# Tolerance for Opiate Analgesia: Complex Effect of Antagonists of Receptors for Excitatory Amino Acids

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The effect of excitatory amino acid receptor antagonists on the development of the conditioned reflex component of the tolerance for morphine analgesia is studied. It is demonstrated that the antagonists of NMDA and nonNMDA receptors reduce the development of associated component of the tolerance, while the magnitude of the nonassociative component changes after co-administration of morphine with NMDA receptor antagonists.

**Key Words:** *antagonists of EAA receptors; opiates; analgesia; tolerance; learning*

The tolerance (T) that develops during chronic administration of opiate analgesic drugs is a major problem associated with their use. The development of T may reflect both conditioned and unconditioned reflex components of an adaptive reaction to a pharmacological stimulus [11,13]. The excitatory amino acid (EAA) system, which is a component of the system of endogenous regulation of pain sensitivity, plays an important role in nervous system plasticity. Agonists of the main EAA receptors (NMDA, AMPA and kainate) produce a specific excitatory effect on the nerve cell. Therefore, when injected into the periaqueductal gray matter (PAG), they produce analgesia [5], but lower pain thresholds after intraperitoneal injection [10]. NMDA receptor antagonists produce opposite effects; the ability to inhibit morphine analgesia has been demonstrated for the noncompetitive NMDA antagonist MK-801 for both systemic and PAG administration [5,6]. MK-801 doses that do not modulate the analgesic effect of morphine prevent the development of T for it [7,8,14].

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In the present study we examined the effects of the broad-spectrum EAA antagonist kynurenic acid (KYNA), the noncompetitive NMDA receptor blocker phencyclidine (PCP), and the specific AMPA antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) on the development of associative and nonassociative forms of morphine tolerance.

## MATERIALS AND METHODS

The experiments were performed on outbred male albino rats purchased from the laboratory animal farm Rappolovo. Tolerance for the analgesic effect of morphine was studied by the method of discriminative control of tolerance [11]. The animals received alternate injections of morphine (20 mg/kg, subcutaneously, 8 injections) and normal saline (1 ml/kg, 8 injections). Morphine was injected in room A and the vehicle was injected in room B. Environmental stimuli in the rooms were different. The animals were kept in the same room for 3 h postinjection. Ten min after each of the 8 morphine injections the rats received an intraperitoneal injection of PCP (2.5 mg/kg, group I, $n$=20) or its vehicle (normal saline, 1 ml/kg, group II, $n$=20), KYNA (150 mg/kg, group III, $n$=20) or its

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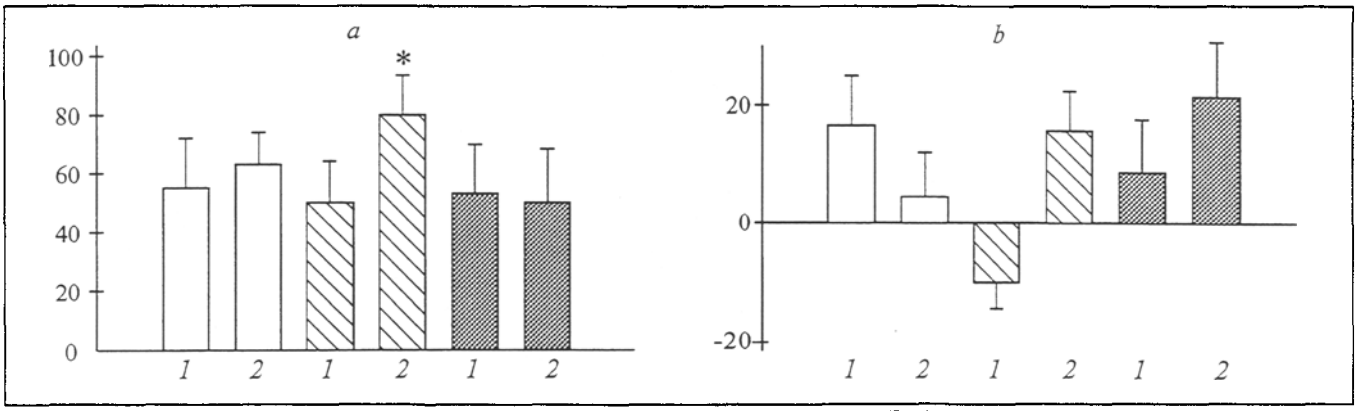


Fig. 1. Changes in analgesic activity of morphine upon co-administration with EAA receptor antagonists. Ordinate: degree of analgesia (%) 30 min after subcutaneous administration of morphine (*a*, 5 mg/kg) or normal saline (*b*, 1 ml/kg) co-administered (10 min after morphine injection) with phencyclidine (2.5 mg/kg intraperitoneally, white bars), kynurenic acid (150 mg/kg intraperitoneally, shaded bars), or DNQX (15 mg/kg intraperitoneally, black bars). The vehicle (*1*) or preparation (*2*) was co-administered with morphine. Asterisk indicates a statistically significant ($p<0.01$) difference between groups I and II.

vehicle (10% ethanol, group IV, $n=20$), DNQX (15 mg/kg, group V, $n=20$) or its vehicle (50 % dimexide, group VI, $n=20$). The analgesic effect of morphine (10 mg/kg subcutaneously, 30 min prior to the test) was assessed by tail-flick latencies before and after subchronic administration. The degree of analgesia (DA) was calculated from the following formula: $DA = [(T_1-T_2)/(T_{lim}-T_2]\times100$, where $T_1$ and $T_2$ are the tail-flick latencies before and after drug administration, respectively, and $T_{lim}$ is the duration of the thermal stimulus (20 sec). The conditioned reflex component of T was revealed after subchronic administration by assessing the effect of the test dose in a "familiar" (associated with the opiate effect) and an "unfamiliar" (associated with the vehicle effect) environment. For this purpose in half of the animals of each group the analgesic effect of the second test dose of morphine was assessed in room A and in the rest of the animals the effect was assessed in room B. In separate experiments we evaluated the changes in the analgesic activity of morphine (5 mg/kg subcutaneously, 30 min before the test) administered in combination with PCP (2.5 mg/kg), KYNA (150 mg/kg), DNQX (15 mg/kg), and their vehicles (1 ml/kg) (all these antagonists were administered 20 min before the test). Control animals were injected with normal saline (1 ml/kg, subcutaneously, 30 min before the test). The effect of each combination was assessed in 8-10 animals. The data were analyzed using Student's *t* test.

## RESULTS

Figure 1 illustrates the effects of EAA antagonists on the analgesic effect of morphine (5 mg/kg) after a single administration with EAA antagonists. Treatment of the rats with KYNA (150 mg/kg), PCP (2.5 mg/kg), and DNQX (15 mg/kg) did not change the tail-flick latency: however, in combination with morphine KYNA, but not PCP or DNQX, significantly potentiated the analgesic ef-

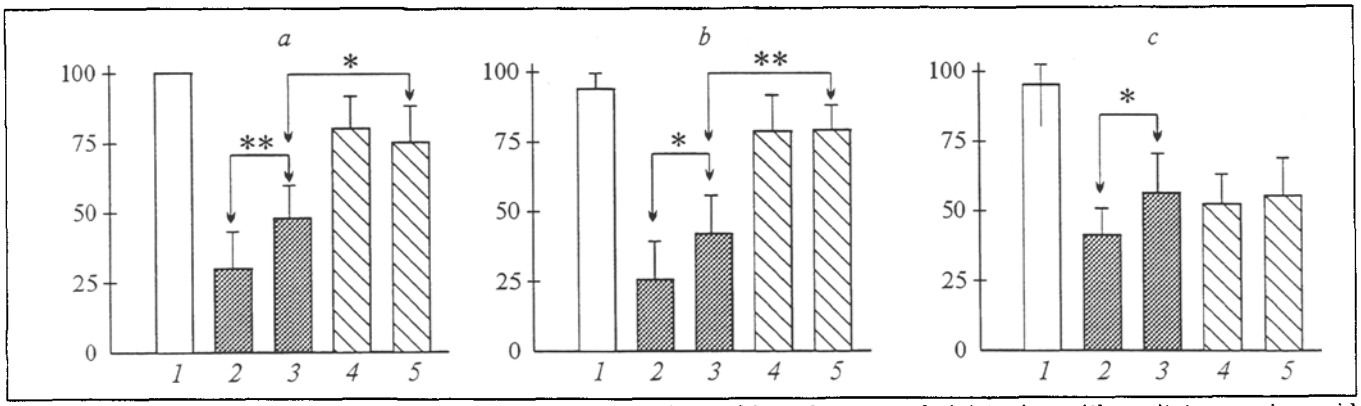


Fig. 2. Development of tolerance for the analgesic effect of morphine after co-administration with excitatory amino acid receptors: phencyclidene (*a*), kynurenic acid (*b*), and DNQX (*c*). Ordinate: analgesic effect (%) of morphine test dose (10 mg/kg, subcutaneously) before (*1*) and after its cubchronic co-administration with the vehicle (*2*, *3*) or the preparation (*4*, *5*). *2* and *4*) morphine injection in a familiar environment (room A); *3* and *5*) morphine injection in an unfamiliar environment (room B). One asterisk indicates $p<0.05$, two asterisks indicate $p<0.01$; the compared groups are indicated by arrows.

fect of the opiate. It should be mentioned that no statistically significant changes in analgesic activity of morphine occurred in animals after long-term administration of EAA antagonists [7].

Figure 2 shows the results of experiments with subchronic administration of morphine. Subchronic administration of morphine with vehicles (groups II, IV, and VI) according to the above-mentioned schedule resulted in the development of T. In the animals which received the test dose of morphine in an unfamiliar environment T was significantly lower, indicating the presence of the conditioned reflex component of T during testing in a familiar environment.

Subchronic co-administration of morphine and PCP (group I) reduced T for morphine analgesia; the magnitude of T was the same upon testing in rooms A and B (Fig. 2, *a*) .

Co-administration with KYNA (group III) prevented the development of T, the effect being independent of the environment (Fig. 2, *b*).

After subchronic co-administration of morphine with DNQX (group V) T was the same as in group VI (co-administration with dimexide). There was no statistically significant change between T in a familiar and unfamiliar environment (Fig. 2, *c*).

Thus, all the compounds studied in combination with morphine abolished the associative component of T, which confirms the involvement of the EAA receptors in learning and memory. It is known that EAA receptors are implicated in the induction and maintenance of long-term potentiation [2]. Since the NMDA receptors interfere with the mechanisms underlying the postconditioning consolidation of memory traces [15], a postponed (2 h) application of EAA antagonists related to morphine did not allow us to assess the conditioning factor in the effect of MK-801 on the development of T [8]. In addition, the NMDA receptor antagonists markedly impair working memory in the radial maze test [4].

A significant reduction in the nonassociative component of T was observed upon co-administration of morphine with PCP and KYNA, which are NMDA antagonists. Two mechanisms may be responsible for this effect. First, a reduction in the analgesic effect of morphine co-administered with these agents is possible at the supraspinal level [5], which is compensated by a summing of the analgesic properties of the compounds at the spinal level. As a result, PCP does not alter and KYNA even potentiates the analgesic effect of morphine and the development of T [1]. Second, at the cell level the NMDA receptor antagonists may interact with adaptive processes that accompany the development of T, which may be explained by the ability of these agents to reduce transmembrane calcium permeability [9]. Moreover, other compounds inhibiting the transmembrane transport of calcium ions (antagonists of slow calcium channels, agonists of κ opioid receptors) also inhibit the development of T for morphine analgesia [3,12].

Thus, EAA receptor antagonists prevent the development of the conditioned reflex component of tolerance for the analgesic effect of morphine. The slowed development of the nonassociative component of T which occurs upon co-administration of morphine with NMDA receptor antagonists may be useful for the improvement of pain management.

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