available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Short Communication

Reduced pain sensitivity in mice lacking latexin, an inhibitor of metallocarboxypeptidases

Minghao Jin[1], *Mami Ishida*, *Yuko Katoh-Fukui*[2], *Reiko Tsuchiya*, *Toru Higashinakagawa*[3], *Shiro Ikegami*[4], *Yasuyoshi Arimatsu*[*]

Mitsubishi Kagaku Institute of Life Sciences, Machida, Tokyo 194-8511, Japan

ARTICLE INFO

Article history:
Accepted 27 December 2005
Available online 15 February 2006

Keywords:
Latexin
Gene targeting
Thermal pain
Metallocarboxypeptidase
Potato carboxypeptidase inhibitor

ABSTRACT

Latexin, the endogenous protein inhibitor of the A/B subfamily of metallocarboxypeptidases, is expressed in small nociceptive neurons in sensory ganglia and in a subset of neurons in the telencephalon. In this study, we generated latexin-deficient mice that exhibited increased tail-flick latency compared to wild-type animals upon noxious heat stimulation. The reduced pain sensitivity in the mutants was rescued by the systemic administration of a plant carboxypeptidase inhibitor that inhibits the A/B subfamily of metallocarboxypeptidases. These findings suggest that latexin is involved in the transmission of pain.

Latexin, the only known endogenous inhibitor of the A/B subfamily of metallocarboxypeptidases, is expressed in various tissues including the brain (Hatanaka et al., 1994; Normant et al., 1995b; Pallarès et al., 2005). Carboxypeptidases A1 and A2–well-known pancreatic digestive enzymes belonging to the A/B subfamily–also are expressed in the brain and other non-pancreatic tissues (Normant et al., 1995a). However, the in vivo functional roles of non-pancreatic metallocarboxypeptidases and latexin remain to be determined.

In the periphery, latexin is expressed in small-diameter nociceptive neurons in sensory ganglia (Takiguchi-Hayashi et al., 1998), suggesting its potential involvement in pain perception. In the CNS, latexin immunoreactivity is localized in a subset of corticocortical projection neurons in the sensory association cortices (e.g. the secondary somatosensory and granular insular areas), as well as in neurons in the claustrum, endopiriform nucleus, and subiculum (Arimatsu et al., 1992, 1999a,b, 2003; Bai et al., 2004). Within the cerebral cortex and other telencephalic structures, the distribution pattern of latexin-expressing neurons is strikingly similar to the distribution pattern of the mRNA of κ-opioid receptors, raising the possibility that latexin may be involved in central pain modulation (DePaoli et al., 1994; Mansour et al., 1994; Arimatsu et al., 1999b, 2003).

To explore the in vivo roles of latexin, we generated latexin-deficient mice using a gene targeting method (Fig. 1). The mouse latexin gene (*Lxn*) was isolated from a genomic DNA library of 129/SvJ mouse (Stratagene, La Jolla, CA) by hybrid-

[*] *Corresponding author*. Fax: +81 427 24 6312.
E-mail address: lspr@libra.ls.m-kagaku.co.jp (Y. Arimatsu).
[1] Current address: Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90095, USA.
[2] Current address: Department of Developmental Biology, National Institute for Basic Biology, Myodaiji-cho, Okazaki, Aichi 444-8585, Japan.
[3] Current address: Department of Biology, School of Education, Waseda University, 1-6-1 Nishi-Waseda, Shinjuku, Tokyo 169-8050, Japan.
[4] Current address: Department of Psychology, Saitama Institute of Technology, Fukaya-si, Saitama 369-0293, Japan.

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.12.099

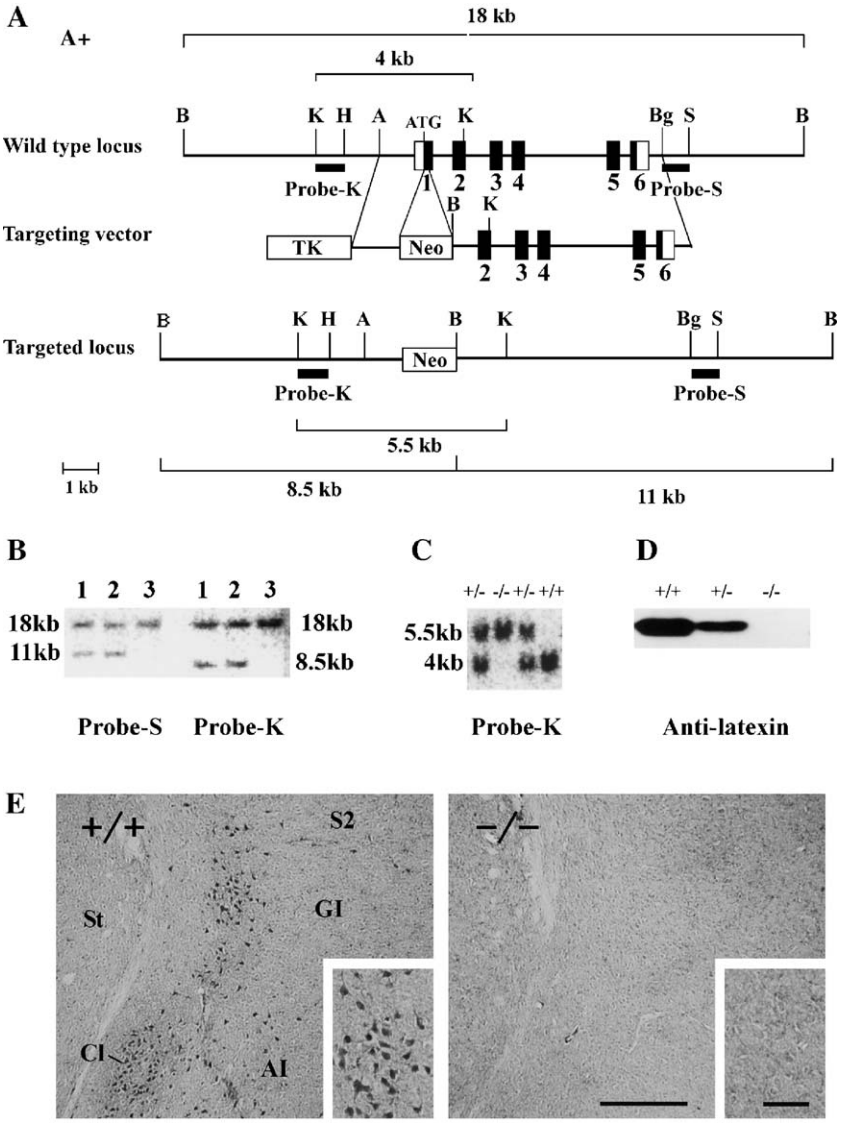

Fig. 1 – Generation of latexin-deficient mice. (A) Wild-type *Lxn* locus (top), targeting vector (middle), and targeted locus (bottom). Coding regions are shown as filled boxes with numerals indicating exon numbers. The ATG in exon 1 indicates the translation initiation codon. The coding region in exon 1 was replaced by the PGK-*neo* cassette (Neo) in the targeting vector. Restriction sites and length (kb) of restriction fragments are shown. A, *Apa*I; B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; S, *Sal*I. (B) Southern blot of *Bam*HI-digested genomic DNA from targeted (lanes 1 and 2) and wild-type (lane 3) embryonic stem cell (ES) clones, hybridized with probe-S and probe-K. Probe-S recognizes both 18-kb and 11-kb fragments in targeted ES clones and an 18-kb fragment in wild-type ES clones. Probe-K recognizes both 18-kb and 8.5-kb fragments in targeted ES clones and an 18-kb fragment in wild-type ES clones. (C) Southern blot of *Kpn*I-digested tail DNA from wild-type (+/+), heterozygous (+/–), and homozygous (–/–) mice, hybridized with probe-K. Probe-K detects a 5.5-kb fragment in the homozygous mice, a 4-kb fragment in wild-type mice, and both fragments in heterozygous mice. (D) Western blot of latexin expression in the forebrain of wild-type (+/+), heterozygous (+/–), and homozygous (–/–) mice. Proteins (20 μg) from forebrain homogenates were fractionated by SDS-PAGE (12.5% gel) and transferred onto an Immobilon-P membrane (Millipore, Tokyo, Japan). The membrane was incubated with rabbit anti-latexin antiserum (Hatanaka et al., 1994) followed by peroxidase-conjugated goat anti-rabbit IgG. Latexin protein was visualized using the ECL system (Amersham Biosciences, Tokyo, Japan). (E) Immunohistochemical analysis of latexin expression in the brain of wild-type (left) and $Lxn^{-/-}$ (right) mice. Latexin is expressed in a subset of neurons in the secondary somatosensory area (S2), agranular (AI) and granular insular (GI) areas, and the claustrum (Cl) of the wild-type, but not of $Lxn^{-/-}$ mice. St, striatum. Scale bar = 200 μm. Insets represent higher magnification images of layer VI of GI (scale bar = 50 μm). The brains were fixed with a solution containing 4% paraformaldehyde and 0.1% picric acid for 2 h. The cryostat sections (20 μm) were stained for latexin immunoreactivity as described previously (Arimatsu et al., 1992). All animal experiments were carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of the Mitsubishi Kagaku Institute of Life Sciences.