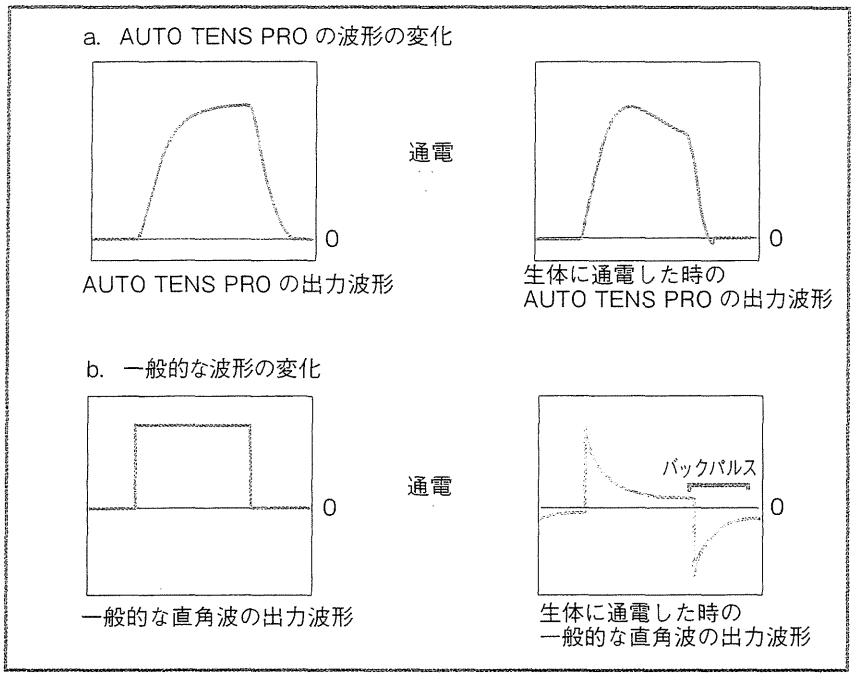

図6　出力波形とバックパルス（オートテンズプロ（ホーマーイオン研究所）のパンフレットを参考に作成）

用いていたが，最近の製品の電極は粘着性の高いパッドタイプが多く，ゲルが不要となっている．しかもパッドの乾燥を防げば，繰り返し利用可能なものが多い．

電極の数は通常2～4つで，個々の症状に合わせて有効な点を探し，その部位に設置することが大切である．設置の際，電極の極性は影響を与えない．

3．TENSの副作用と禁忌

1）副作用

TENSの治療で最もよくみられる副作用は，アレルギー性の皮膚反応である．電極そのものや電極を固定する絆創膏が原因となるが，最近ではアレルギー反応の少ない電極が用いられるようになっている．また，先に述べたように電極と皮膚との間に空気の層である空間があると，熱傷を起こすことがある．副作用ということではないが，TENSの短所として，電気治療特有の「チクチク」,「ピリピリ」感が存在する．これは，皮膚の電気抵抗が高いために生まれるバックパルスによるもので，最近の機器ではバックパルスが生まれないような出力波形を採用しているものもある（**図6**）．

2）禁　忌

TENS治療の禁忌として，ペースメーカー埋め込み患者，妊婦が挙げられる．また，電極を頸動脈の走行上に設置すると，迷走神経が刺激を受け，迷走神経反射による心臓の伝導ブロック（除脈）を起こす危険性があるので禁忌である[25]．

またTENSの鎮痛機序にはアデノシン受容体が関与していることが報告されているため[26]，TENS施行に際してはカフェインの摂取を控

えるべきともいわれている．

文　献

1) Melzack R, Wall PD：Pain mechanism：A new theory. Science 150：971-979, 1965
2) Burton C, Maurer DD：Pain suppression by transcutaneous electrical nerve stimulation IEEE Trans. Biomed. Eng 21：81-88, 1974
3) Kano T：Local electroanalgesia：1. Percutaneous current application to the human forehand to produce local analgesia. Analgesia 27：495-500, 1978
4) Wall PD, Gutnick M：Properties of afferent nerve impulses originating from a neuroma. Nature 248：740-743, 1974
5) Garrison DW, Foreman RD：Decreased activity of spontaneous and noxiously evoked dorsal horn cells during transcutaneous electrical nerve stimulation (TENS). Pain 58：309-315, 1994
6) Garrison DW, Foreman RD：Effects of prolonged transcutaneous electrical nerve stimulation (TENS) and variation of stimulation variables on dorsal horn cell activity in cats. Eur J Phys Med Rehabil 7：87-94 1994
7) Garrison DW, Foreman RD：Effects of transcutaneous electrical nerve stimulation (TENS) electrode placement on spontaneous and noxiously evoked dorsal horn cell activity in the cat. Neuromodulation 5：231-237 2002
8) Sjölund BH, Eriksson MB：The influence of naloxone on analgesia produced by peripheral conditioning stimulation. Brain Res 173：295-301, 1979
9) Abram SE, Reynolds AC, Cusick JF：Failure of naloxone to reverse analgesia from transcutaneous electrical stimulation in patients with chronic pain. Anesth and Analg 60：81-84, 1981
10) Lee KH, Chung JM, et al：Transcutaneous nerve stimulation inhibits spinotharamic tract cells. Ad Pain Res Therap 9：203-209, 1985
11) 烏野　大，千賀富士敏，太田厚美：電気刺激療法の有効性とリスク管理．PTジャーナル 40：99-106, 2006
12) Tsou K, Jang CS：Studies on the site of analgesic action of morphine by intracerebral microinjection. Sci Sin 13：1009-1109, 1964
13) Sluka KA, Walsh D：Transcutaneous electrical nerve stimulation：Basic science mechanisms and clinical effectiveness. J Pain 4：109-121, 2003
14) 濱出茂治，染矢富士子：頸肩腕症候群のリハビリテーション－経皮的末梢神経電気刺激法を中心に－．MB Med Reha 55：65-70, 2005
15) 四方公康：統合失調症の脳卒中片麻痺を合併した対象者への理学療法の試み？鏡治療とTENSを適用した1症例．PTジャーナル 42：533-536, 2008
16) 河野　浩，関　昌彦，細見　僚，原好延：TENSを用いた肘部管症候群の保存治療（第1報）．中部整災誌 47：469-470, 2004
17) 大塚吉則，坂井友吉，阿岸祐幸，他：疼痛を伴う関節可動域制限を有する患者における経皮的電気的神経刺激装置 Soma Dyne の効果．ペインクリニック 26：384-388, 2005
18) Mohamed A, Hamza MD：Effect of the frequency of transcutaneous electrical nerve stimulation on the post-operative opioid analgesic requirment and recovery profile. Anesthesiology 91：1232-1238, 1999
19) Lei Chen MD, Jun Tang MD：The effect of location of transcutaneous electrical nerve stimulation on post-operative opioid analgesic requirement. Acupoint versus nonacupoint stimulation. Anesth Anal 87：1129-1134, 1998
20) Hamza MA, White PF, Ahmed HE, et al：Effet of the frequency of transcutaneous electrical nerve stimulation on the postoperative opioid analgesic requirement and recovery profile. Anesthesiology 91：1232-1238, 1999
21) 石崎直人，矢野　忠：多発性筋炎による手指チアノーゼにTENS及び鍼治療が効果的であった1症例．医道の日本 721：52-56, 2003
22) 堀　享一，渡部一郎，生駒一憲：経皮的電気刺激による脳卒中片麻痺上肢の温度変化．Biomedical Thermology 25：21, 2005
23) 堀　享一：中周波経皮的電気刺激による上肢の温度変化．Biomedical Thermology 26：62-66, 2006
24) 友利幸之介，小林隆司，沖田　実，他：末梢神経圧挫後の脱神経筋に対する経皮的電気刺激が筋萎縮と神経再生におよぼす影響．作業療法 25：230-238, 2006
25) Ottoson D, Lundeberg T：Pain treatment by transcutaneous electrical nerve stimulation (TENS). Berlin, Springer-Verlag, 1988
26) Marchand S, Li J, Charest J：Effects of caffeine on analgesia from transcutaneous electrical nerve stimulation. N Engl J Med 333：325-356, 1995

Molecular Pain

BioMed Central

Research

Open Access

Lysophosphatidic acid-3 receptor-mediated feed-forward production of lysophosphatidic acid: an initiator of nerve injury-induced neuropathic pain

Lin Ma[1], Hitoshi Uchida[1], Jun Nagai[1], Makoto Inoue[1], Jerold Chun[2], Junken Aoki[3] and Hiroshi Ueda*[1]

Address: [1]Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan, [2]Department of Molecular Biology, Helen L. Dorris Child and Adolescent Neuropsychiatric Disorder Institute, The Scripps Research Institute, 10550 North Torrey Pines Road, ICND118, La Jolla, CA 92037, USA and [3]Laboratory of Molecular and Cellular Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

Email: Lin Ma - dm08067h@cc.nagasaki-u.ac.jp; Hitoshi Uchida - huchida@nagasaki-u.ac.jp; Jun Nagai - dm08100b@cc.nagasaki-u.ac.jp; Makoto Inoue - makoto.inoue2@duke.edu; Jerold Chun - jchun@scripps.edu; Junken Aoki - jaoki@mail.pharm.tohoku.ac.jp; Hiroshi Ueda* - ueda@nagasaki-u.ac.jp

* Corresponding author

Published: 13 November 2009

Molecular Pain 2009, **5**:64 doi:10.1186/1744-8069-5-64

Received: 26 August 2009
Accepted: 13 November 2009

This article is available from: http://www.molecularpain.com/content/5/1/64

Abstract

Background: We previously reported that intrathecal injection of lysophosphatidylcholine (LPC) induced neuropathic pain through activation of the lysophosphatidic acid (LPA)-1 receptor, possibly via conversion to LPA by autotaxin (ATX).

Results: We examined *in vivo* LPA-induced LPA production using a biological titration assay with B103 cells expressing LPA_1 receptors. Intrathecal administration of LPC caused time-related production of LPA in the spinal dorsal horn and dorsal roots, but not in the dorsal root ganglion, spinal nerve or sciatic nerve. LPC-induced LPA production was markedly diminished in ATX heterozygotes, and was abolished in mice that were deficient in LPA_3, but not LPA_1 or LPA_2 receptors. Similar time-related and LPA_3 receptor-mediated production of LPA was observed following intrathecal administration of LPA. In an *in vitro* study using spinal cord slices, LPA-induced LPA production was also mediated by ATX and the LPA_3 receptor. Intrathecal administration of LPA, in contrast, induced neuropathic pain, which was abolished in mice deficient in LPA_1 or LPA_3 receptors.

Conclusion: These findings suggest that feed-forward LPA production is involved in LPA-induced neuropathic pain.

Background

Lysophosphatidylcholine (LPC, lysolecithin) is the most abundant lysophospholipid in the blood and tissues [1,2]. LPC, an important biologically active signaling molecule, is generated under specific physiological and pathological conditions, and exerts multiple effects in atherosclerosis and inflammatory diseases [3-9]. LPC is widely reported to possess demyelinating properties, and has been used to study the processes underlying demyelination and remyelination [10-15]. Moreover, recent evi-

dence suggests that LPC has an effect on pain. Topical LPC treatment was found to induce painful sensory phenomena (allodynia and hyperalgesia), focal demyelination and changes in the expression of some pain-related molecules [16]. These LPC-induced effects on pain transmission are similar to the reported effects of lysophosphatidic acid (LPA), since LPA_1 receptor signaling initiates neuropathic pain and its underlying mechanisms, including demyelination, and alters the expression of pain-related molecules [17-19]. We speculate that centrally administered LPC may be converted to LPA by autotaxin (ATX) and cause LPA_1 receptor activation. In accord with this proposal, we recently demonstrated that neuropathic pain induced by intrathecally (i.t.) administered LPC was abolished in mice deficient in LPA_1 receptors (*Lpar1*$^{-/-}$mice), and markedly attenuated in ATX gene heterozygous mutant (*atx*$^{+/-}$) mice [20,21]. Furthermore we demonstrated that intense stimulation of spinal cord slices with pain transmitters or capsaicin, which is thought to release pain transmitters, caused a biosynthesis of LPC. This LPC was subsequently converted to LPA in the presence of recombinant ATX (rATX) [22]. These findings suggest that neuropathic pain induced by i.t. administered LPC occurs after its conversion to LPA and subsequent activation of LPA_1 receptor signaling. The present study was an initial neurochemical examination of the processes underlying LPC-induced LPA production, and provides evidence of feed-forward LPA production through the LPA_3 receptor.

Methods

Animals

Male C57BL/6J mice (Tagawa experimental animal laboratory, Japan), heterozygous mutant mice for the ATX gene (*atx*$^{+/-}$) [23], and homozygous mutant mice for the LPA_1 [24], LPA_2 [25] and LPA_3 [26] receptor genes (*Lpar1*$^{-/-}$, *Lpar2*$^{-/-}$ and *Lpar3*$^{-/-}$), and their sibling wild-type (WT) mice from the same genetic background were used in this experiment, unless stated otherwise. The subjects weighed 20-24 g. They were kept in a room maintained at 21 ± 2°C, 55 ± 5% relative humidity and a 12 h light/dark cycle and had free access to a standard laboratory diet and tap water. The procedures were approved by the Nagasaki University Animal Care Committee, which complied with the fundamental guidelines for proper conduct of animal experiments and related activities in academic research institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Drugs

LPC (18:1), LPA (18:1) and sphingosine-1-phosphate (S1P) were purchased from Sigma (MO, USA). LPC and LPA were dissolved in artificial cerebrospinal fluid (aCSF: NaCl 125 mM, KCl 3.8 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 26 mM, glucose 10 mM) when they were used *in vivo*. In the *in vitro* experiments, LPA and S1P were dissolved in Dulbecco's Modified Eagle's Medium (DMEM) with 0.1% fatty acid-free bovine serum albumin (A-6003 Sigma-Aldrich, St. Louis, MO, USA).

Recombinant ATX

In accord with previous experiments involving rat proteins [27], mouse cDNA for ATX was introduced into the baculovirus transfer vector pFASTBac-1 (Invitrogen, Carlsbad, CA, USA), and recombinant baculovirus was prepared according to the manufacturer's protocol. After purification using a baculovirus system and nickel column chromatography (HisTrap HP; Amersham Biosciences, Osaka, Japan), rATX was obtained from 1 L of culture supernatant of Sf9 insect cells infected with ATX recombinant baculovirus.

Sample preparation from tissues

At different time-points (see Results for details) after the injection of LPC or LPA, mice were anesthetized by pentobarbital (50 mg/kg, i.p.). The bilateral dorsal horn (Lamina I-V) of the lumbar (L4-6) spinal cord (SC), L4-6 dorsal roots (DR), L4-6 dorsal root ganglions (DRG), L4-6 spinal nerves (SPN) and L4-6 sciatic nerves (SCN) were then removed to enable the extraction of LPA, as shown in Fig. 1a. The average wet weights of isolated bilateral SC, DR, DRG, SPN and SCN in each mouse were 8, 4, 4, 4 and 5 mg, respectively. Following isolation, these tissue samples were put into polypropylene 1.5 mL tubes and homogenized by sonication in 300 μL serum-free DMEM solution for approximately 30 sec. To extract LPA from the homogenates using the solid-phase lipid extraction method, the sample was then slowly loaded onto Oasis HLB cartridges (Millipore, Tokyo, Japan), which had been pre-conditioned with 3 mL of methanol, followed by 3 mL of distilled water. The column was washed with 3 mL of distilled water then 1 mL of chloroform. LPA was then eluted with 600 μL methanol and dried up with N_2 gas. The final sample was dissolved with 100 μL of DMEM solution and stored at -80°C until the biological assay.

Biological titration method

B103 cells expressing LPA_1 receptors and enhanced green fluorescence protein [B103 (+) cells] were used for the quantitative measurement of LPA, according to a method [22] modified from the earlier report [28]. The cells were maintained as monolayer cultures on tissue culture dishes in DMEM supplemented with a 10% heat-inactivated fetal bovine serum (Gibco, Carlsbad, CA, USA) that included penicillin and streptomycin (final concentration 100 units/mL). Cells were seeded at 2.5×10^4 cells/cm^2 onto 8-well slide glass coated with poly-L-lysine (Sigma, MO, USA; final concentration 100 mg/L) and collagen (BD Bioscience, San Jose, CA, USA; final concentration 5 μg/cm^2). Next, they were cultured in DMEM containing 10% heat-inactivated fetal bovine serum at 37°C in a 5% CO_2

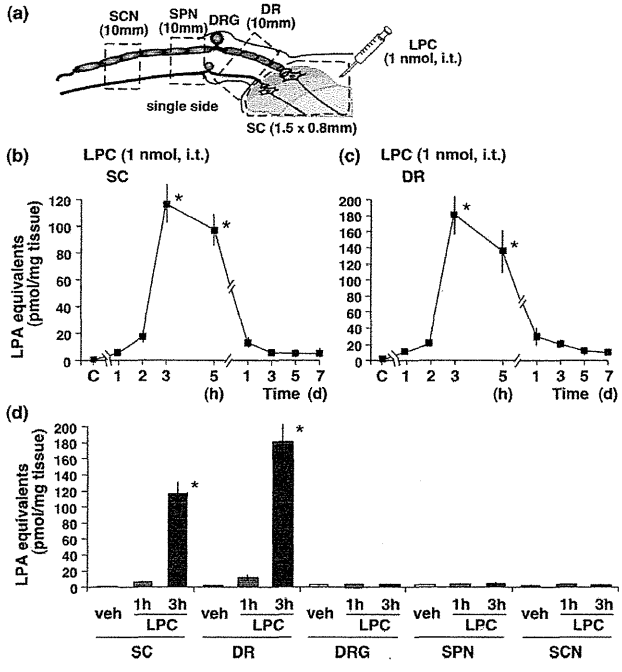

Figure 1

Lysophosphatidylcholine (LPC) induces lysophosphatidic acid (LPA) production in the spinal dorsal horn and dorsal roots. (a) The exact regions of removed samples, including dorsal horn of lumbar spinal cord (SC), dorsal roots (DR), dorsal root ganglions (DRG), spinal nerves (SPN) and sciatic nerves (SCN). (b and c) Quantification of LPA production at different time points after the LPC treatment. The capital letter "C" showed on the time course axis represents the vehicle group. (d) LPC-induced LPA production in different preparations from SC, DR, DRG, SPN and SCN at 1 and 3 h post-treatment. The measurement of LPA was carried out in triplicate of each sample. All data represent the mean ± SEM from three separate experiments. Rounding morphology was evaluated in at least 500 enhanced green fluorescence-positive cells. *$p < 0.05$ compared with the vehicle group.

atmosphere for 10 h. Cells were then cultured in serum-starved DMEM for 15 h, in accord with a previous study [22].

In the biological assay, either standard LPA or a diluted tissue sample was applied to B103 (+) cells. After incubation for 20 min at 37°C, the medium was replaced by 4% paraformaldehyde and incubated for another 60 min at 25°C. The slide glass was then cover-slipped with Fluoromount™ (DBS, Pleasanton, CA, USA) and examined under a fluorescence microscope (Keyence, Osaka, Japan). The percentage of cells that exhibited a rounded morphology was determined in at least 500 cells in each well.

Preparation of spinal cord slices

Mice were anesthetized using pentobarbital (50 mg/kg, i.p.) with 1% xylocaine. The lumbar spinal cord was removed within 5 min and placed in ice-cold Krebs buffer (117.0 mM NaCl, 3.6 mM KCl, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 25.0 mM $NaHCO_3$, 5.0 mM $CaCl_2$ and 11.0 mM glucose) containing EGTA (1 mM), and aerated with 95% O_2 and 5% CO_2 (carbogen) at pH 7.4. After the meninges were carefully removed, the rostral end of the lumbar spinal cord was glued and supported by a block of agar (1%). Transverse slices (500 μm) were then prepared in Krebs buffer, under a controlled temperature of 4°C and constant aeration with carbogen. Cutting was performed using a super microslicer ZERO (DSK, Kyoto, Japan) equipped with a ceramic knife. After sectioning, the slices were washed with Krebs buffer and serum-starved DMEM. Ten slices were then incubated in 100 μl of serum-starved DMEM in 96-well culture dishes at 37°C in 5% CO_2 for 30 min. Just before drug treatment, 50 μl of medium was removed. 50 μl of each drug at double the final concentration was then added to each well. After incubation, the culture medium was collected and filtered using a nylon filter net (NRS-100; 13-XX, 100 μm pore; Nippon Rikagaku Kikai, Tokyo, Japan) to remove any fragments of spinal cord tissue. Half of each culture medium was used in the experiments evaluating the cell rounding effects.

Nociceptive tests

In thermal paw withdrawal tests, nociception was measured as the latency to paw withdrawal evoked by exposure to a thermal stimulus [29,30]. Unanesthetized animals were placed in Plexiglas cages on top of a glass sheet and were allowed an adaptation period of 1 h. A thermal stimulator (IITC Inc., Woodland Hills, CA, USA) was positioned under the glass sheet and the focus of the projection bulb was aimed precisely at the middle of the plantar surface of the animal. A mirror attached to the stimulator permitted visualization of the plantar surface. A cut-off time of 20 sec was set in order to prevent tissue damage. The paw pressure test was performed, as described previously [30]. Mice were placed into a Plexiglas chamber on a 6 × 6 mm wire mesh grid floor and allowed to acclimatize for a period of 1 h. A mechanical stimulus was then delivered onto the middle of the plantar surface of the right hind-paw using a Transducer Indicator (Model 1601, IITC Inc., Woodland Hills, CA, USA). The pressure needed to induce a flexor response was defined as the pain threshold. All behavioral experiments were performed under double blind conditions.

RT-PCR

The expression levels of LPA receptors in SC and DR were evaluated by reverse transcription polymerase chain reac-

tion (RT-PCR), according to described method [31]. The L4-6 SC and DR were removed from naïve mice and lysed with TRIzol (Invitrogen, Carlsbad, CA, USA) for RNA preparation. Total RNA (1 μg/sample) was used for cDNA synthesis with PrimeScript® RT reagent Kit (Takara, Otsu, Japan). A 1: 3 dilution series of the products was amplified by PCR. The cycling conditions for all primers were 3 min at 95°C, then 33 cycles of 30 s at 95°C, 30 s at 55°C and 2 min at 72°C. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used as a control. The PCR primer sequences were as follows: LPA_1, 5'-AT CTTTGGCTATGTTCGCCA-3' (forward) and 5'-TTGCTGT-GAACTCCAGCCA-3' (reverse); for LPA_2, 5'-TG GCCT AC CTCTTCCTCATGTTCCA-3' (forward) and 5'-GTGT CC AGCACACCACAAATGCC-3' (reverse); for LPA_3, 3'-TTGC CTCTGCAACATCTCGG-3' (forward) and 5'-CAT GACG-GAGTTGAGCAGTG-3' (reverse); and for GAPDH, 5'-CA AGGTCATCCATGACAACTTTG-3' (forward) and 5'-GG CCATCCACAGTCTTCTGG-3' (reverse). Then, the PCR products were analyzed by 1.5% agarose gel electrophoresis.

Statistical analysis

Statistical analysis was evaluated using the Student's t-test and Tukey's multiple comparison *post hoc* analysis following one-way ANOVA. The criterion of significance was set at $p < 0.05$. All results are expressed as mean ± SEM.

Results

LPC induces LPA production in the spinal dorsal horn and dorsal roots

For the measurement of LPA production, we adopted a biological titration method using LPA_1 receptor-expressing B103 [B103 (+)] cells, according to the methods outlined in [28] and [22]. Using this method, we evaluated the percentages of cells showing a rounded morphology induced by the addition of LPA, examining at least 500 cells in each well. This measure was found to be specific to LPA, since 100 nM (equivalent: 10 pmol/100 μl) of both LPC [22] and S1P had no effect (Additional file 1, Fig. S1a). In addition, 100 nM LPA and extracts from samples produced no substantial effect on cell rounding in B103 cells without LPA_1 receptor [B103 (-)] cells (Additional file 1; Fig. S1b). In B103 (+) cells, a linear equation of the induction of cell rounding activity was established for LPA from 0.15 to 5 pmol, after subtracting the basal cell rounding activity. Experiments were carried out in 100 μl wells. The equation was defined as $y = 5.454x + 5.66$ ($R^2 = 0.991$; x: $\log_{10}$ [LPA (pmol)]; y: cell rounding percentage; Additional file 1; Fig. S1c). In subsequent studies, LPA equivalents in the extracts from the dorsal horn of the spinal cord or dorsal roots were estimated using this equation based on linear LPA concentration-dependent responses.

The basal level of LPA-equivalents in the spinal dorsal horn (SC) of control mice was 0.66 pmol/mg tissue. The LPA levels were markedly increased when mice were i.t. injected with 1 nmol of LPC. The increase was maximal (117 pmol/mg tissue) at 3 h, slightly declined at 5 h, and had disappeared by day 1 (Fig. 1b). An increase in LPC-induced LPA was observed with a similar time-course in dorsal root (DR) preparations. The maximum level was 181 pmol/mg tissue (Fig. 1c), which was significantly higher than that in the dorsal horn. As the isolated SC (Lamina I-V) and DR (L4-6) weighed 8 and 4 mg respectively, the level of LPA production was approximately 936 pmol for SC and 724 pmol for DR at 3 h after treatment (117 pmol/mg tissue × 8 mg = 936 pmol; 181 pmol/mg tissue × 4 mg = 724 pmol). Therefore, the total synthesis of LPA is likely to have been at least 1.66 nmol (936 pmol + 724 pmol), which is higher than the original level of LPC at 1 nmol. A similarly high level of LPA was also observed at 5 h post-treatment. In contrast, no significant increase in LPA levels was observed in the preparations of DRG, SPN or SCN, as shown in Fig. 1d. In addition, there were no significant differences in the basal level of LPA equivalents among any of these preparations (Fig. 1d).

ATX and LPA_3 receptors are required in LPC-induced LPA production

LPC-induced LPA production in the SC and DR was attenuated in *atx*$^{+/-}$ mice at 1 h after LPC treatment, compared with WT mice (Fig. 2a). As expected, the LPA level in SC and DR of *atx*$^{+/-}$ mice was decreased to 38 and 50% of control (WT) mice, respectively, suggesting that LPC is converted to LPA by the action of endogenous ATX. However, the level in WT mice was markedly increased to approximately 117 and 181 pmol/mg protein in the SC and DR respectively, as long as 3 h after the LPC treatment. Since the LPA level at 1 h was approximately 6 and 11 pmol/mg proteins in the SC and DR respectively, time-dependent changes were calculated as a 20-fold increase in the SC, and a 16-fold increase in the DR (Fig. 2b). Interestingly, the LPA levels in the SC and DR of *atx*$^{+/-}$ mice at 3 h were both below 4% of WT mice (Fig. 2b), while LPC-induced LPA production in the SC and DR was completely absent in *Lpar3*$^{-/-}$ mice, but not in *Lpar1*$^{-/-}$ or *Lpar2*$^{-/-}$ mice (Fig. 2c and 2d). On the other hand, three kinds of LPA_1, LPA_2 and LPA_3 receptors were all expressed in SC and DR by RT-PCR, though the level of LPA_1 transcripts was much higher than the others (Additional file 2; Fig. S2).

LPA induces LPA production through LPA_3 receptor in the spinal dorsal horn and dorsal roots

When 1 nmol LPA was i.t. injected, the LPA level in the SC was increased in a time-dependent manner. A significant increase was observed at 2 h, with a maximum level at 3 h, followed by a slight decline at 5 h. The maximum level

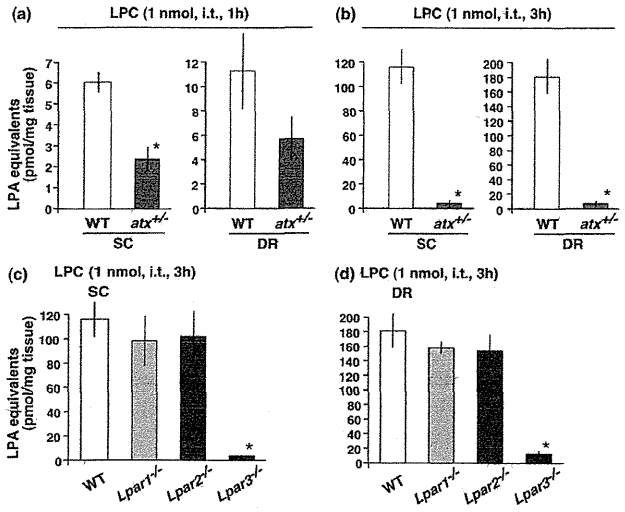

Figure 2
ATX and LPA_3 receptors are required for LPC-induced LPA production. (a and b) Quantification of LPA production by LPC-treated tissue extraction from the SC and DR of wild-type (WT) mice and ATXgene heterozygous mutant ($atx^{+/-}$) mice at 1 (*panel a*) and 3 h (*panel b*) post-treatment. (c and d) LPC-induced LPA production in the SC (*panel c*) and DR (*panel d*) from WT, *Lpar1*$^{-/-}$, *Lpar2*$^{-/-}$ and *Lpar3*$^{-/-}$ mice at 3 h post-treatment. Three female $atx^{+/-}$ mice were used in this experiment, but no significant difference was observed compared with other male mice. *$p < 0.05$ compared with the WT group. Other details are shown in the legend of Figure 1.

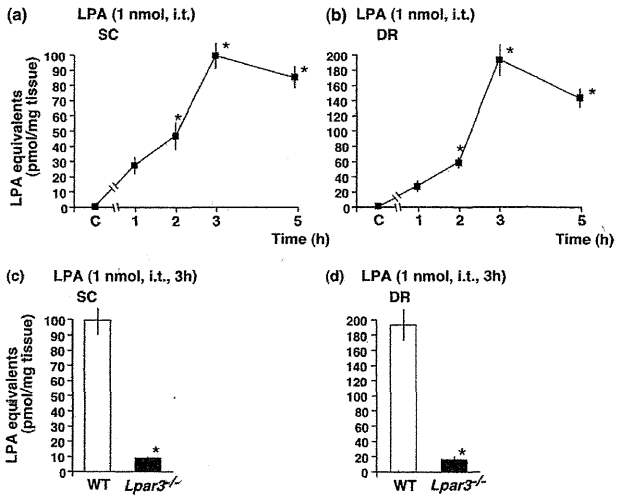

Figure 3
LPA induces amplified LPA production through LPA_3 receptor in spinal dorsal horn and dorsal roots. (a and b) Time course of LPA production induced by LPA-treated tissue extraction from SC and DR. The capital letter "C" showed on the time course axis represents the vehicle group. (c and d) LPA_3 receptor-dependent LPA production by LPA treatment. *$p < 0.05$ compared with the vehicle or WT group. Other details are shown in the legend of Figure 1.

at 3 h (approximately 100 pmol/mg protein) was equivalent to that observed with LPC at 1 nmol (i.t.), as shown in Fig. 3a. Similar levels were observed in the DR (Fig. 3b). Calculations using the method described above suggest that the total level of LPA [100 pmol/mg tissue × 8 mg (SC) + 193 pmol/mg tissue × 4 mg (DR) = 1572 pmol] was higher than the LPA level at 1 nmol. LPA-induced LPA production was also entirely absent in *Lpar3*$^{-/-}$ mice (Fig. 3c and 3d).

In vitro *evidence for LPA-induced LPA production through ATX and LPA_3 receptors*

Ten transverse slices of the lumbar spinal cord were cut and washed using Krebs buffer several times to remove cerebrospinal fluids (CSF), which contain high levels of ATX [22,32]. These slices were incubated in serum-starved DMEM for 30 min at 37°C, followed by LPA application for the indicated time periods, with or without rATX. The supernatant culture medium was then collected from these cultured spinal cord slices. After filtration, the collected medium was applied to B103 (+) cells for 20 min at 37°C, and LPA activity was evaluated (Fig. 4a). In the presence of rATX (30 ng/mL), the addition of LPA at 0.1 pmol increased the LPA level in a time-dependent manner from 30 to 60 min, followed by a slight decline (Fig. 4b). However, no significant increase was observed in the absence of rATX. In addition, LPA-induced LPA production in the presence of rATX was significantly decreased in *Lpar3*$^{-/-}$ mice, but not in *Lpar1*$^{-/-}$ or *Lpar2*$^{-/-}$ mice (Fig. 4c). As the ATX-dependent LPA production was detected in the supernatant of spinal cord slices in the present *in vitro* assay, it appears that this synthesis occurs in the extracellular space.

LPA_1 *and* LPA_3 *receptors are required in LPA-induced hyperalgesia and allodynia*

LPA i.t. injection (1 nmol) in WT mice caused robust thermal hyperalgesia and mechanical allodynia for at least 7 days, while aCSF injection produced no change in thresholds (Fig. 5). These findings are consistent with our previous report [17]. LPA-induced hyperalgesia and allodynia were completely absent in *Lpar1*$^{-/-}$ and *Lpar3*$^{-/-}$ mice at days 3, 5, and 7 after injection (Fig. 5).

Discussion

Here we demonstrated for the first time that i.t. injected LPC is not only converted to LPA through an action of endogenous ATX, but also causes a new synthesis of LPA *in vivo* in a feed-forward manner through the LPA_3 receptor. Previous studies examining the involvement of

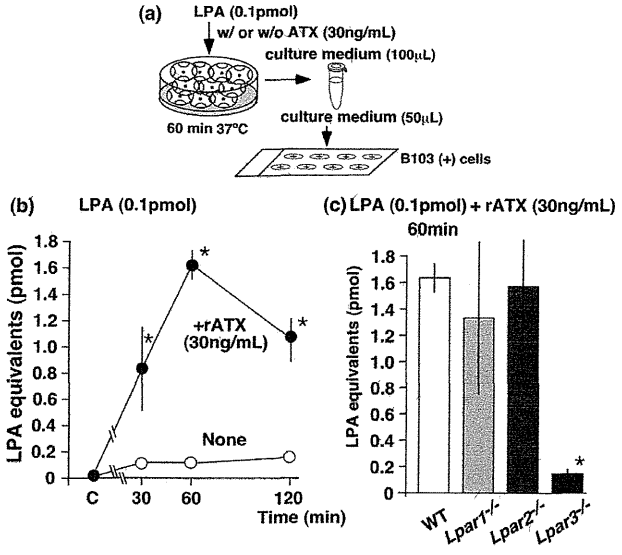

Figure 4
LPA induces ATX-mediated LPA production through the LPA_3 receptor *in vitro*. (a) Schematic representation of the method used. Details are described in the text. (b) Quantification of LPA production by LPA (0.1 pmol)-treated culture media with or without recombinant ATX (rATX, 30 ng/ml). The capital letter "C" showed in the time course axis represents the vehicle group. (c) LPA_3 receptor-dependent LPA production by LPA and rATX. All data represent the mean ± SEM from three to seven separate experiments. *$p < 0.05$ compared with the vehicle or WT group. Other details are shown in the legend of Figure 1.

endogenous ATX reported that it is a crucial enzyme for the conversion of LPC to LPA [21,33-36], and that high levels of ATX are expressed in CSF [22,32]. In this study, we used a biological titration assay, in which LPA_1 receptor-mediated cell rounding activity was measured as a quantitative evaluation of LPA levels in processed extracts from tissue samples. In this assay, LPA concentration was detectable from 0.15 pmol (equivalent: 1.5 nM). This demonstrates the higher sensitivity of this assay compared with the enzymatic cycling method, another widely used method for LPA determination that is only suitable for concentrations over 100 nM LPA [37]. Furthermore, the addition of S1P to B103 (+) cells, and the addition of LPC or LPA to B103 (-) cells did not significantly increase cell rounding. Considering these findings together, the present biological assay method appears to be useful for the quantitation of low levels of LPA in extracts, as previously reported [22,28]. Because the addition of LPC to B103(+) cells did not cause cell rounding activity [22], the increase in LPA levels in the SC and DR after i.t. LPC injection is likely to be caused by the synthesis of new LPA. The lack of LPA production in the DRG, SPN or SCN may be simply related to the topological distance from the injection site of LPC. Since there are several subspecies of LPA [38,39], however, the advanced method for measurement of each species of LPA molecules utilizing mass spectrometry including highly efficient purification and condensation would be the next subject.

The LPA level in *atx*$^{+/-}$ mice, which have been found to express only 50% of both the ATX levels and the lysophospholipase D (lyso-PLD) activity of WT mice [23], was just

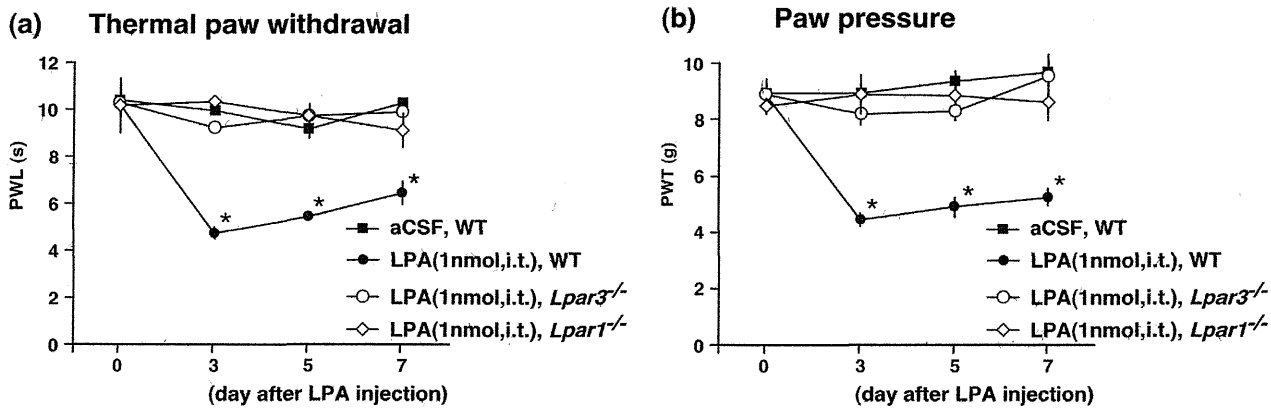

Figure 5
LPA_1 and LPA_3 receptors are involved in LPA-induced neuropathic pain. LPA (1 nmol) or aCSF was i.t. injected, and the thermal paw withdrawal test (*panel a*) and paw pressure test (*panel b*) were performed at days 3, 5 and 7 after injection. Results represent the threshold of latency (s) or pressure (g) to thermal (a) or mechanical (b) stimulus, respectively. All data represent the mean ± SEM from 3 mice. *$p < 0.05$ compared with the aCSF group.

half that of the WT mice at 1 h following LPC injection. These findings suggest that the rapid production of LPA can be attributed to the ATX-mediated conversion of LPC to LPA. Although the experiment using the ATX inhibitor may support this hypothesis, commercially available ATX inhibitor has some affinities to LPA_1, LPA_2 and LPA_3 receptors.

A second important issue is the time-dependent increase in LPA levels caused by injections of LPA as well as LPC. I.t. injection of LPA (1 nmol) caused a time-dependent increase in LPA levels in the SC and DR that lasted until 3 h post-treatment, followed by a slight decline in LPA levels at 5 h. This decline may be caused by the product-inhibition of ATX, since ATX activity is inhibited by high levels of LPA [40]. LPA-induced LPA production was also observed in the presence, but not in the absence of ATX in the *in vitro* experiment using spinal cord slices. LPA-induced production of LPA has also been previously found in an *in vitro* study using ovarian cancer cells. It was reported that phosphatidylcholine was first converted to phosphatidic acid (PA) by phospholipase D (PLD) followed by further hydrolysis to LPA by phospholipase A (PLA_2) [41]. This finding of LPA production independent of ATX in ovarian cancer cells may suggest the existence of a different type of LPA synthetic pathway to that found in the central nervous system in the present study.

A similar level and time-course of LPA synthesis was observed following LPC injection (1 nmol, i.t.). The LPA synthesis induced by LPC in both the SC and DR was reduced to the basal level by 1 day and remained at this level for the succeeding days, possibly because of degradation and diffusion. It should be noted that LPA production induced by LPC markedly increased with time, until 3 h had passed. In contrast, the increase observed in *atx*$^{+/-}$ mice was severely limited, suggesting that LPC-induced LPA production largely depends on the ATX action *in vivo*. This fact may be explained by the possibility that the LPA converted from LPC by ATX causes a new synthesis of LPC and this cycle occurs several times over 3 h.

Finally, it should be noted that LPA-induced *de novo* LPC (or converted LPA) synthesis in SC and DR was completely absent in *Lpar3*$^{-/-}$ mice, but not in *Lpar1*$^{-/-}$ and *Lpar2*$^{-/-}$ mice, though LPA_1, LPA_2 and LPA_3 receptors were all expressed in SC and DR. This finding was observed in both *in vivo* and *in vitro* experiments using spinal cord sections, and was subsequently supported by nociceptive tests showing that LPA-induced thermal hyperalgesia and mechanical allodynia were absent in *Lpar3*$^{-/-}$ mice. This result is consistent with previous reports suggesting that LPA_2 and LPA_3 receptors might contribute to LPA-induced LPA production in ovarian cancer cells, because ovarian cancer cells express high levels of LPA_2 and LPA_3 receptors whereas normal ovarian epithelial cells express low levels of LPA_2 and LPA_3 receptors [41-43]. However, it is currently unclear which cell types in the spinal cord are involved in LPA production. It is particularly difficult to clarify whether the new synthesis of LPA occurs in specific neurons or in highly differentiated cell types. Alternatively, LPA synthesis may occur through neuron-glia interactions as well as in an autocrine manner. In the present study, we found that the LPA_3 receptor is responsible for LPA synthesis. According to previous reports, the LPA_3 gene in mice is expressed in astrocytes, but not microglias [44-46]. There are currently no reports detailing the expression of the LPA_3 receptor in mouse neurons. An important aim of future research will be evaluating the role of LPA_3-related molecular mechanisms of LPA-induced LPA production in single or co-cultured specific cell types.

The present findings can also be considered in relation to the mechanisms underlying neuropathic pain. In a series of studies, we have demonstrated that LPA_1 receptor signaling initiates nerve injury- or LPA -induced (via i.t. injection) neuropathic pain and its underlying mechanisms. These mechanisms include up-regulation of the expression of the voltage-gated calcium channel $\alpha_2\delta$-1 subunit ($Ca\alpha_2\delta$-1) in the DRG, protein kinase Cγ (PKCγ) in the spinal dorsal horn and demyelination of dorsal root fibers as well as Aβ-fiber-mediated spinal reorganization [17-19,47,48]. Most recently, a pharmacological study has shown that LPA_1 signaling within a 3 h timeframe can cause neuropathic pain [49]. As such, it is reasonable to speculate that the LPA_1 and LPA_3 receptors have differential key roles in the biological processes underlying the mechanisms of neuropathic pain and the amplification of LPA production, respectively.

Conclusion

Our study provides the first demonstration that LPA can induce feed-forward LPA synthesis, a result that emerged in both *in vivo* and *in vitro* experiments. This feed-forward synthesis involves ATX-mediated conversion of LPC to LPA and LPA_3 receptor-mediated LPC production. These mechanisms appear to be repeatedly active in a relatively short period within 3 to 5 h. This result is consistent with previous findings that LPA is a key molecule in the initiation of mechanisms underlying neuropathic pain.

List of abbreviations

LPC: lysophosphatidylcholine; LPA: lysophosphatidic acid; ATX: autotaxin; *Lpar1*$^{-/-}$, *Lpar2*$^{-/-}$ and *Lpar3*$^{-/-}$: LPA_1, LPA_2 and LPA_3 receptor deficient mice; *atx*$^{+/-}$: ATX gene heterozygous mutant mice; rATX: recombinant ATX; WT: wild-type mice; i.t.: intrathecal; S1P: sphingosine-1-phosphate; aCSF: artificial cerebrospinal fluid; DMEM: Dulbecco's Modified Eagle's Medium; SC: dorsal horn of

lumbar spinal cord; DR: dorsal roots; DRG: dorsal root ganglions; SPN: spinal nerves; SCN: sciatic nerves; B103 (+): LPA_1 receptor-expressing B103 cells; B103 (-): LPA_1 receptor-lacking B103 cells; CSF: cerebrospinal fluids; RT-PCR: reverse transcription polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; lyso-PLD: lysophospholipase D; PA: phosphatidic acid; PLD: phospholipase D; PLA_2: phospholipase A; $Ca\alpha_2\delta$-1: voltage-gated calcium channel $\alpha_2\delta$-1 subunit; PKCγ: protein kinase Cγ

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LM is responsible for performance of overall experiments and writing the manuscript. H Uchida and JN participated in the RT-PCR study and LPA measurements, respectively. MI participated in the LPA measurement using spinal cord slices. JC generated LPA_1, LPA_2 and LPA_3 receptor deficient mice. JA generated recombinant ATX and ATX gene heterozygous mutant mice. HU is responsible for the experimental design and writing the manuscript. All authors read and approved the final manuscript.

Additional material

Additional file 1

*Specificity of LPA measurements using a measure of cell rounding activity in B103 cells. (a) Cell rounding activity of added LPA or S1P to B103 (+) cells. (b) Cell rounding activity of added LPA or tissue extracts to B103 (-) cells. (c) Linearity ($y = 5.454x + 5.66$, $R^2 = 0.991$) of cell rounding-inducing activity for LPA between 0.15 and 5 pmol in B103 (+) cells, after subtracting the basal activity. All data represent the mean ± SEM from three to four separate experiments. *$p < 0.05$ compared with the vehicle group. Other details are shown in the legend of Figure 1.*

Click here for file
[http://www.biomedcentral.com/content/supplementary/1744-8069-5-64-S1.tiff]

Additional file 2

Gene expression analysis for LPA_1, LPA_2 and LPA_3 receptors in the spinal cord dorsal horn and dorsal root. Expression of LPA_1, LPA_2 and LPA_3 receptors in mouse spinal cord dorsal horn (SC) and dorsal root (DR) by RT-PCR.

Click here for file
[http://www.biomedcentral.com/content/supplementary/1744-8069-5-64-S2.tiff]

Acknowledgements

The authors thank Yosuke Matsushita for technical help. The authors also thank Nobuyuki Fukushima for providing B103 cells expressing LPA_1 receptor. This work was supported by MEXT KAKENHI (17109015 to HU; 18689010 to MI), Health Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan (HU), the President's Discretionary Fund of Nagasaki University, Japan (MI), and by grants from the National Institutes of Health (MH51699 and NS48478).

References

1. Ojala PJ, Hirvonen TE, Hermansson M, Somerharju P, Parkkinen J: **Acyl chain-dependent effect of lysophosphatidylcholine on human neutrophils.** *J Leukoc Biol* 2007, **82(6):**1501-1509.
2. Kishimoto T, Soda Y, Matsuyama Y, Mizuno K: **An enzymatic assay for lysophosphatidylcholine concentration in human serum and plasma.** *Clin Biochem* 2002, **35(5):**411-416.
3. Yokota T, Hansson GK: **Immunological mechanisms in atherosclerosis.** *J Intern Med* 1995, **238(6):**479-489.
4. Murugesan G, Fox PL: **Role of lysophosphatidylcholine in the inhibition of endothelial cell motility by oxidized low density lipoprotein.** *J Clin Invest* 1996, **97(12):**2736-2744.
5. Chisolm GM, Chai Y: **Regulation of cell growth by oxidized LDL.** *Free Radic Biol Med* 2000, **28(12):**1697-1707.
6. Xu Y: **Sphingosylphosphorylcholine and lysophosphatidylcholine: G protein-coupled receptors and receptor-mediated signal transduction.** *Biochim Biophys Acta* 2002, **1582(1-3):**81-88.
7. Meyer zu Heringdorf D, Jakobs KH: **Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism.** *Biochim Biophys Acta* 2007, **1768(4):**923-940.
8. Matsumoto T, Kobayashi T, Kamata K: **Role of lysophosphatidylcholine (LPC) in atherosclerosis.** *Curr Med Chem* 2007, **14(30):**3209-3220.
9. Kougias P, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C: **Lysophosphatidylcholine and secretory phospholipase A2 in vascular disease: mediators of endothelial dysfunction and atherosclerosis.** *Med Sci Monit* 2006, **12(1):**RA5-16.
10. Hall SM: **The response of the (myelinating) Schwann cell population to multiple episodes of demyelination.** *J Neurocytol* 1983, **12(1):**1-12.
11. Ghasemlou N, Jeong SY, Lacroix S, David S: **T cells contribute to lysophosphatidylcholine-induced macrophage activation and demyelination in the CNS.** *Glia* 2007, **55(3):**294-302.
12. Jeffery ND, Blakemore WF: **Remyelination of mouse spinal cord axons demyelinated by local injection of lysolecithin.** *J Neurocytol* 1995, **24(10):**775-781.
13. Kotter MR, Zhao C, van Rooijen N, Franklin RJ: **Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression.** *Neurobiol Dis* 2005, **18(1):**166-175.
14. Ousman SS, David S: **MIP-1alpha, MCP-1, GM-CSF, and TNF-alpha control the immune cell response that mediates rapid phagocytosis of myelin from the adult mouse spinal cord.** *J Neurosci* 2001, **21(13):**4649-4656.
15. Harrison B: **Schwann cell and oligodendrocyte remyelination in lysolecithin-induced lesions in irradiated rat spinal cord.** *J Neurol Sci* 1985, **67(2):**143-159.
16. Wallace VC, Cottrell DF, Brophy PJ, Fleetwood-Walker SM: **Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain behavior that is attenuated by cannabinoids.** *J Neurosci* 2003, **23(8):**3221-3233.
17. Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J, Ueda H: **Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling.** *Nat Med* 2004, **10(7):**712-718.
18. Ueda H: **Molecular mechanisms of neuropathic pain-phenotypic switch and initiation mechanisms.** *Pharmacol Ther* 2006, **109(1-2):**57-77.
19. Ueda H: **Peripheral mechanisms of neuropathic pain - involvement of lysophosphatidic acid receptor-mediated demyelination.** *Mol Pain* 2008, **4:**11.
20. Inoue M, Ma L, Aoki J, Chun J, Ueda H: **Autotaxin, a synthetic enzyme of lysophosphatidic acid (LPA), mediates the induction of nerve-injured neuropathic pain.** *Mol Pain* 2008, **4:**6.
21. Inoue M, Xie W, Matsushita Y, Chun J, Aoki J, Ueda H: **Lysophosphatidylcholine induces neuropathic pain through an action of autotaxin to generate lysophosphatidic acid.** *Neuroscience* 2008, **152(2):**296-298.
22. Inoue M, Ma L, Aoki J, Ueda H: **Simultaneous stimulation of spinal NK1 and NMDA receptors produces LPC which undergoes ATX-mediated conversion to LPA, an initiator of neuropathic pain.** *J Neurochem* 2008, **107:**1556-1565.

23. Tanaka M, Okudaira S, Kishi Y, Ohkawa R, Iseki S, Ota M, Noji S, Yatomi Y, Aoki J, Arai H: **Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid.** *J Biol Chem* 2006, **281(35)**:25822-25830.
24. Contos JJ, Fukushima N, Weiner JA, Kaushal D, Chun J: **Requirement for the lpA1 lysophosphatidic acid receptor gene in normal suckling behavior.** *Proc Natl Acad Sci USA* 2000, **97(24)**:13384-13389.
25. Contos JJ, Ishii I, Fukushima N, Kingsbury MA, Ye X, Kawamura S, Brown JH, Chun J: **Characterization of lpa(2) (Edg4) and lpa(1)/lpa(2) (Edg2/Edg4) lysophosphatidic acid receptor knockout mice: signaling deficits without obvious phenotypic abnormality attributable to lpa(2).** *Mol Cell Biol* 2002, **22(19)**:6921-6929.
26. Contos JJ, Chun J: **The mouse lp(A3)/Edg7 lysophosphatidic acid receptor gene: genomic structure, chromosomal localization, and expression pattern.** *Gene* 2001, **267(2)**:243-253.
27. Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J, *et al.*: **Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production.** *J Cell Biol* 2002, **158(2)**:227-233.
28. Ishii I, Contos JJ, Fukushima N, Chun J: **Functional comparisons of the lysophosphatidic acid receptors, LP(A1)/VZG-1/EDG-2, LP(A2)/EDG-4, and LP(A3)/EDG-7 in neuronal cell lines using a retrovirus expression system.** *Mol Pharmacol* 2000, **58(5)**:895-902.
29. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: **A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia.** *Pain* 1988, **32(1)**:77-88.
30. Rashid MH, Inoue M, Kondo S, Kawashima T, Bakoshi S, Ueda H: **Novel expression of vanilloid receptor 1 on capsaicin-insensitive fibers accounts for the analgesic effect of capsaicin cream in neuropathic pain.** *J Pharmacol Exp Ther* 2003, **304(3)**:940-948.
31. Fujita R, Ma Y, Ueda H: **Lysophosphatidic acid-induced membrane ruffling and brain-derived neurotrophic factor gene expression are mediated by ATP release in primary microglia.** *J Neurochem* 2008, **107(1)**:152-160.
32. Sato K, Malchinkhuu E, Muraki T, Ishikawa K, Hayashi K, Tosaka M, Mochiduki A, Inoue K, Tomura H, Mogi C, *et al.*: **Identification of autotaxin as a neurite retraction-inducing factor of PC12 cells in cerebrospinal fluid and its possible sources.** *J Neurochem* 2005, **92(4)**:904-914.
33. van Meeteren LA, Moolenaar WH: **Regulation and biological activities of the autotaxin-LPA axis.** *Prog Lipid Res* 2007, **46(2)**:145-160.
34. Aoki J, Inoue A, Okudaira S: **Two pathways for lysophosphatidic acid production.** *Biochim Biophys Acta* 2008, **1781(9)**:513-518.
35. Aoki J: **Mechanisms of lysophosphatidic acid production.** *Semin Cell Dev Biol* 2004, **15(5)**:477-489.
36. Xie Y, Meier KE: **Lysophospholipase D and its role in LPA production.** *Cell Signal* 2004, **16(9)**:975-981.
37. Kishimoto T, Matsuoka T, Imamura S, Mizuno K: **A novel colorimetric assay for the determination of lysophosphatidic acid in plasma using an enzymatic cycling method.** *Clin Chim Acta* 2003, **333(1)**:59-67.
38. Sugiura T, Nakane S, Kishimoto S, Waku K, Yoshioka Y, Tokumura A, Hanahan DJ: **Occurrence of lysophosphatidic acid and its alkyl ether-linked analog in rat brain and comparison of their biological activities toward cultured neural cells.** *Biochim Biophys Acta* 1999, **1440(2-3)**:194-204.
39. Liliom K, Guan Z, Tseng JL, Desiderio DM, Tigyi G, Watsky MA: **Growth factor-like phospholipids generated after corneal injury.** *Am J Physiol* 1998, **274(4 Pt 1)**:C1065-1074.
40. van Meeteren LA, Ruurs P, Christodoulou E, Goding JW, Takakusa H, Kikuchi K, Perrakis A, Nagano T, Moolenaar WH: **Inhibition of autotaxin by lysophosphatidic acid and sphingosine 1-phosphate.** *J Biol Chem* 2005, **280(22)**:21155-21161.
41. Eder AM, Sasagawa T, Mao M, Aoki J, Mills GB: **Constitutive and lysophosphatidic acid (LPA)-induced LPA production: role of phospholipase D and phospholipase A2.** *Clin Cancer Res* 2000, **6(6)**:2482-2491.
42. Fang X, Gaudette D, Furui T, Mao M, Estrella V, Eder A, Pustilnik T, Sasagawa T, Lapushin R, Yu S, *et al.*: **Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer.** *Ann N Y Acad Sci* 2000, **905**:188-208.
43. Goetzl EJ, Dolezalova H, Kong Y, Hu YL, Jaffe RB, Kalli KR, Conover CA: **Distinctive expression and functions of the type 4 endothelial differentiation gene-encoded G protein-coupled receptor for lysophosphatidic acid in ovarian cancer.** *Cancer Res* 1999, **59(20)**:5370-5375.
44. de Sampaio ESTC, Choi JW, Gardell SE, Herr DR, Rehen SK, Gomes FC, Chun J: **Lysophosphatidic acid receptor-dependent secondary effects via astrocytes promote neuronal differentiation.** *J Biol Chem* 2008, **283(12)**:7470-7479.
45. Moller T, Contos JJ, Musante DB, Chun J, Ransom BR: **Expression and function of lysophosphatidic acid receptors in cultured rodent microglial cells.** *J Biol Chem* 2001, **276(28)**:25946-25952.
46. Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF, Hepler JR: **Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes.** *Mol Pharmacol* 2003, **64(5)**:1199-1209.
47. Fujita R, Kiguchi N, Ueda H: **LPA-mediated demyelination in ex vivo culture of dorsal root.** *Neurochem Int* 2007, **50(2)**:351-355.
48. Xie W, Matsumoto M, Chun J, Ueda H: **Involvement of LPA1 receptor signaling in the reorganization of spinal input through Abeta-fibers in mice with partial sciatic nerve injury.** *Mol Pain* 2008, **4**:46.
49. Ma L, Matsumoto M, Xie W, Inoue M, Ueda H: **Evidence for lysophosphatidic acid 1 receptor signaling in the early phase of neuropathic pain mechanisms in experiments using Ki-1 a lysophosphatidic acid 1 receptor antagonist.** *J Neurochem* 6425, **109(2)**:603-610.

特集 痛みと遺伝子多型

オピオイド耐性機構に関与するグルタミン酸－NMDA受容体アンチオピオイド機構

植田 弘師　　松下 洋輔

麻　　酔
第58巻第9号別刷
克誠堂出版株式会社

特集　痛みと遺伝子多型

オピオイド耐性機構に関与するグルタミン酸—NMDA受容体アンチオピオイド機構

植田　弘師*　松下　洋輔*

キーワード：BDNF，神経可塑性，エピジェネティック，鎮痛補助薬，クルクミン

はじめに

モルヒネなどのオピオイドは強力な鎮痛作用をもっているものの，長期的投与により耐性や依存が形成される。しかしながら，最近の臨床的知見では耐性はそれほど起こるものではなく，オピオイドが臨床で正しく使用されているかぎり，癌や非悪性組織障害による慢性疼痛治療において耐性・依存は問題にならなくなってきている[1]。しかしながら，末期癌患者では長期間高用量のオピオイドが用いられ，その結果オピオイドに対する感受性低下を招き十分な鎮痛効果を得るために，より高用量を必要とする（耐性）場合があることは念頭に置かなければならない。また，高用量になればそれだけよけいに副作用の危険性が増し，生活の質（quality of life：QOL）をますます悪化させることが予想される。それゆえ，われわれは分子メカニズムに基づいたモルヒネ耐性を抑制する方法を研究していく必要性がある。本項では，著者らが提唱しているモルヒネ耐性におけるグルタミン酸-N-メチル-D-アスパラギン酸（N-methyl-D-aspartate：NMDA）受容体アンチオピオイドシステムの役割について，最近得られた知見だけでなく，このシステムにかかわる因子についても紹介する。

1　オピオイド鎮痛耐性の分子機構

モルヒネ耐性形成の分子機構は急性と慢性で異なることが古くから議論されてきている。この両者の違いを区別することは難しいが，著者らは単一細胞レベルでの応答変化と複雑な神経回路を介した可塑的変化によるものとに分けて議論するべきであり，急性耐性は前者が大きく関与し，慢性耐性は両者がともに関与するという考え方を提唱している。

単一細胞レベルでのモルヒネ耐性機構は受容体のリン酸化とそれに続く内在化に焦点を当てたものが多く，分子機構研究の多くはこの脱感作機構をテーマとしてきた。この詳細については他稿を参照していただきたい[2]。

長期的なモルヒネ耐性・依存性機構に関してはcAMP仮説が知られており[3]，現在でもなお，モルヒネ慢性投与時に認められる新たな分子の発現変化を伴い部分的には受け入れられている。これは*in vitro*において，モルヒネをはじめとする各種オピオイドはセカンドメッセンジャーであるcAMP合成を抑制するのに対し，長期適用するとその合成抑制作用が減弱し（耐性），逆に適用中止や拮抗薬添加により，cAMP合成上昇（依存）が認められるというものである。身体依存ではモルヒネの退薬や阻害薬投与による禁断症状が認められる。痛覚過敏や過呼吸，下痢といった禁断症状はモルヒネ単回投与により認められる鎮痛や呼

* 長崎大学大学院医歯薬学総合研究科分子薬理学分野

麻酔 2009；58：1136—1142.

吸抑制，便秘といった作用と正反対であるため，耐性・依存機構が細胞レベルだけでは説明できないように思われる。Maldonado ら[4]はモルヒネ依存の形成が cAMP 応答配列結合蛋白質（CREB）遺伝子欠損マウスにより有意に抑制されたことを報告している。しかしながら，モルヒネによる Gi を介したアデニル酸シクラーゼの抑制が CREB 活性化につながるとは考えにくく，むしろ G 蛋白質βγサブユニットによる RAS-MAPK 経路の活性化が CREB 活性化につながると推測される[5]。加えて，CREB 活性化がアデニル酸シクラーゼの遺伝子発現を促進することも考慮しなくてはならない[6]。

2 神経回路を介した可塑性

著者らは以前，慢性モルヒネ投与による耐性現象が単一細胞レベルのみでは説明できない重要な知見を報告している[7]。モルヒネ 10 mg・kg^{-1}を 1 日 1 回 5 日間全身投与すると，テイルピンチ試験法によって認められるモルヒネ鎮痛効果はほとんど消失する。しかしながら末梢性のモルヒネ鎮痛効果は影響を受けていなかった[7]。それゆえ，モルヒネ鎮痛耐性におけるこの矛盾は末梢にはなく，中枢に密に存在するシナプス回路の可塑的変調が慢性投与によるモルヒネ耐性現象の中心となることを示唆している。また，末梢性のモルヒネ鎮痛に対する急性耐性が最初のモルヒネ投与 4 時間後だと形成されるのに対し 24 時間後では形成されないことは注目すべきことである[7][8]。そのため，われわれは神経回路のシナプス可塑性の観点では急性と慢性耐性の分子メカニズムを分けて議論しなければならない。モルヒネ耐性と比較し，オピオイド依存形成に関する神経回路のシナプス可塑性を明らかにすることは困難を極める。より複雑なこの神経可塑性を理解することの難しさは，他の非オピオイドシステムの関与[9]もさることながら，薬物依存に関与するさまざまな脳領域にオピオイドシステムが存在するという事実と関係しているかもしれない。これまでの報告によりモルヒネ耐性・依存の責任脳領域が証明されているが，耐性・依存形成に関与する神経回路の可塑的変調が脳局所のオピオイド受容体活性化によって引き起こされるのかどうかはいまだ定かではない。ゆえに，われわれは脳局所での複雑な神経回路の可塑的研究を推進するため，オピオイド耐性・依存の脳特定領域でのメカニズム解明を試みた。

3 グルタミン酸-NMDA 受容体アンチオピオイドシステム

グルタミン酸神経系と耐性・依存形成との関連については，1991 年に Trujillo と Akil[10]によりモルヒネによる鎮痛耐性および身体依存の形成が NMDA 受容体拮抗薬 MK-801 の処置により抑制されることが初めて報告された。この発見により，NMDA 受容体がオピオイドの慢性投与により増強し，オピオイド作用が相殺される，すなわちアンチオピオイド的な機能を有していることが示唆された。アンチオピオイドとはモルヒネ鎮痛作用に拮抗する物質であり，これを神経伝達物質にもつ神経系をアンチオピオイド神経と定義づけている。これまでにいくつかの内因性アンチオピオイドの関与が明らかにされてきており，ノシセプチンやニューロペプチド FF，コレシストキニンといった神経ペプチドがアンチオピオイド作用を有し，オピオイド慢性投与による増強や耐性に関与していることが明らかになっている。これらアンチオピオイドシステムによる神経可塑性が，後に示す特異的な阻害薬や遺伝子欠損マウスを用いた研究により明らかになってきた。

よく知られた競合もしくは非競合性の NMDA 受容体拮抗薬はモルヒネによるカタレプシー，致死性[11]，低体温症[12]や関連する学習行動[13]を増強するため，モルヒネ耐性のみを特異的に抑制する NMDA 受容体拮抗薬の開発が研究されている。特異的な阻害薬を見出すための一つのアプローチにはモルヒネ耐性に関与する NMDA 受容体サブユニット同定が試みられている。著者ら[14]の研究において，NMDA 受容体サブユニット NR2A 遺伝子欠損マウスはテイルピンチ試験法において急性モルヒネ鎮痛効果の増強が認められた。NR2A 遺伝子欠損マウスは投与前の侵害閾値に変化は認めら

れていないので，NR2Aサブユニットを刺激するグルタミン酸神経はオピオイド神経の下流に位置し，内因性および外因性オピオイド作用を抑制する。5日間のモルヒネ10 mg・kg^{-1}慢性投与は野生型マウスにおける6日目のモルヒネ鎮痛効果に耐性を形成する。しかしながら，NR2A遺伝子欠損マウスでは鎮痛耐性が形成されなかった。また，NR2A蛋白質の発現量が中脳水道周囲灰白質（PAG），腹側被蓋野（VTA），側坐核（NAcc）でのみ，コントロールと比較し有意に上昇していたため，われわれはNMDA受容体アンチオピオイドシステムの機能亢進が，モルヒネ慢性投与により生じる鎮痛効果の減弱につながると推測している。この遺伝子をPAGやVTAに*in vivo*エレクトロポレーション法という新しい技術を用いてレスキューするとモルヒネ耐性が再形成されたが，NAccへのレスキューでは成功しなかった。PAGでの蛋白質量は野生型とほぼ同量であり，このレベルがエレクトロポレーション9日後まで持続した。また，このエレクトロポレーション法はPAGでの組織傷害を引き起こさなかった。もっとも興味深いことはモルヒネ慢性投与による神経回路の可塑的変調がNR2Aを介したアンチオピオイドシステムにより調節され，ある程度が脳特定領域において起こっていることである。しかしながら，これらすべての知見は薬理学的手法を用いて得られたものである。モルヒネ鎮痛耐性のメカニズムにNR2Aが非常に重要であるように，おのおののタイプのグルタミン酸受容体機能を解明するため，特異的な受容体遺伝子を欠損させた研究を待たねばならない。

NR2Aによるメカニズムに加えて，NR2Bがモルヒネ耐性機構やオピオイド作用における神経可塑性に寄与していることも提唱されている。NR2Bサブユニット阻害薬であるRo256981はモルヒネ鎮痛耐性の発現を抑制する[15]。前帯状皮質（ACC）におけるNR2Bサブユニットは長期増強や恐怖記憶の確立に重要であるため，モルヒネ耐性動物における全身性，またはACCのNR2B抑制は鎮痛耐性の発現を抑制する。動物実験において，オピオイド慢性投与時に阻害薬を用いたNMDA受容体抑制が耐性を減弱させるという報告も数多く見受けられるが，否定的な報告もある。これはおそらく動物種や鎮痛試験法の違いだけでなく，使用している阻害薬やモルヒネ用量の違いのためだと推測される。さらに，異なる種類のグルタミン酸受容体がオピオイド鎮痛耐性の形成に関与しているという報告もある。Kozelaら[16]はホスホリパーゼCと共役しているグループ1の代謝型グルタミン酸受容体（mGluR）特異的阻害薬MPEPの慢性投与がモルヒネ耐性を著しく抑制したことを示すことで，mGluR5の役割を報告している。

4 NMDA受容体アンチオピオイドシステムを支えるBDNF

NMDA受容体アンチオピオイドシステムに基づくメカニズムにおいて，モルヒネ慢性投与によるNR2A発現上昇が耐性形成の分子機構と考えている。われわれは脳由来神経栄養因子（BDNF）がアンチオピオイドシステムを支持するものと仮説を立てており，これは以下の発見に基づく。1）ラット培養皮質神経細胞へのBDNF適用によりNR2A遺伝子発現が上昇する[17]。2）培養ミクログリアにおいて自己分泌機構を介したモルヒネによるBDNF発現上昇[18]。3）モルヒネ慢性投与がニューロン-ミクログリア間相互作用を介し，*in vivo*における脳神経細胞でBDNF発現を上昇させる[19]。4）BDNF抗体の脳室内投与によりモルヒネ耐性が抑制される[20]。さらに5）モルヒネ身体依存が前脳特異的BDNF欠損マウスで消失する[21]。BDNFによるNR2A遺伝子発現の分子機構はいまだ不明であるが，NR2Aプロモーターにある3つのGC-ボックスがSp1やSp4転写因子と相互作用し，コアプロモーター活性に重要であることが見出されている。興味深いことに，BDNFとその受容体（receptor tyrosine kinase B：TrkB）の活性化がリン酸化を介したSp1のトランス活性化能を増加させる[22]。一方で，モルヒネ慢性投与によりBDNFがどのように発現上昇するかはとても興味深い論点である。モルヒネ10 mg・kg^{-1} 5日間投与後，6日目ではモルヒネ鎮痛効果は消失する。この時期に，モルヒネ鎮痛に重要な

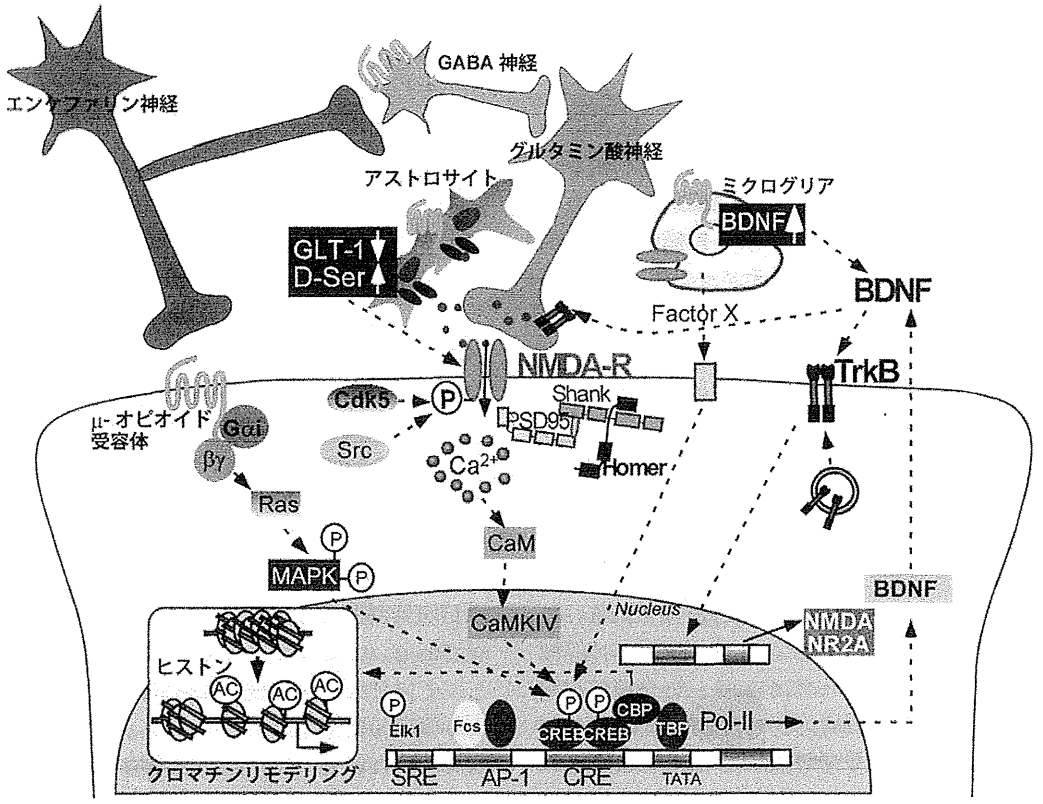

図　グルタミン酸-NMDA 受容体アンチオピオイドシステムにおけるグリアの関与

モルヒネ慢性投与により，グルタミン酸神経伝達や NMDA 受容体下流シグナリングがニューロン-グリア相互作用を介して増強する。アストロサイトにおけるグルタミン酸トランスポーターの発現低下はシナプス間隙でのグルタミン酸量の増加が予想され，D-セリンの増加はアロステリックな機構を介して NMDA 受容体機能を亢進させる。一方で，ミクログリアの活性化がアンチオピオイドシステムに重要な BDNF 発現増加を引き起こし，これが NR2A 発現を誘発する。モルヒネ耐性時の CBP 活性化を介したエピジェネティックコントロールも，BDNF 発現増加に関与している。

（植田弘師，戸田一雄．第四部　鎮痛薬．やさしい痛み学．初版．東京：プレーン出版；2007. p.136-44 より引用）

領域である PAG において有意な BDNF 発現上昇が認められる[20]。モルヒネ慢性投与後，PAG における強い BDNF 免疫活性の多くが神経細胞で認められ，ミクログリアにも少なからず存在する（植田ら，未発表データ）。この選択的な発現はとても興味深いものである。なぜならモルヒネによる BDNF 発現上昇が培養ミクログリアで認められ[18]，培養神経細胞では認められなかったからである。この知見から，われわれはミクログリア由来の生理活性分子の同定を試みており，それが神経細胞で BDNF 発現上昇を起こすと推測している。それゆえ，ニューロン-ミクログリア間相互作用は BDNF を通してモルヒネ鎮痛耐性に重要な役割を果たしていると推測される（図）。しかし，この成果は前脳領域特異的に BDNF を欠損させたマウスにおいてモルヒネ身体依存は消失するが，鎮痛耐性には影響しなかった[21]という報告と一見矛盾しているように思えるが，モルヒネ鎮痛効果はむしろ下位脳幹部に起因する事実を考慮するならば何ら矛盾はない。実際，floxed-BDNF マウスの PAG に Cre 組み換え酵素アデノウイルスを投与するとモルヒネ鎮痛耐性は顕著に減弱する

(植田ら，未発表データ)。また，われわれはCREB結合蛋白質（CBP）抑制によりヒストンアセチルトランスフェラーゼ活性を阻害するクルクミンが，モルヒネ慢性投与により発現上昇するBDNFエキソンⅠとⅣ，さらに鎮痛耐性をも抑制することを見出している[20]。これは健康補助食品として用いられるクルクミンがモルヒネ鎮痛耐性を抑制すること，さらにエピジェネティックなBDNF遺伝子発現制御が耐性を抑制する新しい戦略となりうることを示している。

5 NMDA受容体アンチオピオイドシステムを支えるシステム

ミクログリアの役割に加え，アストロサイトもまたグルタミン酸-NMDA受容体アンチオピオイドシステムにおいて重要な役割を担っている。モルヒネ慢性投与により，アストロサイトと神経細胞でグルタミン酸トランスポーターの発現量が減少する[23]。この現象からシナプス間隙におけるグルタミン酸シグナルを増強することが期待される。すなわちNMDA受容体の刺激頻度の増加が起こると推測される（図）。この観点から，アロステリックな活性化薬としてNMDA受容体を活性化するD-セリンもまた，アンチオピオイドシステムに関与することが推測される。なぜなら，モルヒネ慢性投与により，D-セリン発現を増加させるラセマーゼが発現上昇するためである[24]。さらにNMDA受容体の足場蛋白質であるシナプス後肥厚部蛋白質（PSD)-95の脊髄における欠損はモルヒネ耐性形成を抑制する[25]。一方，マイクロアレイを用いた研究により，Homer-1蛋白質のスプライシングアイソフォームであるAnia-3発現がナロキソン誘発性モルヒネ禁断症状時に増加することが報告されている[26]。Ania-3はNMDAとmGluRをつなぐHomer蛋白質の恒常的活性型である長鎖型の機能を妨げるため，Ania-3発現の変化はモルヒネ依存形成に寄与していることが示唆される。これらの知見はBefortら[27]による最近の研究と一致しており，この報告ではモルヒネ慢性投与が薬物への欲求や探索行動に重要な領域である偏桃体中心体において，PSD-95やHomer-1遺伝子の発現を上昇させることを示している。一方で，Cdk5がオピオイドに耽溺した前頭前野において，またモルヒネを投与したラット大脳皮質において減少するという報告もある[28]。これらの知見はとても興味深い。なぜならCdk5はNR2Aサブユニットをリン酸化し，NMDA受容体機能を活性化することが報告されているためである[29]。一方で，Cdk5の抑制はSrcを介したNR2Bリン酸化を起こし，AP-2への結合を阻害するが，NMDA受容体の細胞膜表面への発現を促進するとも報告されている[30]。しかしながら，モルヒネが投与されたラットの同様な脳領域においてCdk5発現に変化が認められていない，相反する報告もある[27]。

おわりに

現在では慢性的もしくは持続的な痛みはそれ自身が病気であると認められ，適切に治療すべき問題である。オピオイドは末期癌患者の緩和医療としてだけでなく，神経因性疼痛患者の鎮痛など，より幅広く使用され始めている。慢性痛のない動物を用いた研究と異なり，臨床において適切に使用されたオピオイドは安全であるといわれているが，オピオイドの長期使用は鎮痛耐性や依存を含めた危険性の増加が推測される。こうした医療上の背景において，モルヒネを真に安心して使用するには耐性・依存性形成メカニズムの解明とその抑制方法を科学的に明らかにする必要がある。これまでの著者らの知見から，いくつかの鎮痛補助薬となりうる候補物質を見出している。例として，NR2A特異的な阻害薬やクルクミンのようなBDNF転写を抑制する化合物などが良い候補物質だと考えられる。また，より効率的な受容体内在化を引き起こすようなオピオイドの開発も実現可能な手法と考えられる。あるいは，両者を兼ね備えた鎮痛補助薬の開発がより良い手法となりうるかもしれない。今後，さらにオピオイド耐性の情報伝達機構が解明されるとともに，新しい分子標的が見出されるものと期待される。

引用文献

1) Carroll IR, Angst MS, Clark JD. Management of perioperative pain in patients chronically consum-

ing opioids. Reg Anesth Pain Med 2004；29：576-91.
2) 植田弘師, 戸田一雄. 第四部　鎮痛薬. やさしい痛み学. 初版. 東京：プレーン出版；2007. p.136-44.
3) Nestler EJ, Hope BT, Widnell KL. Drug addiction：a model for the molecular basis of neural plasticity. Neuron 1993；11：995-1006.
4) Maldonado R, Blendy JA, Tzavara E, Gass P, Roques BP, Hanoune J, et al. Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 1996；273：657-9.
5) Xing J, Ginty DD, Greenberg ME. Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. Science 1996；273：959-63.
6) Lane-Ladd SB, Pineda J, Boundy VA, Pfeuffer T, Krupinski J, Aghajanian GK, et al. CREB (cAMP response element-binding protein) in the locus coeruleus：biochemical, physiological, and behavioral evidence for a role in opiate dependence. J Neurosci 1997；17：7890-901.
7) Ueda H, Inoue M. Peripheral morphine analgesia resistant to tolerance in chronic morphine-treated mice. Neurosci Lett 1999；266：105-8.
8) Ueda H, Inoue M, Matsumoto T. Protein kinase C-mediated inhibition of mu-opioid receptor internalization and its involvement in the development of acute tolerance to peripheral mu-agonist analgesia. J Neurosci 2001；21：2967-73.
9) Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev 2001；81：299-343.
10) Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. Science 1991；251：85-7.
11) Trujillo KA, Akil H. The NMDA receptor antagonist MK-801 increases morphine catalepsy and lethality. Pharmacol Biochem Behav 1991；38：673-5.
12) Bhargava HN. Enhancement of morphine actions in morphine-naive and morphine-tolerant mice by LY 235959, a competitive antagonist of the NMDA receptor. Gen Pharmacol 1997；28：61-4.
13) Bannerman DM, Rawlins JN, Good MA. The drugs don't work—or do they? Pharmacological and transgenic studies of the contribution of NMDA and GluR-A-containing AMPA receptors to hippocampal-dependent memory. Psychopharmacology (Berl) 2006；188：552-66.
14) Inoue M, Mishina M, Ueda H. Locus-specific rescue of GluRepsilon1 NMDA receptors in mutant mice identifies the brain regions important for morphine tolerance and dependence. J Neurosci 2003；23：6529-36.
15) Ko SW, Wu LJ, Shum F, Quan J, Zhuo M. Cingulate NMDA NR2B receptors contribute to morphine-induced analgesic tolerance. Mol Brain 2008；1：2.
16) Kozela E, Popik P. The effects of NMDA receptor antagonists on acute morphine antinociception in mice. Amino Acids 2002；23：163-8.
17) Small DL, Murray CL, Mealing GA, Poulter MO, Buchan AM, Morley P. Brain derived neurotrophic factor induction of N-methyl-D-aspartate receptor subunit NR2A expression in cultured rat cortical neurons. Neurosci Lett 1998；252：211-4.
18) Takayama N, Ueda H. Morphine-induced chemotaxis and brain-derived neurotrophic factor expression in microglia. J Neurosci 2005；25：430-5.
19) Yonekubo S, Matsushita Y, Ueda H. Microglia activation is involved in the initiation of morphine analgesic tolerance, INRC 2008. Charleston, SC, USA, 2008. p.46.
20) Matsushita Y, Ueda H. Curcumin blocks chronic morphine analgesic tolerance and brain-derived neurotrophic factor upregulation. Neuroreport 2009；20：63-8.
21) Akbarian S, Rios M, Liu RJ, Gold SJ, Fong HF, Zeiler S, et al. Brain-derived neurotrophic factor is essential for opiate-induced plasticity of noradrenergic neurons. J Neurosci 2002；22：4153-62.
22) Solomon SS, Majumdar G, Martinez-Hernandez A, Raghow R. A critical role of Sp1 transcription factor in regulating gene expression in response to

insulin and other hormones. Life Sci 2008 ; 83 : 305-12.
23) Ozawa T, Nakagawa T, Shige K, Minami M, Satoh M. Changes in the expression of glial glutamate transporters in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal. Brain Res 2001 ; 905 : 254-8.
24) Yoshikawa M, Shinomiya T, Takayasu N, Tsukamoto H, Kawaguchi M, Kobayashi H, et al. Long-term treatment with morphine increases the D-serine content in the rat brain by regulating the mRNA and protein expressions of serine racemase and D-amino acid oxidase. J Pharmacol Sci 2008 ; 107 : 270-6.
25) Liaw WJ, Zhang B, Tao F, Yaster M, Johns RA, Tao YX. Knockdown of spinal cord postsynaptic density protein-95 prevents the development of morphine tolerance in rats. Neuroscience 2004 ; 123 : 11-5.
26) Ammon S, Mayer P, Riechert U, Tischmeyer H, Hollt V. Microarray analysis of genes expressed in the frontal cortex of rats chronically treated with morphine and after naloxone precipitated withdrawal. Brain Res Mol Brain Res 2003 ; 112 : 113-25.
27) Befort K, Filliol D, Ghate A, Darcq E, Matifas A, Muller J, et al. Mu-opioid receptor activation induces transcriptional plasticity in the central extended amygdala. Eur J Neurosci 2008 ; 27 : 2973-84.
28) Ferrer-Alcon M, La Harpe R, Guimon J, Garcia-Sevilla JA. Downregulation of neuronal cdk5/p35 in opioid addicts and opiate-treated rats : relation to neurofilament phosphorylation. Neuropsychopharmacology 2003 ; 28 : 947-55.
29) Li BS, Sun MK, Zhang L, Takahashi S, Ma W, Vinade L, et al. Regulation of NMDA receptors by cyclin-dependent kinase-5. Proc Natl Acad Sci USA 2001 ; 98 : 12742-7.
30) Zhang S, Edelmann L, Liu J, Crandall JE, Morabito MA. Cdk5 regulates the phosphorylation of tyrosine 1472 NR2B and the surface expression of NMDA receptors. J Neurosci 2008 ; 28 : 415-24.

ABSTRACT

Anti-opioid Action of Glutamate-NMDA Receptor Systems Underlying Morphine Analgesic Tolerance

Hiroshi UEDA, Yosuke MATSUSHITA

Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523

Although the opioid analgesics such as morphine represent an important class of agents for the treatment of severe clinical pain, the development of tolerance and dependence often overshadows the therapeutic advantages of morphine and related opioids in individuals undergoing long-term treatments. The mechanisms underlying opioid tolerance are not fully understood, but appear to be comprised of two types of plasticity or counter-adaptation, at the cellular level and through neuronal circuits. Current studies mostly emphasize the cellular adaptation mechanisms, which include altered gene expression and receptor desensitization due to phosphorylation and endocytosis. However, the mechanisms underlying opioid tolerance and dependence are not always explained by cellular adaptation mechanisms alone. This review focuses on the plasticity in neuronal circuits achieved through an enhancement of so-called anti-opioid glutamate/NMDA receptor synaptic activities. There have been also conceptual advances in understanding the changes to supporting systems, which include the altered expression of key molecules regulating the anti-opioid system through neuron-glial networks. We also introduce a new approach using *in vivo* electroporation to identify the brain loci responsible for morphine tolerance and dependence.

key words : BDNF, neuronal plasticity, epigenetics, analgesic adjuvant, curcumin

生体の科学　60(5)：490-491，2009

5. 脂質－リゾホスファチジン酸

神経障害性疼痛におけるリゾリン脂質のフィードフォワード性産生制御機構と病態生理機構

植田弘師　関野有紀

[用いられた物質/研究対象となった受容体]
リゾホスファチジン酸/LPA_1受容体

リン脂質(PLs)の2本のアシル基のうち1本が除かれたリン脂質をリゾリン脂質(LPLs)という。このリゾリン脂質は容易に生体膜から離れ，他の膜へと移行し，脂質メディエーターとしての機能を持つことが近年明らかとなってきている。生体内には様々なリゾリン脂質が存在するが，量的に最も多いのはリゾホスファチジルコリン(LPC)であり，哺乳類の血液中には約数100 μMという高濃度のLPCが存在する。一方，生体内での存在量は少ないが，必要時に産生され強力な作用を発揮するリゾリン脂質群があり，その一つにリゾホスファチジン酸(LPA)がある。このLPAは細胞増殖性・運動性の亢進，抗アポトーシス作用，神経突起の退縮など多彩な作用が報告されている。また，LPA受容体，産生酵素のノックアウトマウス，遺伝病の解析から脳の形態形成，神経障害性疼痛，生殖，育毛，血管形成などに関与することが明らかとなってきており，このリゾリン脂質が個体レベルで重要な役割を担う生理活性脂質として注目されている。

●神経障害性神経因性疼痛原因分子としてのLPA

LPAは$G_{q/11/14}$，$G_{12/13}$，$G_{i/o}$などのGタンパク質と共役する7回膜貫通型受容体($LPA_{1\sim5}$)に作用し，多彩な機能を発揮する。神経障害性疼痛との関連で注目されるLPA_1受容体は，$G_{i/o}$を介したPI3キナーゼ-Akt経路の活性化に伴い生存効果を示す一方で，$G_{12/13}$を介したRhoA-Rhoキナーゼ経路の活性化により細胞の形態的変化を誘導する。シュワン細胞やオリゴデンドロサイトに対してもその形態的変化を促し，ミエリンの形成に影響を与えることが報告されている。ミエリン形成異常である脱髄と神経因性疼痛は密接に関与することが知られており，多発性硬化症やギランバレー症候群などさまざまな難治性慢性疼痛疾患で脱髄とともにアロディニアや著しい痛覚過敏現象が確認されている。筆者らは，組織障害時に産生されるLPAが一次知覚神経の脊髄後根においてシュワン細胞に作用し，脱髄現象を伴う慢性的な神経因性疼痛を誘発する原因分子であることを報告している。坐骨神経部分結紮による神経障害性疼痛モデルにおいて，脱髄は障害部位と後根に認められるが，後根神経での脱髄は末梢側よりも中枢側，とりわけ脊髄入力部位で顕著に観察されることから，LPA産生は脊髄で生じることが示唆された。

●神経障害とLPA産生

LPA産生のメカニズムについては未だ不明な点が多く残されている。血漿中ではジアシルグリセロール(DG)あるいはリン脂質(PLs)からホスファチジン酸(PA)が産生され，さらにPAのアシル基が加水分解されLPAに変換される経路と，豊富に存在するリゾホスファチジルコリン(LPC)が刺激を受けて活性化されるリゾホスホリパーゼD(あるいはオートタキシン，ATX)によりLPAが産生される経路が存在するが，脳神経系においてLPC含量は必ずしも高くないことから，LPA産生はLPC生合成に依存していることが予想される。脳脊髄液中には十分なATX量が含まれるが，*in vitro*実験で用いる脊髄スライス標品からは失われることが確認できたので，リコ

Hiroshi Ueda, Yuki Sekino：長崎大学 大学院医歯薬学総合研究科 分子薬理学分野

0370-9531/10/¥500/論文/JCOPY