Feature Articles

新たなる視点で捉えた「侵襲(Stress)と麻酔」
―周術期病態制御の新機軸として
「今」注目される分子・pathway―

ストレスと副腎髄質と麻酔
Stress, Adrenal Medulla and Anesthesia

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様々なストレスによってカテコラミンが分泌され，さらに副腎髄質においてカテコラミン合成酵素のチロシン水酸化酵素のmRNAも増加する．ストレスがなくなった後もカテコラミンの分泌が持続することがある．このためストレス後にもカテコラミン分泌増加による弊害が発生する．同様のことが手術侵襲によっても発生する可能性があり，ストレスによる副腎髄質でのmRNAレベルの変化をコントロールすることが，術後痛や予後の面からも麻酔管理において重要な点の1つだと考えられる．

はじめに

周術期には，手術侵襲（surgical stress）を含めて，不安，緊張や恐怖などのストレスが生体に加わる．これらは生体にとって有害な刺激であり，これに対して神経系，内分泌系，免疫系，および代謝系などが密接に関係して生体防御的に反応する[1]（表1）．ストレスに対する反応は，侵襲の種類や程度，持続時間や個々の生体の予備能などによって規定される．麻酔管理の目標の1つは，手術侵襲による有害な作用を防ぎ，生体反応を調節し手術を安全に終えることである[2]．麻酔は，意識を消失させ呼吸器系や循環器系を抑制しそれ自体も侵襲であるので，手術侵襲とのバランスを考えたうえで統合的に生体を管理しなければならない．麻酔管理の指標は，この手術侵襲に対する生体反応を抑制する程度をもって評価されてきた．本稿では，ストレス時の副腎髄質の変化（カテコラミンの合成や分泌など）に注目して，手術侵襲と麻酔管理について考察する．

表1　手術に対する全身性の反応（文献1より引用・改変）

交感神経系活性化
内分泌系のストレス応答
下垂体ホルモン分泌
インスリン抵抗性
免疫学的および血液学的な変化
サイトカイン産生
急性期反応
好中球増加
リンパ球増加

ストレスに対する交感神経系と副腎髄質の反応

ストレスにより生体に起きる反応は，Cannonが提唱したfight or flight，すなわち戦うか恐怖のため逃げ出すかという反応である．交感神経系が興奮し，瞳孔散大，立毛，血圧上昇，骨格筋への血流増加，体全体の代謝亢進，および血液凝固系亢進などがみられる．エネルギーとしてブドウ糖や脂肪酸を供給するため，グリコーゲン分解により血糖が上昇し，脂肪分解により遊離脂肪酸が増加する．これらの反応は，交感神経系および副腎髄質からのカテコラミン分泌増加を介した急性反応として発生する．副腎髄質は，外部からの種々のストレスに反応してアドレナリンとノルアドレナリンを分泌し，交感神経終末からはノルアドレナリンが分泌される．ストレスに対するこの反応は有用な反応であり，カテコラミン合成酵素を欠損したマウスは成体まで成長できず寒冷ストレスで死亡することが報告[3]され，カテコラミンは生存やストレス反応にとって重要であることが明らかとなった．しかし，ストレスに対するこのカテコラミン分泌増加反応は，反面，その後持続する有害な影響をもたらすことがある．例えば，アドレナリンとノルアドレナリンは心理的ストレスの記憶と関係があり，心的外傷後ストレス障害（Posttraumatic Stress Disorder：PTSD）患者では持続的に増加していることが報告されている[4]．

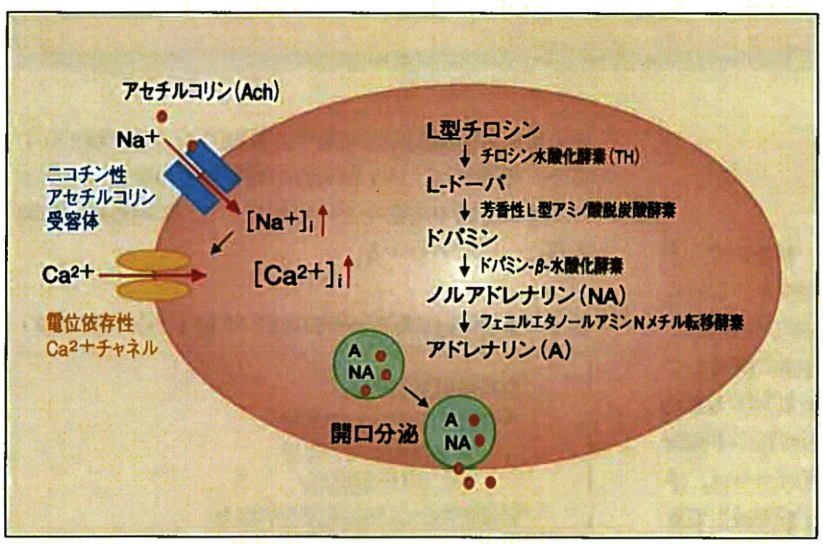

図1　副腎髄質細胞におけるカテコラミンの合成と分泌
大内臓神経終末からのアセチルコリンが神経型ニコチン性アセチルコリン受容体に作用し，細胞内にNa^+が流入し，細胞膜に脱分極が発生し電位依存性Ca^{2+}チャネルから細胞内にCa^{2+}が流入し，細胞内Ca^{2+}の上昇により，顆粒から開口分泌によってカテコラミンが分泌される．

ストレスと副腎髄質の研究

1. 副腎髄質でのカテコラミンの合成

L型チロシンが，チロシン水酸化酵素（Tyrosine Hydroxylase：TH）によりL-ドーパになり，ドパミンを経てノルアドレナリンになる．さらに副腎髄質にはフェニルエタノールアミンNメチル転移酵素があり，アドレナリンが産生され顆粒に貯蔵される．この合成の律速酵素は，THである（図1）．

2. 拘束ストレスによるラット副腎髄質での変化

ラットの四肢を単にテープで30分間拘束するのみで副腎髄質のTHが4倍に増加する[5]．拘束を持続させるとTHのタンパクレベルは急速に増加し，またTHのmRNAレベルは1回拘束するだけでも増加する．2時間の拘束によるTHmRNAの増加は1日以内に元のレベルに戻るが，拘束を繰り返すとTHmRNAの増加は，拘束をやめた後も1週間以上にわたってコントロールの3倍以上の高さで持続した[5]．このストレスに対する副腎髄質のカテコラミン合成酵素のmRNAの調節には，転写レベルでの調節が重要である．副腎髄質細胞において転写調節因子（cAMP Response Element Binding Protein：CREB）がリン酸化されることが，この調節には重要であることが報告された[5]．副腎髄質のCREBのリン酸化はラットを5分間拘束するだけで生じた．同じ拘束ストレスにより上位中枢の青斑核でもTHmRNAが増加し，ノルアドレナリン分泌が増加する[4]．このことはPTSDの発症と関係することが指摘されている．様々なストレスにより副腎髄質細胞でカテコラミン合成酵素mRNA発現量が増加し，

カテコラミンの合成分泌が促進されることから，生体のストレスに対する反応において副腎髄質は重要な役割を果たしている．

副腎髄質細胞を使用した麻酔薬の研究

副腎髄質細胞は，発生学的に神経堤（neural crest）に由来する細胞である．神経堤に由来する細胞にはほかに自律神経，脊髄後根神経節，およびクロマフィン細胞などがある．発生学的には，副腎髄質は交感神経節後線維が変化したものと考えられる．生理学的には，副腎髄質は大内臓神経に支配される．大内臓神経終末から分泌されたアセチルコリンが副腎髄質細胞の神経型ニコチン性アセチルコリン受容体に作用することによって細胞内にNa^+が流入し，細胞膜に脱分極が発生し，電位依存性Ca^{2+}チャネルから細胞内にCa^{2+}が流入する．細胞内Ca^{2+}の上昇により，顆粒から開口分泌によってカテコラミンが分泌される（図1）．副腎髄質細胞は，神経細胞に類似し培養も比較的容易だったので様々な麻酔薬の作用機序の研究に使用されてきた．副腎髄質細胞には，神経型ニコチン性アセチルコリン受容体だけでなく，電位依存性Na^+チャネルの$Na_v1.7$や電位依存性Ca^{2+}チャネル，ノルアドレナリントランスポータ（noradrenaline transporter）など様々な受容体やチャネルが存在する．表2に示した麻酔関連薬のEC_{50}は臨床において麻酔管理中に使用される範囲内の濃度であり，それぞれ副腎髄質細胞においてカテコラミン分泌を低下させた（ロクロニウムのデータは投稿中）．

表2　副腎髄質細胞を使用した麻酔関連薬の研究

	nAChR	NAT	Na channel ($Na_v1.7$)
ハロタン[6] エンフルラン[7] イソフルラン[8]	↓ ↓ ↓		
パンクロニウム[9,10] ベクロニウム[9,10] ロクロニウム	↓ ↓ ↓	↓ ↓	
ペントバルビタール[6] ケタミン[11,12] プロポフォール[13]	↓ ↓ ↓	↓ ↓	
リドカイン[14] ブピバカイン[15]	↓ ↓		↓ ↓

nAChR：ニコチン性アセチルコリン受容体，NAT：ノルアドレナリントランスポータ，↓：抑制作用．

麻酔管理と血中カテコラミン

麻酔管理において交感神経をコントロールすることは重要である[16]．麻酔の目標にストレスフリーがある．手術侵襲によって様々な内分泌反応が生じることが報告されている（表3）[1]．麻酔を管理するうえでの指標としてストレスホルモンの1つである血中カテコラミン濃度が測定された[17]．手術侵襲による血圧上昇や心拍数の増加を50%の患者で防止することができる吸入麻酔薬の肺胞濃度をMAC-BAR（Minimum Alveolar Concentration Blocking Adrenergic Responses）として麻酔管理が行われた[18]．しかし，手術侵襲による副腎髄質からのカテコラミン分泌は，吸入麻酔薬のみでは十分に抑制できず，オピオイドや硬膜外麻酔が併用される．最近では予後も考慮してβ-ブロッカーやクロニジンが使用されている．

麻酔中の手術侵襲を評価することを目的とした様々なモニタリング方法が考案されている．多くは手術侵襲に対する交感神経系の変化を評価するものであり，筋肉の微小交感神経反応，心拍変動解析や圧受容体反射などがあり，麻酔中に応用することが検討されている．最近，Surgical Stress Index（SSI）をHeart Beat Interval（HBInorm）とPlethysmographic Pulse Wave Amplitude（PPGAnorm）からコンピュータで処理し算出する方法[19]や皮膚の伝導度（skin conductance）から評価する方法[20]が紹介されている．しかし，これらのモニタリングは手術侵襲による血圧上昇や心拍数変化を反映させたものであり，副腎髄質でのmRNAレベルでの変化については不明である．ラット拘束ストレスの研究と同様に

手術侵襲によって副腎髄質でTHmRNAが増加すれば，術後カテコラミン合成分泌の増加した状態が持続することになり，術後にβ-ブロッカーやクロニジンなどを投与することによって循環動態をコントロールすることが必要になると考えられる．直接副腎髄質の変化を調べることは困難だが，手術後に自律神経系の変化が持続するという報告がある[21]．ラットにおいて同じ拘束ストレスにより右心室の組織カテコラミンが増加し，β-adrenergic receptorのmRNAレベルとタンパクレベルが変化するという報告がある[22]．この拘束ストレスによる変化は正常ラットでの変化であり，心疾患や高血圧などを合併していればより複雑な変化になると思われる．

一方，免疫系とカテコラミンには密接な関係があり，カテコラミンの分泌増加は細胞性免疫を抑制することが報告されている[23]．術中，硬膜外麻酔によりストレス反応を抑制すれば，ストレスによる周術期の免疫機能低下を予防できると報告されている[24]．

SSIなどの交感神経系の状態から術後痛を評価する方法も報告されている[25]．痛みはそれ以外の様々なストレスによって増強される．例えば，ラットでは侵害性刺激ではない，音によるストレスのみでブラジキニンによる痛みが増強した[26]．この音による痛みの増強効果は，副腎髄質を摘出したり副腎髄質を支配する神経を切除することで改善された．このことからストレスによる副腎髄質からのアドレナリン分泌増加によって，痛みが増強されることが指摘されている[27,28]．硬膜外麻酔を使用したpre-emptive analgesiaは，痛みによって発生する脊髄での神経の可塑的変化を抑制することができ，術後痛のコントロールに有用と考えられている．硬膜外麻酔を併用することで，術後の問題点として注目されているPersistent Postsurgical Pain（PPP）も減少できると指摘されているが明白ではない[29,30]．炎症性の痛みなどにはcyclo-oxygenase（COX）阻害薬やオピオイドも使用したmultimodal analgesiaが必要とされている[30]．交感神経－副腎髄質系は脊髄や脳幹のみならず，より上位の中枢である視床下部，大脳辺縁系，小脳および大脳皮質によって総合的かつ協調的に調節されているため，副腎髄質を高位硬膜外麻酔でブロックしても十分ではない．上腹部や胸部の手術による副腎皮質からのコルチゾール分泌を抑制するには第6頸髄までの硬膜外麻酔によるブロックでも不十分である[31]．より上位の中枢ホルモンである成長ホルモンや副腎皮質刺激ホルモンなどは分泌され続けているからである．硬膜外麻酔の有用性を証明しようとした大規模な研究があるが，術後呼吸不全低減や短期予後には有用性が認められたが，長期予後については認められなかった[32,33]．全身麻酔に硬膜外麻酔だけでなくオピオイドを併用するなどmultimodalな麻酔管理が重要と考えられている．

麻酔管理の問題点と展望

ストレスと副腎髄質でのカテコラミン合成分泌の変化は密接に関係している．最近の麻酔管理目標は，ストレスフリー状態で術後の早期回復をはかることである（enhanced recovery）[34]．さらに麻酔管理によって長期予後（outcome）も改善することがそのエンドポイントとして考えられている．筋弛緩薬による不動状態で術中覚醒が起これば，拘束ラットと同様の状態で術後にカテコラミン分泌が持続し，PTSDを発症する可能性があり

表3　手術による主要なホルモンの反応（文献1より引用・改変）

内分泌器官	ホルモン	変化
下垂体前葉	副腎皮質刺激ホルモン	↑
	成長ホルモン	↑
	甲状腺刺激ホルモン	↑ or ↓
	卵胞刺激ホルモン，黄体形成ホルモン	↑ or ↓
下垂体後葉	バソプレシン	↑
副腎皮質	コルチゾール	↑
	アルドステロン	↑
膵臓	インスリン	↓
	グルカゴン	軽度↓
甲状腺	甲状腺ホルモン	↓

↑：増加，↓：減少．

BIS（Bispectral Index）などによる意識レベルのモニタは必須であろう．BISは麻酔深度の指標であり，手術侵襲（カテコラミン濃度）の状態とは相関しなかった[35]．イソフルランはfear memoryを防御できるようである[36]．

また，術後痛についても鼠径ヘルニアなどの低侵襲手術でも10%に，乳房切断術でも20～30%に術後痛が持続することがあり，このうちの2～10%に重症PPPが発生する．多様な鎮痛手段による管理の必要性が指摘されている[30,37]．

また，麻酔自体もストレスであることを忘れてはならない．イソフルランやプロポフォールは痛みに関係するTRPA（Transient Receptor Potential Ankyrin）1 受容体を活性化し術後の痛みを増強する可能性が指摘されている[38]．新生児に全身麻酔をすると幼若な発達中の神経細胞にアポトーシスを起こし神経障害を起こす可能性を示唆する報告[39]や，けいれんを発症させる報告[40]がある．これらの麻酔薬の負の作用も考慮して，手術侵襲とのバランスを考えて麻酔管理を行わねばならない．

手術侵襲（ストレス）によるカテコラミン合成分泌変化は，術後循環動態や術後痛などにも関係する可能性が示唆され，有害な手術侵襲を減少させ，生体反応を調節し長期予後を見すえた周術期管理が求められている（図2）[41]．

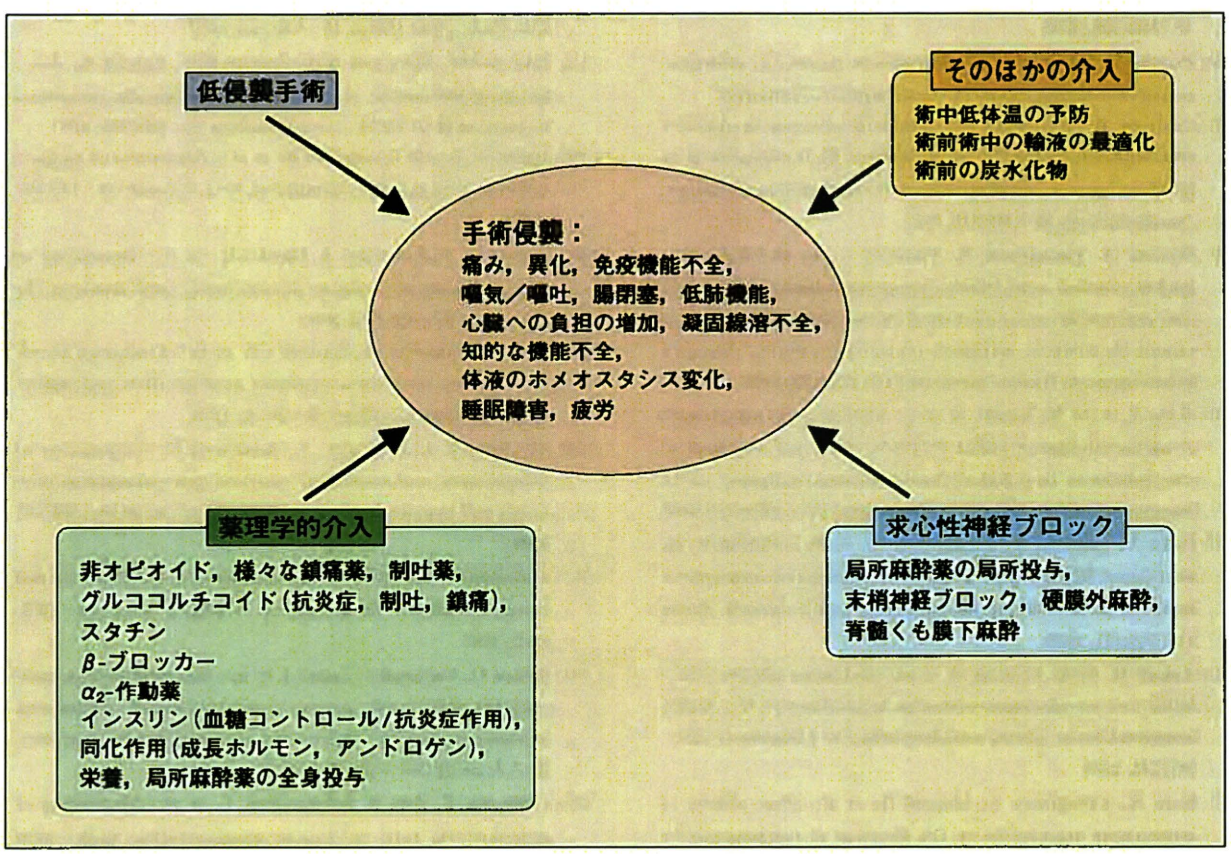

図2　周術期ストレスを減少させるための最近の主流（文献41より引用・改変）

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別紙4

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ

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Narita M et al.	Implication of dopaminergic projection from the ventral tegmental area to the anterior cingulate cortex in m-opioid-induced place preference.	Addict. Biol.	15	434-447	2010

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PRECLINICAL STUDY: BRIEF REPORT

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Epigenetic modulation at the CCR2 gene correlates with the maintenance of behavioral sensitization to methamphetamine

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ABSTRACT

The intermittent administration of methamphetamine produces behavioral sensitization to methamphetamine. In the limbic forebrain, mainly including the nucleus accumbens, of mice that had been intermittently treated with methamphetamine, we found a significant increase in mRNA of a chemokine, CCR2. This increase was accompanied by a significant increase in histone H3 lysine 4 (H3K4) trimethylation at its promoter. Interestingly, the maintenance of sensitization to methamphetamine-induced hyperlocomotion was significantly decreased in CCR2 knockout mice. These findings suggest that increased CCR2 associated with epigenetic modification after the intermittent administration of methamphetamine may be associated with the maintenance of sensitization to methamphetamine-induced hyperlocomotion.

Keywords CCR2, drug abuse, epigenetics, histone modification, methamphetamine, sensitization.

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Methamphetamine is a strongly addictive psychostimulant that dramatically affects the central nervous system (CNS) and is highly abused worldwide. In rodents, it has been shown consistently that repeated exposure to psychostimulants results in a progressive and enduring enhancement of the motor stimulant effect elicited by a subsequent drug challenge, which is called behavioral sensitization. Many studies have suggested that the mesolimbic dopaminergic system, which projects from the ventral tegmental area to the nucleus accumbens, is critical for the initiation of methamphetamine-induced hyperlocomotion (Vanderschuren & Kalivas 2000).

A growing body of evidence suggests that the behavioral sensitization induced by psychostimulants may be accompanied by long-lasting neural plasticity (Robinson & Kolb 1999). The neuronal plasticity has been believed to require diverse alterations in gene expression. Although some of the candidate genes that are involved in behavioral sensitization to psychostimulants have been identified (Ujike *et al.* 2002; Sokolov, Poiesskaya & Uhl 2003), an important step toward unraveling the complex machinery of psychostimulant-induced behavioral sensitization is a multiplex analysis for both gene expression profiling and epigenetic modifications, which exert lasting control over gene expression without altering the genetic code.

Recent evidence has suggested that epigenetic mechanisms contribute to drug-induced transcriptional and behavioral changes (Renthal & Nestler 2008). Such epigenetic modulation is mainly controlled by histone modification. Histones are modified at many sites. Previously published reports have indicated that the increased acetylation of histone H3 or methylation of H3 at K4 (lysine 4) highly predicts gene activation, while increased methylation of H3 at K9 or K27 (lysine 9 or 27) is predictives of gene repression. The triggering of signaling cascades in target neurons leads to more long-lasting effects, including changes in gene expression via the control of transcription and thereby, chromatin remodeling.

In the present study, we report for the first time that the intermittent administration of methamphetamine increases the mRNA level of a C-C chemokine, CCR2, with histone modification in the limbic forebrain including the nucleus accumbens. To address the functional relevance of this increased CCR2 expression, we also investigated whether the increased locomotion observed after the intermittent administration of methamphetamine could be affected in CCR2 knockout mice.

The locomotor activity of mice was measured by an ambulometer (ANB-M20, O'Hara, Tokyo, Japan) as described previously. Briefly, male C57BL/6J mice or CCR2 knockout mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and individually placed in a tilting-type round activity cage. To induce behavioral sensitization to methamphetamine-induced hyperlocomotion, the mice were given five intermittent treatments with methamphetamine (2 mg/kg, s.c.), once every 96 hours. To clarify the maintenance of behavioral sensitization, the mice were again administered methamphetamine (2 mg/kg, s.c.) after seven weeks of withdrawal.

The limbic forebrain area, mainly including the nucleus accumbens, was removed 24 hours after the last injection of methamphetamine. RNA preparation and semiquantitative analysis by reverse transcription polymerase chain reaction (RT-PCR) were performed as described previously. The methods are described in detail in the Supporting Information. The primers used are listed in Table S1.

Chromatin immunoprecipitation (ChIP) was performed as described previously with minor modifications. Soluble chromatin extracted from the mouse limbic forebrain was incubated with specific antibodies against acetylated histone H3 (Millipore; Billerica, MA, USA), H3K4 trimethylation (Wako Pure Chemicals, Osaka, Japan), H3K9 trimethylation (Millipore) and H3K27 trimethylation (Millipore) overnight at 4°C. The immunocomplex was collected by Dynabeads Protein A (Invitrogen Dynal AS, Oslo, Norway), and DNA was recovered by isopropanol precipitation. The methods are described in detail in the Supporting Information. The statistical analysis is also described in detail in the Supporting Information.

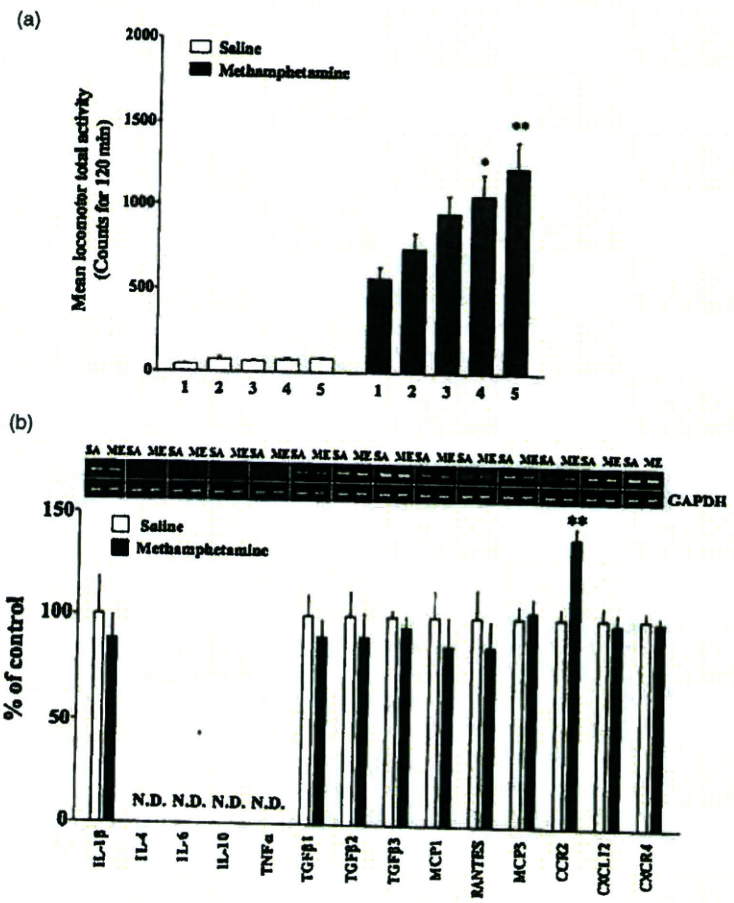

Figure 1 (a) Development of sensitization to methamphetamine in mice. Methamphetamine (2 mg/kg, s.c.) or saline was repeatedly given five times to mice every 96 hours. Total activity was counted for 120 minutes after each injection. Each column represents the mean total counts for 120 minutes with S.E.M. of 20 mice. (*$P<0.01$, **$P<0.001$ versus METH 1st). (b) Upper: Representative RT-PCR with 35 cycles for IL1β, IL-4, IL-6, IL-10, TNFα, TGFβ1, TGFβ2, TGFβ3, MCP1, RANTES, MCP3, CCR2, CXCL12 and CXCR4 mRNAs in the limbic forebrain of mice that have shown behavioral sensitization to methamphetamine (ME). The limbic forebrain sample was prepared 24 hours after the last injection of saline (SA) or ME. Lower: The intensity of the aforementioned bands was semi-quantified using Image J software. The value for mRNA was normalized by that for the internal standard glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The value for mice treated with methamphetamine is expressed as a percentage of the increase in mice treated with saline. Each column represents the mean ± SEM ($n=3$ animals per group; three independent experiments). N.D., not detectable. **$P<0.01$ versus saline-treated mice

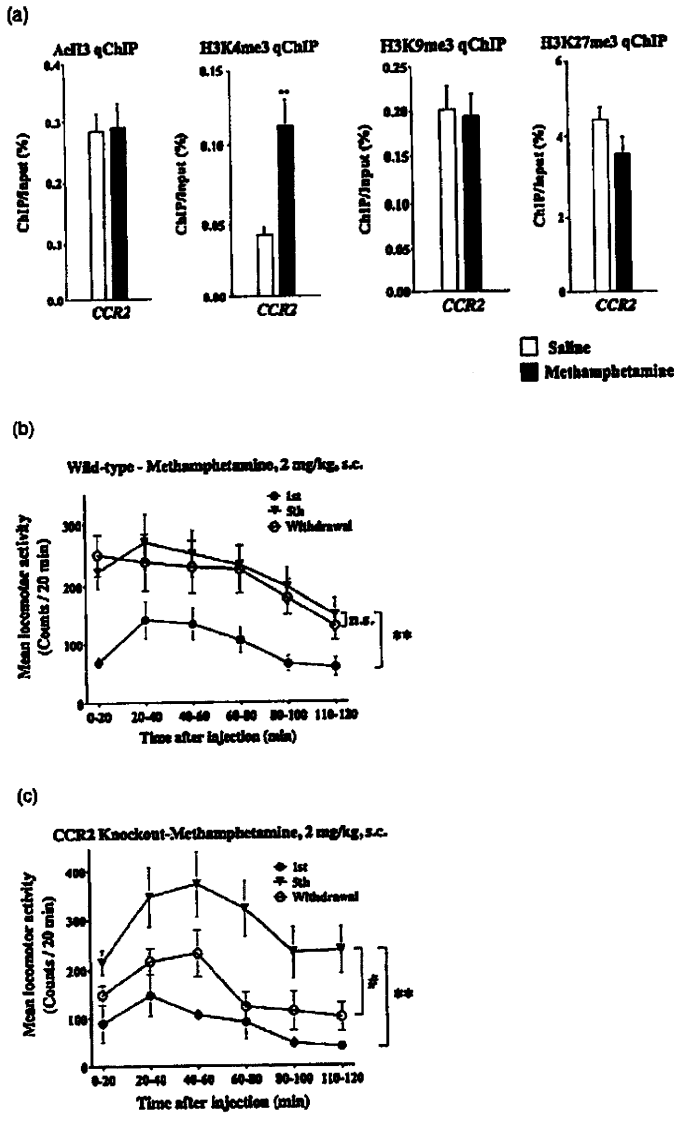

Figure 2 (a) qChIP analysis of acetylated histone H3 (AcH3), histone H3 trimethylated at lysine 4 (H3K4me3), lysine 9 (H3K9me3), and lysine 27 (H3K27me3) at CCR2 loci in the limbic forebrain of mice that had been intermittently treated with methamphetamine. Each column represents the mean ± SEM ($n = 4$ animals per group; three independent experiments). $^{**}P < 0.01$ versus saline-treated mice. (b) Change in locomotor activity (per 20 minutes time intervals) following intermittent administration of methamphetamine (2 mg/kg, s.c.) in wild-type mice (B-i) or CCR2 knockout mice (B-ii). Mice were treated intermittently with methamphetamine every 96 hours for five sessions. '1st' represents the 1st injection group, whereas 5th shows the 5th injection group. Mice described as 'withdrawal' were again administered methamphetamine after seven weeks of withdrawal. $^{**}P < 0.01$, 1st versus 5th, $\#P < 0.05$, 5th versus withdrawal (two-way ANOVA). n.s., not significant. Each point represents the mean ± SEM ($n = 4$–17 mice)

As shown in Fig. 1a, intermittent injection of methamphetamine produced a progressive increase in methamphetamine-induced locomotion, indicating the development of sensitization to methamphetamine (Fig. 1a, $F_{(4, 95)} = 4.940$, $P < 0.01$, first session versus fifth session).

As shown in Fig. 1b, a significant increase in mRNA of CCR2, but not of IL-1β, IL-4, IL-6, IL-10, TNFα, TGFβ1, TGFβ2, TGFβ3, MCP-1, RANTES, MCP-3, CXCL12 or CXCR4, was observed in the limbic forebrain, mainly including the nucleus accumbens, of the mice that had shown behavioral sensitization to methamphetamine (Fig. 1b, $P < 0.01$ versus the saline-treated mice). CCR2 is a seven-transmembrane-spanning Gαi protein-coupled receptor for a member of the C-C chemokine family, MCP-1, and is considered to regulate various brain disorders (Yong & Rivest 2009).

To gain further insight into these phenomena, we next studied histone modifications at the promoter regions of the CCR2 gene (Iida *et al.* 2008). A ChIP assay, where tissue is lightly fixed to crosslink DNA with histones and other DNA-binding proteins and then immunoprecipitated for a protein of interest, can be used to assess the extent to which a given gene is associated with these markers of activation or repression. In this study, we analyzed two active histone modifications [acetylation of

histone H3 and trimethylation of lysine 4 on histone H3 (H3K4)] and two repressive histone modifications [trimethylation of lysine 9 on histone H3 (H3K9) and trimethylation of lysine 27 on histone H3 (H3K27)] at the CCR2 gene promoter in the limbic forebrain of mice that had been intermittently treated with methamphetamine. As a result, intermittent treatment with methamphetamine caused a significant increase in the level of H3K4 trimethylation at the CCR2 promoter in the mouse limbic forebrain (Fig. 2a, $P<0.01$ versus the saline-treated mice; Figs S1 and S2). Methamphetamine did not produce other histone modifications at the CCR2 gene promoter (Fig. 2a). To the best of our knowledge, the present data are the first to indicate that intermittent treatment with methamphetamine induces a dramatic increase in the expression of the CCR2 gene along with epigenetic modifications in the nucleus accumbens.

To address the functional relevance of the increased CCR2 expression after the intermittent administration of methamphetamine, we next investigated whether the reduction of CCR2 expression could affect behavioral sensitization to methamphetamine using CCR2 knockout mice (Fig. S3). As shown in Fig. 2b, the fifth injection of methamphetamine produced a dramatic and significant increase in methamphetamine-induced hyperlocomotion compared with the first injection in both C57BL/6J (wild-type) and CCR2 gene knockout mice to the same degree (wild-type: first versus fifth, $F_{(1,\ 160)} = 12.39$, $P < 0.01$, CCR2 knockout: first versus fifth, $F_{(1,\ 30)} = 20.00$, $P < 0.01$), indicating that lack of the CCR2 gene had little or no effect on the development of sensitization to methamphetamine-induced hyperlocomotion. Intriguingly, the sensitization to methamphetamine was maintained even after seven weeks of withdrawal following intermittent administration of methamphetamine in the wild-type mice (fifth versus withdrawal, $F_{(1,\ 110)} = 0.05$, no significant). However, the methamphetamine-induced sensitization was almost reversed after seven weeks of withdrawal in CCR2 knockout mice (fifth versus withdrawal, $F_{(1,\ 30)} = 8.50$, $P < 0.05$, Fig. 2b). These results indicate that CCR2 is implicated in the maintenance of behavioral sensitization to methamphetamine.

In conclusion, the present study suggests that the intermittent administration of methamphetamine increases the mRNA level of CCR2 in association with epigenetic modification at its promoter in the limbic forebrain including the nucleus accumbens, and this may correlate with the maintenance of sensitization to methamphetamine-induced hyperlocomotion.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 qChIP analysis of acetylation of histone H3 (AcH3), H3K4me3, H3K9me3, and H3K27me3 in the limbic forebrain of mice that had been intermittently treated with methamphetamine (2 mg/kg, s.c., 5 times). Tubulin was used as a control for AcH3 and H3K4me3. High levels of AcH3 and H3K4me3 at the TATA box binding protein (Tbp) gene, which is transcriptionally activated within neurons, H3K9 and K27 trimethylation at silenced genes marker major satellite DNA, which probably comprises the functional centromere, and Gbx2 promoter (a homeobox-containing family of DNA-binding transcription factors) are seen in the limbic forebrain of methamphetamine-treated mice. Each column represents the mean ± S.E.M. ($n = 4$ animals per group; three independent experiments)

Figure S2 Representative PCR product with 40 cycles for CCR2 DNA in the limbic forebrain of mice that have shown behavioral sensitization to methamphetamine (ME). The limbic forebrain sample was prepared 24 hours after the last injection of saline (SA) or ME

Figure S3 Analysis of CCR2 mRNA expression by RT-PCR in the mouse whole brain from wild-type (WT) and CCR2 knockout (KO) mice

Table S1 Comprehensive list of all primer sequences used.

Appendix S1 Supplemental methods

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Neuropathic and chronic pain stimuli downregulate central μ -opioid and dopaminergic transmission

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Although morphine and other μ-opioid agonists are the main analgesics for severe pain, these compounds have potential for abuse and/or addiction. This has complicated the use of μ-agonists in the treatment of chronic pain. However, clinical studies show that when μ-agonist analgesics are appropriately used to control pain, actual abuse or addiction does not usually occur, although some risk factors that increase vulnerability need to be considered, including genetic variation. We review recent findings on molecular adaptations in sustained pain models, and propose how these adaptations (including sustained release of the endogenous μ-agonist β-endorphin) can result in decreased abuse potential of μ-agonists in chronic pain states. We also review data on particular gene polymorphisms (e.g. in the μ-receptor gene) that could also influence the relative abuse potential of μ-agonists in clinical pain populations.

Introduction

Morphine and other μ-opioid agonists (μ-agonists) are frequently used for the treatment of cancer pain and moderate to severe non-cancer pain, as well as post-surgical or traumatic pain [1–3]. Longer-acting opioid medications and formulations such as methadone, buprenorphine and sustained-release oxycodone also have utility in the treatment of neuropathic pain [3–5]. However, μ-agonists also have a constellation of side-effects (e.g. acute respiratory depression, chronic constipation and pruritus), in addition to abuse or addiction potential. Furthermore, chronic administration of μ-agonists results in tolerance and dependence.

There has been a substantial increase in the non-medical use of prescription μ-opioids, possibly because of their widespread availability compared with illicit compounds such as heroin. However, abuse or addiction does not usually occur when μ-agonists are used to treat substantial somatic pain [1,6,7]. Patients do show withdrawal signs when there is abrupt cessation of chronic μ-agonist administration. However, this physical dependence *per se* is not sufficient for a diagnosis of abuse or addiction [7,8]. The relative infrequency of developing an addictive disorder *de novo* in this setting lends support to the safe use of μ-agonists for the treatment of severe acute pain, as well as cancer and non-cancer chronic pain [1,6,7].

Chronic pain, including neuropathic pain, often has a negative effect on quality of life, can function as a stressor and increases the incidence of anxiety and depression. The endogenous opioid system has been strongly implicated in nociception, anxiety and stress-responsive hypothalamic–pituitary–adrenal (HPA) axis modulation. Alterations of the expression of genes involved in stress responsiveness have been reported after chronic intermittent exposure to μ-agonists, cocaine, other stimulants and alcohol in animals and in clinical settings [9].

Glossary

Drug abuse: abuse has been defined by various scientific, national and international policy and clinical groups. Among commonly used diagnostic criteria, the Diagnostic and Statistical Manual IV (DSM-IV) focuses on non-medical use of a particular substance resulting in maladaptive patterns of behavior and leading to clinically significant impairment or distress. It is often considered that reward properties of the drug can drive the initial trajectory of drug abuse from early experimentation to regular usage.
Drug addiction: often described as a clinical disorder with greater severity than abuse and including some cardinal signs such as escalation of drug exposure, compulsive drug use, presence of dependence and withdrawal, and clinical and social consequences. Of note, the specific clinical and neurobiological characteristics of addiction are related to the particular substance that is used (e.g. μ-agonists vs. dopaminergic psychostimulants).
Drug dependence: altered physiological state that develops with persistent drug exposure (e.g. of a μ-agonist). This is observed *in vivo* and clinically as the emergence of a pharmacologically characteristic withdrawal syndrome after sudden drug discontinuation.
Neuropathic pain: pain caused by nerve or neuron injury or its related molecular adaptations.
Nociceptive pain: pain caused by ongoing noxious stimuli, such as heat, cold and chemicals, or acute injury.
Place preference paradigm: an experimental paradigm used to investigate the conditioned reward effects of a drug (e.g. a μ-agonist) by repeated pairing with a novel environment. After repeated pairings, an experimental animal can exhibit drug-induced place preference. In other words, it will spend more time in an environment previously paired with a dose of a rewarding drug than in the complementary environment previously paired with vehicle.
Reward: stimulus (e.g. certain drugs) that can be perceived as pleasant or euphoric. This can result in self-administration of the drug by experimental animals or humans.
Reinforcer: event (in this case, administration of a particular drug) that when paired with a particular behavior, will increase the probability of reoccurrence of that behavior. Reinforcing effects of many drugs abused by humans (including μ-agonists) can thus be studied in self-administration paradigms whereby a subject emits a behavior (e.g. a lever press) that results in drug delivery.

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Neuropathic pain is characterized by burning pain, hyperalgesia (an exaggerated pain in response to painful stimuli) and allodynia (pain evoked by normally innocuous stimuli), and can result from long-term functional alterations of primary afferent neurons and/or spinal dorsal horn neurons (containing diverse receptors, protein kinases and neuropeptides) after nerve injury of different etiologies [10].

In this review, we focus on recent experimental studies detailing molecular and neurobiological adaptations that occur as a result of chronic (e.g. neuropathic) pain stimuli [11–16]. Based on these and prior studies, we propose that these adaptations result in decreased abuse potential of μ-agonists used as analgesics in clinical pain conditions. We present data showing that genetic variation (e.g. in the μ-receptor gene *OPRM1*) can further influence the abuse potential of chronic μ-agonist exposure [17,18].

Role of μ-opioid receptor function in drug reward and in neuropathic pain

The mesolimbic dopaminergic system, projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is a crucial mediator of the reinforcing effects of μ-agonists [19–21] (Box 1). Positron emission tomography studies in humans have mapped μ-opioid receptor distribution in brain and have detected substantial populations in areas involved in pain response (e.g. insular cortex and thalamus) and in reward-related areas (e.g. cingulate cortex, mesolimbic system including NAc) [22]. Intriguingly, release of dopamine in the NAc after morphine treatment is markedly suppressed by sciatic nerve ligation [23], a model of neuropathic pain. μ-Receptor binding sites have also been observed in the pons and medulla regions, critical hindbrain sites that regulate μ-agonist-induced antinociception. We therefore assessed changes in the ability of morphine to activate G proteins in the lower midbrain, including the VTA, limbic forebrain, including the NAc, and pons and medulla regions of sham-operated and sciatic nerve-ligated mice by measuring binding of guanosine-5′-*o*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) to membranes [23]. Morphine-induced [^{35}S]GTPγS binding was decreased in the midbrain, including the VTA, but not in limbic forebrain or pons and medulla of sciatic-nerve-ligated mice [23]. This finding suggests that neuropathic pain induced by sciatic nerve ligation leads to a reduction in μ-opioid receptor function in the midbrain, including the VTA, resulting in inhibition of the reward effect of morphine. This selective reduction in μ-opioid signaling in midbrain, including the VTA, is consistent with a decrease in the reward effects of μ-agonists (and thus their abuse potential) in neuropathic pain states, with relative preservation of analgesic effects.

Molecular adaptations in μ-opioid receptor function due to chronic pain

One mechanism for the aforementioned reduction in μ-opioid receptor signaling in VTA in chronic pain states could be a sustained increase in release of the μ-opioid neuropeptide β-endorphin. Sustained exposure to β-endorphin could result in receptor phosphorylation and uncoupling of receptors from effector systems, and thus desensitization. Of note, β-endorphin tends to cause greater desensitization than exogenous ligands such as morphine [24]. A serine/threonine kinase, G protein receptor kinase 2 (GRK2), promotes μ-agonist-induced phosphorylation [25]. The level of membrane-bound GRK2 in the midbrain, including the VTA, but not in the pons and medulla, was increased in nerve-ligated mice relative to controls [23]. This increase in GRK2 in the midbrain might therefore reduce μ-opioid receptor function during sciatic nerve ligation, leading to decreased morphine-induced place preference (i.e. an apparent decrease in morphine-induced reward effects) [23].

Box 1. Opioid reward and the mesolimbic dopamine system

μ-Agonists have marked effects on mood and motivation. They can produce euphoria in humans and function as positive reinforcers (i.e. they maintain drug-seeking behaviors). These reinforcing effects can become the primary stimuli that motivate behavior, with subsequent compulsive drug-seeking behavior or addiction [50,51]. The mesolimbic dopaminergic system (from the VTA of midbrain, projecting to the NAc) is a critical circuit for this effect [54]. Furthermore, μ-agonists increase dopamine release and dopamine metabolites in mesolimbic terminal fields [21,31,55]. Using the conditioned place preference paradigm, intra-VTA administration of morphine produces a reward effect [21,56]. Morphine-induced place preference is blocked by either dopamine antagonists or neurochemical destruction of the NAc [57]. Dynorphins (endogenous κ-agonists) act on κ-receptors in the NAc, dose-dependently decrease dopamine release [35] and can block the reward effects of μ-agonists (Figure 2).

Changes in β-endorphin levels in chronic pain: impact on abuse potential of exogenous μ-agonists

As alluded to above, the endogenous neuropeptide μ-agonist β-endorphin is released within some brain regions, including the mesolimbic pathway, during pain states [26,27]. Sciatic nerve ligation in rats resulted in suppression of place preference induced by intra-VTA injection of DAMGO (a μ-selective agonist), and intra-VTA injection of a specific antibody to β-endorphin reversed this effect [28]. Furthermore, sciatic nerve ligation also caused suppression of place preference induced by systemic morphine and a parallel decrease in DAMGO-stimulated binding of [^{35}S]GTPγS in the VTA. These phenomena were also abolished in β-endorphin knockout mice [28]. In addition, nerve ligation resulted in inhibition of systemic morphine-induced dopamine release in the NAc, which is consistent with reduced abuse potential of the μ-agonist in this condition; this effect was also abolished in β-endorphin knockout mice [28]. Taken together with data on spinal PKC activation (below), these findings suggest that selective and sustained activation of mesolimbic β-endorphin might be an important proximal mechanism for suppression of the reward effects of exogenous μ-agonists in pain states (Figure 1).

ERK and dopamine neurons in the VTA in neuropathic pain states

Extracellular signal-regulated kinase (ERK) mediates cellular responses to a wide variety of signals. Chronic administration of morphine increases ERK activity in the VTA, and ERK activation in this region is associated with a morphine-induced increase in the activity of tyrosine

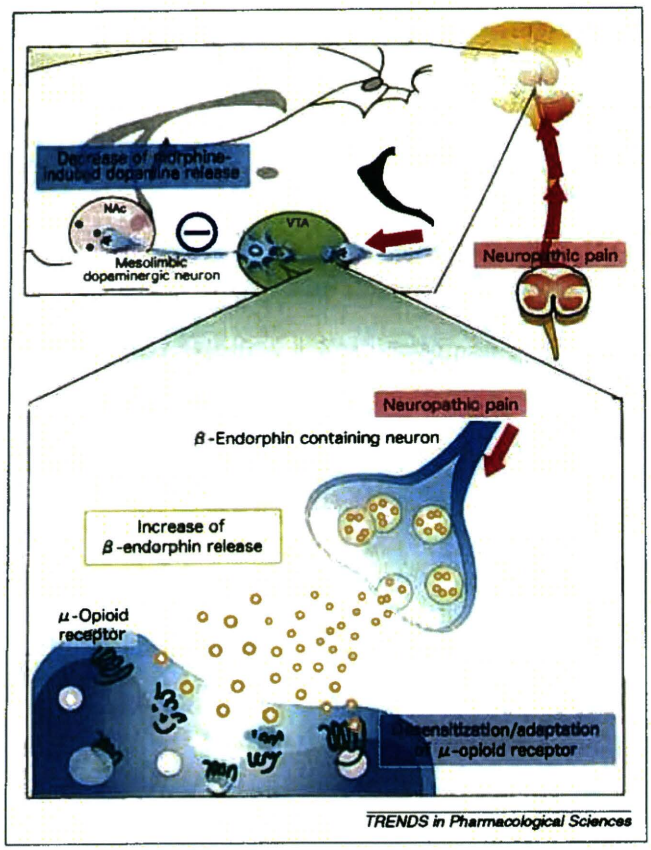

Figure 1. Model of the mechanism of suppression of μ-agonist-induced reward in neuropathic pain. Peripheral nerve injury can cause sustained activation of the endogenous β-endorphinergic system in the brain. β-Endorphin released by chronic nociceptive stimuli can continuously activate μ-opioid receptors in the VTA, thus leading to downregulation of μ-opioid receptor function and resulting in a decrease in dopamine release in the NAc. This phenomenon could explain the mechanism that underlies the suppression of μ-opioid reward under neuropathic pain-like states observed in animal models [28].

hydroxylase (TH) [29], the rate-limiting enzyme in dopamine biosynthesis. We therefore investigated whether ERK could be critical to the reward effects of morphine and whether neuropathic pain could affect ERK in the mouse lower midbrain, including the VTA [15]. Levels of phosphorylated-ERK (p-ERK) in these regions were decreased after sciatic nerve ligation, without changes in basal levels of ERK protein. Furthermore, a double immunolabeling experiment with antibodies against TH and p-ERK demonstrated that almost all of the p-ERK immunoreactivity was localized within TH-positive neurons in the VTA of sham-operated mice. After sciatic nerve ligation, a marked decrease in p-ERK immunoreactivity was detected in the VTA [15]. TH can be phosphorylated at specific serine residues by various protein kinases. The only protein kinase reported to phosphorylate TH at Ser31 *in vitro* are ERKs [30]. Sciatic nerve ligation caused a reduction in p-TH (ser31)-immunoreactivity in NAc projection neurons in the VTA [28]. Taken together, these findings suggest that sustained downregulation of ERK activity in the VTA in neuropathic pain might decrease TH activity and result in decreased dopaminergic tone and potential dysphoria. It can also be hypothesized that this decreases the responsiveness of the mesolimbic system to exogenous μ-agonists during neuropathic pain states.

Upregulation of the κ-opioid–dynorphin system limits drug reward in pain states

κ-Agonists, including the endogenous neuropeptide dynorphin A(1–17), cause a decrease in dopamine dialyzates in terminal fields of the nigrostriatal and mesolimbic systems, and can also block the reward effects of drugs of abuse, including μ-agonists and psychostimulants [21,31–35] (Figure 2). Repeated administration of μ-agonists upregulates expression of the κ-opioid receptor (KOR) and prodynorphin (pDYN) mRNA in brain [36], and this upregulation might decrease the reward effects and abuse potential of chronic μ-agonists in clinical settings. Furthermore, pain stimuli themselves (in the formalin model) decreased reward effects of systemic morphine [12], and this effect was sensitive to κ-receptor antagonism and to dynorphin antibodies in the NAc.

It is also known that both *KOR* and *PDYN* polymorphisms can affect vulnerability to addictive diseases in humans [37,38]. Taken together, these findings suggest that upregulated κ-receptor or dynorphin function, due to chronic pain itself or to chronic μ-agonist therapy, can decrease the abuse potential of μ-agonist analgesics. In addition, genetic variations in *KOR* and *PDYN* could influence the relative impact of these adaptations in clinical pain settings [37,38] (Figure 3).

Role of PKC in neuropathic pain

The protein kinase C (PKC) family of enzymes plays an important role in signal transduction in several physiological processes. PKCγ (the major PKC isoform within mammalian spinal cord) immunoreactivity is clearly increased in the dorsal horn of rat spinal cord after peripheral nerve injury [39]. Furthermore, pain behaviors are decreased after sciatic nerve ligation in mice lacking the PKCγ gene [40,41]. This observation suggests that activated PKC in the spinal cord might result in central sensitization to nociceptive transmission, leading to the development of neuropathic pain.

Interestingly, intrathecal administration of the specific PKC activator phorbol 12,13-dibutyrate (PDBu) induces spontaneous nociceptive pain-like behavior and long-lasting thermal hyperalgesia associated with enhancement of neuronal activity in brain regions related to pain perception [42,43]. In addition, we recently found that intrathecal PDBu-induced activation is observed in brain regions putatively involved with both sensory-discriminative and affective-motivational components of pain (e.g. somatosensory cortex, lateral thalamus, cingulate cortex and medial thalamus) [44] (Box 2). Intrathecal PDBu also induced neuronal activation in the mesolimbic pathway (VTA and NAc) and these effects were abolished in PKCγ-gene knockout mice [44]. We also found that systemic morphine-induced place preference was reduced by intrathecal pretreatment with PDBu [43]. Of interest, this effect of intrathecal PDBu was eliminated in β-endorphin knockout mice, suggesting that spinal PKC activation leads to sustained mesolimbic β-endorphin release, which in turn results in decreased reward by exogenous μ-agonists as detailed above [45]. More broadly, these findings lead to the proposition that activation of spinal PKCγ after sus-

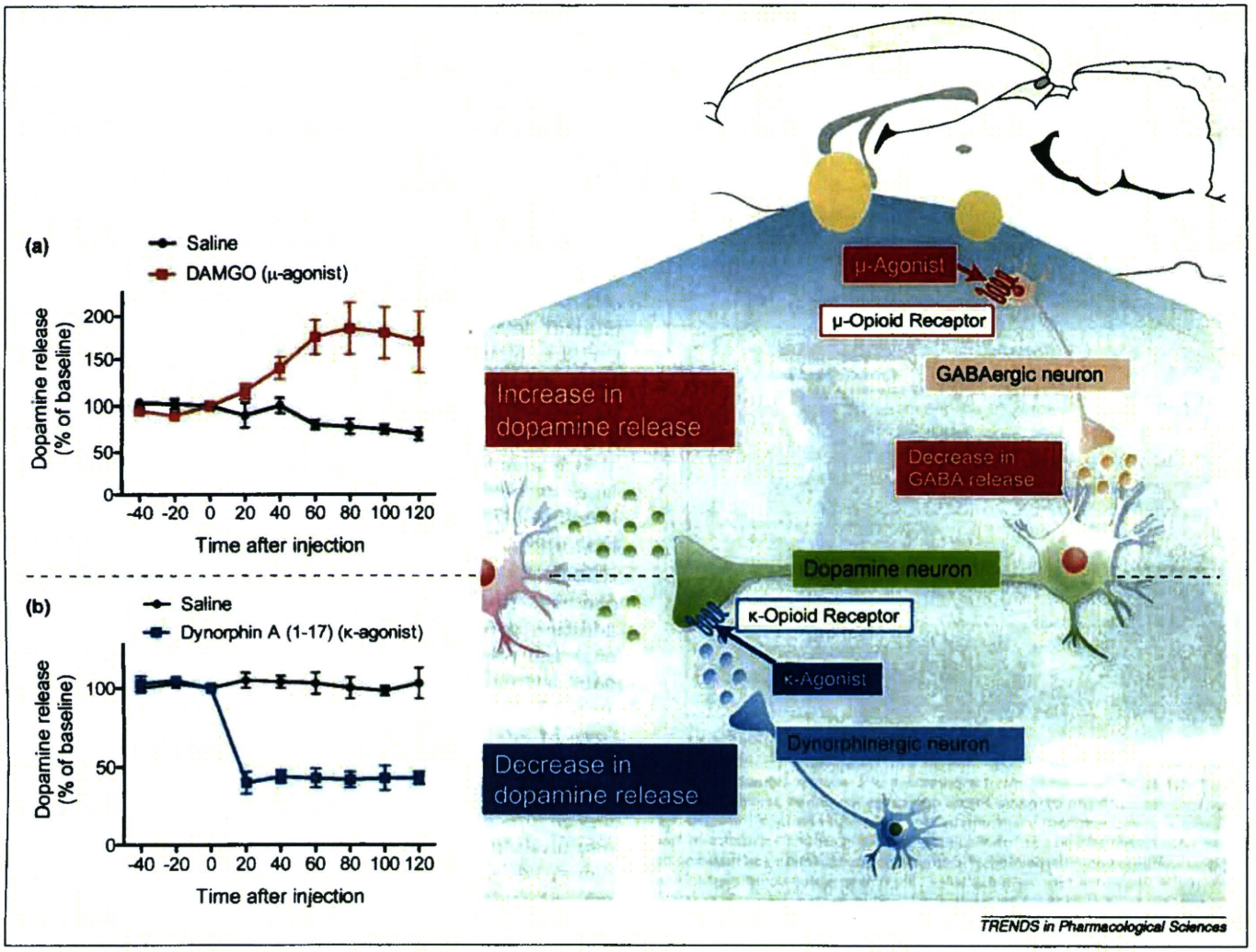

Figure 2. Mechanism of modulation of dopamine release by μ- and κ-opioid systems. **(a)** μ-Agonists inhibit γ-aminobutyric acid (GABA)ergic neurons that normally inhibit dopaminergic neurons in the VTA. This 'disinhibition' leads to an increase in the release of dopamine in the NAc. Inset: DAMGO (0.1 nmol) administered intra-VTA in rats (Narita *et al.*, unpublished observations). **(b)** Dynorphins act through κ-opioid receptors in the NAc as a countermodulatory system. Acute dynorphins (or exogenous κ-agonists) tend to decrease the release of dopamine [35] and the reward effects of μ-agonists [12]. Inset: dynorphin (4.4 nmol) administered intra-striatally in mice; replotted from [35].

tained ascending pain transmission is an important factor in the development of neuropathic pain-like states. Furthermore, by affecting mesolimbic function, this process can lead to changes in emotional and affective function in pain states.

Impact of *OPRM1* genetic variation on clinical analgesia and addiction to μ-agonists

Of relevance to both patient-specific and population-wide patterns of adaptation to chronic pain and μ-agonist administration, a major functional SNP (known as

Box 2. Ascending pain pathways and interactions of pain and reward functions

The axons of second-order spinal nociceptive neurons ascend via the spinothalamic tract and terminate in two different parts of the thalamus. The lateral nociceptive system terminates in the ventrobasal complex, which in turn projects to primary and secondary somatosensory cortices. This system is involved in the sensory-discriminative aspects of responses to noxious stimuli [58]. The medial component of the spinothalamic tract terminates in the medial and intralaminar thalamic nuclei, which project in turn to limbic regions including the anterior cingulate and anterior insular cortices and amygdala. This system is involved in the affective-motivational aspects of pain processing, namely the conscious perception of pain affect, memory and motor outputs associated with prediction and avoidance of noxious stimulation also termed nocifensive behaviors [59,60]. The actions of μ-opioid receptors predominate in areas with the highest densities of such receptors; responses to chronic pain states and surgical treatment are associated with changes in opioid receptor binding in the medial system [61]. The limbic system, including the VTA, is involved in coding the reward properties of appetitive stimuli, and plays a role in selecting between reward and pain resolution outcomes. These dual functions, particularly of the VTA and anterior cingulate cortex, predispose parts of the limbic system to modulation of what at first seem to be diametrically opposed functions: pain and reward. Thus, modulation of dopaminergic functions by chronic pain such as neuropathic pain is a natural outcome of pain and reward processing in the medial pain system and is modulated by opioid compounds.

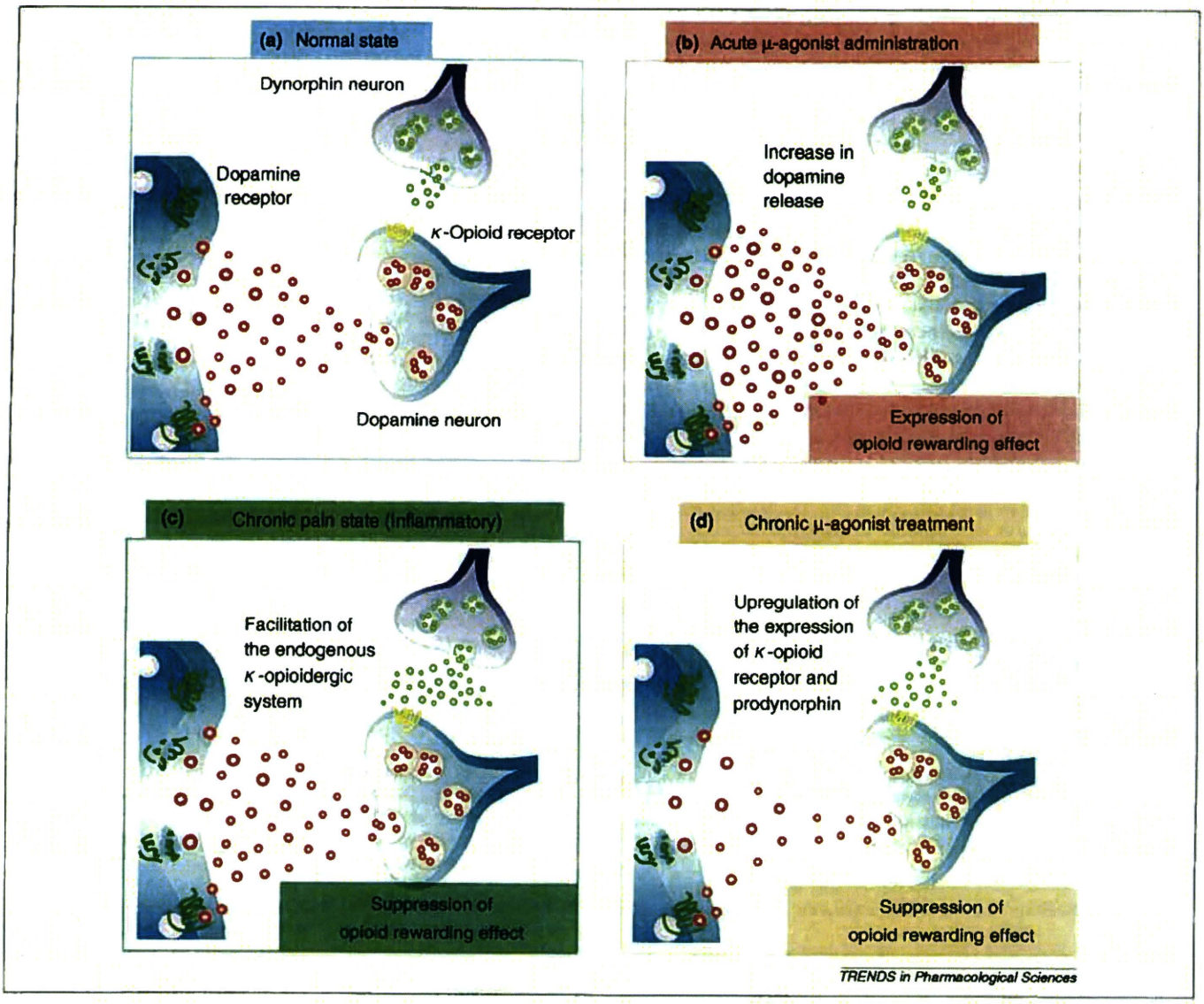

Figure 3. Adaptation of the κ-opioid–dynorphin system limits drug reward in pain states and during chronic μ-agonist administration. **(a)** Normal state: endogenous dynorphin regulates dopamine release as part of a negative feedback system. **(b)** Acute exogenous μ-agonist administration: morphine produces an increase in dopamine release in the NAc, resulting in expression of a reward effect. **(c)** Chronic pain state (inflammatory pain) + μ-agonist administration: the opioid-induced reward effect is suppressed under an inflammatory pain-like state owing to inhibition of dopamine release at dopaminergic terminals caused by facilitation of the endogenous κ-opioid system within the NAc. **(d)** Chronic μ-agonist treatment: repeated administration of a μ-agonist upregulates expression of KOR and pDYN in brain, which in turn might cause inhibition of dopamine release at dopaminergic terminals. In addition, it might cause a decrease in the reward effects of an exogenous μ-agonist.

A118G) in the μ-receptor gene *OPRM1* causes an increase in affinity and potency of β-endorphin [46]. This SNP also leads to lower mRNA and protein expression of μ-opioid receptors *in vitro* [47,48]. As previously discussed, enhanced β-endorphin function can result in decreased abuse potential of exogenous μ-agonists in chronic pain. The A118G SNP is differentially distributed in various ethnic groups, from <2% in African populations to ~50% in Asian populations [37], which is of relevance to population-wide studies of its clinical impact. Consistent with a direct role of this SNP in this area, we found that the A118G SNP imparted substantial attributable risk of developing heroin addiction in a modestly admixed Swedish population [49].

We have long hypothesized that stress responsiveness underlies vulnerability to addiction, adaptation to chronic opioids and neuroendocrine patterns of opioid withdrawal (resulting in prominent activation of the HPA axis) [9,50–52]. Such adaptations might affect vulnerability in the setting of μ-agonist exposure through illicit use or chronic analgesia therapy, and might also be influenced by genetic variation (for recent reviews see [17,37,50,51]). As a direct example, the A118G *OPRM1* SNP imparts greater activation of the HPA stress axis after naloxone challenge in European Americans [53]. Overall, these findings suggest that *OPRM1* SNPs can affect the risk of developing abuse or addiction after chronic μ-agonist exposure in certain patient populations [17,18].

Conclusion

Chronic use of μ-agonists in neuropathic pain has been the subject of several clinical reviews, and its effectiveness with respect to the balance of therapeutic and undesirable

effects (including abuse potential) has been controversial [7]. Work reviewed here reveals that adaptation of specific spinal and supraspinal molecular systems (e.g. PKC and ERK) occurs in neuropathic pain models and that this results in a decrease in the reward effect of exogenous μ-agonists. We further show that functional plasticity occurs in mesolimbic μ- and κ-opioid receptor or neuropeptide systems (involving β-endorphin and dynorphins) as a consequence of pain itself and of repeated exogenous μ-agonist administration. We hypothesize that this plasticity also decreases the reward effects of exogenous μ-agonists, and thus their abuse potential, in chronic pain states. We also propose that variations in specific opioid receptor and neuropeptide genes (e.g. *OPRM1*, *KOR* and *PDYN*) and HPA axis genes can further affect the impact of the aforementioned molecular adaptations on the abuse potential of μ-agonists in the clinical setting of chronic pain.

Acknowledgments

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Implication of dopaminergic projection from the ventral tegmental area to the anterior cingulate cortex in μ-opioid-induced place preference

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ABSTRACT

Despite the importance of prefrontal cortical dopamine in modulating reward, little is known about the implication of the specific subregion of prefrontal cortex in opioid reward. We investigated the role of neurons projecting from the ventral tegmental area (VTA) to the anterior cingulate cortex (ACG) in opioid reward. Microinjection of the retrograde tracer fluorogold (FG) into the ACG revealed several retrogradely labelled cells in the VTA. The FG-positive reactions were noted in both tyrosine hydroxylase (TH)-positive and -negative VTA neurons. The released levels of dopamine and its major metabolites in the ACG were increased by either the electrical stimulation of VTA neurons or microinjection of a selective μ-opioid receptor (MOR) agonist, (D-Ala^2,*N*-MePhe^4,Gly-ol^5) enkephalin (DAMGO), into the VTA. MOR-like immunoreactivity was seen in both TH-positive and -negative VTA neurons projecting to the ACG. The conditioned place preference induced by intra-VTA injection of DAMGO was significantly attenuated by chemical lesion of dopaminergic terminals in the ACG. The depletion of dopamine in the ACG induced early extinction of μ-opioid-induced place preference. The levels of phosphorylated DARPP32 (Thr34) and phosphorylated CREB (Ser133) were increased in the ACG of rats that had maintained the morphine-induced place preference, whereas the increases of these levels induced by morphine were blocked by pre-treatment of a selective dopamine D1 receptor antagonist SCH23390. These findings suggest that VTA-ACG transmission may play a crucial role in the acquisition and maintenance of μ-opioid-induced place preference. The activation of DARPP32 and CREB through dopamine D1 receptors in the ACG could be implicated in the maintenance of μ-opioid-induced place preference.

Keywords Anterior cingulate cortex, dopamine, memory, opioid, μ-opioid receptor, reward.

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INTRODUCTION

Studies on human addicts and behavioural studies in rodent models of addiction have indicated that key behavioural abnormalities associated with addiction are extremely long-lived. Drug of abuse is characterized by behavioural alternations in which compulsive drug seeking plays a central role. It is a chronic brain disorder as the risk of relapse remains high even after years of abstinence (Nestler 2001).

Brain dopamine systems have been the focus of histochemical, biochemical, and pharmacological research on the rewarding effects of and locomotor activity induced by opioids and psychostimulants. Dopaminergic neurons in the ventral tegmental area (VTA) innervate the nucleus accumbens (N.Acc), medial prefrontal cortex (mPFC), amygdala, hippocampus and ventral pallidum (Pierce & Kumaresan 2006). The ascending anatomical dopamine projection from the VTA to the N.Acc and mPFC is composed of mesocorticolimbic dopamine neurons (Koob 1992). Considerable evidence suggests that mesolimbic dopaminergic projections from the VTA to the N.Acc play an important role in the rewarding effects of drugs of abuse, including opioids (Kelley &