Antagonism of N-Methyl-D-aspartate Receptors Reduces the Vulnerability of the Immune System to Stress after Chronic Morphine

NORMA C. ALONZO and BARBARA M. BAYER
Departments of Pharmacology (N.C.A.) and Neuroscience (B.M.B.), Georgetown University, Washington, DC
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ABSTRACT
It has been shown that morphine-tolerant animals have an altered immunological sensitivity to stress. Although the glutamatergic system has been implicated in the neuroadaptive process underlying this tolerant state, its potential role in development of the altered immunological sensitivity consequent to chronic morphine treatment is not known. To determine this, a morphine-tolerant state was induced by 10-day administration of an escalating dose of morphine from 10 to 40 mg/kg (s.c., b.i.d.), and lymphocyte proliferative response to a T-cell mitogen was measured. Morphine challenge (10 mg/kg s.c.) after days of treatment was gradually less immunosuppressive, and this tolerance progression was delayed by concurrent administration of the N-methyl-D-aspartate (NMDA) receptor antagonist (−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) (0.1 mg/kg s.c.) with chronic morphine. The effect was independent of glucocorticoid level changes and was not a result of an acute interaction of the drugs or the prolonged presence of the antagonist alone. Subsequent to chronic treatment, animals were subjected to opioid withdrawal and water stress. Both stressors induced 50% immunosuppression in morphine-tolerant animals compared with saline-treated controls. Increased immunological sensitivity to these stressors was attenuated when MK-801 was administered with chronic morphine as demonstrated by an accelerated recovery rate and lack of immunosuppression from opioid withdrawal and water stress, respectively. Together, these findings provide the first evidence that the neuroadapted state of the immune response after chronic morphine can be modified by NMDA receptor antagonism, as illustrated by a temporal deceleration of the development of immunological tolerance during chronic treatment that is associated with an attenuation of the immunological vulnerability of morphine-tolerant animals to stress.

The outcome of the neuroadaptation thought to underlie opioid-induced tolerance is a modified homeostatic state such that reaction to various stimuli such as drug exposure and stress is altered (Carlson, 1977; Molina et al., 1994; Fitzgerald et al., 1996; Houshyar et al., 2001a,b; Weiss et al., 2001). It has been shown that morphine-tolerant animals have an altered response to cocaine, 3,4-methylenedioxymethamphetamine, cannabinoind, and alcohol administration (Howd and Pryor, 1980; Erdtmann-Vourliotis et al., 2000; Gonzalez et al., 2002), a modified reaction to activators of the hypothalamic-pituitary-adrenal axis such as stress and steroids (Borssook et al., 1994; Houshyar et al., 2001a,b), and a weakened host defense mechanism to pathogens (Bryant et al., 1987; Rouveix, 1992; West et al., 1997). Although extensively studied, the neuroadaptive process thought to be responsible for tolerance is far from elucidated. There is, however, evidence that the central glutamatergic receptor system is a major participant in the development of the tolerant state. Several studies have found that manipulation of central glutamatergic receptors can modulate the development and outcome of tolerance to opioid-induced analgesia (Trujillo and Akil, 1991; Bespalov et al., 1994; Fundytus, 2001), behavioral sensitization (Jeziorski et al., 1994; Iijima et al., 1996), and physical dependence (Fundytus et al., 1997; Gonzalez et al., 1997; Bisaga and Popik., 2000) The additional role of NMDA receptors as a critical factor in neuroplasticity suggests that this receptor system would also affect the resulting altered state of an animal after prolonged opioid treatment (Wang and Feng, 1992; Shors and Servatius, 1995; Krug et al., 2001). Among the acute effects of opioids that have been shown to be diminished subsequent to chronic treatment is immunosuppression. Previous studies have found that the inhibition of natural killer cell activity (Rouveix, 1992; West et al., 1997), humoral and cellular immune responses (Rouveix, 1992; Rahim et al., 2002), and cytokine activity (Chang et al., 1998; Limiroli et al., 2002; Raghavendra et al., 2002) as a

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; MK-801, (−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate.
result of an acute administration of morphine is absent after a prolonged period of morphine management. Studies in this laboratory have shown that morphine administration (10 mg/kg) to a naïve animal significantly suppresses blood lymphocyte proliferative response to a T cell mitogen and that this effect is mediated through central μ-opioid receptors (Bayer et al., 1990; Hernandez et al., 1993; Flores et al., 1995). After chronic morphine treatment, the suppressive effect of morphine administration (10 mg/kg) on the proliferative response is absent (Bayer et al., 1996; Mellon et al., 2001). However, these chronically treated animals were found to be much more sensitive to the immunosuppressive effects of restraint stress, suggesting an altered vulnerability of these animals to the stressor (Bayer et al., 1994).

Similar to other opioidergic effects, the mechanism fundamental to the development of tolerance to immune response to morphine is largely unknown. Surprisingly, although NMDA receptor antagonism has been shown to modulate tolerance of opioid effects such as analgesia and reinforcement, the role of the glutamatergic receptor system in the development and outcome of immunomodulatory tolerance has not been examined. The present study, therefore, endeavors to determine whether NMDA receptor activity has a role in the development of immunological tolerance by comparing mitogen-induced blood lymphocyte proliferative activity after morphine challenge during chronic treatment with either morphine and MK-801 or morphine alone. Additionally, it will be determined whether opioid withdrawal and water stress applied subsequent to chronic morphine treatment induce an altered immune response and whether this immunological vulnerability to these stressors is dependent on NMDA receptor activity during chronic dosing.

Materials and Methods

Animals. Male Sprague-Dawley rats were received from Taconic Farms (Germantown, NY), weighing 200 to 225 g upon arrival. Animals were housed three per cage in polypropylene cages with micro island tops that were contained in a thermoregulated (23 ± 1°C) and light (12-h light/dark)-controlled room. After receipt, animals were allowed to acclimate for 1 week before experimental manipulation. Free access to food (Purina Rat Chow; Purina, St. Louis, MO) and water was provided. In experiments requiring surgery, animals were allowed to recover for an additional week before experimentation. All animal studies have been approved by the Georgetown University Animal Care and Use Committee in accordance with the guidelines adopted by National Institutes of Health.

Drugs. Morphine sulfate was generously provided by the National Institute on Drug Abuse (Research Triangle Park, NC). MK-801 was purchased from Sigma-Aldrich (St. Louis, MO). Equithesin was prepared by dissolving chloral hydrate and magnesium sulfate in a solution of propylene glycol and pentobarbital. All vehicle and drug solutions were made using sterile, 0.9% saline.

Chronic Treatment. Chronic morphine treatment was initiated after acclimatization period with drug administered as follows (doses in milligrams per kilogram morphine s.c.): day of treatment, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10; AM dose, 10, 10, 20, 20, 30, 30, 40, 40, 40, and sacrifice; and PM dose, 10, 20, 30, 30, 40, 40, 40, 40, and 40. Groups receiving MK-801 (0.1 mg/kg s.c.) were administered drug either instead of morphine or immediately before each morphine injection for concurrent treatment.

Plasma Corticosterone Measurement. Heparinized plasma was collected at time of sacrifice and stored at −20°C. Samples were assayed for corticosterone levels using a solid phase 3H radioimmunoassay purchased from ICN Pharmaceuticals (Costa Mesa, CA).

Lymphocyte Proliferation. Animals were decapitated and trunk blood collected in 50-ml polypropylene tubes containing 0.2 ml of heparin (1000 U/ml). Whole blood was diluted 1:5 in sterile RPMI 1640 cell culture media (Invitrogen, Carlsbad, CA) supplemented with 5% fetal bovine serum and 1% gentamicin. Diluted blood (100 μl) was incubated with increasing concentrations of T cell-specific mitogen concanavalin A (at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 μg/well) for 72 h at 37°C with 8% CO2. Samples were pulsed with 0.5 μCi of [methyl-3H]thymidine (6.7 mmol; PerkinElmer Life Sciences, Boston, MA) in a 20-μl volume, and incubated for an additional 24 h at 37°C with 8% CO2. Samples were lysed with distilled water and harvested onto glass fiber filters using a 96-well cell harvester (Brandel, Inc., Gaithersburg, MD). The amount of labeled DNA was determined via liquid scintillation spectrophotometry (Beta Plate, LKB Pharmacia, Piscataway, NJ).

Maximum proliferative response was calculated from the concanavalin A dose-response curves generated. To allow comparisons from experiment to experiment, all data were expressed as a percentage of the response of the saline-treated control group from each individual experiment. All experiments were repeated at least two to three times.

Withdrawal. After 10 days of chronic treatment, as described above, all drug injections were replaced with sterile saline s.c. Body weights were measured at 12-h intervals up to 72 h after initiation of drug withdrawal. Separate groups of animals were sacrificed after 24 and 72 h of withdrawal for corticosterone and blood lymphocyte proliferation analysis.

Water Stress Assay. After 10 days of chronic treatment, as described above, animals were individually placed in polypropylene cages (standard mouse cage) containing 2 cm of water (room temperature) or regular bedding for 1 h. Animals were sacrificed and trunk blood collected for plasma corticosterone and peripheral blood lymphocyte proliferation analysis.

Results

NMDA Receptor Antagonism Alters the Rate of Development of Immunological Tolerance during Chronic Morphine Treatment. To determine whether NMDA receptor antagonism alters the rate of development of morphine-induced tolerance, immune responses were analyzed at various time points throughout chronic treatment with either morphine alone or morphine and MK-801. It has been previously shown in this laboratory that naïve animals challenged with acute morphine showed a suppression of lymphocyte proliferation response to a T cell mitogen compared with saline-treated control animals and that tolerance develops to this immunosuppressive effect after chronic treatment (Bayer et al., 1990, 1994). In animals receiving escalating doses of morphine, an initial 75% inhibition of T cell proliferative ability from a morphine challenge (10 mg/kg s.c.) after 1 day of chronic treatment was gradually less apparent such that peripheral lymphocytes were 70, 80, and 100% responsive to mitogen after 2, 4, and 10 days of treatment (Fig. 1a). Similarly, lymphocyte proliferative response during combined administration of chronic morphine and MK-801 was initially significantly suppressed; however, this suppression was prolonged and responses continued to be suppressed by 75% even after 4 days of treatment (p < 0.01). After 6 days of treatment, animals receiving either concurrent morphine and MK-801 or morphine alone showed lymphocyte activity that was not significantly different from controls (Fig. 1a).
Whether NMDA receptor modulation would affect lymphocyte proliferation alone or alter morphine’s acute effects is not known. Therefore, to determine whether the effect of MK-801 on immunological tolerance is related directly to an acute interaction of the drug with morphine, animals were treated with morphine (10 mg/kg s.c.) and MK-801 (0.1 mg/kg s.c.) for 2 h. Administration of MK-801 did not suppress the proliferative response of lymphocytes to mitogen nor did it affect the immunosuppressive effect of acute morphine (Fig. 2).

Immunosuppression by Acute Morphine Is Not Modified by Chronic Treatment with MK-801. The effect of prolonged antagonism of NMDA receptors on the immune system is unknown. It may be that the chronic antagonism of glutamate receptors alone is sufficient to bring about a delay in immunological tolerance development during chronic morphine. Therefore, the acute effect of morphine in animals chronically treated with MK-801 was studied. Surprisingly, animals receiving twice-daily injections of MK-801 (0.1 mg/kg) alone showed a potentiated mitogenic T cell response compared with control animals. Lymphocyte proliferative response to mitogen in animals administered 10 days of chronic morphine was not suppressed 2 h after a morphine challenge (10 mg/kg). In contrast, a similar dose of morphine in animals that received chronic MK-801 (0.1 mg/kg) for 10 days was still significantly suppressive ($p < 0.001$). Confirming previously shown data (Fig. 1), animals administered both morphine and MK-801 chronically showed no immunosuppression to a morphine challenge dose (10 mg/kg) after 10 days of treatment (Fig. 3).

Morphine Withdrawal Induces Profound Weight Loss That Is Unaffected by Antagonism of NMDA Receptors during Chronic Treatment. Recent evidence has shown that the deprivation of morphine after chronic exposure is likened to a stress response, resulting in significant immunosuppression and a significant elevation of steroid levels (Ignar and Kuhn, 1990; Houshyar et al., 2001b). To determine whether NMDA receptor antagonism would alter the effects and rate of recovery from opioid withdrawal, animals were treated chronically with morphine or morphine and MK-801 as described previously, after which opioid withdrawal was induced by replacing all drug injections with saline or saline and MK-801 (0.1 mg/kg s.c.) at the time of acute morphine administration (+MK-801). Trunk blood was collected for lymphocyte proliferation analysis. Data are expressed as percentage of the mean maximum proliferation ± S.E.M. of the saline-treated control group (32,582 ± 5608 cpm). * $p \leq 0.05$ versus saline-treated control as determined by one-way analysis of variance, Newman-Keuls post test.

The development of tolerance to steroid elevation during chronic morphine with or without concurrent NMDA receptor antagonism was also studied at these different time points. In animals receiving escalating doses of morphine, plasma corticosterone concentrations were 300% over control levels on day 1 of treatment but gradually decreased so that by day 10 of the treatment regime, plasma corticosterone concentrations were not different from those measured in saline-treated control animals. Antagonism of NMDA receptors with MK-801 during chronic morphine did not alter this development of tolerance to the corticosterone elevation caused by morphine challenge. Animals receiving both morphine and MK-801 chronically exhibited plasma corticosterone concentrations that were 250% over control levels with a gradual decrease in levels such that they were no longer significantly different from control animals after 10 days of chronic treatment (Fig. 1b).

**Antagonism of NMDA Receptors Does Not Alter the Immunosuppressive Effect of Acute Morphine.** As previously shown in our laboratory, acute morphine significantly suppresses lymphocyte proliferation in naive animals. Whether NMDA receptor modulation would affect lymphocyte proliferation alone or alter morphine’s acute effects is not known. Therefore, to determine whether the effect of MK-801 on immunological tolerance is related directly to an acute interaction of the drug with morphine, animals were treated with morphine (10 mg/kg s.c.) and MK-801 (0.1 mg/kg s.c.) for 2 h. Administration of MK-801 did not suppress the proliferative response of lymphocytes to mitogen nor did it affect the immunosuppressive effect of acute morphine (Fig. 2).

**Fig. 1.** NMDA receptor antagonism alters the rate of development of tolerance to the immunological, but not steroidal effect of chronic morphine treatment. Animals were chronically treated with morphine (◇) or morphine and MK-801 (▼) as described under Materials and Methods. Animals were sacrificed and trunk blood collected 2 h after a morphine challenge (10 mg/kg s.c.) on treatment days 2, 4, 6, and 10. a, peripheral blood lymphocytes were used in an in vitro proliferation assay. Lymphocyte proliferation data were expressed as percentage of mean maximum proliferation ± S.E.M. of the saline-treated control group for that test day (49,163 ± 3469 cpm; n = 8/group). b, plasma corticosterone levels were measured and data expressed as percentage of mean corticosterone level ± S.E.M. of the saline-treated control group (68.5 ± 22.8 ng/ml; n = 8/group). * $p \leq 0.05$ versus chronic morphine group of respective test day as determined by one-way analysis of variance, Newman-Keuls post test.

**Fig. 2.** Mitogen-induced lymphocyte proliferative response to acute morphine is not altered by acute MK-801. Animals were subcutaneously treated with saline (Ctrl) or acute morphine (10 mg/kg s.c.) (aMS) for 2 h. Half the animals from each group concurrently received an acute dose of MK-801 (0.1 mg/kg s.c.) at the time of acute morphine administration (+MK-801). Trunk blood was collected for lymphocyte proliferation analysis. Data are expressed as percentage of the mean maximum proliferation ± S.E.M. of the saline-treated control group (32,582 ± 5608 cpm). * $p \leq 0.05$ versus saline-treated control as determined by one-way analysis of variance, Newman-Keuls post test.
The withdrawal syndrome was characterized by a dramatic weight loss that peaked after 24 h of drug deprivation ($p < 0.001$) and a return to control levels after 48 h. This characteristic weight loss observed during morphine withdrawal was not altered when MK-801 was administered during chronic morphine treatment (Fig. 4).

**Rate of Recovery from Opioid Withdrawal-Induced Immunosuppression, but Not Plasma Steroid Elevation, Is Accelerated by NMDA Receptor Antagonism during Chronic Treatment.** In addition to its impact on animal body weight, opioid withdrawal had a significant effect on plasma steroid elevation and mitogenic response of peripheral T cells. Lymphocytes isolated from morphine-withdrawing animals had a severe loss of proliferative ability. Mitogen-induced proliferation after 24 and 72 h of opioid withdrawal was 40 and 70% suppressed compared with non-withdrawing controls, respectively. Animals receiving MK-801 during chronic morphine treatment exhibited an altered immune profile. T cell proliferation in animals receiving both saline and MK-801 chronically was significantly suppressed after 24 h of withdrawal. However, unlike animals treated with morphine alone, lymphocyte responses in these animals returned to control levels after 72 h of drug deprivation (Fig. 5b).

Additionally, animals undergoing opioid withdrawal showed an elevation of plasma corticosterone that reached maximum levels after 24 h of withdrawal ($p < 0.001$) and returned to control values after 72 h of abstinence from drug. In contrast to the modulation of the immune response, changes in plasma steroid levels during morphine withdrawal were unaffected by NMDA receptor antagonism during chronic morphine treatment (Fig. 5).

**NMDA Receptor Antagonism Alters Immunological, but Not Steroidal Stress Sensitivity after Chronic Morphine.** We have previously shown that animals receiving chronic morphine exhibit an altered sensitivity to restraint.

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**Fig. 3.** Continual MK-801 treatment does not affect immunosuppression by morphine challenge. Chronic treatment with saline (Ctrl), morphine (cMS), MK-801 (cMK), or morphine and MK-801 (cMS + cMK) was carried out for 10 days as described previously. On test day, groups on right side of graph (+morphine) were administered morphine (10 mg/kg s.c.) and sacrificed 2 h later. Trunk blood was collected for a lymphocyte proliferation assay. Data are expressed as percentage of mean maximum proliferation ± S.E.M. of the saline-treated control group (Ctrl) (53,479 ± 3258 cpm; $n = 6–8$ group). $*, p < 0.05$ versus saline-treated control as determined by one-way analysis of variance, Newman-Keuls post test.

**Fig. 4.** Weight loss precipitated by morphine withdrawal is not altered by concurrent treatment with MK-801 during chronic morphine. Animals were chronically treated for 10 days with saline (○), morphine (□), or morphine and MK-801 (■) as described previously. Withdrawal was induced by replacing all drug injections with sterile saline. Body weight was measured every 12 h. Data are expressed as a mean of the percentage of body weight change compared with starting body weight ± S.E.M. $*$, $p < 0.05$ versus saline-treated control group as determined by one-way analysis of variance, Newman-Keuls post test.

**Fig. 5.** NMDA receptor antagonism during chronic treatment accelerated the rate of recovery of immunosuppression, but not steroid elevation induced by morphine withdrawal. Animals were chronically treated with morphine (cMS), or morphine and MK-801 (cMS + cMK) as described previously. Withdrawal was induced by replacing all drug injections with sterile saline. a, trunk blood was collected for analysis of blood lymphocyte proliferation after 0, 24, and 72 h of withdrawal. Data were expressed as a percentage of the mean maximum proliferative response of the saline-treated control group (37,671 ± 5486 cpm; $n = 7$ group). $*, p < 0.05$ versus cMS group at 72 h as determined by one-way analysis of variance, Newman-Keuls post test. b, plasma corticosterone levels were measured after chronic drug treatment and after 24 and 72 h of withdrawal. Data were expressed as percentage of mean corticosterone level ± S.E.M. of the saline-treated control group (57.9 ± 10.2 ng/ml; $n = 8$ group). $*$, $p < 0.05$ versus nonwithdrawing group of similar drug treatment; $a$, $p < 0.05$ versus cMS group at 72 h as determined by one-way analysis of variance, Newman-Keuls post test.
Stress, suggesting that the immune response to stressors after chronic treatment is altered (Bayer et al., 1994). However, it is not known whether antagonism of glutamate receptors during chronic morphine treatment would alter this vulnerability to stressors. To determine this, animals were chronically treated with morphine or morphine and MK-801 as described previously and underwent a water stress (at room temperature) for 1 h. Characteristic of a stressor, steroid levels of animals that were stressed were elevated irrespective of drug treatment. Animals receiving chronic saline, chronic morphine or chronic morphine and MK-801 all showed a significant increase of plasma corticosterone levels compared with their nonstressed controls \( (p < 0.01) \) (Fig. 6b). In contrast to this steroid response, the water stress protocol did not cause a significant suppression of lymphocyte proliferation in saline-treated control animals. However, water-stressed animals that were chronically treated with morphine showed a significantly inhibited lymphocyte response \( (p < 0.05) \), suggesting an altered immunological vulnerability to the stressor. Conversely, when animals were given concurrent MK-801 during chronic morphine, lymphocyte proliferation was not suppressed and was not significantly different from saline-treated controls, suggesting that NMDA receptor antagonism during chronic morphine treatment did modulate the development of an altered state of sensitivity to the stressor (Fig. 6a).

**Discussion**

The glutamatergic system has been shown to impact the development of tolerance to opioidergic effects. Until now, there has been no evidence to indicate that NMDA receptors are involved in immunosuppressive tolerance after chronic morphine treatment. In the present study, rate of development and expression of immunological tolerance, as measured by mitogen-activated peripheral blood lymphocyte proliferation, was examined after 10-day chronic treatment with morphine with or without the NMDA receptor antagonist MK-801. We find that presence of MK-801 with morphine administration delayed the rate of immunological tolerance progression. Furthermore, we present evidence that NMDA receptor antagonism concurrent to chronic morphine attenuates increased immunological sensitivity of morphine-tolerant animals to two different stressors.

Animals administered twice-daily injections of an escalating morphine dose showed a development of tolerance to the immunosuppressive and steroid-elevating effect of a morphine challenge. This hallmark of tolerance is observed for many other effects of acute morphine (Donovan et al., 1977; Mucha et al., 1978; Milne et al., 1989; Detweiler et al., 1995; Ouellet and Pollack, 1995; Kest and Hopkins, 2001; Bardin et al., 2003). As previously shown, a dose of morphine \( (10\, \text{mg/kg s.c.}) \) that significantly suppresses mitogen-activated lymphocyte proliferation in naive animals had no such effect in chronic morphine-treated animals (Bayer et al., 1996; Mellon et al., 2001). Data presented in this study demonstrate a gradual tolerance progression, occurring throughout the course of chronic morphine. Addition of chronic MK-801 with morphine delayed the rate of tolerance development to the morphine challenge. The question arises as to whether MK-801 itself is altering mitogen-induced T cell function. Our data indicated that administration of acute (Fig. 2) and chronic (Fig. 3) MK-801 was unable to modulate the suppressive effect of acute morphine \( (10\, \text{mg/kg}) \) on lymphocytic proliferation response, suggesting that alteration in the rate of tolerance progression requires the prolonged presence of both morphine and MK-801 and is not due to the actions of MK-801 alone.

Although the presence of an NMDA receptor antagonist delayed the rate of immunological tolerance development, it did not alter steroid responses throughout the course of chronic morphine treatment. Similar to other effects of morphine, elevation of plasma steroid in response to morphine challenge was gradually diminished over the course of chronic treatment. Unlike immunological tolerance, this effect was not altered by administration of MK-801. This suggests a role of NMDA receptors in development of opioid immunological tolerance that is not dependent on actions of the hypothalamic-pituitary-adrenal axis, the pathway mediating release of plasma corticosterone. Additionally, the impact of morphine on proliferative responses of lymphocytes to a T cell mitogen has been shown to be selectively mediated through central μ-opioid receptors, most likely those located

![Fig. 6](image-url). Chronic morphine exposure results in enhanced sensitivity to stress that is prevented by MK-801. Animals were chronically treated with saline (Ctrl), morphine (cMS), or morphine and MK-801 (cMS + cMK) as described previously. On test day, one-half of the animals from each group were placed in 1 inch of room temperature water for 1 h before sacrifice and trunk blood was collected. a, blood lymphocytes were assayed and proliferation expressed as percentage of the mean maximum proliferative response ± S.E.M. of the saline-treated, unstressed group \( (37,671 \pm 5486 \, \text{cpm}; \, n = 7/\text{group}) \). *, \( p < 0.05 \) versus nonstressed group of similar drug-treatment as determined by one-way analysis of variance. b, plasma corticosterone levels were determined and expressed as percentage of mean corticosterone ± S.E.M. \( (180.8 \pm 20.5 \, \text{ng/ml}; \, n = 6/\text{group}) \) of the saline-treated unstressed group.
in the paraventricular nucleus (Mellon et al., 2001). Evidence of NMDA receptors modulating this centrally mediated immune response, but not altering the peripheral effect of steroid level changes, supports the idea that the minimized tolerant state of the immune system is not likely mediated by direct peripheral actions of the glutamatergic system. Rather, the effect of NMDA receptor antagonism during chronic morphine on lymphocyte proliferative tolerance could be the result of an altered neuroadapted state caused by perturbation of the central interaction of these receptor systems. Similar to opioidergic effects such as analgesia, reinforcement, and physical dependence (Iijima et al., 1996; Fundytus et al., 1997; Fundytus, 2001), it may be that the central glutamatergic receptor system is also a participant in the development of the immunologically tolerant state.

It has been reported that subsequent to chronic opioid treatment, response to noxious stimuli or “stressors” is significantly altered. We have previously shown that animals undergoing restraint stress were immunologically vulnerable if they were morphine-tolerant (Bayer et al., 1994; Mellon et al., 2001). Neuroadaptation that occurs during chronic opioid administration is thought to involve changes in levels of neurotransmitters such as norepinephrine and GABA (Jolas et al., 2000; Van Bockstaele et al., 2001), various receptors systems such as orphanin FQ, cholecystokinin, and aminopeptidases (Ding and Bayer; 1993; Laorden et al., 1997; Milanes et al., 1997; Yuan et al., 1999; Brundege and Williams, 2002; Irazusta et al., 2003), and expression of major second messenger signaling factors such as protein kinase C, adenylate cyclase, G protein-coupled receptors, and cAMP response element-binding protein (Parolaro et al., 1993; Lane-Ladd et al., 1997; Bernstein and Welch, 1998; Shen et al., 2000; Tso et al., 2000; Ma et al., 2001; Shichinofe et al., 2001; Ammer and Christ, 2002; Irazusta et al., 2003). It has been theorized that these central neuroadaptations may mediate sensitized responses to stressors and that relapse to drug-taking behavior may be due to this stress vulnerability (Self and Nestler, 1998; Houssyhar et al., 2001a; Mutasa, 2001; Sarnyai et al., 2001; Weiss et al., 2001). Therefore, to extend the idea that NMDA receptors are involved in the immune effect of chronic morphine, we found that immunological sensitivity of morphine-tolerant animals is amplified in response to opioid withdrawal and water stress and this vulnerability is altered by antagonism of NMDA receptors.

After chronic treatment with morphine or morphine and MK-801, opioid withdrawal was induced by replacing drug injections with saline. It has been suggested that opioid withdrawal is likened to a stressor due to its significant physical ramifications including body weight loss and plasma steroid elevation (Mioduszewski et al., 1982; Houshyar et al., 2001b; Sarnyai et al., 2001). In animals chronically receiving morphine with or without MK-801, drug deprivation induced considerable weight loss and plasma corticosterone elevation (Figs. 4 and 5b). However, animals receiving chronic morphine and MK-801 showed an accelerated rate of recovery from the immunosuppressive effect of opioid withdrawal as compared to animals treated with morphine alone (Fig. 5a). These data correlate with previous findings suggesting that administration of an NMDA receptor antagonist during chronic morphine treatment has a “protective” effect by attenuating the severity of several physical manifestations of opioid withdrawal (Tanganelli et al., 1991; Trujillo and Akil, 1991; Fundytus and Codere, 1994; Herman et al., 1995; Gonzalez et al., 1997; Bisaga et al., 2001).

To determine whether this protective effect is primarily on withdrawal alone, or if altered sensitivity of morphine-tolerant animals generalizes to other types of stressors, animals were subjected to water stress subsequent to chronic treatment with morphine and MK-801 or morphine alone. Plasma corticosterone was elevated whether animals were treated chronically with saline, morphine, or morphine and MK-801 (Fig. 6b). Although there was a lack of an effect of water stress on mitogen-induced lymphocyte proliferation in control animals, suppression of blood lymphocyte response was observed in morphine-tolerant animals subjected to water stress that was absent when MK-801 treatment was included with chronic morphine (Fig. 6a). This again suggests a protective effect of NMDA receptor antagonism on immunological vulnerability induced by chronic morphine that corresponds to the ability of MK-801 treatment to decrease peripheral blood lymphocyte sensitivity to the suppressive effect of opioid withdrawal.

At the time that stressors were applied, or subsequent to 10 days of chronic drug treatment, there was no perceived difference in lymphocyte proliferative response between animals treated with morphine or morphine and MK-801. If NMDA receptor antagonism had altered the “tolerant state” that these animals are in, such that neuroadaptive events occurring during chronic treatment is distorted by MK-801 as evidenced by the delayed rate of tolerance progression, decreased immunological vulnerability to stressors is not surprising. It has been shown that in addition to its effect on physical indicators, administration of glutamate receptor antagonists concurrent to chronic opioid can alter neurochemical consequences of opioid dependence and withdrawal and thereby alter the neuroadapted state (Kogan and Aghajanian, 1995; Ozawa et al., 2001; Tokuyama et al., 2001; Vekovisheva et al., 2001; Watanabe et al., 2002). It should also be noted that corresponding to lack of an effect of NMDA receptor antagonism on progression of plasma steroid tolerance during chronic morphine treatment, plasma corticosterone elevation triggered by opioid withdrawal or water stress were resistant to alteration by the presence of MK-801 with chronic morphine (Figs. 1b, 5a, and 6a), thereby discounting a steroid-dependent mechanism by which NMDA receptor antagonism alters these stress responses. The exaggerated immune response to opioid withdrawal and water stress and alteration of this response by concurrent MK-801, therefore, is suggestive of a role of neuroplastic changes that occur as a result of chronic morphine treatment. The relevance of this protection from stress vulnerability is profound in the context of drug addiction treatment. Stress has been shown to increase the risk of relapse of drug abuse after detoxification from alcohol and other drugs, such as opioids (O’Brian et al., 1977; Childress et al., 1986; O’Brien et al., 1998), and it has been suggested that an altered response to stress may predispose animals to self-administration of drugs of abuse (Shaham and Stewart, 1994; Ortiz et al., 1996). If NMDA receptor antagonism can avert the increased sensitivity of morphine-tolerant individuals to stress, it may also be able to convey a protection against initiation of drug abuse behavior or prevent relapse after drug abstinence.

Overall, it can be suggested that modification of the morphine-tolerant “state” by MK-801 is manifested as a delay in
rate of immunological tolerance progression and a subsequent immunological insensitivity to stressors. This ability to alter the tolerant state induced by chronic opioid treatment by NMDA receptor antagonists serves to further elucidate the neuroplastic events that underlie tolerance of immunological, as well as other, opioidergic effects. Further studies addressing central mechanisms by which NMDA receptors mediate these immune responses need to be carried out to fully clarify this modulation of morphine-induced tolerance by the NMDA receptor system.

References