Int. J. Dev. Biol. 45 (S1): S85-S86 (2001)

Short Report

An *in vitro* model to study adult neurogenesis in mammals

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ABSTRACT The wall of the lateral ventricle of bovine brains comprised three distinct layers: ependyma, subependyma (SE) and astroglial net. The wall lining white matter had narrow SE and thin, compacted astroglial net. The wall of the striatum and the anterior horn had a wide SE and a thick and slack astroglial net. The ependyma was S-100 positive and, in rostro-lateral regions, it developed basal processes and reactivity to GFAP and vimentin. The SE and the subjacent astroglial net displayed β3 tubulin small cells and some PCNA positive nuclei. These features suggested that adult neurogenesis takes place in the bovine ventricular walls specially in the striatum and the anterior horn. Explants of the ependyma-SE were cultured in serum free medium. The ependymal cells developed a net of intermingled basal processes that became increasingly GFAP, vimentin and BLBP positive. At the same time SE cells proliferated to produce a population of β3 tubulin-positive cells. These cells synthesised IGF-1 that acted as a survival factor. These explants represent good models to study adult neurogenesis.

Introduction

Neural stem cells persist in the walls of the brain ventricles and the spinal central canal of adult vertebrate central nervous system. But, only in circumscribed regions, the newly generated cells migrate and become functionally active neurons. In these regions it should exist a propitious environment for cell survival, migration and differentiation (Scheffler *et al.,* 1999). In rodents, neural stems have characteristics of ependymal cells (Johansson *et al.,* 1999) and/or SE astrocytes (Doetsch *et al.* 1999). Here a rostral migratory stream of chains of newly formed neurons move through an astroglial net toward the olfactory bulb where they integrate as granular interneurons (Lois *et al.*, 1996)). Experimentally, rodent adult neurogenesis has been shown to be increased by EGF, FGF and αTGF (Craig *et al.*, 1996). Neural stem cells have been reported to exist in other mammals and in humans. However there are not appropriate knowledge of the architecture of the germinative regions in mammals other than rodents. Here we report the histology and immunocytochemical characteristics of the wall of the lateral ventricle of a large mammal, the bovine. By dissecting and culture the ependyma and a part of the SE we developed an *in vitro* model system to study neurogenesis.

Materials and Methods

Bovine brains were obtained from a local slaughterhouse, sliced and put in Bouin fixative. Pieces containing the wall of the lateral ventricles of different regions were dissected, embedded in paraffin, cut and processed for PAP immunocytochemistry. For explants, brains were put in Hank´s solution at 4°C, and the wall of the lateral ventricles exposed. The ependyma was separated and thin sheets were put in fresh DMEM medium without serum or growth factors. At different culture times, explants fixed in Bouin and processed for immunocytochemistry. In some wells BrdU were added. We used primary antibodies against the following substances: S-100 protein, vimentin, glial fibrillary acidic protein (GFAP), brain lipid binding protein (BLBP), β3 tubulin, nestin, proliferating cell nuclear antigen (PCNA) and BrdU.

Results and Discussion

Bovine lateral ventricle displayed anterior, inferior and posterior horns and a central body. The anterior horn ended close to the olfactory bulb. The wall lining white matter, such as the corpus callosum, fornix and septum, corresponding to the dorsomedial walls of the central body and of the posterior horn, displayed similar features as did the walls lining the striatum and the rostral region of the anterior horn. In general, three layers could be distinguished in the ventricular wall: a ciliated cubic ependyma, a SE and a subjacent astroglial layer (Fig. 1a). The structural and immunocytochemical characteristics of these layers varied depending on the region studied. Those lining white matter showed a narrow SE and a thin, compacted astroglial net. At variance, the wall of the striatum and the anterior horn displayed a wide SE and a thick and slack astroglial net. The ependyma was ciliated, cubic, with short basal processes and S-100 (Fig. 1b) and nestin positivity. In the striatum, the basal processes of the ependymal cells were more evident and they became increasingly longer and GFAP, vimentin and BLBP positive in the rostral horn**.** The SE displayed heterogeneous population of cells. Many of them were positive to anti-β3 tubulin (Fig. 1d) and were numerous in the walls of the striatum and the anterior horn. In the SE of all lateral ventricle regions, but especially in the striatum and the anterior horn, PCNA-positive nuclei were found (Fig. 1e). The subjacent astroglial layer was GFAP (Fig. 1c) and vimentin positive. In the striatum, this layer is a thick astroglial net containing bipolar β3 tubulin positive cells. In the cephalic end of the rostral horn, a SE is virtually absent. Here, the ependyma displayed the longest basal processes that penetrated a wide net of GFAP positive fibers intermingled with β3 tubulin positive cells. This net extended ventrally toward the olfactory bulb. According to this description, we think that in the adult bovine lateral ventricles, neurogenesis takes place especially in the walls covering the striatum and the rostral horn. We hypothesise that subventricular

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migratory streams of newly formed neurons around the bovine lateral ventricle wall go toward the olfactory bulb. In preliminary unpublished tracing *in vitro* studies, we have detected small bipolar subventricular cells that appears to migrate along established routes through the subventricular astrocytic layer. The system in bovine appears to be quite more extended that the one described in rodents.

For methodological reasons, we have made explants from the walls of the corpus callosum, and have studied them from 4h to 60 days of culture (Pérez-Martín *et al.*, 2000). Explants did not adhere to the culture dish and formed irregular-shaped structures floating in the culture medium. Recently dissected explants consisted of a sheet of S-100 positive ependymal cells (Fig. 1 f,g) and SE cells, being some of them β3 tubulin-positive. The subjacent glial layer was always absent in explants. With time, explants suffered conspicuous morphological changes. The ependymal cells developed long basal processes that formed a true net and became highly positive to GFAP (Fig. 1j), vimentin and BLBP. Apart from the ependymal cells no other GFAP positive cells have been found in explants. Meanwhile, large cells among the basal processes of the ependymal cells proliferated and gave rise to a population of cells than tended to occupy the centre of the explant (Fig. 1h) but that, with time, all of them leave the explant and invade the culture dish. The great majority of these cells were β3 tubulin positive (Fig. 1i). Accordingly, PCNA positive nuclei were frequently found among the basal processes of the ependymal cells and some of them were seen very close to the ependymal perikarya. Double immunocytochemistry of explants treated with BrdU have demonstrated that virtually all cells produced contained β3 tubulin. Thus it is concluded that in the explants an intense neurogenesis takes place. This means that explants contain cells endowed of proliferative potentiality to generate neurons and all requirements to sustain this activity in the absence of serum or growth factor supplement in the culture medium.

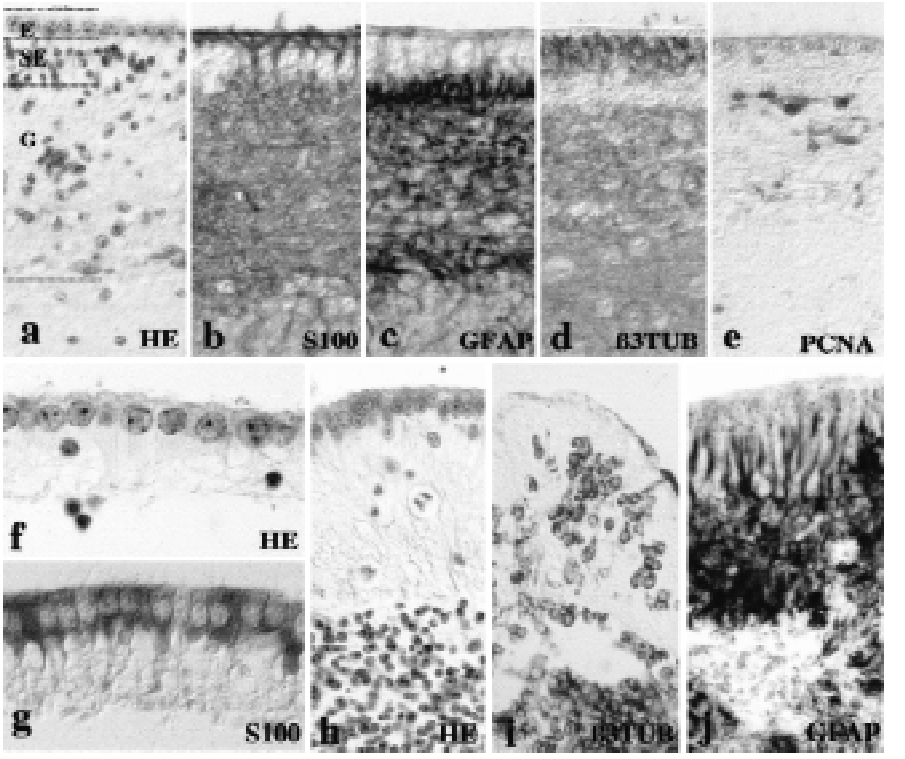

Fig. 1. (a-e) *Wall of the striatum stained with hematoxilyn-eosin and with different antisera. E, ependyma, SE, subependyma, G, glial layer. X 75.* **(f,g)** O*ne day explants, x200.* **(h-j)** *One week explants, x 80.*

Conclusions

From the foregoing, we think that our model system is a good paradigm to study adult neurogenesis. Even more, we are convinced that the system in humans should be more similar to bovines than to rats. Two important tasks should be undertaken, one is to identify and characterise stem cells and the other to study the molecules and mechanisms involved in the control of their activity. Regarding the later, we have evidences that the β3 tubulin newly formed cells produce IGF-1 and that it acts as a survival factor. The identification of neural stem cells in adults and, specially, the knowing of their control will make possible the design of new therapeutical approaches to neurodegenerative disorders.

This work has been supported by grants FIS 01-0949 and DGICYT BFI 2000-1360.

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