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Permanent relief from intermittent cold stress-induced fibromyalgia-like abnormal pain by repeated intrathecal administration of antidepressants

Michiko Nishiyori[1], Hitoshi Uchida[1], Jun Nagai[1], Kohei Araki[1], Takehiro Mukae[1], Shiroh Kishioka[2] and Hiroshi Ueda[1*]

Abstract

Background: Fibromyalgia (FM) is characterized by chronic widespread pain, which is often refractory to conventional painkillers. Numerous clinical studies have demonstrated that antidepressants are effective in treating FM pain. We previously established a mouse model of FM-like pain, induced by intermittent cold stress (ICS).

Results: In this study, we find that ICS exposure causes a transient increase in plasma corticosterone concentration, but not in anxiety or depression-like behaviors. A single intrathecal injection of an antidepressant, such as milnacipran, amitriptyline, mianserin or paroxetine, had an acute analgesic effect on ICS-induced thermal hyperalgesia at post-stress day 1 in a dose-dependent manner. In addition, repeated daily antidepressant treatments during post-stress days 1-5 gradually reversed the reduction in thermal pain threshold, and this recovery was maintained for at least 7 days after the final treatment. In addition, relief from mechanical allodynia, induced by ICS exposure, was also observed at day 9 after the cessation of antidepressant treatment. In contrast, the intravenous administration of these antidepressants at conventional doses failed to provide relief.

Conclusions: These results suggest that the repetitive intrathecal administration of antidepressants permanently cures ICS-induced FM pain in mice.

Keywords: fibromyalgia, cold stress, vicious circle, antidepressant, allodynia, hyperalgesia

2. Background

Fibromyalgia (FM) is characterized by generalized tenderness and chronic widespread pain that affects 2-4% of the population in industrialized nations and primarily affects females [1]. Although its etiology and pathogenesis are largely unknown, emerging evidence indicates that pain amplification within the central nervous system (CNS) plays a critical role in the pathology of FM pain [2]. Recent studies, including functional imaging, have revealed that this central amplification process depends, in part, on deficits in endogenous descending pain inhibitory pathways [3,4] and abnormal pain processing [5]. In addition, FM pain is often refractory to treatment using conventional painkillers, such as non-steroidal anti-inflammatory drugs and opioids [6]. However, numerous studies have demonstrated the effectiveness of antidepressants and antiepileptics, such as gabapentin and pregabalin, in the treatment of FM pain [7,8].

There are several animal models of FM pain, induced by either intramuscular injection of acidic saline [9], vagotomy [10], sound stress [11] or depletion of biogenic amines [12]. However, in order to better understand the molecular basis of the underlying pain mechanisms, it is necessary to establish an animal model which accurately reflects the pathological and pharmacotherapeutic features of the disease.

Recently, we established a mouse model of FM using intermittent cold stress (ICS), which produces long-lasting thermal hyperalgesia and mechanical allodynia, predominantly in females [13]. We found that gabapentin,

* Correspondence: ueda@nagasaki-u.ac.jp
[1]Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan
Full list of author information is available at the end of the article

particularly when injected intracerebroventricularly, had potent anti-hyperalgesic and anti-allodynic effects in this model [13]. In addition, systemically and intracerebroventricularly-administered morphine was found to have no analgesic effect in ICS-exposed mice, due to a failure to activate descending pain inhibitory pathways [14]. These findings indicate that our ICS model might accurately reflect the pathological and pharmacotherapeutic features of FM pain. In this study, we examine whether various antidepressants can ameliorate the abnormal pain sensations in this model.

3. Materials and methods

3.1. Animals

Male C57BL/6J mice weighing 18-22 g were used. They were kept in a room with an ambient temperature of 21 ± 2°C, with free access to a standard laboratory diet and tap water. All procedures were approved by the Nagasaki University Animal Care Committee and complied with the recommendations of the International Association for the Study of Pain [15].

3.2. Drug treatments

Antidepressants were obtained from Sigma (St. Louis, MO, USA). Milnacipran, paroxetine, and amitriptyline were dissolved in artificial cerebrospinal fluid (aCSF; 125 mM NaCl, 3.8 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.2 mM KH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, pH 7.4). Mianserin was dissolved in physiological saline. For vehicle treatments, aCSF or saline was injected. Intrathecal (i.t.) injections were administered according to Hylden and Wilcox [16] using a 30-gauge needle. The site of injection was chosen to be between spinal L5 and L6–near where the spinal cord ends and the cauda equina begins. This allowed us to maximize inter-vertebral accessibility and to minimize the possibility of spinal damage. After sufficient training, the experimenters were able to perform the technique without causing injury to the animals.

3.3. Experimental model of fibromyalgia

ICS exposure and constant cold stress (CCS) were performed as previously reported [13]. Briefly, for the ICS model, mice were placed on stainless mesh plate in a cold room at 4°C overnight (from 4:30 pm to 10:00 am), followed by ICS with environmental temperatures alternating between 24 and 4°C every 30 min, from 10:00 am to 4:30 pm. These procedures were repeated twice. On day 3, the mice were adapted to 24°C for 1 h before behavior testing. We designated day 3 following the onset of stress exposure as day 1 post-stress exposure (P1). For the CCS model, mice were placed in the cold room from 4:30 pm on day 1 to 10:00 am on day 3, followed by adaptation at 24°C for 1 h. Mice in the control group were kept at 24°C for all 3 days (from 4:30 pm on day 1 to 10:00 am on day 3). During the stress period, two mice were kept in each cage (12 × 15 × 10.5 cm), with free access to food and agar as alternate drink water in place of fluid. Although the body weight of mice was decreased during and after the ICS stress, it attained to the control mice level as early as 4 day after the stress (Figure 1).

3.4. Measurement of plasma corticosterone

Plasma corticosterone levels were measured as described previously [17]. Briefly, plasma was separated by centrifugation at 3 000 *g* for 15 min at 4°C and collected into ice-chilled tubes containing 0.1% EDTA and stored at -80°C until use. Blood samples were collected at 9:00 pm in order to exclude the effect of circadian rhythms on circulating plasma corticosterone. The plasma corticosterone level was estimated fluorometrically, according to the method of Zenker and Bernstein [18].

3.5. Assessment of stress-related behaviors

Spontaneous locomotor activity was measured in the open filed (22 × 33 cm) for 3 min, using SCANET apparatus (Melquest, Japan). In the elevated plus-maze test used to estimate anxiety, the time spent in the open arm was recorded during a 6-min period. To assess depression-like behaviors, the tail-suspension test was performed [19,20]. Mice were suspended 30 cm above the floor using adhesive tape, and the total duration of immobility during a 6-min period was measured.

3.6. Nociception tests

In the thermal paw withdrawal test, the nociception threshold was assessed using the latency of paw

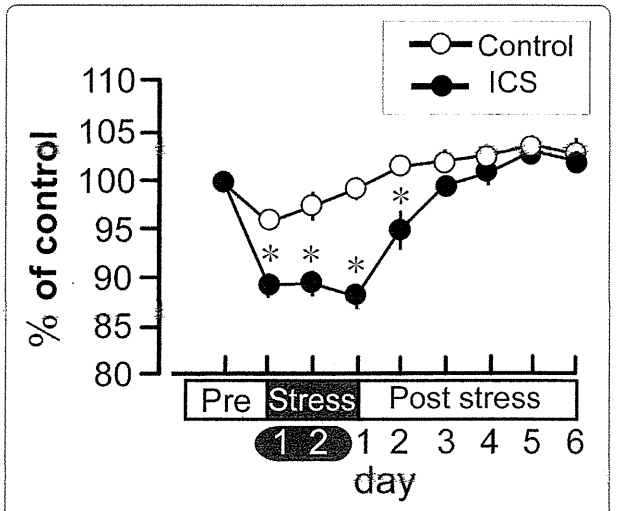

Figure 1 Changes in body weight in ICS treated mice. Results represent the percentage of body weight of mice, compared to the value at 1 day before ICS stress (Pre). *$p < 0.05$, *vs.* control group. Data are the means ± S.E.M; 6 mice per group.

withdrawal upon a thermal stimulus [21,22]. Unanesthetized animals were placed in plexiglass cage on top of a glass sheet and acclimated for 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 exactly 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 s was set to prevent tissue damage.

The mechanical paw pressure test was performed as described previously [22]. Briefly, mice were placed in a plexiglass chamber on a 6 × 6 mm wire mesh grid floor and allowed to acclimate for a period of 1 h. A mechanical stimulus was then delivered to 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. A cut-off pressure of 20 g was set to avoid tissue damage. In these experiments using thermal and mechanical tests, the thresholds were determined from three repeated challenges at 10 min intervals, and the averages were used for statistical analysis. For the time-course experiments, we measured the paw-withdrawal latencies (PWL) at 30, 60, and 180 min after intrathecal injection of antidepressant. In the area under the curve (AUC) analysis of antidepressant-induced analgesia, we calculated the AUC generated by plotting analgesic threshold (after deducting the control threshold from each threshold point) against time, from 30 to 180 min after antidepressant treatment, using a trapezoidal method.

3.7. Statistical analysis

In Figure 2 and Table 1, data were analyzed using Student's *t*-test. In Figures 1, 3, 4, 5 and 6, Tables 2 and 3, the differences between multiple groups were analyzed using a one-way ANOVA with the Tukey-Kramer multiple comparison post-hoc analysis. Significance was set at $p < 0.05$. All results are expressed as means ± S.E.M.

4. Results

4.1. Effects of ICS stress exposure on plasma corticosterone levels and anxiety and depression-like behaviors

We previously designed an improved mouse model for dysautonomia, also referred to as the specific alternation of rhythm in temperature (SART) model [23], and found that ICS, but not CCS, caused long-lasting abnormal pain sensations [13]. In the present study, we used plasma corticosterone levels as a biomarker for stress. As shown in Figure 2A, we found that ICS exposure caused a transient increase in plasma corticosterone levels at P1. In contrast, CCS exposure had no effect on plasma corticosterone levels between P1 and P12 (Figure 2A). ICS had no effect on spontaneous locomotor activity at P1 (Figure 2B). Furthermore there was no significant change in the duration of time spent in the open arm in the elevated plus-maze test or in the total duration of immobility in the tail-suspension test at P1

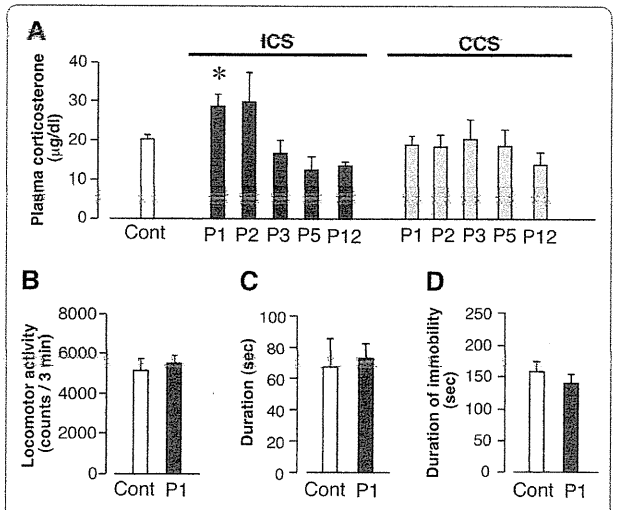

Figure 2 Effects of stress exposure on plasma corticosterone levels, spontaneous locomotor activity, and anxiety and depression-like behaviors. (A) Time-course of plasma corticosterone levels after ICS or CCS exposure. (B-D) Lack of changes in spontaneous locomotor activity in the SCANET apparatus (B), duration of time spent in the open arm of the plus-maze test (C), and total duration of immobility in the tail suspension test (D) at P1. *$p < 0.05$, *vs.* control group. Data are expressed as the means ± S.E.M; 3-5 mice per group.

Table 1 Dose-dependent acute analgesic effects of antidepressants on ICS-induced thermal hyperalgesia

Drug	Dose (μg)	n	AUC
Milnacipran	0.03	4	50.6 ± 21.5
	0.1	6	281.9 ± 71.3*
	0.3	4	166.2 ± 31.3*
Amitriptyline	5	3	51.1 ± 85.2
	15	7	252.6 ± 42.2*
	30	3	235.5 ± 64.4*
Mianserin	10	3	144.7 ± 171.5
	20	4	527.8 ± 103.2*
Paroxetine	2	3	40.7 ± 62.4
	5	7	211.2 ± 38.6*
	10	3	251.3 ± 797*

Thermal pain threshold was assessed at P1, using thermal paw withdrawal tests. Acute anti-hyperalgesic effects of milnacipran, amitriptyline, mianserin, and paroxetine were evaluated by AUC, as described in Materials and Methods. *$p < 0.05$, *vs.* vehicle-treated control group.

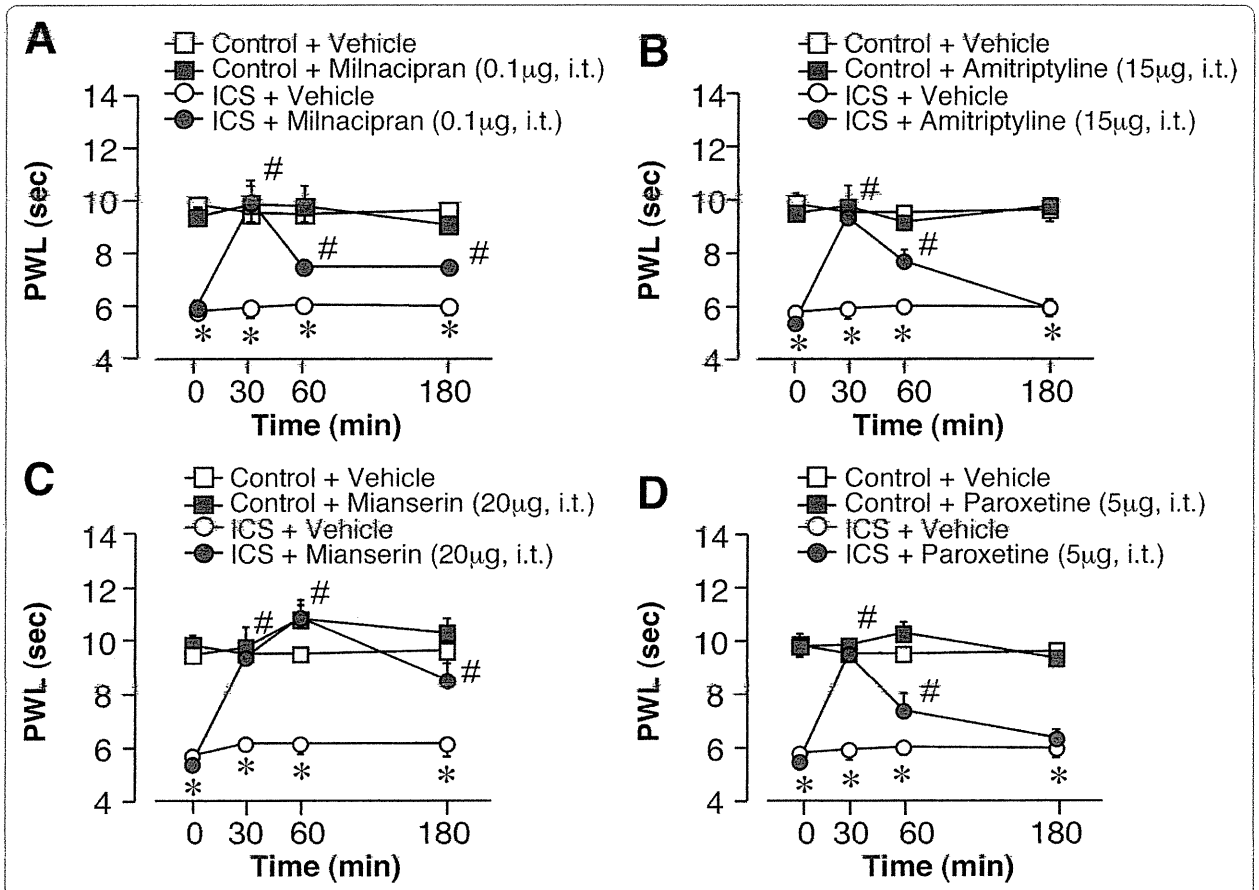

Figure 3 Antidepressant-induced acute analgesic effects in ICS treated mice. Thermal pain threshold was assessed at P1 after control or ICS treatment, using the thermal paw withdrawal test. Results represent the time course of thermal paw withdrawal latencies (PWL, in seconds) after a single intrathecal injection of antidepressants. (A-D) Each data point in [control + vehicle] and [ICS + vehicle] groups is common. *$p < 0.05$, *vs.* vehicle-treated control group; #$p < 0.05$, *vs.* vehicle-treated and ICS-exposed groups. Data are expressed as the means ± S.E.M.; 4-8 mice per group.

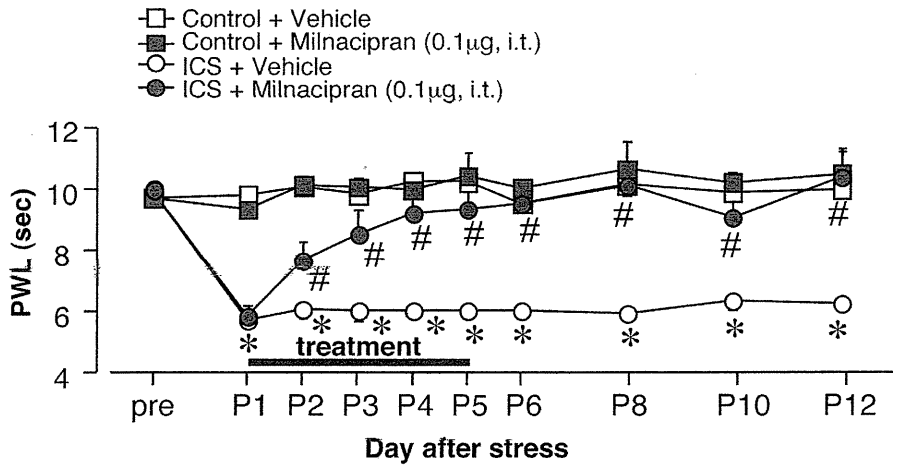

Figure 4 Permanent relief from ICS-induced thermal hyperalgesia by repeated intrathecal administration of milnacipran. Intrathecal injections of milnacipran (0.1 μg) were given once daily at 11:30 a.m. from P1-P5 after assessment of nociceptive thresholds at 11:00 a.m. Results represent the basal threshold as the latency to paw-withdrawal from thermal stimuli (PWL, in seconds), just before the daily injection of vehicle or milnacipran. *$p < 0.05$, *vs.* vehicle-treated control group; #$p < 0.05$, *vs.* vehicle-treated and ICS-exposed groups. Data are expressed as the means ± S.E.M.;4-8 mice per group.

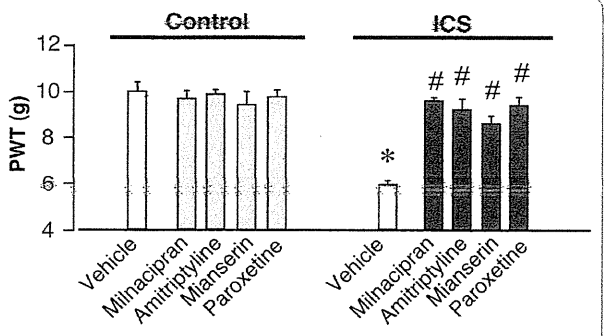

Figure 5 Complete relief from ICS-induced mechanical allodynia. Basal mechanical paw-withdrawal threshold (PWT, in grams) was assessed at P14, using paw pressure tests. Intrathecal injection of milnacipran (0.1 μg), amitriptyline (15 μg), mianserin (20 μg), or paroxetine (5 μg), was given once daily from P1-P5, as described in Figure 4. *$p < 0.05$, *vs.* vehicle-treated control group; #$p < 0.05$, *vs.* vehicle-treated and ICS-exposed groups. Data are expressed as the means ± S.E.M.; 3-6 mice per group.

(Figures 2C, D). In addition, there were no gross behavioral changes in mice as early as 1 h after the transfer from 4°C to 24°C room.

4.2. Antidepressant-induced acute analgesic effects on thermal hyperalgesia in ICS-exposed mice

Previous reports demonstrated that thermal hyperalgesia is elicited at P1 after ICS exposure and lasts for at least 12 days [13,14]. As shown in Figure 3, the nociceptive thermal threshold was significantly reduced and stable throughout experiments for 180 min. A single intrathecal injection of milnacipran (0.1 μg) had no effect on the nociceptive threshold in control mice (Figure 3A), but produced significant anti-hyperalgesic effects that persisted for at least 180 min post-injection at P1 (Figure 3A). This effect of milnacipran was dose-dependent in the range of 0.03-0.1 μg, but declined at 0.3 μg (Table 1). Statistical significance was observed at 0.1 and 0.3 μg. Similar results were observed with other antidepressants, such as amitriptyline (5-30 μg), mianserin (10 and 20 μg), and paroxetine (2-10 μg), as shown in Figures 3B-D and Table 1. However, with 20 μg of mianserin, a significant analgesic effect was observed at 60 min in the control mice, and anti-hyperalgesic effects were observed until 180 min (Figure 3C). Both amitriptyline and paroxetine showed significant anti-hyperalgesic effects, but no significance was observed at 180 min (Figures 3B, D).

4.3. Permanent relief of abnormal pain by repeated central administration

As the anti-hyperalgesic effect of milnacipran remained 180 min after intrathecal administration at day P1 after ICS stress (threshold: ~ 7.46 ± 0.2 s), we measured the nociceptive threshold at 11:00 a.m. on day P2. As seen in Figure 4, a significant anti-hyperalgesic effect still remained (threshold: ~ 7.67 ± 0.6 s). The second administration of milnacipran was performed at 11:30 a.m. The basal nociceptive threshold at 11:00 a.m. on day P3 further increased to 8.56 ± 0.8 s. The increase in basal threshold was maintained by daily administration of milnacipran. Complete recovery to the normal pain threshold was observed on P6, the day following the last administration, and lasted until P12. Similar complete reversals of hyperalgesia on P5 and P12 were observed after 5-day administrations of amitriptyline (15 μg), mianserin (20 μg), and paroxetine (5 μg), as seen in Table 2. Complete recovery was also observed with ICS-induced mechanical allodynia, even on P14, following a 5-day administration of the antidepressants (Figure 5).

4.4. Lack of beneficial effects by repeated systemic administration

When milnacipran was given by intravenous (i.v.) injection (10 mg/kg), there was a significant analgesic effect in the thermal nociception test at 30 min in control mice. However, there was no significant suppression in the ICS mouse model using this dose of antidepressant up to 180 min on P1 (Figure 6A). The absence of an ameliorative effect on ICS-induced hyperalgesia was also observed with amitriptyline (3 mg/kg, i.v.), mianserin (10 mg/kg, i.v.), and paroxetine (1 mg/kg, i.v.), despite producing significant acute analgesia at 30 min in control mice (Figures 6B-D). In addition, the repeated systemic administration of milnacipran for 5 days did not affect the basal threshold throughout the experiment (Figure 6E). Repeated administrations of amitriptyline, mianserin or paroxetine also did not provide relief from ICS-induced hyperalgesia (Table 3).

5. Discussion

Patients with FM exhibit widespread pain, with diverse symptoms, such as fatigue, depression, and sleep disturbance. Although the pathogenesis of FM is not clearly understood, certain biological stressors, such as autonomic nervous system disorder and psychological distress seem to be closely related to the development of FM [24]. An important role for such stressors is supported by studies using animal models in which rats or mice are subjected to stressors, such as chemical, sound, or surgery stress, which induce long-lasting abnormal pain [9-11,25]. Recently, we reported that ICS produces long-lasting thermal hyperalgesia and mechanical allodynia in mice [13,14]. The ICS-induced pain is bilateral and female-predominant (after gonadectomy) [13], which are also features found in FM patients [26].

In this study, mice subjected to ICS exhibited a transient increase in plasma corticosterone levels on P1. In

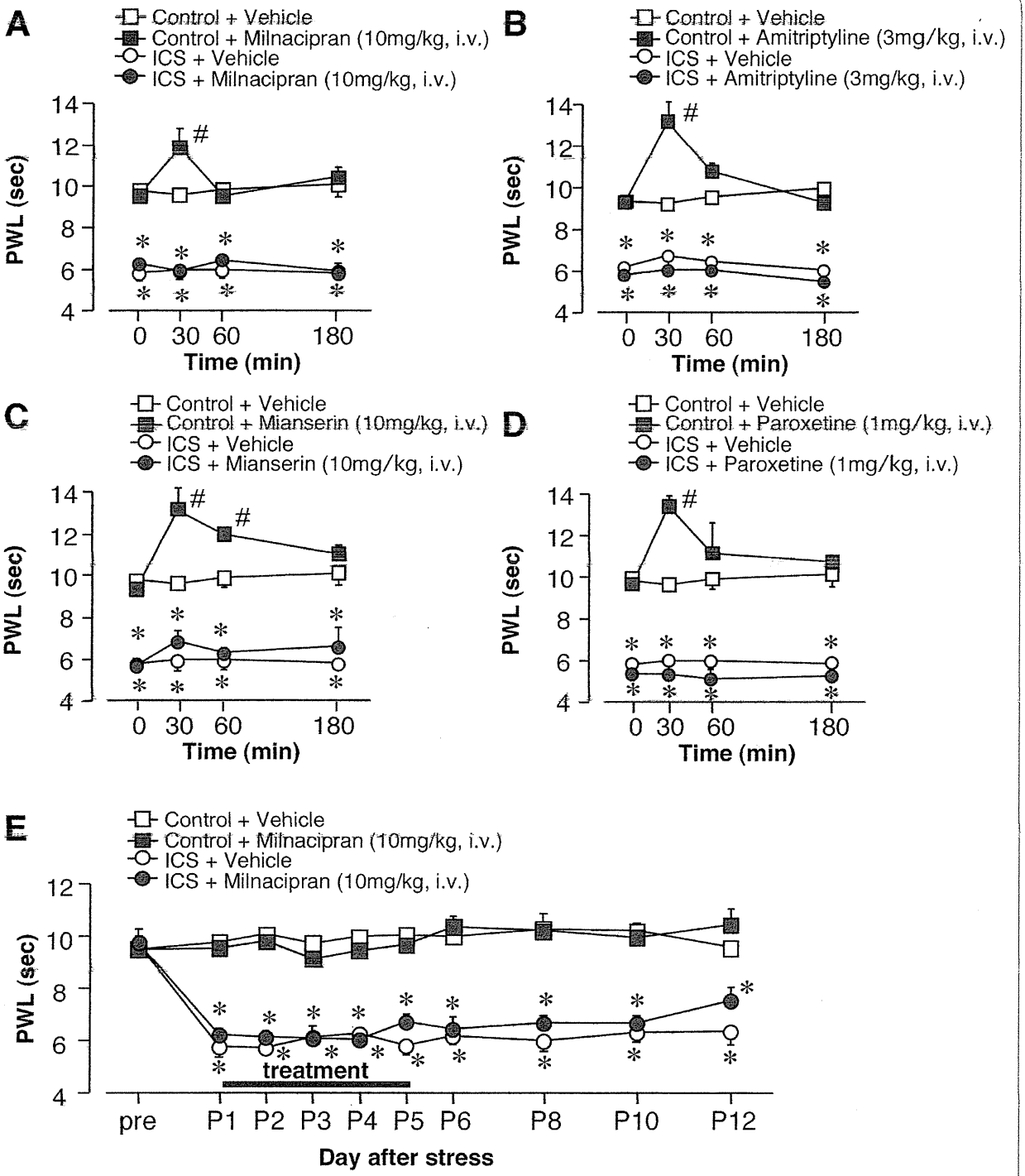

Figure 6 Lack of anti-hyperalgesic effects by systemic administration of antidepressants. Thermal pain threshold was assessed at P1 after control or ICS-treatment, using thermal paw withdrawal tests. (A-D) Results represent the time-course of thermal paw-withdrawal latencies (PWL, in seconds) after a single i.v. injection of antidepressants. (A-D) Each data point in [control + vehicle] and [ICS + vehicle] groups is common. (E) Milnacipran was given i.v. once daily for 5 days, as described in Figure 4. Results represent the basal threshold as the latency to paw-withdrawal from thermal stimuli (PWL, in seconds), just before the daily injection of vehicle or milnacipran. *$p < 0.05$, *vs.* vehicle-treated control group; #$p < 0.05$, *vs.* vehicle-treated and ICS-exposed groups. Data are expressed as the means ± S.E.M.; 3-6 mice per group.

Table 2 Permanent relief from ICS-induced thermal hyperalgesia by repeated intrathecal (i.t.) administration of amitriptyline, mianserin or paroxetine

	n	Pre (sec)	P1 (sec)	P5 (sec)	P12 (sec)
Control-vehicle (i.t.)	8	9.84 ± 0.33	9.81 ± 0.45	10.24 ± 0.23	9.99 ± 0.22
ICS-vehicle (i.t.)	8	10.22 ± 0.28	5.76 ± 0.15*	6.04 ± 0.16*	6.28 ± 0.21*
ICS- amitriptyline (15 μg, i.t.)	4	10.48 ± 0.23	5.35 ± 0.36	8.44 ± 0.16#	9.35 ± 0.66#
ICS-mianserin (20 μg, i.t.)	4	9.68 ± 0.30	5.35 ± 0.36	8.13 ± 0.25#	9.95 ± 0.66#
ICS- paroxetine (5 μg, i.t.)	4	9.82 ± 0.44	5.47 ± 0.13	9.08 ± 0.46#	9.52 ± 0.21#

Antidepressants were administered between P1-P5, as described in Figure 3. Thermal pain threshold was assessed using the thermal paw withdrawal test. *$p < 0.05$, *vs.* vehicle-treated control group; #$p < 0.05$, *vs.* vehicle-treated and ICS-exposed groups.

contrast, there was no significant change in corticosterone levels in mice subjected to CCS. Considering that the abnormal pain in CCS mice was only transient, and not long-lasting [13,14], the rise in corticosterone levels in ICS mice likely played a role in the appearance of abnormal pain. A recent report suggests that the stress-induced increase in corticosterone concentration may be related to abnormal pain behavior in an FM-like animal model, possibly through a mechanism involving epinephrine release [27].

In our ICS model, the mice did not show significant changes in the tail-suspension test, a behavioral test designed to assess depression-like behavior [28]. This is in contrast to a study using less frequent temperature alternation (the SART model), in which mice exhibited hyperalgesia for only a week [29], and there was a transient reduction of immobility duration in forced swimming test, followed by gradual recovery in 5-6 days [30]. As the forced swimming causes a facilitation of immobility in an antidepressant-reversible manner [31], it is not clear whether the transient reduction of immobility duration reflects depression. From this point of view, the tail suspension test seems to be a better method for evaluation of depression-related despair behavior. Gabapentin and pregabalin are widely used to treat FM patients in the clinic [32,33]. These medicines alleviate abnormal pain and the accompanying fatigue and insomnia, without affecting depressive symptoms [33,34]. Therefore, the presence of depression-like behavior is unlikely to be necessary in animal models of FM. Consequently, the ICS model may be more clinically relevant than the SART model for evaluating long-term pain.

Various antidepressants have been used for FM in the clinic [35,36]. Recently milnacipran and duloxetine, serotonin/norepinephrine reuptake inhibitors, and serotonin-specific reuptake inhibitors have been approved for treating FM pain by the United States Food and Drug Administration. As the antinociceptive activities of these compounds are largely independent of their effects on mood, making them potentially efficacious for patients with or without depressive [37], it appears to reflect the

Table 3 Lack of anti-hyperalgesic effects by repeated systemic administration of antidepressants

	n	Pre (sec)	P1 (sec)	P5 (sec)	P12 (sec)
Control-vehicle (i.v.)	8	9.51 ± 0.22	9.80 ± 0.18	10.00 ± 0.29	9.57 ± 0.30
ICS-vehicle (i.v.)	8	9.67 ± 0.23	5.78 ± 0.36	6.02 ± 0.12	6.36 ± 0.21
ICS- amitriptyline (3 mg/kg, i.v.)	4	9.55 ± 0.42	5.85 ± 0.37	5.79 ± 0.36	6.72 ± 0.35
ICS-mianserin (10 mg/kg, i.v.)	4	9.77 ± 0.34	5.14 ± 0.56	5.98 ± 0.39	6.66 ± 0.41
ICS- paroxetine (1 mg/kg, i.v.)	4	9.34 ± 0.36	5.29 ± 0.34	6.89 ± 0.30	6.96 ± 0.65
ICS- milnacipran (10 mg/kg, i.v.)	4	9.75 ± 0.47	6.25 ± 0.25	6.71 ± 0.21	7.54 ± 0.53

Amitriptyline, mianserin, paroxetine or milnacipran were given intravenously (i.v.) and assessment of basal nociceptive thresholds was performed as described in Figure 5. Thermal pain threshold was assessed using the thermal paw withdrawal test. *$p < 0.05$, *vs.* vehicle-treated control group.

importance of central descending monoaminergic pathways in pain regulation [38,39]. Recent studies revealed that polymorphisms in the 5-HT receptor, transporter, and metabolic enzyme can contribute to the etiology of FM [40-42]. The fMRI study also demonstrates that brain regions involved in descending pain inhibitory pathways appear to have decreased activity in FM patients [43]. Although serotonergic and/or noradrenergic pathways are well documented as descending pain inhibitory pathways [39], there is no report that the abnormality of such descending monoaminergic systems is observed in FM patients. However, it would be challenging to examine the effects of representative antidepressants on ICS-induced abnormal pain by introducing the drugs into the intrathecal space, very close to target regions.

Our study shows that the repeated intrathecal administration of different antidepressants gradually suppressed ICS-induced pain. The gradual reversal of abnormal pain may be related to the down-regulation of β-adrenoceptors or abnormal monoaminergic metabolism [44-46]. Alternative mechanisms may include the altered expression of multiple receptors and ion channels, such as the NMDA receptor, opioid receptors, and sodium channels [47-49]. It should be noted that the reversal of abnormal pain continued after the cessation of drug treatment, for each of the antidepressants tested. Although further investigation is required to clarify the molecular mechanisms of antidepressant action and to provide a permanent cure for ICS-induced abnormal pain, it is interesting to speculate that the chronic pain may be due to a vicious cycle of pain elicited by reduced inhibitory input from monoaminergic pathways. Thus, the rescue of pain-inhibitory mechanisms by repeated antidepressant treatment should halt chronic pain. Similar observations were made in our previous study using central administration of gabapentin [13,14]. In that study, using the ICS model, a single intracerebroventricular administration of gabapentin produced a 4-day period of anti-hyperalgesia. As the injection had no effect on peripheral nerve injury-induced neuropathic pain [13,14], and the gabapentin was unlikely to have remained in the brain for 4 days, it is interesting to speculate that the observed effect is due to the inhibition of the pain cycle, possibly through enhancement of inhibitory transmission. However, the present study demonstrates that systemic administration of various antidepressants had no significant beneficial effect on ICS-induced hyperalgesia, though they had a significant acute analgesic effect in control mice. As the clinically beneficial effects of oral antidepressants to FM patients were evident when they are treated for more than several weeks [50], the lack of effects of intravenous antidepressants in the present study may be attributed to the shortage of treatments (5 days). In this meaning it is surprising that only 5 days repetitive intrathecal treatments abolishes abnormal pain even after the cession of treatments. Furthermore, although the mechanisms underlying the lack of antihyperalgesic effect remain elusive, it may be worthwhile to investigate possible involvements of interference of spinal effects by peripheral pain facilitating serotonergic actions or by descending pain facilitating monoaminergic systems [39]. Thus, we expect that repetitive intrathecal administration of antidepressants are likely to be more effective at treating FM-like pain in mouse models.

Finally, this study demonstrates that the ICS model has similarities to clinical features of FM in terms of the sensitivity to analgesics or adjuvant analgesics. In our previous findings, we observed that the effective dose of gabapentin was 3 mg/kg for ICS-induced pain, but was over 30 mg/kg for nerve injury-induced neuropathic pain in mice [13,14], consistent with the fact that the clinically-effective dose of gabapentin for FM patients is lower than that for neuropathic pain [51]. In addition, we observed that ICS-induced thermal hyperalgesia was resistant to morphine treatment [13,14], consistent with the clinical evidence [52]. Considering that other experimental animal models of FM-like pain exhibit morphine analgesia (albeit with low potency) [53-56], the ICS model may be pharmacologically distinct from the others.

6. Conclusion

This study demonstrates that repeated intrathecal antidepressant treatment provides a complete cure of ICS-induced FM-like abnormal pain. Based on the pharmacological similarity of ICS-induced pain to clinical FM, the ICS model appears to be suitable for investigating the pathogenesis of FM and for evaluating therapeutic strategies for this debilitating illness.

List of abbreviations used

aCSF: artificial cerebrospinal fluid; AUC: area under the curve: CCS: constant cold stress; FM: fibromyalgia; ICS: intermittent cold stress; PWL: paw withdrawal latency; PWT: paw withdrawal threshold.

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Author details

[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 Pharmacology, Wakayama Medical University, 811-1 Kimiidera, Wakayama 641-0012, Japan.

Authors' contributions

MN participated in the experimental designing, collection and analyses of data, and drafted the manuscript in equal contribution. HU and JN

performed the statistical analyses and carried out surgical manipulation, data collection, and drafted the manuscript. KA and TM performed stress exposing and participated nociceptive behavior assay. SK measured plasma corticosterone levels. HU conceived of the study, participated in its design and coordination. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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特集「臨床を裏づける神経障害性疼痛の本態」によせて

植田弘師

長崎大学大学院医歯薬学総合研究科分子薬理学

Introduction
Essentials of neuropathic pain which support clinical data

Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences

痛みはあくまでも主観的な感覚であり，病状を視覚的に明らかに示すことはできない．しかも，それが実験動物であり，ヒトでも客観的な評価を行うことが難しいとされる慢性疼痛である場合には，その研究成績の正当性を証明することは困難を極めるといわれている．しかし，慢性痛に限らず創薬を試みるには，この実験動物における基礎研究の段階を素通りしてなし得ることはほとんどない．そんな中で最も現実的に臨床と基礎とを関連づけることができるアプローチは，薬理学的評価である．これまでの先人の優れた研究成果から慢性疼痛，特に神経障害性疼痛の優れた病態モデルが開発され，その病態生理，組織化学，生化学的特徴が報告され，臨床・基礎研究により，病態の鍵を握る重要な因子が明らかにされてきた．本特集では，神経障害性疼痛の病態形成から治療戦略まで，薬理学的視点から最新の話題を解説することを目的としている．

第一稿では，永井・植田により，神経障害性疼痛（術後痛など）のモルヒネによる先制鎮痛機構が述べられている．ここでは，筆者らが一連の研究において明らかにしてきた，神経障害性疼痛の初発原因分子としてのリゾホスファチジン酸（LPA）の生合成と関連づけた研究成果を明らかにしている．第二稿で，大久保・川畑は，硫化水素（H_2S）の疼痛情報伝達の役割を解説している．H_2Sは，知覚神経終末に発現する$Ca_V3.2$T型カルシウムチャネルを活性化し，体性痛や内臓痛を促進することが知られているが，本稿では，末梢神経障害や化学療法薬による神経障害性疼痛時におけるH_2S合成と$Ca_V3.2$T型カルシウムチャネル発現変動との関連が紹介されている．第三稿では，田辺によって，ガバペンチンによる鎮痛効果の上位中枢における新たな作用機構が解説されている．神経障害性疼痛治療としてのガバペンチン/プレガバリンの作用点としては，これまで，末梢知覚神経におけるカルシウムチャネルの$Ca_V\alpha_2\delta$に対する抑制効果として解説されてきたが，本稿では下行性ノルアドレナリン疼痛抑制系やそのGABA神経による制御との関連が紹介されている．第四稿で，柴田・小泉は，臨床的に治療効果が確認されている生薬ブシ末について，神経障害性疼痛抑制効果のメカニズムを，脊髄のアストロサイトの脱活性化との関連で詳細に解説している．第五稿で，宮野・上園・仲田は，化学療法薬であるパクリタキセルにより誘発される末梢神経障害のメカニズムとして，C線維からのサブスタンスP遊離の関与を解説している．このメカニズムは，これまで報告されてきたA線維に対する機構と対比して興味深いものである．第六稿

では，木口・岸岡が，末梢神経障害性疼痛における神経炎症の役割を解説している．本稿では，サイトカイン・ケモカインを中心に，炎症性メディエーターが末梢神経や中枢神経において複雑なネットワークを形成することで慢性神経炎症を誘導することを述べている．従来，神経障害性疼痛は炎症と対比して捉えられてきたことを合わせて考えると，治療薬理学的ツールの活用という観点から本稿は大変興味深い．

近年，治療薬が承認されるようになり，神経障害性疼痛はもはや「時限付き」の難治性疾患になりつつある．本稿で見られるように，臨床で用いられている治療薬が新たな分子機構とともに世界中で認知されることが，今後の慢性疼痛治療克服の確かな道のりであれと祈るばかりである．

※　　※　　※

神経障害性疼痛に対するモルヒネ先制鎮痛

永井　潤　植田弘師
長崎大学大学院医歯薬学総合研究科分子薬理学分野

要　旨
神経障害時に生じる強力な痛み刺激が異常痛の慢性化に至る神経可塑的変化を誘発するならば，あらかじめその痛みを抑制することで慢性痛の記憶を予防できると考えられる．実際，先制鎮痛という治療法において，この概念が術後痛の予防として臨床で証明されている．一方，筆者らは，神経障害性疼痛の初発因子として，脂質メディエーターであるリゾホスファチジン酸（LPA）を同定しており，近年ではこのLPAは強い痛み刺激によって脊髄で産生されることを明らかにしている．本稿では，モルヒネを用いた先制鎮痛のメカニズムについて，痛み刺激によって産生されるLPA合成の観点から解説する．
（ペインクリニック 32：1457-1463，2011）
キーワード：モルヒネ，先制鎮痛，リゾホスファチジン酸

はじめに

神経障害によって引き起こされる痛みの多くは，原因から解除されても持続する長期性であり，記憶機構と深く関連することが知られている．長期化した記憶性の痛みは，痛み自体が誘発する新たな分子機構によって，末梢知覚神経，脊髄および上位脳の神経機能の可塑性を増幅する，いわゆる「痛みの悪循環」によって正常時の痛みと大きく異なった複雑な痛みの伝達がなされている．したがって，原因となる発症初期の段階での疼痛管理は，神経障害性疼痛の予防・抑制にとって非常に重要である．先制鎮痛は，術中などのあらかじめ予想される侵害刺激に対して予防的に鎮痛薬を処置し，神経障害発症初期のメカニズムを抑制することで術後痛などに効果を上げている．しかしながら，先制鎮痛の詳細なメカニズムは依然として不明のままである．筆者らは，神経障害性疼痛の誘発分子として，リゾホスファチジン酸（lysophosphatidic acid：LPA）を発見しているが，最近，この先制鎮痛がLPA産生の抑制と連関することを明らかにできたので，本稿ではこの研究成果を紹介する．

1．神経障害性疼痛に対する先制鎮痛効果

神経障害性疼痛の形成および維持機構の特徴の一つとして，反復痛み刺激によって脊髄後角ニューロンの感受性が亢進する中枢性感作が挙げられる[1]．そこで，この初期に痛み刺激によって引き起こされる神経活動の一連のカスケードを抑制することが，その後，長期的に続く過敏応答を防ぐことができると考えられる．

〈Special Article〉 Essentials of neuropathic pain which support clinical data
Pre-emptive morphine treatment for neuropathic pain
Jun Nagai and Hiroshi Ueda
Division of Molecular Pharmacology and Neuroscience, Nagasaki University Graduate School of Biomedical Sciences

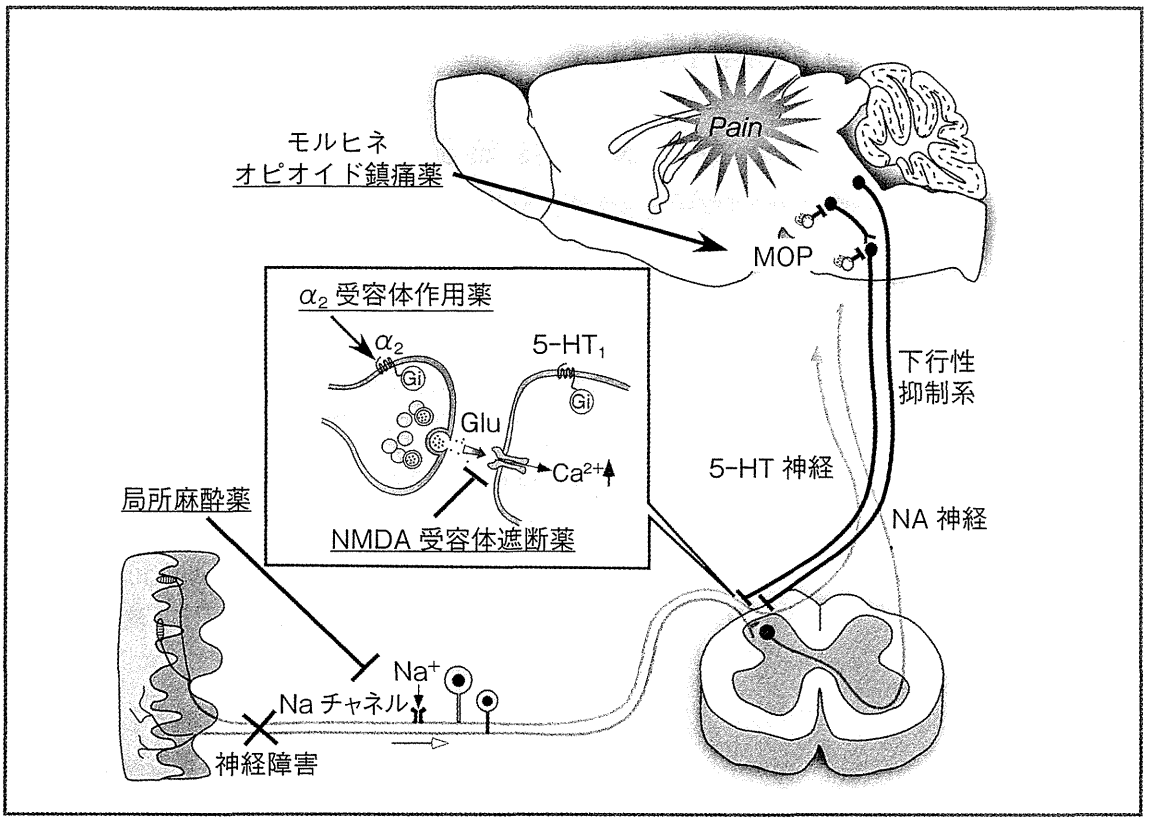

図1　疼痛伝達経路と主な先制鎮痛の標的部位

このような中枢性感作を抑制する試みは，侵害刺激の開始前に鎮痛療法の処置を行う「先制鎮痛」として知られていた[2]．この先制鎮痛の概念は，1900年代前半，Crile[3]によって最初に提唱され，その後1983年に，Woolfら[4]によって動物実験モデルで証明されるようになり，様々な外科的手術後に生じる術後痛をやわらげるために，臨床でもこの療法が使用されるようになった[2,5-8]．実際，臨床の場において，その症状などによって先制鎮痛の有用性にはいくつかの議論が論じられているにもかかわらず，入院期間や術後合併症の減少および生活の質の改善の点から多大な経済的利点が存在すると考えられている[9]．この先制鎮痛の標的は，局所麻酔薬，神経ブロックや硬膜外ブロックなどによる神経遮断，モルヒネなどの鎮痛薬の使用，NMDA受容体拮抗薬による疼痛伝達経路の遮断などが考えられている[2,7-9]（**図1**）．代表的な鎮痛薬であるモルヒネや他のオピオイド鎮痛薬による先制鎮痛効果は古くから研究されており，臨床と前臨床試験の両方において手術前投与によって術後痛を改善することが報告されている[10-12]．さらに近年の研究では，モルヒネをはじめとしたオピオイド鎮痛薬が，実験動物モデルにおける疼痛の中枢性感作を防ぐことが実証されている[13]．

2．下行性抑制機構を介するモルヒネ先制鎮痛

筆者らは，神経障害モデルマウスを用いて，モルヒネの先制鎮痛の責任部位が脊髄より上位，すなわち，脳にその標的が存在することを明らかにした[14]．坐骨神経障害モデルマウスを用いて，神経障害処置30分前にモルヒネの全身投与を行ったところ，その後に続く慢性疼痛

の形成は抑制された．しかしながら，神経障害30分後にモルヒネ投与を行ったマウスにおいては，神経障害性疼痛は抑制されなかった．一方，モルヒネの脳室内投与および脊髄腔内投与をそれぞれ行ったところ，脳室内投与においては顕著に慢性痛の形成を抑制したにもかかわらず，脊髄くも膜下腔内投与を行ったマウスにおいては有意な抑制効果は観察されなかった．そこで，このモルヒネの作用点を明らかにするために，モルヒネ全身投与と同時にMOPの拮抗薬であるナロキソンを脳室内に投与しておくと，モルヒネの先制鎮痛作用は抑制され，一方，脊髄くも膜下腔内にナロキソンを投与しても抑制効果はなかった．これらの結果から，モルヒネによる先制鎮痛の主な標的は脳内にあることが示唆される．モルヒネは下行性のセロトニン神経系あるいはノルアドレナリン神経系の活性化によって，脊髄レベルで疼痛伝達を抑制していると考えられており[15,16]，実際にこのモルヒネ先制鎮痛は非選択性5-HTアンタゴニストのメチセルギドおよびノルアドレナリン受容体アンタゴニストのフェントラミンの脊髄くも膜下腔内への投与により遮断された．興味深いことは，5-HT再取り込み阻害薬であるフルオキセチンおよびα_2アゴニストのクロニジンを前投与しても同様な先制鎮痛が観察されたことである．すなわち，オピオイドを介した下行性抑制系賦活化機構によらなくても，脊髄レベルでノルアドレナリンやセロトニン作用を増強することで同様の先制鎮痛効果が認められたことになる．さらに，筆者らは，生化学的な実験においても先制鎮痛の効果を証明している．神経障害後の脊髄後角において観察される神経発火のマーカー遺伝子である*c-fos*や中枢性感作に関与することが知られているPKCγの発現上昇も，モルヒネ，クロニジンおよびフルオキセチンの前投与によって，ほぼ完全に抑制された[17,18]．

3．神経障害誘発性LPA合成とモルヒネ先制鎮痛の抑制

著者らは，強い痛み刺激が脊髄後角において神経障害性誘発因子LPA産生を誘導し，その産生されたLPAが逆行性シグナルとして神経障害性疼痛の代表的な分子機構であるDRGでの$Cav\alpha_2\delta_1$の発現上昇および後根神経での脱髄，脊髄後角におけるPKCγの発現上昇を誘導することを見い出している[19-22]．$LPA_{1/3}$受容体の拮抗薬を用いた薬理学的実験において，神経障害の2～3時間後におけるLPAシグナリングの阻害は，その後，長期に続く神経障害性疼痛を抑制するので，初期に産生されるLPAが神経障害性疼痛を誘導することを示唆している[23]．

そこで，筆者らは，モルヒネの先制鎮痛がLPAの合成系に連関しているかを検討するために以下の実験を行った．

1）神経障害誘発性LPA産生の定量

筆者らは，LPAを高感度に定量できるバイオアッセイ法を確立し，この方法を用いてLPAの定量を行ったところ，実際に神経障害3時間後を最大値として，LPA産生が障害側の脊髄と後根神経において増加することを明らかにした[24]．また，LPAは体液中ではリゾホスホリパーゼD（LPLD）活性を持つオートタキシン（ATX）によって，リゾホスファチジルコリン（LPC）から産生されることが知られているが[25,26]，ATX遺伝子欠損マウスにおいては，このLPA産生は有意に抑制され，したがって，神経障害時にATXを介してLPAが産生されたと考えられる．

2）脊髄切片培養を用いた疼痛刺激によるLPA産生の定量

脊髄切片培養を用いた*in vitro*の実験におい

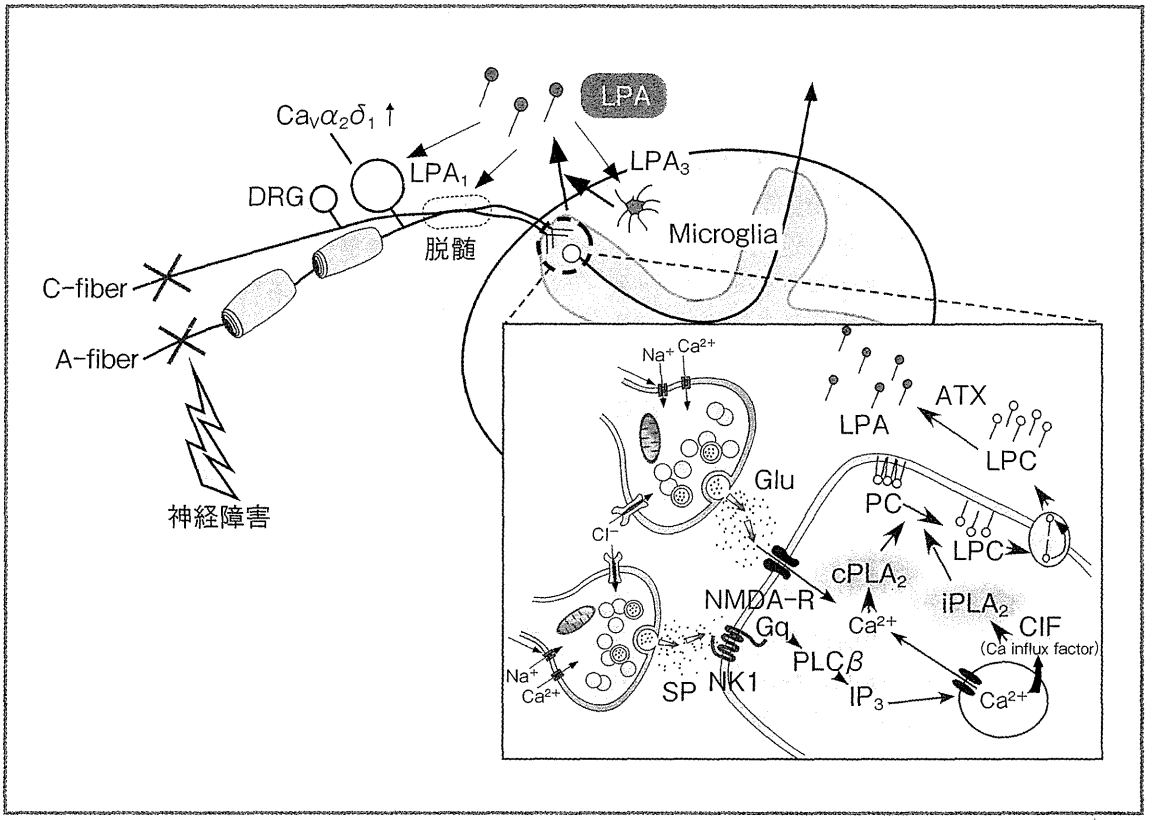

図2　痛み刺激によるLPC/LPA産生

強度な知覚神経刺激によって，伝達物質であるサブスタンスP（SP）およびグルタミン酸（Glu）が同時に放出され，それぞれサブスタンスP受容体（NK1）およびグルタミン酸受容体（NMDA-R）を活性化する．その下流において，$cPLA_2$はCa流入により活性化され，$iPLA_2$は過剰刺激による小胞体（ER）内Ca枯渇に伴い遊離されるCIF（calcium influx factor）により，活性化される．$cPLA_2$および$iPLA_2$は，細胞膜の構成成分であるホスファチジルコリン（PC）の*sn*-2位のアシル基を加水分解して，リゾホスファチジルコリン（LPC）を生成する．細胞外に放出されたLPCは，脳脊髄液中に存在するオートタキシン（ATX）によってLPAへと変換され，脊髄のミクログリアおよびLPA_3受容体を介して自身の産生を増強している．神経障害およびPLA_2によって増加したLPAは，逆行性シグナルとして神経障害性疼痛の分子基盤である$Cav\alpha_2\delta_1$の発現上昇や有髄線維の脱髄を誘発する

て，強い一次求心性神経刺激を擬似する目的でサブスタンスPとグルタミン酸の両方を加えると，ATX存在下でLPAを産生することを明らかにした[27]．特筆すべきことは，サブスタンスPやグルタミン酸をそれぞれ単独で加えても，LPAは産生されないので，LPAは神経障害などの強い刺激や複合の刺激が同時に脊髄に入力した時のみに産生されることを示唆している[27]（**図2**）．

3）LPA誘発性LPA産生機構

また興味深いことに，脊髄くも膜下腔内またはATX存在下の脊髄切片培養にLPAを加えると，LPA産生が増加することを見い出している．このLPA誘発性LPA産生のメカニズムにおいて，ミクログリアの活性化やLPA_3の活性化が関与することを明らかにしている．一方，ミクログリアの阻害薬を神経障害前投与することにより，LPA産生が抑制され，神経障害性疼痛の形成を阻害するが，障害2日目以降

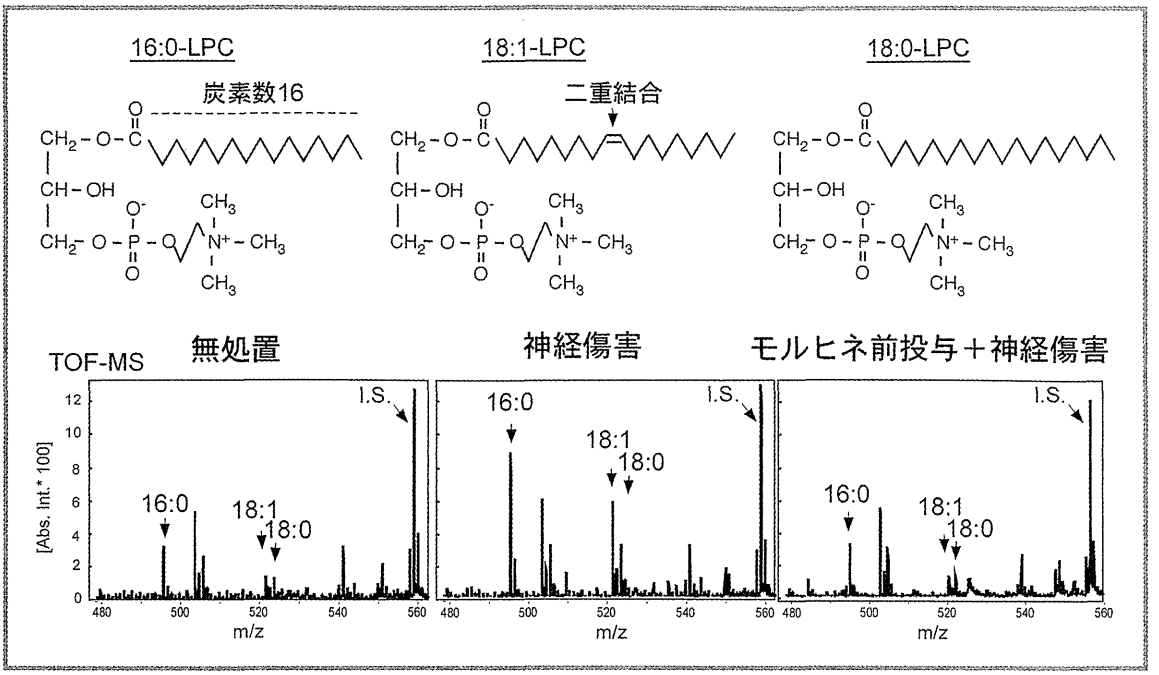

図3　LPC 分子種と神経障害誘発性 LPC 増加

に投与しても神経障害性疼痛を抑制しなかった．このことから，神経障害後すぐに生じるミクログリアを介したLPA産生増幅機構が神経障害性疼痛の記憶機構に重要であると考えられる．

4）LPC 分子種の同定

LPA や LPC は，脂肪酸の長さや不飽和の数によって複数の分子種が存在しており，分子種によってLPA受容体やATXへの結合能が異なることが知られている[28,29]．LPA は酸性リン脂質であり，MS解析には高濃度を必要とするため，筆者らは，LPC を簡便かつ高感度に定量できる方法を確立した[30]．この方法を用いて，神経障害後の脊髄の LPC の定量を行ったところ，神経障害 75 分後において，脂肪酸がパルミチン酸（16：0，炭素数 16 で二重結合 0 という意味），ステアリン酸（18：0）およびオレイン酸（18：1）の LPC が上昇していた[30]（図3）．これらの LPC は主に PLA_2 の活性によって，ホスファチジルコリン（PC）から産生されるリゾリン脂質であり，実際に $cPLA_2$ や $iPLA_2$ 阻害薬の前投与によって神経障害疼痛の形成や LPA 産生が抑制されるという知見と一致している[24]．さらに，ATX 遺伝子ヘテロ欠損マウスにおいてはこれらの LPC 産生ピークは，120 分後にシフトし，LPC 量も増加していたので，神経障害によって産生された LPC は 75～120 分の間に ATX によって LPA へと転換されることを示唆している．

5）モルヒネ先制鎮痛による LPA 合成系の抑制

モルヒネを神経障害前に脳室内投与しておくと，神経障害によって産生される 16：0-，18：0-および 18：1-LPC 産生増加はほぼ完全に抑制され，その他のピークについては有意な変化は観察されなかった[30]．すなわち，モルヒネ先制鎮痛のメカニズムは，脊髄後角における痛み入力を遮断し，LPC/LPA 産生を抑制することで，記憶性の神経障害性疼痛を遮断したことを示唆している．

おわりに

今回，先制鎮痛のメカニズムについて，神経障害誘発性のLPA合成系を抑制するという形で明らかにした．別の言い方をすると，LPA産生は強度の痛み刺激により生じ，それが慢性疼痛という一種の記憶機構を形成する上で重要であることを明らかにした．脊髄で産生されたLPAは，それ自身の産生を増強するようなフィードフォワード機構を介して，痛みの悪循環に関与していることが示唆されているので[20,31]，今後，痛み刺激やLPA自身によって産生されるLPAのメカニズムをより詳細に明らかにしていく必要がある．一方，慢性痛の記憶形成には上位脳の関与が示唆されているが，このような脊髄におけるフィードフォワード機構の分子基盤を基に上位脳への研究に取り組む必要がある．

本研究が，今後の慢性痛研究の基盤となり，世界的な基礎研究活動の活性化を促し，痛みのコントロールが万人において可能になることを願う次第である．

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線維筋痛症の薬物療法

第一薬科大学　助教（薬剤設計学講座）　西依（にしより）　倫子（みちこ）
長崎大学　教授（大学院 分子薬理学分野）　植田（うえだ）　弘師（ひろし）


線維筋痛症は、全身に伴う激しい痛みが少なくとも3ヶ月以上の長期間持続する難治性疼痛疾患である。国内においても推定200万人以上の発症率が報告されており、決して少なくない罹患率である。しかしながら、発症原因は不明で血液検査やバイタルサインに異常が認められず、見た目にも判別の難しい症状である事から、線維筋痛症として診断される事の困難さが問題となっている。そこで近年、線維筋痛症学会を中心に、様々なガイドラインの制定・治療方針等が臨床医師、患者、コメディカル医療スタッフ、研究者らにより検討されており今後の進展が期待されている。

本疾患の重大な問題点は、コントロールが非常に難しい疼痛症状である。一般的に炎症や組織損傷に伴う痛みの多くは原因が治癒されるとともに消失する。これらは発症原因が明らかであるために予防・対処が容易であり、症状が慢性化する事は少ない。一方で線維筋痛症に認められる突如発症型の痛みに対しては、治療のターゲットが不明で、薬物・非薬物療法があくまでも対症療法的に試みられる傾向であった。そこで本稿では本疾患で多く使用される鎮痛薬、鎮痛補助薬を例に薬理学的観点から線維筋痛症の病態を考察する。

1．線維筋痛症の病態分類と疼痛治療アプローチ

一般的に鎮痛薬に分類される薬物には抗炎症薬やモルヒネなどの麻薬性鎮痛薬があげられる。近年では抗うつ薬やガバペンチン、プレギャバリンにおいても鎮痛作用が認められ、補助薬としての使用頻度が高くなっている。これらの薬剤は単一で処方される事は少なく、各々低用量で多剤併用するのが一般的である。これは副作用を最小限に抑えた上、患者個人の病態に最適の治療を行うための薬理学的アプローチである。日本における線維筋痛症ガイドラインでは、病態の特異性ごとに部類分けし、それぞれに適した治療法を提案している。

1）　筋肉緊張亢進型（Ⅰ型）

筋緊張亢進とは、安静状態に『つっぱった感じ（痙縮）』、『手足のこわばり（固縮）』を誘発する状態を主にいう。痛みに転じる原因としては筋や筋以外の組織の損傷や病変に伴う場合、また不安などの精神ストレスによる場合が考えられる。そのためⅠ型にはストレスに伴うような過敏性腸症候群を併発しているケースも多く含まれる。薬物療法にはこれらの筋緊張および筋肥大に対して弛緩作用を持つ抗けいれん薬などが推奨される。また、薬物外療法として筋緊張緩和を目的とした運動療法が行われる事が多い。

2）　筋附着部炎型（Ⅱ型）

線維筋痛症は炎症を伴わない病態と言われているが、線維筋痛症として確定診断された患者の一部で附着部炎を認める症例も報告されている。一般的に、筋肉は慢性的な機械的負荷に対して損傷と修復を繰り返す中で慢性炎症を生じ、これに伴い痛みを生じる。そのためⅡ型の治療には主に炎症を抑えるための抗炎症薬やTNF阻害剤、抗リウマチ薬が用いられる。

3）　うつ状態身体性症状型（Ⅲ型）

Ⅰ型、Ⅱ型では局所の筋肉に痛みが強いのに対し