Neuroscience 280 (2014) 99–110

INTRAVENTRICULAR INJECTION OF 6-HYDROXYDOPAMINE RESULTS IN AN INCREASED NUMBER OF TYROSINE HYDROXYLASE IMMUNE-POSITIVE CELLS IN THE RAT CORTEX

B. WACHTER, [b†‡] **S. CARADONNA,** [a,b*†] **K. GITTINGER,** [a] **A. SCHLÄGER** [a] **AND E. KÜPPERS** [a]

[a] *Institute of Neuroanatomy, University of Tübingen, Österbergstrasse 3, 72074 Tübingen, Germany*

[b] *Graduate School of Cellular & Molecular Neuroscience, University of Tübingen, Österbergstrasse 3, 72047 Tübingen, Germany*

Abstract—Previously we have demonstrated that intraventricular injection of 6-hydroxydopamine (6-OHDA) results in increased proliferation and de-differentiation of rat cortical astrocytes into progenitor-like cells 4 days after lesion (Wachter et al., 2010). To find out if these cells express tyrosine hydroxylase (TH), the rate-limiting enzyme in the catecholamine synthesis pathway, we performed immunohistochemistry in the rat cortex following intraventricular injection of 6-OHDA. Four days after injection we demonstrated a strong emergence of TH-positive (TH^+) somata in the cortices of 6-OHDA-lesioned animals. The number of TH^+ cells in the cortex of 6-OHDA-lesioned animals was 15 times higher than in sham-operated animals, where virtually no TH^+ somata occurred. Combining TH immunohistochemistry with classical Nissl stain yielded complete congruency, and ~45% of the TH^+ cells co-expressed calretinin, which indicates an interneuron affiliation. There was no co-staining of TH with other interneuron markers or with glial markers such as glial fibrillary acidic protein (GFAP) or the neural stem/progenitor marker Nestin, nor could we find co-localization with the proliferation marker Ki67. However, we found a co-localization of TH with glial progenitor cell markers (Sox2 and S100β) and with polysialylated-neural cell adhesion molecule (PSA-NCAM), which has been shown to be expressed in immature, but not recently generated cortical neurons. Taken together, this study seems to confirm our previous findings with respect to a 6-OHDA-induced expression of neuronal precursor markers in cells of the rat cortex, although the TH^+ cells found in this study are not identical with the potentially de-differentiated astrocytes described recently (Wachter et al., 2010). The detection of cortical cells expressing the catecholaminergic key enzyme TH might indicate a possible compensatory role of these cells in a dopamine-(DA)-depleted system. Future studies are needed to determine whether the TH^+ cells are capable of DA synthesis to confirm this hypothesis.

Key words: Parkinson's model, dopamine, astrocytes, progenitor-like cells, immunohistochemistry.

INTRODUCTION

6-Hydroxydopamine (6-OHDA) is a catecholamine-selective neurotoxin that destroys dopaminergic neurons and is commonly used to mimic Parkinson's disease in animal models (Schober, 2004). Dopaminergic neurons in the mammalian brain are mainly located in the midbrain, diencephalon, retina and the olfactory bulb and can be characterized by the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in the catecholamine synthesis pathway (Zeiss, 2005; Bjorklund and Dunnett, 2007). In addition, immunohistochemical studies have provided evidence for TH^+ cells also in the cortical and subcortical telencephalon (Smeets and Gonzalez, 2000). In the striatum of rodents (Meredith et al., 1999; Darmopil et al., 2008) and non-human primates (Betarbet et al., 1997; Mazloom and Smith, 2006; Tande et al., 2006; San Sebastian et al., 2007; DiCaudo et al., 2012) the dopaminergic denervation by either 6-OHDA or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) results in an increase in the number of TH^+ somata, suggesting that either dopamine (DA) itself or other factors released from dopaminergic fibers limit the number of intrinsic TH^+ cells (Huot et al., 2007). Interventions that depleted endogenous DA without affecting the anatomy of the nigrostriatal pathway by using systemic application of alpha-methyl-p-tyrosine (αMpT), an inhibitor of catecholamine synthesis, confirmed a negative regulation of the number of intrinsic TH^+ cells by DA in the striatum (Busceti et al., 2012).

To our knowledge only sparse data (Yan et al., 2001) concerning intrinsic TH^+ cells have been obtained from cortical areas in DA-depletion models so far. Since

*Correspondence to: S. Caradonna, Institute of Neuroanatomy, University of Tübingen, Österbergstrasse 3, 72074 Tübingen, Germany. Tel: +49-7071-29-78243; fax: +49-7071-29-4014.
E-mail address: sandro.caradonna@uni-tuebingen.de (S. Caradonna).
† Authors contributed equally.
‡ Present address: Institute of Clinical Neurobiology, University Hospital Würzburg, Versbacherstrasse 5, 97087 Würzburg, Germany.
Abbreviations: 6-OHDA, 6-hydroxydopamine; CB, calbindin; CNS, central nervous system; CR, calretinin; DA, dopamine; DAB, 3,3′-Diaminobenzidine; GABA, γ-amino-butyric acid; Gad67, glutamic acid decarboxylase 67; GFAP, glial fibrillary acidic protein; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NPY, neuropeptide Y; PSA-NCAM, polysialylated form of the neural cell adhesion molecule; PV, parvalbumin; ROI, region of interest; SST, somatostatin; SVZ, subventricular zone; TH, tyrosine hydroxylase; VIP, vasoactive intestinal peptide.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.010
0306-4522/

we recently demonstrated (Wachter et al., 2010) a de-differentiation of resident mature astrocytes in the parietal cortex into progenitor-like cells 4 days after intraventricular 6-OHDA injection, we were interested, if these cells have a dopaminergic character. Therefore we studied the expression of TH in the rat parieto-temporal cortex cells 4 days after intraventricular injection of 6-OHDA.

EXPERIMENTAL PROCEDURES

Animals and 6-OHDA lesion

All experiments were performed in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the Regierungspräsidium Tübingen (ZV2/09, AT2/12). Adequate measures were taken to minimize pain and discomfort of animals. Fourteen adult male Sprague–Dawley rats (Charles River, Germany) weighing 240–310 g, underwent a stereotactic surgery as described recently (Wachter et al., 2010).

To minimize the number of animals in animal testing, we shared the brain of six rats (three 6-OHDA-lesioned animals and three sham-operated animals) from a parallel study that performed immunohistochemical analyses of the cerebellum 4 days after lesion. Briefly, 30 min before surgery these rats received 30-mg/kg nortriptyline and eight rats (four sham and four 6OHDA-lesioned) received 20-mg/kg desipramine intraperitoneally to prevent degeneration of noradrenergic neurons. Following that pre-treatment, seven animals received bilaterally an intraventricular injection (AP: −0.7; L: ±1.3 and DV: −3.6) according to (Paxinos and Watson, 2007) of 105-μg 6-OHDA (6-OHDA Hydrobromide, Sigma) dissolved in 10 μl 0.1% ascorbic acid solution (Sigma) and seven animals received only 10 μl 0.1% ascorbic acid (sham animals). Two animals died during the surgery.

Tissue processing

At 4 days post-surgery, the rats were sacrificed, transcardially perfused and the brain was cryoprotected as described recently (Wachter et al., 2010). Coronal sections (20 μm) at the level of the anterior commissure (Bregma −0.26 up to −1.4) were prepared with a cryostat (HM560, Micron, Boise, ID, USA).

Biotinylated tyramide immunohistochemistry and Nissl staining

Tyramide signal amplification of TH immunohistochemistry was performed according to (Freedman and Maddox, 2001) as described recently (Wachter et al., 2010). In brief, frozen sections were incubated with anti-TH (1:1000; mouse monoclonal, ImmunoStar, Hudson, NY, USA) overnight at 4 °C. The next day, the sections were subsequently incubated with biotinylated goat-anti-mouse IgG (1:100; Sigma), avidin–biotin peroxidise complex solution (ABC-Kit, Vectastain, Burlingame, CA, USA), biotinylated tyramide (1:50; Molecular Probes, Darmstadt, Germany) and ABC solution before the antibody was visualized with DAB (3,3′-Diaminobenzidine; Serva, Heidelberg, Germany). Sections were dehydrated and mounted in DePex (Serva, Heidelberg, Germany).

For the Nissl staining the frozen sections were treated according to a slightly modified protocol as described in (Powers and Clark, 1955). In brief, frozen sections were shortly rinsed with distilled water and incubated for 30 min in buffered cresyl violet acetate (pH 3.9) (Sigma). Sections were washed in distilled water, air-dried, cleared in xylol (Merck, Darmstadt, Germany) for 5 min and mounted in DePeX (Serva, Heidelberg, Germany).

For the combined staining of Nissl with TH (tyramide signal amplification) the Nissl protocol was added at the end of the TH staining protocol before the sections were mounted in DePeX. TH staining with tyramide signal amplification and/or Nissl staining was investigated with a Zeiss Axioplan2.

For the combined Green CellTracker™ (Molecular Probes) and 6-OHDA injection, equimolar solutions were mixed to a final volume of 10 μl and injected according to the above-mentioned details.

Immunohistochemistry

The following antibodies were used: calbindin (CB) (1:200, mouse monoclonal, Swant, Marly, Switzerland), calretinin (CR) (1:2000, rabbit polyclonal, Millipore, CR (1:1000, mouse monoclonal, Millipore), c-Fos (1:500, rabbit polyclonal, Santa Cruz), glutamic acid decarboxylase 67 (Gad67) (1:1000, mouse monoclonal, Chemicon), glial fibrillary acidic protein (GFAP) (1:400, rabbit polyclonal, Dako, Glostrup, Denmark), Ki-67 (1:50, mouse monoclonal, Dako, Glostrup, Denmark), nestin (1:100, mouse monoclonal, Millipore), neuropeptide Y (NPY) (1:1000, rabbit polyclonal, Chemicon), parvalbumin (PV) (1:1000, mouse monoclonal, Sigma), Pax6 (1:800, rabbit polyclonal, MBL, Woburn, MA, USA), polysialylated-neural cell adhesion molecule (PSA-NCAM) (1:200, mouse monoclonal, Millipore), somatostatin (SST) (1:1000, rabbit polyclonal, ImmunoStar, Hudson, NY, USA), Sox2 (1:50, rabbit polyclonal, Santa Cruz), S100β (1:3000, rabbit polyclonal, Abcam), TH (1:1000, mouse monoclonal, ImmunoStar, Hudson, NY, USA), TH (1:1000, rabbit polyclonal, Abcam) and vasoactive intestinal peptide (VIP) (1:500, rabbit polyclonal, Chemicon). Frozen sections were post-fixed in an ice-cold chloroform-acetone solution (1:1) (both Merck, Darmstadt, Germany) and washed in 10 mM PBS (pH 7.4). Sections were incubated in pre-incubation buffer (5% normal goat serum (Dako, Glostrup, Denmark), 0.5% Triton X-100 (Sigma), 1% bovine serum albumin (Sigma) in PBS for 30 min at RT, before the primary antibody diluted in antibody dilution buffer (5% normal goat serum, 0.5% Triton X-100 in PBS) was added and incubated overnight at 4 °C. The next day sections were washed with PBS, and incubated with the appropriate secondary antibody (anti-rabbit Alexa Fluor 488 nm, anti-mouse Alexa Fluor 488 nm, anti-rabbit Alexa 546 nm, anti-mouse Alexa Fluor 546 nm, 1:400, Molecular Probes) diluted in antibody dilution buffer for 90 min at RT. Subsequently the sections were washed in PBS and if a cell nucleus staining was required the sections were incubated in either Sytox green (1:5000 in PBS,