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Blockade of the development of morphine tolerance by U-50,488, an AVP antagonist or MK-801 in the rat hippocampal slice

Mei-Tsu Su, Wei-Bin Lin, Wei-Ming Lue, [1]Chen-Yu Cheng & [2]Pao-Luh Tao

Department of Pharmacology, National Defense Medical Center and [1]School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan, Republic of China

1 In this study, we investigated the effects of different drugs (a κ-opioid receptor agonist U-50,488, a vasopressin receptor antagonist dPTyr(Me)AVP or an N-methyl-D-aspartate (NMDA) receptor antagonist MK-801) on the development of morphine tolerance in rat hippocampal slices.

2 Hippocampal slices (450 μm) of Sprague-Dawley rats (250–300 g) were used. Slices were continuously superfused with artificial CSF or drugs at 1 ml min^{-1}. Nichrome wire electrodes were placed in the Schaffer-collateral pathway and used to deliver biphasic 0.2 ms pulses of 5–30 V (0.033 Hz). A glass microelectrode was placed in the CA1 area to record population spikes.

3 When the slices were superfused with 10 μM morphine, the amplitude of population spikes increased 2–3 fold in 30–40 min. However, this effect of morphine decreased, i.e. tolerance developed after continuous superfusion of morphine for 2–6 h.

4 When either U-50,488 (200 nM) or dPTyr(Me) AVP (500 pM) or MK-801 (500 pM) was co-superfused with morphine (10 μM), it significantly blocked the development of morphine tolerance. Nor-BNI (a κ-opioid receptor antagonist, 200 nM) significantly reversed the inhibitory effect of U-50,488 but not those of dPTyr(Me)AVP or MK-801 on the development of morphine tolerance.

5 These data indicate that κ-opioid receptors, AVP receptors and NMDA receptors are all involved in the development of morphine tolerance. The suppression of κ-opioid receptor activity after chronic morphine may occur before the activation of AVP receptors or NMDA receptors during the development of morphine tolerance.

Keywords: Morphine; opioid receptor; tolerance; U-50,488; MK-801; N-methyl-D-aspartate; vasopressin

Introduction

Morphine is an important drug in the clinical treatment of severe pain. However, tolerance develops with chronic use of morphine, resulting in decreased effectiveness over time. Understanding of the mechanisms underlying the development of morphine tolerance is very important because it may extend the clinical use and effectiveness of morphine. Morphine tolerance may involve a number of different mechanisms such as uncoupling of opioid receptors and guanine nucleotide-binding proteins (G-proteins) (Tao *et al*., 1993); increased levels of G-proteins, adenylate cyclase, adenosine 3′:5′-cyclic monophosphate (cyclic AMP)-dependent protein kinases and a number of phosphoproteins (Guitart & Nestler, 1993).

U-50,488 (*trans*-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide hydrochloride), a selective κ-opioid receptor agonist, has been shown to suppress the development of antinociceptive tolerance to morphine in rats (Yamamoto *et al*., 1988), mice (Takahashi *et al*., 1991) and guinea-pigs (Tao *et al*., 1994). The mechanism may involve the activation of κ-opioid receptors and an inhibitory effect of U-50,488 on vasopressin release (Tao *et al*., 1997). Xu *et al*. (1992) demonstrated that concomitant intracerebroventricular (i.c.v.) injection of anti-arginine vasopressin (AVP) antiserum dose-dependently suppressed the development of analgesic tolerance to daily morphine (10 mg kg^{-1}, s.c.) in mice. Recent studies in our laboratory have also shown that chronic co-administration of an AVP antagonist (dPTyr(Me)AVP) (i.p. or i.c.v.) with morphine (i.p.) blocked the development of morphine tolerance in rats (Tao *et al*., 1997). Recently, both competitive and non-competitive N-methyl-D-aspartate (NMDA) antagonists (Trujillo & Akil, 1991; Marek *et al*., 1991; Tiseo & Inturrisi, 1993), and nitric oxide synthase inhibitors (Kolesnikov *et al*., 1992; Majeed *et al*., 1994) have been found to prevent the development of morphine tolerance, suggesting that the development of morphine tolerance may also involve the activation of NMDA receptors and NO production. Therefore, κ-opioid receptor suppression, AVP receptor activation or NMDA receptor activation may all be involved in the mechanisms of morphine tolerance.

The aim of this study was to investigate the relationships between a κ-opioid receptor agonist (U-50,488), an AVP antagonist (dPTyr(Me)AVP), an NMDA antagonist (MK-801) and nitric oxide in blocking morphine tolerance. We also wanted to discover whether the blocking effect of U-50,488 on the development of morphine tolerance is mediated by κ-opioid receptors and whether the activation of AVP receptors or NMDA receptors occurs before or after the κ-opioid receptor is affected by morphine.

Methods

Preparations

In vitro hippocampal slice preparations were used (Dunwiddie & Lynch, 1978; Dunwiddie *et al*., 1987). Male Sprague-Dawley rats (200–300 g) were decapitated and the brain was rapidly removed and placed in ice-cold artificial CSF (4°C ACSF, consisting of mM: NaCl 124, KCl 3.3, KH_2PO_4 1.2, $MgSO_4$ 2.4, $CaCl_2$ 2.5, $NaHCO_3$ 25.7 and D-glucose 10) which was pregassed with 95% O_2 and 5% CO_2. The hippocampus was dissected free of the surrounding tissue and transverse slices

[2]Author for correspondence at: Department of Pharmacology, P.O. Box 90048-504, Taipei, Taiwan, R.O.C.

(450 μm) were cut on a Sorval tissue chopper. Then the slices were placed immediately on nylon nets in a recording chamber containing oxygenated ACSF maintained at 33.5 ± 1°C. Before being used for electrophysiological recording, the slices were held at the liquid medium-gas interface in this chamber for at least 60 min. During the recording, the slices were continuously superfused with ACSF, gassed as above, at a rate of 1 ml min^{-1}.

Electrophysiology

Bipolar stimulation was delivered through two-strand twisted Nichrome wire electrodes to stimulate fibres (Schaffer and commissural fibres) in the stratum radiatum near the border of CA1-CA2. Extracellular population spikes (field potentials) in response to stimulation of the stratum radiatum (8–30 V, 0.2 ms duration, 0.033 Hz) were recorded from the CA1 cell layer with glass microelectrodes filled with 3 M NaCl. The amplitude of the population spike was taken as the average of the differences between the spike peak negativity and the preceding and following positivities (Figure 1b). The stimulation voltage was adjusted to elicit a baseline population spike between 0.7 and 1.8 mV. At least 30 min of stable baseline response was obtained in each experiment before drug application.

U-50,488 was prepared according to the literature (Szmuszkovicz, 1979; Szmuszkovicz & Von Voigtlander, 1982) and isolated as the hydrochloride salt, mp 221.5–222.5°C, 99.5% by high performance liquid chromatography (h.p.l.c.) (RP-select B; $H_2O/CH_3OH = 1{:}4$ buffered at pH 7.52; 254 nm). Nor-binaltorphimine (nor-BNI) was prepared according to literature (Portoghese *et al*., 1987; Portoghese & Lipkowski, 1987) and conforms to analytical data. Morphine hydrochloride was purchased from the Narcotics Bureau of the National Health Administration (Taipei, Taiwan, R.O.C.). MK801 ((+)-5-methyl-10,11-dihydro-5H-dibenzyl[a,d]cyclohepten-5,10-imine hydrogen maleate) was purchased from Research Biochemicals International (RBI; MA, U.S.A.). All other chemicals used were reagent grade and supplied by Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Statistical analysis

The results are expressed as the means $\pm$ s.e.mean. Analysis of variance was used to assess the statistical significance by repeated measures of the overall data and differences between the individual mean values in different groups were analysed by use of the Duncan multi-range test. The difference was considered to be significant at $P < 0.05$.

Results

Effect of morphine on the amplitude of the population spikes

The population spikes recorded from stratum pyramidale of the hippocampal CA1 region are shown in Figure 1b. When the slices were superfused with morphine (0.1–50 μM), the amplitude of the population spikes (Figure 1b) increased concentration-dependently (Figure 2a). The maximum effect was obtained by superfusion with 10 μM of morphine for 30–40 min and the amplitude of population spikes rose to over 250% of the control value (Figure 2a). When the slices were continuously superfused with 10 μM morphine for 6 h, the amplitude of population spikes gradually decreased back to the control value indicating that this effect had been tolerant to 10 μM morphine (Figures 1 and 3).

Effect of U-50,488 on the amplitude of the population spikes

In order to find a concentration of U-50,488 which did not have any effect in the population spikes, we performed several concentration-effect experiments. It was found that U-50,488 also increased the amplitude of population spikes if the concentration was higher than 1 μM (Figure 2b). Therefore, in the following studies, we chose 200 nM U-50,488 to co-superfuse with 10 μM morphine. We also found this concentration of U-50,488 (200 nM) did not affect the acute response to 10 μM morphine.

Effect of U-50,488 on the development of tolerance to morphine in hippocampal slices

When the hippocampal slices were continuously superfused with 10 μM morphine for 6 h, the stimulating effect of morphine on the amplitude of population spikes gradually

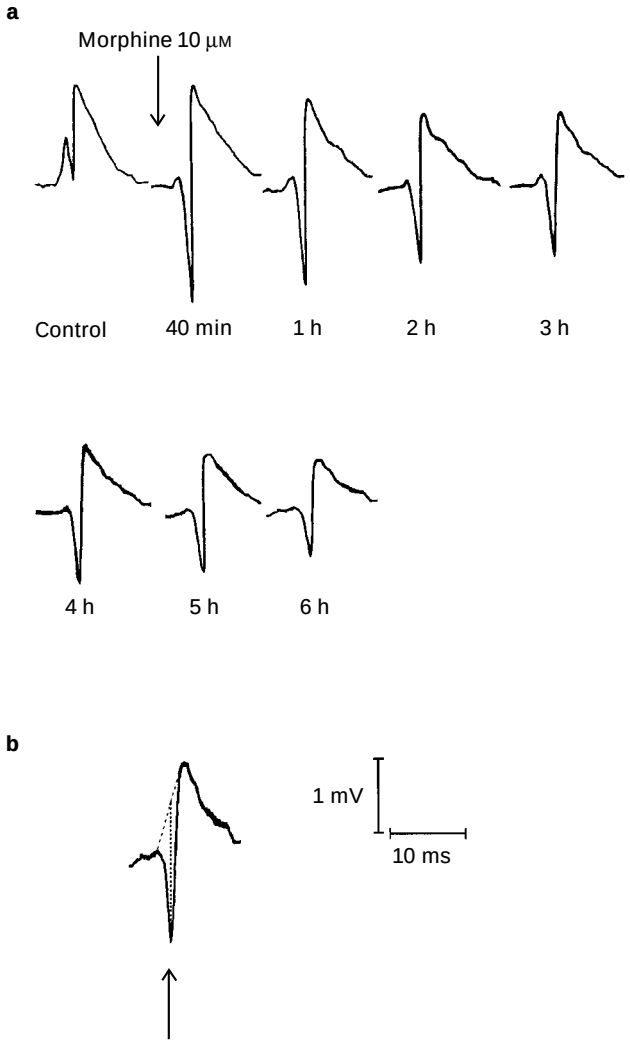

Figure 1 Recordings of population spikes. (a) The acute and chronic effect of 10 μM morphine on the population spike responses recorded from stratum pyramidale of the CA1 region in the superfused hippocampal slices. (b) The amplitude of the negative-going population spike (arrow) was determined as the length of the vertical dashed line illustrated.

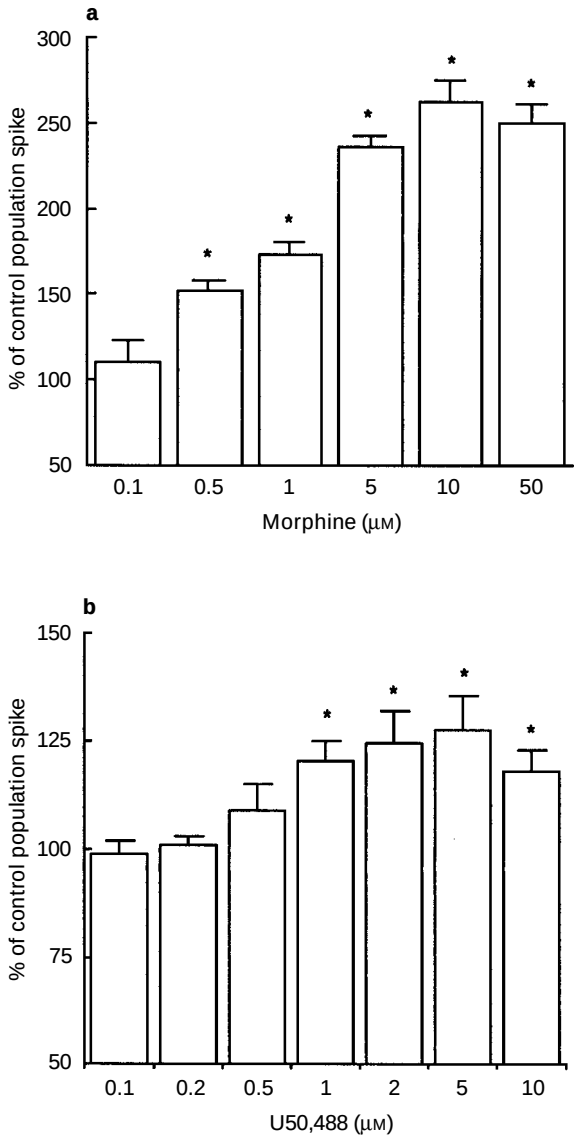

Figure 2 The concentration-effect of (a) morphine ($n=8$) (b) U-50,488 ($n=6$) on the population spikes recorded at 30 min after drug superfusion. Significant differences from control group ($^*P<0.05$) were determined with one-way ANOVA and Duncan multiple range test.

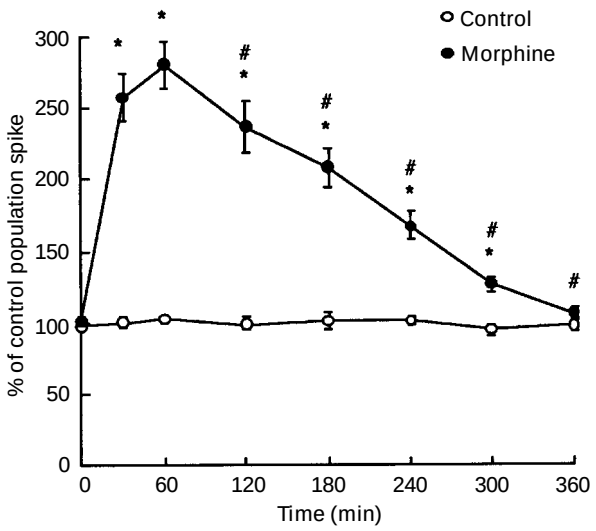

Figure 3 Effect of continuous superfusion of 10 μM morphine on the population spikes. The control group was superfused with ACSF ($n=6$) and the morphine group was superfused with 10 μM morphine ($n=14$). Significant differences from control ($^*P<0.05$); significant differences from the maximum effect of morphine ($\#P<0.05$) were determined with two-way ANOVA and Duncan multiple range test.

decreased, as shown in Figure 4a. When 200 nM U-50,488 was co-superfused with 10 μM morphine, the effect of morphine was maintained for at least 6 h. When the κ-opioid receptor antagonist, nor-BNI (200 nM), was co-superfused with morphine and U-50,488 for 6 h, the effect of morphine still gradually decreased (Figure 4a).

These experiments indicated that continuous superfusion of 10 μM morphine could induce the development of tolerance to morphine in hippocampal slices. Co-superfusion of U-50,488 (200 nM) with morphine could block the development of morphine tolerance. This effect of U-50,488 was antagonized by a κ-opioid receptor antagonist, nor-BNI.

Effect of dPTyr(Me)AVP on the development of morphine tolerance in hippocampal slices

When 500 pM dPTyr(Me)AVP (which had no effect by itself and did not affect the acute response of 10 μM morphine; data not shown) was co-superfused with 10 μM morphine, it significantly blocked the development of morphine tolerance, as shown in Figure 4b. Nor-BNI (200 nM) did not antagonize this effect of dPTyr(Me)AVP (Figure 4b).

Effect of MK-801 on the development of morphine tolerance in hippocampal slices

When 500 pM MK-801 (which had no effect by itself and did not affect the acute response of 10 μM morphine) was co-superfused with 10 μM morphine, it also significantly blocked the development of morphine tolerance, as shown in Figure 4c. Nor-BNI (200 nM) did not antagonize this effect of MK-801 (Figure 4c).

Discussion

In the present study, we chose to use the rat hippocampal slice because it is not only a good model for electrophysiological measurements but it also contains μ and κ-opioid receptors (Mansour *et al*., 1987), vasopressin receptors (Phillips *et al*., 1988) and NMDA receptors (Greenamyre *et al*., 1985). Thus, we could study the effects of morphine (μ-opioid receptor agonist), U-50,488 (κ-opioid receptor agonist), dPTyr(Me) AVP (AVP antagonist) and MK-801 (NMDA antagonist) in this model. The 'tolerance' observed in our model occurred within the hours of the continuous superfusion of a high concentration of morphine. This phenomenon is similar to the acute tolerance induced in animals by continuous infusion of morphine (Ling *et al*., 1989).

It has been found that dynorphin (dyn) A-(1-13) can ameliorate the signs of opioid withdrawal and suppress the expression of opioid tolerance in morphine-dependent mice (Takemori *et al*., 1992). Similar findings have been obtained in rats (Green & Lee, 1988), monkeys (Aceto *et al*., 1982) and man (Wen & Ho, 1982). Dynorphins are a class of endogenous opioids with κ-opioid agonist activity (Huidobro-Toro *et al*.,

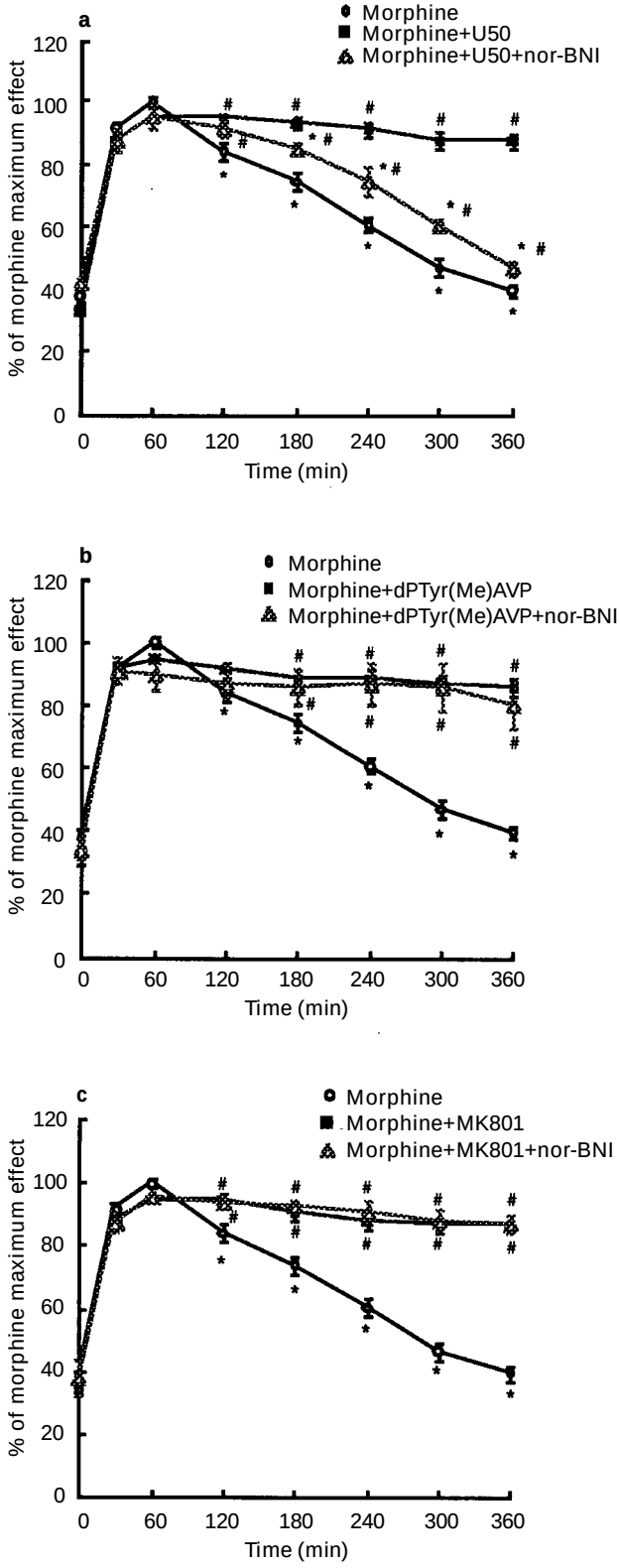

Figure 4 Effect of U-50,488, dPTyr(Me)AVP or MK-801 on the development of morphine tolerance. (a) Effect of U-50,488 (200 nM; $n=8$); nor-BNI (200 nM; $n=6$) or (U-50,488 + nor-BNI; $n=6$) on continuous superfusion of 10 μM morphine ($n=14$). (b) Effect of dPTyr(Me)AVP (500 pM; $n=7$) or (dPTyr(Me)AVP + nor-BNI; $n=6$) on continuous superfusion of 10 μM morphine ($n=14$). (c) Effect of MK-801 (500 pM; $n=9$) or (MK-801 + nor-BNI; $n=6$) on continuous superfusion of 10 μM morphine ($n=14$). Significant differences from maximum effect ($^*P<0.05$) and significant differences from the morphine group ($\#P<0.05$) were determined with two-way ANOVA and Duncan multiple range test.

1981). However, the effect of dyn A-(1-13) in suppressing the expression of opioid tolerance or withdrawal was considered to be a non-opioid effect (Takemori *et al*., 1993; Hooke *et al*., 1995). In the present study, we found that a selective exogenous κ-opioid receptor agonist, U-50,488, could effectively block the development of morphine tolerance in hippocampal slices. This effect of U-50,488 could be antagonized by the κ-opioid receptor antagonist, nor-BNI, indicating that this is an opioid effect (Figure 4a). In 1992, Suzuki *et al*. demonstrated that the development of tolerance to morphine-induced analgesia in mice was significantly potentiated by the pretreatment with nor-BNI. Hence we tested the effect of nor-BNI on the development of morphine tolerance in our preparation. However, we did not find any potentiation of morphine tolerance (data not shown). We consider that even 10 μM morphine may cause a maximum reduction of κ-opioid receptor activity in our model so that nor-BNI cannot show any further action. On the other hand, dPTyr(Me)AVP (an AVP antagonist) or MK-801 (a non-competitive NMDA antagonist), could each block the development of morphine tolerance in the hippocampal slice model, but their effects were not reversibly by nor-BNI (Figure 4b and c). These results imply that κ-opioid receptors, AVP receptors and NMDA receptors are all involved in the development of morphine tolerance.

Since all the drugs we tested in this study could almost completely block the development of morphine tolerance, they may act through a sequence of events that occurs after chronic morphine treatment. Since nor-BNI could not reverse the effect of dPTyr(Me)AVP or MK-801 on blocking the development of morphine tolerance, the activation of AVP receptors or NMDA receptors must have happened after the κ-opioid receptors were suppressed by morphine. The activation of NMDA receptors can induce the synthesis of nitric oxide (NO) through the activation of NO synthase (NOS; Bredt & Snyder,

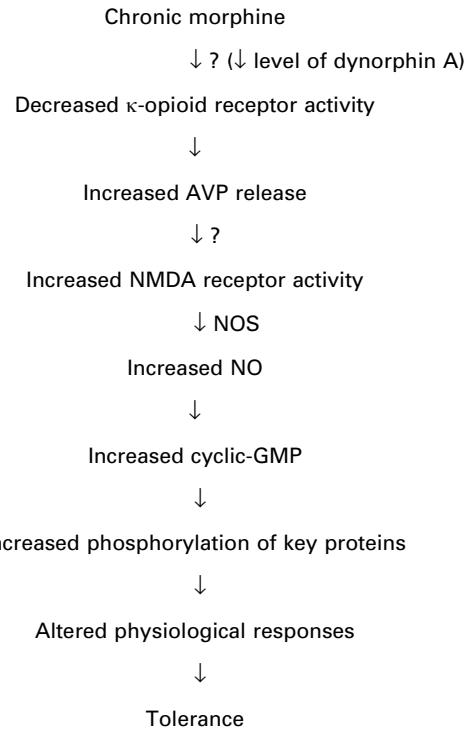

Figure 5 A hypothetical model for the possible mechanism of morphine tolerance.

1992). It has also been found that co-administration of NOS inhibitors, such as N^G-nitro-arginine, along with morphine prevents morphine tolerance (Kolesnikov *et al*., 1992; Majeed *et al*., 1994). The enhanced NOS activity by chronic morphine may increase the levels of cyclic GMP (Bredt & Snyder, 1989; Garthwaite *et al*., 1989) that causes phosphorylation of some key proteins and alters the physiological responses, finally inducing morphine tolerance. Therefore, we propose the model shown in Figure 5. After chronic treatment of morphine, the dynorphin level may decrease in certain brain nuclei (Yukhananova *et al*., 1993; Nylander *et al*., 1995) which in turn decreases the activity of κ-opioid receptors and increases the release of AVP (Firemark & Weitzman, 1979; Leander *et al*., 1987). Through unknown mechanisms, this increase of AVP activity may increase NMDA receptor activity that can induce the synthesis of nitric oxide (NO) and increase the levels of cyclic GMP. Phosphorylation of certain key proteins by cyclic GMP may alter the responses to morphine and thus permit the development of tolerance to morphine.

It is clear that the development of morphine tolerance is a very complex process involving a number of neurotransmitters and neuromodulators. Any drug that can effectively block any step of this process will effectively block the development of morphine tolerance.

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