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Enhancement of morphine-induced antinociception after electroconvulsive shock in mice

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Abstract

Electroconvulsive therapy (ECT) has been applied for chronic pain for decades. The amounts of opioids to treat pain are sometimes reduced after a series of ECT. The effect of ECT on morphine-induced analgesia and its mechanism underlying the reduction of morphine requirement has yet to be clarified. Therefore, we administered electroconvulsive shocks (ECS) to mice and investigated the antinociceptive effect of morphine in a hot plate test. We examined the expression level of μ-opioid receptor in the thalami of mice 25 h after administration of ECS compared to the thalami of mice without ECS administration using western blotting. ECS disturbed the development of a decrease in the percentage of maximal possible effect (%MPE), which was observed 24 h after a morphine injection, when ECS was applied 25, 23, 21, and 12 h before the second administration of morphine. We also examined the effect of ECS on the dose-response curve of %MPE to morphine-antinociception. Twenty-five hours after ECS, the dose-response curve was shifted to the left, and the EC_{50} of morphine given to ECS-pretreated mice decreased by 30.1% compared to the mice that were not pretreated with ECS. We also found that the expression level of μ-opioid receptors was significantly increased after ECS administration. These results confirm previous clinical reports showing that ECT decreased the required dose of opioids in neuropathic pain patients and suggest the hypothesis that this effect of ECT works through the thalamus.

Keywords

opioid, morphine, electroconvulsive shock, pain, electroconvulsive therapy

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Introduction

Electroconvulsive therapy (ECT), a neuro-stimulator for the brain, is a widely used method for treating drug-resistant depression and schizophrenia. Previous studies have reported that ECT has a positive and analgesic effect on chronic pain, including neuropathic pain such as phantom pain,[1,2] although several of these reports[3,4] reviewed by Rasmussen and Rummans[5] remain controversial. Most of the reported patients suffered from depression and pain. Therefore, it was proposed that ECT provided its analgesic effect for chronic pain through a mechanism that depended on its effects on alleviating depression. A case-matching study by Wasan et al.[6] addressed this issue and showed that ECT might have analgesic properties independent of its alleviation of depression, which patients have concurrently with pain. Another report described that the levels of β-endorphins in patients with depression were elevated above pre-ECT levels the day after the sixth ECT.[7] The mechanism by which ECT induces analgesia remains to be elucidated.

Neuropathic pain results from direct injury to nerves in the peripheral or central nervous system and is often accompanied by a burning or electric sensation. There

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are two million patients with neuropathic pain in the United States of America (US).[8,9] Currently available medications for neuropathic pain are mainly anticonvulsants, antidepressants, opioids, and topical agents.[10] Pharmacotherapy using antidepressants or anticonvulsants is known to achieve analgesia in fewer than half of these patients.[11]

Results from controlled trials have indicated a positive effect of opioids in treating neuropathic pain.[12] However, they are often associated with medical problems, including sedation, mental clouding, and constipation. In recent years, an increase in aggregated opioid production and deaths from opioid overdoses in the US has been reported.[13,14] Furthermore, sustained opioid consumption can result in paradoxical pain and opioid-induced hyperalgesia, characterized by nociceptive sensitization and analgesic tolerance. Opioid-induced hyperalgesia may exacerbate neuropathic pain. Therefore, clinicians need to prescribe the lowest opioid dose sufficient to suppress pain.

From the perspective of decreasing the need for opioids, several reports have indicated that some medications, including the selective 5-HT_7 receptor agonist, E-55888,[15] the sigma-1 receptor antagonist, S1RA,[16] and the α2-adrenoceptor agonist, agmatine,[17] may enhance the antinociceptive effect of opioids in mice. The *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, has been shown to potentiate the antinociceptive effect of morphine in rats, and ketamine, a weaker NMDA receptor antagonist, was reported to be successfully used as an adjuvant of opioids.[18] Only ketamine is used in patients with a clinical indication, and the development of a clinical technique to enhance the effects of opioids is therefore needed.

Since neuropathic pain is difficult to treat using pharmacotherapy alone, neurostimulation has often been combined with several pharmaceutical treatment methods. Interestingly, some studies have shown that the amount of opioids required to relieve pain is reduced after a series of ECT.[6,19] Similarly, after administering transcranial magnetic stimulation (TMS), another method for neuro-stimulation, in patients undergoing gastric bypass surgery, the dose of morphine was shown to decrease to 60%.[20] Furthermore, the analgesic effect of TMS was inhibited by pretreatment with naloxone, an antagonist of μ-opioid receptors.[21] We also reported that the more doses of opioids a patient took, the more effectively ECT suppressed neuropathic pain, and the required opioid doses were decreased one or several days after ECT in some patients.[22]

Previously, two groups[23,24] demonstrated recovery from decreased blood flow levels in the thalamus of patients and that neuropathic pain declined after a series of ECT. It has also been reported that decreased blood flow in the thalamus of patients with neuropathic pain may be recovered after TMS.[25] [^{15}O]H_2O positron emission tomography reportedly detects an elevation of cerebral blood flow in the thalamus in patients with depression during and after ECT.[26] Therefore, we focused on the thalamus and examined whether the expression level of μ-opioid receptors would change after ECS treatment in mice.

Hence, we hypothesized that ECT might enhance the analgesic effect of morphine. To determine the effects of ECT on morphine-induced analgesia and elucidate the underlying mechanism, we administered electroconvulsive shocks (ECS) to mice, an analog of ECT in humans, and investigated the antinociceptive effect of morphine in mice.

Several reports have shown that ECS alters the expression levels of neurotransmitters, including those that activate G protein-coupled receptors (reviewed by Newman et al.[27]). ECS may increase the number of 5-HT_{2A} receptors[28] and α_{1B} adrenoreceptors,[29] as well as of the expression levels of Neuropeptide Y,[30] neuronal calcium sensor 1,[31] cyclic adenosine 3', 5'-monophosphate response element-binding protein (CREB),[32] and brain-derived neurotrophic factor/its receptor, TrkB.[33] The magnitude of the alterations in each protein's expression level varies depending on the brain region and ECS dose. Repeated, but not single, ECS was reported to increase the B_{max} for [^{3}H]diprenorphine binding in the olfactory bulb, nucleus accumbens, striatum, hippocampus, amygdala, septum, hypothalamus, and pyriform cortex.[34] Since [^{3}H]diprenorphine may bind to μ, κ, and δ-opioid receptors with equal affinity, the report indicated that repeated ECS might increase the expression level of opioid receptors, but did not determine which opioid receptor subtype shows increased levels in these regions.

ECS reportedly has acute antinociceptive effects in rats and 'post-electroconvulsive shock antinociception,' which is diminished within 60 min[35–38] or 180 min.[39] In this study, we investigated whether ECS's antinociceptive effect lasts more than one day after ECS administration, as clinical reports have shown with ECT.

Methods

Animals

Male C57BL/6J mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Mice between eight and ten weeks of age were used for all experiments. All experimental procedures and conditions were approved by the Animal Experimentation Ethics Committee of Tokyo Metropolitan Institute of Medical Science; approval no. 20-019. All mice were cared for and treated humanely following the animal experimentation procedures outlined by our institution.

ECS

Bilateral ECS (90 V, 0.9 s, 50 Hz of sine pulses) using ear clip electrodes was applied using an electroconvulsive apparatus (Sakai Medical, Tokyo, Japan). The present study parameters were set by referring to a previous study[40] and clinical settings.[41] The control mice which were indicated in the legends of figures were handled similarly to those that underwent ECT except that the former received no electrical stimulation.

Hot-plate tests

Hot-plate testing was performed according to our previously used method with a slight modification.[42,43] The temperature of the metal plate was maintained at 56°C. The latency from the start of the test to the first jumping or licking of the hind legs was measured with a cut-off time of 60 s. The hot-plate response in each mouse in the drug-induced antinociception was converted to the percentage of maximal possible effect (%MPE) according to the following formula:

$$\%\mathrm{MPE} = (\text{post-drug latency} - \text{pre-drug latency})/(\text{cut-off time} - \text{pre-drug latency}) \times 100\%$$

To examine the effects of ECS on morphine-tolerance using the hot-plate test, mice were subcutaneously injected with 10 mg/kg of morphine twice at 24 h intervals, and hot-plate tests were performed before and after each morphine injection. The ECS administration was scheduled for 25 h, 23 h, 21 h, 12 h, or 1 h before the second treatment of morphine (Figure 1(a)). A 30 min interval elapsed between the first morphine injection and the first hot-plate test, between the first hot-plate test and the second morphine injection, and between the third morphine injection and the second hot-plate test, respectively.

To generate the morphine dose-response curves of the %MPE, 7-10 mice treated with or without ECS were independently administered at various concentrations of morphine as indicated in Figure 2(a) and (b). Dose-response curves were fitted using the following equation: $100/\{1 + (\mathrm{M}/\mathrm{EC}_{50})\ \hat{}\ \mathrm{A}\}$

The values of $\mathrm{EC}_{1/2}$ and A were determined from fitting curves used as parameters. 'M' is the concentration of morphine.

Western blot analyses

Thalami were collected from both brain hemispheres of differentially treated mice with/without morphine and with/without ECT and kept at -80°C until subsequent experiments. The thalami were sonicated three times for 1 min each in 750 μL of homogenate buffer containing 10 mM Tris pH 7.4, 2 mM EDTA, 0.25 M sucrose, and protease inhibitor cocktail tablets (Roche Diagnostics GmbH, Indianapolis, IN, USA). The resulting homogenate was centrifuged at 1000 × g for 5 min at 4°C. The supernatant was transferred to another tube, and 300 μL of homogenate buffer was added to each tube, followed by centrifugation at 100,000 × g for 60 min at 4°C. The supernatant was then removed, and the pellet was stored at -80°C until subsequent procedures were performed. The pellet was resuspended to the appropriate protein concentration by homogenate buffer II containing 10 mM Tris, pH 7.4, 2 mM EDTA (Ethylene Diaminetetraacetic Acid), and protease inhibitor cocktail tablets. Laemli's sample buffer (120 mM Tris/HCl, pH 6.8, 6% SDS [Sodium Dodecyl Sulfate], 20% glycerol, 0.01% bromophenol blue, 10% 2-mercaptoethanol; 50 μL) was added to 50 μL of diluted samples and the mixture was kept for 60 min at room temperature. Protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The incubated samples were subjected to SDS/PAGE using 5-20% gradient SDS-polyacrylamide gel (ATTO, Tokyo, Japan) followed by electroblotting onto Pure Nitrocellulose Membrane (Bio-Rad Laboratories) using Trans-Blot Turbo (Bio-Rad Laboratories). The membranes were subsequently incubated with Blocking One (Nacalai Tesque, Inc., Kyoto, Japan) for 1 h, and then incubated with a μ-opioid receptor antibody (Merck Millipore, Billerica, MA, USA) diluted 1:20000 for four days at 4°C in Canget Signal (Toyobo, Osaka, Japan) containing 5% Blocking One. After washing six times with Tris-buffered saline containing 0.05% Tween-20 (TBST), the membrane was incubated with anti-rabbit IgG, and horseradish peroxidase-conjugated antibody (Cell Signaling, Danvers, MA, USA) diluted 1:20000 for 1 h. After washing six times with TBST, membranes were incubated with Immobilon Western (Millipore Corporation, Billerica, MA, USA) for 5 min and exposed to electrogenerated chemiluminescence (ECL) films (Amersham Biosciences, Piscataway, NJ, USA). ECL films were scanned densitometrically, and the density of bands was quantified using ImageJ 1.46r software.

Statistical analysis

A two-way analysis of variance (ANOVA) followed by post hoc multiple comparisons were performed for statistical analysis (IBM SPSS 26, IBM, Inc., NY, USA). The homogeneity of the variances was analyzed by the Levence test. In cases where the variances were unequal, t-tests were used to analyze the differences between the two groups. Statistical significance was set at $P < 0.05$ (indicated by * in figures). P-values of less than 0.01 and 0.0001 are indicated by ** and *** in figures, respectively.

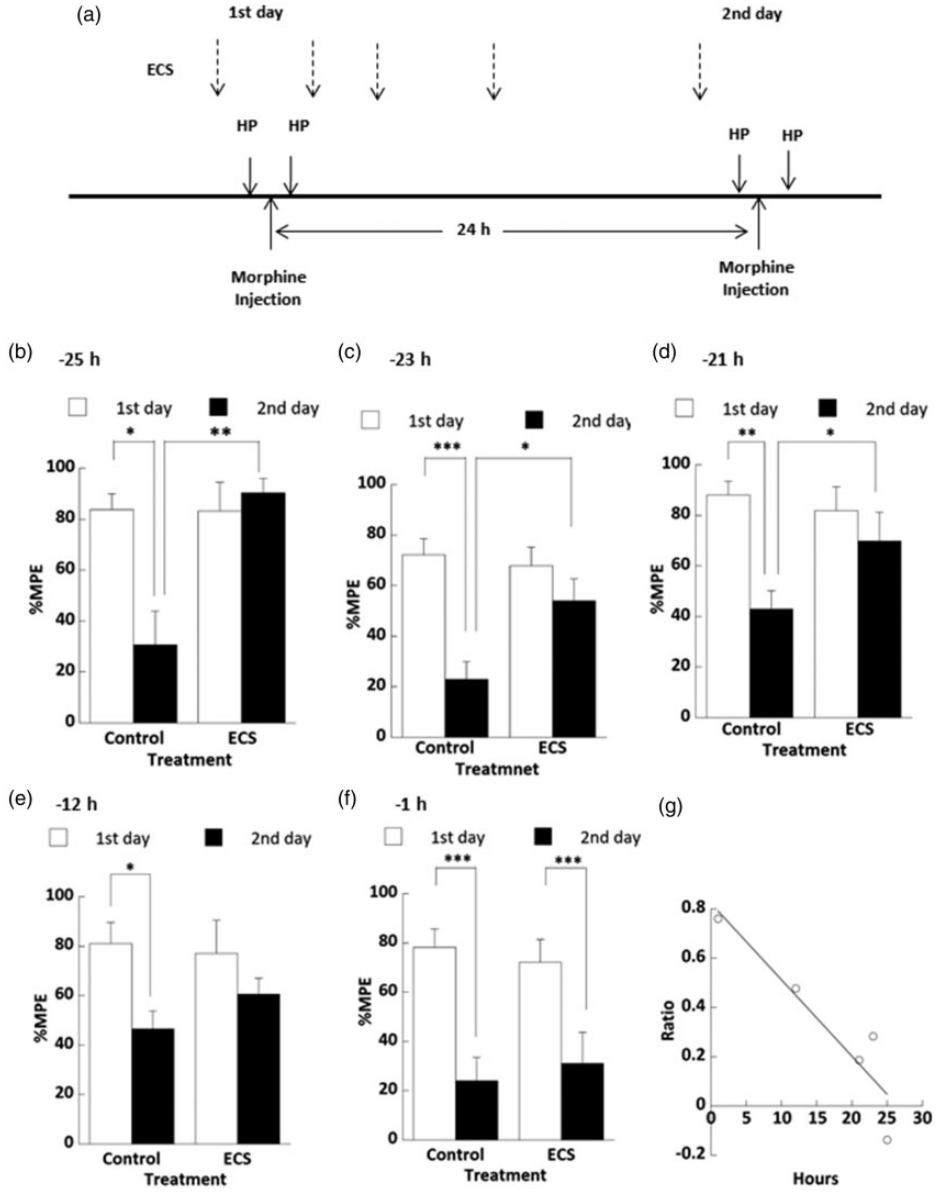

Figure 1. The effect of ECS on the development of morphine tolerance. The schedule for morphineadministration and hot-plate tests (indicated in the figure as HP) is shown in (*a*). Morphine (10 mg/kg) was subcutaneously administered twice with a 24 h interval. The hot-plate tests were performed 30 min before and after each injection (a). ECSs were performed at the following varying times before the second injection: 25 h before (b), 23 h before (c), 21 h before (d), 12 h before (e), and 1 h before (f). The bars in 'ECS' are from mice treated with ECS and the bars in control are from mice treated with only handling. The white bar represents an averaged value of %MPE on the first day. The black bar represents the average value of %MPE on the second day. Each bar indicates the average of data from 8-11 mice. Error bars represent standard error (S.E.). A two-way ANOVA followed by post hoc multiple comparisons were performed for statistical analysis. The relationship between time and the ratio of the difference between %MPE of the ECS group on the first day and on the second day to the difference between %MPE of the control group on the first day and on the second day (*g*).
Ratio $= 0.8229 - 0.031035 \times$ Time (h), Correlation coefficient: $r = 0.92315$, T distribution: $t = 4.159148$, $P < 0.05$.

Results

Effects of ECS on the development of morphine-tolerance

We examined the effects of ECS on morphine-tolerance using a hot-plate test, the schedule of which is shown in Figure 1(a) and in the Methods section. ECS-untreated mice (Control) and ECS-treated mice (ECS) underwent the hot-plate tests twice, once each on two consecutive days. A two-way ANOVA (ECS as the grouping factor $\times$ point of measuring %MPE as the repeated factor) was performed, revealing significant main effects of ECS [$F(1, 15) = 8.657$, $P = 0.010$] and point of

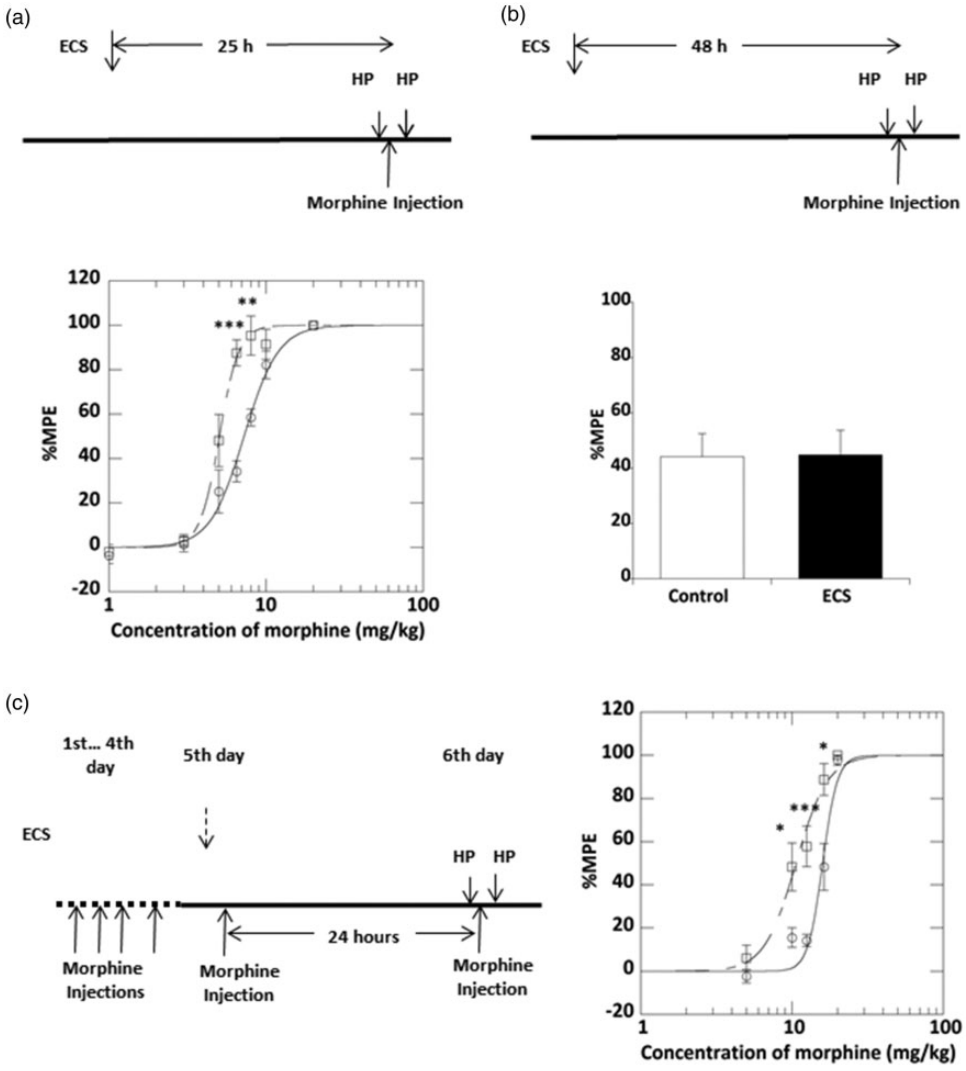

Figure 2. Effect of ECS on the dose-response curve for morphine-antinociception in the hot-plate test. Mice treated with ECS (ECS, the interrupted line) or without ECS (Control, the solid line) were administered at various concentrations (1, 3, 5, 6.5, 8, 10, or 20 mg/kg) of morphine 25 h after ECS administration, in which morphine dose-response curves of %MPE were generated (a). Mice treated with ECS (ECS, the interrupted line) or without ECS (Control, the solid line) were administered 6.5 mg/kg morphine 48 h after ECS administration (b). Mice were injected with 10 mg/kg morphine every 24 h for five consecutive days, followed by ECS (ECS) or no ECS (Control) on day five. On day six, mice were administered various concentrations of morphine (5, 10, 12.5, 16.25, or 20 mg/kg) and dose-response curves of %MPE were generated (c). Data represent the mean ± S.E. of the results from 7–10 mice. $P < 0.05$, $P < 0.01$, and $P < 0.005$ are indicated by *, **, and ***, respectively. T-tests were used to analyze the statistical differences.

measuring %MPE [F (1, 15) = 6.125, P = 0.025], and interaction effect of ECS and point of measuring % MPE 25 h [F (1, 15) = 10.953, P = 0.0048] when ECS was applied 25 h before the second administration of morphine. There was also a significant main effect of point of measuring %MPE when ECS was applied 23 h [F (1, 19) = 16.175, P = 0.001], 21 h [F (1, 14) = 15.445, P = 0.0015], 12 h [F (1, 14) = 6.727, P = 0.021], and 1 h [F (1, 19) = 29.797, P = 0.000029] before the second administration of morphine. Furthermore, there was a significant interaction effect of ECS and point of measuring %MPE when ECS was applied 23 h [F (1, 19) = 5.035, P = 0.037) and 21 h (F (1, 14) = 5.251, P = 0.038), but not 12 h (F (1, 14) = 0.844, P = 0.374) or 1 h (F (1, 19) = 18.470, P = 0.897], before the second administration of morphine. There was no significant main effect of ECS when ECS was applied 23 h [F (1, 19) = 3.850, P = 0.065], 21 h [F (1, 14) = 1.099, P = 0.312], 12 h [F (1, 14) = 0.324, P = 0.578], or 1 h [F (1, 38) = 0.484, P = 0.491] before the second administration of morphine. There was also no significant interaction effect of ECS and point of measuring %MPE, 12 h

[F (1, 14) = 0.324, P = 0.578] or 1 h [F (1, 38) = 0.018, P = 0.894] before the second administration of morphine.

When significant interactions were detected, post hoc multiple comparisons were made with the use of the Bonferroni method. The analysis showed that without the administration of ECS, %MPE was significantly decreased 24 h after an injection of 10 mg/kg of morphine when ECS was applied 25, 23, and 21 h before the second administration of morphine [Controls in Figure 1(b) to (d), from 83.7 ± 6.3% to 30.8 ± 13.1% (P = 0.01), from 72.2 ± 6.4% to 22.9 ± 7.0% (P = 0.000021), and from 88.0 ± 5.6% to 43.4 ± 7.3% (P = 0.001), respectively]. In contrast, no significant decrease in %MPE was observed when ECS was applied 25, 23, or 21 h before the second administration of morphine ['ECS's in Figure 1(b) to (d), from 83.1 ± 11.4% to 90.3 ± 5.6% (P = 0.585), from 68.0 ± 7.3% to 54.4 ± 8.8% (P = 0.197), and from 81.8 ± 9.6% to 70.0 ± 11.2% (P = 0.343), respectively]. In addition, the % MPE of the 'ECS' group was significantly increased compared with the %MPE of the control group on the second day when ECS was applied 25, 23, and 21 h before the second administration of morphine (P = 0.00013, P = 0.04994, and P = 0.036, respectively).

To evaluate whether the timing of ECS was important for the negative effect of ECS on acute morphine-tolerance, we calculated the ratio of the difference between the %MPE of the ECS-applied group on the first day and second day to the difference between % MPE of the control group on the first day and second day. The ratio decreased linearly in a time-dependent manner, and acute morphine-tolerance was abrogated 25 h after ECS treatment (Figure 1(g)). This result indicates that the negative effect of ECS on acute morphine-tolerance increases in a time-dependent manner for 25 h.

Effects of ECS on the dose-response curve for morphine-antinociception in the hot-plate test

We examined the effects of ECS on the dose-response curve of %MPE to morphine-antinociception. Twenty-five h after ECS, the dose-response curve had shifted to the left, and the EC_{50} of morphine given to ECS-pretreated mice decreased by 30.1% to 5.0 ± 0.1 mg/kg from 7.2 ± 0.2 mg/kg compared to mice that were not pretreated with ECS. However, the maximum %MPE was not inhibited by pretreatment with ECS (Figure 2 (a)). This result shows that ECS enhances the antinociceptive effect of morphine.

To determine whether this effect continued for a longer period, ECS was performed 48 h before morphine injection (Figure 2(b)). The ECS did not change the % MPE at 6.5 mg/kg of morphine. This implies that this enhancement by ECS may have deteriorated within 48 h.

We also examined whether ECS may change the dose-response curve of %MPE in mice with chronic morphine-tolerance. We injected morphine in each mouse daily for five consecutive days to generate mice with chronic morphine-tolerance; this caused the dose-response curve to shift to the right, and the EC_{50} of morphine was increased by 124% to 16.1 ± 0.7 mg/kg compared to mice without morphine injection (Figure 2(a) and (c)). With ECS administrated on the 5th day, the dose-response curve shifted to the left, and the EC_{50} of morphine in ECS-pretreated mice decreased by 34% to 10.6 ± 0.6 mg/kg compared to mice that were not pretreated with ECS (Figure 2(c)).

Expression levels of μ-opioid receptor expression in the thalamus of mice with or without ECS

We investigated the expression levels of μ-opioid receptors in the thalami of mice with or without ECS using western blotting. To elucidate how the specific band for the μ-opioid receptor protein on the membrane was stained by western blotting, we performed the blotting of the protein from a wild-type mouse and a μ-opioid receptor knockout mouse with an anti-μ-opioid receptor antibody.[44,45] The bands at 51-71 kDa that were not detected in the lane of the protein from the μ-opioid receptor knockout mouse were identified as the specific band for the μ-opioid receptor (Figure 3(a)). In western blotting experiments, we used the corresponding bands at 51-71 kDa as those resulting from μ-opioid receptor proteins.

We examined the expression level of μ-opioid receptors 25 h after ECS administration in the thalami of mice compared to those without ECS administration, which are indicated as controls in Figure 3(b) and (c). We found that the expression level of the μ-opioid receptor was significantly increased by 1.23 ± 0.01 times after ECS administration using a t-test (P=0.038).

Next, we investigated the effects of pretreatment with morphine, which is thought to make a mouse acquire acute tolerance for morphine at the expression level of the μ-opioid receptor in the thalamus. An elevation in the expression level of the μ-opioid receptor (1.35 ± 0.19) in the thalamus after ECS administration compared with mice without ECS administration (1.03 ± 0.05) was observed (P=0.046) (Figure 3(b) and (c)).

Additionally, we found that pretreatment with morphine did not increase the expression level of the μ-opioid receptor protein irrespective of ECS treatment.

Discussion

The results of this study suggest that ECS may inhibit the development of acute morphine-tolerance or enhance the anesthetic effect of morphine in a time-dependent manner

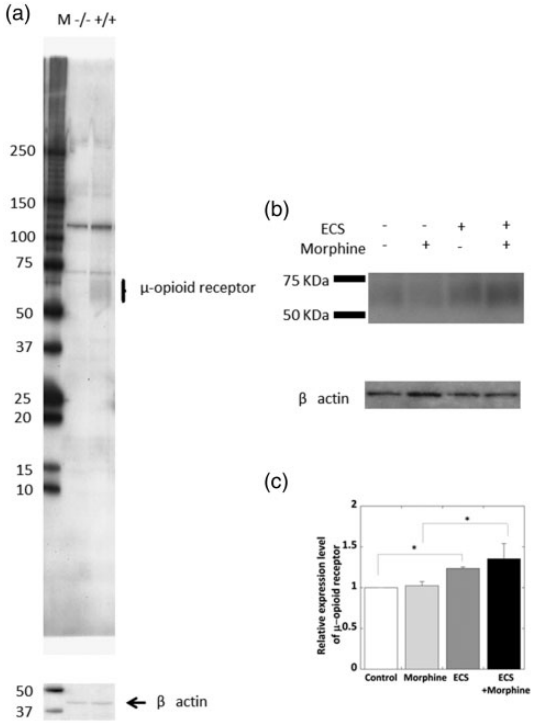

Figure 3. Effects of ECS and morphine on the expression level of μ-opioid receptors in the thalami of mice. The membranes on which crude soluble fractions from the thalami of a wild-type mouse (+/+) and an μ-opioid receptor homozygous knockout mouse (-/-) were used for western blotting using an anti-μ-opioid receptor antibody (a). Crude soluble fractions from the thalamus of mice injected with Ringer's solution, injected with morphine only, or injected with morphine, followed by ECS are presented. Next, the membrane was applied with a specific μ-opioid antibody followed by the application of horseradish peroxidase-conjugated anti-rabbit secondary antibody (b, upper). The blots were also stripped and probed with an anti-actin antibody (b, below). Immunoblotting was performed using NIH imaging. Each expression level of μ-opioid receptor was corrected by the corresponding expression level of actin and expressed as the ratio relative to the control (c). Data in 'ECS,' 'Morphine,' and in 'ECS + Morphine' represent the means ± S.E. from four, eight and eight independent experiments, respectively. T-tests were used to analyze the statistical differences.

(Figure 1). The results also suggest that ECS can enhance the antinociceptive effect of morphine in mice with both acute and chronic morphine-tolerance (Figure 2) and that ECS may increase the expression level of the μ-opioid receptor in the thalamus, whether morphine has been injected or not (Figure 3(c)). A previous study reported an enhancement in the analgesic effect of morphine in rats, 24 h after repeated ECS, although the study did not interpret this result.[46]

The results of the present study of the morphine-dose-response curves in mice in the hot-plate test, which were left-shifted 25 h after ECS (Figure 2) correspond well to our[22] and other previous[1,2] clinical observations, which indicate that some patients with neuropathic pain may require lower amounts of opioids for pain control after a series of ECT. In our clinical case report,[22] we noted that the more doses of opioids patients took, the more efficiently ECT appeared to suppress their neuropathic pain. The positive correlation between opioid dosage and decrease in pain following ECT was seen when data derived from patients who took markedly higher doses of opioids, equivalent to 25 mg/kg/day morphine, were excluded. In the present paper, we showed that the dose-response curve of morphine in the hot-plate test was left-shifted and that the maximum analgesic effect of morphine did not change after ECS. This result may explain the effects of ECT on patients with neuropathic pain and the reason for this exemption. We speculate that the analgesic effect of opioids in patients taking an extremely high dose of opioids, whose analgesic effect is maximized, may not be enhanced by ECT. Meanwhile, the analgesic effect of opioids in patients taking a lower dose of opioids may be enhanced in proportion to their doses, similar to mice that underwent the hot-plate tests injected with a morphine dose lower than its EC_{50}.

We also showed that ECS enhances morphine's antinociceptive effect on both mouse groups, regardless of developing acute or chronic morphine-tolerance (Figures 1 and 2). This action of ECS is unique, because to our knowledge, it is difficult to enhance opioid antinociceptive or analgesic actions in rodents or humans with opioid tolerance. ECT is a safe procedure since its primary side-effect is amnesia, which is mostly recovered within six months.[47] Thus, ECT might be used as a clinical method to enhance the analgesic effect of morphine and recover the effects of opioids in patients who have developed a tolerance. Furthermore, our results introduce the possibility that ECT may enhance morphine's analgesic effect in patients inexperienced with morphine administration and decrease the opioid dose required to suppress pain in these patients. Morphine is a strong agonist of μ-opioid receptors but not of δ-opioid receptors. Therefore, we examined the expression level of μ-opioid receptors but not δ-opioid receptors, although δ-opioid receptors in the thalamus reportedly play crucial roles in inflammatory pain.[48]

We did not observe a change in basal responding time on a hot-plate in mice 1 h to 25 h after the administration of ECS, and this finding is consistent with previous reports indicating that ECS has an antinociceptive effect on rats that have experienced pain within the last hour[35–38] but is inconsistent with a report in which post-electroconvulsive shock analgesia was observed for 120 min, but not 180 min, after ECS in rats.[39]

Our results also suggest that it takes no more than 21 h for the enhancement of morphine by ECS to occur and that this enhancement is observed for 4 h following

ECS (Figure 1). We also demonstrated that the expression level of μ-opioid receptors in the thalamus significantly increased after ECS (Figure 2). These results imply that the effect of ECS might work through the expression of certain proteins. Repeated ECS has been shown to increase the expression levels of CREB in a number of brain regions, including the frontal cortex, hippocampus, piriform cortex, amygdala, and brainstem.[33,49] Recently, Liang et al. reported that CREB triggers the expression of μ-opioid receptors in primary sensory neurons.[50] These results suggest that repeated ECS may induce an increase in the expression level of CREB in the thalamus, which leads to an increase in the expression level of μ-opioid receptors. An increase in μ-opioid receptors has been reported in humans with epilepsy and mice in which epilepsy was pharmacologically induced. The expression level of μ-opioid receptors in the hippocampus of patients with pharmaco-resistant medial temporal lobe epilepsy has been reported to be increased compared with non-epileptic autopsy samples.[51] It has also been reported that the mRNA level of μ-opioid receptors in the mouse cortex was increased after epilepsy was induced by kainic acid treatment.[52]

Several reports have indicated that the μ-opioid receptor in the thalamus is involved in pain modulation in animals, including humans. Microinjection of morphine into the rat thalamus produces antinociception,[53] which is inhibited by pretreatment with naloxone, an antagonist for the μ-opioid receptor.[54] The μ-opioid receptor-stimulated [^{35}S] GTPγS binding in the thalamus, but not in the periaqueductal gray or anterior cingulate cortex, was decreased in mice in which the sciatic nerve was ligated, a model of neuropathic pain.[55] The thalamus is the relay point of nociception to the cortex. It has been reported that μ-opioid receptors are activated in the thalamus of humans suffering from sustained pain. This activation is associated with both the sensory and affective ratings in pain experiments.[56] Meanwhile, the amygdala is involved in the former, and the anterior cingulate in the latter dimension of pain suppression.[56] ECT has been reported to have no positive effect on patients with thalamic pain,[3–5] suggesting that the thalamus may be an essential component for ECT to induce its effects. The increase in the expression level of μ-opioid receptors after ECS might enhance morphine's effects following ECS. However, the expression level of μ-opioid receptors in other parts of the brain must still be studied. It remains to be determined whether ECT increases the expression of another type of opioid receptor in the thalamus, the δ-opioid receptor, which has reportedly been implicated in chronic inflammatory pain.[48]

It was reported that β-endorphin levels in patients with depression were elevated above pre-ECT levels the day after the sixth ECT.[7] Together with the results of the present study, this suggests that ECT may effectively provide an analgesic effect in patients suffering from intractable chronic pain. ECT may have induced both an elevation in the β-endorphin level and an increase in the expression level of μ-opioid receptors.

In conclusion, we show that ECS may enhance the antinociceptive effect of morphine in mice regardless of acute tolerance to morphine. ECS may also increase the expression level of μ-opioid receptors in the thalamus of mice receiving ECS treatment. These results confirm previous clinical reports which have shown that ECT decreased the required dose of opioids in patients with neuropathic pain and suggest a possible mechanism underlying these clinical observations.

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Author Contributions

Dr. Takamatsu acquired data from a subset of hot-plate tests. Dr. Iwata acquired and analyzed the data from the rest of the experiments. Drs. Iwata, Doi, and Ikeda conceptualized the research. Dr. Ikeda and Dr. Iwata designed the research.

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