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Reduced Supraspinal Nociceptive Responses and Distinct Gene Expression Profile in CXBH Recombinant Inbred Mice

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Abstract: CXBH mice, known as an "opioid receptor-rich" strain, are a recombinant inbred mouse strain established by crossing the C57BL/6By and BALB/cBy strains. In the present study, we investigated nociceptive and antinociceptive sensitivity in CXBH mice and elucidated the underlying molecular mechanisms. CXBH mice exhibited slightly higher morphine-induced antinociception compared with C57BL/6J and BALB/cBy mice in the hot-plate test but not tail-flick test. CXBH mice exhibited a marked reduction of nociceptive sensitivity, regardless of the type of nociceptive stimulus, with the exception of tail stimulation. Changes in gene expression that corresponded to reduced nociceptive sensitivity in the brains of CXBH mice were observed in 62 transcripts, including pain- and analgesia-related transcripts, in a whole-genome expression assay. The total mRNA expression of opioid receptors was higher in CXBH mice than in C57BL/6J and BALB/cBy mice. However, the expression levels of *MOR-1* mRNA, a major transcript of the μ opioid receptor gene, were not different among the C57BL/6J, BALB/cBy, and CXBH strains. In conclusion, supraspinal nociceptive responses were reduced in the CXBH mouse strain, and the expression levels of transcripts were altered in the brain of this strain.

***Perspective:** This article presents the nociceptive and antinociceptive properties of CXBH recombinant inbred mice and gene expression differences that may underlie nociceptive tolerance in the strain. The CXBH mouse strain may be a useful animal model to investigate the molecular basis of individual differences in supraspinal pain sensitivity.*

***Key words:** Gene expression, morphine, nociceptive tolerance, opioid receptor, recombinant inbred strain.*

Pain sensation is an essential sensory modality that protects the body from excessive nociceptive stimuli and further injury. However, considerable variability exists in the sensitivity to pain across individuals, which has been an unresolved clinical issue in pain management.[11]

The heritability of pain sensitivity has been estimated by human twin studies to be .57 (irritable bowel syndrome), .55 (dysmenorrhoea), .50 (back pain), and .34 to .57 (migraine).[19,28] Numerous polymorphisms in the genes that encode cytokines (eg, interleukin-1β and interleukin-6), neuropeptides (eg, brain-derived neurotrophic factor and prodynorphin), and receptors (eg, α_{2A} adrenergic receptor and μ opioid receptor) are reportedly associated with interindividual differences in pain- and analgesic-related clinical traits.[6,13,18,28]

Genetic factors mainly cause variability between inbred strains because these animals are isogenic within each strain, barring the very rare occurrence of a new mutation. Therefore, the use of inbred animals has been an effective strategy for investigating the heritability of traits associated with interindividual differences. To date, various inbred and recombinant inbred mouse strains have been established, and interstrain differences among laboratory mice, such as BALB/c, C3H/HeJ, and C57BL/6, have been reported in nociception,[20,25-27] antinociception,[5,24,40,41] reward,[32] antinociceptive tolerance,[15] and physical dependence.[16]

CXB mice comprise recombinant inbred strains established by crossing the C57BL/6By and BALB/cBy inbred strains. Among the seven CXB mouse strains, the CXB7/ByJ (CXBK) strain exhibited the lowest naloxone-binding affinity and a marked reduction of morphine-induced antinociception compared with its progenitor strains.[1,9] We previously identified

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abnormally long μ opioid receptor mRNA and its attenuated expression caused by insertion of the intracisternal A-particle (IAP) transposon in the 3′ untranslated region (UTR) of the μ opioid receptor gene in CXBK mice.[7,10] In contrast to CXBK mice, CXB4/ByJ (CXBH) mice have the highest naloxone-binding affinity among its progenitor strains and 7 CXB mouse stains and higher antinociceptive effects induced by Mr2096 (ie, a diastereoisomeric pair of *N*-tetrahydrofurfurylnormorphones), ethanol, and stress than its progenitor strains.[1,21-23,36,43] Therefore, CXBH mice have been known as an "opioid receptor-rich" strain. However, the antinociceptive effect of morphine was lower in CXBH mice than in its progenitor strains.[1,39] U-50488H, a κ opioid receptor-selective agonist, did not produce antinociception in CXBH mice,[35] results that are inconsistent with naloxone-binding affinity. Therefore, the behavioral characteristics of CXBH mice and molecular bases of this strain's nociceptive and antinociceptive sensitivity have been unclear.

In the present study, we investigated nociceptive and antinociceptive sensitivity in a CXBH recombinant inbred strain and the molecular bases of alterations in nociceptive and antinociceptive sensitivity.

Methods

Animals

C57BL/6By, BALB/cBy, and CXBH mice were purchased from Jackson Laboratory (Bar Harbor, ME). C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). C57BL/6By, C57BL/6J, BALB/cBy, and CXBH mice were used in the genetic experiments, and male C57BL/6J, BALB/cBy, and CXBH mice were used for the behavioral and gene expression analyses. All of the mouse strains were maintained at the Tokyo Metropolitan Institute of Medical Science in a temperature-controlled room at 22 ± 2°C under a 12 hour/12 hour light/dark cycle (lights on 8:00 AM–8:00 PM) and had ad libitum access to a standard laboratory diet and water. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the Tokyo Metropolitan Institute of Medical Science.

Behavioral Tests

The behavioral responses to 3 types of nociceptive stimuli (ie, thermal, mechanical, and visceral chemical) were examined in the tail-flick and hot-plate tests, Randall-Selitto test,[37] and abdominal constriction test,[3] respectively. The tail-flick and hot-plate tests were slightly modified from the original methods developed by D'Amour and Smith[4] and by Woolfe and MacDonald,[42] respectively. Male mice of each strain were used in each behavioral test (n = 11–14). All of the behavioral tests were performed from 9:00 AM to 6:00 PM.

In the tail-flick test, the mice were loosely wrapped in a velvet towel and placed on the tail-flick apparatus (Model MK-330B; Muromachi Kikai, Tokyo, Japan). A light beam was focused on the tail approximately 1 to 3 cm from the base, and the latency to flick the tail vigorously in response to the heat stimulus was measured with a 15-second cut-off time to minimize tissue damage. Tail-flick latencies were measured 3 times per mouse with different light beam foci, and the average was considered the tail-flick latency. In the hot-plate test, the mice were placed on the hot-plate apparatus (20-cm width × 25-cm length × 25-cm height: Model MK-350B; Muromachi Kikai) that was maintained at 52 ± .2°C. The latency to lick the hindpaws or jump in response to the heat stimulus was measured with a 180-second cut-off time to minimize tissue damage. Hot-plate latencies were measured twice per mouse at a 5-minute interval, and the average was considered the latency. In the Randall-Selitto test, the mice were gently handled, and force was applied to the hindpaw on a silicon stage under a cone-shaped pusher with a rounded tip using a Randall-Selitto apparatus (Model MK-201D; Muromachi Kikai) at a constant rate of 10 mm Hg/second. The withdrawal threshold to hindpaw struggling or attacking the silicon stage or pusher was measured with a 250 mm Hg pressure cut-off to avoid tissue damage. The withdrawal thresholds in the Randall-Selitto test were measured once for each hindpaw, and the average was considered the threshold. In the abdominal constriction test, the mice were intraperitoneally (i.p.) injected with 10 mL/kg of a .6% acetic acid solution and placed in a breeding cage. The number of writhes was counted for 30 minutes after the injection, and the total number of writhes was considered the response of the mouse.

The analgesic (antinociceptive) effects of morphine were assessed using the tail-flick and hot-plate tests. Morphine hydrochloride solution (10 mg/mL; Takeda Chemical Industries, Osaka, Japan) was diluted to 1 mg/mL with sterile saline on every experimental day. Morphine was injected intraperitoneally at 20-minute intervals using an ascending cumulative dose regimen that produced total drug doses of 3, 10, 30, and 100 mg/kg. The tail-flick and hot-plate tests were performed 10 minutes after the morphine injection at each dose point described above. Antinociceptive effects are expressed as the percentage of the maximal possible effect (%MPE):

$$\%\text{MPE} = (\text{latency after morphine injection} - \text{baseline latency})/(\text{cut-off latency} - \text{baseline latency}) \times 100\ (\%)$$

The ED_{50} was calculated by constructing cumulative dose-response curves for morphine antinociception in each mouse strain. Computer-assisted probit and relative potency analyses were used to calculate ED_{50} values.

Whole-Genome Expression Assay and Microarray Data Analysis

Whole-genome gene expression profiles in the brains of each mouse strain were analyzed using an