

Efek baclofen, agonis selektif reseptor GABA_B pada analgesik dan ketergantungan secara psikologis dari opioid

Effect of a selective GABA_B receptor agonist, baclofen, on the opioid-induced antinociception and rewarding effect

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Abstrak

Penatalaksanaan nyeri dan efek samping yang tidak diinginkan masih merupakan problem besar dalam klinik. Kesuksesan penatalaksanaan nyeri dengan obat opioid diperlukan suatu efek analgesik yang kuat tanpa adanya efek samping yang berarti. Pada penelitian ini dirancang untuk meneliti efek baclofen, agonis selektif reseptor GABA_B pada efek analgesik dan ketergantungan secara psikologis dari obat-obat opioid. Pada penelitian ini dilaporkan bahwa pemberian morfin atau fentanyl menghasilkan efek analgesik yang tergantung dosis pada mencit menggunakan *tail-flick test*. Hasil penelitian juga menunjukkan bahwa pemberian baclofen bersama dengan morfin atau fentanyl menghasilkan sinergistik efek analgesic dari obat-obat opioid. Pada *place preference study* kami menemukan bahwa baclofen menghambat efek ketergantungan psikologis dari obat-obat opioid. Hasil tersebut menunjukkan bahwa pemberian baclofen bersama dengan obat-obat opioid menghasilkan efek sinergistik analgesik tanpa adanya efek ketergantungan psikologis yang berarti. Kami menyarankan bahwa pemberian baclofen bersama dengan obat-obat opioid mungkin dapat digunakan sebagai strategi baru untuk penatalaksanaan nyeri dan merekomendasikan baclofen sebagai *adjuvant drug*.

Kata kunci : opioid, *place preference*, ketergantungan psikologis, baclofen

Abstract

The management of excessive adverse effects is a major clinical problem. Multiple approaches have been described to address this problem. Successful pain management with opioids required the adequate analgesia without excessive side effects. The present study was designed to investigate the effect of a selective GABA_B receptor agonist baclofen on the opioid induced antinociception and rewarding effect. In the present study, we confirmed that either morphine or fentanyl produced a dose dependent antinociceptive effect in mice using tail-flick test. The results demonstrated that co-administration of baclofen with morphine, fentanyl or oxycodone produced the synergistic effect on antinociception in mice. In the place preference study, we found that baclofen inhibited on morphine or fentanyl-induced place preference in rats. These results suggest that co-administration of baclofen with opioids produce synergistic antinociception with less effects of place preference. We propose here that co-administration of baclofen with opioids may pave the way for the new strategy for the control of pain and recommended for the adjuvant drug.

Key words : opioid, place preference, rewarding effects, baclofen

Introduction

According to the World Health Organization (WHO) guidelines for patients with moderate or severe pain, morphine has been a "gold standard" for treatment of moderate to severe cancer pain. However, the use of morphine for the treatment of cancer pain is sometimes accompanied by side effects such as emesis, constipation and drowsiness. Although misplaced fears of the danger of dependence potential and abuse liability may lead doctors underprescribe and nurse to underdose, clinical studies have demonstrated that when opiates are used to control pain, psychological dependence and analgesic tolerance are not a major concern and patients rarely show withdrawal signs when the pain is relieved and the drug is gradually withdrawn. The management of excessive adverse effects is a major clinical problem. Multiple approaches have been described to address this problem. Successful pain management with opioids required the adequate analgesia without excessive side effects. Therefore, the detailed understanding of opioid-related side effects and the strategies used to prevent and manage them are essential skills for all in pain management. In general, four different approaches to the management of opioid adverse effects have been described (Cherny *et al.*, 2001): (1) dose reduction of systemic opioid; (2) symptomatic management of the adverse effects; (3) opioid rotation; (4) switching route of systemic administration. Fentanyl, which is a one series of potent opioids synthetic analgesic, has a high affinity for μ -opioid receptor and exhibits 50-100 times more potent analgesic activity than that of morphine. In the clinic, fentanyl is mainly used as epidural anesthetic and it has been used for the opioid rotation.

γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the CNS and has been implicated in the regulation of many physiological processes including pain, drug dependence, learning, memory and sensory motor integration. Three types GABA receptors, i.e., GABA_A, GABA_B and GABA_C have been distinguished on the basis of distinct pharmacological and physiological properties (Macdonald and Olsen., 1994; Misgeld *et al.*,

1995; Johnston., 1996). GABA_A and GABA_C receptors belong to the class of ligand-gated ion channels that mediates fast inhibitory postsynaptic currents (Macdonald and Olsen., 1994; Johnston., 1996). In contrast, GABA_B receptor belongs to the superfamily of seven-transmembrane and G protein-coupled receptors and it shows the highest sequence homology to metabotropic glutamate receptors (Kaupmann *et al.*, 1997). A stimulation of GABA_B receptors in cell bodies and dendrites elicits slow inhibitory postsynaptic potentials, and their activation at presynaptic nerve terminals inhibits neurotransmitter release (Misgeld *et al.*, 1995).

Considerable progress has recently developed highly specific GABA_B receptor agonist and antagonist, and these drugs have been explored in a number of animal models. However, only baclofen (*Lioresal*[®]), a selective GABA_B receptor agonist, is available and used in the clinic. Baclofen is used to treat migraine headache, musculoskeletal pain, and the pain associated with trigeminal neuralgia, stroke and spinal cord injury (Loubser and Akman., 1996; Hering-Hanit., 1999 and Becker *et al.*, 2000). Baclofen also reduces the reinforcing effects of drugs of abuse including opioids, cocaine, nicotine, and ethanol in human and animal models (Cousins *et al.*, 2002).

Therefore, the present study was designed to examine the effect of baclofen on the morphine or fentanyl -induced antinociception and rewarding effect, in order to investigate the advantages for treatment of baclofen with opioid for new therapeutic strategies in the pain management.

Methodology

The present study was conducted in accordance with Guiding Principles for the Care and Use of Laboratory Animals Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animal used in the following experiments.

Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo Co., Tokyo, Japan), fentanyl citrate (gift kindly from Hisamitsu Pharmaceutical Co. Inc., Tokyo, Japan) and (\pm)

baclofen (Sigma Chemical Co, St. Louis, MO, USA). All drugs were dissolved in saline (Otsuka Pharmaceutical Co. Inc., Tokyo, Japan).

Animals

Male SD rats and ICR mice weighing 250-300 g and 20-25 g, respectively were obtained from Tokyo Laboratory Animals Science Co., Ltd (Tokyo, Japan). Animals were housed in a room maintained at $23 \pm 1^\circ\text{C}$ under a 12 hr light/dark cycle (light on 08.00-20.00 hr). Rats and mice were given a standard food and water available ad libitum.

Antinociceptive assay (Tail-flick test)

The antinociceptive response produced by morphine or fentanyl were evaluated by recording the tail-flick test (Tail Flick Analgesia Meter Model MK 300B, Muromachi Kikai Co. Ltd., Tokyo, Japan). To prevent tissue damage, we established a 10 sec cut-off time. The tail-flick latency was measured both before and after the challenge with morphine or fentanyl. Antinociceptive response was calculated as a percentage of maximum possible effect (percentage of antinociception) according the following formula: % antinociception = (test latency - predrug latency)/(cut off time - predrug latency) \times 100. The treatment with baclofen for the morphine or fentanyl-induced antinociception was conducted 30 or 45 min before morphine injection in mice.

Conditioned place preference assay

Place conditioning was performed according to our previous report. The apparatus consisted of a shuttle box (30 x 60 x 30 cm: w x l x h) that was divided into two compartment of equal size. One compartment was white with a textured floor and the other was black with a smooth floor. For conditioning, rats were confined to one compartment after drug injection and the other compartment after saline injection for 1 hr. The orders of the injection (drug or saline) and the compartment (white or black) were counter balanced across the subjects.

Conditioning sessions were conducted once daily for 6 days (three days for drug: three days for saline). Immediately after s.c. injection of morphine (4 or 8 mg/kg) or fentanyl (30 or 56 $\mu\text{g}/\text{kg}$), animals were placed in one compartment for 1 hr. On alternate days, animals receiving with saline were placed the other compartment for 1 hr. Baclofen was administered 30 min before each conditioning. On day 7, test conditioning were performed as follows: the partition separating the two compartments was raised to 12 cm above the floor, and the neutral platform was inserted along the

seam separating the compartments. The rats which had treated with neither drugs nor saline on day 7, placed on the platform. The time spent in each compartment during a 900 sec in the test session was then recorded automatically in blinded fashion using an infrared beam sensor (KN-80, Natsume Seisakusyo Co., Ltd, Tokyo, Japan). All sessions were conducted under condition of dim illumination (28 lux lamp) and white masking noise (Suzuki, 1996).

Statistical analysis

The data are represented as the mean \pm S.E.M. The statistical significance of differences between the groups was assessed with a two-way ANOVA, followed by Bonferroni/Dunn or Student's t-test.

Results And Discussion

Antinociceptive effect of opioids following s.c. injection in the mouse tail-flick test

Either morphine (1, 1.7, 3 and 5.6 mg/kg, s.c.) or fentanyl (10, 17, 30 and 56 $\mu\text{g}/\text{kg}$, s.c.) produced a dose-dependent antinociception in the mouse tail-flick test. It is of interest to note that the antinociceptive effect of fentanyl observed in mice was much potent than that induced by morphine. The maximal antinociceptive responses induced by morphine or fentanyl was reached at 30 min and 15 min after the injection, respectively (Fig. 1). The ED_{50} values for the antinociception induced by morphine or fentanyl were 2.12 (1.31-3.28) mg/kg and 25.31 (12.76-47.29) $\mu\text{g}/\text{kg}$, respectively. These findings are consistent with the experiences in the clinic.

Effect of baclofen on the opioid -induced antinociception in mice

We also demonstrated that co-administration of baclofen with morphine or fentanyl produced the synergistic effect on antinociception in mice. Pretreatment with baclofen enhanced the antinociceptive effect induced by morphine or fentanyl and the ED_{50} value of morphine, 2.08 (1.89-2.27) mg/kg, s.c. or fentanyl, 25.31 (12.75-47.29) $\mu\text{g}/\text{kg}$, s.c. was significantly shifted to the left (ED_{50} = 0.88 (0.62-1.25) mg/kg, s.c and ED_{50} = 14.70 (5.67-33.08) $\mu\text{g}/\text{kg}$, s.c, respectively, Fig. 2). The antinociceptive activity of baclofen in model of acute and chronic pain is well established (Dickenson *et al.*, 1985; Thomas *et al.*, 1996 and Malan *et al.*, 2002). It was reported that the

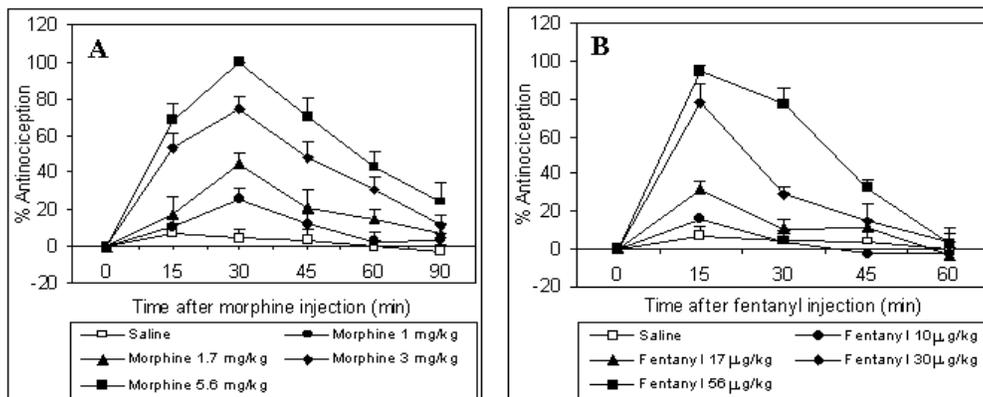


Figure 1. Antinociceptive effect of morphine (A) or fentanyl (B) following s.c. administration in the mouse tail-flick test. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represents the mean \pm S.E.M. of 8-12 mice

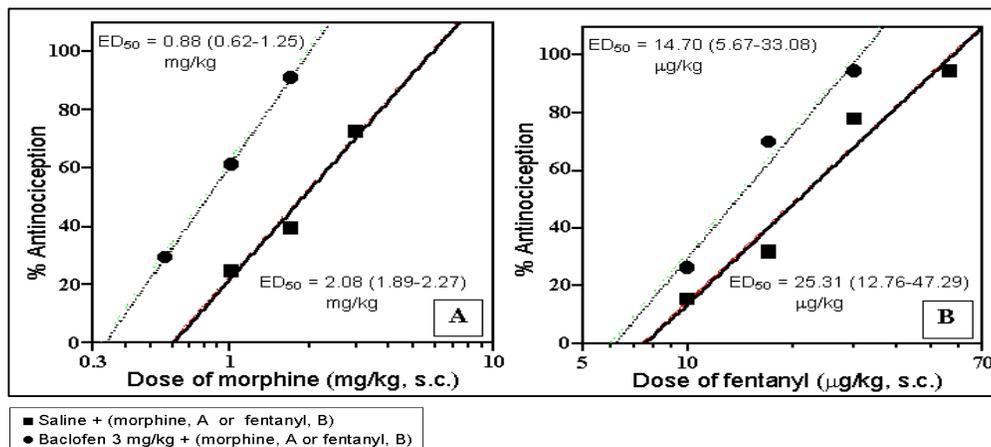


Figure 2. Effects of baclofen on the dose-response curve for the morphine-induced antinociception in mice. Groups of mice were pretreated with baclofen (3 mg/kg s.c.) 30 and 45 min before morphine or fentanyl injection. The data represent the result of 30 or 15 min after morphine or fentanyl injection. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). The ED₅₀ value for antinociception induced by morphine or fentanyl with or without baclofen are shown inside the graph

antinociceptive effect of systemic administration of baclofen is blocked by intrathecal injection of a GABA_B receptor antagonist CGP 35348, while its effect was only modestly attenuated by microinjection of CGP 35348 into the ventromedial medulla (VMM). These results suggest that systemic administration of baclofen may act at sites in both the spinal cord and the VMM, but its antinociceptive effects are likely to be mediated mainly through the spinal site (Thomas *et al.*, 1996). Thus, it is likely that the synergistic antinociception induced by systemic administration of baclofen with

morphine or fentanyl may result from the potentiation of opioid receptor-dependent inhibition of pain-like stimulation mainly through the spinal GABA_B receptor.

Effect of baclofen on the opioid-induced rewarding effect

Either morphine (8 mg/kg, s.c.) or fentanyl (56 µg/kg, s.c.) produced a significant place preference in rats. In the present study, we found that baclofen inhibited either morphine- or fentanyl-induced place preference in rats. Pretreatment with baclofen (1.5 and 3

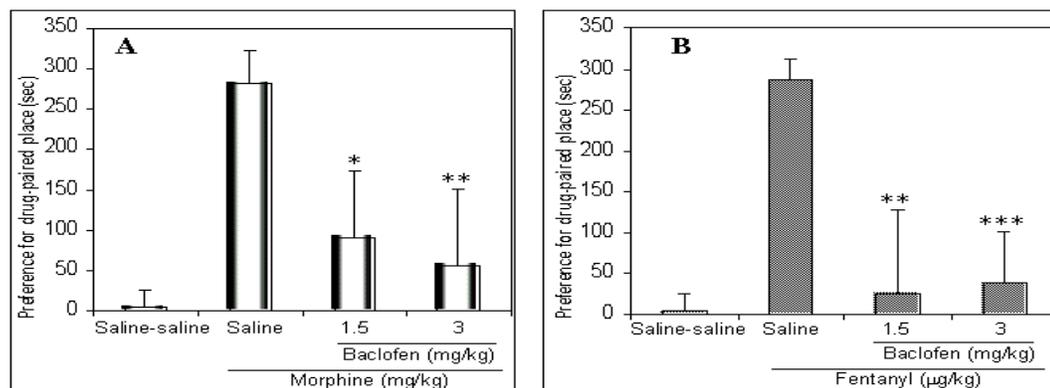


Figure 3. Effect of baclofen on the morphine (A)- or fentanyl (B)-induced place preference in rats using conditioned place preference assay. Groups of rats were pretreated with baclofen (1.5 and mg/kg) 30 min before morphine (8 mg/kg s.c.) or fentanyl (56 mg/kg, s.c.) injection, respectively. Ordinate: mean difference(s) between times spent in the drug- and saline-paired sides of the test box. Each point represents the mean \pm S.E.M. of 6-12 rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. saline + morphine or saline + fentanyl group.

mg/kg, s.c.) significantly suppressed both fentanyl and morphine-induced rewarding effects in rats (Fig. 3). Many studies have been pointed out that the mesolimbic dopamine system is a critical pathway for the initiation of opioid-induced reinforcement (Funada *et al.*, 1995; Koob *et al.*, 1998 and Narita *et al.*, 2001). Various reports have indicated that the dopamine neuronal activity in the VTA is modulated by GABA-containing inhibitory interneurons (Koob, 1992 and Kalivas *et al.*, 1990). Intra-VTA microinjection of DAMGO produced a decrease in the extracellular GABA level in the VTA (Garzon and Pickel, 2001), resulting in the expression of rewarding effect. Behavioral study has clearly demonstrated that microinjection of the GABA_B receptor agonist baclofen into the VTA significantly suppressed the morphine-induced place preference (Tsuiji *et al.*, 1996).

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Finally, we conclude that fentanyl produced potent antinociception than that of morphine. Therefore, these findings may provide evidence for benefit an usefulness of fentanyl for clinical frame on the management of pain treatment. Co-administration of baclofen with morphine or fentanyl produced a significant potentiation of antinociception and suppression of rewarding effect induced by those opioids. We therefore, propose here that co-administration of baclofen with opioids may pave the way for the new strategy for the control of pain and recommended for the adjuvant drug.

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