Neuroscience 132 (2005) 777–788

THE DISTRIBUTION OF PROGENITOR CELLS IN THE SUBEPENDYMAL LAYER OF THE LATERAL VENTRICLE IN THE NORMAL AND HUNTINGTON'S DISEASE HUMAN BRAIN

M. A. CURTIS,[a,b] E. B. PENNEY,[a] J. PEARSON,[c] M. DRAGUNOW,[b] B. CONNOR[b] AND R. L. M. FAULL[a]*

[a]*Department of Anatomy with Radiology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

[b]*Department of Pharmacology and Clinical Pharmacology, The University of Auckland, Auckland, New Zealand*

[c]*Department of Statistics, The University of Auckland, Auckland, New Zealand*

Abstract—The recent demonstration of endogenous stem/progenitor cells in the adult mammalian brain raises the exciting possibility that these undifferentiated cells may be able to generate new neurons for cell replacement in neurodegenerative diseases such as Huntington's disease (HD). Previous studies have shown that neural stem cells in the rodent brain subependymal layer (SEL), adjacent to the caudate nucleus, proliferate and differentiate into neurons and glial cells and that neurogenesis occurs in the hippocampus and the SEL of the caudate nucleus in the adult human brain, but no previous study has shown the extent to which progenitor cells are found in the SEL in the normal and diseased human brain with respect to location. From detailed serial section studies we have shown that overall, there is a 2.7-fold increase in the number of proliferating cell nuclear antigen positive cells in HD (grade 2/3); most notably, the ventral and central regions of the SEL adjacent to the caudate nucleus contained the highest number of proliferating cells and in all areas and regions examined there were more cells in the HD SEL compared with the normal brain. Furthermore, progenitor cells colocalized with βIII tubulin in a subset of cells in the SEL indicating neurogenesis in the HD brain. There was a 2.6-fold increase in the number of new neurons that were produced in the Huntington's disease SEL compared with the normal SEL; however, the Huntington's disease SEL had many more proliferating progenitor cells; thus, the proportion of new neuron production relative to the number of progenitor cells was approximately the same. This study provides new evidence of the pattern of neurogenesis in the normal and HD brain.

Key words: neurogenesis, caudate nucleus, subventricular zone, proliferating cell nuclear antigen, cell replacement therapy.

A particularly exciting and novel development in the treatment of neurodegenerative diseases is the demonstration from both animal and human studies that the endogenous progenitor cells may offer a potential treatment strategy for neurodegenerative disorders such as Parkinson's disease, Huntington's disease and Alzheimer's disease (Craig et al., 1996; Luskin et al., 1997; Svendsen et al., 1997; Gage et al., 1998; Nait-Oumesmar et al., 1999; Svendsen and Smith, 1999). Thus, the demonstration of endogenous stem/progenitor cells in the hippocampus and the subependymal layer (SEL) overlying the caudate nucleus in the adult mammalian brain has raised the exciting possibility that these undifferentiated cells may be able to generate new neurons for cell replacement in neurodegenerative diseases such as Huntington's disease (HD; Eriksson et al., 1998; Curtis et al., 2003). Indeed, neural stem cells in the rodent brain SEL adjacent to the caudate nucleus have recently been shown to proliferate and differentiate into striatal neurons suggesting they may provide a source of replacement neurons (Morshead et al., 1994; Arvidsson et al., 2002; Parent et al., 2002; Tattersfield et al., 2004). In this regard it is especially interesting that recent studies on the normal adult human brain have shown evidence of neurogenesis in the hippocampus, in their study 2-bromodeoxyuridine (BrdU; a thymidine analog) was used to detect dividing cells that could subsequently be labeled with neuronal or glial markers to determine the phenotype of the labeled cell (Eriksson et al., 1998). We have recently demonstrated cell proliferation and neurogenesis in the normal brain and also increased cell proliferation in the HD SEL adjacent to the caudate nucleus (Connor et al., 2001; Curtis et al., 2003). In these studies proliferating cell nuclear antigen (PCNA), a cell cycle marker, was detected co-localized with either neuronal or glial cell markers in the SEL in the normal and HD brain SEL.

Cytoarchitectural studies of the mammalian SEL of the lateral ventricle have demonstrated that the SEL is a heterogeneous layer comprised of neuroblasts, glial cells, precursor cells and a minority of other cell types. In particular, Doetsch and colleagues (1997) demonstrated that in the normal mouse brain there was a 3:2:1 ratio of migrating neuroblasts (type A cells):glial cells (type B cells):precursor cells (type C cells). However, they did not study the localization of these cells throughout the wall of the lateral ventricle. In the bovine brain, Rodríguez-Pérez and colleagues (2003) undertook a very detailed regional analysis of the SEL by dividing the lateral ventricle into a rostral horn, central body, temporal (inferior) horn and occipital horn. The wall of the lateral ventricle was characterized by dividing the lateral ventricle into different wall types based on the presence, and orientation of the different cell types

*Corresponding author. Tel: +64-9-373-7599; fax: +64-9-373-7484.
E-mail address: rlm.faull@auckland.ac.nz (R. L. M. Faull).
Abbreviations: BrdU, 2-bromodeoxyuridine; EPL, ependymal layer; HD, Huntington's disease; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; SEL, subependymal layer.

0306-4522/05$30.00+0.00 © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2004.12.051

present. Type 1 ventricle wall consisted mostly of astrocytes and a few subependymal neuroblasts; very few cells were PCNA positive. Type 1 ventricle wall was located on the upper and medial surfaces of the lateral ventricle in the rostral half of the lateral ventricle wall and the entire caudal half of the lateral ventricle was made up of the type 1 ventricle wall architecture (Rodríguez-Pérez et al., 2003). Type 2 ventricle wall was composed of subependymal neuroblasts and PCNA positive cells in the region close to the ependymal layer (EPL). Further away from the EPL there were many astrocytes that were interspaced with subependymal neuroblasts and PCNA positive cells. Type 2 ventricle wall was located on the rostral half of the ventricle, but only on the lateral aspect of the ventricle wall overlying the caudate nucleus (Rodríguez-Pérez et al., 2003). Type 3 ventricle wall was composed of radially oriented astrocytes and subependymal neuroblasts; close to the EPL were PCNA positive cells and subependymal neuroblasts. The location of type 3 ventricle wall was restricted to a small segment of the rostro-ventral SEL in an area equivalent to the beginning of the rostral migratory stream described in rodents (Lois et al., 1996; Doetsch et al., 1997).

The area of particular interest in this paper is the type 2 region which overlies the caudate nucleus which is the brain region most affected in HD. The human SEL and ventricle wall has not been characterized as well as has the bovine and rodent brain. However, a recent study has demonstrated some characteristics of the human SEL. Bernier and colleagues (2000) reported the presence of the neuroepithelial marker 'nestin' throughout the SEL and showed it to stain four major cell types identified in the rodent SEL by Doetsch et al. (1997). Nestin was used as a general marker of the SEL and with this marker variations in the thickness of the human SEL were noted from ventral to dorsal. The thickest part of the SEL was at the level of the anterior commissure and the ventral striatum (Bernier et al., 2000). Some of the nestin staining was also present in the temporal and occipital horns of the lateral ventricle. Bernier and colleagues (2000) also demonstrated immunoreactivity for βIII-tubulin, GFAP, PSA-NCAM and PCNA; however the localization of these cell types throughout the lateral ventricles was not detailed. Thus, the detailed distribution of proliferating cells throughout the lateral ventricle has not been reported. Although, the presence of cells that proliferate in the SEL has been previously demonstrated in the human brain (Eriksson et al., 1998; Bernier et al., 2000; Curtis et al., 2003), their distribution in the normal brain and their presence and distribution in the HD brain has not been previously studied.

The present study was aimed at mapping the location of PCNA positive cells in the lateral ventricle that overlies caudate nucleus. This is the first study looking at the localization and number of proliferating cells in the normal and HD brain SEL. The first part of this study will demonstrate the ventral–dorsal distribution of PCNA positive cells followed by the rostral–caudal localization; the results from the two parts have then been combined to give a three-dimensional demonstration of the distribution of PCNA positive cells in the SEL of the lateral ventricle overlying the caudate nucleus. Furthermore, the present study quantitatively investigates the number of proliferating cells that co-label with neuronal markers demonstrating neurogenesis in the normal and HD SEL.

Table 1. Table of normal and Huntington's disease cases examined for the distribution of PCNA positive cells in the SEL

Case number	Age	Sex	Postmortem delay (h)	CAG repeats	HD grade
N1	69	Female	11.5	Unavailable	Normal
N2	70	Male	16	Unavailable	Normal
N3	87	Female	11	Unavailable	Normal
HC1	66	Female	12	21/39	2
HC2	47	Male	24	17/50	3
HC3	65	Male	10	17/42	3

EXPERIMENTAL PROCEDURES

Human tissue collection

For this study the basal ganglia from six postmortem human brains were obtained from the Neurological Foundation of New Zealand Human Brain Bank in the Department of Anatomy with Radiology, University of Auckland, Auckland, New Zealand (see Table 1). The full consent of all families was obtained at the time of autopsy and the University of Auckland Human Subjects Ethics Committee approved the protocols used in these studies. Three normal brains were received from cases with no history of neurological disease and on pathological examination showed no neurological abnormalities (average age 75.3 years; average postmortem delay 12.8 h). Three HD brains were received from cases with a family and clinical history of HD. The diagnosis of HD was confirmed by genetic analysis of the CAG repeat length in both alleles of the IT15 gene (average age 59.3 years; average postmortem delay 15.3 h). The HD brains used were grades 2 and 3 because they best represent the average presentation of HD cases at postmortem.

For the immunohistochemistry studies, the brains were fixed by perfusion through the basilar and internal carotid arteries. Initially the brains were perfused with phosphate-buffered saline (PBS) with 1% sodium nitrite to clear the brain's circulation, followed by 15% formalin in 0.1 M phosphate buffer pH 7.4. Following the perfusion, the brains were dissected into regions and the basal ganglia were removed intact and post-fixed in the same fixative for 24 h. Subsequently these blocks were cryo-protected in 20% sucrose in 0.1 M phosphate buffer with 0.1% sodium-azide for 4–5 days, and then in 30% sucrose in 0.1 M phosphate buffer with 0.1% sodium-azide for a further 4–5 days. The basal ganglia blocks were then sectioned on a freezing microtome (HM 440, Zeiss, Walldorf, Germany) at a thickness of 50 μm and the sections were collected in PBS and 0.1% sodium-azide and stored in individual wells at 4 °C for further immunohistochemical, histochemical or immunofluorescent processing.

Immunohistochemistry

Section preparation. The basal ganglia sections were taken from the rostral pole of the caudate nucleus to the caudal part of the body of the caudate nucleus; approximately 600 sections were cut at a thickness of 50 μm on a freezing sledge microtome (Zeiss HM440) from each basal ganglia specimen. In order to preserve the exact serial order of the sections, each section was stored in an individual well in a solution of PBS and 0.1% sodium-azide. A