Pharmacology Biochemistry and Behavior, Vol. 46, pp. 145–148, 1993
Printed in the U.S.A.

0091-3057/93 $6 00 + .00

BRIEF COMMUNICATION

Naloxone-Induced Analgesia: Involvement of κ-Opiate Receptors

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Received 12 August 1992

BIANCHI, M. AND A. E. PANERAI. *Naloxone-induced analgesia: Involvement of κ-opiate receptors.* PHARMACOL BIOCHEM BEHAV **46**(1) 145–148, 1993. – Rats treated with an acute high dose (30 mg/kg) or 4 days with a lower dose (5 mg/kg) of naloxone or naltrexone show an analgesic response at the hot-plate test. This paradoxical analgesic effect of the two μ-opiate receptor antagonists is blocked by the κ opiate receptor antagonist MR 1452, and is modulated by the κ opiate receptor agonist U 50-488. Our results suggest that κ opiate receptors are involved in naloxone-induced analgesia and are consistent with a high degree of plasticity of the opiatergic system.

Naloxone Naltrexone κ-Opiate receptors MR 1452 U 50-488 Analgesia Hot plate

NALOXONE has been the first opiate receptor antagonist widely used in research and clinical practice. For many years naloxone has been considered a pure antagonist, and its effects have been thought to be mediated mainly through an interaction with the μ-opiate receptor. As a consequence of these assumptions, many biological effects have been labeled opiate or nonopiate accordingly to their reversibility or not by naloxone (24). However, the affinity studies have demonstrated that naloxone binds not only μ sites, but, although with lower affinity, also σ and κ receptors (14). Moreover, as a pure receptor antagonist, naloxone should be devoid of any intrinsic activity. In the last years, it became evident, however, that in well-defined experimental conditions, naloxone could induce an analgesic effect (8). Naloxone-induced analgesia has been shown in the experimental animal after chronic treatment with the drug (9) and after the administration of different stress paradigms (18). In the human, the analgesic effect of naloxone was mainly shown in the postoperative period (10).

Several evidences support a role of κ-opiate receptors in the analgesic effect of naloxone. It was shown, in fact, that the chronic administration of naloxone induces an increase of the analgesic effect of κ-opiate receptor agonists in the rat (23), and that naloxone-induced analgesia after stress can be prevented by treatment with κ-opiate receptor antagonists (18). Along the same line, naloxone potentiates the analgesia induced in man by the κ-opiate receptor agonist pentazocine (11). In the present study we investigated the possible role of κ receptors in the analgesia induced by naloxone after acute and chronic administration in the rat. We tested in the same experimental conditions naltrexone, the second most used opiate receptor antagonist, which is also thought to interact mainly with μ-opiate receptors as a pure antagonist (3). These experiments were conducted to investigate whether the same effects were shared by the two drugs.

Finally, we evaluated the interaction of the κ-opiate receptor agonist U 50-488 (12) on the analgesia induced by the acute administration of naloxone.

METHOD

Male Sprague Dawley rats, 200–250 g b.w. (Charles River, Calco, Italy) were used in all experiments, 10 in each experimental group. In the chronic study, two groups of rats received naloxone and two groups naltrexone; on the fourth day one group in each treatment was administered the κ opiate receptor antagonist MR 1452 acutely. Naloxone-hydrochloride (Salars, Como, Italy) and naltrexone-hydrochloride (Chiesi, Parma, Italy) were used at the dose of 30 mg/kg acutely, or 5 mg/kg for 4 days. The dose of naloxone for the acute treatment (30 mg/kg) was chosen after a preliminary dose finding study (Bianchi et al., unpublished), in which it appeared to consistently increase nociceptive thresholds without inducing evident behavioral changes. The dose of 5 mg/kg was chosen for the chronic study because it had been shown

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to induce analgesia with this administration schedule (9). Doses of naltrexone were chosen on the basis of the equipotency of the two antagonists.

The κ receptor agonist U 50-488 (Upjohn, Kalamazoo, MI) was administered acutely at the doses of 1.25, 2.5, and 5.0 mg/kg alone or together with naloxone 30 mg/kg. The κ-opiate receptor antagonist MR 1452 (Boheringer Ingelheim, Ingelheim, Germany) (13,15,20) was always administered at the dose of 5.0 mg/kg. All drugs were administered SC. All experiments were conducted in the morning beginning between 0900–1000 h, and the nociceptive thresholds were always measured by the same operator.

Nociceptive thresholds were measured by the hot plate (58°C) method, as previously described (2), and the end point used was the licking of the hind paws. The results are expressed as percentage of the maximal possible effect (% MPE). MPE expresses the equation [(TL-BL)/(ML-BL)] × 100, where BL is the mean basal latency (4.0–8.0 s) measured before the first treatment was applied; TL is the test latency measured after treatments; ML is the maximal latency accepted (20 s), chosen to avoid tissue damage to the footpads. To evaluate a role of the hot-plate test as a stressful cue, the chronic study was performed either measuring hot plate latencies every day, or only on the last day of the experiment.

Statistical analysis of results was obtained by the Kruskal-Wallis analysis of variance (ANOVA) of ranks, followed by the Dunnet's test for multiple comparisons.

RESULTS

The acute administration of naloxone (Fig. 1, upper panel) or naltrexone (lower panel) induced a statistically significant increase of nociceptive thresholds that was maximal 30 min after the administration of the drugs. In the same figure it is also shown that the effect of naloxone and naltrexone was abolished by the concomitant treatment with the κ-opiate receptor antagonist MR 1452.

Treatment for 4 days with either naloxone or naltrexone induced an increase of analgesic thresholds that was statistically significant starting on day 3 (Fig. 2). The same effect was obtained in rats administered the hot-plate test only on the last day (data not shown). Under these experimental conditions too, the maximal effect of naloxone was observed 30 min after the drug administration. For this reason, we report in the figure the values measured at this time interval.

In Fig. 2 it is also shown that the chronic administration of MR 1452 alone did not elicit any effect, whereas the acute administration of MR 1452 on the last day abolished the analgesic effect elicited by chronic treatment with naloxone or naltrexone.

The κ-opiate receptor agonist U 50-488 elicited an analgesic effect that peaked 30 min after administration, as it is shown in Fig. 3. The figure shows that U 50-488 modulates the analgesic effect of naloxone 30 mg/kg. While the lowest (1.25 mg/kg) and the middle dose (2.5 mg/kg) induce a decrease of the analgesia induced by naloxone alone, but at the highest dose (5.0 mg/kg).

DISCUSSION

Although the evidences contributed by several reports indicate that naloxone exerts pharmacological actions unrelated to opiate binding (6,19,22) the data presented indicate that naloxone and naltrexone can exert an analgesic activity through the κ-opiate receptor. Some Authors (22) suggest that

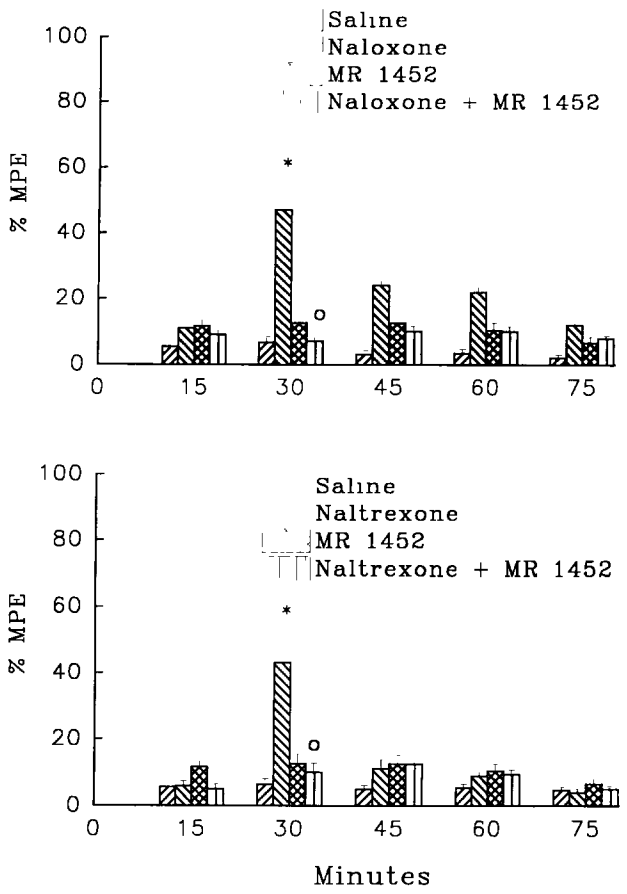

FIG. 1. Analgesic effect of naloxone (upper panel) or naltrexone (lower panel) (30 mg/kg SC); the effect is blocked by MR 1452 at the dose of 5.0 mg/kg SC. Values are mean ± SE; $*p < 0.01$ vs. saline; o = $p < 0.01$ vs. naloxone or naltrexone. The analgesic effect is expressed as % MPE (maximum possible effect). Basal latencies (s) for the different experimental groups were: Saline: 5.2 ± 0.6; Naloxone: 5.0 ± 0.4; Naltrexone: 5.7 ± 0.6; MR 1452: 5.9 ± 0.5; Naloxone + MR 1452: 6.1 ± 0.6; Naltrexone + MR 1452: 6.0 ± 0.4.

naloxone induces analgesia by blocking presynaptic autoinhibition of enkephalin release. However, these findings were obtained in vitro by using low concentrations of the drug. In the same experimental conditions, higher concentrations of naloxone induce an opposite effect. Thus, it is difficult to interpret our results in a similar way. At high doses, naloxone has also been reported to behave as a GABA antagonist (4,6). However, a recent report suggests that the GABA receptor complex is not involved in mediating naloxone-induced analgesia in the rat (17).

Our hypothesis is consistent with the reversibility of the effect of naloxone by the κ-opiate receptor antagonist MR 1452. These data are also consistent with the block by the κ-opiate receptor inhibitor MR 1452 of the analgesic effect exerted by naloxone or naltrexone after stress (18). However, the results presented suggest that the κ agonistic effects observed after acute or chronic administration of naloxone or naltrexone have different origins.

We hypothesize that both drugs possess the well-known μ-opiate receptor antagonist activity, and a less marked κ agonistic effect, similarly to what is known for pentazocine or

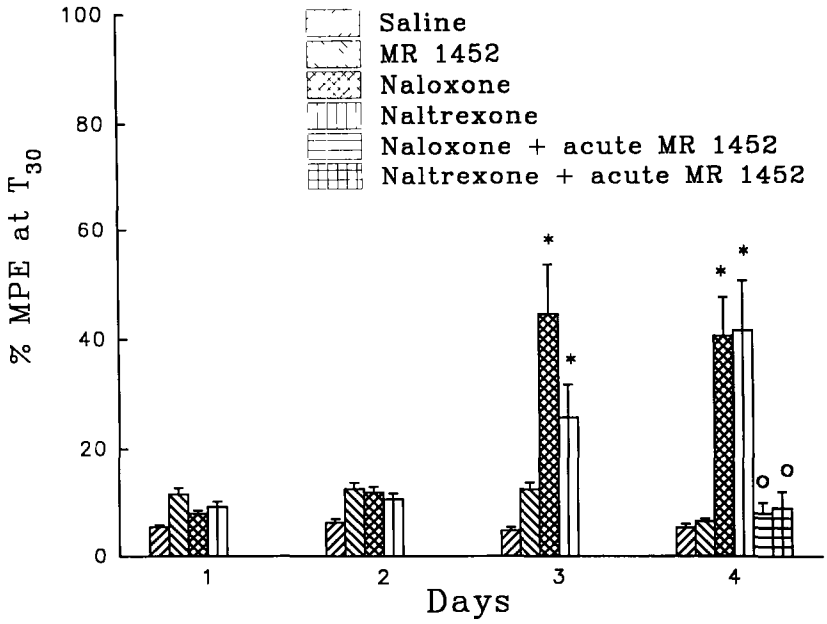

FIG. 2. Effect on analgesic thresholds induced by the daily administration for four days of naloxone, naltrexone or MR 1452 (5.0 mg/kg SC) alone, and blockade of the analgesic effect of naloxone or naltrexone by the administration of MR 1452 on day four. Values are mean ± SE; *$p < 0.01$ vs. saline; o = $p < 0.01$ vs. naloxone or naltrexone. The analgesic effect is expressed as % MPE (maximum possible effect). Basal latencies (s) for the different experimental groups were: Saline: 6.2 ± 0.6; Naloxone: 6.1 ± 0.3; Naltrexone: 6.4 ± 0.3; MR 1452: 5.7 ± 0.4; Naloxone + MR 1452: 6.5 ± 0.2; Naltrexone + MR 1452: 6.6 ± 0.2.

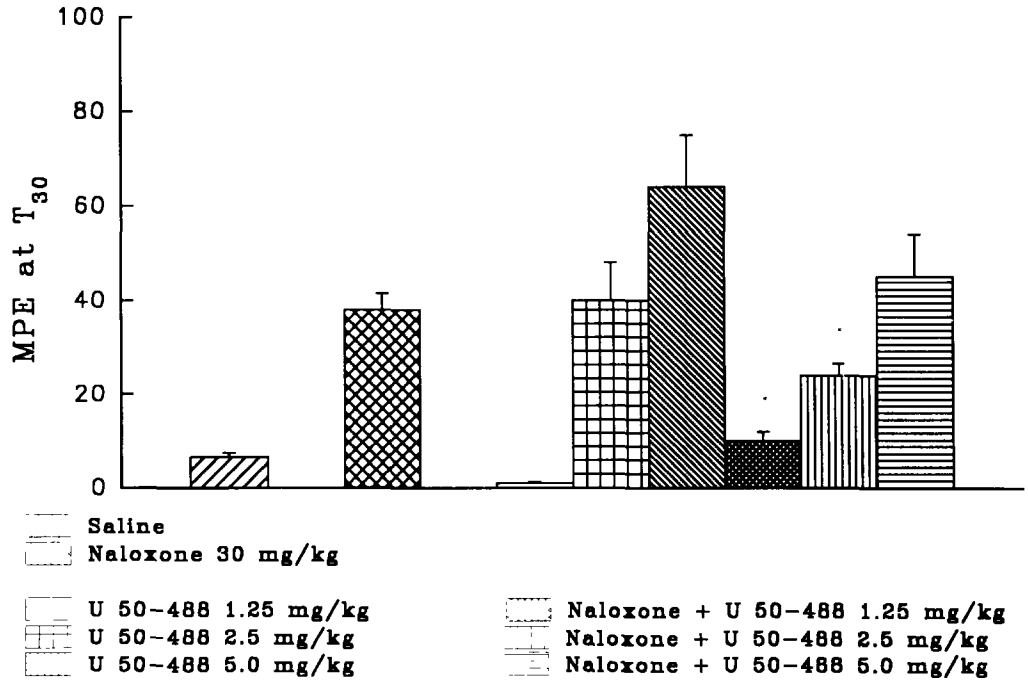

FIG. 3. Dose-dependent analgesic effect of the acute administration of U 50-488, and interference of the κ opiate agonist with the effect of acute naloxone. Values are mean ± SE; *$p < 0.01$ vs. naloxone. The analgesic effect is expressed as % MPE (maximum possible effect). Basal latencies (s) for the different experimental groups were: Saline: 5.9 ± 0.4; Naloxone: 5.8 ± 0.2; U 50488 1.25 mg: 5.7 ± 0.4; U 50488 2.5 mg: 5.8 ± 0.5; U 50488 5.0 mg: 6.1 ± 0.4; U 50488 1.25 mg + Naloxone: 6.6 ± 0.4; U 50488 2.5 mg + Naloxone: 5.9 ± 0.6; U 50488 5.0 mg + Naloxone: 6.4 ± 0.3.

nalorphine (11,21). The μ antagonist or κ agonist characteristics of the drug prevail according to the doses and the experimental conditions. In this context, the acute effect of high doses of naloxone or naltrexone can be explained by the fact that the administration of the two drugs allows the detection of the κ agonist component. For the effect after prolonged administration, one has to take into consideration several data concerning the interplay between μ- and κ-opiate receptors. It was previously shown that acute or chronic treatment with μ antagonists enhances the effects of κ agonists (11,23), thus suggesting a counterbalance between the two receptors. This possibility is consistent with the observation that κ agonists can affect analgesia induced by μ agonists (5,16). In our case, the analgesic effect of naloxone and naltrexone after prolonged treatment can therefore be explained by the development of a functional supersensitivity to the κ-agonist effect, induced by the blockade of μ receptors exerted by the two drugs themselves. This mechanism could explain, in the chronic treatment, their analgesic effect also at doses much lower than those necessary for the acute effect (5.0 mg/kg vs. 30 mg/kg).

Our data do not rule out the possibility of an indirect effect of naloxone or naltrexone on κ-opiate receptors. For example, the two antagonists could induce the release of the endogenous kappa agonist dynorphin and through this mechanism induce analgesia. MR 1452, in fact, fully prevents the analgesic effect of dynorphin (20). However, although clonidine induces the release of dynorphin in the rat (1), naloxone induced analgesia has been shown to be unaffected (7) or reduced (17) by the administration of clonidine; that is, of a drug that indirectly acts on the κ-opiate receptor. The results obtained with the κ agonist U 50-488 are difficult to be fully explained on the basis of simple pharmacological principles. However, although a clear trend in responses does not prevail, these results show that the two drugs significantly modulate each other, probably competing on the same receptor.

The observation that the paradoxical analgesic effect of naloxone is evident after the acute administration of the drug or, chronically, also without the exposure of rats to the hot plate, suggests that the naloxone-induced analgesia is not related to a possible stress induced by repeated exposure to the hot plate.

In conclusion, we suggest that the κ-opiate receptors are involved in naloxone- and naltrexone-induced analgesia.

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