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Blockade of Tolerance to Stress-Induced Analgesia by MK-801 in Mice

A. L. VACCARINO,[1] AND M. C. CLAVIER

Department of Psychology, University of New Orleans, New Orleans, LA 70148

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VACCARINO, A. L. AND M. C. CLAVIER. *Blockade of tolerance to stress-induced analgesia by MK-801 in mice.* PHARMACOL BIOCHEM BEHAV **56**(3) 435–439, 1997.—The *N*-methyl-D-aspartate (NMDA) receptor has been implicated in mechanisms of tolerance to morphine-induced analgesia. The present study examined the role of the NMDA receptor in the development of tolerance to stress-induced analgesia (SIA). In the first experiment, mice were exposed to a stressor (a 3-min forced swim in water maintained at 32°C) once daily for 15 consecutive days. Analgesia was measured 2 min after stress on the first and last day using the hot-plate test. To examine the role of the NMDA receptor in the development of tolerance to SIA mice were treated daily with the non-competitive NMDA receptor antagonist, MK-801, 15 min before swimming. Pretreatment with MK-801 was found to block both analgesia and tolerance. In a second experiment, to examine whether SIA and tolerance to SIA are mediated by similar or different mechanisms, mice were injected daily with MK-801 after analgesia had dissipated (1 h following swim). Tolerance to SIA was blocked by delayed injections of MK-801. These results suggest that the NMDA receptor is involved in mechanisms of tolerance to SIA, independent of its role in analgesia.

Swim stress-induced analgesia *N*-methyl-D-aspartate (NMDA) MK-801 Tolerance

STUDIES have indicated that the *N*-methyl-D-aspartate (NMDA) receptor plays a role in mediating morphine analgesia and some forms of stress-induced analgesia (SIA). For example, analgesia produced by microinjections of morphine or NMDA into the periaqueductal gray (PAG) are blocked by NMDA receptor antagonists (14). Furthermore, NMDA antagonists have been shown to attenuate both morphine-induced analgesia (18,19) and SIA (18,25,28,36).

Tolerance to the analgesic effects of both morphine and stress can develop following repeated exposures. Recent evidence suggests that the NMDA receptor is involved in mechanisms of tolerance to morphine analgesia. It has been shown that competitive and non-competitive NMDA antagonists prevent the development of tolerance, without affecting acute morphine analgesia (2,23,24,31,32). However, the role of the NMDA receptor in mediating tolerance to SIA has not been examined. In the present study we examined the role of the NMDA receptor in the development of tolerance to SIA.

EXPERIMENT 1

The non-competitive NMDA antagonist, MK-801, has been demonstrated to prevent the development of tolerance to morphine analgesia (2,23,24,32). Tolerance to SIA produced by a 3-min forced swim occurs after 2 weeks of daily swims (5,26). The present experiment examined the effect of MK-801 on the development of tolerance to swim stress-induced analgesia (SSIA).

METHOD

Subjects

Male Swiss Webster mice (Harlan Laboratories, IN) weighing 20–30 g at the time of testing served as subjects. The mice were allowed free access to food and water and were maintained on a 12-h light cycle (light onset at 7:00 am).

Drugs

MK-801 (Research Biomedical Inc., MA) was dissolved in physiological saline and administered in a volume of 1ml/100 g of body weight. Saline was used as a vehicle control. Drugs were injected interperitoneally.

Procedure

To examine the development of tolerance to SSIA mice were individually swum for 3 min in water maintained at 32°C

[1]To whom requests for reprints should be addressed.

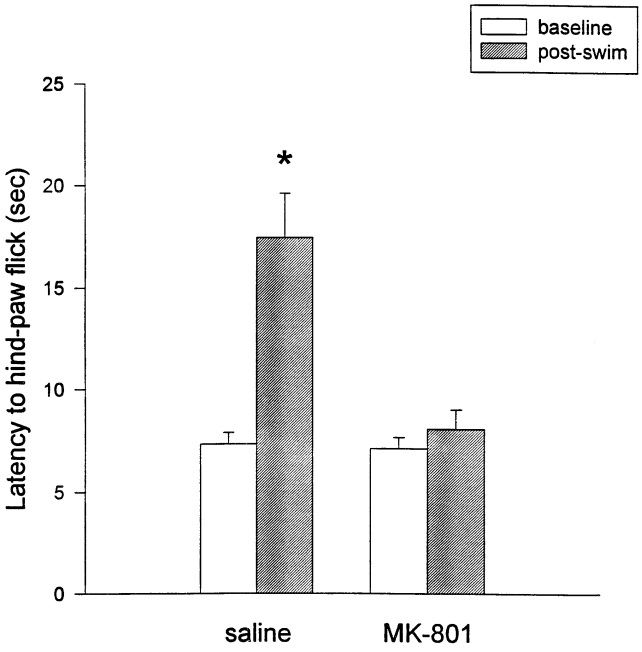

FIG. 1. Effects of MK-801 (0.075 mg/kg) on swim stress-induced analgesia. Data are expressed as mean latency to hind-paw flick in seconds (sec) (± SEM) in the hot-plate test before (baseline) and after (post-swim) swimming on day 1. *$p < 0.01$ compared to baseline.

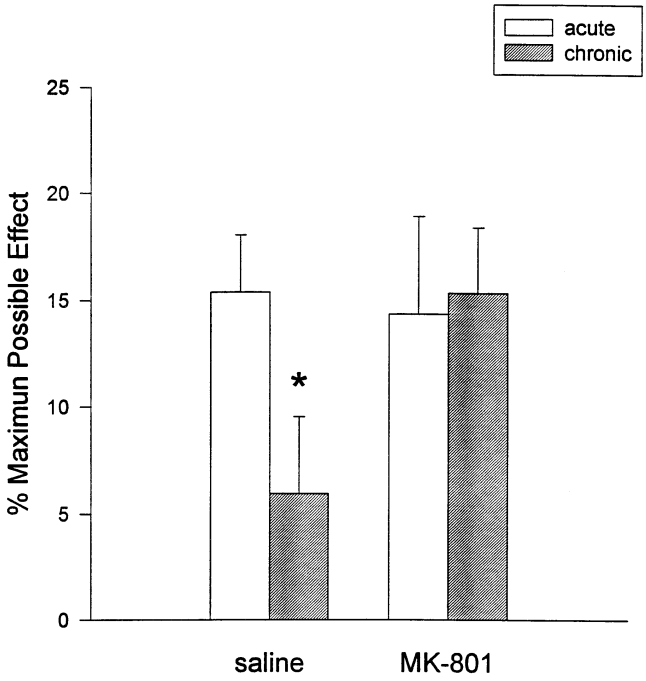

FIG. 2. Effects of MK-801 (0.075 mg/kg) on tolerance to swim stress-induced analgesia. Data are expressed as % maximum possible effect (± SEM) in the hot-plate test on day 15. Mice were exposed to daily swims for 15 consecutive days (chronic) or on day 1 and day 15 only (acute). *$p < 0.025$ compared to acute/saline group.

for 15 days (CHRONIC group). A control group of acutely stressed mice were swum on day 1 and day 15 only (ACUTE group). Pain sensitivity was assessed on day 1 and day 15. Mice were placed individually on a hot-plate maintained at 52 ± 1°C and the latency to produce a characteristic hind-paw flick was measured before (baseline) and 2 min after swimming. A cut-off period of 60 s was imposed to avoid tissue damage.

To examine the role of the NMDA receptor on SSIA and tolerance to SSIA, mice were injected with 0.075 mg/kg of MK-801 (CHRONIC/MK-801) or saline (CHRONIC/SAL) 15 min before swimming on days 1–14. Acutely stressed mice also received daily injections of MK-801 (ACUTE/MK-801) or saline (ACUTE/SAL).

Statistics

Data obtained in the hot-plate test were expressed as latency to hind-paw flick in seconds, or as percentages of maximum possible effect (%MPE) using the following formula:

$$\%\text{MPE} = \frac{(\text{post-swim latency} - \text{baseline})}{(\text{cut-off latency} - \text{baseline latency})} \times 100$$

The results were evaluated using analysis of variance (ANOVA), and one-tailed Student t-tests with the appropriate Bonferroni adjustments. Post-hoc comparisons of group means were made using Tukey's test after ANOVA.

RESULTS

On day 1, mice displayed a significant degree of SSIA ($t(23) = 5.48$, $p < 0.01$), which was antagonized by MK-801 ($t(23) = 1.08$, n.s., Fig. 1). On day 14, chronically swum mice displayed significantly less analgesia as compared with the acutely swum mice (ACUTE/SAL vs CHRONIC/SAL: $t(22) = 2.12$, $p < 0.025$, Fig. 2), indicating that significant tolerance developed to SSIA. Tolerance to SSIA produced by chronic swims, however, was prevented in mice that received MK-801 (ACUTE/MK-801 vs CHRONIC/MK-801: $t(22) = 0.18$, n.s.).

EXPERIMENT 2

Since MK-801 blocked SSIA (see Fig. 1), it could be argued that the blockade of SSIA by MK-801 might also be responsible for the blockade of tolerance to SSIA (see Fig. 2). In this experiment, to examine whether the inhibitory effects of MK-801 on SSIA and tolerance to SSIA are mediated by similar or different mechanisms, MK-801 was administered 1 h following stress. Therefore, the analgesic effects of stress would have dissipated by the time MK-801 was administered, and any effect of MK-801 on tolerance would occur independent of the effects of MK-801 on analgesia.

METHODS

Mice were treated the same as in Experiment 1 except that they were injected with MK-801 or saline 1 h following swim stress on days 1–14. To verify that SSIA dissipates within an hour after the stress, an additional analgesic measure was taken 45 min after swim stress on day 1.

RESULTS

Mice displayed a significant degree of analgesia 2 min after the stress, but not 45 min after the stress as compared to

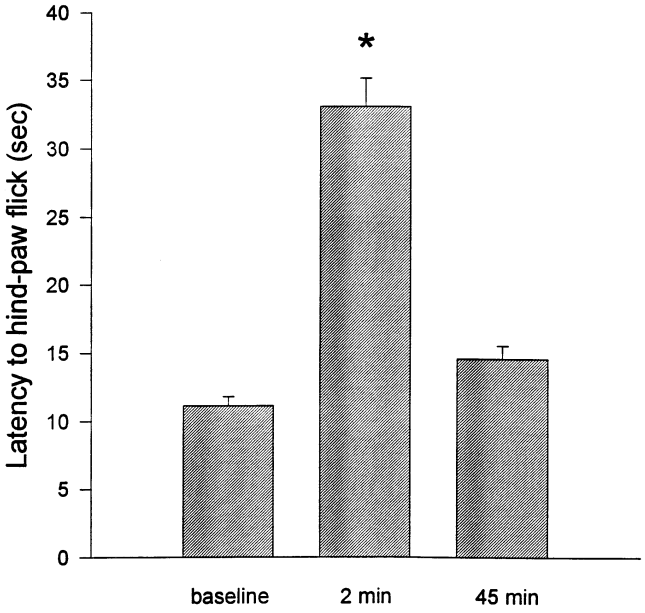

FIG. 3. Analgesic effects of swim stress in the hot-plate test. Data are expressed as mean latency to hind-paw flick in seconds (sec) (± SEM) before (baseline), 2 minutes, and 45 minutes after swimming on day 1. *$p < 0.05$ compared to baseline.

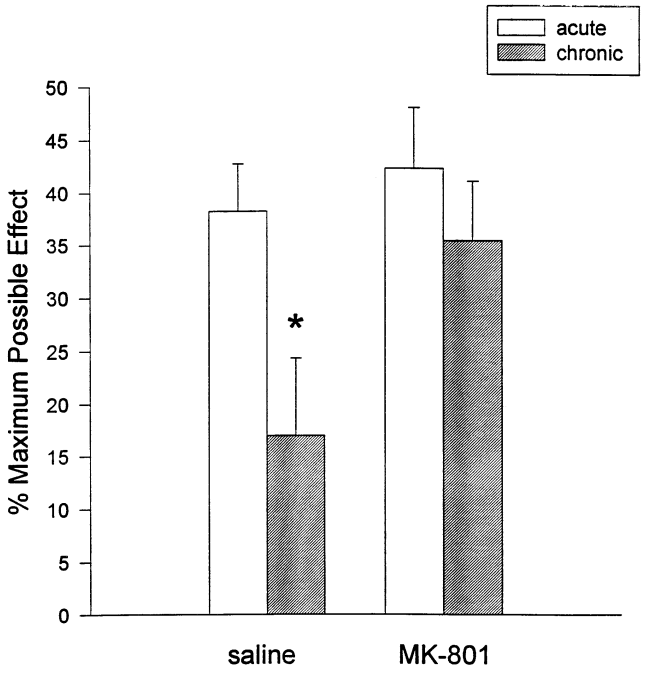

FIG. 4. Effects of delayed injections (1 hour following swimming) of MK-801 (0.075 mg/kg) on tolerance to swim stress-induced analgesia. Data are expressed as % maximum possible effect (± SEM) in the hot-plate test on day 15. Mice were exposed to daily swims for 15 consecutive days (chronic) or on day 1 and day 15 only (acute). *$p <$ 0.025 compared to acute/saline group.

baseline ($F(2,78) = 91.36, p < 0.001$, Fig 3). On day 14, chronically swum mice displayed a significant degree of tolerance (ACUTE/SAL vs CHRONIC/SAL: $t(18) = 2.45$, $p < 0.025$, Fig. 4), which was prevented in mice that received daily post-swim injections of MK-801 (ACUTE/MK-801 vs CHRONIC/MK-801: $t(22) = 0.71$, n.s.).

DISCUSSION

This is the first study to show that MK-801 blocks the development of tolerance to SIA. These findings are consistent with the effects of MK-801 on tolerance to morphine analgesia (2,23,24,32). Furthermore, the antagonistic effect of MK-801 on tolerance to SSIA appears to be independent of SSIA, as MK-801 prevented tolerance development when administered after SSIA had dissipated (i.e., 1 h following stress, Experiment 2). This is similar to the finding by Marek et al (24) that MK-801 was effective in preventing the development of tolerance to morphine analgesia when administered 2 h following morphine. Studies have shown that the activity of NMDA synapses outlasts the initial activation of the receptor (6,8), and the NMDA receptor has been implicated in mechanisms of central neural plasticity (see 22). Taken together, these results suggest that MK-801 may prevent the development of tolerance to both morphine analgesia and SSIA by preventing the development of delayed or enduring changes that occur at the NMDA receptor (23,24).

Morphine analgesia has been attributed in part to an indirect activation of NMDA receptors within the PAG (14). Furthermore, overactivation of NMDA receptors has been shown to result in cell degeneration, which is prevented by delayed application of MK-801 (8). Marek et al (24) suggested chronic morphine administration may lead to a similar overactivation of NMDA receptors which leads to long-lasting changes in pain inhibitory systems. It is possible, therefore, that stress-induced activation NMDA receptors within the PAG produce analgesia, and that chronic activation of NMDA receptors by stress produces delayed and enduring changes within this pain inhibitory system. Such a hypothesis provides a means of reconciling the data that MK-801 blocks tolerance to SSIA by a mechanism that is independent of its ability to block of SSIA.

The development of morphine tolerance has been suggested to involve associative learning in which environmental cues may signal the subsequent administration of the drug, resulting in compensatory responses to the drug's effect thus reducing its effectiveness (20). One possibility, therefore, is that MK-801 disrupted the development of the association between the environment in which the stress was delivered (e.g., handling procedure, drug injections, room, etc.) and the subsequent analgesic effects of stress. However, MK-801 has been shown to block learning when administered before training, but not when delivered after training (15,29). Therefore, the finding MK-801 blocked tolerance when delivered 1 h after the stressor suggests that MK-801 did not disrupt associative learning. Furthermore, recent studies have suggested that MK-801 disrupts non-associative opiate tolerance, but not associative opiate tolerance (2,33).

In the present study it was found that MK-801 completely abolished SSIA (Experiment 1). Previous studies have reported that a 32°C swim produces a mixed opioid/non-opioid analgesia blocked by both MK-801 and naloxone (36), or a predominately opioid analgesia blocked by naloxone but not MK-801 (25). In the present experiment, we found that SSIA was also antagonized by naloxone (data not shown). It is unlikely that the effect of MK-801 on SSIA is through an opioid mechanism, however, as binding studies have shown

that MK-801 has a greater than 1000-fold lower affinity for opioid receptors as compared with naloxone (19). Therefore, it would appear that inhibitory effect of MK-801 and naloxone on SSIA produced at this temperature (32°C) occur via independent mechanisms.

Stress (29) and pain (7,34,35) have been shown to prevent the development of tolerance to morphine analgesia, which appears to be related to neuroendocrine activity (12,30,34). Beta-endorphin and adrenocorticotropin hormone (ACTH) are released concomitantly from some pituitary cells during stress (11). Beta-endorphin is suggested to mediate some forms of SIA (1,16), and ACTH has been shown to prevent the development of tolerance to morphine analgesia (12). This raises the possibility that two parallel systems may be activated during stress: an endogenous opiate-mediated analgesic system (i.e., SIA) and a non-opiate system that prevents the development of 'rapid' tolerance to SIA. Indeed, adrenalectomy and hypophysectomy have been shown to attenuate SIA (3,17,21) and potentiate the rate of tolerance development to opiate analgesia (13,37). The existence of such a dual system would have clear adaptive advantages during prolonged stress. NMDA antagonists prevent the development of tolerance to both morphine analgesia (2,23,24,32) and SIA (as shown in this study). Interestingly, kynurenic acid is an endogenous metabolite of tryptophan that acts as a post-synaptic NMDA antagonist (10), and has also been shown to prevent the development of tolerance to morphine analgesia (23). Glucocorticoids, which are elevated during stress (9,27), may also indirectly increase levels of kynurenic acid by stimulating the production of tryptophan pyrrolase, which is responsible for the metabolism of tryptophan (4). Therefore, it is possible that stress/pain prevents the development of tolerance (to both SIA and morphine-induced analgesia) indirectly via the NMDA receptor.

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