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Short communication

Riluzole decreases the abstinence syndrome and physical dependence in morphine-dependent mice

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Abstract

The effects of the antiglutamatergic agent, riluzole, were examined on the antinociceptive action of morphine, on the induction of physical dependence, and on the expression of the abstinence syndrome to the opiate in mice. Morphine was administered as a single dose (200 mg/kg) of a slow-release preparation. Acute and chronic administration of riluzole decreased the analgesic response to morphine, the intensity of abstinence behavior (administered 30 min before a dose of naloxone), and the development of physical dependence (repeatedly administered during the period of chronic morphine treatment). © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

It is now accepted that glutamatergic functions are involved in the development of tolerance to opiate actions and in the induction of dependence in a way similar to that of a number of transmitters and modulators acting in the central nervous system. Thus, it has been shown that glutamate receptor antagonists reduce the induction of physical dependence, and tolerance to the opiate, and decrease the symptoms of naloxone-precipitated withdrawal syndrome in mice (Trujillo and Akil, 1991; Gonzalez et al., 1997).

Riluzole is a new antiglutamatergic agent that interferes with responses mediated by excitatory amino acids, even though it does not interact with any known binding site on NMDA, kainate or AMPA glutamate receptors (Debono et al., 1993). Riluzole inhibits the electrophysiological responses mediated by rat kainate and NMDA receptors expressed in Xenopus oocytes (Debono et al., 1993). In vivo data show that riluzole, after peripheral administration, inhibits the neuronal excitation induced by the iontophoretic application of excitatory amino acids to motoneurons of the facial nucleus in the rat (Girdlestone et al., 1989). The drug also blocks the convulsions induced after the intracerebroventricular (i.c.v.) administration of kainate and NMDA in the rat (Koek and Colpaert, 1990).

Recently, Tzschentke and Schmidt (1998) demonstrated that riluzole blocks morphine-induced conditioned place preference in the rat, suggesting that it interferes with the process of adaptation to chronic opiate administration.

In view of these data, the present study was undertaken to determine the effects of riluzole on the antinociceptive action of morphine, on the induction of physical dependence, and on the naloxone-precipitated withdrawal syndrome in morphine-dependent mice.

2. Materials and methods

2.1. General

Female adult albino Swiss Webster mice 12–15 weeks of age, weighing 26–33 g, from the animal reproduction laboratories of the Department of Pharmacology of the University of Concepción were used in all experiments. Mice were housed in groups of 10 and maintained on a 12/12-h light/dark cycle at constant room temperature (22 ± 2°C) with free access to food and water. Determina-

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tions of antinociceptive responses were carried out in the period between 14.30 and 18.00 h under normal room light and temperature (22 ± 2°C) conditions. Each animal was used for only one experimental condition.

All experiments were performed in accordance with institutional guidelines and with the National Institutes of Health Guide for the Care and Use of Laboratory animals.

2.2. Drugs

The drugs used were morphine HCl (May and Baker, Dagenham, England), naloxone (Sigma, St. Louis, MO, USA) and riluzole (Research Biochemicals, Natick, MA, USA). For the induction of chronic morphine effects, the opiate was administered in a suspension of the following composition: morphine, as the base form, 200 mg, 4.2 ml liquid paraffin, and 0.8 ml sorbital sesquioleate mixed with 5 ml saline. In all experiments riluzole was dissolved in 10% Tween 80.

2.3. Analgesic test

The hot-plate test described by Eddy and Leimbach (1953) was used for assessing analgesia (temperature kept at 55 ± 0.5°C). The end points considered were jumping off the plate or leg kicking. Each mouse was tested twice before drug administration and the values were averaged to obtain a baseline. In order to avoid severe leg burning, a cut-off time of 25 s was used. To determine the effects of riluzole on the reaction time to thermal stimulation, the drug was given 30 min before the hot-plate test, and the reaction times were determined at 30-min intervals over 90 min. The total antinociceptive response was obtained as the area under the time–response curve. To study the effects of riluzole on the analgesic response to morphine, a test dose of 5 mg/kg of morphine was used. Antinociception induced by this dose allows one to evaluate either an increase or a decrease in the responses elicited by the concomitant administration of riluzole without interference with the cut-off imposed in the test. For this purpose, the drug was given 30 min before the s.c. test dose of the opiate and under a schedule similar to that described for the groups of mice used for physical dependence (30, 27, 21 and 7 h before the test dose of morphine). Control groups were injected with the vehicle instead of riluzole under the schedules of acute and chronic treatments.

2.4. Induction of morphine dependence

To study morphine dependence, a single s.c. dose of morphine (200 mg/kg, in a suspension) was administered 30 h before the i.p. administration of 4 mg/kg naloxone. To test the effect of riluzole on the induction of dependence, the drug was administered during chronic morphine treatment according to the following schedule: 30 min before and 3, 9 and 23 h after the priming dose of morphine. Results for these groups are referred to as development of physical dependence. Riluzole was also administered in an additional scheme, which differed from the former, in which mice received a single dose of the drug 30 min before they were given an abstinence behavior-precipitating naloxone dose. Results for the latter groups are referred to as effects on withdrawal syndrome. Control groups were injected with the morphine suspension and the vehicle used for riluzole treatments in schedules similar to those for riluzole administration.

In all cases, the withdrawal syndrome precipitated by 4 mg/kg naloxone was characterized by diarrhea, micturition, piloerection, body shakes, running, paw tremors, and jumping. The number of mice presenting this syndrome was recorded after a 20 min observation period. Comparisons were made by assigning the following withdrawal scores: no appreciable effects, 0; micturition, 1; running, 2; piloerection, 2; diarrhea, 3; paw tremors, 3; body shakes, 4 and jumping, 5. These scores were assigned according to the severity of the observed signs. The relative frequencies of withdrawal signs were calculated by summing the number of mice presenting a sign during the observation period. The mean withdrawal scores were also calculated.

2.5. Statistical analysis

The significance of the differences in the mean responses to a test dose of morphine in tolerance experiments was determined by analysis of variance (ANOVA) and confirmed with the Student–Newman–Keul's test. Statistical analysis of the withdrawal syndrome was done

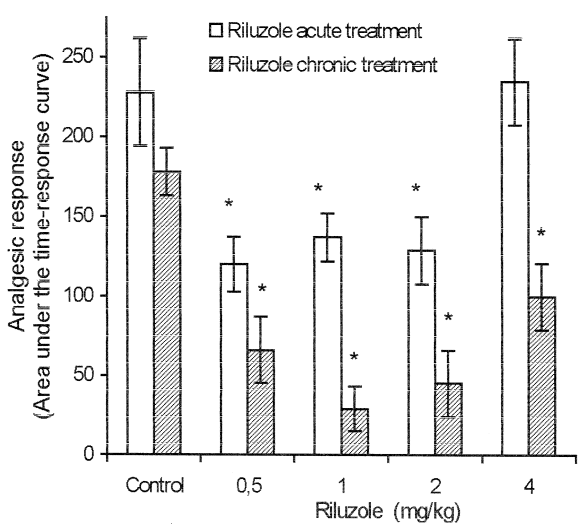

Fig. 1. Effect of riluzole on morphine-induced antinociception. The hot-plate test was used for assessing the analgesic response to morphine (5 mg/kg). Riluzole was administered as an acute treatment, 30 min before opiate injection, or as a chronic treatment (see text). Control groups were treated with riluzole vehicle. $^{*}P < 0.05$ compared with control group, ANOVA (F values for acute and chronic treatments, 13.08 and 4.81, respectively) followed by Student–Newman–Keul's test. $N = 10$ mice per group.

Table 1
Effects of riluzole on the development of physical dependence and on the withdrawal syndrome of morphine-treated mice
Mice were treated with a slow-release preparation of morphine (200 mg/kg, s.c.) 30 h before the precipitation of the withdrawal syndrome by naloxone (4 mg/kg, i.p.). For the study of effects on the development of physical dependence to morphine, riluzole was administered during the course of morphine pretreatment at different time intervals (see text). Control groups were treated with morphine and the vehicle used for riluzole treatments. To study the effects on the withdrawal syndrome, the drug under study was administered 30 min before naloxone administration. $N = 10$ mice per group.

Withdrawal sign	Relative frequencies of withdrawal signs as percentage of the maximum											
	Development of physical dependence to morphine						Withdrawal syndrome of morphine-treated mice					
	Control[a]	Riluzole (mg/kg)					Control[a]	Riluzole (mg/kg)				
		0.5[b]	1.0	2.0	3.0[a]	4.0[b]		0.5	1.0	2.0	3.0[a]	4.0
Micturition	89	63	70	60	67	75	78	50	60	50	78	80
Running	33	0	0	0	0	38	33	40	20	0	0	0
Piloerection	100	88	80	50	78	88	89	70	60	80	67	60
Diarrhea	55	50	40	40	67	75	33	80	50	30	33	50
Paw tremors	89	100	70	50	67	75	89	80	70	60	55	70
Body shakes	100	75	90	50	55	50	55	30	60	0[c]	10	60
Jumping	55	50	10	10	33	63	89	40	20[c]	10[c]	11[c]	40
Withdrawal Score ± S.E.M.	12.4 ±2.5	12.4 ±3.8	9.3 ±3.7	6.8[d] ±4.0	10.1 ±4.8	11.4 ±4.8	13.6 ±2.8	10.7 ±4.0	9.2 ±5.3	5.3[e] ±3.5	5.8[f] ±3.4	10.4 ±5.3

[a]One animal died during the treatment.
[b]Two animals died during morphine treatment.
[c]Significantly different from control mice, $P < 0.05$, χ^2 test.
Significantly different from control mice, [d]$P < 0.05$; [e]$P < 0.01$; [f]$P < 0.001$, Kruskal–Wallis analysis of variance (K values for development of physical dependence and withdrawal syndrome were 13.29 and 20.57, respectively) followed by Dunn's multiple comparison test.

with a Kruskal–Wallis ANOVA. When a significant K value was found by ANOVA, Dunn's multiple comparison test was carried out to identify differences among the groups. The original scores obtained for each animal were used in the evaluation. A level of probability of 0.05 was accepted as statistically significant.

3. Results

3.1. Effects of riluzole on morphine antinociception

The doses of the compounds tested did not affect gross animal behavior. The drug decreased the time response to thermal stimulation, but a dose related response was not observed. Acute (0.5, 1 and 2 mg/kg) or chronic administration of riluzole (0.5, 1, 2 and 4 mg/kg given 30, 27, 21 and 7 h before the dose test of morphine) significantly decreased the antinociceptive effects of morphine (Fig. 1).

3.2. Effects of riluzole on morphine dependence

The effects of riluzole on morphine dependence are shown in Table 1. The dose of 2 mg/kg significantly decreased the development of physical dependence. The signs tremors, body shakes, and jumping were affected the most by drug administration.

3.3. Effects of riluzole on the intensity of the abstinence behavior

Riluzole was also assayed in single doses (0.5, 1, 2, 3 and 4 mg/kg) administered 30 min before induction of the withdrawal syndrome by naloxone. The results of this treatment are shown in Table 1. As in the case of chronic administration, riluzole significantly decreased the expression of the withdrawal syndrome.

4. Discussion

Our results demonstrate that the antiglutamatergic agent, riluzole, significantly decreases the antinociceptive effects of morphine. This effect was more effective under a chronic treatment than in an acute treatment protocol. This antagonistic effect is in accordance with other studies that have shown a decrease in morphine analgesia after the administration of non-competitive glutamate receptor antagonists (Jacquet, 1988; Lufty et al., 1993).

In this regard, Jacquet (1988) hypothesized that the morphine analgesic response is produced, at least in part, by the activation of the endogenous descending inhibitory system by stimulation of glutamate receptors. Our present results may reflect an antagonistic action between riluzole and morphine at this level.

Riluzole co-administered with morphine decreased the intensity of the withdrawal syndrome, reflecting a reduction of physical dependence. This effect was observed only with 1 and 2 mg/kg doses; higher doses (3 and 4 mg/kg) were ineffective. Furthermore, riluzole, in doses of 1, 2 and 3 mg/kg, given 30 min before naloxone induced withdrawal behavior, reduced the intensity of the syndrome. Jumping, paw tremors and body shakes were the most affected signs. In reference to the results obtained with riluzole co-administered with morphine or given in a single dose before naloxone, it is of interest to note that these effects showed a bell-shaped response. Similar re-

sponses have been reported after the administration of glutamate receptor antagonists (Sanjay et al., 1994; Gonzalez et al., 1997). A number of studies have demonstrated that glutamate receptor antagonists reduce the intensity of the withdrawal syndrome as well as the development of physical dependence, demonstrating that glutamatergic transmission plays an important role in these processes. Therefore, it seems reasonable to postulate that the effects of riluzole on the development of physical dependence and on the withdrawal syndrome may be related to its antiglutamatergic actions. Direct evidence for the role of glutamate in abstinence behavior has been reported by Tokuyama et al. (1996), who showed that i.c.v. administration of the amino acid to morphine-tolerant rats elicited withdrawal signs similar to those evoked by naloxone. In addition, Sepulveda et al. (1998) reported an increased release of glutamate in areas of the limbic system of morphine tolerant rats following naloxone administration. Although riluzole is recognized as an antiglutamate agent, the drug induces other actions that could contribute to modify the effects of morphine. In this regard, it has been reported that riluzole induces a blockade of GABA uptake (Mantz et al., 1994), which may also reduce withdrawal signs. In addition, the inactivation of sodium channels produced by the drug (Benoit and Escande, 1991) might reduce neuron excitability and, consequently, attenuate the intensity of abstinence behaviour.

The present findings demonstrate that riluzole decreases the antinociceptive effect of morphine, and that its co-administration with morphine reduces the intensity of physical dependence to the opiate. Riluzole also decreases the intensity of the withdrawal syndrome when given in single doses a few minutes before naloxone administration to morphine-dependent mice.

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