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THE DIFFERENTIAL RESPONSE OF ASTROCYTES WITHIN THE VESTIBULAR AND COCHLEAR NUCLEI FOLLOWING UNILATERAL LABYRINTHECTOMY OR VESTIBULAR AFFERENT ACTIVITY BLOCKADE BY TRANSTYMPANIC TETRODOTOXIN INJECTION IN THE RAT

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Abstract—In this study, we investigated whether changes in the vestibular neuronal activity per se influence the pattern of astrocytes morphology, glial fibrillary acidic protein (GFAP) expression and ultimately their activation within the vestibular nuclei after unilateral transtympanic tetrodotoxin (TTX) injections and after unilateral inner ear lesion. The rationale was that, theoretically the noninvasive pharmacological functional blockade of peripheral vestibular inputs with TTX, allowed us to dissociate the signals exclusively related to the shutdown of the resting activity of the first-order vestibular neurons and from neuronal signals associated with transganglionic changes in first order vestibular neurons induced by unilateral labyrinthectomy (UL). Since the cochlea was removed during the surgical procedure, we also studied the astrocytic reaction within the deafferented cochlear nuclei. No significant changes in the distribution or relative levels of GFAP mRNA expression, relative levels of GFAP protein or immunoreactivity for GFAP were found in the ipsilateral vestibular nuclei at any post-TTX injection times studied. In addition, no sign of microglia activation was observed. In contrast, a robust increase of the distribution and relative levels of GFAP mRNA expression, protein levels and immunoreactivity was observed in the deafferented vestibular and cochlear nuclei beginning at 1 day after inner ear lesion. GFAP mRNA expression and immunoreactivity in the cochlear nucleus was qualitatively stronger than in the ipsilateral vestibular nuclei. The results suggest that astrocyte activation in the vestibular nuclei is not related to drastic changes of vestibular nuclei neuronal activity per se. Early trans-ganglionic changes due to vestibular nerve dendrites lesion provoked by the mechanical destruction of vestibular receptors, most probably induced the glial reaction. Its functional role in the vestibular compensation process remains to be elucidated. © 2004 Published by Elsevier Ltd on behalf of IBRO.

Key words: glia, neuronal activity, post-lesional plasticity, vestibular compensation, deafferentation, axotomy.

Vestibular compensation following unilateral labyrinthectomy (UL) is an interesting model for studying the cellular and molecular mechanisms underlying post-lesional plasticity of the adult CNS. Unilateral removal of peripheral vestibular receptors (UL) induces a massive oculomotor and postural syndrome, which largely disappears over time in all species of vertebrates studied including humans because of the vestibular compensation process (Smith and Curthoys, 1989; Curthoys and Halmagyi, 1995; Dieringer, 1995). These static deficits are caused by the immediate depression of the resting discharge of the ipsilateral second-order vestibular neurons (Smith and Curthoys, 1988; Ris et al., 1995) whereas the resting discharge on the contralateral side is unchanged or increased (Smith and Curthoys, 1988; Ris et al., 1995). The deafferented second order vestibular neurons progressively recover a quasi-normal resting activity within a week, which contributes to abating the static vestibular syndromes (Smith and Curthoys, 1989; Ris et al., 1995, 1997) although there is some dissociation between the time-courses of the two processes (Ris et al., 1997).

Several hypotheses have been proposed to explain vestibular compensation (Smith and Curthoys, 1989; Dieringer, 1995); however, the cellular and molecular mechanisms involved remain incompletely understood. Collateral sprouting has been shown in amphibians after UL (Dieringer, 1995), although it does not occur in vertebrates brainstem at any post-lesional time (Gacek et al., 1988; de Waele et al., 2000). In contrast, modulation of the sensitivity of vestibular nuclei neurons receptors to various neurotransmitters and neuromodulators is involved (Smith and Darlington, 1991; Vidal et al., 1999; Yamanaka et al., 2000). Finally, plastic changes in the intrinsic membrane properties of the deafferented vestibular nuclei neurons may also contribute to the vestibular compensation process (Vibert et al., 1999; Him and Dutia, 2001).

Astrocytes are the major glial cell type of CNS gray matter and play an active role in synaptic transmission and plasticity by supporting neuronal and synaptic function during development and in the adult CNS (Araque et al., 1999; Carmignoto, 2000). They also play a role in the preservation of the host tissue integrity following injury

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Abbreviations: IL, interleukin; OD, optical density; PBS, phosphate buffer; RT, reverse transcription; RT-PCR, reverse transcriptase–polymerase chain reaction; TTX, tetrodotoxin; UL, unilateral labyrinthectomy.

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(Ridet et al., 1997) and in the CNS immune response (Dong and Benveniste, 2001). As in microglia, the astrocyte response to injury proceeds through several gradual stages and depends on the characteristics and intensity of the lesion (Raivich et al., 1999a,b). In addition, astrocytes morphology and GFAP expression has been shown to be regulated by neuronal activity (Tweedle and Hatton, 1980; Hatton, 1988; Canady and Rubel, 1992; Rubel and MacDonald, 1992; Canady et al., 1994) and by several postinjury signals (Ridet et al., 1997).

In earlier studies we showed the presence of a glial reaction within the deafferented vestibular nuclei (de Waele et al., 1996; Campos Torres et al., 1999). We hypothesized that the shut down of the resting activity of the first- and/or second-order vestibular neurons and/or trans-neuronal signals other than rapid degenerative events or programmed neuronal death, most probably triggered the astrocyte reaction. In this study we investigated whether the changes of the first- and/or second-order vestibular neurons activity per se, contribute to the induction of the vestibular nuclei astrocyte activation. We therefore studied the astrocytes activation after transtympanic TTX injections, which block the primary vestibular neurons activity (Saxon et al., 2001). The rationale was that, the noninvasive pharmacological functional blockade of peripheral vestibular inputs with TTX, would allow us to dissociate the signals exclusively related to the shut down of the resting activity of the first-order vestibular neurons, from neuronal signals associated with trans-ganglionic changes in first order vestibular neurons induced by UL.

EXPERIMENTAL PROCEDURES

Surgical procedures

Adult male-pigmented Long-Evans rats, weighing between 200 and 300 g were divided into three groups: labyrinthectomized group (n=95), tetrodotoxin (TTX) group (n=56) and a control group (n=26). All studies were carried out in accordance with the European Communities Council directive of November 24th, 1986, and following the procedures issued by the French Ministère de l'Agriculture. All efforts were made to minimize the number of animals used and their suffering. Global left UL under halothane anesthesia inhalation was performed using a retro-auricular approach as previously described (Campos Torres et al., 1999). Briefly, the entire length of the ventral edge of the meatus was removed and the facial nerve sectioned. The tympanic membrane, malleus and incus were extirpated to expose and coagulate the pterygopalatine artery caudal to the stapes, the latter was then removed and the oval window opened. The cochlea was then entirely destroyed and the vestibule was drilled and aspirated using a suction tube. After recovery from anesthesia, animals which did not exhibit the typical postural and oculomotor syndromes (De Waele et al., 1989) were not used. The animals were then left free in normal visual conditions until compensation of the vestibular deficits was completed. Finally, 10 unoperated and 10 sham-operated rats served as controls: Facial nerve transection and tympanic membrane and bullae were opened in five animals and facial nerve transection and the opening of the bullae with associated electro-coagulation of the pterygopalatine artery was performed in the five other sham-operated animals.

Trans-tympanic TTX injections

TTX, a specific blocker of voltage-gated sodium channels, which prevents occurrence of action potentials, has been already used as an effective tool for blocking vestibular nerve action potentials (Saxon et al., 2001). Vestibular afferent blockade was induced in halothane-anesthetized adult Long-Evans rats (n=56) by left trans-tympanic injection of 50 μl of 3 mM TTX (Sigma Aldrich-Chimie, Lyon, France) in PBS using a 100 μl Hamilton syringe (Saxon et al., 2001). After recovery from anesthesia, vestibular afferent TTX blockade was monitored and the ocular nystagmus quantified (protocol described below). In preliminary experiments we observed that three successive trans-tympanic TTX applications 6 h apart mimicked the time course disappearance of spontaneous nystagmus observed after UL. Thus the interval between TTX injections prevented the occurrence of an acute systemic effect of the toxin. Controls consisted of animals (n=8) injected with PBS.

Eye movements recordings

As an index of development of vestibular compensation, we characterized the time course of disappearance of horizontal spontaneous nystagmus after UL (Goto et al., 1997; Li et al., 2001). Eye movements recording was performed in the dark in UL rats (n=8) and in TTX-injected rats (n=8) by using a magnetic search coil system (0.02 mm diameter; Sokimat; Kasper et al., 1987). Spontaneous nystagmus was measured immediately after the anesthesia recovery and at 1, 2, 4, 6, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 40, 44, 48, and 52 h after UL or TTX injection. Experimental procedures have been described previously (Kasper et al., 1987). During recordings rats were placed in a vestibular turntable (Toennis) using a Plexiglas cylinder allowing the animals to stand up freely. They were positioned in the center of the magnetic field generated by two pairs of field coils set up perpendicular to the turntable. The head was positioned with a holder device which had been attached to the skull with dental acrylic and screws 1 week before UL or TTX injection. Eye position signals were recorded on a DAT recorder for off-line analysis or printed on paper on an electrostatic chart recorder (Gould ES 1000; Gould, Laguna Niguel, CA, USA). Spontaneous nystagmus was evaluated by counting nystagmus beats per minute from the recorded eye position signal.

In situ hybridization histochemistry and autoradiography

The distribution of GFAP-encoding mRNA expression was studied within the deafferented vestibular and cochlear nuclei and through out the different brain areas related to vestibular pathways in labyrinthectomized (n=24) and TTX-injected (n=15) rats. In particular, we were interested in the oculomotor nucleus (III), the trochlear nucleus (IV) and the abducens nucleus (VI), the thalamus and the cerebellum; these CNS areas are known to be related to vestibular nuclei. Controls consisted of normal (n=3), sham-operated (n=2) and PBS-injected rats (n=2).

Experimental animals (n=3 at all time points) were deeply anesthetized with an overdose of chloral hydrate, at 2, 6, 12 h, 1, 3, 8, 14 and 21 days after the lesion or at 3, 6, 12 h, 1 and 3 days after TTX injection. They were then killed by decapitation and their brains were rapidly removed, immediately frozen on powdered dry ice and kept at −80 °C until use. Coronal sections (14 μm) were cut in a cryostat at −20 °C and thaw mounted on Superfrost plus slides (Polylabo, Fontenay Sous Bois, France) and immediately fixed for 20 min in 2% paraformaldehyde in phosphate buffer (PBS; 0.1 M, 0.9% NaCl, pH 7.4), rinsed in PBS (3×5 min) dehydrated in a graded ethanol series and stored at −80 °C until use.