available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Hearing development and spiral ganglion neurite growth in VASP deficient mice

Stefan Dazert[a,*], *Bernhard Schick*[b], *Rene Hartensuer*[c], *Stefan Volkenstein*[a], *Christoph Aletsee*[c], *Stefan Hansen*[a], *Wafaa E. Shehata-Dieler*[c], *Martin Eigenthaler*[d], *Ulrich Walter*[d], *Allen F. Ryan*[e], *Dominik Brors*[a]

[a]*Department of Otorhinolaryngology-Head and Neck Surgery, Ruhr-Universitaet Bochum, St. Elisabeth-Hospital Bochum, Germany*
[b]*Department of Otorhinolaryngology, Universitaet Erlangen-Nürnberg, Erlangen, Germany*
[c]*Department of Otorhinolaryngology, Universitaet Wuerzburg, Germany*
[d]*Institute for Clinical Biochemistry and Pathobiochemistry, Universitaet Wuerzburg, Germany*
[e]*Department of Surgery, Division Otolaryngology and Neuroscience, UCSD School of Medicine and VA Medical Center, La Jolla, CA 92093, USA*

ARTICLE INFO

Article history:
Accepted 7 June 2007
Available online 5 July 2007

Keywords:
Hearing development
Spiral ganglion
VASP
Knockout mice

ABSTRACT

Vasodilator-stimulated phosphoprotein (VASP) has been found to be involved in intracellular signalling pathways and to play an important role in the actin associated organization and formation of the cytoskeleton. Since differential VASP expression was noted in inner ear tissues, the present study was performed to investigate the hearing development in VASP deficient mice. Hearing development in VASP–/– mice and wild type animals was investigated by auditory brain stem (ABR) measurements. In addition, inner ear tissues of wild type animals were tested for VASP expression using PCR, Western blot analysis, *in situ* hybridisation, and immunohistochemistry. To compare spiral ganglion (SG) neurite growth, SG explants from VASP–/– and wild type mice were analyzed under cell culture conditions. The electroacoustical results of the present study indicate that VASP deficient mice present with a later onset of hearing during postnatal development compared to wild type animals. Transient VASP expression was detected in neonatal SG of wild type mice. Tissue culture experiments with SG explants from VASP–/– animals revealed significant alterations in SG neurite extension compared to wild types. The present findings suggest a role for VASP during neonatal development of the mammalian cochlea and allow speculation on a possible delayed innervation of cochlear hair cells due to changes in SG neurite growth in VASP-deficient mice. Temporary VASP deficits in the neonatal inner ear may be compensated by related proteins like MENA leading to a delayed but complete development of hearing function in VASP–/– animals.

* *Corresponding author*. Department of Otorhinolaryngology, Ruhr-Universitaet Bochum, St. Elisabeth-Hospital, Bleichstrasse 15, D-44787 Bochum, Germany. Fax: +49 234 612 279.
E-mail address: Stefan.Dazert@ruhr-uni-bochum.de (S. Dazert).

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.06.041

1. Introduction

Vasodilator-stimulated phosphoprotein (VASP) is the founding member of the proline-rich Ena (Enabled)/VASP protein family and has been identified to be a common substrate of cAMP- and cGMP-dependent protein kinases (Butt et al., 1994). Besides their involvement in signalling pathways, Ena/VASP proteins have been found to play an important role in the organization and formation of the cytoskeleton and during neuronal development of eucaryotic cells (Lanier et al., 1999; Lanier and Gertler, 2000; Reinhard et al., 2001). They take part in the regulation of actin-associated processes, being located at major subcellular sites with high actin dynamics in various cell types. Members of the Ena/VASP family are involved in focal adhesion mechanisms of the cell matrix and also take part in cell–cell adherence junctions of the actin cytoskeleton (Gertler et al., 1996; Ahern-Djamali et al., 1998; Lambrechts et al., 2000; Vasioukhin et al., 2000). They have been demonstrated to be necessary to seal opposing membranes into epithelial sheets, a process that requires directed actin polymerization (Vasioukhin et al., 2000). Ena/VASP proteins facilitate actin filament formation and are involved in integrin regulation, cell motility processes as well as axon guidance and axon repulsion (Colavita and Culotti, 1998; Bashaw et al., 2000). Thus, they are prominent promoters of actin-based motility but there is growing evidence that these proteins also exert inhibitory effects in the mentioned biological functions (Aszodi et al., 1999; Hauser et al., 1999; Bear et al., 2000).

Filamentous actin expression was shown at several sites in the mammalian inner ear and has been suggested to play a role in pillar cell stiffness affecting cochlear mechanical properties (Pack and Slepecky, 1995; Kuhn and Vater, 1996; Tolomeo and Holley, 1997). Recent investigations revealed differential expression of VASP and zyxin, a partner of VASP activity in various inner ear tissues. VASP and zyxin expression were noted in cochlear pillar cells as well as in limbal fibroblasts, in the stria vascularis and in the vascular endothelium of the spiral ganglion (SG) in the adult rat cochlea. VASP expression was interestingly noted in pillar cells coincident with the onset of hearing (Schick et al., 2003).

Expecting a role for VASP during the maturation of the mammalian inner ear, the present study was performed to investigate hearing development in neonatal VASP-deficient mice. The finding of a delayed hearing onset in VASP–/– mice compared to wild type mice in our study prompted us to analyse VASP expression in the neonatal cochlea of wild-type mice. The observation of VASP expression in the SG during cochlear development stimulated us to compare SG neurite growth of both wild type and VASP–/– mice to test for the possibility of delayed hearing onset caused by alterations in SG neurite growth.

2. Results

Electrophysiological investigations of adult animals (3 months old) using ABR responses did not show any significant differences in hearing performance between VASP deficient and wild type animals. Also, there were no signs of vertigo or disequilibrium in either group.

Newborn animals were investigated daily by ABR beginning at postnatal day 5–6 (P5–P6). Significant differences in the onset of hearing were found between VASP deficient and wild type animals. The first ABR responses (onset of hearing) in wild type animals were observed at P9, with the majority of animals hearing at P12. The first ABR responses (onset of hearing) in VASP deficient animals were observed at P11, with the majority of animals hearing at P13. Between P10 and P14 VASP deficient mice showed higher ABR thresholds compared to wild type mice. The differences were statistically significant (Fig. 1).

The VASP–/– mice showed no evidence for a general developmental delay by weight and behaviour compared to the wild type mice. Detailed anatomical dissection of the ear canal and the middle ear at different stages (P6, P10, P12, and P15) provided a regular development of these structures in VASP–/– mice. Thus the observed delay of hearing development in VASP–/– mice was found not to be related to any developmental delay in the middle ear/outer ear canal.

The finding of a delayed onset of hearing in VASP deficient mice in the absence of an obvious developmental delay prompted us to analyze VASP expression in the neonatal mouse cochlea as VASP expression in the organ of Corti has been reported to arise first with the onset of hearing (Schick et al., 2003). From four wild type mice, cochlear mRNA was extracted at the day of birth (P0). RT-PCR analysis proved VASP mRNA expression in the neonatal mouse cochlea (Fig. 2a). Similar findings were obtained at P4 and P6 (data not shown). On the protein level, western blot analysis in neonatal cochleae (P0) showed mainly the dephosphorylated 46 kDa that was seen in the Hela cells used for control and only slightly the phosphorylated 50 kDa VASP protein was detected (Fig. 2b). Similar findings were observed at P4 and P6 (data not shown). Previously the cochlear expression of VASP protein at day P4 and P10 has been shown already by Western blot analysis (Schick et al., 2003). mRNA *in situ* hybridisation and immunohistochemistry were used in order to analyse cochlear VASP expression within the time period P0 to P6

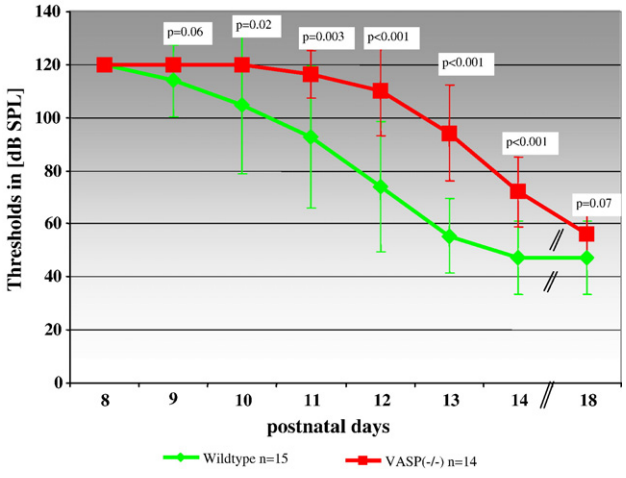

Fig. 1 – ABR analysis showed a significantly delayed onset of hearing in VASP–/– mice.