Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pain 11 (2007) 535–541
www.EuropeanJournalPain.com

Possible role of NMDA receptors in antinociception induced by rilmenidine in mice in the formalin test

Masoomeh Sabetkasaie *, Niaz Khansefid, Mohammad Ali Raies Sadat Ladgevardi

Department of Pharmacology and Neuroscience Research Center, School of Medicine, Shaheed Beheshti University of Medical Sciences, P.O. Box 19835-355, Tehran, Iran

Received 13 October 2005; received in revised form 20 June 2006; accepted 10 July 2006
Available online 24 August 2006

Abstract

Objectives: The aim of the study was to investigate the possible role of MK-801, an NMDA antagonist, in analgesia induced by rilmenidine, an imidazoline (I_1) agonist, in mice in the formalin test.
Methods: 25 μl of formalin 2.5% was injected into the dorsal surface of the right hind paw of the mouse. Pain response was scored after formalin injection for a period of 50 min. A weighted average of nociceptive score, ranging from 0 to 3, was calculated. The mean ±SEM of scores between 0–5 and 15–40 min after formalin injection was presented.
Results: The study showed that rilmenidine (1.25, 2.5 and 5 mg/kg, i.p.) produced analgesia dose-dependently ($p < 0.001$) in formalin test. In addition, the results demonstrated that efaroxan (0.1 and 1 mg/kg, i.p.) could reduce the antinociceptive effect of rilmenidine (2.5 mg/kg, i.p.) ($p < 0.01$) in animals, however, yohimbine (0.1 and 0.2 mg/kg, i.p.) could not block the analgesia induced by rilmenidine (2.5 mg/kg, i.p.) ($p > 0.05$). On the other hand, MK-801 (0.05 mg/kg, i.p.) reduced the pain related behaviors in mice ($p > 0.05$). Moreover, our findings demonstrated that MK-801 (0.01 mg/kg, i.p.) could potentiate the analgesic effect of rilmenidine (1.25 mg/kg, i.p.) significantly ($p < 0.01$).
Conclusions: The present study suggests that imidazoline (I_1) receptors play an important role in mediating the antinociception induced by rilmenidine in formalin test. Furthermore, it may be concluded that there is an interaction between NMDA receptors and imidazoline (I_1) binding sites.

Keywords: Rilmenidine; MK-801; Antinociception; Formalin test; Mice

1. Introduction

Rilmenidine, a selective imidazoline (I_1) receptor agonist (Ernsberger et al., 1992; Reis, 1996) has recently been introduced to physicians as a new centrally-acting antihypertensive agent (Fillastre et al., 1988; Verbeuren et al., 1990). It exhibits fewer side-effects compared with α_2-adrenergic agonists such as clonidine (Haxhiu et al., 1994; Van Zwieten et al., 1986).

Previous studies showed that clonidine had antinociceptive effects in the formalin test in rats and mice after systemic administration (Dennis et al., 1980; Taskar and Melzack, 1989). In addition, it has been reported that rilmenidine is as effective as clonidine without lowering blood pressure. But, it has a reduced frequency of side-effects such as sedation and dry mouth, which are due to α_2-adrenoceptor stimulation (Bousquet, 1997; Bousquet et al., 2000; Eglan et al.,

* Corresponding author. Tel./fax: +98 21 22403154.
E-mail address: fkasaei@yahoo.com (M. Sabetkasaie).

1090-3801/$32
doi:10.1016/j.ejpain.2006.07.004

1998; Milligan and MacKinnon, 1997). Therefore, if rilmenidine also produces antinociception in the formalin test, then it might represent an antinociceptive effect with an improved side-effect profile relative to clonidine.

On the other hand, several studies have demonstrated that imidazoline compounds elicit central and peripheral effects through the interaction with various non-adrenoceptor sites including imidazoline receptors (Bousquet, 1995; French, 1995; Molderings, 1997; Regunathan and Reis, 1996) and various cation channels, including N-methyl-D-aspartate (NMDA) receptors (Olmos et al., 1996).

According to previous work, however, non-competitive NMDA antagonists have also been found to reduce pain related behaviors induced by formalin injection (Coderre and Melzack, 1992; Yamamoto and Yaksht, 1992), and both competitive and non-competitive NMDA antagonists decrease persistent dorsal horn neuronal activity associated with peripheral formalin injection (Haley et al., 1990). Nevertheless, it was also demonstrated that these antagonists only produce antinociceptive effects at doses which produce motor dysfunction as well (Coderre and Van Empel, 1994b). Although high antinociceptive doses of non-competitive NMDA antagonists induce motor dysfunction, it may be possible to obtain antinociceptive effects with lower doses of these agents by using them with compounds which act at different binding sites such as imidazoline receptors.

The present study, however, was designed to (1) determine if rilmenidine produces antinociception in the formalin test in mice, (2) establish which receptor type, α_2-adrenoceptors or imidazoline (I_1) receptors, may be implicated in the effects of rilmenidine on pain and (3) assess if non-competitive NMDA antagonism is involved in mediating antinociception induced by imidazoline (I_1) receptor agonist. Throughout the work, the guidelines proposed by the Committee on Research and Ethical Issues of IASP (Zimmermann, 1983) for investigation in experimental pain in animals were followed.

2. Materials and methods

2.1. Animals

Male NMRI mice (Institute Pasteur, Iran) weighing 25–30 g were used for all studies. The animals were housed in groups of 4 or 5 mice per cage at room temperature (21 ± 1 °C) under a regular light/dark schedule (light 8.00 AM-8.00 PM). Food and water were unlimited. Testing took place during the light phase. The mice were habituated to the test room for 30 min on the day before testing and 2 h before the test started. The animals were used only once and were humanely killed upon completion of the experiment.

2.2. Formalin test

Each mouse was placed in a transparent acrylic cage ($30 \times 30 \times 30$ cm), and was allowed to move freely for 15–20 min. A mirror was arranged in a 45° angle under the cage to allow an unimpeded view of the animal's hind paws. Fifteen minutes after an intraperitoneal (i.p.) injection of either saline or one of the agonist, each mouse was given a formalin (2.5%, 25 μl) injection into the dorsal surface of the right hind paw.

Pain response was scored immediately after formalin injection for a period of 50 min. A nociceptive score was determined for each 5-min time block by measuring the amount of time spent in each of four behavioral categories: 0 is when the injected paw bears the animal's weight on the floor, 1 is when the animal lightly rests its injected paw on the ground, bearing only some of its weight, 2 is when the animal elevates the injected paw off of the ground, and 3 is when the animal licks, bites, or shakes the injected paw. A weighted average of nociceptive score, ranging from 0 to 3, was calculated by multiplying the time spent in each category by the category weight, and then dividing by the total time for each 5-min time block (Coderre and Melzack, 1992). The mean ± SEM of scores between 0–5 and 15–40 min after formalin injection were presented.

2.3. Treatment

Experimental groups of six mice were tested: control group and groups treated with: rilmenidine (1.25, 2.5 and 5 mg/kg) and MK-801 (0.01,0.025 and 0.05 mg/kg) 15 min before formalin injection; efaroxan (0.1 and 1 mg/kg) and yohimbine (0.1 and 0.2 mg/kg) 30 min before formalin administration.

2.4. Drugs

The chemicals used were: rilmenidine, efaroxan and MK-801 (Tocris, England), yohimbine (Boehringer Ingelheim, Germany). All agents were dissolved in saline except rilmenidine, which was dissolved first in alcohol and then in saline.

2.5. Statistical analysis

ANOVAs followed by Newman–Keuls test were used for analysis of the data. Differences between means were considered statistically significant if $p < 0.05$. Each point is the mean ± SEM of the six mice.

3. Results

3.1. Antinociception induced by rilmenidine in the formalin test

In Fig. 1, the effects of rilmenidine in comparison with those of saline on chronic pain are shown. In the early phase of the formalin test (Fig. 1a), rilmenidine with the dose of 5 mg/kg had antinociceptive effects in animals ($P < 0.01$). Fig. 1b showed that the intraperitoneal injection of rilmenidine (1.25, 2.5 and 5 mg/kg) to mice produced dose-dependent antinociception in the second phase of the formalin test ($P < 0.001$). The maximum response was obtained with 5 mg/kg of the drug.

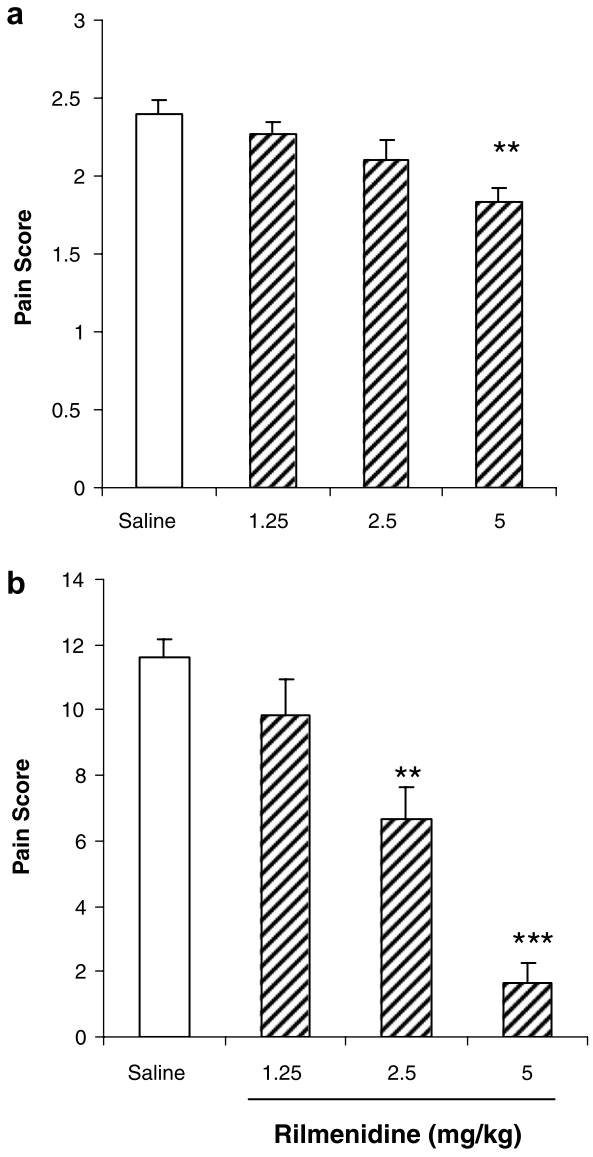

Fig. 1. (a, b) Antinociceptive effect of different doses of rilmenidine in the formalin test. Animals were administered (i.p.) with saline (5 ml/kg), different doses of rilmenidine (1.25, 2.5 and 5 mg/kg) 15 min before formalin injection. Antinociception during 0–5 min (a first phase) and 15–50 min (b second phase) after formalin administration was recorded. Each point is the mean ± SEM of six mice. $^{**}P < 0.01$, $^{***}P < 0.001$, different from saline control group.

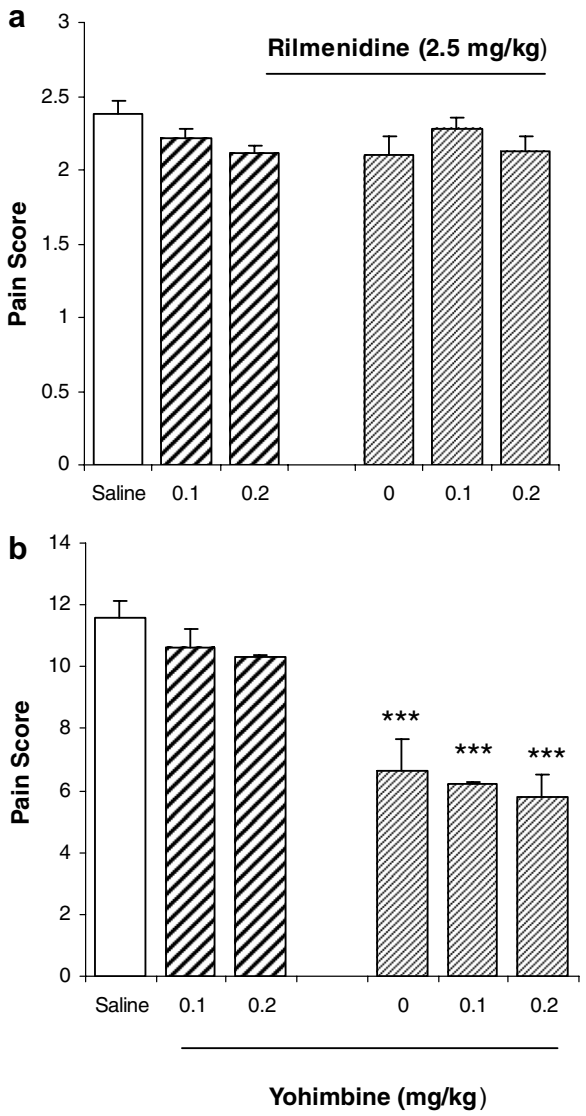

Fig. 2. (a, b) Antinociceptive effect of different doses of rilmenidine in the presence or absence of yohimbine in the formalin test. Mice were injected (i.p.) with saline (5 ml/kg), rilmenidine (2.5 mg/kg) 15 min and yohimbine (0.1 and 0.2 mg/kg), 30 min before formalin administration. Antinociception during 0–5 min (a first phase) and 15–50 min (b second phase) after formalin injection was recorded. Each point is the mean ± SEM of six mice. $^{***}P < 0.001$, different from saline control group.

3.2. Effects of yohimbine on antinociception induced by rilmenidine in the formalin test

In Fig. 2, yohimbine did not reduce the antinociceptive activity of rilmenidine in both phases of the formalin test ($P > 0.05$).

3.3. Efaroxan effects on antinociception produced by rilmenidine

In Fig. 3, the antinociceptive effects of rilmenidine are shown in the presence or absence of efaroxan. One-way ANOVA indicated that efaroxan (0.1 and 1 mg/kg) decreased the antinociceptive effect of rilmenidine (2.5 mg/kg) significantly in the late phase ($P < 0.01$) but had no effect in the early phase of the formalin test ($P > 0.05$).

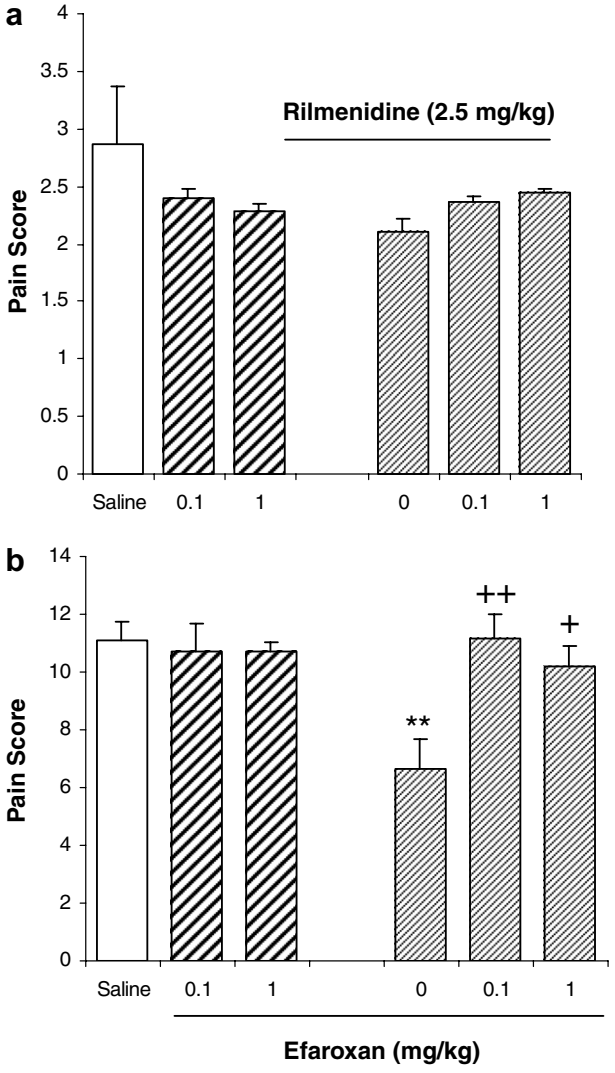

Fig. 3. (a, b) Effect of efaroxan on antinociception induced by rilmenidine in the formalin test. Mice were treated (i.p.) with saline (5 ml/kg), rilmenidine (2.5 mg/kg) 15 min and efaroxan (0.1 and 1 mg/kg), 30 min before formalin administration. Antinociception during 0–5 min (a first phase) and 15–50 min (b second phase) after formalin injection was recorded. Each point is the mean ± SEM of six mice. $^{**}P < 0.01$, as compared with saline control group. $^{+}P < 0.05$, $^{++}P < 0.01$ as compared with rilmenidine control group.

3.4. Effects of MK-801 on chronic pain in the formalin test

In Fig. 4a, MK-801 did not reduce the antinociception in the early phase of the formalin test ($P > 0.05$). However, one-way ANOVA indicated that MK-801 (0.05 mg/kg) produced antinociception significantly in the late phase of the formalin test ($P < 0.05$) (Fig. 4b).

3.5. Effects of concurrent administration of the non-antinociceptive doses of rilmenidine and MK-801 on nociception in the formalin test

In Fig. 5, the effects of concurrent administration of the non-antinociceptive doses of rilmenidine (1.25 mg/kg) and MK-801 (0.01 mg/kg) are compared with those of saline and each drugs alone. The injection of non-antinociceptive dose of rilmenidine and MK-801

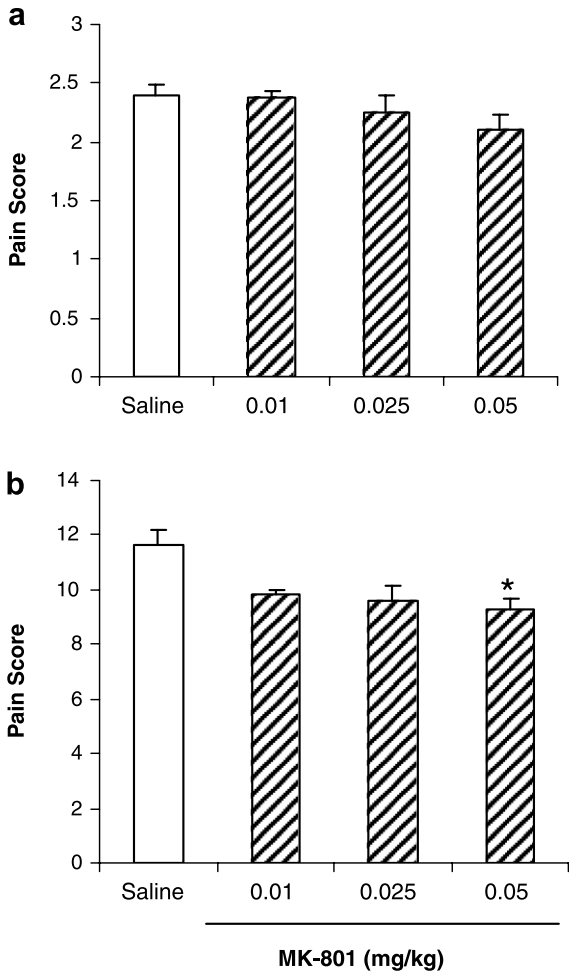

Fig. 4. (a, b) Antinociceptive effect of different doses of MK-801 in the formalin test. Mice were administered (i.p.) with saline (5 ml/kg), different doses of MK-801 (0.01, 0.025 and 0.05 mg/kg) 15 min before formalin injection. Antinociception during 0–5 min (a first phase) and 15–50 min (b second phase) after formalin administration was recorded. Each point is the mean ± SEM of six mice. $^{*}P < 0.05$, different from saline control group.

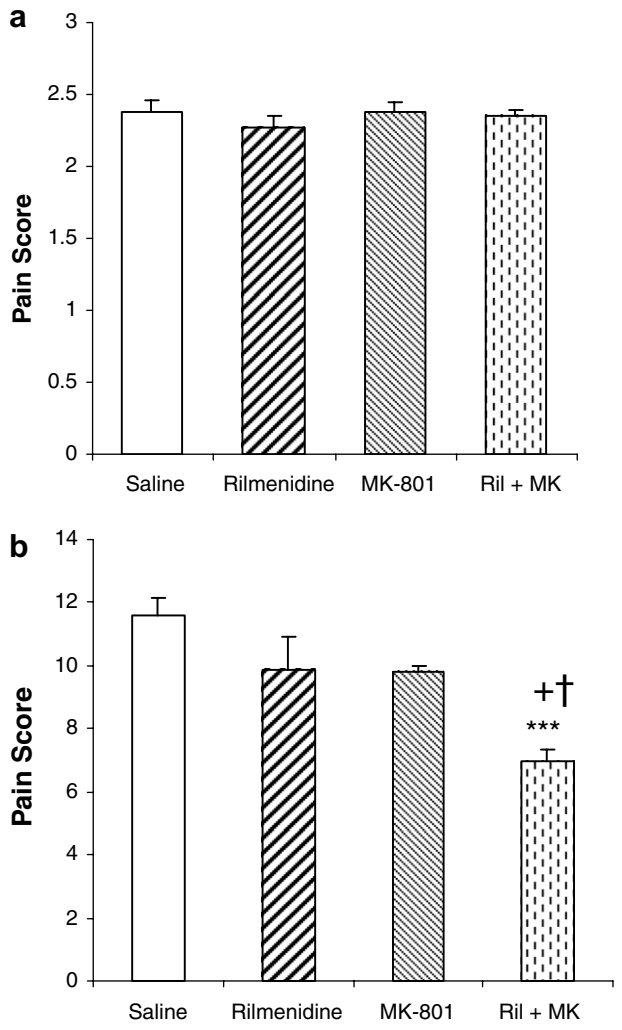

Fig. 5. (a, b) Antinociception induced by simultaneous administration of rilmenidine and MK- 801 in the formalin test. Mice were treated (i.p.) with saline (5 ml/kg), rilmenidine (2.5 mg/kg) and MK-801(0.01 mg/kg), 15 min before formalin administration. Antinociception during 0–5 min (a first phase) and 15–50 min (b second phase) after formalin injection was recorded. Each point is the mean ± SEM of six mice. $^{***}P < 0.001$, as compared with saline group. $^{+}P < 0.05$, as compared with rilmenidine control group. $^{\dagger}P < 0.01$, as compared with MK-801 control group.

simultaneously produced a significant antinociception in the late phase of the formalin test ($P < 0.001$). Two-way ANOVA indicated an interaction between rilmenidine and MK-801 in the late phase of the formalin test ($P < 0.05$) (Fig. 5b).

4. Discussion

The first aim of the present study was to evaluate the antinociceptive activity of rilmenidine in the presence or absence of yohimbine and efaroxan in the formalin test in mice.

The formalin test is used to assess the mechanism of moderate and continuous pain generated by the injured tissue in animals (Abbott et al., 1995; Dubuisson and Dennis, 1977; Tjolsen et al., 1992). This test is characterized by two phases. The early phase is caused by C-fiber activation due to the peripheral stimulus. The late phase seems to depend on the combination of an inflammatory reaction in the peripheral tissue (Davidson and Carlton, 1998; Omote et al., 1998, 2000) and functional changes in the dorsal horn of the spinal cord (Abbott et al., 1995).

Our data demonstrated that different doses of rilmenidine (half-life: 8 h), an imidazoline (I_1) receptor agonist, could produce dose-dependent antinociception in the second phase but is able to induce antinociception only with a dose of 5 mg/kg in the first phase of the formalin test. Such an effect, however, has been reported by other imidazoline compounds such as moxonidine and clonidine. Previous studies showed that moxonidine, a mixed imidazoline I_1/α_2-adrenergic receptor agonist induced antinociception in formalin (Shannon and Lutz, 2000), tail-flick and the substance P nociceptive tests (Fairbanks and Wilcox, 1999).

However, it has been reported that clonidine had antinociceptive effects in visceral pain induced by intracolonic instillation of formalin (Sabetkasaie et al., 2004) as well as formalin (Shannon and Lutz, 2000) and tail-flick tests (Kumar et al., 1993; Ossipov et al., 1984).

In addition, our findings showed that yohimbine, an α_2-antagonist, could not reduce the antinociceptive effects of rilmenidine in both phases of the formalin test.

As demonstrated previously by Piletz et al. in 1996, yohimbine did not bind to imidazoline receptors (Piletz et al., 1996).

Our data also indicated that pretreatment of animals with efaroxan, an imidazoline I_1 antagonist, decreased the antinociception induced by rilmenidine in the second phase of the formalin test. Our results are consistent with those of previous work which demonstrated that the antinociceptive effects of moxonidine were antagonized by the imidazoline I_1 receptor preferring antagonist, efaroxan (Haxhiu et al., 1994).Therefore, the present study may indicate that imidazoline (I_1) receptors play a greater role than α_2-receptors in mediating antinociception induced by rilmenidine in the formalin test.

The second aim of our research was to determine if MK-801, a non-competitive NMDA antagonist, in involved in the antinociceptive effect of rilmenidine in the formalin test in mice.

The present study showed that MK-801 (half-life: 2.05 h) with a dose which does not produce motor dysfunction (Berrino et al., 2003), was able to produce antinociception in the late phase of the formalin test. Our findings are in agreement with previous work showing that the administration of MK-801 produced

antinociception significantly during the second phase after formalin injection (Berrino et al., 2003; Chung et al., 2000; Coderre and Van Empel, 1994a,b).

In our research, MK-801 did not have any antinociceptive effects, either alone or with rilmenidine on the early phase of the formalin injection. The lack of effect of NMDA antagonists in the early phase of the formalin test is consistent with the results reported previously (Coderre and Melzack, 1992; Coderre and Van Empel, 1994a,b; Haley et al., 1990; Yamamoto and Yaksht, 1992). It is the late phase of the formalin test that the significant antinociceptive activity of MK-801, alone or with rilmenidine becomes evident.

However, concurrent administration of the non-antinociceptive dose of rilmenidine (1.25 mg/kg) and MK-801 (0.01 mg/kg) induced significant antinociception in the second phase of the formalin test. This may indicate an interaction between imidazoline (I_1) and NMDA receptors in mediating antinociception.

There are several lines of evidence showing that imidazoline drugs, independent of their affinity on imidazoline receptors, also interact with several cation channels, including NMDA receptors (Olmos et al., 1996). In recent studies, some researchers attributed the neuroprotective effects of imidazolines to a voltage-dependent, fast and fully reversible blockade of NMDA receptors at a phencyclidine (PCP)-like site (Mihaud et al., 2000; Olmos et al., 1999).

On the other hand, agmatine is an endogenous imidazoline receptor ligand which binds to α_2-adrenergic and imidazoline receptors with high affinity (Piletz et al., 1995). Agmatine can evoke a non-competitive voltage- and concentration-dependent block of the N-methyl-D-aspartate (NMDA) ionophore (Yang and Reis, 1999). Besides, Aricioglu and Altunbas suggested that a part of agmatine's antidepressant action in animals might result from the blockade of NMDA receptors (Aricioglu and Altunbas, 2003).

In general, if rilmenidine also produces antinociception in humans, then it will be a good alternative for clonidine because of its improved side-effect profile. In addition, by simultaneous use of rilmenidine and MK-801, it may be possible to produce more effective antinociception in the formalin test at doses comparable to NMDA antagonists, which do not produce motor dysfunction. Although combining the non-antinociceptive doses of rilmenidine and MK-801 might suggest an interaction in the formalin test, further analysis and dose-effect determination are necessary to support such a claim.

Acknowledgements

The authors wish to thank S. Ghafari and F. Masoudnia for their assistance in preparing this manuscript.

References

Abbott FV, Franklin KB, Westbrook RF. The formalin test: scoring properties of the first and second phases of the pain response in rats. Pain 1995;60:91–102.

Aricioglu F, Altunbas H. Is agmatine an endogenous anxiolytic/antidepressant agent? Ann NY Acad Sci 2003;1009:136–40.

Bousquet P, Bruban V, Schann S, Feldman j. Imidazoline receptors: a challenge. Pharm Acta Helv 2000;74:205–9.

Bousquet P. Imidazoline receptors: from basic concepts to recent developments. J Cardiovasc Pharmacol 1995;26:S1–6.

Bousquet P. Imidazoline receptors. Neurochem Int 1997;30:3–7.

Berrino L, Oliva P, Massimo F, Aurilio C, Maione S, Grella A, et al. Antinociceptive effect in mice of intraperitoneal N-methyl-D-aspartate receptor antagonists in the formalin test. Eur J Pain 2003;7:131–7.

Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci 1992;12:3665–70.

Coderre TJ, Van Empel I. The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. Pain 1994a;59:345–52.

Coderre TJ, Van Empel I. The utility of excitatory amino acid (EAA) antagonists as analgesic agents. II. Assessment of the antinociceptive activity of combinations of competitive and non-competetive NMDA antagonists with agents acting at all allosteric-glycine and polyamine receptor sites. Pain 1994b;59:353–9.

Chung KM, Song DK, Huh SO, Kim YH, Choi MR, Suh HW. Supraspinal NMDA and non-NMDA receptors are differently involved in the production of antinociception by morphine and β-endorphin administered intracerebroventricularly in the formalin pain model. Neuropeptides 2000;34:158–66.

Dubuisson D, Dennis SG. The formalin test: aquantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977;4:161–74.

Dennis SG, Melzack R, Gutman S, Boucher F. Pain modulation by adrenergic agents and morphine as measured by three pain tests. Life Sci 1980;26:1247–59.

Davidson EM, Carlton SM. Intraplantar injection of dextrophan, ketamine, or memantine attenuates formalin-induced behaviours. Brain Res 1998;785:136–42.

Ernsberger P, Westbrooks KL, Christen MO. A second generation of centrally acting antihypertensive agents acts on putative I_1-imidazoline receptors. J Cardiovasc Pharmacol 1992;20:S1–S10.

Eglan RM, Hudson AL, Kendall DA, Nutt DJ, Morgan NG, Wilson VG, et al. Seeing through a glass darkley: Casting light on imidazoline 'I' sites. TiPS 1998;19:381–90.

French N. α_2-adrenoceptores and I_2 sites in the mammalian central system. Pharmacol Ther 1995;68:175–208.

Fairbanks CA, Wilcox GL. Moxonidine, a selective α_2-adrenergic and imidazoline receptor agonist, produces spinal antinociception in mice. J Pharmacol Exp Ther 1999;290:403–12.

Fillastre JP, Lectac B, Galinier F, Le Bihan G, Schwartz J. A multicenter double-blind study of rilmenidine and clonidine in 333 hypertensive patients. Am J Cardiol 1988:61D–81D.

Haley JE, Sullivan AF, Dickenson AH. Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res 1990;518:218–26.

Haxhiu MA, Dreshaj I, Schafer SG, Ernsberger P. Selective antihypertensive action of moxonidine is mediated mainly by I_1-imidazoline receptors in the rostral ventrolateral medulla. J Cardiovasc Pharmacol 1994;24:S1–8.

Kumar A, Ragubir R, Dhawan BN. Analgesic effect of morphine, clonidine and serotonin microinjected in the PTN of rats. NeuroReport 1993;4:944–6.

Mihaud D, Fagni L, Bockaert J, Lafon-cazal M. Imidazoline-induced neuroprotective effects result from blockade of NMDA receptor channels in neuronal cultures. Neuropharmacology 2000;39:2244–54.

Milligan CM, MacKinnon. Imidazoline receptor ligand. DN& P 1997;10:74–84.

Molderings GJ. Imidazoline receptors: basic knowledge, recent advances and future prospects for therapy and diagnosis. Drug Fut 1997;22:757–72.

Omote K, Kawamata T, Kawamata M, Namili A. Formalin-induced release of excitatory amino acid in the skin of the rat hindpaw. Brain Res 1998;787:161–4.

Omote K, Kawamata T, Kawamata M, Nakayama Y, Hazama K, Namiki A. Activation of peripheral NMDA-nitric oxide cascade in formalin test. Anesthesiology 2000;93:173–8.

Olmos G, De Gregorio-Rocosolano N, Paz Regalado M, Gassul T, Assumpcio Boronat M, Trullas R, et al. Protection by imidazol (ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br J Pharmacol 1999;127:1317–26.

Olmos G, Ribera J, Garcia-Sevilla JA. Imidazoline compounds interact with the phencyclidine site of NMDA receptors in the rat brain. Eur J Pharmacol 1996;310:273–6.

Ossipov MH, Malseed RT, Eisenman LM, Goldstein FJ. Effects of α_2-adrenergic agents upon central etorphine antinociception in the cat. Brain Res 1984;309:135–42.

Piletz JE, Chikkala DN, Ernsberger P. Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and α_2-adrenergic receptors. J Pharmacol Exp Ther 1995;272:581–7.

Piletz JE, Zhu H, Chikkala DN. Comparison of ligand binding affinities at human I_1-imidazole binding sites and the high affinity state of α_2-adrenoceptor subtypes. J Pharmacol Exp Ther 1996;279:694–702.

Regunathan S, Reis DJ. Imidazoline receptors and their endogenous ligands. Annu Rev Pharmacol Toxicol 1996;36:511–44.

Reis DJ. Neurons and receptors in the rostroventrolateral medulla mediating the antihypertensive actions of drugs acting at imidazoline receptors. J Cardiovasc Pharmacol 1996;27: S11–8.

Sabetkasaie M, Vala S, Khansefid N, Hossein AR, Raies sadat MA. Clonidine and guanfacine-induced antinocicepyion in visceral pain: possible role of α_2/I_2 binding sites. Eur J Pharmacol 2004;501: 95–101.

Shannon HE, Lutz EA. Effects of the I_1 imidazoline/α_2-adrenergic receptor agonist, moxonidine in comparison with clonidine in the formalin test in rats. Pain 2000;85:161–7.

Taskar RAP, Melzack R. Different α-receptor subtypes are involved in clonidine-produced analgesia in different pain tests. Life Sci 1989;44:9–17.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.

Van Zwieten PA, Thoolen MJ, Jonkman FA, Wilffert B, De Jonge A, Timmermans PB. Central and peripheral effects of S 3341 [(N-dicyclopropylmethyl)-amino-2-oxazoline] in animal models. Arch Int Pharmacodyn Ther 1986;279:130–49.

Verbeuren TJ, Dihn Xuan AT, Koenig-Berard E, Vitou P. Rilmenidine. Cardiovasc Drug Rev 1990;8:56.

Yamamoto T, Yaksht L. Comparison of the antinociceptive effects of pre- and post-treatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology 1992;77:757–63.

Yang X, Reis DJ. Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J Pharmacol Exp Ther 1999;288:544–9.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.