) ($P<0.001$ genotype × clonidine interaction) (Fig. 2).

4. Discussion

Our results from tail-flick and hot-plate tests indicate that the acute analgesic effect of amitriptyline is lost in α_{2A}-adrenoceptor knockout mice. Also, the locomotor inhibitory effect of amitriptyline was attenuated in α_{2A}-adrenoceptor knockout mice, indicating that α_{2A}-adrenoceptors contribute to the sedative effects of amitriptyline.

Tricyclic antidepressants appear to induce antinociception through mechanisms related to the endogenous central pain-modulating systems (Sawynok et al., 2001). Descending noradrenergic and serotonergic pathways participate in the antinociception induced by tricyclic drugs. Depletion of central noradrenaline with α-methyl-*p*-tyrosine in mice (Valverde et al., 1994) and depletion of 5-HT with *p*-chlorophenylalanine in rats (Tura and Tura, 1990) inhibit antinociception by antidepressants. The descending inhibitory pathways have important roles in pain modulation as they project to the spinal dorsal horn where they inhibit nociceptive neurons. Lesions of the dorsolateral funiculus, through which descending bulbospinal pathways traverse, have been shown to inhibit analgesia produced by clomipramine in rats (Ardid et al., 1995). Descending central antinociception can be antagonized by intrathecal (Sawynok and Reid, 1992) and systemically administered α_2-adrenoceptor antagonists (Liu and Zhao, 1992; Schreiber et al., 1998, 1999) and 5-HT receptor antagonists (Liu et al., 2002). The principal α_2-adrenoceptor subtype mediating antinociception has recently been identified as the α_{2A}-adrenoceptor (Lakhlani et al., 1997; Stone et al., 1997). In the dorsal horn of the spinal cord, activation of both noradrenergic and 5-HT receptors produces analgesia, and noradrenergic and 5-HT mechanisms interact in the modulation of pain. Antinociception by 5-HT is blocked with α_2-adrenoceptor antagonists and by depletion of endogenous noradrenaline by 6-hydroxydopamine (Archer et al., 1986; Sawynok and Reid, 1996). The baseline analgesic scores were similar between α_{2A}-adrenoceptor knockout and wild-type control mice, as also reported earlier (Lähdesmäki et al., 2003b; Malmberg et al., 2001), indicating that the α_{2A}-adrenoceptors are not tonically active. It is therefore possible that the increased descending noradrenergic transmission after amitriptyline leads to activation of α_{2A}-adrenoceptors possibly at the spinal level, which could at least partly underlie its acute analgesic effect.

In addition to supraspinal pain-modulating mechanisms, amitriptyline also has antihyperalgesic effects at spinal and peripheral levels in neuropathic animals (Abdi et al., 1998; Esser and Sawynok, 1999).

Clonidine is a classical α_2-adrenoceptor agonist, but it also activates α_1-adrenoceptors in higher doses. Clonidine is a potent sympatholytic drug and inhibits adrenergic outflow in the brain by an action on α_2-adrenoceptors. Clonidine has analgesic and sedative effects that are probably mediated by α_{2A}-adrenoceptors, like those of the more selective α_2-adrenoceptor agonist, dexmedetomidine (Lähdesmäki et al., 2003a,b; Lakhlani et al., 1997; Stone et al., 1997). The analgesic effect of clonidine was lost and the locomotor inhibitory effect of clonidine was decreased in α_{2A}-adrenoceptor knockout mice.

We conclude that α_{2A}-adrenoceptors have a significant role in mediating the acute analgesic effect of amitriptyline, even though amitriptyline analgesia seems to involve multiple receptor systems. The mechanisms involved in the mediation of the analgesic effect of tricyclic antidepressant drugs in acute and chronic pain conditions may, however, differ. Still, in rodent models, these agents seem to be

efficacious both in acute and chronic pain, at least upon thermal stimulation (Sawynok et al., 2001). In addition to mediating antinociception, α_{2A}-adrenoceptors appear to have a major role in the locomotor inhibitory activity of amitriptyline, indicating that α_{2A}-adrenoceptors contribute also to the sedative effects of amitriptyline.

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