発する要因は，①摂取していた薬物と類似した作用を有する薬物の使用，②薬物関連刺激，ならびに③ストレス暴露の3種類と考えられている．また，薬物探索行動誘発試験法として，3つの方法がある．ヒトでの渇望再燃過程をよく反映した方法として，between-session法がある．この方法は，薬物自己投与実験，消去実験ならびに薬物探索行動の誘発試験の順にそれぞれ異なる日に行うものである．しかしながら，本実験法は試験の繰り返しにより次第にレバー押し反応が減弱する傾向があり，探索行動誘発試験として繰り返し使用が出来ない点に難点がある．この点を改善したのが，within-session法である．この方法では，薬物自己投与行動を獲得させた後に，1週間に2～3回の割合で薬物自己投与実験，消去実験に続いて薬物探索行動の誘発試験を1日で行う．この方法では繰り返しの使用が可能となるが，体内に薬物が残存した状態で薬物探索行動の試験を行う点で臨床との相関性が得られ難い．さらに近年用いられるようになった方法がbetween-within-session法である．薬物自己投与行動の獲得後の，特定の退薬時に消去実験と薬物探索行動の誘発試験を同日で行う方法である．この方法は，消去実験に日数を要さない事から退薬期間と薬物探索行動発現の関係を調べる場合に有用な方法として用いられている．

① 薬物探索行動の誘発因子

薬物プライミング投与；摂取していた薬物と類似した作用を有する薬物の使用によって，ヒトでは容易に渇望が再燃する．動物実験においては，同一または類似した作用を持つ薬物の少量投与（薬物プライミング投与）によって薬物探索行動（レバー押し行動）が誘発される．薬物プライミング投与による薬物探索行動の発現は，これまで薬物弁別刺激効果や薬物報酬効果を介して起こると考えられてきた．しかしながら，コカイン弁別刺激はドパミンD_1受容体作動薬に般化するが，コカイン探索行動はドパミンD_1受容体作動薬によって誘発されない[8]．また，モルヒネの腹側被蓋野内微量注入により，ヘロイン探索行動が誘発されるが，モルヒネはヘロイン弁別刺激には般化しない[9]．このように，薬物弁別特性が同じでも，薬物探索行動を必ずしも誘発する訳ではなく，またその逆も同様である事が分かる．また，コカインプライミング投与によるコカイン探索行動はカンナビノイドCB_1受容体拮抗薬によって抑制されるが，コカイン自己投与行動は抑制されない[10]．このように，薬物プライミング投与による薬物探索行動の発現機構は，薬物摂取行動の発現機構と必ずしも同一ではない事が示唆される．

薬物関連刺激（cue）；薬物関連刺激としては，discrete cue，discriminative cueならびにcontextual cueの3種類が用いられている．薬物自己投与行動獲得過程において薬物注入と同時に特定の音／光の関連刺激を与える．薬物が得られない消去過程では，関連刺激も呈示されない．この状況下で再び薬物関連刺激として音／光を与えると薬物探索行動が誘発される[11]．この実験系で用いる薬物関連刺激をdiscrete cueと呼ぶ．一方，薬物探索行動が特定の関連刺激にのみ対応する事をより鮮明にするのがdiscriminative cueである．薬物自己投与行動の獲得過程においてレバー押し反応が薬物獲得に繋がる関連刺激（S^+）ならびにレバー押し反応が生理食塩液注入に繋がる関連刺激（S^-）との2つの刺激を与える．このような条件下では，薬物探索行動はS^+刺激呈示により発現し，S^-刺激呈示では発現しない[12]．薬物摂取環境そのものをcueとするのが，contextual cueである．薬物自己投与行動獲得過程ではdiscrete cueと同様のスケジュールで実験するが，消去過程は薬物摂取時と全く異なる実験装置を用いて行う．その後，薬物摂取時と同一の装置（薬物関連刺激）に入れて薬物探索行動の誘発試験を行うと，同

一装置への暴露により薬物探索行動が誘発される[13]．この実験から，薬物探索行動の発現には薬物摂取環境が重要な因子となる事が分かる．

ストレス；ストレスがもたらす不安や絶望感を緩和する手段として，飲酒や薬物乱用に手を出すケースがある．また，震災などの非日常的なストレス体験は，アルコール・薬物乱用を含む精神疾患の誘発・再発に関わる事も知られている．このような状況が繰り返されると，ストレス遭遇に限らず，ストレスが予測される事態だけでも薬物探索・薬物摂取行動が誘発されるようになる．動物を用いた薬物自己投与実験法においても，ストレスによって薬物探索行動が誘発される事が知られている．ストレスの中で最も一般的に頻用されているのはfoot shockストレスである．foot shockストレスを退薬時に与える事で著明な薬物探索行動が誘発される．Shalevらは，薬物自己投与実験装置内でfoot shockストレスを暴露した場合でのみ薬物探索行動が惹起される事を報告し，ストレスを暴露する環境が重要であると結論付けている[14]．さらに，ストレス誘発性のヘロイン探索行動は，退薬後すぐに現れず，経日的に増強され12日目にピークに達する事も明らかにされている．一方，ヨヒンビンはノルアドレナリン（NA）遊離を促進し，不安様症状を起こす事が知られている．このヨヒンビンは薬物性ストレッサーとして用いられ，ヘロインおよびコカイン探索行動を誘発する事が分かっている[15]．一方，コルチコステロン／コルチゾールの遊離を調節する副腎皮質刺激ホルモン放出因子（corticotropin releasing factor; CRF）は，視床下部－下垂体－副腎系（hypothalamic-pituitary-adrenal system; HPA系）の重要な構成要因である．近年，我々はこのCRFが覚せい剤メタンフェタミン（methamphetamine; MAP）探索行動を誘発する事を明らかにした．このCRF誘発性MAP探索行動は，コルチコステロン合成酵素阻害薬メチラポンによって拮抗されない事から，HPA系を介さないCRF自身の作用である可能性が示唆される．

一方，ストレスは薬物探索行動のみならず，薬物自己投与行動にも影響を与える．食餌制限ストレス[16]，敗北／恐怖ストレス[17]，foot shockストレス[18]，社会的隔離ストレス[19]，胎児期ストレス[20]ならびにtail pinchストレス[21]は，薬物自己投与行動の獲得を促進させ，また薬物の摂取量を増加させる事が知られている．逆に，コルチコステロンの分泌の低下または分泌不能によって，コカイン自己投与行動は抑制される[22]．さらに，コルチコステロン分泌機能不全動物を用いた実験では，オピオイドまたは中枢興奮薬による側坐核内ドパミン遊離量が減少する事が報告されている[23]．これらの知見から考えると，コルチコステロン分泌低下によるコカイン自己投与行動の抑制作用は，側坐核内ドパミン遊離抑制に基づく報酬効果の減弱に基因するのかもしれない．

② 薬物探索行動の脳内責任部位

表2では，MAP探索行動の発現の脳内責任部位に関する我々の実験結果を示した．この実験では，MAPプライミング投与ならびに薬物関連刺激によって誘発されるMAP探索行動に対するリドカインの脳実質内微量注入による影響を調べている[11]．前頭前皮質，側坐核ならびに海馬へのリドカイン注入により，MAP探索行動の発現はいずれも抑制され，脳内責任部位である事が分かる．扁桃体は，薬物関連刺激によるMAP探索行動の発現に重要な役割を演じているが，薬物プライミング投与によるMAP探索行動の発現には関与していない．一方，脳内のNA神経系は，起始核である青斑核より腹側神経束を経由し，扁桃体，中隔ならびに分界上床核へと投射しており，ストレス反応に関与する事が知られている．ストレス誘発性の薬物探索

表2 リドカイン脳内局所注入法によるMAP探索行動発現の抑制

Initiating Factor	Prefrontal Cortex	Nucleus Accumbens	Amygdala	Hippocampus
MAP priming	+	+	−	+
MAP associated cue	+	+	+	+

+ : inhibition
− : no effect

行動は，このNA神経投射部位に焦点を当てその脳内責任部位の追究がなされている．foot shockにより誘発されるヘロイン探索行動は，腹側神経束，扁桃体ならびに分界上床核への6-OHDAまたはテトロドトキシン注入により抑制される[24]．中隔への注入では，逆にヘロイン探索行動が誘発される[25]．また，コカイン探索行動における前頭前皮質の重要性も指摘されている[26]．

おわりに

本稿では，薬物自己投与実験法を用いての薬物探索行動の発現に焦点を当て，3つの誘因を中心に概説した．近年では，陽電子放射断層撮影法（Positron Emission Tomography; PET）や機能的磁気共鳴映像法（functional magnetic resonance imaging; fMRI）を用いたヒトでの薬物への渇望再燃に関わる脳部位の同定も試みられ[27,28]，行動薬理学的アプローチで得られた薬物探索行動の発現の脳内責任部位との融合／再構築が今後の課題である．

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分担研究報告書

乱用薬物による依存形成における視床下部神経ペプチドの役割

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[研究要旨]

本研究では、視床下部神経ペプチドである orexin ならびに leptin に焦点を当て、これら視床下部ペプチドが脳内報酬系に及ぼす影響について分子生物学的ならびに機能解剖学的な検討を行った。まず、逆行性輸送マーカーである fluoro-gold (FG) を側坐核領域に微量注入し、腹側被蓋野において dopamine 生合成の律速段階酵素である tyrosine-hydroxylase (TH) ならびに orexin 1 受容体の特異的抗体を用いて免疫染色を行った。その結果、FG 陽性反応を有する TH 陽性細胞上に orexin 1 受容体の発現が認められた。次に、腹側被蓋野-側坐核 dopamine 神経が orexin-A により活性化されるか否かを検討する目的で、腹側被蓋野に orexin-A を微量注入し、側坐核における dopamine 遊離量の変化を *in vivo* microdialysis 法に従い検討した。その結果、腹側被蓋野に orexin-A を微量注入したところ側坐核において dopamine ならびにその代謝物である DOPAC、HVA 遊離量の有意な増加が認められた。さらに、腹側被蓋野に leptin を微量注入し、側坐核における dopamine 遊離量の変化について同様に検討を行ったところ、側坐核において dopamine 遊離量の増加傾向が認められた。

以上、本研究の結果より orexin は腹側被蓋野において dopamine 神経上に存在する orexin 受容体を介して腹側被蓋野-側坐核 dopamine 神経を活性化させる可能性が示唆された。また、leptin は腹側被蓋野に存在する leptin 受容体を介して腹側被蓋野-側坐核 dopamine 神経を活性化させる可能性が示唆された。

A. 研究目的

オーファンレセプターの内因性リガンドとして同定された orexin は、前駆体タンパク質である prepro-orexin から生成され、7 回膜貫通型 G タンパク共役型受容体に結合し、摂食や覚醒などの生理作用を示す神経ペプチドとして知られている。また、leptin は主として脂肪組織より循環血液中に分泌される16 kDa のタンパク質であり，摂食量抑制・体重増加抑制作用により生体のエネルギー代謝調節や神経内分泌調節に関与することが知られている。Leptin はトランスポーターを介して脳内に移行し、主に視床下部において leptin receptor (Ob-R) に結合することによりその作用を発現すると考えられている。一方、dopamine は依存性薬物による精神依存形成のみならず情動反応や認知、さらには摂食などの多くの中枢神経機能を制御する重要な神経伝達物質

として知られているが、orexin ならびに leptin の中脳辺縁 dopamine 神経系に及ぼす影響についての報告はほとんどなされていないのが現状である。そこで本研究では、視床下部神経ペプチドである orexin ならびに leptin に焦点を当て、これら視床下部ペプチドが脳内報酬系に及ぼす影響について分子生物学的ならびに機能解剖学的な検討を行った。

B. 研究方法

実験には SD 系雄性ラットならびに C57BL/6J 系雄性マウス、ob/ob マウス、db/db マウスを使用した。*In vivo* microdialysis 法は、ペントバルビタール麻酔下、動物を動物脳定位固定装置に固定し、ステレオ用ガイドを用いてガイドカニューレを目的とする脳部位に植え込み、固定した後、ダミーカニューレを挿入した。カニュレーション 3-5 日後、orexin (1nmol/0.3μL) または leptin (2μg/0.3μL) を腹側被蓋野領域に微量注入し、HPLC-ECD システムを用いて側坐核領域における dopamine およびその代謝物の分離定量を行った。報酬効果は、conditioned place preference (CPP) 法に従い測定し、自発運動量は tilting cage 法に従って評価した。また、orexin 受容体ならびに leptin 受容体の発現分布は、RT-PCR 法および免疫組織染色法に従い確認した。さらに、脳神経回路の解析は、イソフルラン (3%, 吸入) 麻酔下において神経軸索輸送物質である fluoro-gold (FG) を側坐核に注入することにより行った。なお、本研究を遂行するにあたり、星薬科大学動物実験指針に従い、本学の動物実験委員会で承認を得たうえで、動物に対する倫理面を十分に考慮し、使用動物数を最小限にしてすべての実験を行った。

C. 研究結果および考察

まず初めに、腹側被蓋野を含む中脳領域における orexin 受容体ならびに leptin 受容体の発現分布を RT-PCR 法に従い検討した。その結果、腹側被蓋野被蓋野を含む中脳領域において orexin 1 receptor、orexin 2 receptor ならびに Ob-Ra、Ob-Rb の発現が認められた。また、腹側被蓋野における orexin 1 receptor の分布を確認する目的で、FG を側坐核領域に微量注入し、dopamine 生合成の律速段階酵素である tyrosine hydroxylase (TH) ならびに orexin 1 receptor の特異的抗体を用い、免疫染色を行った。その結果、腹側被蓋野領域において側坐核領域から逆行性に輸送されてきた FG の自家発光ならびに TH、orexin 1 receptor 陽性細胞がそれぞれ認められた。さらに、FG 陽性反応を有する TH 陽性細胞上に orexin 1 receptor の発現が認められた。以上の結果より、腹側被蓋野から側坐核に投射する dopamine 神経上に orexin 1 receptor が発現していることが明らかとなった。そこで次に、腹側被蓋野から側坐核に投射している dopamine 神経が orexin A により活性化されるか否かを *in vivo* microdialysis 法に従い検討した。その結果、腹側被蓋野に orexin A を微量注入したところ、側坐核において dopamine ならびにその代謝物の遊離量の有意な増加が認められた。さらに、腹側被蓋野に leptin を微量注入し同様に検討したところ、側坐核において dopamine ならびにその代謝物の遊離量の増加が認められた。以上の結果より、腹側被蓋野から側坐核に投射している dopamine 神経が orexin A ならびに leptin により活性化される可能性が示唆された。また、morphine 誘発自発運動量促進作用に対する leptin の影響を leptin 欠損マウスである ob/ob マウスならびに leptin 受容体欠損マウスである db/db マウスを用いて検討した。その結果、wild type (WT) 群で認められた morphine 誘発自発運動量促進作用は、ob/ob マウスならびに db/db マウスにおいて有意に抑制された。以上の結果より、morphine 誘発自発運動量促進作用に

対して脳内 leptin は促進的な調節を行っている可能性が示唆された。

D. 結論

本研究の結果より、脳内 orexin ならびに leptin は腹側被蓋野領域に存在する orexin ならびに leptin 受容体を介して直接的かつ促進的に dopamine 神経系の活性を調節し、その投射先である側坐核領域において dopamine の遊離を促すことで、自発運動量などの dopamine 関連行動の発現を一部調節している可能性が示唆された。

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薬物依存の研究 (第 434 報): 脳内報酬系における腹側被蓋野-帯状回 dopamine 神経系の役割・松島勇紀、成田　年、成田道子、新倉慶一、朝戸めぐみ、倉橋香菜、高木茂実、葛巻直子、鈴木　勉・第 18 回日本臨床精神神経薬理学会・第 38 回日本神経精神薬理学会合同年会・品川・2008 年 10 月 1-3 日

G. 知的財産権の出願・登録状況（予定も含む）

1．特許取得
なし

2．実用新案登録
なし

3．その他
なし

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Behavioural Pharmacology

Suppression of dopamine-related side effects of morphine by aripiprazole, a dopamine system stabilizer

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ABSTRACT

Dopamine receptor antagonists are commonly used to counter the adverse effects of opioids such as hallucinations, delusions and emesis. However, most of these agents themselves have side effects, including extrapyramidal symptoms. Here, we investigated the effect of the dopamine system stabilizer aripiprazole on morphine-induced dopamine-related actions in mice. Morphine-induced hyperlocomotion and reward were significantly suppressed by either the dopamine receptor antagonist prochlorperazine or aripiprazole. Catalepsy was observed with a high dose of prochlorperazine, but not with an even higher dose of aripiprazole. The increased level of dialysate dopamine in the nucleus accumbens stimulated by morphine was significantly decreased by pretreatment with aripiprazole. These results suggest that the co-administration of aripiprazole may be useful for reducing the severity of morphine-induced dopamine-related side effects.

1. Introduction

According to the World Health Organization (WHO) guidelines, morphine is considered the "gold standard" for the treatment of patients with moderate to severe pain due to cancer. However, the use of morphine for the treatment of cancer pain is sometimes accompanied by side effects, such as emesis, constipation, drowsiness and delirium (Aparasu et al., 1999; McNicol et al., 2003).

In the management of cancer pain, dopamine receptor antagonists such as haloperidol are used to protect against opioid-induced delirium (Ross and Alexander, 2001). In addition to delirium, dopamine receptor antagonists such as prochlorperazine have been considered by most clinicians to be the drug of choice to combat opioid-induced nausea and vomiting (Aparasu et al., 1999; McNicol et al., 2003).

These dopamine receptor antagonists are frequently associated with adverse effects, including extrapyramidal symptoms (Casey, 1995; Tonini et al., 2004). Therefore, new strategies for the prevention of opioid-induced delirium and emesis are required, along with a working knowledge of the proposed mechanism of drug action.

Aripiprazole is a novel dopamine D_2 receptor partial agonist that has a different pharmacological profile than currently marketed typical and atypical antipsychotics (Winans, 2003; Naber and Lambert, 2004). As a result, aripiprazole seems to provide a way to fine-tune the treatment of psychiatric disorders by maximizing the treatment effect while minimizing undesirable adverse events (Ohlsen and Pilowsky, 2005). Against this background, the present study was undertaken to evaluate whether aripiprazole could affect the severity of morphine-induced dopamine-related actions with fewer side effects.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at 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 animals used in the following experiments.

2.1. Animals

Male ICR mice (20–25 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) were used in the present study. Animals were housed in a room maintained at 22±1 °C with a 12 h light–dark cycle.

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Food and water were available ad libitum. Each animal was used only once.

2.2. Locomotor activity

The locomotor activity of mice was measured by an ambulometer as described previously (Narita et al., 1993). Briefly, a mouse was placed in a tilting-type round activity cage 20 cm in diameter and 19 cm high. Any slight tilt of the activity cage, which was caused by horizontal movement of the mouse, was detected by three microswitches. Total activity counts in each 10-min segment were automatically recorded for 180 min following the administration of saline (10 ml/kg, s.c.) or morphine (10 mg/kg, s.c.). Aripiprazole (3, 10, 20 mg/kg) or prochlorperazine (0.1, 0.3, 1 mg/kg) was co-administered with morphine s.c. 30 min prior to morphine treatment.

2.3. Place conditioning

Place-conditioning studies were conducted using a shuttle box (15×30×15 cm: $w \times l \times h$) that was made of an acrylic resin board and divided into two equal-sized compartments. One compartment was white with a textured floor and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test). The pre-conditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co. Ltd, Tokyo, Japan). Conditioning sessions (three for morphine: three for saline) were started the day after the pre-conditioning test and conducted once daily for 6 days. Groups of mice were pretreated with aripiprazole (10 or 20 mg/kg, s.c.), prochlorperazine (0.1or 0.3 mg/kg, s.c.) or their vehicle (saline or 5% dimethyl sulfoxide (DMSO), respectively) at 30 min before morphine or saline injection. Immediately after s.c.-injection of morphine at 5 mg/kg, these animals were placed in the compartment opposite that in which they had spent the most time in the pre-conditioning test for 1 h. On alternate days, these animals were treated with saline after the pretreatment with aripiprazole, prochlorperazine or their vehicle and placed in the other compartment for 1 h. On the day after the final conditioning session, a post-conditioning test that was identical to the pre-conditioning test was performed.

2.4. Horizontal bar test

To minimize the effects of arousal and stress, mice were handled gently and exposed to the testing site several times before measurements. Catalepsy was evaluated by placing the animal with both forelegs over a horizontal bar elevated 5 cm from the floor. The time (s) for which the mouse maintained this position was recorded for up to 60 s. Catalepsy was considered to be finished when the forepaw touched the floor or when the mouse climbed on the bar. A score was assigned to each test based on the duration of the cataleptic posture (score 1, between 15 and 29 s; score 2, between 30 and 59 s; score 3, 60 s or more).

2.5. In vivo microdialysis study and quantification of dopamine release

Stereotaxic surgery was performed under sodium pentobarbital (70 mg/kg, intraperitoneal injection) anesthesia. Mice were placed in a stereotaxic apparatus and the skull was exposed. A small hole was then made using a dental drill. A microdialysis probe (D-I-6-01; 1 mm membrane length; Eicom) was implanted into the nucleus accumbens (from bregma: anterior, +1.5 mm; lateral, −0.9 mm; ventral, −4.9 mm) according to the atlas of Paxinos and Franklin (Paxinos and Franklin, 1997). The microdialysis probe was fixed to the skull with cranioplastic cement. At 24 h after implantation, mice were placed in experimental cages (30 cm wide×30 cm long×30 cm high). The probe was perfused continuously at a flow rate of 2 ml/min with aCSF containing 0.9 mM $MgCl_2$, 147.0 mM NaCl, 4.0 mM KCl, and 1.2 mM $CaCl_2$. Outflow fractions were taken every 20 min. After three baseline fractions were collected, mice were given morphine (10 mg/kg, s.c.) or saline (1 ml/kg, s.c.). Aripiprazole (20 mg/kg, s.c.) or vehicle (saline; 1 ml/kg, s.c.) was injected 30 min before treatment with morphine or saline. For this experiment, dialysis samples were collected for 180 min after treatment with morphine or saline. Dialysis fractions were then analyzed using HPLC (Eicom) with ECD (HTEC-500; Eicom). Dopamine was separated by a column with a mobile phase containing sodium acetate (3.76 g/l), citric acid monohydrate (6.74 g/l), sodium 1-decane sulfonate (170 mg/l), EDTA (2Na; 5 mg/l), and 22% methanol. The mobile phase was delivered at a flow rate of 300 ml/min. Dopamine was identified according to the retention times of a dopamine standard, and the amounts of dopamine were quantified based on calculations using the peak areas.

2.6. Drugs

The drugs used in the present study were morphine hydrochloride (Daiichi-Sankyo Co., Tokyo, Japan), aripiprazole (Toronto Research Chemicals Inc., Ontario, Canada) and prochlorperazine (Sigma-Aldrich Co., St. Louis, MO, USA). Prochlorperazine was dissolved in 5% DMSO containing physiological saline, and morphine hydrochloride and aripiprazole was dissolved in physiological saline.

2.7. Statistical analysis

All data are presented as the mean±S.E.M. The statistical significance of differences between groups was assessed with Student's *t*-test or two-way ANOVA, followed by the Bonferroni/Dunnett test.

3. Results

3.1. Suppression of morphine-induced hyperlocomotion by pretreatment with either aripiprazole or prochlorperazine

Treatment with morphine (10 mg/kg, s.c.) produced a locomotor-enhancing effect. Groups of mice were pretreated with aripiprazole (3–20 mg/kg, s.c.) or prochlorperazine (0.1–1 mg/kg, s.c.) 30 min before morphine (10 mg/kg) injection. Pretreatment with either aripiprazole or prochlorperazine caused a dose-dependent suppression of morphine-induced hyperlocomotion (***$P<0.001$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ or ###$P<0.001$ vs. saline- or vehicle-morphine) (Fig. 1A, B). At the dose of aripiprazole which dramatically reduced the increase in locomotion caused by morphine, no hyper- or hypo-locomotion was observed compared to the saline group.

3.2. Suppression of morphine-induced place preference by pretreatment with aripiprazole or prochlorperazine

We next investigated whether pretreatment with aripiprazole (10 or 20 mg/kg) or prochlorperazine (0.1 or 0.3 mg/kg) could affect the rewarding effect of morphine. Pretreatment with aripiprazole or prochlorperazine inhibited the morphine-induced place preference (*$P<0.05$ or **$P<0.01$ vs. saline- or vehicle-saline, #$P<0.05$ or ##$P<0.01$ vs. saline- or vehicle-morphine) (Fig. 1C, D).

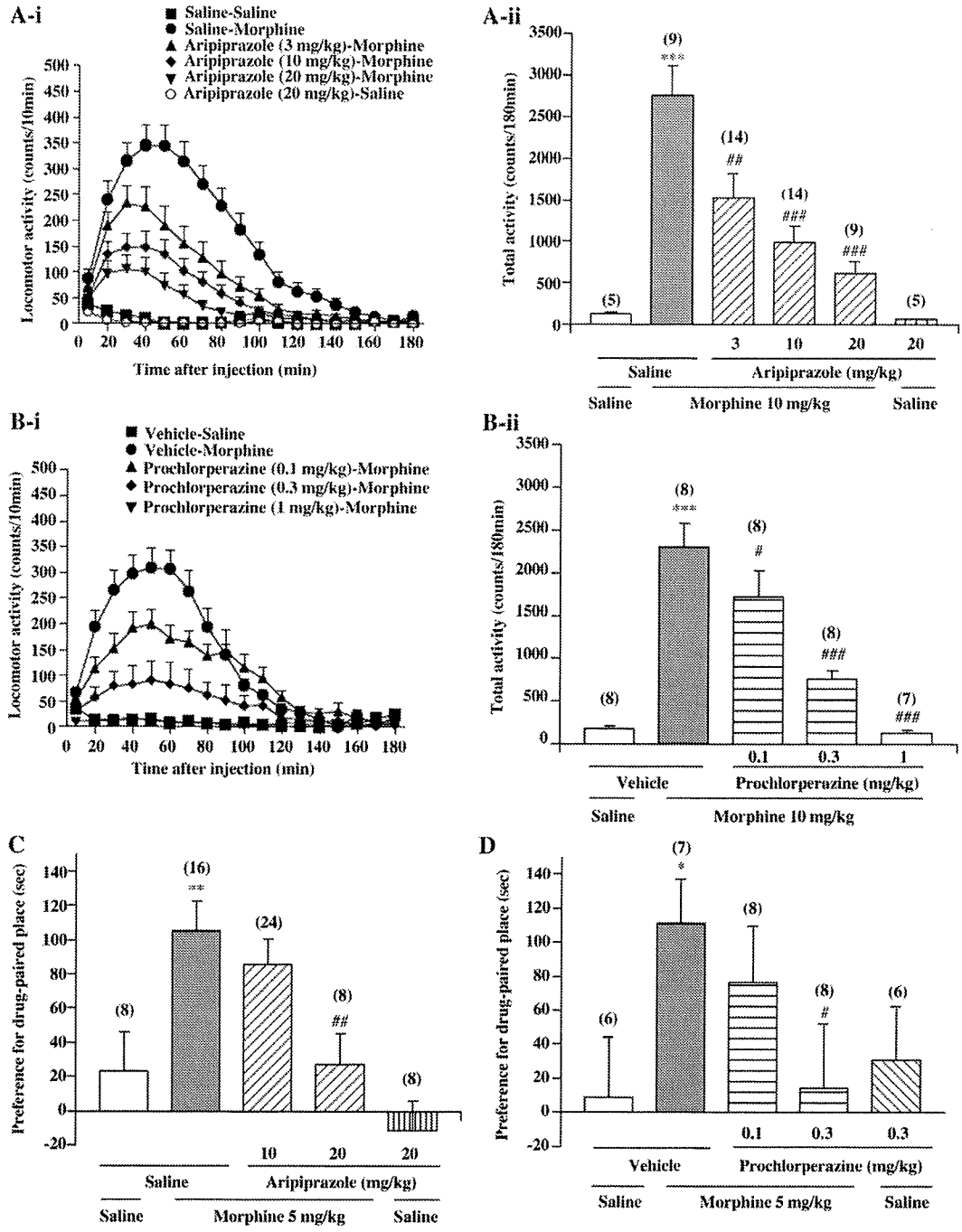

Fig. 1. Effects of aripiprazole or prochlorperazine on s.c. morphine-induced hyperlocomotion and conditioned place preference. (A) Groups of mice were pretreated with either aripiprazole (3–20 mg/kg, s.c.) or saline 30 min before morphine (10 mg/kg) injection. (B) Groups of mice were pretreated with prochlorperazine (0.1–1 mg/kg, s.c.) or vehicle (5% DMSO) 30 min before morphine (10 mg/kg) injection. Each column represents the mean total activity for 180 min with S.E.M. ***$P<0.001$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ or ###$P<0.001$ vs. saline- or vehicle-morphine. (C) Groups of mice were pretreated with either aripiprazole (10–20 mg/kg, s.c.) or saline 30 min before morphine (5 mg/kg) injection. (D) Groups of mice were pretreated with prochlorperazine (0.1–0.3 mg/kg, s.c.) or vehicle (5% DMSO) 30 min before morphine (5 mg/kg) injection. Immediately after s.c. injection of morphine, mice were placed and conditioned in either compartment for 1 h. Ordinate: mean differences between time spent in post-conditioning test and pre-conditioning test. Each column represents the mean with S.E.M. *$P<0.05$, **$P<0.01$ vs. saline- or vehicle-saline, #$P<0.05$, ##$P<0.01$ vs. saline- or vehicle-morphine. Numbers in parentheses indicate the number of mice tested.

3.3. Effects of aripiprazole or prochlorperazine on catalepsy

Catalepsy values were obtained at 15, 30, 45 and 60 min after s.c. administration of saline, aripiprazole (3–40 mg/kg) or prochlorperazine (0.1–1 mg/kg). The catalepsy scores were not changed by a single s.c.-injection of aripiprazole (20 or 40 mg/kg), whereas a significantly high score was noted with prochlorperazine (1 mg/kg) (*$P<0.05$, **$P<0.01$ or ***$P<0.001$ vs. saline) (Fig. 2A, B).

3.4. Change in the increased dialysate dopamine level induced by morphine upon pretreatment with aripiprazole

In the microdialysis study, the dialysate dopamine levels in the mouse nucleus accumbens were markedly increased by s.c. injection of morphine at 10 mg/kg compared with that induced by saline ($F_{(1,66)}$= 15.47, $P<0.01$) (Fig. 2C). Under these conditions, the increased level of dialysate dopamine in the nucleus accumbens stimulated by morphine

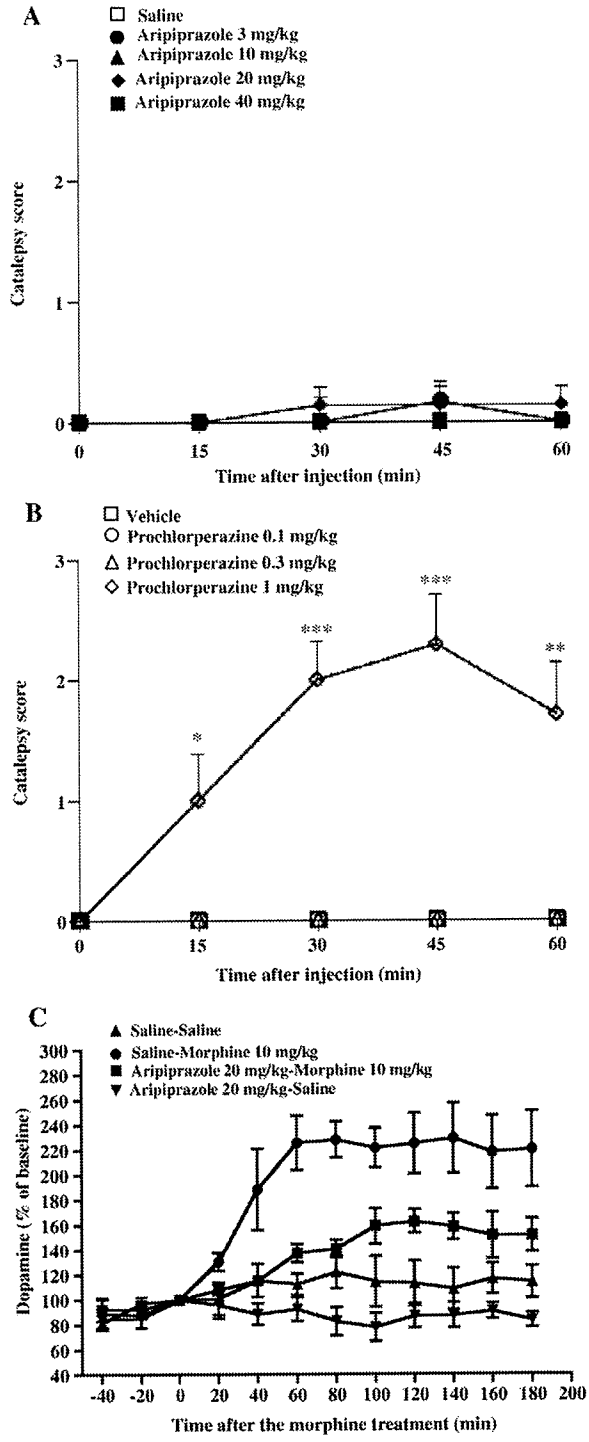

Fig. 2. Time-course of the catalepsy caused by aripiprazole (A) or prochlorperazine (B) in mice. Catalepsy values were obtained at 15, 30, 45 and 60 min after s.c. administration of saline, aripiprazole (3–40 mg/kg, s.c.), vehicle or prochlorperazine (0.1–1 mg/kg, s.c.), respectively. The horizontal bar test was performed by placing the forepaws of the mouse on a 5 cm-high bar. The time until the forepaw touched the floor or when the mouse climbed upon the bar was measured up to 60 s. A score was assigned to each test based on the duration of the cataleptic posture (score 1, between 15 and 29 s; score 2, between 30 and 59 s; score 3, 60 s or more). Each column represents the mean with S.E.M. of 6–7 mice. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. vehicle. (C) Effects of aripiprazole on the influence of s.c. morphine on the dialysate dopamine level in the nucleus accumbens. After baseline fractions were collected, mice were pretreated with either aripiprazole (20 mg/kg, s.c.) or saline 30 min before morphine (10 mg/kg) injection at time 0 to evoke the release of dopamine. Data are expressed as percentages of the corresponding baseline levels with S.E.M. for 4 mice. Saline–saline vs. saline–morphine 10 mg/kg, $F_{(1,66)}=15.47$, $P<0.01$; saline–morphine 10 mg/kg vs. aripiprazole 20 mg/kg-morphine 10 mg/kg, $F_{(1,66)}=9.836$, $p<0.05$.

was significantly decreased by pretreatment with aripiprazole at 20 mg/kg ($F_{(1,66)}=9.836$, $P<0.05$, saline-pretreated vs. aripiprazole-pretreated) (Fig. 2C). By itself, aripiprazole did not affect dopamine levels.

4. Discussion

Several clinical studies have suggested that undue anxiety about psychological dependence on opioids in cancer patients has caused

physicians and patients to use inadequate doses of opioids (WHO, 1996). It is widely accepted that the enhanced dopamine neuronal activity is a key factor in the development of psychological dependence on opioids (Narita et al., 2001).

Delirium is an organic psychiatric syndrome characterized by fluctuating consciousness and impaired perception, cognition, and behavior (Sipahimalani and Masand, 1998; Trzepacz, 2000). Exposure to opioids is associated with an increased risk of delirium in hospitalized cancer patients (Gaudreau et al., 2007). An excess of dopamine, glutamate, and norepinephrine, diminution of cholinergic function, and disturbances in gamma-aminobutyric acid (GABA) and serotonergic (5-hydroxytryptamine, 5-HT) activity have been implicated in its pathogenesis (Trzepacz, 2000).

In the management of cancer pain, nausea and vomiting are some of the most distressing adverse effects induced by opioids. Opioids induce emesis through various mechanisms: i.e., via stimulation of the chemoreceptor trigger zone in the brainstem and through enhanced vestibular sensitivity (Costello and Borison, 1977; Rubin and Winston, 1950). Although several agents that act on receptors in the chemoreceptor trigger zone are available, it has been determined that dopamine transmission is mainly responsible for opioid-induced nausea and vomiting.

Conventional antipsychotics that mainly act as dopamine D_2 receptor antagonists have been considered by most clinicians to be drugs of first choice for protecting against opioid-induced dopamine-related symptoms (Aparasu et al., 1999; Ross and Alexander, 2001; McNicol et al., 2003). However, most of these dopamine receptor antagonists are frequently associated with extrapyramidal symptoms (Tonini et al., 2004).

Aripiprazole is the first dopamine system stabilizer to mainly act as a dopamine D_2 receptor partial agonist, which is active against both positive and negative symptoms of schizophrenia. Uniquely, aripiprazole has a low propensity for extrapyramidal side effects, causes minimal weight gain or sedation, and produces no elevation in serum prolactin levels or prolongation of the QTc interval on electrocardiogram (Naber and Lambert, 2004). It has been clearly shown that aripiprazole has potent partial agonist activity at dopamine D_2 and 5-HT_{1A} receptors and antagonist activity at 5-HT_{2A} receptors (Li et al., 2004). In the present study, we first evaluated whether aripiprazole could affect the severity of morphine-induced dopamine-related actions.

It has been widely accepted that central dopaminergic systems contribute to hyperlocomotion induced by morphine in mice (Narita et al., 1993). As with the standard central dopamine D_2 receptor antagonist prochlorperazine, which has been widely administered as an anti-emetic drug for use with opioids, pretreatment with aripiprazole caused a dose-dependent suppression of morphine-induced hyperlocomotion. Similarly, we found that aripiprazole inhibited the morphine-induced place preference. Various studies have pointed out that the mesolimbic dopamine system is a critical pathway for the initiation of opioid-induced reinforcement (Funada and Shippenberg, 1996; Koob et al., 1998; Narita et al., 2001). In our biochemical experiments, aripiprazole failed to displace [tylosil-3,5-(3)H(N)]-[D-Ala(2), N-MePhe(4),Gly-ol(5)]enkephalin ([^{3}H]DAMGO) binding, which is a selective μ-opioid receptor ligand, in the mouse brain membrane, whereas it partially inhibited the guanosine 5′-o-(3-thio) triphosphate ([^{35}S]GTPγS) binding by dopamine but not morphine in the membrane of the mouse limbic forebrain including the nucleus accumbens, which is a terminus for the mesolimbic dopaminergic pathway (Narita et al., personal communication). In the present study, we found that pretreatment with aripiprazole caused a significant suppression of morphine-induced dopamine release in the nucleus accumbens. These findings suggest that a partial agonistic effect of aripiprazole on central dopamine D_2 receptors may suppress hyperlocomotion and the rewarding effects of morphine through both blockade of the firing of mesolimbic dopaminergic neurons in the ventral tegmental area and the competitive blockade of dopamine D_2 receptors in the nucleus accumbens.

In the present study, catalepsy was not observed after a single s.c. injection of aripiprazole at high doses, whereas it was produced by high doses of prochlorperazine. In a previous study, we demonstrated that aripiprazole at doses lower than those used here significantly suppressed morphine-induced retching or vomiting in ferrets (Shiokawa et al., 2007). Furthermore, aripiprazole did not appear to have any effect on morphine-induced antinociception in mice (Narita et al., personal communication). Taken together, these findings suggest that aripiprazole may have a low propensity for extrapyramidal side effects and may inhibit some of the distressing adverse effects of opioids.

In conclusion, we demonstrated here that morphine-induced hyperlocomotion, reward and dopamine release were significantly suppressed by pretreatment with the novel dopamine system stabilizer aripiprazole, whereas catalepsy was not produced by aripiprazole itself. Although more biochemical and clinical studies are required, we propose that the combination of aripiprazole with opioids may pave the way for a new strategy for controlling pain and suppressing the dopamine-related adverse effects of opioids.

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Comparative Pharmacological Profiles of Morphine and Oxycodone under a Neuropathic Pain-Like State in Mice: Evidence for Less Sensitivity to Morphine

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The present study was undertaken to investigate pharmacological actions induced by morphine and oxycodone under a neuropathic pain-like state. In the μ-opioid receptor (MOR) binding study and G-protein activation, we confirmed that both morphine and oxycodone showed MOR agonistic activities. Mice with sciatic nerve ligation exhibited the marked neuropathic pain-like behavior. Under these conditions, antinociception induced by subcutaneously (s.c.) injected morphine was significantly decreased by sciatic nerve ligation, whereas s.c. injection of oxycodone produced a profound antinociception in sciatic nerve-ligated mice. There were no significant differences in spinal or supraspinal antinociception of morphine and oxycodone between sham operation and nerve ligation. Moreover, either morphine- or oxycodone-induced increase in guanosine-5′-*o*-(3-thio) triphosphate ([^{35}S]GTPγS) binding in the spinal cord, periaqueductal gray matter and thalamus in sciatic nerve-ligated mice was similar to that in sham-operated mice. Antinociception induced by s.c., intrathecal, or intracerebroventricular injection of the morphine metabolite morphine-6-glucuronide (M-6-G) was significantly decreased by sciatic nerve ligation. Furthermore, the increase in the G-protein activation induced by M-6-G was eliminated in sciatic nerve ligation. In addition, either morphine- or oxycodone-induced rewarding effect was dramatically suppressed under a neuropathic pain-like state. The increased [^{35}S]GTPγS binding by morphine or oxycodone was significantly lower in the lower midbrain of mice with sciatic nerve ligation compared with that in control mice. These findings provide further evidence that oxycodone shows a profound antinociceptive effect under a neuropathic pain-like state with less of a rewarding effect. Furthermore, the reduction in G-protein activation induced by M-6-G may, at least in part, contribute to the suppression of the antinociceptive effect produced by morphine under a neuropathic pain-like state.

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INTRODUCTION

Most of the opioids used clinically have been classified as μ-opioid receptor (MOR) agonists, including morphine and fentanyl. However, recent studies have shown that these MOR agonists and metabolites have interesting pharmacological differences (Peckham and Tratnor, 2006; Lemberg *et al*, 2006a, b). Oxycodone has been in clinical use since 1917. Oxycodone, which is a semisynthetic opioid analgesic derived from the naturally occurring alkaloid, thebain, has good oral bioavailability and seems to provide analgesic action that is as potent as that of morphine. It has been demonstrated that both oxycodone and its active metabolite showed MOR agonistic activities (Lemberg *et al*, 2006a). Several clinical studies have suggested that oxycodone may be useful for the treatment of neuropathic pain (Watson and Babul, 1998; Satthl *et al*, 2006). Although oxycodone has been clinically used for many years, its pharmacological properties are still very poorly characterized.

A growing body of clinical evidence suggests that when opioid analgesics including morphine are used to control pain in patients, psychological dependence is not a major concern. We previously reported that morphine failed to induce rewarding effects in rats that had been injected with formalin or carrageenan into the hind paw (Suzuki *et al*, 1996, 1999, 2001; Narita *et al*, 2005a). Furthermore, it has been documented that chronic pain attenuates the development of tolerance to the antinociceptive effect of morphine in rats (Vaccarino *et al*, 1993). These findings suggest the possibility that pain could lead to physiological

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changes at supraspinal levels associated with the suppression of opioid dependence.

Neuropathic pain can elicit abnormal pain characterized in part by hyperalgesia, so that noxious stimuli are perceived as more painful and allodynia. Neuropathic pain is particularly difficult to treat clinically, as it is only partially relieved by high doses of morphine. Many studies have focused on the long-term changes in the functions of the spinal cord dorsal horn neurons, which include some receptors, protein kinases, and peptides, following nerve injury (Nichols *et al*, 1995; Mayer *et al*, 1999; Narita *et al*, 2000). However, the mechanism of the reduced sensitivity to morphine-induced antinociceptive effect under a neuropathic pain is not fully understood.

The aim of the present study was to further compare pharmacological profiles of morphine and oxycodone following sciatic nerve ligation and investigate the mechanisms underlying less sensitivity to morphine under a neuropathic pain-like state.

MATERIALS AND METHODS

The present study was conducted in accordance with the 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 animals used in the following experiments.

Animals

Male ICR mice (20–25 g) and male guinea pig (250–300 g) (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) were used in the present study. Animals were housed in a room maintained at 22 ± 1°C with a 12 h light–dark cycle. Food and water were available *ad libitum*. Each animal was used only once.

Receptor Binding Assay

For membrane preparation, the mouse brain without cerebellum and the guinea pig cerebellum were quickly removed after decapitation, and rapidly transferred to a tube filled with an ice-cold buffer. The homogenate was centrifuged at 4°C for 10 min at 1000*g* and the surpernatant was centrifuged at 4°C for 20 min at 48 000*g*. The pellet was resuspended at 4°C for 20 min at 48 000*g*. The resulting pellet was resuspended and retained as membrane fraction. The μ-, δ-, or κ-opioid receptor (KOR) binding assays were performed in duplicate with [tylosil-3,5-(3)*H*(*N*)]-[D-Ala(2),*N*-MePhe(4),Gly-ol(5)]enkephalin ([^{3}H]DAMGO) (specific activity, 59.0 Ci/mmol; Amersham Biosciences, Arlington Heights, IL) at 1 nM, (2-D-penicillamine, 5-D-penicillamine)-enkephalin ([^{3}H]DPDPE) (specific activity, 45.0 Ci/mmol; PerkinElmer Life science, Arlington Heights, IL) at 2 nM, or [phenyl-3,4-(3)*H*]-(5α,7α,8β)-3,4-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide ([^{3}H]U69,593) (specific activity, 41.7 Ci/mmol; PerkinElmer Life science, Arlington Heights, IL) at 2 nM in a final volume of 1.0 ml that contained 50 mM Tris-HCl buffer, pH 7.4, and 0.1 ml of the membrane fraction. The amount of membrane proteins used in each assay was in the range of 90–140 μg, as determined by the method of Narita *et al* (2001a). The test tubes were incubated for 1 h at 25°C. Specific binding was defined as the difference in bindings observed in the absence and presence of 1 μM unlabeled DAMGO, DPDPE, or U50,488. Incubation was terminated by collecting membranes on Whatman GF/B filters using a Brandel cell harvester. The filters were then washed three times with 5 ml Tris-HCl buffer, pH 7.4, at 4°C and transferred to scintillation vials. Then, 4 ml of clear-sol 2 (Nacalaitesque Inc. Kyoto, Japan) was added to the vials. After a 12 h equilibration period, radioactivity in the samples was determined in a liquid scintillation analyzer.

Guanosine-5′-*o*-(3-thio) Triphosphate Binding Assay

For membrane preparation, the mouse spinal cord, thalamus, periaqueductal gray matter (PAG), a section of the lower midbrain that included the ventral tegmental area (VTA), which described previously (Ozaki *et al*, 2004), and the guinea pig cerebellum were quickly removed after decapitation, and rapidly transferred to a tube filled with ice-cold buffer. The membrane homogenate (3–8 μg protein/assay) was prepared as described previously (Narita *et al*, 2001b) and incubated at 25°C for 2 h in 1 ml of assay buffer with various concentrations of each agonist, 30 μM guanosine-5′-diphosphate (GDP) and 50 pM guanosine-5′-*o*-(3-thio) triphosphate ([^{35}S]GTPγS) (specific activity, 1000 Ci/mmol; Amersham, Arlington Heights, IL, USA). The reaction was terminated by filtration using Whatman GF/B glass filters (Brandel, Gaithersburg, MD, USA) that had been presoaked in 50 μM Tris-HCl, pH 7.4, and 5 μM $MgCl_2$ at 4°C for 2 h. The filters were washed three times with 5 ml of ice-cold Tris-HCl buffer, pH 7.4, and then transferred to scintillation-counting vials containing 0.5 ml of Soluene-350 (Packard Instrument Co., Meriden, CT, USA) and 4 ml of Hionic Fluor (Packard Instrument Co.) and equilibrated for 12 h. The radioactivity in the samples was determined with a liquid scintillation analyzer. Nonspecific binding was measured in the presence of 10 μM unlabeled GTPγS. In the present study, sample preparation was performed 7 days after partial sciatic nerve-ligation.

Neuropathic Pain Model

The mice were anesthetized with sodium pentobarbital (70 mg/kg, i.p.) or 3% isoflurene. We produced a partial sciatic nerve injury by tying a tight ligature with a 8–0 silk suture around approximately one-third to one-half the diameter of the sciatic nerve on the right side (ipsilateral side) under a light microscope (SD30, Olympus, Tokyo, Japan) as described previously (Seltzer *et al*, 1990; Malmberg and Basbaum, 1998). In sham-operated mice, the nerve was exposed without ligation. We previously reported that the sciatic nerve-ligated mice exhibit the thermal hyperalgesia and the mechanical hyperalgesia on the ipsilateral side, indicating the state of neuropathic pain hypersensitivity. These persistent painful states caused by sciatic nerve ligation lasted for more than 15 days (Narita *et al*, 2005b).

Inflammatory Pain Model

A persistent inflammatory pain model was produced by unilateral intraplantar injection of complete Freund's adjuvant (CFA; Mycobacterium tuberculosis; Sigma, St Louis, MO, USA) in 20 μl into the plantar surface of the right hind paw (ipsilateral side) of mice under the anesthesia with sodium pentobarbital (70 mg/kg, i.p.) or 3% isoflurene (Ohsawa *et al*, 2000). The control mice were given saline into the plantar surface of the right hind paw.

Intrathecal Injection

Intrathecal (i.t.) administration was performed following the method described previously (Hylden and Wilcox, 1980) using a 25-μl Hamilton syringe with a 30-gauge needle. The injection volume was 4 μl for each mouse. Each solution was injected without injection cannulae.

Intracerebroventricular Injection

Intracerebroventricular (i.c.v.) administration was performed following the method described previously with modifications (Haley and McCormick, 1957; Narita *et al*, 2003). To make a hole in the skull for injection, 1 day before the administration of each drug, mice were briefly anesthetized with ether and a 2-mm double-needle (tip: 27 G × 2 mm and base: 22 G × 10 mm, Natsume Seisakusyo Co. Ltd, Tokyo, Japan) attached to a 25-μl Hamilton microsyringe was inserted into a unilateral injection site using a V-shaped holder to hold the head of the mouse. The unilateral injection site was approximately 2 mm from either side of the midline between the anterior roots of the ears. On the day of the tail-flick assay, the head of the mouse was held against a V-shaped holder and the drugs were injected into the hole. The injection volume was 4 μl for each mouse. Each solution was injected without injection cannulae.

Measurement of Thermal Hyperalgesia

To assess the sensitivity to thermal stimulation, each of the hind paws of mice was tested individually using a thermal stimulus apparatus (UGO-BASILE, Biological research apparatus, VA, Italy). The intensity of the thermal stimulus was adjusted to achieve an average baseline paw withdrawal latency of approximately 8–10 s in naive mice. Only quick hind paw movements (with or without licking of the hind paws) away from the stimulus were considered to be a withdrawal response. Paw movements associated with locomotion or weight shifting were not counted as a response. The paws were measured alternating between the left and right with an interval of more than 3 min between measurements. The latency of paw withdrawal after the thermal stimulus was determined as the average of three measurements per paw.

Assessment of Antinociception

Antinociception induced by oxycodone or morphine was determined by the tail-flick test (Tail Flick Analgesia Meter Model MK 330B, Muromachi Kikai Co. Ltd, Tokyo, Japan). The intensity of the heat stimulus was adjusted, so that the animal flicked its tail after 3–5 s. When the intensity of stimulation was enough to produce a basal movement within 3–5 s in mice, it was defined that pharmacological observation results from the spinal reflex and supraspinal modulations (Le Bars *et al*, 2001). The inhibition of this tail-flick response was expressed as a percentage of the maximum possible effect (%MPE), which was calculated as $((T_1-T_0) \times 100/(T_2-T_0))$, where T_0 and T_1 were the tail-flick latencies before and after the administration of each MOR agonist and T_2 was the cut-off time (set at 10 s) in the tests to avoid injury to the tail. In the present study, the antinociceptive assay was performed 7 days after partial sciatic nerve-ligation. Each group consisted of 8–11 mice.

Place Conditioning

Place conditioning studies were conducted using a shuttle box (15 × 30 × 15 cm, w × l × h) that was made of an acrylic resin board and divided into two equal-sized compartments. One compartment was white with a textured floor and the other was black with a smooth floor to create equally inviting compartments. The place-conditioning schedule consisted of three phases (pre-conditioning test, conditioning, and post-conditioning test). The pre-conditioning test was performed as follows: the partition separating the two compartments was raised to 7 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and mice that had not been treated with either drugs or saline were then placed on the platform. The time spent in each compartment during a 900-s session was then recorded automatically using an infrared beam sensor (KN-80, Natsume Seisakusyo Co. Ltd, Tokyo, Japan). Immediately after subcutaneously (s.c.) injection of morphine (3–10 mg/kg) or oxycodone (0.3–3 mg/kg), these animals were placed in the compartment opposite that in which they had spent the most time in the pre-conditioning test for 1 h. On alternate days, these animals received vehicle and were placed in the other compartment for 1 h. On the day after the final conditioning session, a post-conditioning test that was identical to the pre-conditioning test was performed.

Drugs

The drugs used in the present study were morphine hydrochloride (Daiichi-Sankyo Co., Tokyo, Japan), oxycodone hydrochloride (a kind gift from Shionogi Pharmaceutical Co. Inc., Osaka, Japan), morphine-6-glucuronide (M-6-G) (Sigma-Aldrich Co., St Louis, MO, USA), [D-Ala^2, *N*-Me-Phe^4, Gly^5-ol] enkephalin (DAMGO; Sigma-Aldrich Co., St Louis, MO, USA), (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-metyoxybenzyl]-*N*,*N*-diethylbenzamide] (SNC80; Tocris Cookson Ltd, Ballwin, MO, USA), (−)trans-(1S,2S)-U-50,488 (U50,488; Sigma-Aldrich Co., St Louis, MO, USA), ICI 199,441 hydrochloride (Tocris Cookson Ltd, Ballwin, MO, USA), β-funaltrexamine hydrochloride (β-FNA), naltrindol hydrochloride (NTI; Tocris Cookson Ltd, Ballwin, MO, USA) and nor-binaltrophimine dihydrochloride (nor-BNI; Tocris Cookson Ltd, Ballwin, MO, USA). All drugs were dissolved in 0.9% physiological

saline (Otsuka Pharmaceutical Co. Inc., Tokyo, Japan) for *in vivo* experiments or assay buffer for *in vitro* experiments.

Statistical Analysis

The data for antinociceptive response were shown as the mean ± SEM of % MPE. The data for [^{35}S]GTPγS binding assay were expressed as the mean ± SEM of % Stimulation. Receptor binding curves were fitted using the GraphPad Prism 4.0 program. The statistical significance of differences between the groups was assessed with a two-way ANOVA followed by Bonferroni/Dunn multiple comparison test or Student's *t*-test.

RESULTS

Binding Properties of Oxycodone with Opioid Receptor

To evaluate the specific involvement of the opioid receptor types in oxycodone-induced pharmacological actions, we performed the competitive displacement-binding assay. At first, we determined the competitive displacement binding of the MOR ligand [^{3}H]DAMGO (Figure 1a), the δ-opioid receptor (DOR) ligand [^{3}H]DPDPE (Figure 1b) or the KOR ligand [^{3}H]U69,593 (Figure 1c) with graded concentrations (10^{-11}–10^{-7} M) of unlabeled opioid agonists in membranes of the mouse brain without cerebellum (Figure 1a and b) and the guinea pig cerebellum, which is relatively rich in KOR sites (Figure 1c). As shown in Table 1, IC_{50} values were determined by the displacement of [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69,593 (Table 1). The [^{3}H]DAMGO binding was clearly displaced by morphine or oxycodone in a concentration-dependent manner. The affinity of oxycodone to the MOR binding was about 10 times lower than that of morphine. In contrast, the binding of either [^{3}H]DPDPE or [^{3}H]U69,593 was not affected by morphine or oxycodone, whereas [^{3}H]DPDPE or [^{3}H]U69,593 binding was displaced by increasing concentrations of either a selective DOR agonist SNC80 or a specific KOR agonist U50,488, respectively.

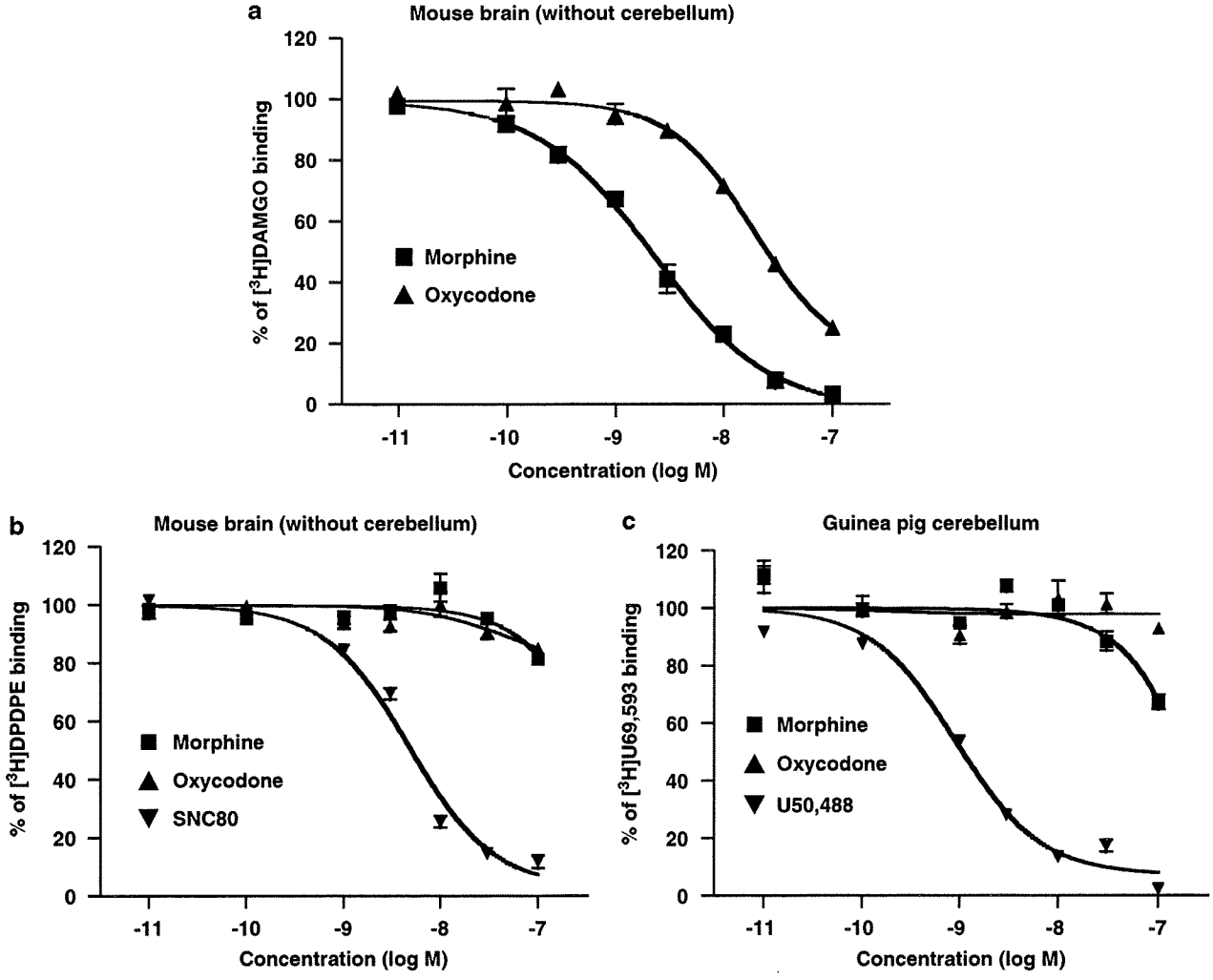

Figure 1 Displacement of the MOR ligand [^{3}H]DAMGO (a), DOR ligand [^{3}H]DPDPE (b), or the KOR ligand [^{3}H]U69,593 (c) binding in membranes of the mouse brain without cerebellum (a or b) and the guinea pig cerebellum (c) by morphine, oxycodone, SNC80, or U50,488. Experiments were performed in the presence of either [^{3}H]DAMGO (1 nM), [^{3}H]DPDPE (2 nM), or [^{3}H]U69,593 (2 nM) and increasing concentrations of morphine, oxycodone, SNC80, or U50,488. The data represent the mean ± SEM of three to four samples.

Table 1 Binding Property of Oxycodone for the μ-, δ-, or κ-Opioid Receptor Determined by Displacement of [^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69,593

	Morphine	Oxycodone	Fentanyl	SNC80	U50,488
[^{3}H]DAMGO (nM)	2.29 (1.97–2.67)	20.26 (15.02–27.32)	0.64 (0.58–0.71)	ND	ND
[^{3}H]DPDPE (nM)	<500	<500	<500	5.17 (3.90–6.84)	ND
[^{3}H]U69,593 (nM)	161.3 (69.62–373.7)	<500	<500	ND	1.16 (0.96–14.12)

IC_{50} values were determined using the analysis of variance and linear regression techniques. Groups were treated with morphine, oxycodone, fentanyl, SNC80, or U50,488. To calculate IC_{50} values, at least seven drug doses were used and three to four samples were used for each dose. Values in parenthesis indicate the 95% confidence range.

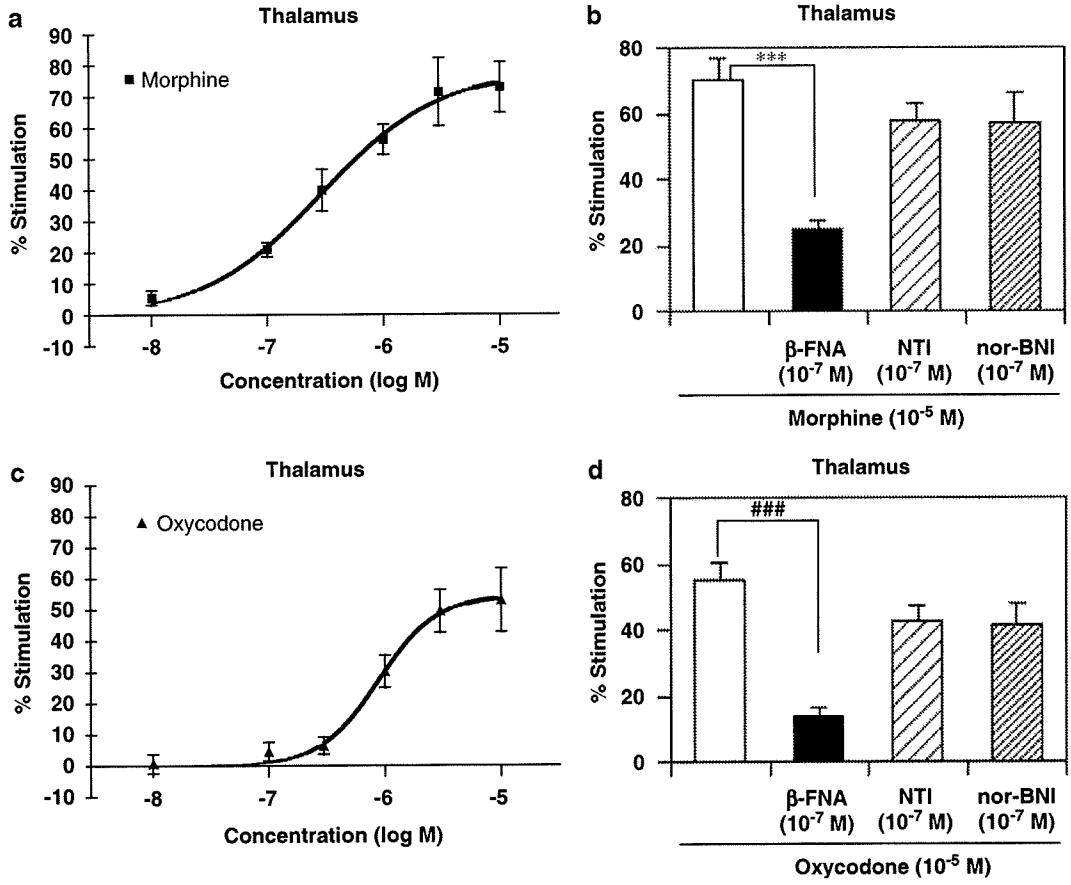

Figure 2 Concentration–response curve of [^{35}S]GTPγS binding to membranes induced by morphine (a) or oxycodone (c) in the mouse thalamus. Either morphine- (b) or oxycodone (d)-induced increase in [^{35}S]GTPγS binding to membranes of the mouse thalamus was blocked by the selective MOR antagonist β-FNA, but not the DOR antagonist NTI or the KOR antagonist nor-BNI. Membranes were incubated with [^{35}S]GTPγS and GDP with morphine or oxycodone in the presence or absence of β-FNA, NTI or nor-BNI. The data are shown as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of morphine or oxycodone. Each column represents the mean ± SEM of three to six samples. $^{***}p<0.001$ β-FNA-morphine *vs* morphine alone. $^{###}p<0.001$ β-FNA-oxycodone *vs* oxycodone alone.

We next investigated the ability of oxycodone to activate G-proteins in the mouse thalamus, spinal cord and the guinea pig cerebellum membranes. Either morphine (10^{-8}–10^{-5} M) or oxycodone (10^{-8}–10^{-5} M) showed a concentration-dependent increase in the binding of [^{35}S]GTPγS to membranes of the mouse thalamus (Figure 2a and c) and spinal cord (Figure 3a and c). Co-incubation with a MOR antagonist β-FNA (10^{-7} M) significantly attenuated either morphine- or oxycodone-induced G-protein activation, whereas either a DOR antagonist NTI (10^{-7} M) or a KOR antagonist nor-BNI (10^{-7} M) failed to affect those of morphine and oxycodone ($^{***}p<0.001$ β-FNA-morphine *vs* morphine alone, $^{###}p<0.001$ β-FNA-oxycodone *vs* oxycodone alone, Figures 2b and d, 3b and d). Conventional KOR agonists, U50,488 and ICI-199,441 (10^{-8}–10^{-5} M) produced a concentration-dependent increase in [^{35}S]GTPγS binding to membranes of the guinea pig cerebellum region (Figure 4). In contrast, morphine (10^{-8}–10^{-5} M) showed relatively smaller increase in the binding of [^{35}S]GTPγS than that of KOR agonists. Further-

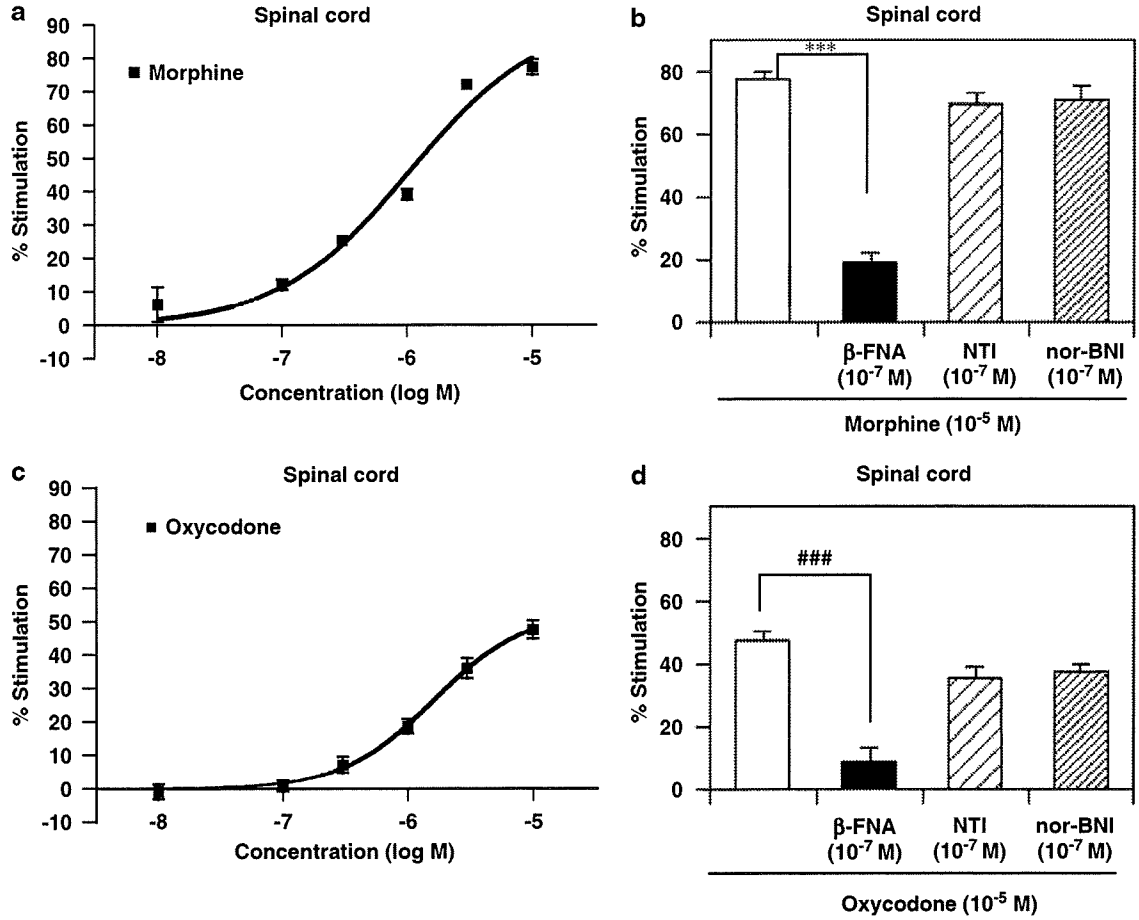

Figure 3 Concentration–response curve of [^{35}S]GTPγS binding to membranes induced by morphine (a) or oxycodone (c) in the mouse spinal cord. Either morphine- (b) or oxycodone (d)-induced increase in [^{35}S]GTPγS binding to membranes of the mouse spinal cord was blocked by the selective MOR antagonist β-FNA, but not the DOR antagonist NTI or the KOR antagonist nor-BNI. Membranes were incubated with [^{35}S]GTPγS and GDP with morphine or oxycodone in the presence or absence of β-FNA, NTI, or nor-BNI. The data are shown as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP and absence of morphine or oxycodone. Each column represents the mean ± SEM of four to six samples. $^{***}p<0.001$ β-FNA-morphine *vs* morphine alone. $^{###}p<0.001$ β-FNA-oxycodone *vs* oxycodone alone.

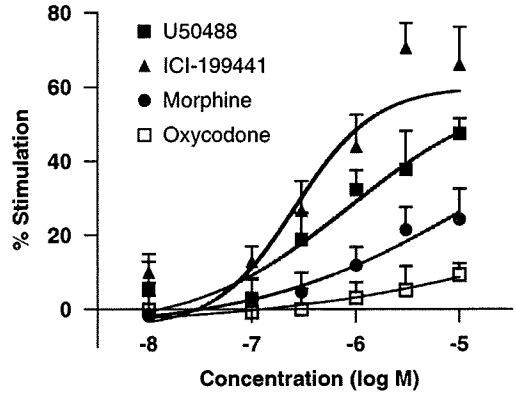

Figure 4 Concentration–response curve of [^{35}S]GTPγS binding to membranes obtained from the guinea pig cerebellum by U50,488, ICI-199,441, oxycodone, or morphine. The columns are expressed as the percentage of basal [^{35}S]GTPγS binding. The data represent the mean ± SEM of four samples.

more, a little change in the binding of [^{35}S]GTPγS to membranes of the guinea pig cerebellum region was noted by oxycodone (10^{-8}–10^{-5} M) (Figure 4).

We next investigated the role of MOR in oxycodone (3 mg/kg, s.c.)-induced antinociception using the tail-flick assay. Pretreatment with β-FNA (40 mg/kg, s.c.) significantly attenuated the oxycodone-induced antinociception (Figure 5a, $^{*}p<0.05$, $^{***}p<0.001$ *vs* saline-pretreated group), whereas either nor-BNI (5 mg/kg, s.c.) or NTI (3 mg/kg, s.c.) had no effect on the antinociception of oxycodone (Figure 5b and c).

Effect of s.c. Injection of Morphine or Oxycodone on Thermal Hyperalgesia Induced by Sciatic Nerve Ligation or Intraplantar Injection of CFA in Mice

Sciatic nerve-ligated mice produce the state of neuropathic pain-like hypersensitivity. The persistent painful state caused by sciatic nerve ligation lasted for more than 21 days (data not shown). In the present study, mice with partial sciatic nerve ligation exhibited marked neuropathic pain-like behavior only ipsilateral side at 7 days after the nerve ligation ($^{**}p<0.01$, $^{***}p<0.001$ *vs* sham-saline group) (Figure 6a and b). Unilateral intraplantar injection of CFA into the mouse hind paw produces the state of inflammatory pain-like hypersensitivity, which caused a