J Med Sci 2007;27(1):015-022
http://jms.ndmctsgh.edu.tw/2701015.pdf

U-50488 Decreases the Rewarding Effect and Mesolimbic Dopamine Neuron Activity Induced by Morphine in Rats

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Background: Morphine is one of the most effective analgesics used to treat postoperative or cancer pain. A major drawback of its continuous use is the development of tolerance and dependence. U-50488, a selective κ-opioid receptor agonist has been reported to suppress the development of antinociceptive tolerance to morphine in animals. Here we further investigated its effect on morphine addiction. **Methods:** Conditioned place preference (CPP) test and behavioral sensitization of locomotor activity were used to investigate drug-seeking related behaviors associated with psychological dependence in Sprague Dawley rats. Microdialysis and high-performance liquid chromatography were used to determine the extracellular level of dopamine metabolites in the shell region of the nucleus accumbens (NAc). **Results:** Coadministered U-50488 was able to abolish both the CPP effect and behavioral sensitization induced by morphine. A significant increase of dopamine metabolites following morphine administration was demonstrated in the NAc. This increase by morphine could be attenuated by coadministered U-50488, whereas U-50488 itself did not show significant effects. **Conclusions:** U-50488 might effectively attenuate morphine-induced psychological dependence. Neurochemical analysis suggested that U-50488 could be acting by inhibiting the dopaminergic mesolimbic pathway activated by morphine, which is believed to cause rewarding.

Key words: Morphine; U-50488; Microdialysis; Nucleus accumbens; Mesolimbic pathway

INTRODUCTION

Morphine and other opioids are still the most effective analgesics used to treat postoperative and cancer pain clinically. The major drawback of their continuous use is the development of tolerance and dependence (including physical and psychological dependence). Trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide hydrochloride (U-50488), a selective kappa-opioid receptor agonist, has been reported to suppress the development of antinociceptive tolerance to morphine in rats, mice and guinea pigs[1-3]. In 1997, Kuzmin et al. reported that U50488H could decrease the intake of morphine in experiments using self-administration in mice[4]. Therefore, it is possible that U-50488 can attenuate morphine-induced rewarding effects, which could be linked with the development of psychological dependence by morphine. In this study, we used the conditioned place preference (CPP) paradigm to investigate the effects of U-50488 on rewarding induced by morphine, which can also relate to drug-seeking behaviors and psychological dependence[5,6]. Behavioral sensitization of locomotor activity was examined in parallel with CPP experiments, as it is viewed as an important factor in the development and maintenance of addiction[7].

In conjunction with the behavioral study, microdialysis experiments were performed at the shell region of the nucleus accumbens (NAc) to determine the neurochemical changes caused by morphine and the impact of U-50488 coadministration. The concentrations of dopamine and their metabolites were determined to evaluate the action of U-50488 on morphine-induced activation of the mesolimbic pathway. Because the dopaminergic neuropathways of the mesolimbic system, especially the neurons at the ventral tegmental area (VTA) projecting to the NAc, have been recognized as a major target of addictive drugs[8,9], this investigation of microdialysis in free-moving animals could provide more information about the possible action of U-50488 on the neural circuits responsible for morphine-induced rewarding effects.

Received: July 5, 2006; Revised: August 4, 2006; Accepted: August 24, 2006
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METHODS

Animals

Male Sprague Dawley rats, weighing 350-400 g, were purchased from the National Experimental Animal Center, Taipei, Taiwan. All rats were kept in an animal room with a 12 h light/dark cycle, at a temperature of 25±2°C and humidity of 55%. Standard diet and water were provided ad libitum. The animals were acclimated for at least one week before the experiments were commenced. The care of animals was carried out in accordance with institutional and international standards (Principles of Laboratory Animal Care, NIH) and the protocol received prior approval from the Institutional Animal Care and Use Committee of the National Defense Medical Center, Taiwan, R.O.C.

Schedule of Drug Administration

As shown in Fig. 1A, a nine-day schedule was employed for CPP tests. On day-1, the animals were placed in an isolated dark room for 60 min for habituation. On day 0, predrug place preference was tested in the dark room. Drug conditionings were carried out on days 1, 3, 5 and 7, immediately following drug administration: morphine (10 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) + U-50488 (8 mg/kg, i.p.). Postdrug place preference was measured and recorded on day 9. On days 2, 4, 6 and 8, control saline injections were performed and the rats were placed in the saline-paired compartment for 40 min conditioning.

In another set of experiments, the total time of the schedule for drug administration and behavioral tests was 20 days (Fig. 1B). Drug administrations consisting of morphine (10 mg/kg, i.p.) or morphine (10 mg/kg, i.p.) + U-50488 (8 mg/kg, i.p.) were conducted on days 1, 3, 5, 7, 9, 10, 11, 12, 13 and 20. On day-1, the animals were brought to the room and placed into the test cages for 60 min to adapt to the environment for the locomotor activity test. Following the injection of drugs, the rats were immediately put into the cages for locomotor activity determination on days 1, 7, 12 and 20. On days 2, 4, 6 and 8, saline was given to the animals at the same volume used to dissolve and deliver the drugs.

Conditioned Place Preference (CPP) Testing

The CPP apparatus, made of an acrylic plastic box (70×25×25 cm), was divided into three compartments. Two identical-sized compartments (30×25×25 cm) were constructed, separated by a narrower corridor (10×25×25 cm). The compartments were connected by guillotine doors (10×10 cm) between the central unit and the larger two at each end. One of the large compartments was

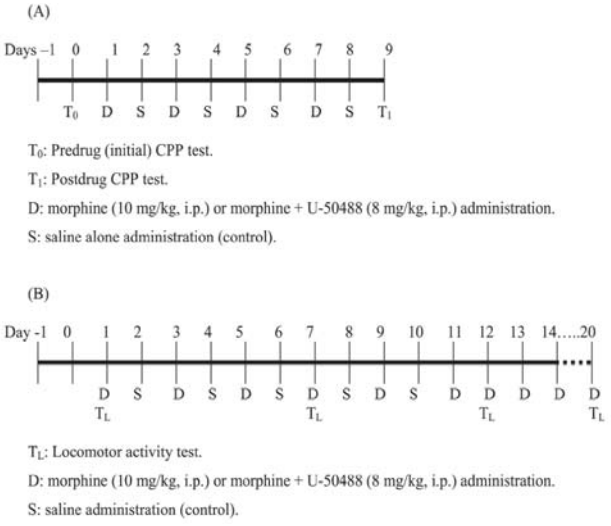

Fig. 1 (A) Time schedule of the conditioned place preference (CPP) test. (B) Time schedule of the locomotor activity test.

covered by a mosaic-type paper (checkered, 2.5×2.5 cm black and white squares) on the three walls and the floor as a visual cue; the other large compartment was covered by pure white paper. To aid the visual cues, a blue light bulb and a red light bulb were hung separately above the two large compartments. During the experiments, the CPP apparatus was kept in an isolated room with only the colored light bulbs on. After each behavioral test or place conditioning, the whole box was cleaned thoroughly to prevent any interference from the smell of feces and urine.

As described above, the predrug and postdrug place preferences of the animals were determined, giving the rats free access to the entire box for 15 min. During conditioning, the animals were kept for 40 min in the corresponding compartment.

Locomotor Activity Testing

The total activity of rats was measured in transparent standard polypropylene animal cages (38×22×15 cm). The test cages were placed in a photobeam activity system (San Diego Instruments, San Diego, CA, USA). A computer control unit registered interruptions to the photobeams from five individual cages. Any break of the photobeam was recorded as total activity. Activity was recorded in 5 min periods for 2 h immediately after drug or saline administration. The experiments of locomotor activity were performed in an isolated noise-free room.

Microdialysis Experiments

Under pentobarbital anesthesia (50 mg/kg, i.p.), differ-

ent groups of rats were implanted stereotaxically with guide cannulae (MAB 6 Guide Cannula for MAB 6.20 Microdialysis Probes, re-usable, Microbiotech, Stockholm, Sweden) at the shell of the nucleus accumbens (coordinates: AP, +1.7 mm; L, +1.0 mm with respect to the bregma; V, -7.2 mm from skull). The cannulae were fixed firmly to the skull using two metal screws and dental cement. Animals were allowed three days for recovery. On the day of the experiment, a microdialysis probe (MAB 6.20, Microbiotech) was inserted. The probe contains 1 mm of exposed dialysis membrane (outer diameter 0.6 mm; molecular weight cutoff 15,000 Da) protruding from the guide cannula. The dialysis solution (artificial cerebrospinal fluid: NaCl 145 mM, KCl 2.8 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.2 mM, D-glucose 0.25 mM, pH 7.4) was advanced through the probe via Santopren tubing (Microbiotech) using a microsyringe pump (CMA 102, Microbiotech, Stockholm, Sweden) at a flow rate of 1 μL/min. Before the collection of dialysates, 2.5 μL of antioxidants (oxalic acid 1 mM and acetic acid 0.1 M) were added to the sampling tubes to prevent the rapid oxidation of monoamines. After 60 min of equilibration, dialysis samples were collected every 15 min and measured by high-performance liquid chromatography (HPLC). If the levels of measured monoamines remained stable (i. e., the difference between the three sequential dialysates was within 15%), the last two values were averaged to define the basal efflux. Subsequently, animals were injected with saline (control), with morphine (10 mg/kg, i. p.), with U-50488 (8 mg/kg, i.p.), or with both drugs, and the dialysates were collected every 30 min for 225 min, except for the first sample, which was collected 15 min after drug injection. The samples were immediately subjected to HPLC to analyze the monoamines (described below). After each microdialysis experiment, the location of the probe was verified histologically in a series of 50 μm sections.

Monoamine Analysis

The HPLC system comprised a reverse-phase C-18 column (MD-150, RP-C-18, 3 μm, length: 15 cm, ESA Biosciences, Chelmsford, MA, USA), a high-pressure pump (LC-10AD, Shimadzu Corporation, Japan) and connected with an electrochemical detector (ECD) coupled with three electrodes (Coulochem II, ESA Biosciences). The electrode of the guard cell was set at 350 mV and electrodes 1 and 2 (for detection) were set at 40 mV and 250 mV, respectively. Under isocratic conditions, the mobile phase solvent (MD-TM, ESA Biosciences) was circulated at a flow rate of 0.5 mL/min. To quantify the sample peaks, each chemical [3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA)] was compared with the external standards, which were freshly prepared and injected every fifth sample run.

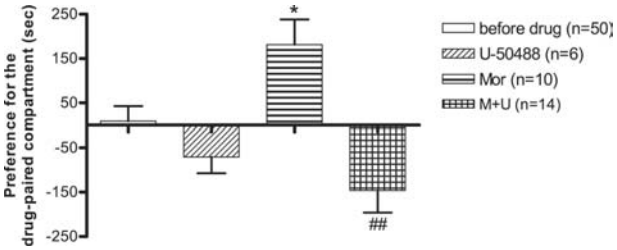

Fig. 2 Effect of U-50488 on the development of morphine-induced conditioned place preference. Data are expressed as preference for the drug-paired compartment as determined by the time spent in the drug-paired compartment minus the time spent in the saline-paired compartment. One-way ANOVA followed by post-hoc Newman-Keuls test was used to analyze the data. $^{*}P < 0.05$ compared with the place preference before drug; $^{\#\#}P < 0.01$ compared with the place preference of the morphine group ($n \geqq 6$).

Statistical Analysis

Two-way repeated measures ANOVA followed by Bonferroni post-testing were used to analyze the data from the locomotor activity tests. One-way ANOVA followed by a post-hoc Newman-Keuls test was used to analyze the data from the CPP test and the area under the curve (AUC) of the microdialysis curves. The AUC was obtained from the total peak area above the horizontal line of 100%, in summation with the peak areas below the line, which were considered as negative values. $P < 0.05$ was considered significant.

Materials

U-50488 was prepared according to the literature[10,11] and isolated as the hydrochloride salt, purity at 221.5-222.5 ^{o}C, 99.5% by HPLC (RP-select B; H_2O/CH_3OH = 1:4 buffered at pH 7.52; 254 nm). Morphine hydrochloride was purchased from the National Bureau of Control Drugs, National Health Administration, Taipei, Taiwan, R.O.C. Other chemicals used were reagent grade and were supplied by Sigma-Aldrich (St. Louis, MO, USA).

RESULTS

Effect of U-50488 on Rewarding Induced by Morphine

As shown in Fig. 2, time spent in the drug-paired compartment minus the time spent in the saline-paired

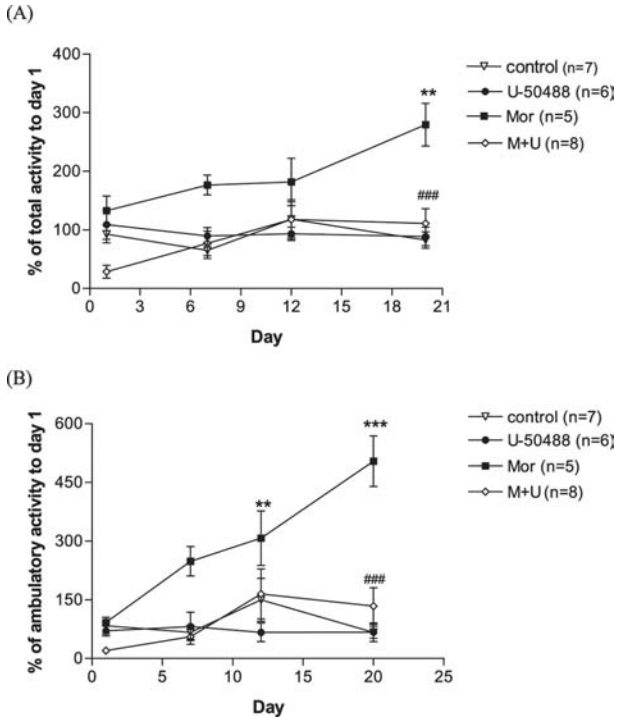

Fig. 3 The effect of U-50488 on the development of morphine-induced behavioral sensitization in (A) total activity of locomotion and (B) ambulatory activity of locomotion. The durations of activity of the animals were all converted to the percentage of the activity on day 1. Two-way ANOVA followed by a post-hoc Bonferroni test was used to examine the significance of the results. $^{**}P < 0.01$, $^{***}P < 0.001$ when each result was compared with the activity on day 1; $^{###}P < 0.001$ when the M + U group was compared with the morphine alone group (n ≧ 6).

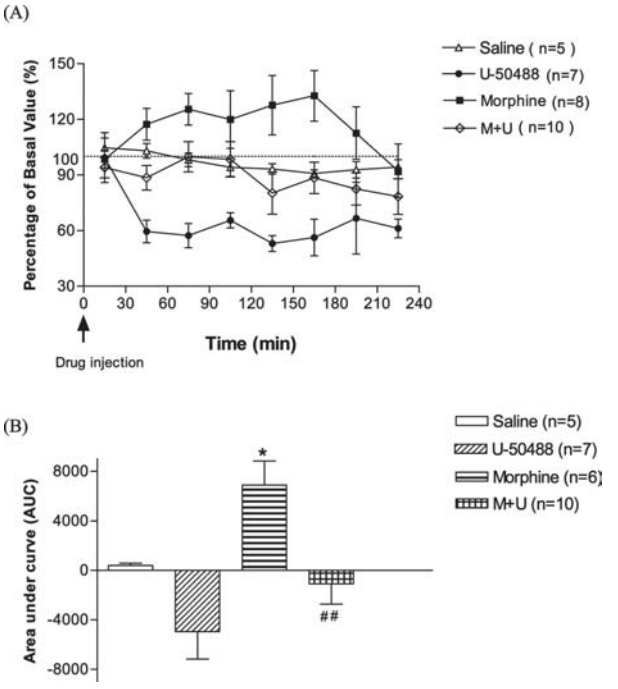

Fig. 4 (A) Time-dependent change of DOPAC in the shell region of the nucleus accumbens after drug administration. The concentration of DOPAC was measured every 30 min and converted to a percentage of the basal value. (B) A bar graph presenting the area under curve (AUC) of the microdialysis curve of DOPAC from 0 to 195 min, as shown in (A). One-way ANOVA followed by a post-hoc Newman-Keuls test was used to analyze the data. $^{*}P < 0.05$ when compared with the AUC of the saline group; $^{##}P < 0.01$ when the M + U group was compared with the morphine alone group (n ≧ 7).

compartment was used to express the place preference induced by drugs. All rats showed no significant place preference (9.1±33.6 sec) for the drug-paired compartment before drug conditioning, which indicated that the CPP apparatus that we used is in a nonbiased design[5]. The U-50488-treated rats exhibited no rewarding effect measured by the CPP test (-70.5±36.6 sec). Administration of morphine (10 mg/kg, i.p., 4 times in 8 days) resulted in a marked increase in time spent in the drug-paired compartment (+181.3±57.0 sec), indicating that morphine produced a significant rewarding effect. When U-50488 was coadministered with morphine, the rewarding effect of morphine was completely abolished (-146.0±50.0 sec).

Effects of U-50488 on Behavioral Sensitization Induced by Morphine

Repeated administration of morphine or psychostimulants, such as cocaine or amphetamine, results in the augmentation of locomotor and/or stereotypical behavior patterns[12-16]. This persists for a long period after drug abstinence and is termed behavioral sensitization[17]. In the present study, rats were treated (saline, morphine alone, U-50488 alone, or morphine plus U-50488) ten times in 14 days and then withdrawn from the drug for five days. Locomotor activity was determined on days 1, 7, 12 and 20. For total activity (Fig. 3A), the rats of the control or U-50488 groups did not show any significant change from day 1 to day 21. On the other hand, the rats of the morphine-treated group showed a significantly increased activity on day 20 compared with the activity induced by morphine on day 1 ($P < 0.01$), which means the animals had developed behavioral sensitization. When U-50488 was coadministered with morphine (M + U group), the total activity was not increased on day 20; there was a significant difference between the

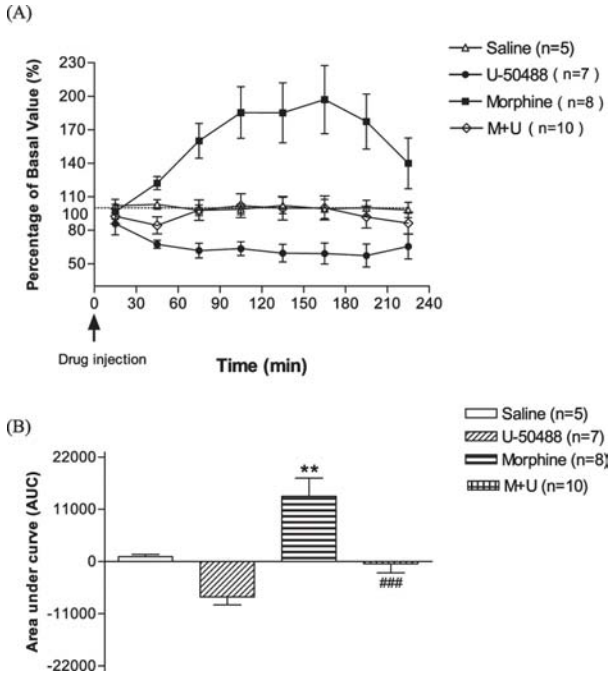

Fig. 5 (A) Time-dependent change of HVA level in the shell region of the nucleus accumbens after drug administration. The concentration of HVA was measured every 30 min and converted to a percentage of the basal value. (B) A bar graph presenting the area under curve (AUC) of the microdialysis curve of HVA from 0 to 195 min, as shown in (A). One-way ANOVA followed by post-hoc Newman-Keuls test was used to analyze the data. ** $P < 0.01$ when compared with the AUC of the saline group; $^{###}$ $P < 0.001$ when the M + U group was compared with the morphine alone group (n $\geqq$ 7)

M + U and morphine-treated groups ($P < 0.001$). The ambulatory activity displayed a similar pattern to the total activity (Fig. 3B). Thus, rats developed sensitization to the effect of the same doses of morphine (10 mg/kg) on locomotor activity. Coadministration of U-50488 effectively suppressed this behavioral sensitization induced by morphine.

Effect of Morphine or Morphine plus U-50488 on Dopamine Neuronal Activity in the Shell Region of NAc

A microdialysis probe was put into the shell region of the NAc to collect the extracellular fluid. The metabolites of dopamine (DOPAC and HVA) were determined by HPLC and assumed to represent the activity of dopaminergic neurons. As shown in Figs 4 and 5, saline by itself did not increase or decrease the extracellular levels of DOPAC or HVA at 195 min. U-50488 had a trend to decrease the extracellular levels of DOPAC and HVA although this was not statistically significant. Morphine treatment increased the extracellular level of DOPAC significantly (maximum increase 132.7±13.6% of basal value at 165 min) and HVA (maximum increase 197.1±30.5% of basal value at 165 min). Coadministration of U-50488 completely reversed the effect of morphine.

DISCUSSION

We have reported previously that a low dose of U-50488 could prevent the development of morphine tolerance to antinociception in guinea pigs[3]. The mechanisms may involve decreases in glutamate and nitric oxide (NO) levels stimulated by chronic morphine treatment[18]. Opioid κ-receptor agonists could offer possible remedial pharmacotherapy for drug dependence, including addiction to amphetamine, methamphetamine and alcohol[19]. These reports all pointed to a possible inhibitory effect of κ-opioids on the mesocorticolimbic system. In 1992, Spanagel et al. reported that the increase in the extracellular level of dopamine produced by morphine is attenuated by the microinjection of κ-opioid agonist into the NAc, but not into the VTA[20]. Therefore, unlike μ-opioid receptors at the VTA, the κ-opioid receptors are thought to be located at the NAc, where they exert direct inhibition on dopaminergic neurotransmission[21]. However, several reports also indicated the actions of κ-opioids at the VTA[22,23]. VTA neurons have been classified as principle, secondary, or tertiary according to their electrophysiological and pharmacological properties[24]. Both principle and tertiary GABAergic neurons can be hyperpolarized directly by μ-opioid agonists. However, κ-opioid agonists inhibit a subset of principle and tertiary neurons postsynaptically: an effect limited to dopaminergic neurons of each class[21]. The difference between the actions of μ- and κ-opioids-GABA disinhibition and direct dopamine inhibition-at the VTA could be one of the mechanisms by which κ-agonists block morphine-induced rewarding effects. Using the selective κ-opioid agonist U50488H, the effects of κ-opioids on morphine-induced rewarding effects have been investigated widely. In CPP tests, U50488H, at a dose that alone did not produce place aversion, suppressed both place preference and the levels of dopamine metabolites in the rat limbic forebrain produced by morphine[25,26]. In experiments using self-administration, treatment with U50488H decreased the intake of morphine in a dose-dependent manner when offered in doses that readily initiated and sustained self-administration behavior in mice[4].

To further investigate the effect of U-50488 on the mesolimbic system, we analyzed the concentrations of the

dopamine metabolites, DOPAC and HVA, in the NAc following drug or saline administration. It has been reported that the NAc shell is highly correlated with rewarding and responds sensitively to stimulation at the VTA, whereas the NAc core shows less relevance[27]. We were unable to detect the concentration of dopamine consistently, which was often below the detection limit of our system. Although several recent reports have described the determination of extracellular dopamine level in the NAc, the dialysis windows of the microdialysis probe that they used were larger and with a membrane length of 2 to 2.5 mm[28-30]. In our system, the low level of dopamine measured was possibly because of the small dialysis window (1 mm) used and the fast oxidation of dopamine in the microdialysis system. As described in our previous study, the levels of dopamine metabolites (DOPAC and HVA) could also be positively associated with the activity of dopaminergic neurons[6]. Thus, using microdialysis in the shell region of the NAc, we observed that DOPAC and HVA levels both increased with time following morphine administration. These increases were blocked completely by coadministration of a low dose of U-50488 (8 mg/kg). U-50488 alone at this dose did not cause statistically significant changes in the levels of DOPAC and HVA. Nevertheless, a tendency to decrease the level of DOPAC and HVA could be observed, as shown in Figs 4 and 5, although these were not statistically significant. Therefore, it is also possible that there was a simple additive effect of a reduction caused by U-50488, plus an elevation caused by morphine, resulting in no effect for the combination. These neurochemical results are consistent with the behavioral findings in CPP and locomotor activity testing, showing that the inhibitory effect of U-50488 on morphine-induced rewarding and behavioral sensitization can be highly linked with its impact on the dopaminergic pathway of the mesolimbic system.

In summary, we demonstrated here that U-50488 coadministration could effectively block the rewarding effect and behavioral sensitization induced by morphine in rats. This was probably via the inhibition of U-50488 on the dopaminergic neuroactivity of mesolimbic pathways. Thus, low doses of U-50488 may have a potential for the development of new therapies to prevent morphine addiction during chronic pain control.

ACKNOWLEDGMENTS

This study was supported by grants from the National Health Research Institute (NHRI-EX-94-9401NP), Taipei, Taiwan, R.O.C.

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