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Short Report

A temporo-spatial programmed ependymal denudation leads to hydrocephalus in the *hyh* mutant mouse

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ABSTRACT *Hyh* is a lethal autosomal recessive mouse mutation located in chromosome 7. Newborn homozygous mutants presented moderate hydrocephalus, and then a severe hydrocephalus develops during the first postnatal days (Pérez-Fígares *et al.,* 1998). Mutant embryos showed a well-defined pattern of ependymal cell denudation in floor and in basal plate derivatives and later in alar plate derivatives. A relationship between ependymal denudation and degree of ependymal differentiation can be observed. Ependymal denudation precedes hydrocephalus; an increased bulk flow of brain fluid may be causing the hydrocephalus developed in these embryos. The floor plate of hindbrain detached whereas that of the spinal cord did not, further supporting the functional zonation of the floor plate.

Introduction

Fetal ependyma play a role in developmental processes of the brain and its differentiation follows a temporo-spatial pattern. Neuroepithelial cells differentiates late, thus allowing neurogenesis to proceed for a relatively long period during ontogeny. Floor plate differentiates very early and extends from the most caudal level of the spinal cord to the mesencephalic-diencephalic boundary (Sarnat, 1992). In the spinal cord the ependyma of the basal plate is the next region to differentiate, followed by that of the alar plate. A similar sequence occurs later in the fourth ventricle and the cerebral aqueduct. In third and lateral ventricles ependymal differentiation occurs at later. The expression of intermediate filament citosqueletal proteins (vimentina, glial fibrillary acidic protein, GFAP and cytokeratins) and of some ependymal secretory molecules (proteoglycans and S-100β protein) has been used to define the early and late stages of ependymal development (Sarnat, 1998).

Congenital hydrocephalus may course with a normal, stenosed, or obliterated cerebral aqueduct. Most studies support the view that aqueductal stenosis is a key event for the development of congenital hydrocephalus (Pérez-Fígares *et al.,* 2001). The aim of the present investigation was to study the mechanisms of hydrocephalus starting in the fetal life coursing with an open cerebral aqueduct. In the present study it has been observed that detachment of ependymal cells lineage occurring during the embryonic life, precedes the development of a congenital hydrocephalus.

Material and Methods

Heterozygous mice of the C57BL/10J strain purchased from Jackson Laboratory (Bar Harbor, Me, USA) were bred in the animal house of the University of Malaga. Handling, care and processing of the animals were carried out according to Spanish national laws. Normal and mutant embryos were obtained after 12, 13, 14, 15, 16, 17, 18 days of pregnancy and 1, 3, 11, 30 and 60 days after birth. Material was processed for immunocytochemical studies in serial transversal and sagittal paraffin sections and for scanning electron microscopy. Antibodies used to immunotype the ependymal cells were against vimentin (Sigma, Spain), GFAP (Biogenesis, England), S-100 (Biogenesis), NCAM (Developmental Studies Hybridoma Bank, 5B8 clone) and Reissner´s fibre (obtained in our laboratories).

Results

A developmental pattern of vimentin, GFAP and S-100 expression following a caudo-rostral and a ventro-dorsal pattern was found (Fig. 1). Detachment of ependymal cells followed a similar pattern (Fig. 2). Thus, denudation initiated in E-12 in the floor of the fourth ventricle, at the pontine flexure. At the level of the basal and alar plates detached abruptly between E-14 and E-15, but floor plate was unaffected (Fig. 3). From E-14 to E-16 the ventral ependymal wall (basal plate) of the cerebral aqueduct showed a symmetrical pattern of detachment, including the mesencephalic floor plate (Figs. 4 and 5). In the third ventricle the basal plate extending between the subthalamic sulcus and the dorsal border of the ventromedial hypothalamic nucleus was denuded of ependyma at E-15 (Fig. 2). In the lateral ventricles denudation took place in late gestational days and few days after birth. In all brain regions, detachment of alar plate ependymal walls was delayed as compared to that of the basal plate. Tanycytes and ependymocytes of the choroid plexus, subcommissural organ and roof plate (Fig. 5) did not detach. In hydrocephalic *hyh* embryos the process of ependymal denudation started earlier that dilatation of ventricular brain cavities. The first sign of a moderate dilatation was observed at E-15 in the third ventricle and later in the cerebral aqueduct. In contrast, in the central canal of the spinal cord denudation in basal and alar plates led to a fusion of the lateral walls (Fig. 3).

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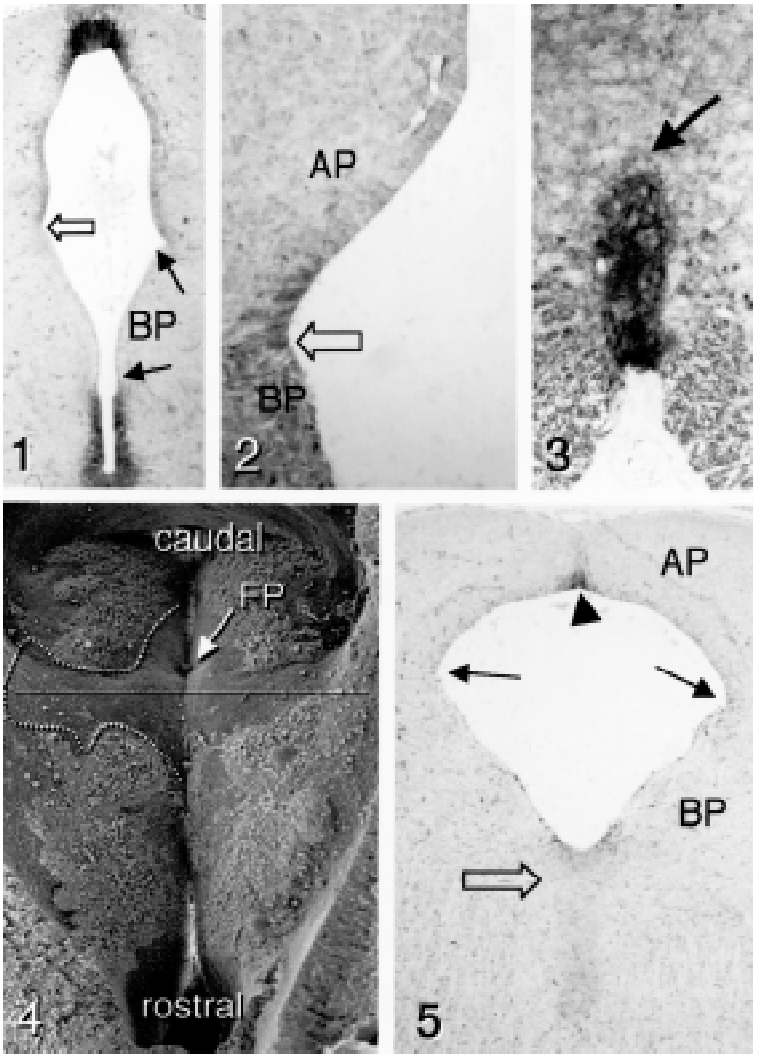

Fig. 1. Transversal section of the third ventricle in a non-mutant (control) mouse at E-15. *Anti-vimentin immunostaining. The areas of future tanycytes, ventrally, and in the subcommissural organ in the epithalamus, dorsally, present strong immunopositivity. In the basal (BP, between arrows) and alar plate, at both sides of the subthalamic sulcus (open arrow), ependymal cells display a different pattern of immunoreactivity. x40.*

Fig. 2. Transversal section of the subthalamic sulcus (open arrow) in the third ventricle. *Homozygous mutant* hyh *mouse at E-16. Anti-vimentin immunostaining. The basal plate (BP) ependymal wall is denuded in contrast of the alar plate (AP) wall. x145.*

Fig. 3. Transversal section of an E-18 hydrocephalic mouse, immunostained for NCAM, *showing a detail of an intact spinal cord floor plate. The central canal lumen lacks an alar plate and basal plate ependymal wall and is obliterated (arrow). x300.*

Fig. 4. Panoramic view using scanning electron microscopy of the cerebral aqueduct floor (floor and basal plates) in an E-15 homozygous mutant mouse. *A circumscribed area of the floor plate (FP) and in its lateral area in the basal plate (framed with a broken line) was unaffected by ependymal denudation. Symmetrical pattern of denudation at both sides of the rostro-caudal axis can be observed. A transversal section of the area crossed with a line is shown in the next figure. x90.*

Fig. 5. Transversal section of a hydrocephalic E-16 embryo at the level of the cerebral aqueduct shown with a line in Fig. 4. *Vimentin immunostaining. Arrows point to the sharp limit between the alar plate (AP) and the denuded basal plate (BP). Arrowhead indicates the strongly immunoreactive roof plate. This remnant area of the hindbrain floor plate is immunoreactive (open arrow). x40.*

Conclusions

The present results point to a key role of the ventricular lining in the aetiology of hydrocephalus developing with a normally open cerebral aqueduct. In these mutant mice the overlapped temporo-spatial patterns of ependymal differentiation and of ependymal detachment suggest that both processes are genetically driven and related. The different behaviour in the *hyh* mutant mice of the spinal cord floor plate and that of the hindbrain support the view of a functional zonation of this region of the neural tube (Rodríguez *et al.,* 1996; del Brío *et al.,* 2001).

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References

DEL BRÍO, M.A., RIERA, P., PERUZZO, B. and RODRÍGUEZ, E.M. (2001). Hindbrain floor plate of the rat. Ultrastructural changes occurring during development. *Microsc. Res. Tech.* 52: 615-626.

PÉREZ-FÍGARES, J.M., JIMÉNEZ, A.J., PÉREZ-MARTÍN, M., FERNÁNDEZ-LLEBREZ, P., CIFUENTES, M., RIERA, P., RODRÍGUEZ, S. and RODRÍGUEZ E.M. (1998). Spontaneous congenital hydrocephalus in the mutant mouse hyh. Changes in the ventricular system and the subcommissural organ. *J. Neurophathol. Exp. Neurol.* 57:188-202.

PÉREZ-FÍGARES, J.M., JIMÉNEZ, A.J. and RODRÍGUEZ, E.M. (2001). Subcommissural organ, cerebrospinal fluid circulation and hydrocephalus. *Microsc. Res. Tech.* 52: 591-607.

RODRÍGUEZ, E.M., DEL BRIO LEÓN, M.A., RIERA, P., MENÉNDEZ, J. and SCHOEBITZ, K. (1996). The floor plate of the hindbrain is a highly specialized gland. Immunocytochemical and ultrastructural characteristics. *Dev. Brain. Res.* 97: 153-168.

SARNAT, H.B. (1992). Role of human fetal ependyma. *Pediatric. Neurology* 8:163-176.

SARNAT, H.B. (1998). Histochemistry and immunocytochemistry of the developing ependyma and choroid plexus. *Microsc. Res. Tech.* 41:14-28.