ため、モルヒネの精神依存形成に及ぼす脳内の COX の役割について検討することは、臨床での NSAIDs とモルヒネの併用意義を明確にする上で非常に重要であると思われる。そこで本年度は、モルヒネの精神依存形成ならびに自発運動促進作用における脳内の COX の関与について検討した。モルヒネの鎮痛作用を増強させる用量のジクロフェナクを脳室内に投与したところ、モルヒネの精神依存形成ならびに自発運動促進作用は全く抑制されなかった。一般的に、依存性薬物の精神依存形成ならびに自発運動促進作用の発現には、中脳辺縁ドパミン神経系が深く関与していることが知られている。モルヒネは中脳辺縁ドパミン神経系の起始核である腹側被蓋野の g-aminobutyric acid (GABA) 神経上に存在する m-オピオイド受容体に作用し、GABA 神経の抑制作用に起因した脱抑制機構によりドパミン神経を興奮させ、投射先におけるドパミンの遊離量を増加させることで、精神依存や自発運動促進作用を誘発することが知られている。これらの結果から、脳内の COX はモルヒネによる中脳辺縁ドパミン神経系の活動性にほとんど関与していないと考えられる。一方、前年度までに我々は、正常時ならびに炎症性疼痛時にモルヒネと NSAIDs を併用することにより、モルヒネの鎮痛作用が増強することを見出している。したがって、NSAIDs との併用はモルヒネの精神依存形成を直接的には抑制しないものの、十分な鎮痛作用を維持しながらモルヒネの用量を軽減させることができると考えられる。

E. 結論

以上本研究の結果より、NSAIDs を併用してもモルヒネの精神依存形成は抑制されなかったことから、脳内の COX はモルヒネの精神依存形成にはほとんど関与していない可能性が示唆された。

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H.知的財産権の出願・登録状況

1.特許取得：なし

2.実用新案登録：なし

3.その他：なし

研究成果の刊行に関する一覧表

雑誌

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著者氏名	書籍全体の監修	書　籍　名	発行	出版地
平賀　一陽	国立がんセンター	「痛み止めの薬」のやさしい知識 ～あなたの痛みを上手に取り除くために～	財団法人　がん研究振興財団	大阪

緩和医療学

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放射線科医がはじめた緩和医療

本家好文

はじめに

「放射線治療を担当する医者は，どうせ放射線の“かけ屋”だ．患者からの苦情を聞くのは自分たちだし，治療方針には口を挟まないで欲しい」．約25年前に，ある医師からいわれた言葉です．いまでも忘れられない言葉ですが，筆者が「チーム医療」が重要なことを実感し，ベッドサイドで「患者さんの声に耳を傾けることの大切さ」を認識させられた言葉でもあります．

ベッドサイドで患者さんの声を聴きつづけたことから，痛みをとることの大切さを学び，放射線治療医から緩和医療を専門とする医師に転身したといっても過言ではありません．

放射線治療医として

「何もしないわけにはいかないし，放射線でもかけておくか」．これも放射線治療医時代の自分にとっては，忘れられない言葉です．放射線治療には臓器の形態を保って機能が温存できることや，身体への負担が小さいといったメリットがあります．喉頭癌や舌癌では手術よりも放射線治療で機能を温存することで，患者さんのQOL（quality of life）が維持されることはよく知られています．

最近では，乳癌治療で乳房温存手術と放射線を用いることによって乳房を温存し，美容面や精神面でよい結果が残せるようになりました．機能が温存できるだけでなく，負担の軽い放射線治療の役割は，今後ますます大きくなることが予測されています．

しかし，一般的には放射線治療というと「副作用が強い」ことばかりが強調されて，十分に活用されていないのが現状です．ひと昔前までは，手術ができない患者さんに「仕方なく」実施することや，再発や転移巣への治療を依頼されることが多かったのです．

骨転移の痛みに対しては，放射線治療をおこなうことによって身体が動かせるようになったり，オピオイド鎮痛剤を減量できるといったメリットがあります．しかし，以前には治癒が望めない状態で「痛みをとるためだけ」の治療に対して，放射線治療医も「姑息的放射線治療」と称して，あまり関心をもってきませんでした．最近になって苦痛の緩和を目的とする「緩和的放射線治療」の大切さが，ようやく理解されるようになりました．

大学病院から第一線病院へ

卒業して3年目からの2年間，放射線医学総合研究所（放医研：千葉市）で放射線治療の基

本家好文（Honke Yoshifumi）/広島県緩和ケア支援センター

礎を学ぶ機会を得ました．その後，広島に戻ってからの５年間は地域のがん治療医に放射線治療を正しく理解してもらい，手術療法や化学療法と連携して集学的治療を実践することに力を注ぎました．不治の病といわれていた「がん」を放射線で治すことにエネルギーを注いだ時期でもありました．

卒業して10年目にあたる1985年に，厚生連広島総合病院に新しく放射線治療部門が設立されて赴任しました．放射線治療医は患者さんを診ないといわれたことへの反発心から，広島総合病院では放射線治療中の患者さんは自らが主治医となって治療をおこないました．

大学病院の放射線治療部門では，完全に治癒できる可能性のある患者さんも数多くおられました．しかし，第一線病院では８～９割の患者さんたちは，紹介された当初から治癒が望めない進行がんという状況でした．

大学病院時代には，患者さんやご家族の声が届きにくい立場にいましたが，再び第一線病院に勤務することになり，主治医として直接患者さんやご家族の声を聴く機会が増えました．がんに罹患したことによる不安や恐怖だけでなく，痛みが改善しないことに対する辛い気持ちを毎日聴くようになりました．

その当時から，患者さんのところにうかがうときには，必ず腰をかけて座って話し合うように心がけました．最初は照れ臭くて抵抗がありましたが，じっくり患者さんの話を「聴く」ことは，患者さんに大変喜ばれたのでいまでもつづけています．

疼痛治療の重要性

多くの医師は，自分の将来の方向性を左右するような忘れられない患者さんとの出会いを体験しています．筆者にとっては15年前に出会った50歳代の乳癌患者さんとの出会いが，緩和医療を志すきっかけになりました．

まだ硫酸モルヒネ徐放剤が発売されて間もないころ，自分自身のモルヒネ使用方法に関する知識が未熟で経験が不足していたために，十分な量のモルヒネを使わず「痛みと向き合う毎日」を余儀なくさせてしまいました．その結果，最終的には病棟から投身自殺をされてしまい，スタッフも自分自身も大きなショックを受けました．その患者さんの体験をきっかけにして，病棟内で医師・看護師・薬剤師とで疼痛治療の勉強会をはじめました．勉強会を通じて学ぶことによって，徐々に痛みを抱えた患者さんへの治療が上手くいくようになりました．その後，疼痛治療の勉強会を病棟から病院全体の「院内ターミナルケア研究会」に発展させていきました．

病院内部で研究会を開催することによって，疼痛治療に対する病院内の医療者の意識が大きくかわりました．モルヒネの具体的な使用方法を学ぶことによって，病院全体のがん性疼痛治療のレベルが明らかに改善しました．

病院から地域へ

さらに広島県内の医療機関にも声をかけて「ターミナルケアを考える会・広島」を発足させました．この会は，自分にとって緩和医療をめざす基盤となる研究会となっています．発足以来10年以上が経過していますが，「継続は力なり」の言葉を信じていまもつづけています．会

の活動は地域のメディアにも注目されるようになり，社会的な支援を受けたことも大きな励みになりました．

「ターミナルケアを考える会・広島」がスタートした1993年には，ホスピスをみたことがありませんでした．そこでデーケン氏（元上智大学）の主催するヨーロッパのホスピス視察ツアーに参加して，はじめて英国のホスピス施設を見学して基本的な考え方に接したり，全国から集まった人たちとの意見交換ができたことや，英国の大学医学部で「緩和医療学」が講座として確立していることを知ったことなどが，自分を「緩和医療」に向かわせる大きな刺激になりました．

放射線治療と緩和医療の両立

緩和ケアへの関心が高まるにつれて，逆に放射線治療への関心が徐々に薄れていく自分を感じていました．緩和医療と放射線治療とを両立させるむずかしさを悩んでいたときに，国立呉病院（現：独立行政法人国立病院機構呉医療センター）に緩和ケア病棟が開設され，担当医師を探しているという話が舞い込みました．放射線治療医として全身の悪性腫瘍にかかわってきた25年間の経験を生かしながら，緩和医療を専門にする医師に転身することを決意しました．

2000年1月からは国立呉病院緩和ケア病棟に勤務しました．一般病棟に勤務しているときには，医師と看護師が患者さんのケアについて10分間のカンファレンスをもつことも簡単ではありませんでしたが，緩和ケア病棟ではカンファレンスを開催できることが当たり前という状況でした．痛みを緩和するためにはどんなアプローチが必要か，鎮痛剤は有効か，副作用の問題は生じていないか，身体的痛み以外の問題を抱えていないかといったことを話し合っていると，チーム医療を実践していることを実感できました．

しかし一方で，緩和ケア病棟に勤務していると，一般病棟の感覚とのずれを感じることもあって戸惑いもありました．緩和ケア病棟に入院するのだから，積極的な治療をおこなうことは認めないといった雰囲気を感じることもありました．患者さんの心理状態を考えると，自分が積極的ながん治療が困難で緩和ケアの対象となる病状であることは，説明を受けて理屈では理解していても，何とかならないだろうかという期待感をもっていることも多いのです．そのことを認めないような姿勢で入院の判断をすることもあり反省させられました．

現在のがん医療では，積極的ながん治療の効果が得にくくなった時期の患者さんへの援助が欠落しているように感じます．緩和ケア病棟や在宅ケアという選択をする前段階の患者さんで，将来の方向性について一番迷っている時期の患者さんたちへの支援が必要だと思います．そのような時期の患者さんに対して緩和医療がもっとかかわる必要性があると感じています．

広島県緩和ケア支援センター

最近の5年間に広島県に8つの緩和ケア病棟が整備されました．最も新しく2004年9月に開設したばかりの広島県緩和ケア支援センターでは，県内8番目の緩和ケア病棟の運用だけでなく，広島県全体の緩和ケアの推進を目標とした緩和ケア支援室の運用をおこなっています．

緩和ケア支援室の事業としては，地域で独自に緩和ケアを担う人材を育成するための教育研修事業，患者さんや医療関係者から直接相談を受ける電話相談や面談窓口，また直接県内の各

地域との連携を図り具体的な援助をおこなうアドバイザー派遣事業や，在宅緩和ケアを推進するための「デイホスピス」事業などをおこなっています．

少し長期的な展望で，地域の在宅緩和ケアを中心とした緩和ケアの推進に取り組んでいく予定です．

おわりに

緩和医療は医療の分野ではいぜんとしてマイナーな分野で，決して十分な理解が得られているとはいえない状況にあります．しかし，徐々に関心が高まっていることも事実です．今後とも，一人ひとりの患者さんを苦痛から解放することを積み重ねながら，緩和医療の重要性について啓発活動をつづけていきます．

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Molecular mechanism of changes in the morphine-induced pharmacological actions under chronic pain-like state: Suppression of dopaminergic transmission in the brain

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Abstract

In the present study, we demonstrated whether a neuropathic pain-like state induced by sciatic nerve ligation in rodents could cause a long-lasting change in intracellular signaling in both supraspinal and spinal cord related to the suppression of morphine's effect. Mice with sciatic nerve ligation exhibited a significant suppression of the morphine-induced antinociception. Under this condition, phosphorylated-conventional protein kinase C-like immunoreactivity (p-cPKC-IR) and phosphorylated-μ-opioid receptor (p-MOR)-IR were clearly increased on the ipsilateral side in the dorsal horn of the spinal cord of nerve-ligated mice. It is of interest to note that astroglial hypertrophy as well as its proliferation was also noted in this area of sciatic nerve-ligated mice. Like nerve injury, the increase in cPKC activities and astroglial hypertrophy/proliferation in this region was observed by repeated morphine treatment. These findings suggest that the phosphorylation of both cPKC and MOR in the dorsal horn of the spinal cord by sciatic nerve ligation may play a substantial role in the suppression of morphine-induced antinociception under a neuropathic pain-like state. Sciatic nerve injury also caused a significant inhibition of MOR-mediated G-protein activation onto GABAergic neurons and a dramatic reduction in ERK activities onto dopaminergic neurons in the ventral tegmental area (VTA) regulating the rewarding effect of opioids. Furthermore, we found that the inhibition of ERK cascade in the VTA by treatment with specific inhibitors suppressed the morphine-induced rewarding effect in normal mice. These findings provide evidence that the direct reduction in MOR function and the persistent decrease in ERK activity of dopaminergic neurons in the VTA may contribute to the suppression of the morphine-induced rewarding effect under a neuropathic pain-like state. Conclusively, our

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recent findings provide novel evidences for the mechanism underlying the less sensitivity to opioids under a neuropathic pain-like state.

Keywords: Neuropathic pain; Morphine dependence; Protein kinase C

Introduction

Pain can be an adaptive sensation, an early warning to protect the body from tissue injury. Multiple molecular and cellular mechanisms operate alone and in combination within the peripheral and central nervous systems to produce the different forms of pain. Pharmacological studies have helped to identify many neurotransmitters and neuromodulators involved in pain processes in the dorsal horn of the spinal cord. The excitatory amino acids and several kinds of peptides released by primary afferents play a major role in nociception (Hunt and Mantyh, 2001). Increases in synaptic transmission in the dorsal horn can begin almost immediately as a result of activity-dependent phosphorylation and trafficking of receptors or ion channels. Primary afferent nociceptors terminate primarily in laminae I, II and V, where they connect with several classes of second-order neurons in the dorsal horn of the spinal cord. Structural alterations in the synaptic contacts of low-threshold afferents with pain transmission neurons, or a reduction of inhibitory mechanisms due to a loss of interneurons, and represent persistent changes in the central nervous system (CNS) that eventually results in a fixed state of sensitization. The resultant action potentials are conducted to the dorsal horn of the spinal cord, and the input is conveyed via the spinothalamic and spinoparabrachial pathways to higher centers (Hunt and Mantyh, 2001). Activity in the spinaothalamic tract relays through the thalamus to the somatosensory cortex and associated areas. The parabrachial nucleus of the brainstem has connections to the ventral medial nucleus of the hippocampus and the central nucleus of the amygdala, and the brain regions involved in the affective response to pain. Impulses from supraspinal centers are integrated in the midbrain periaqueductal gray, which is pivotal in modulating descending facilitation and inhibition of nociceptive input mainly via the nucleus raphe magnus (NRM).

A growing body of clinical evidence suggests that when opioid analgesics including morphine and fentanyl 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). 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 changes at supraspinal levels associated with the suppression of opioid dependence.

It has been widely recognized that neuropathic pain, which is characterized by spontaneous burning pain, hyperalgesia (an exaggerated pain in response to painful stimuli) and allodynia (a pain evoked by normally innocuous stimuli), may result from hypersensitivity due to alteration of primary afferent neurons and/or spinal dorsal horn neurons followed by nerve injury. Neuropathic pain is particularly difficult to treat in the clinic, as it is only partially relieved by high doses of opioids such as morphine

and fentanyl. There are many studies focused on the long-term changes in functions of the spinal cord dorsal horn neurons, containing some receptors, protein kinases and peptides following nerve injury (Petersen-Zeitz and Basbaum, 1999; Scholz and Woolf, 2002). However, little is known about the molecular mechanism of the down-regulation of μ-opioidergic function associated with synaptic plasticity under chronic pain (Bessou and Perl, 1969; Beitel and Dubner, 1976; Woolf, 1983). It, therefore, is worthwhile to investigate whether a neuropathic pain-like state induced by sciatic nerve ligation in rodents could cause a long-lasting change in intracellular signaling in both supraspinal and spinal cord related to the suppression of morphine's effect. This review attempts to summarize the molecular mechanism underlying the suppression of morphine's effect under a neuropathic pain-like state.

Change in the spinal transmission under a neuropathic pain-like state

Increased spinal proten kinase C (PKC) activity and astrocyte under a neuropathic pain-like state and morphine-tolerant state

Several lines of evidence have demonstrated that the activation of PKC plays a critical role in the modulation of synaptic plasticity as characterized by long-term potentiation (Abellovich et al., 1993). PKC is a key regulatory enzyme that modulates both pre- and post-synaptic neuronal function, synthesis and release of neurotransmitters, and the regulation of receptors. It has been recognized that PKC family consists of at least 12 isoforms that possess distinct differences in structure, substrate requirement, expression and localization, therefore, may underlie diverse physiological functions (Nishizuka, 1992; Way et al., 2000). Recent studies have provided evidence for an important role of PKC expressed on dorsal horn neurons in regulating pain hypersensitivity in a number of different pain models (Codderre, 1992; Sluka and Willis, 1997; Ohsawa et al., 2000). It is considered that the activation of PKC in the dorsal horn of the spinal cord may be responsible for the release of excitatory amino acids and neuropeptides, resulting in the initiation of central sensitization. We documented that thermal hyperalgesia induced by sciatic nerve ligation was markedly suppressed by repeated i.t. pretreatment with the selective PKC inhibitor (Fig. 1), but not the specific protein kinase A (PKA) inhibitor, in mice (Yajima et al., 2003). We also found that the level of membrane-bound PKCγ isoform, which is identified in neurons of the brain and inner part of laminae II of the spinal cord, was significantly increased in the ipsilateral side of the spinal cord in sciatic nerve-ligated mice (Fig. 2, Yajima et al., 2002). It is of interest to note that mice lacking PKCγ isoform exhibit normal responses to acute pain stimuli, but they almost completely inhibit the development of neuropathic pain-like behaviors after sciatic nerve ligation (Malmberg et al., 1997; Ohsawa et al., 2001). We recently reported that the immunoreactivity to activated form of conventional PKC (cPKC), including PKCα, PKCβI, PKCβII and PKCγ, was clearly increased on the ipsilateral side of the superficial layers of the L5 lumbar spinal dorsal horn in sciatic nerve-ligated mice (Fig. 3). The increased phosphorylated-cPKC-like immunoreactivity (p-cPKC-IR) observed in the spinal dorsal horn was obviously overlapped with microtubule-associated protein 2a/b (MAP2a/b), which confined to neuronal cell bodies and dendrites (unpublished data). Collectively, these findings provide further evidence that the activition of neuronal cPKC in the dorsal horn of the spinal cord by nerve injury may play a key factor for the development of neuropathic pain-like state in mice.

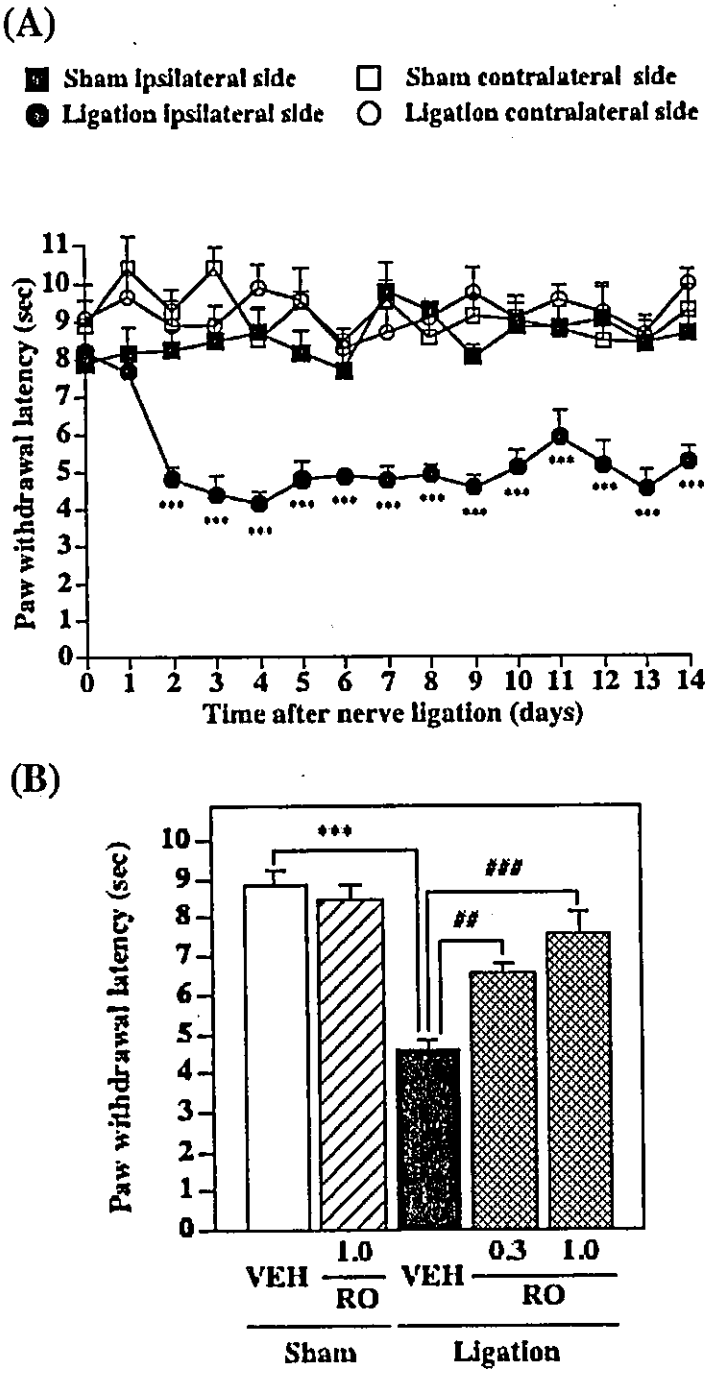

Fig. 1. (A) Time course changes in the latency of paw withdrawal from a thermal stimulus induced by partial sciatic nerve ligation in mice. Partial ligation of sciatic nerve caused a marked decrease in the latency of paw withdrawal from a thermal stimulus only on the ipsilateral (nerve-ligated) side of the hind paw of mice. Each point indicates the mean ± S.E.M. of 6–7 mice. ***p < 0.001: Sham-Ipsilateral side vs. Ligation-Ipsilateral side. (B) Effect of repeated intrathecal (i.t.) injection of the selective PKC inhibitor RO-32-0432 on latencies of paw withdrawal from a thermal stimulus on the ipsilateral side of nerve-ligated mice. Groups of mice were injected i.t. with RO-32-0432 (RO; 0.3 and 1.0 nmol/mouse) or its vehicle (VEH) 30 min prior to nerve ligation and once a day for 7 consecutive days. Each column indicates the mean ± S.E.M. of 4–8 mice. ***p < 0.001: VEH-Sham group vs. VEH-Ligation group, ##p < 0.01 and ###p < 0.001: VEH-Ligation group vs. RO-Ligation group.

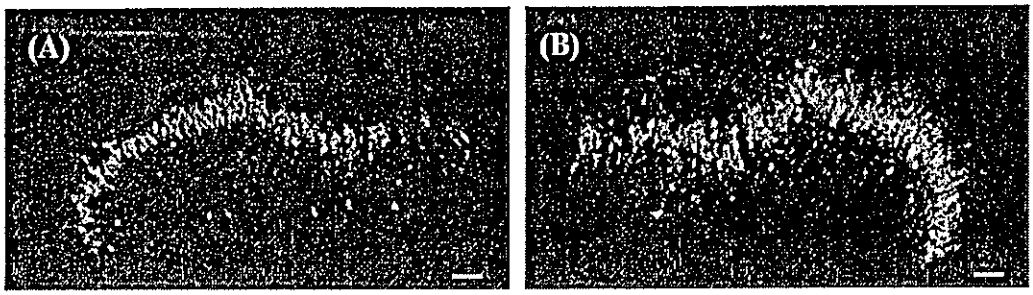

Fig. 2. Immunofluorescent staining for protein kinase Cγ (PKCγ)-IR on the dorsal horn of the L5 lumbar spinal cord in nerve-ligated rats. The PKCγ-IR was clearly increased on the ipsilateral side in the superficial laminae of the L5 lumbar spinal dorsal horn in nerve-ligated rats (B) as compared to that observed on the contralateral side (A). Scale bars; 50 μm.

For years, astrocytes were considered only to have supportive and nutritive functions in the CNS. However, advanced imaging methods show that glia communicates with one another and with neurons primarily through chemical signals. The activated glial cells are characterized by decreased ramification, hypertrophy, proliferation, and the up-regulation of immunoregulatory molecules, including nitric oxide, prostaglandins, excitatory amino acids and nerve growth factors (Raivich et al., 1999). A growing body of evidence suggests that synaptic astrocytes regulate synaptic transmission by responding to signaling molecular. Recently, there are several lines of evidence supporting the hypothesis that spinal cord glia are implicated in exaggerated pain states created by such diverse manipulations as subcutaneous inflammation, neuropathy, and spinal immune activation (Watkins et al., 2001). We recently found that the level of glial fibrillary acidic protein (GFAP)-IR, a specific astrocyte marker, was elevated mostly in the ipsilateral side of the spinal dorsal horn in sciatic nerve-ligated mice (Fig. 4). The apparent each individual astrocyte labeled by GFAP was hypertrophied with an enlarged cell body and was not co-localized with the activated form of cPKC-IR in the spinal dorsal horn of nerve-ligated mice. These findings suggest that the enhanced cPKC activity in the dorsal horn of the spinal cord is located within the primary afferent and/or dorsal horn neurons, but not within astroglial cells. It is worthwhile to note that, like nerve injury, the increase in cPKC activity and the astroglial hypertrophy in this area were

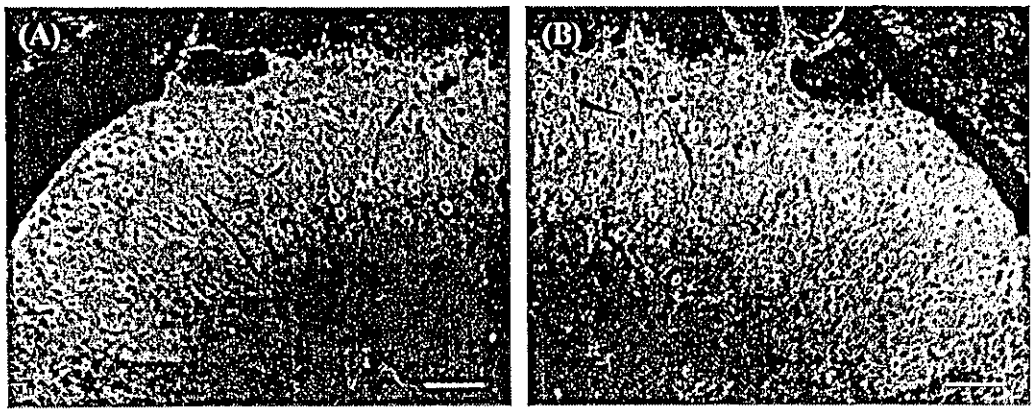

Fig. 3. Immunofluorescent staining for phosphorylated-conventional PKC-IR (p-cPKC-IR) on the dorsal horn of the L5 lumbar spinal cord in nerve-ligated mice. The p-cPKC-IR was clearly increased on the ipsilateral side in the superficial laminae of the L5 lumbar spinal dorsal horn in nerve-ligated mice (B) as compared to that observed on the contralateral side (A). Scale bars; 50 μm.

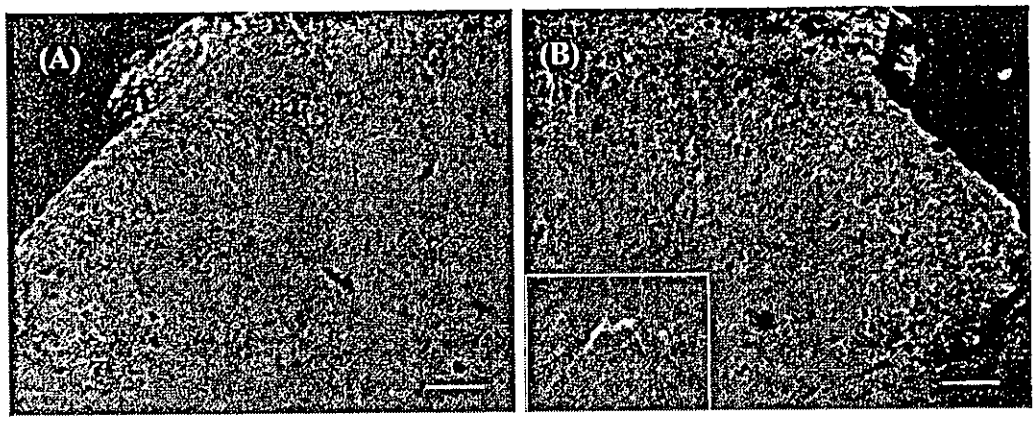

Fig. 4. Immunofluorescent staining for GFAP-IR on the dorsal horn of the L5 lumbar spinal cord in nerve-ligated mice. The GFAP-IR was clearly increased with morphologic differentiation on the ipsilateral side in the superficial laminae of the L5 lumbar spinal dorsal horn in nerve-ligated mice (B) as compared to that observed on the contralateral side (A). Scale bars; 50 μm.

observed by repeated morphine treatment (Narita et al., 2004). Several studies have demonstrated that neuronal plasticity associated with hyperalgesia and morphine tolerance has similar cellular and molecular mechanisms, suggesting predictable interactions between hyperalgesia and morphine tolerance through the common mechanism. Taken together, these findings support the possibility that astroglial hypertrophy and increase in neuronal cPKC activity in the dorsal horn of the spinal cord induced by either neuropathy or chronic treatment with morphine leads to the change in synaptic transmission.

Direct evidence for the suppression of morphine analgesia under a neuropathic-pain like state

Although pain produced by tissue injury can usually be controlled by opioids, neuropathic pain is often refractory to such treatment. This clinical experience can be supported by the finding that the antinociceptive effect by either s.c. or i.t. treatment with morphine is attenuated in rodents with sciatic nerve ligation (Mao et al., 1995; Nichols et al., 1995; Ossipov et al., 1995; Yaksh et al., 1995). We also comfirmed that sciatic nerve ligation caused a significant suppression of the antinociception induced by s.c. administration of morphine in the mouse (Fig. 5). Furthermore, we found that the antinociceptive potency and efficacy induced by i.c.v.-administered morphine were not changed by sciatic nerve ligation (Ozaki et al., 2003), indicating the importance of the spinal area for this suppression.

It is well-known that prolonged exposure to opioids induces adaptive changes, resulting in tolerance or reduced responsiveness to opioids. Recent pharmacological and molecular biological approaches have suggested that the functional change in μ-opioid receptor (MOR) is one of the considerable mechanisms underlying opioid-induced tolerance (Keith et al., 1998). The cloning of MOR reveals several phosphorylation sites (Knapp et al., 1995). Phosphorylation of MOR at diverse sites of its intracellular domain by PKC, G-protein-coupled receptor kinase (GRK), mitogen-activated protein kinase (MAPK) or protein tyrosine kinase has been shown to trigger the phosphorylated (p)-MOR internalization from the cell surface to the cytosol (Narita et al., 1995, 2001b; Kovoor et al., 1998; Polakiewicz et al., 1998; Kramer et al., 2000), which is thought to be an important step toward desensitization (Narita et al.,

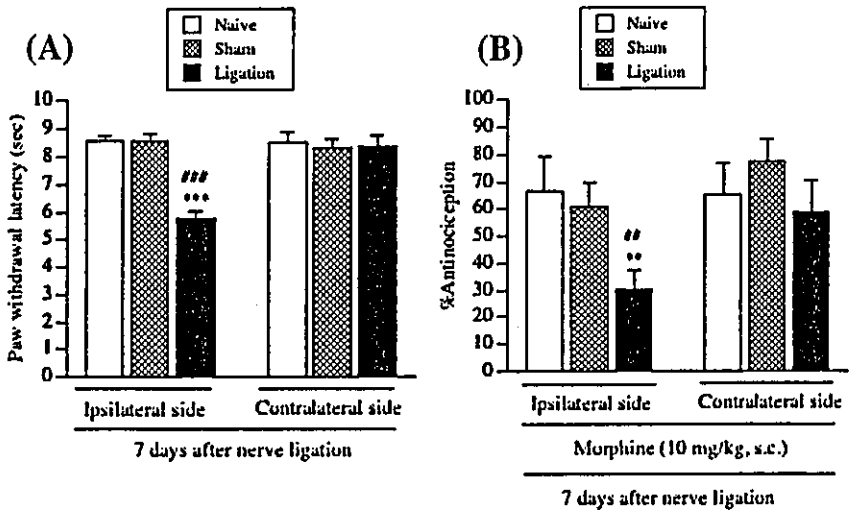

Fig. 5. (A) Changes in thermal paw withdrawal latencies of nerve-ligated mice. The measurement of thermal threshold was performed 7 days after nerve ligation. Each column represents the mean ± S.E.M. of 9–12 mice. ***$p < 0.001$: Ipsilateral side of Naive and Sham group vs. Ipsilateral side of ligation group, $^{###}p < 0.001$: Contralateral side of all groups vs. Ipsilateral side of ligation group. (B) Antinociceptive effect produced by morphine under a neuropathic pain-like state. Groups of mice were treated with morphine (10 mg/kg, s.c.) 7 days after nerve ligation. The antinociception was measured at 30 min after morphine injection using the thermal hyperalgesic test. Each column indicates the mean ± S.E.M. of 9–12 mice. **$p < 0.01$: Ipsilateral side of Naive and Sham group vs. Ipsilateral side of ligation group, $^{##}p < 0.01$: Contralateral side of all groups vs. Ipsilateral side of ligation group.

1996). In particular, agonist-specific phosphorylation of Ser375 in the mouse MOR is essential for its internalization (Kouhen et al., 2001). In our recent study, we found using p-MOR (Ser375) antibody that p-MOR-IR was clearly increased on the ipsilateral side in the superficial laminae of the L5 lumbar spinal dorsal horn in nerve-ligated mice as compared to that found on the contrateral side (Fig. 6). These findings suggest that, although we cannot completely exclude the possibility of long-lasting changes in

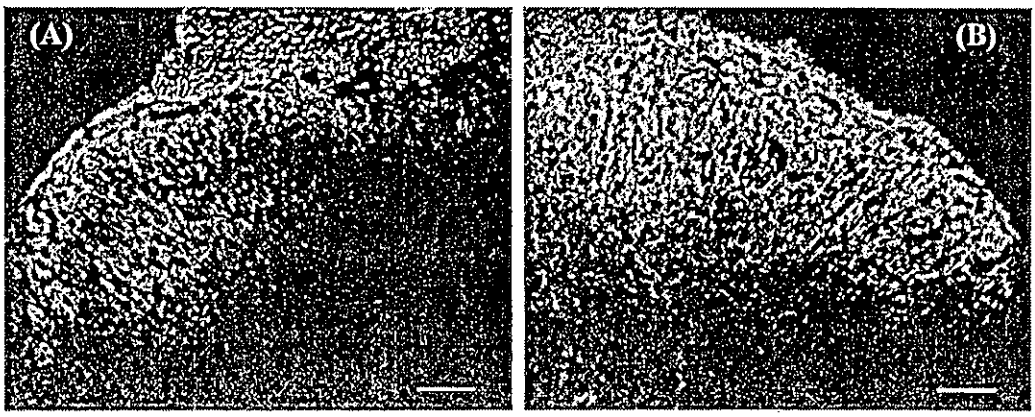

Fig. 6. Immunofluorescent staining for phosphorylated-μ opioid receptor-IR (p-MOR-IR) on the dorsal horn of the L5 lumbar spinal cord in nerve-ligated mice. The p-MOR-IR was clearly increased on the ipsilateral side in the superficial laminae of the L5 lumbar spinal dorsal horn in nerve-ligated mice (B) as compared to that observed on contralateral side (A). Scale bars; 50 μm.

the neuronal transmission at the supraspinal site under the neuropathic pain-like state, the phosphorylation of the MOR in the spinal cord under a neuropathic pain-like state may, at least in part, contribute to the suppression of the antinociceptive effect produced by morphine.

Change in brain dynamics under chronic pain-like state

Direct evidence for spinal PKC in the expression of chronic pain-like state

The neuropathic pain following nerve injury or tissue inflammation depends both on an increase in the sensitivity of these first synapses at the site of injury and on an increase in the excitability of neurons in the CNS (Bessou and Perl, 1969; Beitel and Dubner, 1976; Woolf, 1983). A growing body of evidence suggests that several second messenger systems have been implicated in the development or maintenance of hyperalgesia induced by nerve injury. As mentioned previously, we demonstrated that the up-regulation of cPKC in the spinal cord was observed following sciatic nerve ligation in mice. Furthermore, a specific PKC activator, phorbol 12,13-dibutyrate (PDBu), when given i.t., produced a long-lasting hyperalgesic behavior as indicated by severe tail-shaking, vocalization, scratching and biting behaviors in a dose-dependent manner in mice (unpublished data). Collectively, these findings provide further evidence that the activation of spinal PKC is closely related to the development or maintenance of central sensitization to nociceptive transmission.

It is considered to be worthwhile to investigate the ascending nociceptive transmission from the dorsal horn to brain areas involved in the processing of noxious stimuli. Second-order neurons ascend the spinal cord to terminate in many supraspinal structures throughout the brain stem, thalamus and cortex. In the thalamus, it is well-known that these systems are divided into two main groups such as ventrobasal complex and intralaminar nuclei (Siddall and Cousins, 1998). Former including the ventral posterolateral nuclei and ventral posteromedial nuclei, is involved in the sensory discriminative component of pain and further projects to the somatosensory cortex (Yen et al., 1989; Casey et al., 1994; Siddall and Cousins, 1998). Latter including the central medial nuclei and parafascicular nucleus (PF), is associated with the affective motivational aspects of pain and projects to the cingulated gyrus (CG) (Yen et al., 1989; Siddall and Cousins, 1998). To date, many researchers have expected prompt changes at the supraspinal site during a persistent pain-like state. Previous immunohistochemical study demonstrated that the expression of COX-2-IR in vascular endothelial cells throughout the CNS is enhanced during capsaicin-induced allodynia, indicating that the expression of COX-2 in the brain may be involved in induction of the inflammation-induced hyperalgesia (Ibuki et al., 2003). Furthermore, recent evidence has indicated that peripheral inflammation accompanied with hyperalgesia also alters the structure and increases the permeability of the blood brain barrier (BBB) (Wolka et al., 2003). Taken together, these findings suggest that a persistent pain-like state may lead to functional changes at the supraspinal level as well as spinal level.

Augmentation of c-fos expression is a well-established as a marker of neuronal activation in response to noxious stimuli (Dragunow and Faull, 1989). We previously found that a single i.t. injection of a specific PKC activator, PDBu, caused a marked increase in the number of c-fos-IR expressing cells in the PF, CG and amygdala, but not hippocampus (unpublished data). These findings provide evidence that noxious stimuli activates neurons in the PF, CG and amygdala. Our data support the possibility that

physiological and functional changes in neurotransmission in the PF, CG and amygdala can be occurred by spinal PKC-dependent noxious stimulation.

Influence of activated spinal PKC in the morphine-induced rewarding effect

It has been documented that chronic pain attenuates the development of tolerance to the antinociceptive effect of morphine and some naloxone-precipitated withdrawal signs in rats repeatedly treated with morphine (Vaccarino and Couet, 1993; Vaccarino et al., 1993). Furthermore, various number of clinical studies have suggested that there are only few cases that psychological dependence on opioids is considered to be a serious side-effect, when patients were suffered from severe pain. These findings gave us the idea that pain could lead to physiological and functional changes associated with the decrease in morphine's effect at supraspinal levels in mice. We, therefore, investigated whether direct activation of spinal PKC by PDBu could suppress the place preference induced by morphine in mice using conditioned place preference (CPP) paradigm. It is of interest to note that, s.c. morphine-induced place preference was significantly suppressed by a single i.t. pretreatment with PDBu, (unpublished observation).

Suppression of the rewarding effect and G-protein activation induced by morphine following nerve injury

We first assessed whether morphine could produce rewarding effects and supraspinal antinociception in partial sciatic nerve-ligated mice. As a result, the s.c.-administered morphine-induced place preference was significantly attenuated following nerve ligation (Fig. 7), whereas the supraspinal antinociception induced by i.c.v.-administered morphine was not affect by nerve ligation.

It has been reported that the mesolimbic dopaminergic (DAergic) system, projecting from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens (N.Acc.), has been identified as the critical substrate of the reinforcing effects of morphine (Funada et al., 1993; Narita et al., 2001a). It should be mentioned that the released dopamine in the N. Acc. following morphine treatment is dramatically suppressed by sciatic nerve ligation (Ozaki et al., 2003). It is well documented that MOR located in the VTA has been shown to be critical for opioid reward (Bals-Kubik et al., 1993; Devine and Wise, 1994). In contrast, high density of MOR binding site has also been observed in the pons/medulla regions including the NRM, which is considered to be critical sites to regulate the antinociception of MOR agonists, in rodents (Goodman and Pasternak, 1985; Moskowitz and Goodman, 1985). Considering these backgrounds, we next assessed changes in the ability of morphine to activate G-proteins in the lower midbrain including the VTA, limbic forebrain including the N.Acc. and pons/medulla regions of sham-operated and sciatic nerve-ligated mice by monitoring the binding of guanosine-5′-*o*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) to membranes. Morphine produced a concentration-dependent increase in the binding of [^{35}S]GTPγS to lower midbrain, limbic forebrain and pons/medulla membranes obtained from sham-operated mice. Interestingly, the increased binding of [^{35}S]GTPγS stimulated by morphine in the lower midbrain, but not limbic forebrain or pons/medulla of sciatic nerve-ligated mice, was significantly decreased as compared to that in sham-operated mice (Fig. 8A). However, there was no significant difference in MOR production in the lower midbrain between sham-operated and sciatic nerve-ligated mice (Ozaki et al., 2003). These findings suggest that the MOR function in the VTA area is down-regulated by sciatic nerve injury.

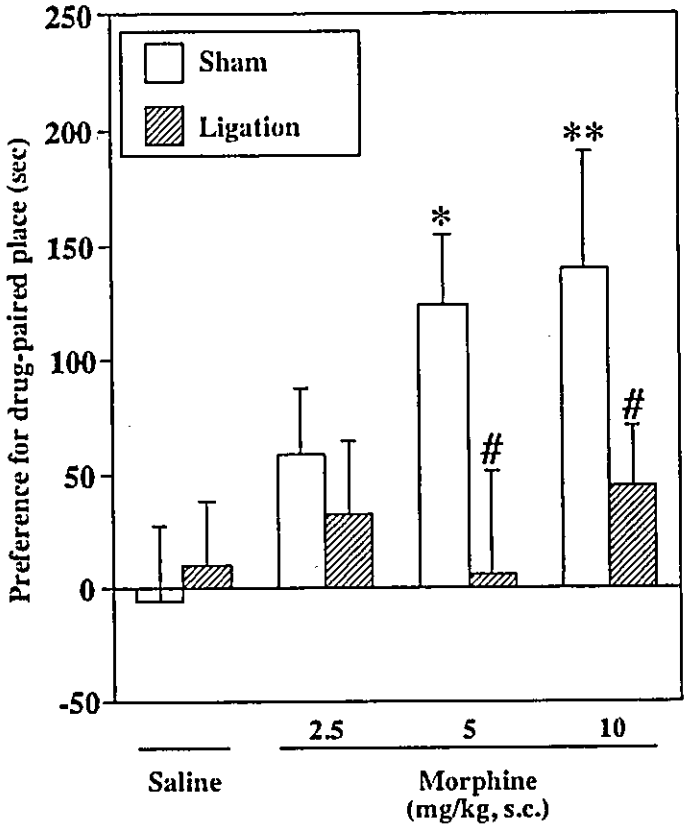

Fig. 7. (A) The place preference produced by s.c. administration of morphine (2.5, 5 or 10 mg/kg) in sham-operated and sciatic nerve-ligated mice using conditioned place preference paradigm. Conditioning sessions (3 for morphine: 3 for saline) were started at 4 days after surgery and conducted once daily for 6 days. Ordinate: mean difference (s) between time spent during the post-conditioning test and pre-conditioning test. Immediately after s.c. injection of morphine or saline, mice were placed and conditioned in either compartment for 1 hr. The data represent the mean ± S.E.M. of 12–16 mice. *$p < 0.05$, **$p < 0.01$ vs. saline group. #$p < 0.05$ vs. sham group.

A myriad of MOR-mediated responses are diminished by repeated exposure to selective agonists (Chavkin et al., 2001). One mechanism of the molecular basis of reduction in MOR function may be the uncoupling of a receptor from its effector system due to receptor phosphorylation. The decrease in the MOR function is referred to as MOR desensitization. It has been proposed that PKC is implicated in the desensitization of MOR-mediated actions. μ-Opioids have been shown to stimulate the βγ subunits of their G-proteins and PKC (Kramer and Simon, 1999). It is of interest to note that repeated intrathecal administration of the MOR agonist activates PKC and in turn causes the desensitization of MOR-mediated G-protein activation in the mouse spinal cord, indicating the negative feedback modulation of MOR-mediated responses by PKC (Narita et al., 2001b). A serine/threonine kinase, GRK2, has also been shown to promote agonist-induced phosphorylation and to lead to an attenuation of the morphine-mediated inhibition of adenylyl cyclase (Zhang et al., 1998). It should be mentioned that GRK2 induces the homologous desensitization of MORs onto the GABAergic neurons in the NRM (Li and Wang, 2001). In our recent study, we observed that the level of membrane-bound GRK2 in the lower midbrain of sciatic nerve-ligatied mice was significantly greater than that in sham-operated mice, whereas no change in the protein level of GRK2 was observed in membranes of the pons/medulla

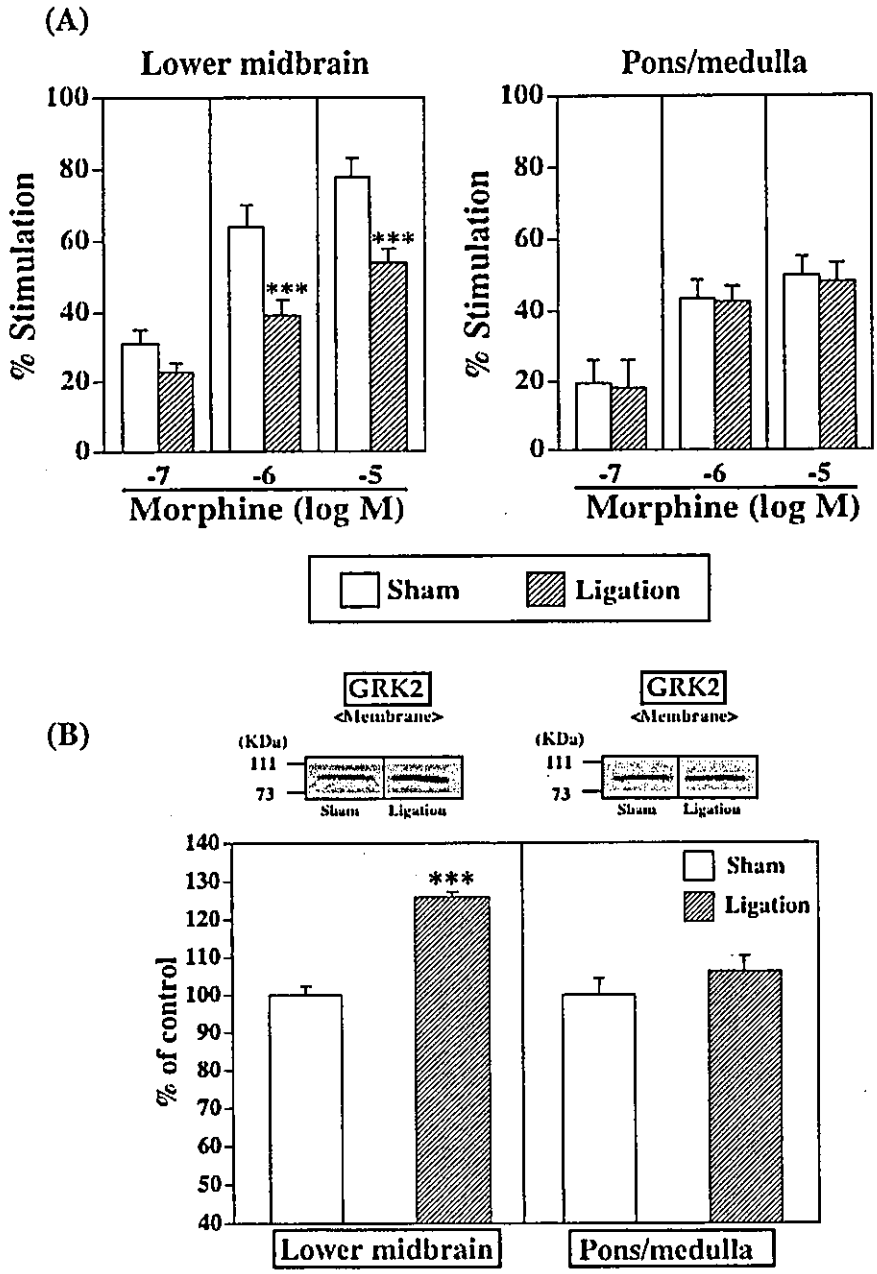

Fig. 8. (A) Effect of morphine on the binding of guanosine-5′-*o*-(3-[^{35}S]thio) triphosphate ([^{35}S]GTPγS) to membranes of the lower midbrain (left) and pons/medulla (right) obtained from sham-operated and sciatic nerve-ligated mice. ***$p < 0.001$ vs. sham groups. (B) Immunoblot analysis of protein levels of the membranous fraction of G-protein-coupled receptor kinase 2 (GRK2) in the lower midbrain (left) and pons/medulla (right) obtained from sham-operated and sciatic nerve-ligated mice. Each column represents the mean ± S.E.M. of three samples. ***$p < 0.001$ vs. sham group.

following sciatic nerve ligation (Fig. 8B, Ozaki et al., 2003). On the contrary, the level of p-cPKC in membranous fractions of the lower midbrain was not changed by sciatic nerve ligation. These findings suggest that the increased level of GRK2 in membranes of the lower midbrain may reduce MOR function in this area under sciatic nerve ligation, leading to the inability of morphine to induce a place preference.

Role of extracellular signal-regulated kinase (ERK) in the suppression of morphine-induced rewarding effect following nerve injury

MAPKs, which include ERK, p38 and c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK), are serine/threonine kinases that play a critical role in cell growth and survival (Davis, 1993). MAPK signals, especially ERK and p38, have been shown to be directly regulated by opioid receptors (Belcheva et al., 1998; Zhang et al., 1999). It has been reported that chronic administration of morphine increases ERK activity in the VTA, and ERK activation in this region is associated with the morphine-induced increase in activities of tyrosine hydroxylase (TH), which is the rate-limiting enzyme in dopamine (DA) biosynthesis (Berhow et al., 1996). Therefore, we investigated whether ERK could be essential for the rewarding effects of morphine, and sciatic nerve ligation could affect the activities of ERK in the mouse lower midbrain area including the VTA.

Immunoblot analysis with the cytosolic fraction showed that the level of phosphorylated-ERK (p-ERK) in the region was significantly and maximally decreased at 4 days after sciatic nerve ligation without any changes in basal protein levels of ERK. The next study was then to investigate whether the activation of ERK can be directly associated with the development of the morphine-induced rewarding effect in mice. The i.c.v. pretreatment with PD98059 (2′ -amino-3′ -methoxyflavone) and U0126 (1,4-diamino-2,3-dicyano-1,4-*bis*(2-aminophenylthio)butadiene), which are the specific inhibitors of ERK kinase (MEK) and an upstream regulators of ERK, caused a significant reduction in levels of p-ERK in the lower midbrain of normal mice. Under these conditions, both PD98059 and U0126 significantly inhibited the place preference induced by morphine in a dose-dependent manner in normal mice (Fig. 9A, Ozaki et al., 2004). These findings indicate the possible involvement of ERK activation in rewarding processes of morphine. Furthermore, double-immunolabeling experiment with antibodies against the DAergic neuron marker TH and p-ERK demonstrated that almost all of p-ERK immunoreactivity was expressed within TH-positive neurons in the VTA of sham-operated mice. Following sciatic nerve ligation, the drastic decrease in p-ERK immunoreactivity was detected in the VTA (Fig. 9B and C, Ozaki et al., 2004). The up-regulation of TH activity would be expected to increase the activity of DAergic neurons, resulting in the substantial increase of DA release. It should be pointed out that activated ERK can directly activate TH and also regulate TH expression (Guo et al., 1998; Lindgren et al., 2002). Taken together, these findings rise the possibility that the sustained down-regulation of ERK activity in the VTA after sciatic nerve injury may decrease the TH activity, resulting in a significant reduction in the morphine-induced DA release in the N.Acc.

As previously shown in Fig. 8A, we found that sciatic nerve ligation reduced MOR function to activate G-proteins in the VTA. This functional reduction of MOR may lead to the inhibition of ERK activity, because the stimulation of MOR can directly activate ERK in a Ras-dependent manner (Belcheva et al., 1998). However, the double-labeling experiment indicates that many positive responses for p-ERK-IR were seen within TH-positive cells in the VTA. In contrast, various studies provide evidence that MORs in the VTA are located mainly within non-DA (TH-negative) neurons (Garzon and Pickel, 2001). These findings suggest that the reduced ERK activity in the VTA by sciatic nerve ligation may not be directly linked to the down-regulation of MOR functions under a state of neuropathic pain.

The specific reason why sciatic nerve ligation caused the decrease in ERK activity in the VTA DAergic neurons remains unclear, however, this could be explained by the fact that the VTA region is involved in the processing of nociceptive information. Indeed, neurochemical lesion of the VTA DA neurons produced by injection of 6-hydroxydopamine increases the behavioral response to pain triggered