Molecular and Cellular Neuroscience 45 (2010) 439–448

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Adenylate Cyclase 1 modulates peripheral nerve branching patterns

Corinna Haupt, Jana Langhoff, Andrea B. Huber *

Institute of Developmental Genetics, Helmholtz Zentrum München — German Research Center for Environmental Health, Ingolstädter Landstrasse 1, D-85764 Munich/Neuherberg, Germany

ARTICLE INFO

Article history:
Received 19 May 2010
Revised 9 July 2010
Accepted 2 August 2010
Available online 6 August 2010

Keywords:
Adenylate cyclase 1
Motoneurons
Mouse
Nerve branching
Spinal cord
Dorsal root ganglia

ABSTRACT

The Ca^{2+}-stimulated adenylate cyclase 1 (AC1) is a key mediator of retinotopic map refinement and is required for the retraction response of retinal growth cones to the guidance cue ephrin-A5. We show here that *AC1* is dynamically expressed in subpopulations of motor neurons in the spinal cord and sensory neurons of the dorsal root ganglia during development. AC1 was first detected around E12.5 in motoneurons of the medial aspect of the lateral motor column (LMCm) and the lateral region of the medial motor column (MMCl), which project to the ventral limb and body wall musculature, respectively. Expression levels gradually increased until they reached a maximum at a time when peripheral sensory and motor axons branch and establish connections with their targets. In *barrelless* mice, where a mutation inactivates the *AC1* gene, sensory projections to the skin in the limbs and trunk region as well as innervations of the intercostal musculature provided by MMCl axons show increased branching. These results suggest a function of AC1 in the formation of peripheral nerve trajectories such as branching and pruning, after the initial projections have been laid down.

Introduction

During development growing axons are guided by a large number of spatially and temporally tightly organized cues that act in concert to create functional neuronal circuitries (Huber et al., 2003; O'Donnell et al., 2009). After initial establishment of axon-target contacts a refinement of these immature circuitries takes place. These restructuring and remodeling events have been extensively studied in the visual system. Retinal ganglia neurons project their axons in a topographic manner to the lateral geniculate nucleus (LGN) so that nasal axons target to the anterior LGN and temporal axons to posterior LGN (for review see Haupt and Huber, 2008; Wong, 1999). Nasal axons, however, overshoot their correct targets during development and also establish branches in posterior regions of the LGN. As the visual system matures, these exuberant branches are eliminated in a process termed pruning (Low and Cheng, 2006; Wong, 1999). The Ca^{2+}-stimulated adenylate cyclase 1 (AC1) plays an essential role in the fine patterning of the retinal map. Indeed, in *barrelless* mice (Welker et al., 1996), where a spontaneous mutation leads to inactivation of the *AC1* gene, retinal projections initially develop normally, subsequent refinement, however, is disturbed including the establishment of eye-specific domains in the LGN and superior colliculus (SC) and the patterning of the retinotectal map (Ravary et al., 2003). It was shown that AC1 mediates regionally selective branching and retraction of exuberant retinal branches in an ephrin-A5 dependent manner (Nicol et al., 2006). The establishment of precise topographic maps in the mammalian visual system, however, requires not only the coordinated effects of molecular guidance cues but also the interplay with spontaneous neural activity that further contributes to the refinement of the map (Torborg et al., 2005). Ephrin-A5 might present the molecular link between spontaneous rhythmic retinal activity and axon pathfinding during the establishment of topographic neural circuits (Nicol et al., 2007).

Rhythmic bursts of spontaneous activity reminiscent of the retinal waves have also been observed throughout the spinal cord of mouse and chick embryos, where they may control the molecular signaling cascades that regulate downstream guidance decisions (Hanson et al., 2008; Milner and Landmesser, 1999). Consequently, blocking or slowing this rhythmic activity at a time when motor axons are navigating the important decision region of the plexus prevented the expression of the axon guidance receptor EphA4 and of polysialic acid on NCAM (neural cell adhesion molecule), thereby likely causing altered fasciculation and dorsal–ventral pathfinding errors (Hanson and Landmesser, 2004). Increasing the frequency of the spontaneous activity on the other hand had no effect on the dorsal–ventral guidance but severely disrupted the subsequent sorting of motor axons into target specific fascicles and resulted in pathfinding errors in the anterior–posterior orientation (Hanson and Landmesser, 2006).

Abbreviations: AC1, adenylate cyclase 1; DRG, dorsal root ganglia; LGN, lateral geniculate nucleus; LMC, lateral motor column; LMCl, lateral motor column lateral; LMCm, lateral motor column medial; MMC, medial motor column; MMCl, medial motor column lateral; MMCm, medial motor column medial; NCAM, neural cell adhesion molecule; PFA, parafomaldehyde; PKA, cAMP-dependent protein kinase; SC, superior colliculus; TrkA, neurotrophic tyrosine kinase receptor type 1; TrkC, neurotrophic tyrosine kinase receptor type 3.

* Corresponding author. Fax: +49 89 3187 3099.
E-mail address: andrea.huber@helmholtz-muenchen.de (A.B. Huber).

1044-7431/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.mcn.2010.08.003

Table 1
AC1 expression in spinal cord and DRG during development.

	E10.5	E11.5	E12	E12.5	E13.5	E14.5	E15.5	P0
DRG	0	0	+	++	++	++	++	++
Spinal cord	0	0	+/−	+	+	+	++	++
Ventral horn	0	0	+	++	++	++	++	+
Roof plate	0	0	+/−	++	+	+	++	++
Dorsal horn	0	0	+/−	++	+	+	++	++

0, not detected; +/−low expression; + moderate expression; ++ high expression.

Rhythmic bursting activity strongly depolarizes motor neurons, which together with ensuing Ca^{2+} transients may regulate neuronal gene expression (Dolmetsch et al., 1997; Itoh et al., 1997; Watt et al., 2000). The molecular nature of the guidance cues that are located downstream of the rhythmic bursting activity and mediate the motor pool-specific pathfinding is currently unknown.

Here we show that *AC1* mRNA is dynamically expressed in mouse spinal cord and dorsal root ganglia (DRG) during development in a pattern consistent with a role in motor and sensory axon patterning. We further show that AC1 is required *in vivo* to regulate branching

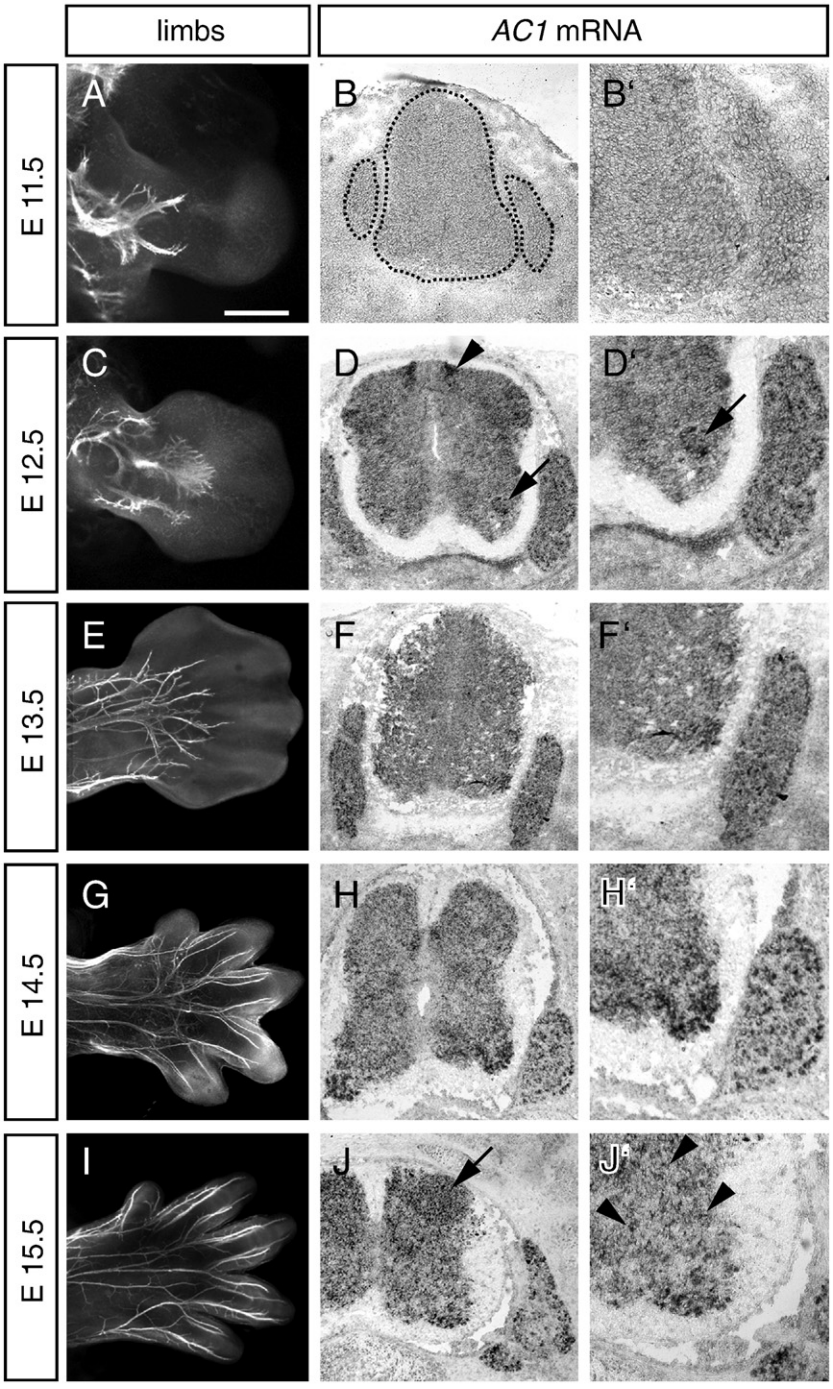

Fig. 1. *AC1* mRNA expression in the spinal cord and DRG during development. *AC1* mRNA expression is very low at E11.5 (A, B). Spinal cord and DRG are outlined with dotted black lines (B, B′). From E12.5 to E14.5 transcript levels of *AC1* are increased in the ventral horn of the spinal cord (arrow in D), cells adjacent to the roof plate (arrowhead in D) and a subpolulation of cells in DRG (C–H). At E15.5, in addition to sensory and motoneurons, *AC1* is also found in the dorsal horn (arrow in J) and in cells scattered throughout the spinal cord (arrowheads in J′). The first column (A, C, E, G, and I) displays photomicrographs of whole mount immunohistochemistry for neurofilament showing the stage specific progression of the spinal nerves into the forelimb. Scale bar: 500 μm for G and I; 460 μm for E; 370 μm for C; 325 μm for D, F, and H; 320 μm for A; 280 for J, 270 for B; 190 for D′, F′, H′; and 170 for J′; 140 for B′.