J. Toxicol. Pub. Health
Vol. 19, No. 4, pp. 311-314 (2003)

Official Journal of
Korean Society of Toxicology

Available online at http://www.toxmut.or.kr

Effects of Glycine on the Development of Analgesic Tolerance to and Physical Dependence on Morphine in Mice

Jong-Won Baik[1], Jin-Tae Hong[1], Young-Won Yun[2] and Ki-Wan Oh[1]

[1]College of Pharmacy, Chungbuk National University, Cheongju, 361-763, Korea
[2]College of Veterinary Medicine, Chungbuk National University, Cheongju, 361-763, Korea

Received November 4, 2003; Accepted December 2, 2003

ABSTRACT. This study was performed to investigate the effects of glycine on the development of tolerance to and physical dependence on morphine. Repeated administration of morphine (10 mg/kg) developed tolerance and physical dependence. Glycine (100, 200 and 400 mg/kg) was administered intraperitoneally (i.p.) to mice for 7 days prior to the morphine injection. Analgesic effects were estimated by the tail flick methods. The inhibitory degree of the development of morphine-induced analgsic tolerance by i.p. administration of glycine was evidenced by the increase in analgesic response to morphine. Glycine inhibited the development of tolerance to morphine. In addition, we separately measured jumping response as the naloxone-precipitated withdrawal sign in mice that had received the same morphine. Glycine reduced the number of jumping behaviors in morphine dependent mice. These results suggest that glycine might be useful the prevention or treatment of morphine tolerance and physical dependence.

Keywords: Morphine, Glycine, Analgesic tolerance, Physical dependence, Jumping.

INTRODUCTION

Although opioid-drugs such as morphine are widely used for the management of pain, their clinical usefulness is limited by development of tolerance and dependence that occurs after repeated treatment. Thus, there is considerable interest in the search of drugs that delay, inhibit and reverse the development of morphine tolerance and dependence. Recent studies have implicated the excitatory amino acids and their receptors in the chronic action of opioids (Bhargava, 1994; Herman *et al.*, 1995; Inturrisi, 1997) and nociception (Dickinson, 1994; Dickinson *et al.*, 1997). Many behavioral studies have demonstrated that competitive and noncompetitive N-methyl-D-aspartate (NMDA) antagonists attenuate or reverse the development of analgesic tolerance to and physical dependence on morphine in rodents (Trujillo, 1995; Elliott *et al.*, 1995; Herman *et al.*, 1995). Therefore, NMDA receptors are involved in the development of analgesic tolerance to and physical dependence on morphine. Pharmacological modulation of the NMDA receptors at the glycine recognition site is a recent and potentially more attractive therapeutic approach of psychosis such as schizophrenia than the dopamine (DA) receptors. It is generally accepted that glycine is required for the activation of NMDA receptors (Johnson and Ascher, 1987). Glycine also acts a modulator of excitatory amino acid transmission mediated by NMDA receptors. However, glycine is one of the major inhibitory neurotransmitters in the mammalian central nervous systems (CNS), predominantly active in the spinal cord and brain stem. Until now, there are contraversal opinions that glycine agonists and antagonists inhibited the development of analgesic tolerance to and physical dependence on morphine.

Therefore, we are interested in whether glycine itself inhibits the development of analgesic tolerance to and physical dependence on morphine in mice.

Correspondence to: Ki-Wan Oh, College of Pharmacy, Chungbuk National University, Cheongju, 361-763, Korea
E-mail: kiwan@chungbuk.ac.kr

MATERIALS AND METHODS

Animals

White ICR male mice, weighing 25~30 g, in a group of 10~15, were used in all experiments. The animals are housed in plastic cages in a group of 5 per cage, and unlimited access water and lab chow. The mice

were kept under constant temperature (20±22°C) and on a controlled 12 hours light/dark cycle. The animals were adapted to the laboratory conditions for at least 4 days.

Measurement of Analgesic Tolerance

To induce morphine tolerance and dependence in mice, morphine hydrochloride (Sam-Sung Pharm. Co., Korea) was administered (10 mg/kg/day, s.c.) in a volume of 0.1 ml/kg, every 24 hours for a period of 7 days. Glycine 100, 200 and 400 mg/kg (G 100, G 200 and G 400) were pretreated every 30 min before morphine injection.

The inhibitory degree of morphine tolerance development of the test drugs was evidenced by the increase in analgesic response to morphine hydrochloride 5 mg/kg as an analgesic response, estimated at 0. 30, 60, 90 and 120 min by the tail flick method's 24 hours after the final injection of morphine and calculated as area under the curve (DAmour and Smith, 1941; Kaneto *et al.*, 1987). The tail-flick latencies to thermal stimulation were determined in second prior to and at 30, 60, 90 and minutes after the morphine injection. A value of 10 seconds was used as a cut-off point to avoid damage to the tail. The analgesic response for each mouse was calculated by the following formula;

$$\text{Percent analgesia (\%)} = T_t - T_o / T_c - T_o \times 100$$

Where T_o is the base line or pre-morphine tail flick reaction time. T_t is the reaction time at t minutes after morphine injection, Tc is cut-off time. The base lines of tail flick latencies in different groups were around 2±0.2 seconds. The effects were calculated as area under the curve (A.U.C.) that was obtained by plotting the analgesia percent on the ordinate and the time intervals (min) on the abscissa, and expressed as a percent of the effects obtained in control animals treated only with morphine 5 mg/kg.

Measurement of Naloxone-Precipitated Jumping Behaviors

The inhibition of naloxone induced jumping behavior in mice treated with morphine alone and in morphine treated mice with test drugs was estimated by the decreased number of jumping response induced by naloxone 5 mg/kg (i.p.) for 30 minutes, 24 hours just after the final injection of morphine on the 8th day. The jumping response was quantified by placing animals on a diaphanous circular cylinder 35 cm in diameter and 70 cm in height.

Statistics

The data were expressed as mean±SEM. The significant of drug effects was assessed by an analysis of variance (ANOVA). In case of significant variation, the individual values were compared with Dunnett's t-test.

RESULTS

Inhibitory Effects of Glycine on Morphine-Induced Analgesic Tolerance

Repeated administration of morphine for 7 days caused remarkable reduction of analgesic effects of single administration of morphine. Concomitant administration of glycine 400 mg/kg resulted in the inhibition of the development of analgesic tolerance in mice (Fig. 1). The analgesia of glycine 400 mg/kg calculated by the AUC to morphine 5 mg/kg showed the comparative value of 1.6 times, compared with that of control group ($P<0.05$).

Inhibitory Effects of Glycine on Naloxone-Precipitated Jumping Behavior

Repeated administration of morphine for 7 days also developed the naloxone-precipitated withdrawals, dramatically increasing jumping behavior. We measured this behavior because jumping is a typical withdrawal sign at morphine-dependent mice (Fig. 2). Glycine 400 mg/kg significantly decreased naloxone-precipitated jumping behavior ($P<0.05$).

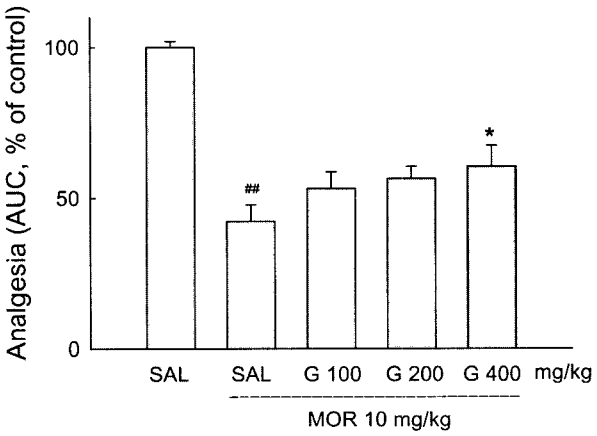

Fig. 1. Inhibitory effects of glycine on the development of analgesic tolerance induced by morphine. Morphine hydrochloride (MOR) 10 mg/kg was administered every 24 hours for a period of 7 days. Glycine 100, 200 and 400 mg/kg (G 100, G 200 and G 400) were pretreated every 30 min before morphine injection for 7 days. Morphine 5 mg/kg was given as test dose to measure analgesic tolerance, 24 hours after the final morphine 10 mg/kg. *$P<0.05$, compared with that of SAL group. ##$P<0.01$, compared with that of morphine control.

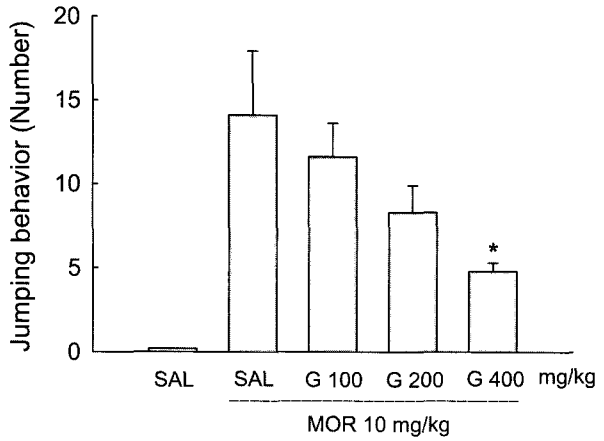

Fig. 2. Inhibitory effects of glycine on the development of physical dependence induced by morphine. Morphine hydrochloride (MOR) 10 mg/kg was administered every 24 hours for a period of 7 days. Glycine 100, 200 and 400 mg/kg (G 100, G 200 and G 400) were pretreated every 30 min before morphine injection for 7days. Naloxone 5 mg/kg (i.p.) was injected 24 hours after the final injection of morphine on the 8th day. The number of jumping behavior was observed for 30 min just after naloxone injection by placing animals on a diaphanous circular cylinder. *P<0.05, compared with that of SAL control group.

DISCUSSION

From these experiments, we confirmed that glycine inhibited the development of tolerance to and dependence on morphine. It has been reported that NMDA antagonists attenuate the development of tolerance to and dependence on morphine. In contrast, glycine site NMDA antagonist failed to alter the development of tolerance following chronic morphine administration (Trujillo and Akil, 1991; Trujillo and Akil, 1994; Guen *et al.*, 1999). Pharmacological modulation of the NMDA receptor at the glycine recognition site is a recent and potentially more attractive therapeutic approach than the glutamate binding site. It is now generally accepted that glycine is required for the activation of NMDA receptors (Johnson and Ascher, 1987). This discovery causes a stir, because glycine had hitherto been recognized as an inhibitory transmitter, so to find it facilitating excitation ran counter to the prevailing doctrine. Competitive NMDA antagonists are known, which block this action of glycine, thus indirectly inhibit the action of glutamate. The physiological role of glycines action at NMDA receptors is still uncertain. The concentration required is low in relation to the concentration of glycine normally present in the brain, suggesting that it may serve as a constant enabling factor for NMDA-receptor-mediated effects of glutamate, rather than as a regulatory mechanism.

Therefore, glycine is one of the major inhibitory neurotransmitters in the mammalian central nervous systems (CNS), predominantly active in the spinal cord and brain stem. The hyperactivity induced by phencyclidine (PCP) was inhibited by pretreatment with glycine (Javitt *et al.*, 1997). Glycine at the higher dosage than we used in these experiments inhibited the action of psychostimulant, suggesting that higher dosage of glycine would be required to inhibit the action of simulative drugs. In microdialysis studies, glycine significantly inhibited PCP-induced stimulation of subcortical DA release in a dose-dependent manner. In behavioral studies, glycine also reversed the effects of PCP in rodents (Daniel *et al.*, 1999).

Overall, we confirmed that glycine inhibited the development of tolerance to and dependence on morphine, suggesting that glycine inhibits dopaminergic functions. High doses of glycine are needed to inhibit the analgesic tolerance to and physical dependence on morphine, Glycine may be useful for the treatment or prevention of the adverse action of morphine. Further studies are needed to understand the possible mechanism of the effects of glycine.

ACKNOWLEDGEMENT

This work was supported by the Brain Korea 21 project (2003-2004).

REFERENCES

Bhargava, H.N. (1994): Diversity of agents modify opioid tolerance, physical dependence, abstinance syndrome, and self-administrative behavior. *Pharmacol. Rev.*, **46**, 293-324.

Damour, F.E. and Smith, D.L. (1941): A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther.*, **72**, 74-79.

Daniel, C.J., Andrea, B., Henry, S. and Abel, L. (1999): Reversal of phencyclidine-induced effects by glycine and glycine transport inhibitors. *Biol. Psychiatry*, **45**, 668-679.

Dickinson, A.H. (1994): NMDA receptor antagonists as analgesics, In: Fields, H.L. and Liebeskind, J.C. (Eds.), Progress in Pain Research and Management. IASP Press, Seattle, pp. 173-187.

Dickinson, A.H., Chapman, V. and Green, G.M. (1997): The pharmacology of excitatory and inhibitory amino acids-mediated events in the transmission and modulation of pain in the spinal cord. *Gen. Pharrmacol.* **28**, 633-638.

Elliott, K.J., Kest, B., Man, A., Kao, B. and Inturrisi, C.E. (1995): N-Methyl-D-Aspartate (NMDA) receptors, mu, and kappa opioid tolerance, and perspectives on new analgesic drug development. *Neuropsychopharmacol.*, **13**, 347-

356.

Guen, S.L., Catheline, G. and Besson, J.-M. (1999): Effects of NMDA receptor antagonists on morphine-tolerance: a c-fos study in the lumbar spinal cord of the rat. *Eur. J. Pharmacol.*, **373**, 1-11.

Herman, B.H., Vocci, F. and Bridge, P. (1995): The effects of NMDA receptor antagonists and nitric oxide synathase inhibitors on opioid tolerance and withdrawal. *Neuropsycopharmcol.*, **13**, 269-293.

Inturrisi, C.E. (1997): Preclinical evidence for a role of glutaminergic systems in opioid tolerance and dependence. *Semin. Neurosci.*, **9**, 110-119.

Javitt, D.C., Sershen, H. and Hashim, A. (1997): Reversal of phencyclidine-induced hyperactivity by glycine and the glycine uptake inhibitor glycyldodecylamide. *Neuropsychpharmacol.*, **17**, 202-204.

Johnson, J.W. and Ascher, P. (1987): Glycine potentiates the NMDA responses in cultured mouse brain. *Nature*, **325**, 529-531.

Kaneto, H. Kosaka, N. and Hirota, N. (1982): Timing of cycloheximide administration and ibhibition of the development of analgesic tolerance to morphine. *Life Sci.*, **31**, 2351-2354.

Trujillo, K.A. and Akil, H. (1991): Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science*, **251**, 85-87.

Trujillo, K.A. and Akil, H. (1994): Inhibition of opiate tolerance by non-competitive N-Mehtyl-D-Aspartate receptor antagonist. *Brain Res.*, **633**, 178-188.

Trujillo, K.A. (1995): Effects of noncompetitive N-methyl-D-Aspartate receptor antagonists on opiate tolerance and physical dependence. *Neuropsychopharmacol.*, **13**, 301-307.