visceral and somatic pain have several differences [25]. Notably, treatments with KOP but not MOP or DOP receptor agonists have been shown to attenuate the responses of afferent fibers to colorectal distension [26]. The KOP receptor may play a primary role in the antinociceptive effect of opioid agonists on visceral pain via peripheral mechanisms, and MOP and KOP receptors may play a role via central mechanisms. The present results, together with previous studies, suggest that pain induced by various visceral stimuli can be better controlled by a nonselective opioid that acts at both MOP and KOP receptors. Further studies on the receptor mechanisms that underlie the analgesic effects of opioids will lead to the development of better clinical treatments of various types of pain.

Sex differences in the antinociceptive effects of (-)-pentazocine were also demonstrated in the present study. The antinociceptive effects of (-)-pentazocine were significantly higher in male than in female mice in both the hot-plate and writhing tests and tended to be high in the tail-flick and formalin tests. These sex differences appear to be pronounced in heterozygous MOP-KO mice, although sex differences in the antinociceptive effects of (-)-pentazocine in wildtype mice might not be noticeable because of a possible ceiling effect in the present nociceptive tests. The present results are consistent with previous reports. Pentazocine has been shown to exert more potent antinociception in males than in females in both mice [27] and rats [28]. These reports also showed that U50488H, a selective KOP receptor agonist, and other opioids (e.g., U69593, bremazocine, and butorphanol) are more effective in males than in females. Furthermore, with regard to MOP receptor-selective agonists, morphine exerted greater antinociceptive effects in male than in female mice [29], rats [30,31], and monkeys [32]. Additionally, female mice may differentially respond in pain tests during different phases of their estrous cycle [18]. In humans, males required less morphine or fentanyl than females for postoperative pain relief [33,34]. In contrast, some inconsistent human studies have reported that the antinociceptive effects of pentazocine on postoperative pain were higher in females than in males [35-37]. The discrepancy between these studies might be attributable to differences in the body weight-adjusted dose of pentazocine, although other factors (e.g., type of nociceptive stimulus, type of clinical surgery, estrous cycle phase, patient race, and ethnicity) might affect these results. Thus, the present results, together with previous reports, suggest that not only MOP receptor-selective opioids, but also other subtype-nonselective opioids such as pentazocine, are more effective in males than in females.

Conclusions

The present study demonstrated the abolition of the thermal, mechanical, and somatic chemical antinociceptive effects of (-)-pentazocine in male and female MOP-KO mice, suggesting that thermal, mechanical, and somatic chemical antinociception induced by (-)-pentazocine is completely mediated by the MOP receptor partial agonist effects of (-)-pentazocine. We also demonstrated the retention of (-)-pentazocine-induced visceral chemical antinociception in MOP-KO mice and abolition of (-)-pentazocine-induced visceral chemical antinociception by pretreatment with nor-BNI. Our *in vitro* data showed that (-)-pentazocine more strongly acted at KOP and MOP receptors than DOP receptors, suggesting that (-)-pentazocine-induced visceral chemical antinociception is mediated by its MOP receptor partial agonist effects and full KOP receptor agonist effects. In the clinic, (-)-pentazocine may effectively control visceral pain. Future studies will elucidate the precise molecular mechanisms that underlie the antinociceptive effects of (-)-pentazocine and will contribute to the better use of opioid drugs for pain management.

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Authors' contributions

The study was conceived and the experiments were designed by SI, MM, MS, and KI. SI performed the experiments, performed the statistical analyses, and wrote the manuscript. MOP-KO mice were developed by IS and GRU. KI supervised the experiments and finalized the manuscript. All authors contributed to writing the manuscript, and all authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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References

1. Gutstein H, Akil H: **Opioid analgesics.** In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*.. 10 edition. Edited by: Hardman JG, Limbird LE, Goodman-Gilman A. New York: McGraw-Hill; 2001:569-619.
2. Chien CC, Pasternak GW: **(-)-Pentazocine analgesia in mice: interactions with a σ receptor system.** *Eur J Pharmacol* 1995, 294:303-308.

3. Suzuki T, Narita M, Misawa M, Nagase H: **Pentazocine-induced biphasic analgesia in mice.** *Life Sci* 1991, **48**:1827-1835.
4. Bidlack JM, McLaughlin JP, Wentland MP: **Partial opioids: medications for the treatment of pain and drug abuse.** *Ann N Y Acad Sci* 2000, **909**:1-11.
5. Newman LC, Sands SS, Wallace DR, Stevens CW: **Characterization of μ, κ, and δ opioid binding in amphibian whole brain tissue homogenates.** *J Pharmacol Exp Ther* 2002, **301**:364-370.
6. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL: **Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the μ-opioid-receptor gene.** *Nature* 1996, **383**:819-823.
7. Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, Miner LL, Uhl GR: **Opiate receptor knockout mice define μ receptor roles in endogenous nociceptive responses and morphine-induced analgesia.** *Proc Natl Acad Sci USA* 1997, **94**:1544-1549.
8. Sora I, Elmer G, Funada M, Pieper J, Li XF, Hall FS, Uhl GR: **μ Opiate receptor gene dose effects on different morphine actions: evidence for differential *in vivo* μ receptor reserve.** *Neuropsychopharmacology* 2001, **25**:41-54.
9. Loh HH, Liu HC, Cavalli A, Yang W, Chen YF, Wei LN: **μ Opioid receptor knockout in mice: effects on ligand-induced analgesia and morphine lethality.** *Brain Res Mol Brain Res* 1998, **54**:321-326.
10. Ide S, Minami M, Satoh M, Uhl GR, Sora I, Ikeda K: **Buprenorphine antinociception is abolished, but naloxone-sensitive reward is retained, in μ-opioid receptor knockout mice.** *Neuropsychopharmacology* 2004, **29**:1656-1663.
11. Ide S, Minami M, Ishihara K, Uhl GR, Satoh M, Sora I, Ikeda K: **Abolished thermal and mechanical antinociception but retained visceral chemical antinociception induced by butorphanol in μ-opioid receptor knockout mice.** *Neuropharmacology* 2008, **54**:1182-1188.
12. Woolfe G, MacDonald A: **The evaluation of the analgesic action of pethidine hydrochloride (demerol).** *J Pharmacol Exp Ther* 1944, **80**:300-307.
13. D'Amour F, Smith D: **A method for determining loss of pain sensation.** *J Pharmacol* 1941, **72**:74-79.
14. Randall LO, Selitto JJ: **A method for measurement of analgesic activity on inflamed tissue.** *Arch Int Pharmacodyn Ther* 1957, **111**:409-419.
15. Collier HO, Dinneen LC, Johnson CA, Schneider C: **The abdominal constriction response and its suppression by analgesic drugs in the mouse.** *Br J Pharmacol Chemother* 1968, **32**:295-310.
16. Tanimoto S, Nakagawa T, Yamauchi Y, Minami M, Satoh M: **Differential contributions of the basolateral and central nuclei of the amygdala in the negative affective component of chemical somatic and visceral pains in rats.** *Eur J Neurosci* 2003, **18**:2343-2350.
17. Katsumata S, Minami M, Nakagawa T, Iwamura T, Satoh M: **Pharmacological study of dihydroetorphine in cloned μ-, δ- and κ-opioid receptors.** *Eur J Pharmacol* 1995, **291**:367-373.
18. Mogil JS, Chesler EJ, Wilson SG, Juraska JM, Sternberg WF: **Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype.** *Neurosci Biobehav Rev* 2000, **24**:375-389.
19. Birch PJ, Hayes AG, Sheehan MJ, Tyers MB: **Norbinaltorphimine: antagonist profile at κ opioid receptors.** *Eur J Pharmacol* 1987, **144**:405-408.
20. Spanagel R, Almeida OF, Shippenberg TS: **Evidence that nor-binaltorphimine can function as an antagonist at multiple opioid receptor subtypes.** *Eur J Pharmacol* 1994, **264**:157-162.
21. Zhu Y, King MA, Schuller AG, Nitsche JF, Reidl M, Elde RP, Unterwald E, Pasternak GW, Pintar JE: **Retention of supraspinal δ-like analgesia and loss of morphine tolerance in δ opioid receptor knockout mice.** *Neuron* 1999, **24**:243-252.
22. Simonin F, Valverde O, Smadja C, Slowe S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL: **Disruption of the κ-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective κ-agonist U-50,488H and attenuates morphine withdrawal.** *EMBO J* 1998, **17**:886-897.
23. Larsson MH, Bayati A, Lindstrom E, Larsson H: **Involvement of kappa-opioid receptors in visceral nociception in mice.** *Neurogastroenterol Motil* 2008, **20**:1157-1164.
24. Riviere PJ: **Peripheral kappa-opioid agonists for visceral pain.** *Br J Pharmacol* 2004, **141**:1331-1334.
25. Al-Chaer ED, Traub RJ: **Biological basis of visceral pain: recent developments.** *Pain* 2002, **96**:221-225.
26. Sengupta JN, Su X, Gebhart GF: **Kappa, but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon.** *Gastroenterology* 1996, **111**:968-980.
27. Mogil JS, Wilson SG, Chesler EJ, Rankin AL, Nemmani KV, Lariviere WR, Groce MK, Wallace MR, Kaplan L, Staud R, Ness TJ, Glover TL, Stankova M, Mayorov A, Hruby VJ, Grisel JE, Fillingim RB: **The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans.** *Proc Natl Acad Sci USA* 2003, **100**:4867-4872.
28. Craft RM, Bernal SA: **Sex differences in opioid antinociception: κ and 'mixed action' agonists.** *Drug Alcohol Depend* 2001, **63**:215-228.
29. Kest B, Palmese C, Hopkins E: **A comparison of morphine analgesic tolerance in male and female mice.** *Brain Res* 2000, **879**:17-22.
30. Bobeck EN, McNeal AL, Morgan MM: **Drug dependent sex-differences in periaqueducatal gray mediated antinociception in the rat.** *Pain* 2009, **147**:210-216.
31. Craft RM, Ulibarri C, Leitl MD, Sumner JE: **Dose- and time-dependent estradiol modulation of morphine antinociception in adult female rats.** *Eur J Pain* 2008, **12**:472-479.
32. Negus SS, Mello NK: **Opioid antinociception in ovariectomized monkeys: comparison with antinociception in males and effects of estradiol replacement.** *J Pharmacol Exp Ther* 1999, **290**:1132-1140.
33. Aubrun F, Salvi N, Coriat P, Riou B: **Sex- and age-related differences in morphine requirements for postoperative pain relief.** *Anesthesiology* 2005, **103**:156-160.
34. Fukuda K, Hayashida M, Ide S, Saita N, Kokita Y, Kasai S, Nishizawa D, Ogai Y, Hasegawa J, Nagashima M, Tagami M, Komatsu H, Sora I, Koga H, Kaneko Y, Ikeda K: **Association between OPRM1 gene polymorphisms and fentanyl sensitivity in patients undergoing painful cosmetic surgery.** *Pain* 2009, **147**:194-201.
35. Gear RW, Gordon NC, Heller PH, Paul S, Miaskowski C, Levine JD: **Gender difference in analgesic response to the kappa-opioid pentazocine.** *Neurosci Lett* 1996, **205**:207-209.
36. Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD: **Kappa-opioids produce significantly greater analgesia in women than in men.** *Nat Med* 1996, **2**:1248-1250.
37. Gear RW, Miaskowski C, Gordon NC, Paul SM, Heller PH, Levine JD: **The kappa opioid nalbuphine produces gender- and dose-dependent analgesia and antianalgesia in patients with postoperative pain.** *Pain* 1999, **83**:339-345.

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Inhibitory Role of Inducible cAMP Early Repressor (ICER) in Methamphetamine-Induced Locomotor Sensitization

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Abstract

Background: The inducible cyclic adenosine monophosphate (cAMP) early repressor (ICER) is highly expressed in the central nervous system and functions as a repressor of cAMP response element-binding protein (CREB) transcription. The present study sought to clarify the role of ICER in the effects of methamphetamine (METH).

Methods and Findings: We tested METH-induced locomotor sensitization in wildtype mice, ICER knockout mice, and ICER I-overexpressing mice. Both ICER wildtype mice and knockout mice displayed increased locomotor activity after continuous injections of METH. However, ICER knockout mice displayed a tendency toward higher locomotor activity compared with wildtype mice, although no significant difference was observed between the two genotypes. Moreover, compared with wildtype mice, ICER I-overexpressing mice displayed a significant decrease in METH-induced locomotor sensitization. Furthermore, Western blot analysis and quantitative real-time reverse transcription polymerase chain reaction demonstrated that ICER overexpression abolished the METH-induced increase in CREB expression and repressed cocaine- and amphetamine-regulated transcript (CART) and prodynorphin (Pdyn) expression in mice. The decreased CART and Pdyn mRNA expression levels *in vivo* may underlie the inhibitory role of ICER in METH-induced locomotor sensitization.

Conclusions: Our data suggest that ICER plays an inhibitory role in METH-induced locomotor sensitization.

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Introduction

The inducible cyclic adenosine monophosphate (cAMP) early repressor (ICER) is the collective name for a group of proteins produced from the cAMP response element modulator (CREM)/ICER gene driven by the P2 internal promoter located in an intron of the CREM gene [1]. Lacking the CREM *N*-terminus, ICER only contains two DNA binding domains (DBD I and DBD II) and lacks the activation and kinase-inducible domains. Consequently, ICER functions as an endogenous repressor of transcription of several cAMP response element (CRE)-containing genes [1–3]. The P2 promoter of the ICER gene contains two pairs of CRE sequences. The phosphorylated CRE-binding protein (CREB) can induce transcription of the ICER gene from the P2 promoter. The increased ICER competes with CREB in binding with the CRE sequence, blocking transcription from CRE-containing promoters, including ICER's own promoter, and functioning as a potent endogenous CREB antagonist [1,4].

Four ICER isoforms have been identified: ICER I, ICER Iγ, ICER II, and ICER IIγ. ICER I mRNA contains DBD I and DBD II, but DBD II is absent in the ICER I protein because a stop codon exists at the end of DBD I. The ICER II isoform contains only DBD II. ICER Iγ and ICER IIγ are characterized by a deficiency of exon γ from ICER I and ICER II, respectively [4].

Numerous reports have shown that CREB in the nucleus accumbens (NAc) is associated with responses to drugs of abuse and emotional responses. Chronic drug administration increases levels of CREB immunoreactivity and CRE-binding activity [5–6]. Overexpression of CREB by introducing herpes simplex virus-CREB into the NAc decreases behavioral responses to drug administration, whereas blockade of CREB transcription via introducing a dominant-negative CREB mutant or via genetic knockout increases behavioral responses to drug administration [7–10]. However, other studies showed that genetic ablation of CREB did not affect the rewarding effects of psychostimulants [11–13], indicating that the role of CREB in drug-induced responses is debatable. Recent findings suggest that ICER mRNA expression was threefold higher in the striatum after amphetamine injection [14], suggesting that the endogenous functional CREB antagonist ICER may participate in the mechanisms that underlie the effects of drugs of abuse.

The prodynorphin (Pdyn) peptide is an endogenous ligand of the κ opioid receptor. Cocaine- and amphetamine-regulated transcript (CART) was first sequenced as a peptide with unknown function [15], and previous studies revealed that the CART

peptide is co-localized with Pdyn in brain regions associated with drug reward, including the NAc and ventral tegmental area (VTA) [16–17]. Both CART and Pdyn play roles as psychostimulant neuromodulators [8,18–20]. CART and Pdyn mRNA are suggested to be CRE-mediated transcripts regulated by CREB *in vitro* and *in vivo* [8,21–23].

Kojima *et al.* [24] generated two types of ICER mutant mice—ICER knockout mice and ICER-overexpressing mice—and suggested a negative role for ICER in regulating long-term fear memory and kindling epileptogenesis. The present study used two types of transgenic mice with opposite genetic alterations of ICER gene expression (i.e., ICER knockout and ICER I-overexpressing mice) and investigated the role of ICER in methamphetamine (METH)-induced locomotor sensitization. Locomotor sensitization is characterized by the progressive enhancement of locomotor activity after repeated psychostimulant exposure [25–26]. The augmentation of this behavioral response can be maintained for several months after the cessation of drug treatment [27]. We observed an inhibitory effect of ICER on METH-induced locomotor sensitization. To identify the downstream components of ICER-mediated gene transcription *in vivo* and provide a possible mechanism that contributes to the inhibitory role of ICER in METH-induced locomotor sensitization, we determined METH-induced CREB and phosphorylated CREB (pCREB) levels using Western blot analysis and further determined CART and Pdyn mRNA expression levels in the striatum (caudate putamen [CPu], which mediates locomotor activity) but not in the NAc (which mainly mediates the rewarding effects of drugs of abuse) in ICER I-overexpressing mice and their littermates using real-time reverse transcription polymerase chain reaction (RT-PCR).

Results

METH-induced locomotor sensitization in ICER I-overexpressing mice

Consistent with a previous study [28], on Day 1, the initially elevated levels of locomotor activity in wildtype mice were reduced to near-zero levels after 180 min habituation. ICER I-overexpressing mice displayed a similar pattern of locomotor activity as wildtype mice (Fig. 1*a*). No significant difference in baseline locomotion was observed between genotypes ($n = 7$ for wildtype mice; $n = 9$ for ICER I-overexpressing mice; $F_{1,16} = 0.49$, $p = 0.49$; Fig. 1*a*). On Day 20, ICER I-overexpressing mice displayed decreased levels of spontaneous locomotor activity during the 180 min habituation period compared with wildtype mice ($F_{1,14} = 9.934$, $p = 0.007$; Fig. 1*b*). After a METH injection (1 mg/kg), a significant difference was observed between the two genotypes ($F_{1,14} = 14.566$, $p = 0.0019$; Fig. 1*b*). Repeated administration of METH (1 mg/kg) on Days 1, 3, 5, 7, 9, 11, 13, and 20 significantly increased locomotor activity in both wildtype and ICER I-overexpressing mice (Fig. 1*c*). A two-way, mixed-design analysis of variance (ANOVA; Genotype×Day) revealed a significant effect of Day ($F_{7,98} = 19.13$, $p<0.0001$), indicating the presence of METH-induced locomotor sensitization. METH-induced locomotor sensitization in ICER I-overexpressing mice significantly decreased compared with wildtype mice ($F_{1,14} = 12.54$, $p = 0.0033$; Fig. 1*c*), and a significant Genotype×Day interaction was observed ($F_{7,98} = 6.52$, $p<0.0001$; Fig. 1*c*). From Day 5, locomotor activity in ICER I-overexpressing mice was significantly lower than in wildtype mice (Student's *t*-test).

METH-induced locomotor sensitization in ICER knockout mice

On Day 1, the levels of locomotor activity in wildtype and ICER knockout mice were reduced to near-zero after 180 min habitua-

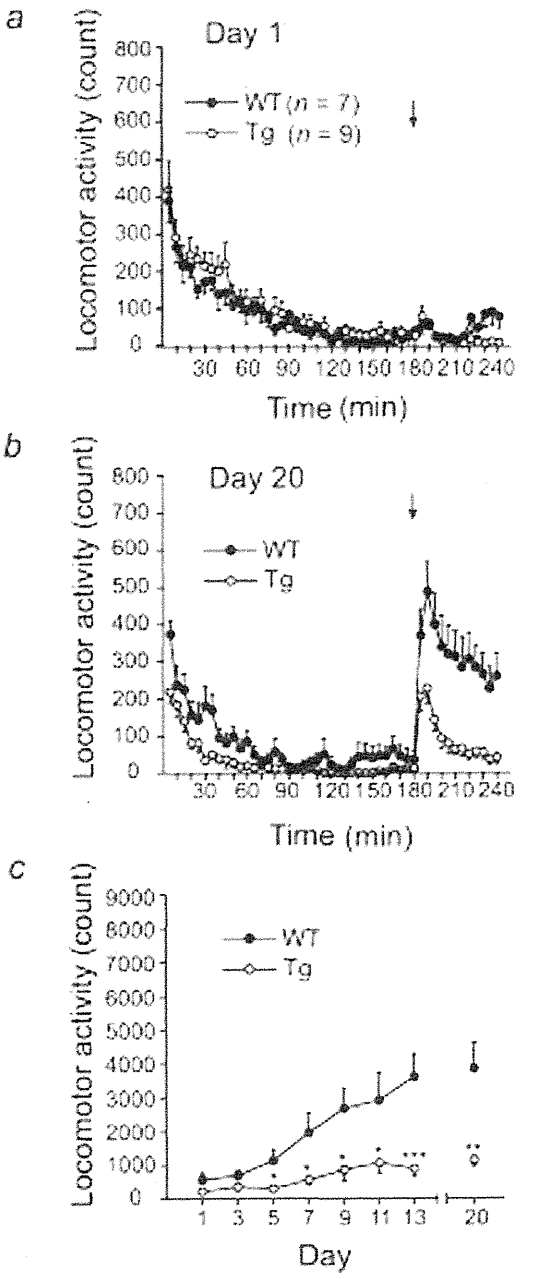

Figure 1. Spontaneous and METH-stimulated locomotor activity in wildtype mice (WT) and ICER I-overexpressing mice (Tg). METH (1 mg/kg) was administered once per day on Days 1, 3, 5, 7, 9, 13, and 20 in WT ($n = 7$) and Tg ($n = 9$) mice. *a.* Time-course of spontaneous locomotor activity before and after METH administration on Day 1. The data are expressed as mean ± SEM beam breaks in 5 min bins. The arrow indicates the start of a METH injection. *b.* Time-course of spontaneous locomotor activity before and after METH administration on Day 20. The data are expressed as mean ± SEM beam breaks in 5 min bins. The arrow indicates the start of a METH injection. *c.* METH-induced locomotor sensitization. The data are expressed as mean ± SEM beam breaks during the 60 min period after METH injection (1 mg/kg) on Days 1, 3, 5, 7, 9, 13, and 20. *$p<0.05$, **$p<0.01$, ***$p<0.001$, significant difference in locomotor activity scores between WT and Tg mice.

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tion. No significant difference in baseline locomotion was observed between genotypes ($n = 13$ for both wildtype and knockout mice; $F_{1,24} = 0.27$, $p = 0.61$; Fig. 2*a*). After repeated procedures on Days 1, 3, 5, 7, 9, 11, and 13 and a 7 day drug-free period, on Day 20, the levels of locomotor activity in the two genotypes were reduced but did not reach near-zero levels after 180 min habituation, which might have been caused by the repeated METH administration. No significant difference was detected between the two genotypes during the habituation period ($F_{1,24} = 2.731$, $p = 0.12$; Fig. 2*b*). After a METH injection (1 mg/kg), locomotor activity in both genotypes increased significantly. No significant difference was observed between the two genotypes ($F_{1,24} = 2.071$, $p = 0.16$; Fig. 2*b*). Repeated administration of METH (1 mg/kg) significantly increased locomotor activity in both wildtype and ICER knockout mice (Fig. 2*c*). A two-way, mixed-design ANOVA (Genotype×Day) revealed a significant effect of Day ($F_{7,168} = 25.88$, $p<0.0001$), indicating the presence of METH-induced locomotor sensitization. ICER knockout mice showed a tendency toward higher locomotor activity compared with their wildtype littermates ($F_{1,24} = 2.96$, $p = 0.098$). ICER knockout mice displayed greater locomotor activity on Day 3 and Day 11 compared with wildtype mice ($p<0.05$; Tukey-Kramer *post hoc* test). No significant Genotype× Day interaction was observed ($F_{7,168} = 0.62$, $p = 0.74$).

METH-induced CREB expression and phosphorylation in the CPu was abolished in ICER I-overexpressing mice

Two-way ANOVA revealed marginal differences between genotypes in CREB and pCREB protein levels in the CPu after repeated METH treatment (CREB: $F_{1,40} = 3.76$, $p = 0.06$; pCREB: $F_{1,40} = 3.51$, $p = 0.07$). No significant difference in the effect of METH was found (CREB: $F_{3,40} = 1.28$, $p = 0.29$; pCREB: $F_{3,40} = 1.38$, $p = 0.26$), and no Genotype×METH interaction was observed (CREB: $F_{3,40} = 1.90$, $p = 0.15$; pCREB: $F_{3,40} = 1.79$, $p = 0.16$). The Dunnett *post hoc* test revealed that repeated METH/saline challenge significantly increased CREB protein levels in wildtype mice compared with the saline group ($n = 6$ per group, $p<0.05$; Fig. 3*a*). The level of activated CREB protein (pCREB) in the repeated METH/saline challenge group also significantly increased in wildtype mice ($n = 6$ per group, $p<0.05$, Dunnett *post hoc* test; Fig. 3*b*). However, the levels of CREB and pCREB protein were not significantly altered after repeated METH injection in ICER I-overexpressing mice (Fig. 3).

ICER overexpression significantly reduced CART and Pdyn mRNA expression in the CPu

To identify the downstream components of CRE-mediated gene transcription that contribute to reduced METH-induced locomotor sensitization in ICER I-overexpressing mice, real-time RT-PCR was conducted. First, ICER mRNA levels were evaluated using ICER-specific primers. Significant effects were found for Genotype ($F_{1,24} = 1850.5$, $p<0.001$, two-way ANOVA; Fig. 4*a*). However, METH injection did not significantly affect ICER mRNA levels in wildtype mice ($n = 4$ per group, $p>0.05$, Dunnett *post hoc* test). Furthermore, we evaluated CART and Pdyn mRNA levels because they are suggested to be CRE-mediated transcripts and psychostimulant neuromodulators. Although METH did not alter CART or Pdyn mRNA expression in ICER I-overexpressing mice and their littermates (CART: $F_{3,24} = 0.31$, $p = 0.81$; Pdyn: $F_{3,24} = 0.38$, $p = 0.77$; two-way ANOVA), CART and Pdyn mRNA expression levels were significantly reduced in ICER I-overexpressing mice compared with their littermates (CART: $F_{1,24} = 17.25$, $p<0.01$; Pdyn: $F_{1,24} = 12.21$, $p<0.01$; two-way ANOVA; Fig. 4*b*, *c*). No significant Genotype×METH interaction was observed (CART: $F_{3,24} = 0.21$, $p = 0.89$; Pdyn: $F_{3,24} = 0.17$, $p = 0.92$).

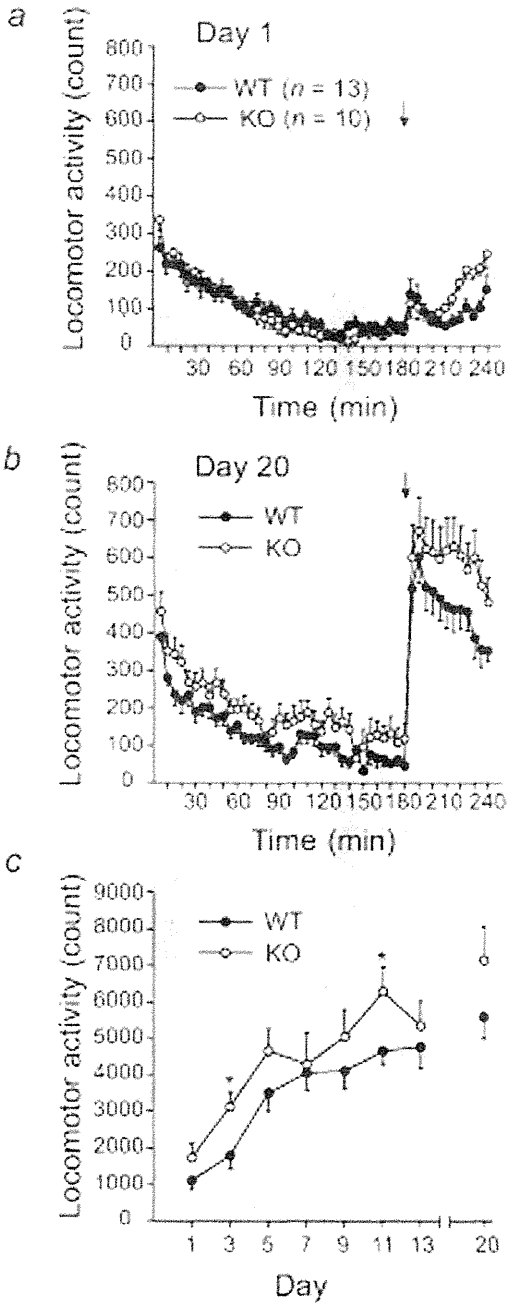

Figure 2. Spontaneous and METH-stimulated locomotor activity in wildtype (WT) and ICER knockout (KO) mice. METH (1 mg/kg) was administered once per day on Days 1, 3, 5, 7, 9, 13, and 20 in WT ($n = 13$) and ICER-KO ($n = 13$) mice. *a.* ime-course of spontaneous locomotor activity before and after METH administration on Day 1. The data are expressed as mean ± SEM beam breaks in 5 min bins. The arrow indicates the start of a METH injection. *b.* Time-course of spontaneous locomotor activity before and after METH administration on Day 20. The data are expressed as mean ± SEM beam breaks in 5 min bins. The arrow indicates the start of a METH injection. *c.* METH-induced locomotor sensitization. The data are expressed as mean ± SEM beam breaks during the 60 min period after METH injection (1 mg/kg). *$p<0.05$, significant difference in locomotor activity scores between WT and KO mice.
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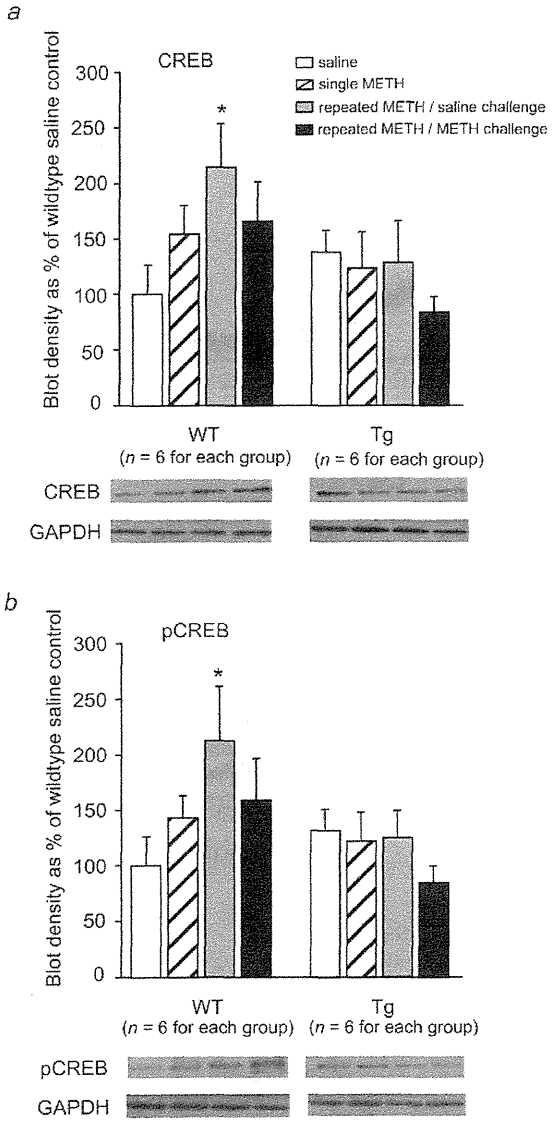

Figure 3. CREB expression and phosphorylation in the CPu after single and repeated METH treatment. The mice were administered METH (1 mg/kg, i.p.) or saline once or received METH (1 mg/kg, i.p.) once every other day from Day 1 to Day 13 and challenged with saline or METH (1 mg/kg, i.p.) on Day 20 after a 7 day drug-free period. The mice were decapitated 1 h after the last METH or saline treatment. The blot density of each group was normalized to that of the wildtype saline group and is expressed as mean ± SEM ($n=6$). *a.* METH-induced CREB expression in the CPu in wildtype mice (WT) and ICER I-overexpressing mice (Tg). *$p<0.05$, significant difference in normalized CREB blot density compared with wildtype saline group. *b.* METH-induced CREB phosphorylation in the CPu in wildtype mice (WT) and ICER I-overexpressing mice (Tg). *$p<0.05$, significant difference in normalized pCREB blot density compared with wildtype saline group.
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Discussion

The present study investigated the role of ICER in long-lasting METH-induced behavioral alterations by evaluating METH-induced locomotor sensitization in ICER knockout and ICER-overexpressing mice. The major findings of the present study were that ICER I overexpression significantly inhibited METH-induced locomotor sensitization and blocked METH-induced increases in CREB and pCREB protein levels. Additionally, CART and Pdyn mRNA expression levels in the CPu were significantly reduced in ICER-overexpressing mice. ICER knockout mice displayed a tendency toward higher activity after repeated METH administration compared with their wildtype littermates, although no significant difference was detected between ICER knockout mice and their wildtype littermates. Considering the negative regulatory role of CREB in the effects of psychostimulants [18,29–30], the reduction in METH-induced locomotor sensitization in ICER-overexpressing mice may be attributable to reduced CART and Pdyn mRNA expression, rather than attributable to increased CREB and pCREB protein levels.

Inhibitory role of ICER in METH-induced locomotor sensitization

Although the mechanisms that underlie locomotor sensitization are not fully understood, it is hypothesized to reflect neuronal adaptations in several brain regions, including in dopamine neurons and the CPu [25]. In the present study, ICER I-overexpressing mice exhibited a significant reduction in METH-induced locomotor sensitization compared with wildtype mice (Fig. 1*c*), whereas ICER knockout mice showed a minimal enhancement of METH-induced locomotor sensitization compared with wildtype mice (Fig. 2*c*). Altogether, these results suggest that ICER plays an inhibitory role in METH-induced locomotor sensitization.

CREB overexpression in the NAc reportedly decreased cocaine- and morphine-induced conditioned place preference (CPP), and decreased CREB in the NAc increased cocaine- and morphine-induced CPP [7–8], suggesting that increased CREB in the NAc has an inhibitory effect on the induction of CPP. However, recent studies have reported conflicting results, in which genetic ablation of CREB did not affect the rewarding properties of psychostimulants [11–13]. Similarly, some studies demonstrated an inhibitory role of CREB in cocaine-induced sensitization [31–32], whereas other studies with CREB mutant mice suggested either minor effects [33] or no effects [11] of CREB on cocaine-induced sensitization. In the present study, overexpression of the endogenous CREB repressor ICER inhibited METH-induced locomotor sensitization. Thus, the inhibitory effect of CREB on the psychostimulant-induced response is debatable. A possible explanation for these discrepant results may include the different gene manipulations (i.e., forebrain- or NAc-specific gene manipulation), different drug types (i.e., METH or cocaine/morphine), and different targeted genes (i.e., ICER or CREB).

Enhanced pCREB in the striatum is a molecular marker of neuroadaptations to chronic psychostimulant-induced plasticity [8,21,29,34–35]. In the present study, both CREB and pCREB levels increased in wildtype mice after repeated METH injection. The increased CREB and subsequent pCREB induced by repeated METH might homeostatically oppose the effect of METH [29]. However, the repeated METH-induced increases in CREB levels were blocked by ICER I overexpression, suggesting that the negative regulation of the CREB pathway was absent in ICER I-overexpressing mice. Therefore, the CREB pathway may not be involved in the reduced locomotor sensitization observed in ICER I-overexpressing mice. Additionally, ICER expression was 60-fold greater in ICER overexpressing mice than in wildtype mice, which may not occur under physiological conditions. The 60-fold increase in expression may interfere with the CREB signaling pathway and homeostatic regulation of CREB.

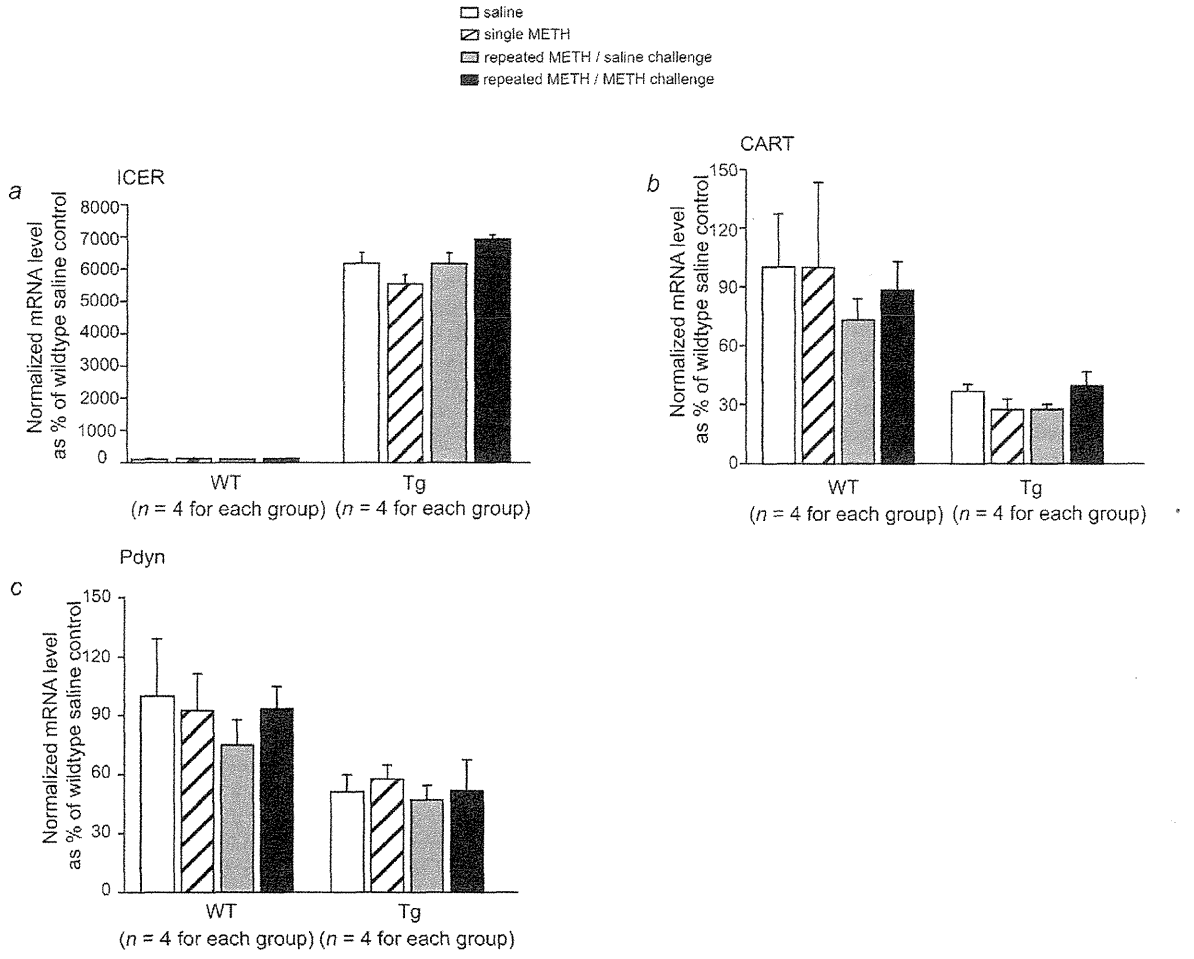

Figure 4. ICER, CART, and Pdyn mRNA levels in the CPu after single and repeated METH treatment. The mice were administered METH (1 mg/kg, i.p.) or saline once or received METH (1 mg/kg, i.p.) once every other day from Day 1 to Day 13 and challenged with saline or METH (1 mg/kg, i.p.) on Day 20 after a 7 day drug-free period. The mice were decapitated 1 h after the last METH or saline treatment. *a.* ICER mRNA expression in the CPu in wildtype (WT) and ICER I-overexpressing (Tg) mice. The data are expressed as mean ± SEM ($n = 4$). *b.* CART mRNA expression in the CPu after single and repeated METH treatment. The data are expressed as mean ± SEM ($n = 4$). *c.* Pdyn mRNA expression in the CPu after single and repeated METH treatment. The data are expressed as mean ± SEM ($n = 4$).
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Inhibitory role of ICER in regulating CART and Pdyn mRNA expression

CART and dynorphin are peptidergic neurotransmitters expressed in the CPu and other brain regions and modulate the rewarding effects of drugs of abuse [17,26,36]. CART's involvement in the actions of psychostimulants was first noted in a study that demonstrate that acute cocaine and amphetamine upregulated CART mRNA in the rat brain [37]. However, this report has been controversial because this finding has been difficult to replicate [38–40]. Other studies found that binge cocaine exposure, rather than acute administration, reliably increases CART expression [38,41]. Additionally, Pdyn mRNA has been reported to increase or not change in response to binge cocaine administration [42,43]. In the present study, neither acute nor repeated administration of METH (1 mg/kg) altered CART and Pdyn mRNA expression in wildtype mice. Furthermore, METH administration (1 mg/kg) did not alter ICER mRNA expression in wildtype mice. A possible reason for this might be that the 1 mg/kg dose of METH may not have been sufficient to induce detectable alterations of ICER, CART, and Pdyn mRNA. However, CART and Pdyn mRNA expression levels significantly decreased as ICER mRNA levels significantly increased, suggesting an inhibitory role of ICER in CART and Pdyn expression. Both the CART and Pdyn genes contain a CRE site in their promoter regions [21–22], and CART and Pdyn mRNA levels are regulated by CREB *in vitro* [21,44] and *in vivo* [8,23]. Therefore, as a CRE-mediated gene transcription repressor, ICER may inhibit the expression of CART and Pdyn *in vivo*. Our studies using ICER I-overexpressing mice support this hypothesis.

CART and Pdyn as neuromodulators of the behavioral effects of psychostimulants

The CART and Pdyn peptides are neurotransmitters expressed in brain regions associated with drug reward, including the NAc and VTA [16–17]. Numerous studies have suggested that CART and Pdyn play a homeostatic role in the NAc to oppose the effects of cocaine. For example, pretreatment with Dyn A (1–17) is effective at decreasing striatal dopamine levels and attenuating

cocaine-induced CPP in mice [45]. Overexpression of CREB, with resulting increases in Pdyn gene expression, in the NAc has been shown to decrease the rewarding effects of cocaine [8]. Microinjection of CART peptide 55–102 into the NAc blocked the rewarding effects of cocaine and amphetamine [46–48]. CREB overexpression increases CART mRNA levels in the NAc and decreases the rewarding effects of drugs [23]. However, studies in knockout mice have reported conflicting results. CART knockout mice exhibited attenuated locomotor sensitization induced by amphetamine [18], and Pdyn knockout mice showed decreased locomotor activity evoked by cocaine [30]. ICER I-overexpressing mice with decreased CART and Pdyn expression levels displayed attenuated METH-induced locomotor sensitization in the present study. These discrepant results among CART and Pdyn studies may be attributable to differences between systemic and NAc-specific downregulation of CART or Pdyn. Further studies are needed to clarify the effects of CART and Pdyn in brain regions other than the NAc.

Conclusion

The present study suggests that ICER plays an inhibitory role in METH-induced locomotor sensitization. Our results support the modulatory effects of the ICER pathway in regulating the effects of drugs of abuse and provide an incentive for exploring the therapeutic potential of stimulating the ICER pathway in the treatment of drug abuse.

Materials and Methods

Ethics statement

The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee (Animal Experimentation Ethics Committee of Tokyo Metropolitan Institute of Medical Science, Approval ID: 11-029), and all animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines.

Animals

Wildtype, ICER knockout, and ICER I-overexpressing mice were produced by conventional gene targeting and transgenic methods [24]. Briefly, the P2 exon encoding the 5′ coding sequence of ICER was deleted to generate ICER-specific knockout mice. To generate ICER I-overexpressing mice, the entire coding sequence of cDNA was subcloned into a pNN265 vector, and the promoter for Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα) was used to express ICER I in the forebrain. The expression patterns of other CREB/CREM family members are not altered in either ICER knockout mice or ICER I-overexpressing mice. ICER knockout mice and their littermates were produced by heterozygote-heterozygote mating. ICER I-overexpressing mice and their wildtype littermates were produced by mating ICER I-overexpressing mice (line I-19) and C57BL/6 mice (CLEA Japan Inc., Shizuoka, Japan) because C57BL/6 is the genetic background strain of ICER I-overexpressing mice. Only naive male mice were used for the experiments. The mice were housed five per cage in a temperature- (22±2°C) and humidity-controlled (55±5%) environment on a 12 h/12 h light/dark cycle (lights on 8:00 a.m. to 8:00 p.m.). The mice had *ad libitum* access to a standard laboratory diet and water. All animal experiments were conducted during the light phase of the cycle, between 9:00 a.m. and 5:00 p.m.

Drugs

Methamphetamine hydrochloride (Dainippon-Sumitomo Pharmaceuticals, Osaka, Japan) was dissolved in saline (0.9% sodium chloride) and administered intraperitoneally (i.p.) in a volume of 10 ml/kg.

Locomotor activity

Locomotor activity corresponding to distance travelled was evaluated in a test chamber (25 cm diameter, 27 cm height) and measured in 5 min bins using digital counters with passive infrared sensors (Supermex system, Muromachi Kikai, Tokyo, Japan). Wildtype littermates of ICER knockout mice ($n = 13$), ICER knockout mice ($n = 13$), wildtype littermates of ICER I-overexpressing mice ($n = 7$), and ICER I-overexpressing mice ($n = 9$) were used. The mice were first habituated to the apparatus for 180 min and then injected with METH (1 mg/kg, i.p.). Locomotor activity was then measured for 60 min after the injection. The procedure was repeated seven times, once every other day from Day 1 to Day 13. After a 7 day drug-free period, locomotor activity was measured again after an injection of METH (1 mg/kg, i.p.) on Day 20.

Western blot analysis

The experiment involved four groups of ICER I-overexpressing mice and wildtype mice: Saline, Single METH, Repeated METH/Saline Challenge, and Repeated METH/METH Challenge. Saline and METH (1 mg/kg, i.p.) were administered once to Saline and Single METH mice, respectively, and the mice were decapitated 1 h after the injection. The Repeated METH/Saline Challenge and Repeated METH/METH Challenge groups received METH (1 mg/kg, i.p.) once every other day from Day 1 to Day 13 and were challenged with saline and METH (1 mg/kg, i.p.), respectively, on Day 20 after a 7 day drug-free period. The mice were decapitated 1 h after the last METH or saline treatment. The brains were removed in less than 45 s and cooled rapidly in ice-cold saline for 30 s. The CPu was then dissected. The tissue was quickly frozen on dry ice, stored at −80°C, and homogenized in 100 µl phosphate-buffered saline containing protease inhibitors (Roche Applied Science, Mannheim, Germany) and PhosStop phosphatase inhibitors (Roche Applied Science, Mannheim, Germany). The homogenate was diluted to 4 µg/µl with 2× Laemmli buffer, heated to 95°C for 2 min, and loaded (20 µg of protein) onto 5–20% gradient polyacrylamide gels. The proteins from eight groups were loaded onto the same gel and separated at 50 mA for approximately 1 h and then transferred onto polyvinylidene membranes in a semi-dry blotter. Nonspecific protein binding sites were blocked by incubating in Blocking One Solution (Nakalai Tesque Inc., Kyoto, Japan). The membranes were incubated overnight at 4°C with phosphor (Ser133) CREB (pCREB) antibody (1:2000; Millipore, Billerica, MA, USA). After incubation in secondary antibody (horseradish peroxidase-conjugated goat antibody to rabbit, 1:50,000; Zymed Labs, South San Francisco, CA, USA) for 1 h, the membrane was treated with chemiluminescent substrate (Millipore, Billerica, MA, USA) and visualized by exposure to Hyperfilm electrochemiluminescence film (GE Healthcare Bio-Sciences, Tokyo, Japan). pCREB blots were stripped with 10% acetic acid solution for 15 min at room temperature. The membranes were reprobed for CREB antibody (1:2000; Cell Signaling Technology, Tokyo, Japan). Finally, the blots were stripped and reprobed for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The blots were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and the sizes were compared with prestained molecular-weight standards. Individual CREB and pCREB values were divided by their respective sample GAPDH values to obtain CREB/GAPDH and pCREB/GAPDH ratio

values for each sample. The CREB/GAPDH and pCREB/GAPDH ratio values from the wildtype saline group were averaged, and the mean was used as a control value. Therefore, the CREB/GAPDH and pCREB/GAPDH ratio values of each sample were calculated as a percentage of this control.

Quantitative real-time reverse transcription polymerase chain reaction

The experiment involved four groups of ICER I-overexpressing mice and wildtype mice: Saline, Single METH, Repeated METH/Saline Challenge, and Repeated METH/METH Challenge. The saline and METH treatments, euthanasia, brain dissection, and storage of brain tissues were the same as described above for Western blot. Total RNA was isolated using Trizol reagent (Invitrogen Life Technology, Tokyo, Japan) and converted into cDNA using a SuperScript VILO cDNA Synthesis Kit (Invitrogen Life Technology, Tokyo, Japan). The real-time RT-PCR reaction was conducted using a LightCycler 480 Instrument (Roche Applied Science, Mannheim, Germany). The ICER, CART, Pdyn, and β-actin primers for real-time RT-PCR were the following: ICER (5′-GCTGAGGCTGATGAAAAACA-3′ and 5′-GCCACACGATTTTCAAGACA-3′), CART (5′-CGAGAAGAAGTACGGCCAAG-3′ and 5′-CACACAGCTTCCCGATCC-3′), Pdyn (5′-TTATGGCGGACTGCCTGT-3′ and 5′-CACTCCAGGGAGCAAATCAG3′), and β-actin (5′-CTAAGGCCAACCGTGAAAAG-3′ and 5′-ACCAGAGGCATACAGGACA-3′). Universal Probes #4, #108, #99, and #64 (Roche Applied Science, Mannheim, Germany) were used for ICER, CART, Pdyn, and β-actin, respectively. Amplification consisted of a preincubation step (95°C for 10 min), 45 cycles of denaturation for 10 s at 95°C, and annealing for 30 s at 60°C. Amplification curves were produced to calculate the crossing point at which the fluorescence of a sample rises above the initial lag phase. Absolute quantification analysis was performed using LightCycler 480 software (Roche Applied Science, Mannheim, Germany). Serial dilutions of an external standard with a predefined, known concentration were used to create a standard curve. The standard dilutions were amplified in separate wells but within the same instrument as the target samples. The crossing points of standards and unknown samples were then used to determine the concentration of the target mRNA. ICER, CART, and Pdyn mRNA levels were normalized according to β-actin mRNA levels. The ICER/β-actin, CART/β-actin, and Pdyn/β-actin values from the wildtype saline group were averaged, and the mean was used as a control value. Therefore, the relative expression levels of CART and Pdyn were calculated as a percentage of this control.

Statistical analysis

The data are expressed as mean ± SEM. The data for the Western blot, real-time RT-PCR, and locomotor sensitization experiments were analyzed by two-way, mixed-design ANOVA and repeated-measures ANOVA followed by the Dunnett *post hoc* test (for the Western blot analysis and real-time RT-PCR experiments) or Tukey-Kramer *post hoc* test (for the locomotor sensitization experiment). Values of $p<0.05$ were considered statistically significant.

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Author Contributions

Conceived and designed the experiments: NK KI. Performed the experiments: WH YT HY SK NK. Analyzed the data: WH YT KI. Contributed reagents/materials/analysis tools: SE TS NK KI. Wrote the paper: WH NK KI.

References

1. Molina CA, Foulkes NS, Lalli E, Sassone-Corsi P (1993) Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. Cell 75: 875–886.
2. Jaworski J, Mioduszewska B, Sanchez-Capelo A, Figiel I, Habas A, et al. (2003) Inducible cAMP early repressor, an endogenous antagonist of cAMP responsive element-binding protein, evokes neuronal apoptosis *in vitro*. J Neurosci 23: 4519–4526.
3. Tinti C, Conti B, Cubells JF, Kim KS, Baker H, et al. (1996) Inducible cAMP early repressor can modulate tyrosine hydroxylase gene expression after stimulation of cAMP synthesis. J Biol Chem 271: 25375–25381.
4. Mioduszewska B, Jaworski J, Kaczmarek L (2003) Inducible cAMP early repressor (ICER) in the nervous system: a transcriptional regulator of neuronal plasticity and programmed cell death. J Neurochem 87: 1313–1320.
5. Carlezon WA, Jr., Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci 28: 436–445.
6. Widnell KL, Russell DS, Nestler EJ (1994) Regulation of expression of cAMP response element-binding protein in the locus coeruleus *in vivo* and in a locus coeruleus-like cell line *in vitro*. Proc Natl Acad Sci U S A 91: 10947–10951.
7. Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, et al. (2002) CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad Sci U S A 99: 11435–11440.
8. Carlezon WA, Jr., Thome J, Olson VG, Lane-Ladd SB, Brodkin ES, et al. (1998) Regulation of cocaine reward by CREB. Science 282: 2272–2275.
9. Maldonado R, Blendy JA, Tzavara E, Gass P, Roques BP, et al. (1996) Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 273: 657–659.
10. Pliakas AM, Carlson RR, Neve RL, Konradi C, Nestler EJ, et al. (2001) Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. J Neurosci 21: 7397–7403.
11. Bilbao A, Parkitna JR, Engblom D, Perreau-Lenz S, Sanchis-Segura C, et al. (2008) Loss of the Ca^{2+}/calmodulin-dependent protein kinase type IV in dopaminoceptive neurons enhances behavioral effects of cocaine. Proc Natl Acad Sci U S A 105: 17549–17554.
12. Kreibich AS, Blendy JA (2004) cAMP response element-binding protein is required for stress but not cocaine-induced reinstatement. J Neurosci 24: 6686–6692.
13. Valverde O, Mantamadiotis T, Torrecilla M, Ugedo L, Pineda J, et al. (2004) Modulation of anxiety-like behavior and morphine dependence in CREB-deficient mice. Neuropsychopharmacology 29: 1122–1133.
14. Green TA, Alibhai IN, Hommel JD, DiLeone RJ, Kumar A, et al. (2006) Induction of inducible cAMP early repressor expression in nucleus accumbens by stress or amphetamine increases behavioral responses to emotional stimuli. J Neurosci 26: 8235–8242.
15. Spiess J, Vale W (1980) Multiple forms of somatostatin-like activity in rat hypothalamus. Biochemistry 19: 2861–2866.
16. Dallvechia-Adam S, Kuhar MJ, Smith Y (2002) Cocaine- and amphetamine-regulated transcript peptide projections in the ventral midbrain: colocalization with γ-aminobutyric acid, melanin-concentrating hormone, dynorphin, and synaptic interactions with dopamine neurons. J Comp Neurol 448: 360–372.
17. Hubert GW, Kuhar MJ (2006) Colocalization of CART peptide with prodynorphin and dopamine D1 receptors in the rat nucleus accumbens. Neuropeptides 40: 409–415.
18. Couceyro PR, Evans C, Mckinzie A, Mitchell D, Dube M, et al. (2005) Cocaine- and amphetamine-regulated transcript (CART) peptides modulate the locomotor and motivational properties of psychostimulants. J Pharmacol Exp Ther 315: 1091–1100.
19. Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc Natl Acad Sci U S A 89: 2046–2050.
20. Vicentic A, Jones DC (2007) The CART (cocaine-and amphetamine-regulated transcript) system in appetite and drug addiction. J Pharmacol Exp Ther 320: 499–506.
21. Cole RL, Konradi C, Douglass J, Hyman SE (1995) Neuronal adaptation to amphetamine and dopamine: molecular mechanism of prodynorphin gene regulation in rat striatum. Neuron 14: 813–823.
22. Dominguez G, Lakatos A, Kuhar MJ (2002) Characterization of the cocaine- and amphetamine-regulated transcript (CART) peptide gene promoter and its

activation by a cyclic AMP-dependent signaling pathway in GH3 cells. J Neurochem 80: 885–893.
23. Rogge GA, Jones DC, Green T, Nestler E, Kuhar MJ (2009) Regulation of CART peptide expression by CREB in the rat nucleus accumbens in vivo. Brain Res 1251: 42–52.
24. Kojima N, Borlikova G, Sakamoto T, Yamada K, Ikeda T, et al. (2008) Inducible cAMP early repressor acts as a negative regulator for kindling epileptogenesis and long-term fear memory. J Neurosci 28: 6459–6472.
25. Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 25: 192–216.
26. Stewart J, Badiani A (1993) Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 4: 289–312.
27. Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396: 157–198.
28. Fukushima S, Shen H, Hata H, Ohara A, Ohmi K, et al. (2007) Methamphetamine-induced locomotor activity and sensitization in dopamine transporter and vesicular monoamine transporter 2 double mutant mice. Psychopharmacology 193: 55–62.
29. McClung CA, Nestler EJ (2003) Regulation of gene expression and cocaine reward by CREB and ΔFosB. Nat Neurosci 6: 1208–1215.
30. Chefer VI, Shippenberg TS (2006) Paradoxical effects of prodynorphin gene deletion on basal and cocaine-evoked dopaminergic neurotransmission in the nucleus accumbens. Eur J Neurosci 23: 229–238.
31. Fasano S, Pittenger C, Brambilla R (2009) Inhibition of CREB activity in the dorsal portion of the striatum potentiates behavioral responses to drugs of abuse. Front Behav Neurosci 3: 29.
32. Sakai N, Thome J, Newton SS, Chen J, Kelz MB, et al. (2002) Inducible and brain region-specific CREB transgenic mice. Mol Pharmacol 61: 1453–1464.
33. Walters CL, Blendy JA (2001) Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. J Neurosci 21: 9438–9444.
34. Turgeon SM, Pollack AE, Fink JS (1997) Enhanced CREB phosphorylation and changes in c-Fos and FRA expression in striatum accompany amphetamine sensitization. Brain Res 749: 120–126.
35. DiRocco DP, Scheiner ZS, Sindreu CB, Chan GCK, Storm DR (2009) A role for calmodulin-stimulated adenylyl cyclases in cocaine sensitization. J Neurosci 29: 2393–2403.
36. Shippenberg TS, Zapata A, Chefer VI (2007) Dynorphin and the pathophysiology of drug addiction. Pharmacol Ther 116: 306–321.
37. Douglass J, McKinzie AA, Couceyro P (1995) PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 15: 2471–2481.
38. Hunter RG, Lim MM, Philpot KB, Young LJ, Kuhar MJ (2005) Species differences in brain distribution of CART mRNA and CART peptide between prairie and meadow voles. Brain Res 1048: 12–23.
39. Marie-Claire C, Laurendeau I, Canestrelli C, Courtin C, Vidaud M, et al. (2003) *Fos* but not *Cart* (cocaine and amphetamine regulated transcript) is overexpressed by several drugs of abuse: a comparative study using real-time quantitative polymerase chain reaction in rat brain. Neurosci Lett 345: 77–80.
40. Vrang N, Larsen PJ, Kristensen P (2002) Cocaine-amphetamine regulated transcript (CART) expression is not regulated by amphetamine. Neuroreport 13: 1215–1218.
41. Fagergren P, Hurd YL (1999) Mesolimbic gender differences in peptide CART mRNA expression: effects of cocaine. Neuroreport 10: 3449–3452.
42. Spangler R, Unterwald EM, Kreek MJ (1993) "Binge" cocaine administration induces a sustained increase of prodynorphin mRNA in rat caudate-putamen. Brain Res Mol Brain Res 19: 323–327.
43. Maiya R, Zhou Y, Norris EH, Kreek MJ, Strickland S (2008) Tisue plasminogen activator modulates the cellular and behavioral response to cocaine. Proc Natl Acad Sci U S A 106: 1983–1988.
44. Dominguez G, Kuhar MJ (2004) Transcriptional regulation of the CART promoter in CATH.a cells. Brain Res Mol Brain Res 126: 22–29.
45. Zhang Y, Butelman ER, Schlussman SD, Ho A, Kreek MJ (2004) Effect of the endogenous κ opioid agonist dynorphin A(1–17) on cocaine-evoked increases in striatal dopamine levels and cocaine-induced place preference in C57BL/6J mice. Psychopharmacology 172: 422–429.
46. Jaworski JN, Hansen ST, Kuhar MJ, Mark GP (2008) Injection of CART (cocaine- and amphetamine-regulated transcript) peptide into the nucleus accumbens reduces cocaine self-administration in rats. Behav Brain Res 191: 266–271.
47. Kim S, Yoon HS, Kim JH (2007) CART peptide 55–102 microinjected into the nucleus accumbens inhibits the expression of behavioral sensitization by amphetamine. Regul Pept 144: 6–9.
48. Yoon HA, Kim S, Park HK, Kim JH (2007) Microinjection of CART peptide 55–102 into the nucleus accumbens blocks both the expression of behavioral sensitization and ERK phosphorylation by cocaine. Neuropharmacology 53: 344–351.

Diversity of Opioid Requirements for Postoperative Pain Control Following Oral Surgery—Is It Affected by Polymorphism of the μ-Opioid Receptor?

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We experience individual differences in pain and sensitivity to analgesics clinically. Genetic factors are known to influence individual difference. Polymorphisms in the human *OPRM1* gene, which encodes the μ-opioid receptors, may be associated with the clinical effects of opioid analgesics. The purpose of this study was to determine whether any of the 5 common single-nucleotide polymorphisms (SNPs) of the *OPRM1* gene could affect the antinociceptive effect of fentanyl. Fentanyl was less effective in subjects with the G allele of the *OPRM1* A118G SNP than in those with the A allele, and subjects with the G allele required more fentanyl for adequate postoperative pain control than those with the A allele. In the future, identifying SNPs might give us information to modulate the analgesic dosage of opioid individually for better pain control. Factors underlying individual differences in sensitivity to pain other than genetic factors may include environmental and psychological factors. We therefore examined the effects of preoperative anxiety on the analgesic efficacy of fentanyl in patients undergoing sagittal split mandibular osteotomy (SSMO). From among the patients enrolled in the study, 60 patients (male/female: 18/42, age: 24.6 ± 6.7 years) who gave informed consent were examined for correlations between preoperative trait/state anxiety, as measured by the state-trait anxiety inventory (STAI) on the day before surgery, and postoperative consumption of patient-controlled analgesia (PCA) fentanyl and visual analog scale (VAS) assessment by patients. Levels of trait and state anxieties measured by the STAI were correlated with neither the consumption of PCA fentanyl nor postoperative VAS assessment. These findings suggest that psychological factors are unlikely to affect postoperative pain or the use of analgesics.

Key Words: Polymorphism; μ-Opioid receptor; Postoperative pain; Patient-controlled analgesia; Preoperative anxiety.

Individual differences in sensitivity to pain and to analgesics are known to vary among patients in daily clinical practice. The amount of analgesics, such as opioids, used for postoperative pain control, even following the same surgery, also varies substantially among patients. Factors possibly underlying these individual differences include environmental, psychological, and genetic factors. We conducted a study involving patients undergoing oral surgery to identify genetic and psychological factors responsible for the diversity of opioid requirements by examining, respec-

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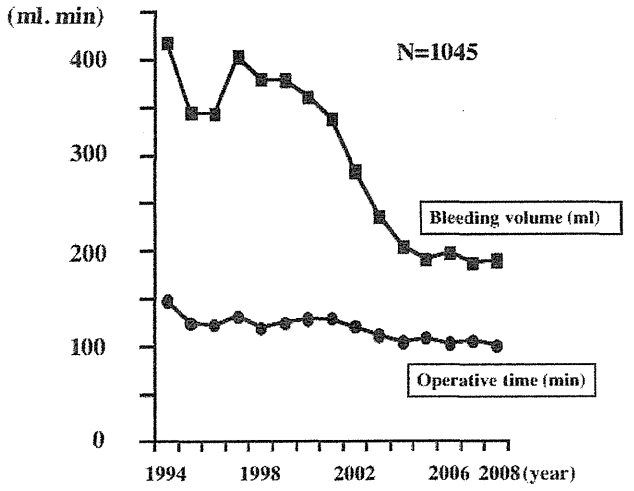

Figure 1. Changes in operative time for sagittal split mandibular osteotomy and volume of intraoperative bleeding are shown.

tively, the effect of polymorphisms in the μ-opioid receptor gene and that of preoperative anxiety on postoperative pain (fentanyl requirement). This report describes the background and current progress of our study.

BACKGROUND

Selection and Setting of Target Surgery

Individual differences in sensitivity to pain are preferably examined in healthy subjects. If sensitivity to analgesics for postoperative pain control is to be examined, pathological condition, degree of surgical invasiveness, surgical site, surgical procedure, and the surgeon's level of skill should to the extent possible be consistent across subjects. The target surgery we selected was sagittal split mandibular asteotomy (SSMO), a procedure in orthognathic surgery. Almost all patients undergoing SSMO are young and healthy people aged 10–30 years. Patients undergoing this surgery have an asymmetric facial appearance. Because patients have no inflammation or injury, the pathological condition of patients undergoing SSMO should be almost the same as those of patients undergoing tumor resection, although some morphological differences may exist between the 2 patient populations. The degree of surgical invasiveness, surgical site, and surgical procedure are highly consistent across cases. At Tokyo Dental College, a total of 300–400 cases of SSMO (total from 3 hospitals in Chiba, Ichikawa, and Suidobashi) are performed annually using nearly the same procedure. Figure 1 shows the changes in operative time for SSMO and volume of intraoperative bleeding at the hospitals over the past 15 years. Both have decreased annually overall but have remained constant over the past few years. Although increased use of propofol for maintenance of general anesthesia may have contributed to the decrease in bleeding volume,[1] the decrease in operative time is probably attributable to the surgical procedure used in our hospitals. Hypotensive anesthesia, which used to be essential for SSMO, is currently required in almost no patients. In addition, the present study involved only patients for whom SSMO was performed by 1 of 3 experienced surgeons. As described above, the target surgery was selected so that intraoperative factors affecting postoperative pain would be as consistent as possible across patients.

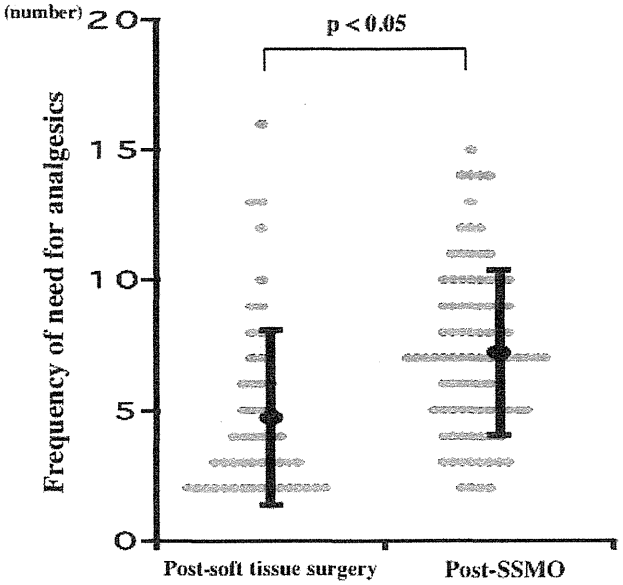

Figure 2. Frequency of need for postoperative analgesics following sagittal split mandibular osteotomy (SSMO) compared with that following oral soft tissue surgery.

Range of Postoperative Pain Following SSMO

A certain level of pain is required for comparison of postoperative pain. Figure 2 shows a comparison of the level of postoperative pain following SSMO and that following oral soft tissue surgery (in terms of frequency of requirement for postoperative diclofenac). SSMO involves osteotomy, and thus causes somewhat more pain than other oral surgery procedures. Levels of postoperative pain following SSMO have been measured by various indicators, as follows: 0–100 mm on visual analog scaler (VAS) at 3 hours postoperatively,[2] 0–400 mg diclofenac requirement in the first 72 hours postoperatively,[2] 0–92 mg consumption of morphine by patient-controlled analgesia (PCA) in the first 72 hours postoperatively,[3] and 0–640 μg consumption of fentanyl in the first 24 hours post-

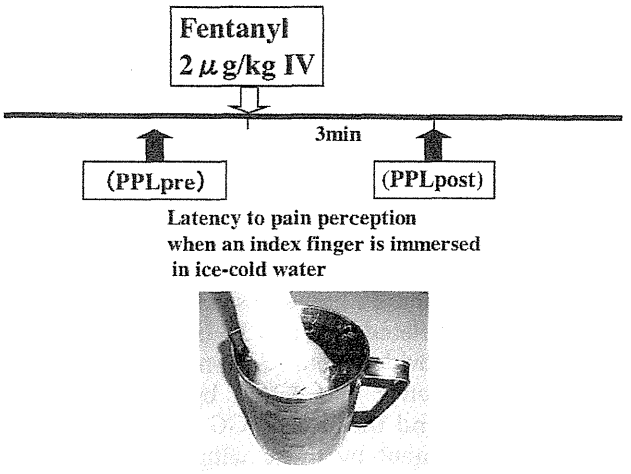

Figure 3. Measurement of latency to pain perception before (PPLpre) and after (PPLpost) administration of fentanyl. (A patient's hand was immersed in ice-cold water so that more than half of the area of the dorsum of the hand was underwater.)

operatively (from a study listed below). These findings indicate substantial variation and diversity as well as apparent individual differences in both the level of postoperative pain and the consumption of analgesics used to control postoperative pain.

STUDY 1

Genetic Factors Underlying Individual Differences in Sensitivity to Pain

Genetic factors are responsible in part for individual differences in sensitivity to pain. Whereas 99.9% of the human genome sequence is shared among all individuals, the remaining 0.1%, ie, polymorphisms, contributes to individual differences. Single-nucleotide polymorphisms (SNPs) account for the largest number of polymorphisms. An SNP involves the alteration of a single nucleotide base; for example, adenine may be replaced by guanine, or thymine may be replaced by cytosine. These SNPs are one type of individual difference. About 3–6 million base pairs are thought to differ from person to person. Provided below are the results of our study on the relationship between polymorphisms and individual differences in sensitivity to pain following SSMO.

Methods

We examined the effects of 5 SNPs representative of 4 linkage disequilibrium blocks of the human μ-opioid

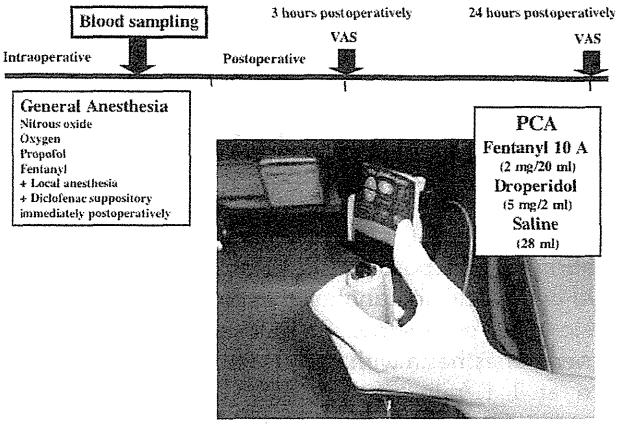

Figure 4. Intraoperative and postoperative procedures.

receptor gene (A118G, IVS2 + G691, IVS3 + G5953A, IVS3 + A8449G, and TAA + A2109) on the analgesic efficacy of fentanyl in patients undergoing SSMO. Performance of the study was approved by the Ethics Committee of Tokyo Dental College. We enrolled 108 patients scheduled to undergo SSMO at Tokyo Dental College Suidobashi Hospital who provided written informed consent to participate in the study.

After being taken into the operating room, each patient was instructed to immerse his or her finger into ice-cold water, and the amount of time until the patient felt cold stress–induced pain (latency to pain perception) was measured before (PPLpre) and 3 minutes after (PPLpost) the intravenous administration of fentanyl at 2 μg/kg (Figure 3). The PPLpre value was regarded as an index of sensitivity to cold stress–induced pain. With the cutoff value of latency to pain perception (maximum effect) set to 150 seconds,

Table 1. Patient Background and Surgical Data†

Age	25.7 ± 6.7 (18 to 47)
Male/female	40/68
Body weight (kg)	58.2 ± 10.3 (42 to 97)
PPLpre (s)	17.2 ± 1.8 (3 to 130)*
PPLpost (s)	41.8 ± 4.0 (4 to 150)*
%MPE	20.7 ± 2.7 (−19 to 100)*
Anesthesia time (min)	167 ± 23 (101 to 243)
Operative time (min)	106 ± 19 (70 to 177)
Total dose of propofol (mg/kg)	27.0 ± 3.7 (17 to 38)
Total fentanyl dose (μg/kg)	3.31 ± 1.82 (0 to 10.3)
Total PCA fentanyl dose (μg/kg)	3.16 ± 2.67 (0 to 13.8)
VAS at 3 h postoperatively (mm)	31.2 ± 23.5 (0 to 90)
VAS at 24 h postoperatively (mm)	27.4 ± 18.7 (0 to 73)

† Data are expressed as mean ± SD. PPLpre indicates latency to pain perception before administration of fentanyl; PPLpost, latency to pain perception after administration of fentanyl; %MPE, analgesic efficacy of fentanyl; PCA, patient-controlled analgesia; VAS, visual analog scale.

* $P < .05$.

Table 2. Frequencies of 5 single-nucleotide polymorphisms (SNPs)*

A118G	AA: 31 (28.7%)	AG: 54 (50.0%)	GG: 23 (21.3%)
IVS2+G691C	GG: 7 (6.5%)	GC: 34 (31.5%)	CC: 67 (62.0%)
IVS3+G5953A	GG: 87 (80.6%)	GA: 18 (16.7%)	AA: 3 (2.8%)
IVS3+A8449G	AA: 86 (79.6%)	AG: 17 (15.7%)	GG: 5 (4.6%)
TAA+A2109G	AA: 92 (85.2%)	AG: 14 (13.0%)	GG: 2 (1.9%)

* SNP indicates single-nucleotide polymorphism.

%MPE = (PPLpost − PPLpre)/(150 − PPLpre) × 100 was calculated and used as an index of the analgesic effect of fentanyl. SSMO was performed under general anesthesia with nitrous oxide at 2 L/min, oxygen at 1 L/min, propofol (3.5 μg/mL, by target-controlled infusion), and fentanyl (290–1020 μg). Ten milliliters of whole blood samples for DNA extraction was collected from each patient during surgery. After surgery, postoperative pain was controlled by PCA with intravenous fentanyl. A PCA pump was filled with 2 mg/20 mL fentanyl, 5 mg/2 mL droperidol, and 28 mL saline. The consumption of fentanyl within the first 24 hours postoperatively and the levels of spontaneous pain as measured by VAS at 3 and 24 hours postoperatively were recorded (Figure 4). Data were examined by analysis of variance.

Results

Patient background data and surgical data are summarized in Table 1 and the frequencies of the 5 SNPs in Table 2. PPLpre was significantly correlated with A118G (P = .0032); patients with the G allele exhibited significant shortening of PPLpre. This finding indicates the involvement of A118G in individual differences in sensitivity to pain. %MPE was also significantly correlated with A118G (P = .0109); those with a G allele exhibited a significantly lower %MPE than those without it (Figure 5). Consumption of PCA fentanyl within the first 24 hours postoperatively was also significantly correlated with A118G (P = .0695). When patients were divided into 3 genotype groups, a significant difference was found by t test between those with AA and those with GG (P = .0398); the fentanyl requirement by those with GG was almost double that by those with AA (Figure 6). These findings indicate that A118G reduces the analgesic effect of fentanyl and increases postoperative fentanyl requirement.

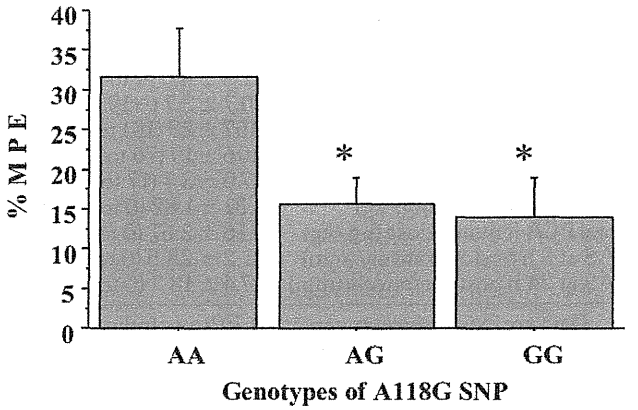

Figure 5. Relationships between analgesic efficacy of fentanyl (%MPE) and A118G genotypes.

STUDY 2

Psychological Factors Underlying Individual Differences in Sensitivity to Pain

Factors underlying individual differences in sensitivity to pain other than genetic factors may include environmental and psychological factors. Healthy patients who have never experienced surgery under general anesthesia and are scheduled to undergo SSMO may experience preoperative anxiety and wonder how painful it will be to have the bone of their face cut. We therefore examined the effects of preoperative anxiety on the analgesic efficacy of fentanyl in patients undergoing SSMO.

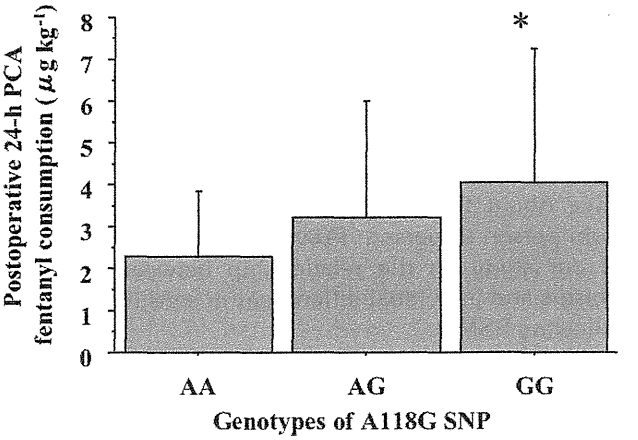

Figure 6. Relationships between postoperative consumption of fentanyl (patient-controlled analgesia [PCA]) and A118G genotypes.

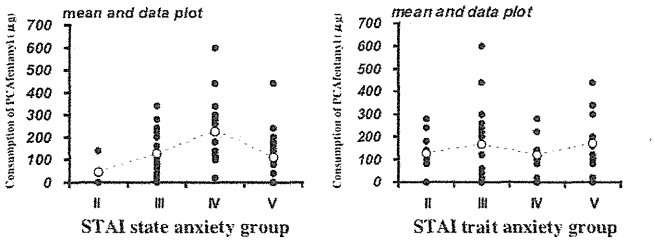

Figure 7. Relationship between postoperative consumption of fentanyl (patient-controlled analgesia [PCA]) and preoperative anxiety level (state-trait anxiety inventory [STAI]).

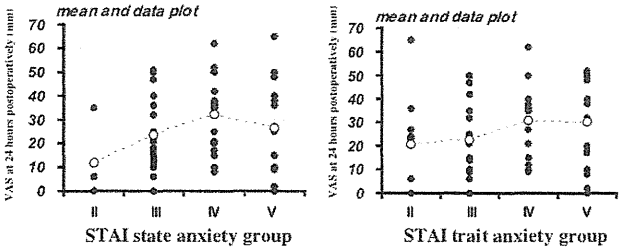

Figure 8. Relationship between visual analog scale (VAS) at 24 hours postoperatively and preoperative anxiety level (state-trait anxiety inventory [STAI]).

Methods

From among the patients enrolled in the study, 60 patients (male/female: 18/42, age: 24.6 ± 6.7 years) who gave informed consent were examined for correlations between preoperative trait/state anxiety, as measured by the state-trait anxiety inventory (STAI) on the day before surgery, and postoperative consumption of PCA fentanyl and VAS assessment by patients. Patients were grouped based on levels of trait and state anxiety according to the Japanese version of the STAI assessment and grading criteria. Postoperative data were then compared among preoperative anxiety groups by the Kruskal-Wallis test.

Results

Patients were grouped by levels of trait and state anxieties measured by the STAI as follows: 9 patients in group II, 20 in group III, 15 in group IV, and 16 in group V by trait anxiety level, and 4 patients in group II, 19 in group III, 20 in group IV, and 17 in group V by state anxiety level. Levels of trait and state anxieties measured by the STAI were correlated with neither the consumption of PCA fentanyl nor postoperative VAS assessment (Figures 7 and 8).

These findings suggest that psychological factors are unlikely to affect postoperative pain or the use of analgesics.

DISCUSSION AND CONCLUSION

We examined whether genetic and psychological factors are involved in individual differences in sensitivity to pain and the use of analgesics. The present study involved healthy patients undergoing oral surgery in which the effects of intraoperative factors such as surgical invasiveness were minimized, and demonstrated that A118G, an SNP of the human μ-opioid receptor gene, is responsible for individual differences in the analgesic efficacy of fentanyl. Although A118G has been shown to be related to the amount of morphine required for pain control,[4] the present study revealed that it also affects the analgesic efficacy of fentanyl. Fentanyl requirement for pain control may thus be predicted by determining this polymorphic A118G genotype. Many other SNPs have been identified in the human μ-opioid receptor gene as well. More case studies will be needed for the realization of "tailor-made pain control" in the future.

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REFERENCES

1. Ahn HJ, Chung SK, Dhong HJ, et al. Comparison of surgical conditions during propofol or sevoflurane anesthesia for endoscopic sinus surgery. *Br J Anaesth*. 2008;100:50–54.

2. Nagatsuka C, Ichinohe T, Kaneko Y. Preemptive effects of a combination of preoperative diclofenac, butorphanol, and lidocaine on postoperative pain management following orthognathic surgery. *Anesth Prog*. 2000;47:119–124.

3. Handa T, Fukuda K, Hayashida M, Koukita Y, Ichinohe T, Kaneko Y. Effects of intravenous adenosine 5′-triphosphate on intraoperative hemodynamics and postoperative pain in patients undergoing major orofacial surgery: a double-blind placebo-controlled study. *J Anesth*. 2009;23:315–322.

4. Rekvag TT, Klepstad P, Baar C. The Val158Met polymorphisms of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. *Pain*. 2005;116:73–78.

Int. J. Environ. Res. Public Health **2011**, *8*, 3922-3937; doi:10.3390/ijerph8103922

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Article

Family Dysfunction Differentially Affects Alcohol and Methamphetamine Dependence: A View from the Addiction Severity Index in Japan

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Abstract: We investigated the differential influence of family dysfunction on alcohol and methamphetamine dependence in Japan using the Addiction Severity Index (ASI), a useful instrument that multilaterally measures the severity of substance dependence. The participants in this study were 321 male patients with alcohol dependence and 68 male patients with methamphetamine dependence. We conducted semi-structured interviews with each patient using the ASI, which is designed to assess problem severity in seven functional domains: Medical, Employment/Support, Alcohol use, Drug use, Legal, Family/Social relationships, and Psychiatric. In patients with alcohol dependence, bad relationships with parents, brothers and sisters, and friends in their lives were related to current severe psychiatric problems. Bad relationships with brothers and sisters and partners in their lives were related to current severe employment/support problems, and

bad relationships with partners in their lives were related to current severe family/social problems. The current severity of psychiatric problems was related to the current severity of drug use and family/social problems in patients with alcohol dependence. Patients with methamphetamine dependence had difficulty developing good relationships with their father. Furthermore, the current severity of psychiatric problems was related to the current severity of medical, employment/support, and family/social problems in patients with methamphetamine dependence. The results of this study suggest that family dysfunction differentially affects alcohol and methamphetamine dependence. Additionally, family relationships may be particularly related to psychiatric problems in these patients, although the ASI was developed to independently evaluate each of seven problem areas.

Keywords: alcohol dependence; methamphetamine dependence; Addiction Severity Index; family relationship

1. Introduction

In 2003, approximately 800,000 adults of the Japanese general population of 120 million could be classified with alcohol dependence, making this group one of the largest among the various mental disorders [1]. Additionally, stimulant dependence is a serious problem not only for patients, but also for Japanese society [2]. For example, approximately 25% of convicted prisoners committed offenses under the Stimulant Control Law [3].

Previous studies have suggested that social support is an important factor for improving the symptoms of substance dependence. Coping and social support are related to substance use behavior and treatment outcomes in adolescents [4,5]. Social support also plays an important role in relapse avoidance efforts for individuals who undergo substance use treatment. Social support is a "social fund" from which individuals draw assistance when confronting stressors [6].

On the other hand, bad relationships may be an aggravating factor. Previous studies have reported an association between familial relationships and substance dependence. Multidimensional Family Therapy is uniquely suited to address adolescent substance abuse and related disorders, given its comprehensive interventions that systematically target the multiple interacting risk factors that underlie many of the developmental disruptions of adolescence [7]. A previous study of alcohol dependence suggested that among the many biological, morphological, and social markers of increased maturation, visible signs of maturity are important triggers of alcohol use and alcohol use disorders, especially when they occur early and in young people with conduct problems, deviant peers, problem families, and inadequate parental supervision [8]. Another study of drug dependence reported that drug use prevention should not simply focus on reducing drug availability, but also help young people develop good family/peer relationships and find healthy ways to enjoy themselves [9].

The Addiction Severity Index (ASI) is a semi-structured clinical research interview widely used in substance abuse treatment settings in the United States and many other countries. This instrument was designed to assess problem severity in seven functional domains: Medical, Employment/Support,

Alcohol use, Drug use, Legal, Family/Social relationships, and Psychiatric [10]. Therefore, family relationships are an important factor in assessing the severity of substance dependence using the ASI.

A comparison of the characteristics of family relationships and the association between family relationships and various problems related to substance dependence in patients with alcohol and drug dependence using the ASI may be useful for establishing personalized programs for individuals with substance dependence. However, no study of which we are aware has compared the differences in the association between family dysfunction and problems related to substance dependence between alcohol and drug dependence. Moreover, the ratio of individuals who use methamphetamine is the highest in individuals with drug dependence in Japanese hospitals, suggesting that it may be meaningful to focus on the characteristics of individuals with methamphetamine dependence. Therefore, we investigated the differences in the influence of family dysfunction on alcohol dependence and methamphetamine dependence in Japanese patients using the ASI as an exploratory survey. We hypothesized that family dysfunction in patients with alcohol and patients with methamphetamine dependence may be related to different aspects of problems related to substance dependence. The present exploratory study may provide future direction for more detailed investigations that lead to the development of more effective methods for finding appropriate psychological interventions for each patient.

2. Methods

2.1. Participants

We surveyed 370 patients with alcohol dependence and 83 patients with drug dependence. Valid data were obtained from 321 male patients with alcohol dependence (86.76%; mean age, 49.7 ± 11.0 years) and 80 male patients with drug dependence (96.39%; mean age, 32.9 ± 9.4 years). The participants with alcohol dependence were recruited from nine nationwide hospitals or recovery facilities for addiction treatment located in Japan: National Hospital Organization Kurihama Alcoholism Center, Kanagawa (n = 91), Wakamiya Hospital, Yamagata (n = 55), Komakino Hospital, Tokyo (n = 50), Mie Prefectural Mental Medical Center, Mie (n = 42), Asahiyama Hospital, Hokkaido (n = 26), Ishikawa Prefectural Takamatsu Hospital, Ishikawa (n = 17), National Hospital Organization Hizen Psychiatric Center, Saga (n = 14), Akagi-Kohgen Hospital, Gunma (n = 13), and Tohokukai Mental Hospital, Miyagi (n = 12). The participants with drug dependence were recruited from five nationwide hospitals or recovery facilities for addiction treatment in Japan: Tokyo Metropolitan Matsuzawa Hospital, Tokyo (n = 37), Self-Support Services (*i.e.*, a recovery facility run by a non-profit organization for addiction recovery), Tokyo (n = 16), National Center of Neurology and Psychiatry Musashi Hospital, Tokyo (n = 17), GAIA (i.e., a recovery facility run by a non-profit organization for addiction recovery), Okinawa (n = 8), and Fukko-kai Tarumi Hospital, Hyogo (n = 2).

2.2. Methods

The Japanese version of the ASI [11,12] was used in the present study. The ASI is a semi-structured clinical research interview designed to assess problem severity in seven functional domains: Medical status, Employment/Support status, Alcohol use, Drug use, Legal status, Family/Social relationships,

and Psychiatric status [10]. The Medical status domain gathers basic information about medical history. It addresses information about lifetime hospitalizations, long-term medical problems, and recent physical ailments. The Employment/Support status domain gathers basic information about work experience and current sources of income. The Drug/Alcohol use domain gathers basic information about the patient's substance abuse history. It addresses information about current and lifetime substance abuse, the consequences of abuse, periods of abstinence, treatment episodes, and the financial burden of substance abuse. The Legal status domain gathers basic information about the patient's legal history. It addresses information about probation or parole, legal charges, convictions, incarcerations or detainments, and illegal activities. The Family/Social relationship domain assesses relationship problems with family members or friends. The Psychiatric status domain is used not to diagnose psychiatric disorders but to assess the experience of various psychiatric symptoms other than those associated with the effects of alcohol or drugs.

Acceptable reliability and validity of the ASI were confirmed in patients with drug [11] and alcohol dependence [12]. The ASI provides a composite score (CS). The CS in each problem area is a mathematically calculated score mainly based on patient responses to sets of items that ask the patient to report behaviors during the 30 days prior to the interview. The CS is calculated using a weighted formula designed to provide an equal contribution from each item and varies from 0 to 1, with a higher score indicating greater problem severity. Additionally, we analyzed the items of the ASI related to education years, employment status, marital status, cohabitation, years of current cohabitation, experience of abuse, family history of substance dependence or psychiatric disorders, and family relationships in their life.

2.3. Procedure

The recruitment criteria were the following: at least 18 years of age, a history of substance addiction problems diagnosed as alcohol dependence or drug dependence based on the criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV), and the ability to understand Japanese.

The inpatients with alcohol dependence were provided an average 80-day treatment program (e.g., group meetings, alcohol education, family treatment programs, psychotherapy, and so on) after detoxification. After recovery from serious physical and mental instability (nearly 1 month after hospitalization), informed consent was obtained from the subjects, excluding the patients who had serious cognitive impairment and psychiatric problems.

The participants with drug dependence were inpatients or outpatients at a Japanese mental hospital or recovery facility or non-patients who were recovering from stimulant abuse in a recovery facility. Considering the time required for an interview and the reliability of the responses, we excluded patients in a state of acute drug-induced psychosis.

The ASI was administered by psychiatrists and clinical psychologists who were experts in alcohol or drug dependence, carefully read the ASI manual [13], and learned the interview methods themselves. The average time required for administration of the questionnaire was 60 min. Inpatient subjects were requested to answer the questions during the 30 days prior to the start of inpatient treatment. The

Institutional Review Board of each institution approved the study, and all of the participants provided written informed consent.

2.4. Statistical Analysis

Comparisons between groups with regard to age, number of convictions, and ASI CS were conducted using the *t*-test. Comparisons between groups with regard to the characteristics of education, employment, marital status, cohabitation, experience of abuse, and psychiatric symptoms were performed using the x^2 test and Fisher exact test (multiple comparisons were performed using residual analysis). The relationships between ASI CSs were analyzed using partial correlation analysis. The significance level was set at less than 0.05 or 0.01. Statistical analyses were performed with SPSS 18 (SPSS Inc., Chicago, IL).

3. Results

3.1. Participant Characteristics

Table 1 shows the substances that the participants with drug dependence in this study mainly used. Most of the patients with drug dependence (85.00%) used methamphetamine, and others used cannabis (6.25%), inhalants (3.75%), analgesics/hypnotics/tranquilizers (2.50%), antitussive drugs (1.25%), or hallucinogens (1.25%). We performed the subsequent statistical analysis only in individuals who mainly used methamphetamine as patients with methamphetamine dependence ($n = 68$).

Table 1. Substances that participants with drug dependence in this study mainly used.

Drug	%
Methamphetamine	85.00
Cannabis	6.25
Inhalants	3.75
Analgesics/hypnotics/tranquilizers	2.50
Antitussive drugs	1.25
Hallucinogens (e.g., lysergic acid diethylamide)	1.25

Table 2 shows the characteristics of the participants. The mean age of the patients with alcohol dependence was significantly higher than that of patients with methamphetamine dependence ($t = 12.31$, $p < 0.0001$). Significant differences were found in educational background ($z = 17.72$, $p = 0.003$). Patients with alcohol dependence had a higher ratio of being a junior high school graduate ($p < 0.05$), and patients with drug dependence had a higher ratio of being a high school dropout ($p < 0.05$). Significant differences were found in employment status ($z = 36.26$, $p < 0.0001$). Patients with alcohol dependence had higher ratios of full-time employment ($p < 0.05$) and retirement ($p < 0.05$). Patients with methamphetamine dependence had higher ratios of part-time employment ($p < 0.05$) and unemployment ($p < 0.05$). A significant difference was found in marital status ($z = 64.08$, $p < 0.0001$). Patients with alcohol dependence had a higher ratio of being