ELSEVIER

European Journal of Pharmacology 298 (1996) 51–55

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Blockade of the polyamine site of NMDA receptors produces antinociception and enhances the effect of morphine, in mice

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Received 17 August 1995; revised 9 November 1995; accepted 14 November 1995

Abstract

The possible effect of ifenprodil – a potent antagonist at the polyamine site of the NMDA receptor complex – on nociceptive threshold and morphine analgesia was investigated in mice. In the hot plate test, the intraperitoneal (i.p.) injection of ifenprodil significantly prolonged the reaction time of mice at the dose of 30 mg/kg, and increased the analgesic effect of morphine. In the phenylquinone writhing test, ifenprodil reduced the number of abdominal constrictions of mice starting from the dose of 2.5 mg/kg i.p., and increased the effect of morphine. The effect of ifenprodil on pain threshold was prevented by naloxone. Moreover, ifenprodil antagonized the pain threshold-reducing effect of α-melanocyte-stimulating hormone (0.05 μg/mouse, intracerebroventricularly). These data show that blockade of the polyamine site of the NMDA receptor complex produces analgesia and increases the analgesic effect of morphine.

Keywords: Pain; Analgesia; Polyamine; NMDA receptor; Ifenprodil; Morphine; Naloxone; α-MSH (α-melanocyte-stimulating hormone)

1. Introduction

The intracerebroventricular (i.c.v) injection of melanocortin peptides (neuropeptides of the family of α-melanocyte-stimulating hormone/adrenocorticotropic hormone) produces a complex and typical behavioral picture characterized by excessive grooming and by recurrent episodes of stretching, yawning and penile erection (Ferrari, 1958; Ferrari et al., 1963; Bertolini et al., 1969; Gispen et al., 1975; Bertolini and Gessa, 1981; Bertolini et al., 1988). Moreover, the i.c.v. injection of melanocortin peptides lowers pain threshold (Bertolini et al., 1979; Amir, 1981) and antagonizes the analgesic effect of opioids (Gispen et al., 1976; Fratta et al., 1981) as well as stress-induced analgesia (Amir and Amit, 1979; Grisswell and David, 1979). Finally, pain threshold is significantly reduced in adrenalectomized animals, and a relationship exists between increased pain sensitivity and adrenocorticotropic hormone (ACTH) levels in these animals (Heybach and Vernikos-Danellis, 1978).

These and many other experimental data have led to the hypothesis that melanocortin peptides play a physiological role in the complex modulation of pain sensitivity, usually opposing the effect of endogenous opioids (for reviews see: Bertolini and Gessa, 1981; De Wied and Jolles, 1982; O'Donohoue and Dorsa, 1982; Bertolini et al., 1986).

The brain ornithine decarboxylase-polyamine system seems to be involved in the melanocortin-induced central effects, because α-difluoromethyl-ornithine, an irreversible inhibitor of mammalian ornithine decarboxylase activity, dose dependently antagonizes the behavioral symptoms induced by the i.c.v. administration of ACTH-(1–24) in rats (Genedani et al., 1984). More recently (Genedani et al., 1994) it has been shown that these symptoms are also prevented by blockade of the polyamine modulatory site of the NMDA receptor complex.

Our present research was aimed at investigating a possible effect of such a blockade on pain threshold, morphine activity, and α-melanocyte-stimulating hormone-induced hyperalgesia both in the hot-plate test and in the phenylquinone-writhing test, in mice.

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Elsevier Science B.V.
SSDI 0014-2999(95)00778-4

2. Materials and methods

2.1. Animals

Swiss albino male mice (Charles River, Calco, Como, Italy), weighing 24–30 g were used. They were housed in climatized colony rooms (21 ± 1°C; 60% humidity) with tap water and standard food available ad libitum, on a natural light-dark cycle, strictly in compliance with the guidelines of the CEE ethical regulations for animal research (CEE Council 86/609, and D.L. 27/01/92 No. 116). A group of mice was prepared for intracerebroventricular treatment by stereotaxically implanting stainless-steel guide cannulae (23 gauge; Plastic Products Co., Roanoke, VA, USA) into a brain lateral ventricle (Paxinos and Watson, 1982), under ketamine plus xylazine anesthesia (115 + 2 mg/kg i.p.; Farmaceutici Gellini, Aprilia, Italy and Bayer, Milan, Italy, respectively), and by fixing them to the skull with a plastic cap and dental acrylic. A removable plug, which extended 0.5 mm below the tip of the guide cannula was kept in place until drug injection. Correct placement was verified at the end of the experiment by injecting 3 μl of toluidine blue dye through an internal cannula used for drug (or solvent) injection, followed by decapitation under ethyl ether anesthesia and dissection of the brain. Data obtained from improperly implanted animals (12 out of 100) were discarded.

2.2. Antinociceptive tests

2.2.1. Hot plate test (Woolfe and MacDonald, 1944; Eddy and Leimbach, 1953)

Animals were placed one at a time on a metal plate which was thermostatically controlled at 50 ± 0.2°C (Basile, Comerio, Varese, Italy). Latency to paw licking was recorded and a 60 s cut-off time was used in order to prevent tissue damage. Each animal served as its own control, the latency to response being measured both before and after drug administration. The analgesic activity was calculated as percentage of maximum possible effect (MPE), using the general equation:

$$\%\,\mathrm{MPE} = \frac{\text{postdrug latency} - \text{predrug latency}}{\text{cut-off latency} - \text{predrug latency}} \times 100$$

Predrug latency was the mean of three values for each animal, measured at 30 min intervals. Groups of 9–10 mice per treatment were used, and each animal was used only for one treatment.

2.2.2. Phenylquinone-induced writhing (Siegmund et al., 1957; Collier et al., 1968)

Mice were intraperitoneally (i.p.) injected with 0.3 ml of a 0.02% solution of phenyl *p*-benzoquinone in alcoholic solution and the number of abdominal constrictions (writhings) was counted for each mouse during the subsequent 30 min. Groups of 10–23 mice per treatment were used, and each animal was used only for one treatment.

2.3. Drugs and treatments

Ifenprodil tartrate, a potent non-competitive antagonist that binds the polyamine modulatory site of the NMDA receptor complex (Carter et al., 1989; Schoemaker et al., 1990), was purchased from Research Biochemicals, Natick, MA, USA; it was freshly dissolved in distilled water and i.p. injected at the doses of 1.25, 2.5, 5.0, 10.0 or 30.0 mg/kg, in a volume of 2.5 ml/100 g body weight. Morphine sulphate was purchased from Salars, Como, Italy; it was freshly dissolved in distilled water and subcutaneously (s.c.) injected at the doses of 0.25 or 5 mg/kg, in a volume of 2.5 ml/100 g body weight. α-Melanocyte-stimulating hormone was purchased from Sigma Chemical Co., St. Louis, MO, USA; it was freshly dissolved in saline and injected into a brain lateral ventricle (i.c.v.) via preimplanted cannulae at the doses of 0.005, 0.05, 0.2, 0.5, 1.0 or 10.0 μg/animal, in a volume of 3 μl. Naloxone hydrochloride was purchased from Sigma Chemical Co., St. Louis, MO, USA; it was freshly dissolved in saline and i.p. injected at the dose of 5 mg/kg, in a volume of 2.5 ml/100 g body weight. Control animals received an equal volume of the solvents by the same routes.

2.4. Data analysis

Statistical analysis of data was performed by analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple comparison test.

3. Results

Ifenprodil reduced nociceptive sensitivity both in the hot-plate test and in the phenylquinone-writhing test. In the hot-plate test, latency to paw licking was significantly increased by the dose of 30 mg/kg i.p., 30 min after treatment. The effect was no more observed 60 min after treatment, but still at this time ifenprodil – although inactive per se – potentiated the analgesic effect of morphine. Likewise, ifenprodil at the inactive dose of 10 mg/kg potentiated the analgesic effect of morphine 30 min after treatment (Fig. 1). On the other hand, the effect of ifenprodil on pain threshold was prevented by naloxone (5 mg/kg) i.p. injected 15 min after ifenprodil treatment (Fig. 2).

In the phenylquinone-writhing test, ifenprodil reduced the number of abdominal constrictions, the effect being significant starting from the dose of 2.5 mg/kg; at the dose of 10 mg/kg the abdominal constrictions were almost completely prevented (Fig. 3a). The combination of a subactive dose of ifenprodil (1.25 mg/kg) with a subactive

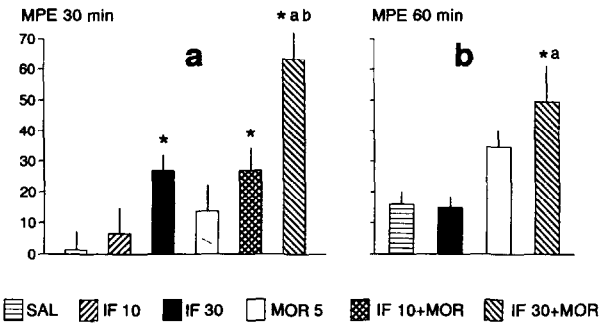

Fig. 1. Influence of i.p. ifenprodil, alone or in combination with morphine, on pain threshold in the hot plate test (50°C) 30 (a) and 60 (b) min after administration (SAL = saline; IF10 = ifenprodil 10 mg/kg; IF30 = ifenprodil 30 mg/kg; MOR5 = morphine 5 mg/kg s.c.; IF10+MOR = ifenprodil 10 mg/kg + morphine 5 mg/kg; IF30+MOR = ifenprodil 30 mg/kg + morphine 5 mg/kg). MPE = percentage of maximum possible effect. Data are means ± S.E.M. (9–10 animals per group). * $P < 0.05$ vs. saline; [a] $P < 0.05$ vs. ifenprodil 30 mg/kg; [b] $P < 0.05$ vs. morphine 5 mg/kg.

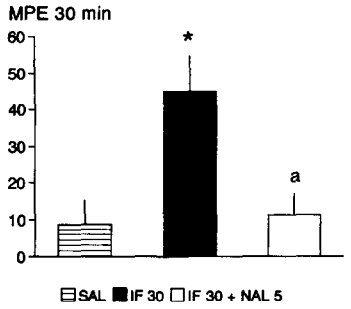

Fig. 2. Influence of naloxone (5 mg/kg i.p. injected 15 min after ifenprodil 30 mg/kg i.p.) on the reduction of pain sensitivity induced by ifenprodil (hot plate test 50°C) (SAL = saline; IF30 = ifenprodil 30 mg/kg; IF30+NAL 5 = ifenprodil 30 mg/kg + naloxone 5 mg/kg). MPE (percentage of maximum possible effect) evaluated 30 min after ifenprodil administration. Data are means ± S.E.M. (10 animals per group). * $P < 0.05$ vs. saline; [a] $P < 0.05$ vs. ifenprodil 30 mg/kg.

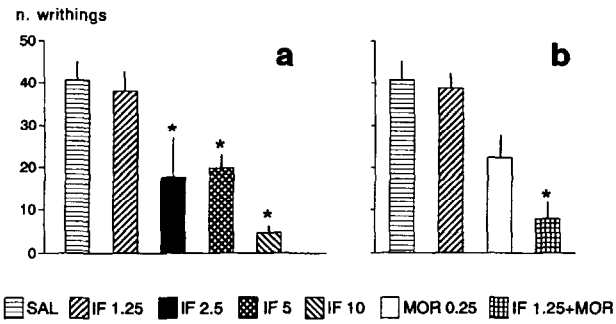

Fig. 3. Influence of i.p. ifenprodil, alone (a) or in combination with morphine (b), on the number of writhings in the phenylquinone-writhing test (SAL = saline; IF1.25 = ifenprodil 1.25 mg/kg; IF2.5 = ifenprodil 2.5 mg/kg; IF5 = ifenprodil 5 mg/kg; IF10 = ifenprodil 10 mg/kg; MOR0.25 = morphine 0.25 mg/kg s.c.; IF1.25+MOR = ifenprodil 1.25 mg/kg + morphine 0.25 mg/kg). Data are means ± S.E.M. of the number of writhings (10–18 animals per group). * $P < 0.05$ vs. saline.

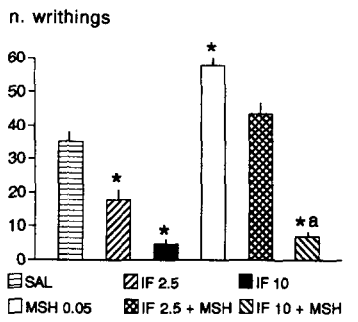

Fig. 4. Influence of i.p. ifenprodil on the pain threshold lowering effect of α-MSH (0.05 μg/mouse) in the phenylquinone-writhing test (SAL = saline; IF2.5 = ifenprodil 2.5 mg/kg; IF10 = ifenprodil 10 mg/kg; MSH0.05 = α-MSH 0.05 μg/animal; IF2.5+MSH = ifenprodil 2.5 mg/kg + α-MSH 0.05 μg/animal i.c.v.; IF10+MSH = ifenprodil 10 mg/kg + α-MSH 0.05 μg/animal). Data are means ± S.E.M. of the number of writhings (10–23 animals per group). * $P < 0.05$ vs. saline; [a] $P < 0.05$ vs. α-MSH.

dose of morphine (0.25 mg/kg) produced a significant reduction of the number of abdominal constrictions (Fig. 3b). Finally, ifenprodil (2.5 mg/kg) prevented the pain threshold-lowering effect of α-melanocyte-stimulating hormone (α-MSH), i.c.v. injected at the dose of 0.05 mg/mouse (Fig. 4) (the dose of α-MSH was chosen on the basis of dose-response experiments).

4. Discussion

Melanocortin peptides (α-MSH, ACTHs) lower pain threshold, as previously shown (Bertolini et al., 1979; Amir, 1981) and as confirmed by our present data, and it has been suggested that they play a role opposite to endogenous opioids in many body functions, including pain sensitivity (for a review see: Bertolini et al., 1986; Bertolini, 1995). The polyamine domain at the NMDA receptor complex plays an important role in the central nervous system (CNS) effects of melanocortin peptides. Indeed, ifenprodil, an antagonist at the polyamine recognition site of the NMDA receptor, dose-dependently prevents the most typical signs (excessive grooming, stretching, yawning, penile erection) of the ACTH-induced behavioral syndrome (Genedani et al., 1994).

The present data show that ifenprodil completely prevents also the α-melanocyte-stimulating hormone-induced reduction of pain threshold. On the other hand, ifenprodil produces per se a reduction of pain sensitivity and increases the analgesic effect of morphine in two different animal models of nociception. Moreover, the effect of ifenprodil on pain threshold is antagonized by naloxone.

Overall, on the basis of our present data, it seems reasonable to infer that the effect of ifenprodil on pain threshold and morphine analgesia is attributable to the functional blockade of melanocortin peptides at the CNS level with consequent functional prevalence of endogenous

opioids (the effect of ifenprodil is in fact blocked by naloxone). Of course, it cannot be ruled out that also some other pharmacodynamic properties of ifenprodil may contribute to its effects on pain sensitivity and morphine activity. In particular, it has been shown that ifenprodil has Ca^{2+} antagonistic properties (Adeagbo and Magbagbeola, 1985; Honda and Sakai, 1987) and it has been repeatedly described that Ca^{2+} antagonists increase morphine- and opioid peptide-induced analgesia (Contreras et al., 1988; Wang et al., 1989). However, the Ca^{2+} antagonistic activity of ifenprodil (blockade of potential-sensitive channels) has been demonstrated only in vascular or muscle preparations (Adeagbo and Magbagbeola, 1985; Honda and Sakai, 1987), while in hippocampal neurons ifenprodil inhibits NMDA receptor-mediated Ca^{2+} influx but not Ca^{2+} influx induced by depolarizing stimulation (Chida et al., 1992).

Available data concerning the possible involvement of excitatory amino acids in the modulation of pain sensitivity are rather inconsistent: antinociception, no effect, or hyperalgesia have been described for the same antagonists of excitatory amino acid receptors, depending on species, route of administration, and nociceptive test (Jacquet, 1988; Lipa and Kavaliers, 1990; Marek et al., 1991; Kest et al., 1992; Yamamoto and Yaksh, 1992; Näsström et al., 1992; Galea et al., 1993; Lufty et al., 1993; Yukhananov and Larson, 1994; Saucier and Kavaliers, 1994; Elliott et al., 1994; Kristensen et al., 1994; Shu et al., 1995). However, from a thorough examination of the available litearature, it would seem that while pain threshold can be modified either by NMDA or by non-NMDA antagonists, morphine activity is affected (increased or reduced) only by NMDA antagonists (Lufty et al., 1993; Yukhananov and Larson, 1994; Saucier and Kavaliers, 1994; Shu et al., 1995). In conclusion, our present results indicate that the polyamine site of the NMDA receptor is involved in pain modulation and in morphine activity.

Acknowledgements

This work was supported in part by grants from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (40 and 60%) and by Consiglio Nazionale delle Ricerche, Rome.

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