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G. 知的財産権の出願・登録状況

1. 特許取得

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2. 実用新案登録

なし

3. その他

特記すべきことなし

II. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著書氏名	論文のタイトル	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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研究成果の刊行に関する一覧表

記事

雑誌名	巻号	ページ	出版年
Japan Medicine	631		2003/12/12
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日経バイオビジネス	2004 4月号	19	2004/4/1

III. 研究成果の刊行物・別刷

快情動発現における
オピオイドシステムの役割
—ドーパミンシステムとの比較—

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1 はじめに

オピオイドは強い鎮痛作用を有し，古くより疼痛治療に用いられてきた。現在でも広く臨床現場で用いられており，ペインコントロールの重要性が認知されてきた今日では，その使用量はますます増加している。鎮痛作用のメカニズムに関する研究も盛んに行われており，多くのことが解明されてきた。一方，オピオイドは鎮痛作用だけではなく，情動にも大きな影響を与える。モルヒネ，ヘロインに代表されるオピオイドが，強い報酬効果を有する依存性薬物であることはよく知られており研究がなされてきたが，そのメカニズムは必ずしもよくわかっていない。オピオイドシステムはドーパミンシステムを制御することによって快情動を発現させることを支持する実験結果がある一方，ドーパミンシステムとは独立したメカニズムである可能性を示唆する実験結果もあり，定まっていない。本総説では，オピオイドシステムによる快情動発現メカニズムについて，ドーパミンシステムとの関連を中心に，筆者らの最近の研究成果を交えて概説したい。

2 オピオイドシステムと快情動発現

オピオイドが生体内の極微量で特異的な受容体に作用することは1973年にSnyderらによって報告された[1]。その後，モルヒネやヘロインと類似の作用を持つエンドルフィン，

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エンケファリンなどのオピオイドペプチドが生体内に存在することが明らかになった[2]。リガンドと受容体を備えたオピオイドシステムが生体内に形成されていることから，オピオイドの作用は，薬物による特殊な現象だけではなく，生理的な鎮痛や快情動を作り出していると考えられる。長年の薬理学的な解析，および近年の遺伝子欠損マウスを用いた解析により，モルヒネやヘロインの報酬効果ではμオピオイド受容体が中心的な役割を果たしていることが明らかになっている[3-6]。しかも，非選択的オピオイド拮抗薬であるナロキソンは野生型マウスでは嫌悪作用を持つのに対して，μオピオイド受容体欠損マウスではこの作用を持たないことから[7]，通常の状態で内在性のオピオイドペプチドがμオピオイド受容体に作用して情動に影響していることが考えられる。また，κオピオイド受容体は逆に嫌悪を引き起こすことが知られており[8]，δオピオイド受容体欠損マウスは動物のうつ様行動を示すことから感情障害との関係が注目されている[9]。さらに，最近我々はオピオイドの鎮痛効果と報酬効果の分子メカニズムが異なることを示唆する実験結果を得た[10]。部分的オピオイド作動薬であるブプレノルフィンの鎮痛効果はμオピオイド受容体欠損マウスでは消失していたが，ブプレノルフィンの報酬効果はこの欠損マウスにおいても残存しており，その報酬効果は非選択的オピオイド拮抗薬前処置により消失した。δオピオイド受容体作動薬やκオピオイド受容体作動薬の鎮痛効果が，μオピオイド受容体欠損マウスやμオピオイド受容体の発現量が減少しているCXBKマウスで減弱していることからも[11-14]，オピオイドの鎮痛効果ではμオピオイド受容体が中心的役割を担っていると考えられる。これに対して，オピオイドの報酬効果は，μオピオイド受容体欠損時でも他のオピオイド受容体によって担われうると考えられる。このようにオピオイドによる快情動発現のオピオイドシステム内でのメカニズムは解明されつつあるが，他のシステムとの関係は不明な点が多く，様々な仮説が唱えられている[15]。

3　ドーパミンシステムと快情動

脳の電気刺激により快情動が発現することが1954年にOldsとMilnerによって示された[16]。電気刺激によって強い快情動が引き起こされ，動物が自分で自分の脳を刺激する現象（脳内自己刺激；ICSS: Intracranial self-stimulation；図１）が現れる脳領域は，腹側被蓋野，外側視床下部，側坐核，前頭前野などであり，カテコラミン神経系の支配領域である。実際，6-hydroxydopamine（6-OHDA）によってこれらカテコラミン神経細胞を破壊すると脳内自己刺激が起こらなくなる[17]。また，快情動を引き起こす覚醒剤やコカイン

は，モノアミンの再取り込みを阻害して遊離モノアミン量を増加させる。その後，ノルアドレナリン神経の主要な起始核である青斑核を破壊しても脳内自己刺激が消失しないことや，ノルアドレナリン再取り込みを選択的に阻害する薬物には報酬効果がないことから，快情動発現を担うシステムはノルアドレナリン神経系ではないと考えられた。一方，もう一つのカテコラミンシステムであるドーパミンシステムに関しては，その起始核の一つである腹側被蓋野を6-OHDAによって選択的に破壊すると，脳内自己刺激もコカインの報酬効果も消失する[18,19]。また，強い脳内自己刺激が起こる脳領域は，Ungerstedtが組織蛍光法を用いて分類したA10神経に一致する[20]。A9神経が黒質を起始核として主に線条体に神経線維を伸ばしているドーパミン神経であるのに対して，A10神経は腹側被蓋野を起始核とし，側坐核や前頭前野に神経線維を伸ばしている。脳内自己刺激はA10神経のドーパミン放出を引き起こすことによってドーパミン信号を増強し，コカインはA10神経の終末でドーパミンの再取り込みを阻害することでこの終末でのドーパミン信号を増強して，それぞれ快情動を発現させると考えられる。電気刺激や薬物による報酬だけでなく自然報酬においてもA10神経が重要であることが示されている。Schultzらは，サルのA10神経の神経細胞の活動を測定し，報酬を得るときや報酬が期待できるときに，これらの神経細胞が相動して発火することを報告している[21]。このような50年に渡る多くの実験から，A10神経が快情動発現において中心的役割を担うことが裏付けられている。

図1　脳内自己刺激試験

床にあいた穴をマウスが覗くと内側前脳束（外側視床下部）が電気刺激されるようにすると，マウスは高頻度に穴を覗いて自分で自分の脳を刺激する。

4　オピオイドによる快情動発現のメカニズム

Wiseは，覚醒剤やコカインだけでなく，ドーパミンシステムに作用する薬物以外の依存性物質（モルヒネ，エタノール，マリファナなど）もすべてA10神経を活性化することで報酬効果を発揮すると考えている[22]。一方，Steinらは，オピオイドペプチドを脳内に注入すると，脳内自己刺激が抑制されることを示し，オピオイドシステムとドーパミンシステムは異なるメカニズムによって快情動を発現させると考えている[23]。オピオイドは

欲求レベルを下げて満足感を引き起こすような快情動を作り出し，ドーパミンは欲求レベルを上げて興奮性の快情動を引き起こすという仮説を彼らは提唱している。最近我々はμオピオイド受容体欠損マウスが正常マウスよりも亢進した脳内自己刺激を示すことを見いだしており，Steinらの仮説を強く支持する実験結果を得ている。野生型マウスおよびμオピオイド受容体欠損マウスの外側視床下部に刺激電極を植え込み，穴のぞき行動によって脳を刺激できるようにすると，野生型マウスの自己刺激頻度よりもμオピオイド受容体欠損マウスの自己刺激頻度は有意に高かった。穴のぞき行動は能動的な行動であり，快情動ではなく衝動性や強迫性が高まることによって行動を引き起こした可能性が考えられるので，刺激領域に滞在すれば滞在時間に応じて受動的に刺激を得られるようにした脳内自己刺激試験（場所滞在法）も同時に行うことが望ましい[24]。場所滞在法においてもμオピオイド受容体欠損マウスが刺激領域により長く滞在したことから，このマウスでは脳内自己刺激が亢進していると結論づけられる。μオピオイド受容体は快情動を引き起こす分子であるが，ドーパミンシステムによる快情動発現に対してはむしろ抑制的な制御をしている可能性が考えられる。図2に示すとおり，オピオイドとドーパミンは，どちらも快情動を引き起こすが，快情動の種類が違っており，欲求レベルという観点からはむしろ逆の作用をもつのかも知れない。

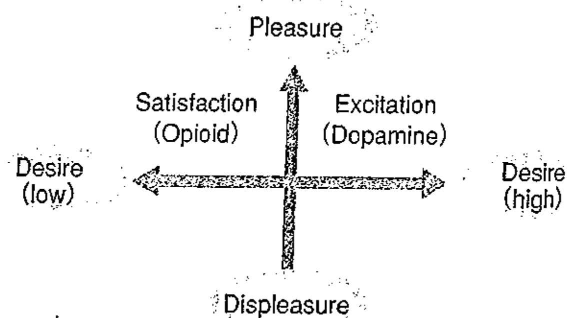

図2　多次元的快情動発現の可能性
快情動発現と欲求レベルとの関係に関する仮説。ドーパミンシステムは，欲求レベルを高め，興奮性の快情動を発現する。一方，オピオイドシステムは，欲求レベルを下げて，満足しリラックスするような快情動発現を担う。

5　おわりに

モルヒネ，ヘロインなどのオピオイド類が，覚醒剤，コカインなどのドーパミン神経亢進薬と並んで深刻な薬物依存問題を引き起こしていることからも，オピオイドシステムとドーパミンシステムは2つの重要な生体内報酬システムであると考えられる。これらのシ

ステムの異同は今後さらなる研究によって明らかになるであろうが，本総説ではオピオイドシステムとドーパミンシステムの差異に注目して論じた。オピオイドシステムはドーパミンシステムに影響することで快情動を発現するという仮説に対して否定的な実験結果が出されてきているが，オピオイドシステムによる快情動発現のメカニズムをうまく説明する他の仮説はまだ出されていない。精神的豊かさやQOL向上が注目される今日では，快情動発現メカニズムの解明は特に重要な研究課題であると考えられる。オピオイド研究は，受容体，神経ペプチド，鎮痛などの研究分野で従来から先導的な役割を果たしてきたが，今後も引き続き，心，情動の研究分野を牽引していく中心的な研究テーマであると考えられる。

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Update article

Molecular mechanisms of analgesia induced by opioids and ethanol: is the GIRK channel one of the keys?

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Abstract

Opioids and ethanol have been used since ancient times for pain relief. Opioid signaling is mediated by various effectors, including G protein-activated inwardly rectifying potassium (GIRK) channels, adenylyl cyclases, voltage-dependent calcium channels, phospholipase Cβ(PLCβ), and mitogen-activated protein kinases, although it has been unclear which effector mediates the analgesic effects of opioids. Ethanol induces a variety of physiological phenomena via various proteins, including GIRK channels rather than via membrane lipids. GIRK channel activation by either G proteins or ethanol is impaired in *weaver* mutant mice. The mutant mice may therefore serve as a useful animal model for studying the role of GIRK channels in vivo. Reduced analgesia by using either opioids or ethanol in *weaver* mutant mice suggests that GIRK channels are important effectors in both opioid- and ethanol-induced analgesia. This hypothesis is supported by similar findings in GIRK2 knockout mice. Among the various effectors coupled with opioid receptors and various targets of ethanol, GIRK channels are the only molecules whose involvement in opioid- and ethanol-induced analgesia has been demonstrated in vivo. The GIRK channel is potentially one of the key molecules in furthering the understanding of the pain control system and in developing advanced analgesics with fewer adverse effects. © 2002 Elsevier Science Ireland Ltd. and the Japan Neuroscience Society. All rights reserved.

Keywords: G protein-activated inwardly rectifying potassium channel; Kir3; Opioid; Ethanol; Analgesia; Opioid receptor; Pain

1. Analgesia induced by opioids and ethanol

Persistent or recurrent pain can degrade the sufferer's quality of life due to discomfort, distraction, and decreased volition, although pain is a crucial alert signal for our body (Wall and Melzack, 1999). Opioids and ethanol have been used widely for relieving pain since ancient times, although the mechanisms of their action remain poorly understood.

Opioids include endogenous peptides, such as enkephalin, endorphin, and dynorphin, and exogenous substances, such as morphine, heroin and codeine. Morphine, the main effective substance in opium, has been used as a potent analgesic in clinical practice and is still the primary analgesic for severe pain, including that caused by terminal cancer and myocardial infarction. Pain control has become an important part of patient treatment, resulting in increased clinical usage of morphine. Unfortunately, opioids have adverse side effects, including constipation, nausea, respiratory depression, psychological dependence, and physical dependence, which limit their use. Moreover, the users readily develop a tolerance to opioids. Improved understanding of the mechanisms underlying opioid-induced analgesia should lead to advanced strategies for pain

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control and to the development of novel analgesics with fewer adverse effects.

Ethanol affects many functions of the central nervous system (CNS), resulting in analgesia, sedation, hypnosis, motor disturbance, memory disturbance, confusion, neurodegeneration, and/or dependence (Deitrich et al., 1989; Fadda and Rossetti, 1998). Ethanol was considered to alter the functions of relatively non-selective membrane proteins as a result of ethanol-induced perturbations of the membrane lipid order (Deitrich et al., 1989; Peoples et al., 1996). However, the behavioral effects of *n*-alcohols become weak or absent (cutoff phenomenon) when the carbon-chain lengths become greater than seven, although the effects of *n*-alcohols on the membrane increase with the length (McCreery and Hunt, 1978; Lyon et al., 1981). Furthermore, the effects of ethanol on membrane lipids are quite small at clinically and pharmacologically relevant concentrations (Deitrich et al., 1989; Peoples et al., 1996). Recent studies have shown that the functions of a variety of proteins, such as some ion channels and enzymes, are altered by ethanol at such concentrations. Cutoff phenomena have also been observed in the effects of *n*-alcohol on the functions of these proteins (Covarrubias et al., 1995; Peoples et al., 1996; Chu and Treistman, 1997; Kobayashi et al., 1999; Lewohl et al., 1999). It is likely that the analgesic effects of ethanol are mediated by some of these target proteins.

2. Molecules in the opioid system

A number of molecules in the opioid system have been identified, and their functions have been investigated extensively (Table 1).

2.1. Opioid peptides

There are more than 20 opioid peptides, and most of them are produced from three precursor proteins: proopiomelanocortin, preproenkephalin (PPE), and preprodynorphin (PPD) (Vaccarino and Kastin, 2000). While dynorphin is relatively selective for kappa-opioid receptors (KORs), β-endorphin and enkephalins activate mu- and delta-opioid receptors (MORs and DORs, respectively). It has been demonstrated that β-endorphin knockout (KO) mice show enhanced stress-induced analgesia (Rubinstein et al., 1996) and that supraspinal responses to pain are increased in PPE-KO mice (Konig et al., 1996). Although dynorphin induces analgesia, PPD-KO mice do not show any differences in opioid-induced analgesia (Sharifi et al., 2001; Zimmer et al., 2001). Endomorphins, selective MOR agonists, are amidated tetrapeptides whose genes have not yet been identified (Zadina et al., 1997). There are other gene-unidentified opioid peptides, such as the peptides in the MIF-1 (melanocyte-stimulating hormone release inhibiting factor 1) family (Erchegyi et al., 1992) and morphiceptin (Chang et al., 1981). Although nociceptin/orphanin FQ structurally resembles dynorphin, the peptide is not classified into the opioid peptide family (Meunier et al., 1995; Reinscheid et al., 1995).

2.2. Opioid receptors

The existence of opioid receptors was first reported in 1973 (Pert and Snyder, 1973). The cloning of the genes for opioid receptors have been triggered by the cloning of DOR cDNA (Evans et al., 1992; Kieffer et al., 1992). A number of pharmacological studies have suggested that there are three opioid receptor genes, and molecular biological studies have confirmed the existence of MOR, DOR and KOR genes (Kieffer, 1995; Minami and Satoh, 1995). All of these receptors are members of the G protein-coupled receptor (GPCR) family. Although pharmacological investigations have indicated that there are several subtypes of each opioid receptor, molecular biological evidence for the existence of the subtypes has not yet been obtained.

Analyses of opioid receptor KO mice have clearly shown that MOR plays a central role in opioid-induced analgesia (Matthes et al., 1996; Sora et al., 1997b). MOR-KO mice show reduced analgesia after administration of morphine, an MOR agonist, but also after administration of DOR (Sora et al., 1997a, 1999; Matthes et al., 1998; Fuchs et al., 1999) and KOR (Sora et al., unpublished data) agonists. However, no compensatory changes have been found in either DOR or KOR ligand binding or distribution, in G-protein activation, or in peptide message levels in MOR-KO mice (Matthes et al., 1996; Kitchen et al., 1997; Sora et al., 1997b; Matthes et al., 1998; Narita et al., 1999). KOR-agonist-induced analgesia is also reduced in CXBK mice that have an abnormal MOR gene (Ikeda et al., 1999, 2001). The formation of opioid receptor heterodimers (Jordan and Devi, 1999) might be one of the mechanisms underlying this cross-communication between different opioid signals. DOR-KO mice show retained supraspinal delta-like analgesia and intact spinal analgesia (Zhu et al., 1999). KOR-KO mice show no analgesia after administration of KOR agonists, whereas the analgesic effects of morphine are intact (Simonin et al., 1998).

The nociceptin/orphanin FQ receptor structurally resembles opioid receptors (approximately 60% amino acid similarities) and is coupled with proteins in the Gi family, as are all opioid receptors (Mollereau et al., 1994; Ikeda et al., 1997). However, it is not classified as an opioid receptor because of its insensitivity to naloxone and because it induces hyperalgesia, which is opposite to the analgesic effects of opioids (Meunier et al., 1995; Reinscheid et al., 1995). The differences in the

Table 1
Molecules in opioid systems

Molecule	Function/property	Analgesia in mutant mice	Reference
Opioid peptides			
β-Endorphin	MOR, DOR agonist	Enhanced stress-induced analgesia (part-of-POMC-KO)	Rubinstein et al. (1996)
Enkephalin	DOR, MOR agonist	Increased supraspinal responses to pain (PPE-KO)	Konig et al. (1996)
Dynorphin	Selective KOR agonist	Normal acute analgesia by opioids (PPD-KO)	Sharifi et al. (2001), Zimmer et al. (2001)
Endomorphin	Selective MOR agonist	Gene unidentified	Zadina et al. (1997)
Opioid receptors			
Mu (MOR)	Target of morphine, Analgesia, euphoria	Lack of morphine analgesia (MOR-KO), Reduced morphine analgesia (CXBK)	Matthes et al. (1996), Sora et al. (1997b) Ikeda et al. (2001)
Delta (DOR)	Analgesia, anti-depression	Retention of delta-like analgesia (DOR-KO)	Zhu et al. (1999), Filliol et al. (2000)
Kappa (KOR)	Analgesia, disphoria	Lack of KOR agonist analgesia (KOR-KO)	Simonin et al. (1998)
G-proteins			
Gi/o	PTX sensitive	No data	
Gz	PTX insensitive	Hypertolerance to morphine (Gz-alpha KO)	Hendry et al. (2000)
Effectors			
GIRK channels	Hyperpolarization	Reduced morphine analgesia (*weaver*)	Ikeda et al. (2000)
Adenylyl cyclase	cAMP production	No data	
VDCC	Transmitter releasae	Altered pain responses Cav2.3-KO)	Saegusa et al. (2000)
		Altered nociceptive response (Cav2.2-KO)	Kim et al. (2001), Hatakeyama et al. (2001) Saegusa et al. (2001)
PLCβ	PI turnover	Enhanced morphine analgesia (PLCβ3-KO)	Xie et al. (1999)

MOR: mu-opioid receptor; DOR: delta-opioid receptor; KOR: kappa-opioid receptor; POMC: preopiomelanocortin; PPE: preproenkephalin; PPD: preprodynorphin; KO: knockout; PTX: pertussis toxin; GIRK: G protein-activated inwardly rectifying potassium channel; VDCC: voltage-dependent caicium channel; PLCβ: phospholipase Cβ; PI: phosphatidylinositol.

functions of opioid and nociceptin/orphanin FQ receptors are probably due to their different neuronal distributions (Ikeda et al., 1998).

2.3. G proteins

There are four major families of G proteins: Gi, Gs, Gq, and G_{12} (Simon et al., 1991; Linder and Gilman, 1992). Opioid receptors are coupled with six members of Gi family proteins, Gi_{1-3}, $Go_{1,2}$ and pertussis-toxin (PTX)-insensitive Gz (Jeong and Ikeda, 1998). Mice lacking the Go protein α subunit (Goα) show hyperalgesia (Jiang et al., 1998), indicating the importance of this protein in pain perception. Mice lacking the Gz protein α subunit (Gzα) are hypertolerant to morphine, although acute morphine administration results in the same degree of antinociception in both wild-type and Gzα-deficient mice (Hendry et al., 2000). In early studies, the βγ subunits of G proteins (Gβγ) were thought to be inactive proteins that merely sequester active α subunits or anchor them to the plasma membrane. However, it has become clear that Gβγ subunits are highly active biological molecules that play important roles in several cellular functions (Nestler and Duman, 1999).

2.4. GIRK channels

G protein-activated inwardly rectifying potassium (GIRK) channels (also known as Kir3 channels) are activated by various GPCRs, such as MOR, DOR, KOR, and nociceptin/orphanin FQ, M_2 muscarinic, α_2 adrenergic, and D_2 dopaminergic receptors (North, 1989; Ikeda et al., 1995, 1996, 1997). GIRK channel opening is triggered by the direct action of Gβγ released from PTX-sensitive G proteins, including Gi and Go (Reuveny et al., 1994). Activation of GIRK channels induces membrane hyperpolarization of the neurons via efflux of potassium ions and ultimately reduces neural excitability and heart rate (North, 1989; Brown and Birnbaumer, 1990; Signorini et al., 1997; Wickman et al., 1998). GIRK channels are members of a family of inwardly rectifying potassium (IRK) channels which include seven subfamilies (Reimann and Ashcroft, 1999).

The cDNAs for four GIRK channel subunits have been cloned from mammalian tissues (Kubo et al., 1993; Doupnik et al., 1995; Reimann and Ashcroft, 1999). Neuronal GIRK channels in most CNS regions are predominant heteromultimers composed of GIRK1 and GIRK2 subunits (Kobayashi et al., 1995; Lesage et al., 1995; Liao et al., 1996), whereas atrial GIRK channels are heteromultimers composed of GIRK1 and GIRK4

subunits (Krapivinsky et al., 1995). GIRK1, GIRK2, and GIRK3 subunits are widely and distinctively expressed in the CNS (Kobayashi et al., 1995; Karschin et al., 1996; Liao et al., 1996), indicating their possible involvement in various CNS functions such as cognition, memory, emotion, and motor coordination. GIRK channels coexist with opioid receptors in various neurons (Ikeda et al., 1996). It is most likely that the immediate analgesic effects of opioids are mediated by rapid signal transduction similar to the direct activation of GIRK channels by G proteins in a membrane-delimited pathway.

2.5. Other effectors

Opioid receptors couple with various other effectors besides GIRK channels (Law et al., 2000). Opioid receptor activation leads to inhibition of adenylyl cyclase. The role of adenylyl cyclase in opioid-induced analgesia is largely unknown, whereas its role in opioid dependence via the cyclic AMP response element binding protein (CREB) has been clarified (Nestler, 2001).

N-, P/Q-, and R-type voltage-dependent calcium channels (VDCCs) are directly inhibited by Gi-family-protein $\beta\gamma$ subunits (Herlitze et al., 1996; Ikeda, 1996). These channels contain α_{1B} (Cav2.3), α_{1A} (Cav2.1), and α_{1E} (Cav2.2) subunits, respectively. Because presynaptic calcium ion influx is essential for neurotransmitter release, inhibition of VDCC should inhibit neural transmission. MOR activation inhibits calcium channel current in the periaqueductal gray region, which is a crucial region of the endogenous pain control system in the brain (Connor et al., 1999). In several VDCC-KO mice, nociceptive responses are generally reduced (Saegusa et al., 2000, 2001; Hatakeyama et al., 2001; Kim et al., 2001; Muth et al., 2001). However, there is no direct evidence for the involvement of VDCCs in opioid-induced analgesia.

Opioid receptors are also coupled with PLCβ via Gi/o and Gq proteins (Ueda et al., 1995; Lee et al., 1998). Because PLCβ-KO mice exhibit enhanced morphine-induced analgesia (Xie et al., 1999), it is unlikely that PLCβ is the main mediator of the analgesic effects of morphine, although PLCβ certainly plays a role in the nociceptive responses.

Mitogen-activated protein kinases (MAP-kinases), the major effectors for growth-factor receptors, are activated by opioid receptors via G$\beta\gamma$ (Fukuda et al., 1996; Li and Chang, 1996). The role of MAP-kinases in analgesic effects of opioids remains unknown.

3. Molecules mediating ethanol effects

Ethanol affects the functions of a variety of proteins, such as GIRK channels, some ligand-gated ion channels, some voltage-gated ion channels, and some enzymes (Table 2).

3.1. GIRK channels

Ethanol activates both brain-type GIRK1/2 and cardiac-type GIRK1/4 channels at clinically and pharmacologically relevant concentrations, whereas channels in other IRK subfamilies, such as ROMK1 (Kir1.1), IRK1 (Kir2.1), and IRK3 (Kir2.3), are not affected by ethanol, even at high concentrations (Kobayashi et al., 1999; Lewohl et al., 1999). The *n*-alcohols in a homologous series have distinctive effects on GIRK1/2 and GIRK1/4 channels (different cutoff phenomena), suggesting that there is a hydrophobic region sensitive to ethanol in GIRK channels (Kobayashi et al., 1999). GIRK channels are activated by ethanol, even though Gi/o proteins are inhibited by PTX or the antisense for the G-protein β subunit (Kobayashi et al., 1999). Furthermore, single-channel analyses have revealed that activation of GIRK channels by ethanol is not mediated via cytosolic messengers (Kobayashi et al., 1999). Therefore, it is quite likely that ethanol directly activates GIRK channels. The distal C-terminal domain between amino acids 331 and 373 of the GIRK2 subunit may be crucial for ethanol sensitivity of the GIRK channel (Lewohl et al., 1999).

It has recently been shown that phosphatidylinositol 4,5-bisphosphate (PIP_2) is an important regulator of IRK channels (Huang et al., 1998; Zhang et al., 1999). GIRK channels have lower affinity for PIP_2 than IRK1 or ROMK1 channels. Chimeric GIRK channels with a high affinity domain for PIP_2 are inhibited by ethanol (Zhou et al., 2001). The interaction of GIRK channels with PIP_2 may be related to the ethanol activation of GIRK channels.

3.2. Ligand-gated ion channels

Other ion channels are also sensitive to ethanol at clinically relevant concentrations (Table 2). In neurotransmitter-gated ion channels, ethanol inhibits currents mediated by *N*-methyl-D-aspartate (NMDA)-type glutamate receptor channels, whereas α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid- and kainate-type glutamate receptor channels are generally less sensitive to ethanol (Lovinger et al., 1989; Woodward, 1999). The ethanol sensitivity of NMDA receptor channels is affected by subunit composition, phosphorylation of the receptors, and interaction of the receptors with intracellular proteins (Woodward, 1999, 2000). Because NMDA receptor channels are involved in excitatory neurotransmission and calcium signaling, the channels are thought to be important sites of ethanol action, including pain sensation.

Table 2
Molecules mediating ethanol signaling

Molecule	Function	Ethanol effects	Reference
Ion channels			
GIRK	Membrane hyperpolarization	Activation (analgesia)	Kobayashi et al. (1999), Lewohl et al. (1999)
NMDA-R	Excitatory neurotransmission, Synaptic plasticity	Inhibition	Lovinger et al. (1989), Woodward (1999)
$GABA_A$-R	Inhibitory neurotransmission	Potentiation (anesthesia)	Mihic (1999)
Gly-R	Inhibitory neurotransmission	Potentiation	Mihic (1999)
nAChR	Excitatory neurotransmission	Modulation	Narahashi et al. (1999)
$5\text{-}HT_3$-R	Excitatory neurotransmission	Potentiation	Lovinger and White (1991)
ATP-R	Excitatory neurotransmission	Inhibition	Li et al. (1994), Weight et al. (1999)
VDCC	Transmitter release	Inhibition	Walter and Messing (1999)
Kv	Regulation of action potential	Inhibition	Covarrubias and Rubin (1993)
BK	Membrane hyperpolarization	Potentiation	Dopico et al. (1999)
TASK-1	Setting of resting potential	Inhibition	Leonoudakis et al. (1998)
Enzymes			
Fyn	Tyrosine kinase	Increased LORR in KO mice	Miyakawa et al. (1997)
PKCγ	Protein kinase	Decreased LORR in KO mice	Harris et al. (1995)
PKCε	Protein kinase	Increased LORR in KO mice	Hodge et al. (1999)
PKA	Protein kinase	Decreased LORR in KO mice	Thiele et al. (2000), Wand et al. (2001)

GIRK: G protein-activated inwardly rectifying potassium channel; nAChR: nicotinic acetylcholine receptor channel; NMDA-R: *N*-methyl-D-aspartate receptor channel; $GABA_A$-R: γ-amino butylic acid type A receptor channel; Gly-R: glycine receptor channel; $5\text{-}HT_3$: 5-hydroxytryptamine type 3 receptor channel; ATP-R: ATP receptor channel; VDCC: voltage-dependent calcium channel; Kv: voltage-gated potassium channel, BK: large-conductance calcium-activated potassium channel; TASK: TWIK-related acid-sensitive potassium channel; PKC: protein kinase C, PKA: cAMP-dependent kinase; LORR: loss of righting reflex; KO: knockout.

Activity of γ-aminobutyric acid type A ($GABA_A$) receptor channels is potentiated by ethanol (Mihic, 1999). The phosphorylation state of $GABA_A$ receptor channels has been shown to be involved in regulating ethanol sensitivity (Harris et al., 1995; Diamond and Gordon, 1997; Weiner et al., 1997; Hodge et al., 1999; Mihic, 1999). In addition, ethanol potentiates the function of glycine receptor channels (Mihic, 1999). Because $GABA_A$ and glycine receptor channels mediate the major inhibitory neurotransmission in the CNS, ethanol potentiation of these receptor functions may be involved in ethanol-induced CNS depression.

Nicotinic acetylcholine (nACh) receptor channels are homo- or hetero-pentamers composed of several subtype subunits and modulate synaptic function (Narahashi et al., 1999). In cortical neurons, ethanol inhibits α-bungarotoxin sensitive currents mediated by nACh receptor channels, whereas it potentiates α-bungarotoxin insensitive currents (Narahashi et al., 1999). The opposite effects of ethanol on different nACh receptor channels are also observed in nACh receptor channels composed of different recombinant subunits on *Xenopus* oocytes (Covernton and Connolly, 1997; Narahashi et al., 1999). The 5-hydroxytryptamine type-3 ($5\text{-}HT_3$) receptor channel, which is located primarily at inhibitory interneurons of the hippocampus (Tecott et al., 1993), mediates fast excitatory responses (Lovinger, 1999). The current responses mediated by the $5\text{-}HT_3$ receptor channel are potentiated by ethanol (Lovinger and White, 1991; Lovinger, 1999). Ethanol inhibits current responses mediated by ATP receptor channels designated as P2X receptors (Li et al., 1994; Weight et al., 1999; Xiong et al., 2000). Modulation by ethanol of nACh, $5\text{-}HT_3$, and ATP receptor channels may also mediate some of the ethanol-induced alterations in CNS functions.

3.3. Other membrane proteins

In voltage-sensitive ion channels, ethanol inhibits L-type VDCC (Diamond and Gordon, 1997; Walter and Messing, 1999). Inhibition of N- and T-type channels by intoxicating concentrations of ethanol is less potent than that of L-type channels (Walter and Messing, 1999). Inhibition of VDCCs may contribute to ethanol-induced analgesia because they play a crucial role in neurotransmitter release.

Among many voltage-gated potassium (Kv) channels, the *Drosophila* Shaw2 channel, a delayed rectifier type, is inhibited by ethanol (Covarrubias and Rubin, 1993). However, the Kv3.4 channel, a human homologue of Shaw2, is insensitive to ethanol (Covarrubias et al., 1995). In addition, ethanol potentiates the current responses of large-conductance Ca^{2+}-activated potassium (BK) channels (Chu and Treistman, 1997; Dopico et al., 1999). Lastly, ethanol modestly inhibits the two-pore domain, pH-sensitive TASK-1 channel, a member of the family of background potassium channels, which are defined by a lack of voltage- and time-dependency (Leonoudakis et al., 1998; Patel and Honore, 2001). TASK-1 channels play an essential role in setting the resting membrane potential.

In addition to the ion channels, ethanol inhibits one class of adenosine transporter, which results in the extracellular accumulation of adenosine on the cell membrane (Krauss et al., 1993). Additionally, the transporter becomes tolerant to acute effects after prolonged exposure to ethanol (Nagy et al., 1990).

3.4. Intracellular signal transduction molecules

Protein phosphorylation can affect sensitivities to ethanol in vitro (Diamond and Gordon, 1997). Recently, involvement of protein phosphorylation in the behavioral, biochemical, and physiological effects of ethanol has been demonstrated by using mutant mice deficient in a specific gene (Table 2).

Mutant mice lacking Fyn, a non-receptor type tyrosine kinase, are hypersensitive to the ethanol-induced hypnotic effect; the sensitivity was evaluated by the duration of loss of righting reflex (Miyakawa et al., 1997). Fyn enhances tyrosine phosphorylation of the NMDA receptor by ethanol and is involved in an acute tolerance to ethanol inhibition of NMDA-receptor-mediated excitatory postsynaptic potentials in the hippocampus, suggesting that Fyn indirectly regulates the ethanol sensitivity of animals by modulating the functions of the NMDA receptors.

Ethanol inhibits protein kinase C (PKC) (Slater et al., 1993), which regulates a number of functions in neurons, including ion channel activity, neurotransmitter release, receptor desensitization, and differentiation (Tanaka and Nishizuka, 1994). Mutant mice lacking the neuronal-specific γ isoform of PKC (PKCγ) show reduced sensitivity to the sedative-hypnotic effects of ethanol (Harris et al., 1995) and consume more ethanol (Bowers and Wehner, 2001). Potentiation of the $GABA_A$ receptor function by ethanol is lower in brain tissue preparations from these mutant mice (Harris et al., 1995). This implies that PKCγ may indirectly regulate ethanol sensitivity via phosphorylation of $GABA_A$ receptors.

In contrast to PKCγ mutant mice, mutant mice lacking PKCε, which is widely expressed in the brain, show increased sensitivity to the sedative-hypnotic effects of ethanol and reduced ethanol self-administration (Hodge et al., 1999). Potentiation of the $GABA_A$ receptor function by ethanol is doubled in brain preparations from these mutant mice. In addition, because ethanol causes translocation of PKCε from perinuclear regions to the cytoplasm (Gordon et al., 1997), PKCε may modulate the pleiotropic effects of ethanol via phosphorylation of ion channels and receptors on the membrane and intracellular proteins. Each PKC isoform might distinctly modulate various effects of ethanol.

Mutant mice lacking the regulatory IIβ (RIIβ) subunit of cAMP-dependent protein kinase (PKA) are less sensitive to the sedative effect of ethanol and consume more ethanol (Thiele et al., 2000). In contrast, in mice overexpressing a PKA regulatory-subunit, PKA activity in the brain is reduced (Abel et al., 1997) and sensitivity to the sedative effects of ethanol increases (Wand et al., 2001). Furthermore, mice with the targeted disruption of one allele of the α subunit of the stimulatory G protein (Gsα) show increased sensitivity to the sedative effects of ethanol and consume less ethanol (Wand et al., 2001). The mice exhibit reduced adenylyl cyclase activity in the brain, indicating downregulation of cAMP-PKA signaling. In contrast, constitutive active Gsα transgenic mice, which exhibit increased adenylyl cyclase activity, are less sensitive to the sedative effects of ethanol (Wand et al., 2001).

In spite of the extensive progress in understanding the molecular mechanisms underlying various ethanol effects, the molecules involved in the analgesic effect of ethanol have not been identified, except for the GIRK channel.

4. Role of GIRK channel in analgesia

Because analgesia is observed in animals, not in cells, animal models are needed to investigate the roles of molecules in analgesia. In the case of GIRK channels, there are two useful animal models: *weaver* mutant mice and GIRK2-KO mice.

4.1. Weaver mutant mice

Weaver mutant mice which show motor ataxia possess a missense point mutation in the pore-forming region of the GIRK2 subunit (Patil et al., 1995). The mutant channel is constitutively active and lacks a hyperpolarizing function because of its permeability to not only potassium but also sodium ions (Slesinger et al., 1996). Furthermore, the activity of the mutant channel is not regulated by G proteins (Navarro et al., 1996), indicating that the pathway of opioid signaling via the GIRK channel is impaired in *weaver* mutant mice (Fig. 1, red pathway). Interestingly, *weaver* mutant mice show reduced analgesia after either morphine or KOR agonist administration (Ikeda et al., 2000), although a non-steroidal anti-inflammatory drug (NSAID) induces analgesia normally (Kobayashi et al., 1999). Among the number of effectors mediating opioid signaling, GIRK channels are the only molecules whose involvement in opioid-induced analgesia has been demonstrated in vivo. The *weaver* mutant GIRK2 channel is also insensitive to ethanol (Kobayashi et al., 1999), indicating that the pathway of ethanol signaling via the GIRK channel is also impaired in *weaver* mutant mice (Fig. 1, yellow pathway). Ethanol-induced analgesia is reduced in *weaver* mutant mice, whereas other

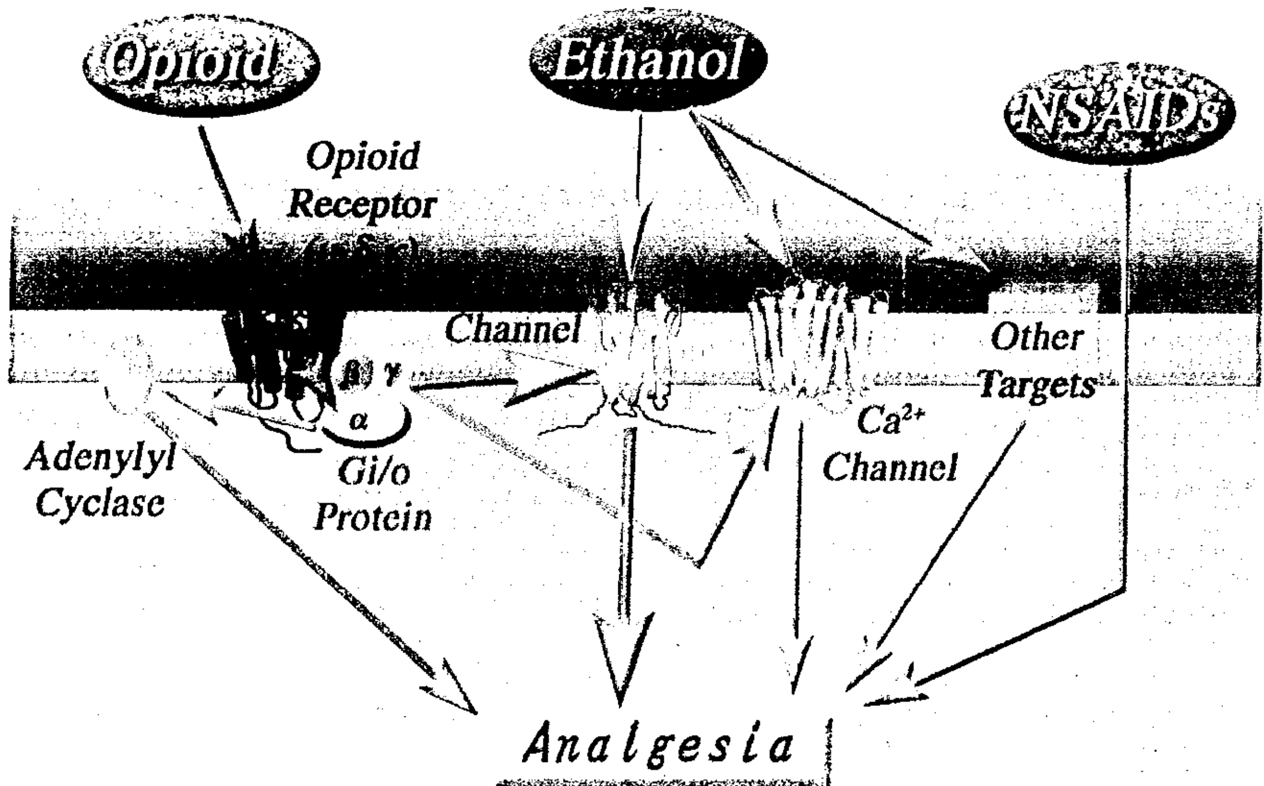

Fig. 1. Signal pathways mediating analgesia. Each opioid distinctively activates MOR, DOR, and KOR. The activation of the three opioid receptors leads to Gi/o protein activation. The activated Gi/o protein activates the GIRK channel and inhibits the function of adenylyl cyclase and calcium channels. Ethanol activates the GIRK channel directly and modulates the functions of other target molecules. NSAIDs induce analgesia in a GIRK channel independent fashion. In *weaver* mutant mice, GIRK channel activation either by Gi/o protein (red pathway) or by ethanol (yellow pathway) is impaired, and both opioid- and ethanol-induced analgesia is reduced, whereas NSAIDs normally induce analgesia.

physiological effects of ethanol, such as hypothermia, bradycardia, hyperactivity, sedation, and hypnosis, are normal (Kobayashi et al., 1999). Therefore, among the large number of molecules mediating ethanol signaling, GIRK channels are the only molecules whose involvement in ethanol-induced analgesia has been demonstrated in vivo.

4.2. GIRK2-KO mice

GIRK2-KO mice are also useful in the investigation of the role of GIRK channels in vivo. Although the mice show an almost normal phenotype under drug free conditions (Signorini et al., 1997), opioid-induced analgesia is reduced in the mice (Mitrovic et al., 2000). The involvement of GIRK channels in several ethanol effects including motor activity in the home cage, anxiolytic action, and handling-induced convulsions has also been shown (Blednov et al., 2001). Ethanol-induced analgesia would be reduced in GIRK2-KO mice.

4.3. Possible mechanisms underlying analgesia mediated by GIRK channels

Pain is modulated by descending neural pathways from the periaqueductal gray to the dorsal horn of the spinal cord via the raphe magnus nucleus and from the locus coeruleus to the dorsal horn of the spinal cord (Wall and Melzack, 1999). Opioid receptors are highly expressed in these brain regions (Mansour et al., 1995), and microinjection of opioids into these brain regions produces analgesia (Basbaum and Fields, 1984). GIRK channels are also highly or moderately expressed in these regions (Karschin et al., 1994, 1996; Liao et al., 1996), although the distribution of GIRK channels in the spinal cord has not yet been well characterized. Furthermore, electrophysiological studies using brain slices have revealed that opioids hyperpolarize subsets of neurons in these brain regions through an increase in potassium conductance (North, 1989; Pan et al., 1990), suggesting that opening of GIRK channels mediates descending analgesic signals. In *weaver* mutant mice, histological abnormality has not been reported in the

descending neural pathways, although neuronal degeneration has been observed in the cerebellum, substantia nigra, and pontine nucleus (Liao et al., 1996; Ozaki et al., in press). The reduced analgesia induced by opioid and ethanol in the mutant mice might be due not to histological abnormality but to alterations in the functional properties of the neural pathways. It is suggested that GIRK channels play a role in the descending analgesic pathways. In addition, GIRK channels might be involved in analgesia produced via functions of other CNS regions, because GIRK channels are widely expressed in the CNS, and induction of analgesia is related to other brain regions, including the amygdala and thalamus.

5. Conclusion

GIRK channels are functionally coupled with MOR, DOR, and KOR, and are directly activated by ethanol. Recent studies using *weaver* mutant mice or GIRK2-KO mice have shown the involvement of GIRK channels in analgesia induced either by opioids or ethanol. Opening of the GIRK channels by using opioids or ethanol might lead to analgesia through activation of the descending analgesic pathways in which GIRK channels are expressed. While there is only indirect evidence of the involvement in opioid-induced analgesia of the effectors coupled with opioid receptors, such as adenylyl cyclases, VDCCs, PLCβ, and MAP-kinases, the involvement of GIRK channels in opioid-induced analgesia has been demonstrated in vivo. Similarly, among a variety of target molecules of ethanol, such as NMDA, $GABA_A$, glycine, nACh, 5-HT_3, and ATP receptor channels and VDCCs, GIRK channels are the only molecules shown to mediate at least a part of ethanol-induced analgesia. Other effectors and other targets might be separately involved in analgesia or involved cooperatively with GIRK channels. Consequently, the GIRK channel is potentially one of the key molecules in furthering the understanding of the pain control system and in developing advanced analgesics with fewer adverse effects.

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