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Barium potentiates the conditioned aversion to, but not the somatic signs of, morphine withdrawal in mice

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Abstract

The effect of barium, a putative blocker of G-protein-activated inwardly rectifying potassium (GIRK) channels, on naltrexone-precipitated withdrawal signs in morphine-dependent mice was investigated. Mice were chronically treated with morphine (8–45 mg/kg) for 6 days. The morphine-dependent mice were then given naltrexone (1 and 3 mg/kg), after which they showed several somatic signs of withdrawal, as well as conditioned aversion, increased cortical noradrenaline turnover, and decreased dopamine turnover in the limbic forebrain. Pretreatment with barium (1.25 and 2.5 nmol) significantly potentiated the naltrexone-precipitated conditioned aversion and augmented the decrease in dopamine turnover in the limbic forebrain. However, barium pretreatment did not affect the naltrexone-precipitated somatic signs of withdrawal and increased cortical noradrenaline turnover. These findings suggest that modification of GIRK channels may be involved in the expression of aversion to morphine withdrawal mediated through the dopaminergic system but it is not involved in the somatic signs of morphine withdrawal mediated through the noradrenergic system.

Keywords: Conditioned place aversion; Dopamine; G-protein-activated inwardly rectifying potassium channel; Morphine; Withdrawal

1. Introduction

G-protein-activated inwardly rectifying potassium (GIRK) channels are involved in diverse cellular functions, including the maintenance of resting conductance, K^+ homeostasis, pacemaker activity, synaptic inhibition, and neuronal firing rates (Isomoto et al., 1997). The GIRK-type subfamily (GIRK 1, 2, 3, and 4) is comprised of four channel subunits that are subject to G-protein activation (Kubo et al., 1993; Krapivinsky et al., 1995; Lesage et al., 1994). GIRK channels are regulated by a variety of G_i/G_o-coupled inhibitory neurotransmitter receptors such as opioid (Chen and Yu, 1994; Lesage et al., 1994; Kovoor et al., 1995), M_2-muscarinic (Kubo et al., 1993; Krapivinsky et al., 1995), and gamma aminobutyric acid type B ($GABA_B$) receptors (Lewohl et al., 1999; Slesinger et al., 1997). It has been reported that weaver mutant mice with mutant GIRK channels display significantly lower analgesia after the administration of an opiate, suggesting that GIRK channel activation is important for opiate-induced analgesia (Ikeda et al., 2000).

Chronic use of opiates such as morphine is known to lead to physical and psychological dependence, which is characterized by a withdrawal syndrome when drug administration stops. This syndrome, which includes both somatic and affective components, is caused by adaptations in specific brain neurons after repeated exposure to the drug (Nestler and Aghajanian, 1997). It is well established that the noradrenergic system, which originates in the locus ceruleus and projects to the prefrontal cortex, plays an important role in physical dependence on opiates (Funada et al., 1993; Suzuki et al., 1995). Furthermore, the mesolimbic dopaminergic system, which originates in the ventral tegmental area and projects to the nucleus accumbens,

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plays an essential role in mediating drug reinforcement (Devine and Wise, 1994; Phillips and Le-Piane, 1980; Druhan et al., 1993).

It has been reported that ethanol, a frequently abused drug, enhances the function of GIRK channels coupled to $GABA_B$ receptors (Lewohl et al., 1999; Kobayashi et al., 1999). Recently, it was reported that GIRK2 channel null mutant mice showed weaker conditioned taste aversion and weaker conditioned place preference than did wild-type mice (Hill et al., 2003). Blednov et al. (2001) have demonstrated that in GIRK2 mutant mice ethanol neither induces motor activation nor reduces anxiety, and that the effects of ethanol on both sleep time and performance on the rotarod test were the same for GIRK2 mutant mice and wild-type mice. These reports suggest that GIRK channels may be involved in the motivational effect of an abused drug. However, the involvement of GIRK channels in the interaction between morphine withdrawal and changes in the central nervous system has not been documented.

Barium chloride is frequently used as a tool for studying GIRK channels. Barium has been shown to block the GIRK channel current induced by opioid and $GABA_B$ receptor agonists (Kobayashi et al., 1999; Slesinger et al., 1997; Svoboda and Lupica, 1998). To investigate the role of GIRK channels in naltrexone-precipitated behavioral and biochemical changes, here we evaluated the effects of barium pretreatment on naltrexone-precipitated withdrawal signs and conditioned place aversion, and on naltrexone-induced neurochemical changes in morphine-dependent mice.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g) were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The mice were maintained on a 12-h light, 12-h dark schedule, and laboratory mouse chow and water were provided ad libitum. All procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society.

2.2. Chronic morphine treatment

Morphine was injected s.c. twice daily, at 9 AM and 7 PM. According to the schedule described by Funada et al. (1993, 2001), the morphine dose was increased progressively from 8 to 45 mg/kg over a period of 6 days as follows (in mg/kg for AM and PM): 1st day (8, 15), 2nd day (20, 25), 3rd day (30, 35), 4th day (40, 45), 5th day (45, 45), and 6th day (45 at 9 AM only). The control mice were chronically treated with saline (10 ml/kg, s.c.) for 6 days.

2.3. Locomotor activity

The locomotor activity of the mice was measured with an activity monitoring system (NS-AS01, Neuroscience Co., Ltd., Tokyo, Japan) according to Narita et al. (2002). Briefly, the activity monitor is composed of an infrared ray sensor placed over a box ($18.2 \times 26 \times 12.8$ cm, $w \times l \times h$), a signal amplification circuit, and a control circuit. The sensor can detect the movement of animals, based on released infrared rays associated with their temperature. Locomotor activity was counted for 80 min before treatment for habituation and for 2 h after the administration of barium (2.5 nmol, i.c.v.) or saline in a volume 10 μl. The dose of barium (2.5 nmol) did not significantly affect on morphine analgesia (Harris et al., 1975). The data were processed by a computerized analytical system (Multidigital 16-port Counter System, Neuroscience Co., Ltd., Tokyo, Japan).

2.4. Effect of barium on naltrexone-induced conditioned place aversion

The experimental apparatus consisted of a shuttle box ($15 \times 30 \times 15$ cm, $w \times l \times h$, Neuroscience Co., Ltd., Tokyo, Japan), which was divided into two compartments of equal size. One compartment was white with a textured floor while the other was black with a smooth floor. Place conditioning was conducted as previously described (Watanabe et al., 2003). Barium chloride (1.25 and 2.5 nmol, i.c.v.) was administered in a volume 10 μl 2 h after the final injection of morphine (45 mg/kg, s.c.). Control animals were given an equal volume of saline. Fifteen minutes after barium or saline was administered, the mice were injected with naltrexone (1 and 3 mg/kg, s.c.) and immediately confined to either the black or white compartment of the test apparatus for 30 min. After at least 5 h, in the afternoon, the mice were administered saline (10 μl, i.c.v.). Fifteen minutes after the saline injection, the mice were treated with saline (10 ml/kg, s.c.) and confined to the other compartment for 30 min. The sequence of compartment assignation (from white to black or vice versa) was counterbalanced across the subjects overall. For the test session, the center wall used for the conditioning session was replaced by a partial wall in the shape of a T. The mice were allowed free access to both compartments (each was 6×6 cm, $w \times h$) on either side of the partial wall. The test session was carried out 1 day after the final conditioning session, with the mice in a drug-free state. The mice were placed in the center of the shuttle box and allowed free access to both compartments. The time a mouse spent in each compartment during a 900-s session was measured automatically in a blind fashion, using an infrared beam sensor (Neuroscience Co., Ltd., Tokyo, Japan). All sessions were conducted under the conditions of dim illumination (18 lx) and white noise.

2.5. Effect of barium chloride on naltrexone-precipitated withdrawal signs

Barium chloride (2.5 nmol, i.c.v.) was administered in a volume of 10 μl 2 h after the final injection of morphine (45 mg/kg, s.c.). Control animals were given an equal volume of saline. Fifteen minutes after barium or saline was administered, the mice were treated with naltrexone (1 and 3 mg/kg, s.c.) and placed in a clear plastic cylinder (12×30 cm, diameter × height). Naltrexone-precipitated behavioral changes were recorded using a digital video camera (DCR-PC100, Sony) for 30 min. Body weight loss was measured 5 min before and at 15, 30, 45, and 60 min after the administration of naltrexone. The naltrexone-precipitated jumps and body shakes were counted for 30 min. The number of mice that expressed withdrawal signs, such as jumping, shaking,

diarrhea, and ptosis, were counted according to Funada et al. (2001).

2.6. Effect of barium on naltrexone-induced monoamine turnover in the limbic forebrain and cerebral cortex

Using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD), the concentrations of noradrenaline, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), dopamine, 3,4-dihydroxyphenyl-acetic acid (DOPAC), homovanillic acid, serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were determined as reported previously (Funada et al., 1993, 2001; Suzuki et al., 1995). The morphine-dependent mice were administered barium chloride (1.25 and 2.5 nmol, i.c.v.) or saline (10 μl) 15 min prior to injection with naltrexone (3 mg/kg, s.c.) or saline. The mice were killed 60 min after the administration of naltrexone and then immersed in a dry ice-ethanol solution. The brain was quickly removed, and the limbic forebrain (including the nucleus accumbens) and the cerebral cortex were removed onto an ice-cold plate, as described by Funada et al. (1993, 2001). The tissues were frozen to −80 °C and stored until analysis. Each frozen sample was homogenized in 250 μl of 0.2 M perchloric acid containing 100 mM EDTA-2Na and 100 ng isoproterenol (as an internal standard). The homogenates were centrifuged at 10,180 × *g* for 20 min at 4 °C, and the supernatants were maintained at pH 3.0 using sodium acetate. Each sample (10 μl) was analyzed by HPLC and ECD. HPLC consisted of a delivery system (EP-300, Eicom, Kyoto, Japan), an analytical column (SC-5ODS, Eicom, Kyoto, Japan), and a guard column. The ECD system (EC-300, Eicom, Kyoto, Japan) had a graphite electrode and was used at a voltage of +0.75 V, with Ag/AgCl as a reference electrode. The mobile phase consisted of a 0.1 M sodium acetate/0.1 M citric acid buffer, pH 3.9, containing 14% methanol, sodium 1-octanesulfonate, and EDTA-2Na. The flow rate was set to 0.23 ml/min with a column temperature of 25 °C (ATC-300, Eicom, Kyoto, Japan).

2.7. Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo Co., Tokyo, Japan), naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO, USA), and barium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) dissolved in sterile saline.

2.8. Data analysis

Statistical analysis was performed using commercially available software (StatView 4.5 for Macintosh, SAS Institute, Cary, NC, USA). Data are expressed as the means ± S.E.M. Total locomotor activity was statistically evaluated using a one-way random factorial analysis of variance (ANOVA), followed by Fisher's least significant difference test to determine whether or not individual treatment produced a significant difference. Conditioning scores represent the time the mouse spent in the compartment where it had been injected with the drug minus the time spent in the compartment where it had been injected with saline. These conditioning scores were statistically evaluated using one-way ANOVA, followed by Fisher's least significant difference test to determine whether individual treatment produced significant conditioning. For the data on the changes in naltrexone-precipitated body weight loss, two-way ANOVA with time as the repeated measures was used to compare the barium-treated and saline-treated groups. The incidence of withdrawal signs was statistically evaluated using the chi-square test. Biochemical data were statistically evaluated with one-way ANOVA followed by the Fisher's least significant difference test. The turnover of dopamine, noradrenaline, and 5-HT was determined as a ratio: dopamine ratio = (DOPAC + homovanillic acid) (ng/mg of tissue)/dopamine (ng/mg of tissue); noradrenaline ratio = MHPG (ng/mg of tissue)/noradrenaline (ng/mg of tissue); and 5-HT ratio = 5HIAA (ng/mg of tissue)/5-HT (ng/mg of tissue). The percentage change in these ratios was calculated by taking the mean dopamine, noradrenaline, and 5-HT ratios for the saline–saline challenge group as 100%.

3. Results

3.1. Gross behavior in the barium-treated mice

The barium (2.5 nmol, i.c.v.)-treated mice did not exhibit abnormal behavior such as convulsions or wild running. After 80 min of habituation, the barium-treated mice showed no difference in locomotion as compared to the saline-treated mice; the mean total activity count for 120 min was 931.0 ± 370.4 (saline) and 605.9 ± 229.7 (barium) ($F[1, 19] = 0.58$, $P < 0.46$). After chronic treatment with morphine for 6 days, the gross behavior of the barium (2.5 nmol)-treated mice did not differ significantly from that of the saline-treated mice.

3.2. Effect of barium on naltrexone-induced conditioned place aversion

Using this schedule, the administration of naltrexone (3 mg/kg) to mice chronically administered saline, did not produce significant place aversion (47.5 ± 46.7, $P = 0.76$).

As shown in Fig. 1, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice produced significant place aversion when compared with the effect of saline (1 mg/kg: −129.2 ± −80.3, $P = 0.03$; 3 mg/kg: −121.5 ± −43.5, $P = 0.02$). In the barium (2.5 nmol, i.c.v.)-pretreatment group, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice also produced significant place aversion when compared with the effect of saline (1 mg/kg: −197.3 ± −78.9, $P = 0.02$; 3 mg/kg: −261.6 ± −23.7, $P < 0.001$). Barium had no significant effect on the morphine-dependent saline-challenge mice (−3.2 ± 56.5, $P = 0.35$). However, pretreatment with barium (1.25 and 2.5 nmol) potentiated the place aversion induced by the administration of naltrexone (3 mg/kg) in the morphine-dependent mice in a dose-dependent manner ($F[2, 36] = 3.49$, $P = 0.04$), and pretreatment with barium (2.5 nmol) significantly potentiated place aversion induced by the administration of naltrexone ($P = 0.01$, Fisher's test).

3.3. Effect of pretreatment with barium on naltrexone-precipitated withdrawal signs

Pretreatment with barium (2.5 nmol) had no effect on naltrexone (1 and 3 mg/kg)-precipitated body weight loss. Two-way ANOVA indicated that time had a significant effect (1 mg/kg; $F[4, 88] = 139.31$, $P < 0.0001$, 3 mg/kg; $F[4, 96] = 139.70$, $P < 0.0001$), unlike either barium (1 mg/kg; $F[1, 22] = 1.05$, $P = 0.32$, 3 mg/kg; $F[1, 24] = 0.00025$, $P = 0.99$) or the interaction

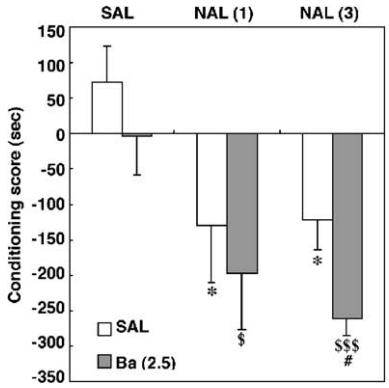

Fig. 1. Effect of barium on naltrexone-induced conditioned place aversion. Barium chloride (Ba, 2.5 nmol, i.c.v.) or saline (SAL, i.c.v.) was administered 2 h after the final injection of morphine (45 mg/kg, s.c.). Fifteen minutes after barium or saline was administered, the mice were injected with naltrexone (NAL, 1 and 3 mg/kg, s.c.). Each column represents the conditioning scores, expressed as means ± S.E.M. for 7–15 animals. *$P<0.05$ as compared with the SAL-SAL group of the morphine-dependent mice. \$$P<0.05$, \$\$\$$P<0.001$ as compared with the SAL-Ba group of the morphine-dependent mice. #$P<0.05$ as compared with the NAL-SAL group of the morphine-dependent mice.

(1 mg/kg; $F[4, 88]=0.52$, $P=0.72$, 3 mg/kg; $F[4, 96]=0.11$, $P=0.98$). Changes in the incidence of each withdrawal sign are shown in Table 1. Pretreatment with barium did not modify the incidence of jumping, body shakes, diarrhea or ptosis, when compared to the effect of pretreatment with saline (chi-square, ns). Pretreatment with barium did not modify the number of withdrawal jumping and body shakes precipitated by naltrexone (3 mg/kg) (Jumping; 65.8 ± 14.7 vs. 72.2 ± 20.6 ns., Body shakes; 5.3 ± 1.1 vs. 4.2 ± 0.6 ns.)

3.4. Effect of barium on naltrexone-induced monoamine turnover in limbic forebrain

As shown in Fig. 2A, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice significantly, and dose dependently, decreased dopamine turnover in the limbic forebrain when compared with the effect of saline ($F[2, 26]=5.61$, $P=0.0094$). Fisher's least significant difference test demonstrated a significant effect at both 1 mg/kg ($P=0.017$) and 3 mg/kg ($P=0.0041$). In the barium (2.5 nmol, i.c.v.)-pretreatment group, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice also significantly and dose dependently decreased dopamine turnover ($F[2, 19]=18.00$, $P<0.0001$). Fisher's least significant difference test demonstrated a

Table 1
Effect of barium on naltrexone-precipitated withdrawal signs

Withdrawal signs		Positive mice/Total mice			
Challenge	Pretreatment	Jumping	Body shakes	Diarrhea	Ptosis
Naltrexone (1)	Saline	11/12	11/12	8/12	5/12
	Barium	12/12	12/12	10/12	6/12
Naltrexone (3)	Saline	12/12	12/12	10/12	1/12
	Barium	12/12	12/12	11/12	5/12

Mice were treated with morphine twice a day for 6 days. Barium chloride (2.5 nmol) or saline was administered intracerebroventricularly 2 h after the final injection of morphine (45 mg/kg, s.c.). Withdrawal was precipitated by naltrexone (1 and 3 mg/kg, s.c.) 15 min after barium or saline administration.

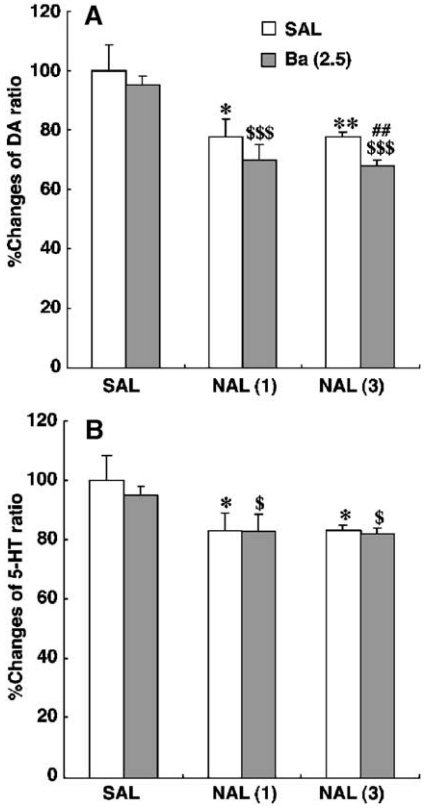

Fig. 2. Effects of barium on decreased dopamine (DA, A) and serotonin (5-HT, B) ratios in the limbic forebrain of morphine-dependent mice. Barium chloride (Ba, 2.5 nmol, i.c.v.) or saline (SAL, i.c.v.) was administered 2 h after the final injection of morphine (45 mg/kg, s.c.). Fifteen minutes after barium or saline was administered, the mice were injected with naltrexone (NAL, 1 and 3 mg/kg, s.c.) or saline (SAL, 10 ml/kg, s.c.). The mice were killed 60 min later. Each column represents the percent change in the dopamine and 5-HT ratios, expressed as the mean ± S.E.M. for 6–14 animals. *$P<0.05$, **$P<0.01$ as compared with the SAL-SAL group of the morphine-dependent mice. \$$P<0.05$, \$\$\$$P<0.001$ as compared with the SAL-Ba group of the morphine-dependent mice. ##$P<0.01$ as compared with the NAL-SAL group of the morphine-dependent mice.

significant effect at both 1 mg/kg ($P=0.0002$) and 3 mg/kg ($P<0.0001$). Pretreatment with barium (1.25 and 2.5 nmol) significantly, and dose dependently, augmented the decrease in dopamine turnover produced by naltrexone (3 mg/kg) in the limbic forebrain ($F[2, 28]=7.12$, $P=0.0032$, 2.5 nmol; $P=0.0012$, Fisher's test). Barium had no significant effect on dopamine turnover in the saline administration group ($F[1, 13]=0.19$, $P=0.67$).

As shown in Fig. 2B, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice significantly and dose dependently decreased 5-HT turnover in the limbic forebrain when compared with the effect of saline ($F[2, 26]=4.22$, $P=0.026$) Fisher's least significant difference test demonstrated a significant effect at both 1 mg/kg ($P=0.036$) and 3 mg/kg ($P=0.011$). In the barium (2.5 nmol, i.c.v.)-pretreatment group, naltrexone (1 and 3 mg/kg) given to morphine-dependent mice also significantly and dose dependently decreased 5-HT turnover ($F[2, 19]=3.97$, $P<0.036$). Fisher's least significant difference test demonstrated significant effect at both 1 mg/kg ($P=0.041$) and 3 mg/kg ($P=0.015$). However, pretreatment with barium did not modify 5-HT turnover in the limbic forebrain in the saline administration group or in the

naltrexone administration group (saline administration group; $F[1, 13]=0.29$, $P=0.60$; naltrexone 1 mg/kg; $F[1, 10]=0.001$, $P=0.98$, naltrexone 3 mg/kg; $F[2, 28]=2.80$, $P=0.078$).

3.5. Effect of barium on naltrexone-induced monoamine turnover in cerebral cortex

As shown in Fig. 3A, naltrexone (3 mg/kg) given to morphine-dependent mice significantly elevated cortical noradrenaline turnover when compared with the effect of saline ($F[1, 23]=15.3$, $P=0.0007$). Pretreatment with barium did not modify noradrenaline turnover in the cerebral cortex in the saline administration group or in the naltrexone administration group (saline administration group: $F[1, 19]=0.49$, $P=0.49$; naltrexone administration group: $F[2, 30]=0.18$, $P=0.83$).

As shown in Fig. 3B, naltrexone (3 mg/kg) given to morphine-dependent mice had no effect on cortical 5-HT turnover when compared with the effect of saline ($F[1, 23]=0.09$, $P=0.77$). Pretreatment with barium did not modify 5-HT turnover in the cerebral cortex in the saline administration group or in the naltrexone administration group (saline administration group: $F[1, 19]=0.47$, $P=0.50$; naltrexone administration group: $F[2, 30]=0.37$, $P=0.69$).

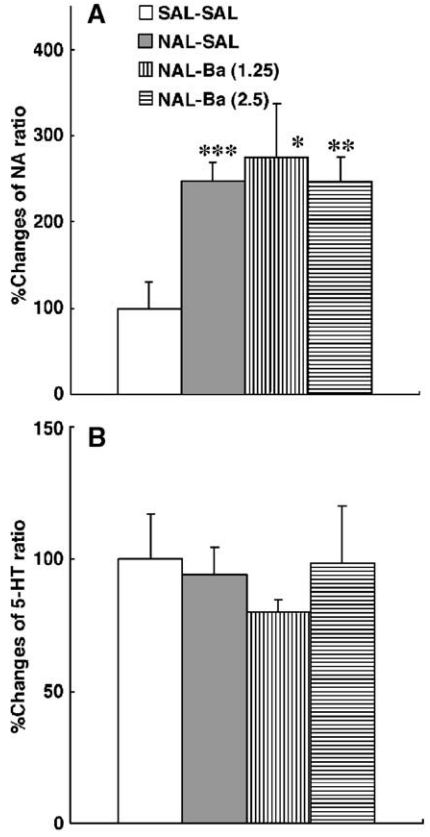

Fig. 3. Effects of barium on cortical noradrenaline (NA, A) and serotonin (5-HT, B) ratios in morphine-dependent mice. Barium chloride (Ba, 1.25 and 2.5 nmol, i.c.v.) or saline (SAL, i.c.v.) was administered 2 h after the final injection of morphine (45 mg/kg, s.c.). Fifteen minutes after barium or saline was administered, the mice were injected with naltrexone (NAL, 3 mg/kg, s.c.) or saline (SAL, 10 ml/kg, s.c.). The mice were killed 60 min later. Each column represents the percent change in the noradrenaline and 5-HT ratios, expressed as the mean±S.E.M. for 8–15 animals. *$P<0.05$, **$P<0.01$, ***$P<0.001$ as compared with the SAL-SAL group of the morphine-dependent mice.

4. Discussion

This study demonstrates that barium significantly potentiates naltrexone-precipitated morphine withdrawal aversion in morphine-dependent mice. Furthermore, the neurochemical experiments show that barium significantly augments the naltrexone-precipitated decrease in dopamine turnover in the limbic forebrain in morphine-dependent mice under the same conditions.

It was previously demonstrated that the systemic administration of the opioid receptor antagonist naloxone to morphine-dependent rats produced conditioned place aversion (Watanabe et al., 2002, 2003). Our findings are consistent with these findings in that the injection of naltrexone, another opioid receptor antagonist, into morphine-dependent mice produced significant conditioned place aversion.

The nucleus accumbens is implicated in the aversive stimulus properties of morphine withdrawal but not in the expression of the somatic signs of morphine withdrawal. It has been reported that the administration of naloxone reduces the extracellular dopamine concentration in the nucleus accumbens of morphine-dependent animals (Pothos et al., 1991). The administration of naloxone to morphine-dependent rats causes a decrease in mesolimbic dopaminergic neuronal activity (Diana et al., 1995). In addition, it has been reported that dopamine turnover in the limbic forebrain, including the nucleus accumbens and olfactory tubercle, significantly decreases during naloxone-precipitated morphine withdrawal, indicating that dopaminergic activity in the nucleus accumbens decreases during naloxone-precipitated morphine withdrawal (Suzuki et al., 1995). The present results are consistent with these earlier findings in that the administration of naltrexone to morphine-dependent mice significantly decreased dopamine turnover in the limbic forebrain.

In the present study, we found that pretreatment with barium significantly augmented the naltrexone-precipitated decrease in dopamine turnover in the limbic forebrain of morphine-dependent mice. Moreover, we found that pretreatment with barium in morphine-dependent mice potentiated naltrexone-induced conditioned aversion to withdrawal under the same conditions. These results suggest that the enhancement by barium of the decrease in limbic dopamine turnover can mediate the potentiation of morphine withdrawal aversion. Thus, pretreatment with barium may potentiate naltrexone-induced morphine withdrawal aversion.

Caille et al. (2003) reported that a rat bearing a 6-hydroxydopamine-induced lesion of the nucleus accumbens displayed naloxone-precipitated conditioned place aversion, similar to a sham-operated rat, and that apomorphine, a

dopamine agonist, did not reduce naloxone-precipitated conditioned place aversion. The findings of present study are not consistent with the findings of the previous study in that dopamine turnover in the nucleus accumbens altered naltrexone-precipitated conditioned place aversion. However, there is a report that naloxone-precipitated place aversion becomes increasingly intense as the number of conditioning sessions is increased (Mucha and Iversen, 1984). In the present study, we used only one conditioning session, as compared with Caille et al. who used three. Also, the induction of morphine dependence (pellets vs. injection) and the state of morphine dependence in conditioning and test sessions (dependent state vs. drug-free state) were different in that study and the present study, which may account for the different results. Likewise, as Caille et al. suggested that multivariable mechanisms, such as the dopaminergic, noradrenergic, GABA, glutamatergic and cholinergic systems, are involved in morphine withdrawal aversion, it is possible that these systems were also changed in present study.

In the present study, the administration of naltrexone to morphine-dependent mice significantly decreased 5-HT turnover in the limbic forebrain. Several items of evidence suggest that a change in serotoninergic neurons may be involved in opioid dependence and withdrawal (Carboni et al., 1989; Harris and Aston-Jones, 2001). Acute morphine administration increases 5-HT transmission within the nucleus accumbens and dorsal raphe magnus (Tao and Auerbach, 2002). In contrast, 5-HT transmission is depressed during withdrawal from chronic morphine treatment (Tao et al., 1998; San-Martin-Clark et al., 1996). The results of the present study are consistent with these previous reports in that they provide evidence for reduced 5-HT transmission during morphine withdrawal. Interestingly, pretreatment with barium did not affect 5-HT transmission in the limbic forebrain in morphine-dependent mice or in morphine withdrawal mice. These results indicate that the potentiation by barium of morphine withdrawal aversion may involve the dopaminergic system but apparently not the serotonergic system.

In this study, we used barium to block the GIRK channel. Another type of inwardly rectifying K+ channel (IRK1-3), which is expressed in the brain, is also blocked by extracellular barium (Karschin et al., 1996; Isomoto et al., 1997). In addition, it has been reported elsewhere that barium blocks voltage-dependent K+ channels (Svoboda and Lupica, 1998). To our knowledge, no selective blockers of GIRK channels have been found, and we are unable to confirm whether or not GIRK channels play an important role in morphine withdrawal aversion and in the decrease in dopamine turnover as compared with other types of K+ channels. However, it has been shown that opioids do not activate IRK1-3 or voltage-dependent K+ channels (Isomoto et al., 1997). Thus, the blockade by barium of GIRK channels may be involved in both morphine withdrawal aversion and the decrease in dopamine transmission. However, another experiment using a selective ligand of GIRK channels would be necessary to confirm this.

It has been hypothesized that the central noradrenergic system is involved in the development of morphine dependence and the expression of withdrawal signs (Rasmussen et al., 1990; Lane-Ladd et al., 1997). The systemic administration of morphine inhibits the firing rate of locus ceruleus neurons, and tolerance to the inhibitory effects of morphine develops after chronic morphine treatment (Aghajanian, 1978). Moreover, the firing rate of locus ceruleus neurons increases during morphine withdrawal precipitated by naltrexone (Rasmussen et al., 1990). In previous neurochemical experiments, a naloxone-precipitated increase in the level of noradrenaline turnover was found in the cerebral cortex innervated by the locus ceruleus in morphine-dependent mice (Funada et al., 1993, 2001; Suzuki et al., 1995). The results of the present study are consistent with these previous reports in that naltrexone injection in morphine-dependent mice markedly increased noradrenaline turnover in the cerebral cortex, without altering 5-HT turnover. These findings suggest that the naltrexone-precipitated activation of the noradrenergic system, but not the serotonergic system, in the cerebral cortex is involved in the expression of somatic signs of morphine withdrawal.

In the present study, we found that pretreatment with barium of morphine-dependent mice did not affect the naltrexone-precipitated increase in cortical noradrenaline turnover or the expression of somatic signs of morphine withdrawal induced by naltrexone. It has been reported elsewhere that morphine withdrawal signs, such as jumping and body shakes, are elicited after microinjection of the hydrophilic opioid antagonist methylnaloxonium into the locus ceruleus (Maldonado et al., 1992). Because of the high incidence of the morphine withdrawal signs of jumping and body shakes, they may have been limited by a possible ceiling effect. However, barium did not modify the number of jumping and body shakes. Under the same conditions, pretreatment with barium did not affect the naltrexone-precipitated increase in cortical noradrenaline turnover. These results suggest that the lack of effect of barium on the increase in cortical noradrenaline turnover may be associated with the lack of effect of barium on the expression of morphine withdrawal signs, such as jumping and body shakes.

The diarrhea or body weight loss caused by morphine withdrawal did not appear after the injection of methylnaloxonium intracerebroventricularly, suggesting that peripheral mechanisms are important in the expression of these signs (Stinus et al., 1990; Maldonado et al., 1992). Moreover, morphine withdrawal ptosis was noted after the microinjection of methylnaloxonium into the amygdala, anterior hypothalamus and nucleus raphe magnus (Maldonado et al., 1992). These results suggest that not only the noradrenergic system projecting from the locus coeruleus

but also another system in another brain area are important in the expression of some morphine withdrawal signs. The blockade of GIRK channels in the brain with barium seems not to affect the expression of morphine withdrawal signs.

In conclusion, we found that the blockade of GIRK channels by barium potentiated naltrexone-induced conditioned aversion in morphine-dependent mice but not the expression of naltrexone-precipitated withdrawal signs. Furthermore, the GIRK channel blockade augmented the decrease in naltrexone-induced dopamine turnover in the limbic forebrain without any effects on 5-HT or noradrenaline turnover. These results suggest that the reduction in dopamine transmission due to GIRK channel blockade may be involved in the expression of morphine withdrawal aversion in mice. Our results suggest that the GIRK channel activator might be of value in the treatment of morphine withdrawal.

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