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Research Report

Natural and lesion-induced apoptosis in the rat striatum during development

K. Mellios[a], T. Zacharaki[a], S. Sophou[a], M. Latsari[a], J. Antonopoulos[a], A. Dinopoulos[a], J.G. Parnavelas[b], I. Dori[a,*]

[a]Department of Anatomy Histology and Embryology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

[b]Department of Anatomy and Developmental Biology, University College London, London WC1E 6BT, UK

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ABSTRACT

We evaluated the pattern of apoptosis in the rat striatum during normal development and in two models of lesion-induced cell death. Lesions included i) unilateral ablations of the cerebral cortex at different postnatal ages, and ii) early postnatal lesions of the catecholaminergic afferent systems of the striatum with 6-hydroxydopamine (6-OHDA). Dying cells were identified as apoptotic using the TUNEL (terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick end labeling) method at the light and electron microscopic levels. Moreover, we used immunohistochemistry for the apoptotic markers active caspase-3 and fractin. TUNEL+ cells were present in the striatum during the first four postnatal weeks. Their frequency was high during the first postnatal week and peaked at postnatal day (P)5. Cortical lesions at birth, in contrast to those performed at later stages, induced a significant increase in the frequency of TUNEL+ cells in the ipsilateral striatum, which peaked at seven days postlesion. 6-OHDA lesions resulted in a similar and significant increase in the frequency of TUNEL+ cells in the striatum, which also peaked at P7. We also showed that cortical lesions at P0 and 6-OHDA lesions resulted in a reduction in the frequency, as well as in alterations of the morphology of γ-aminobutyric acid (GABA)-immunoreactive (ir) neurons in the striatum. We suggest that: i) apoptosis in the striatum is temporally coordinated with maturation events in this area and ii) early developmental lesions of major afferent pathways to the striatum affect both the survival and phenotype of striatal neurons.

* Corresponding author. Fax: +30 2310999842.
E-mail address: idori@vet.auth.gr (I. Dori).

Abbreviations: ABC, avidin–biotin peroxidase complex; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; E, embryonic day; GABA, γ-aminobutyric acid; HD, Huntington's disease; ir, immunoreactive; MSNs, medium spiny neurons; NGS, normal goat serum; NT-3, neurotrophin-3; 6-OHDA, 6-hydroxydopamine; P, postnatal day; PB, 0.1 M phosphate buffer; PBS, phosphate-buffered saline; PD, Parkinson's disease; PLD, postlesion day; TB, 0.1 M Tris buffer; TdT, terminal transferase; TH, tyrosine hydroxylase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling

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1. Introduction

Apoptosis is a type of programmed cell death that plays a key role in the control of cell numbers and the establishment of neuronal circuitry during brain development (Clarke, 1985; Oppenheim, 1991). It is regulated by the competition of neurons for trophic support provided either by target areas or by their afferent inputs (Linden, 1994; von Bartheld et al., 2001). Some studies suggest that apoptosis is also involved in the pathogenesis of acute and chronic neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) disease (Barinaga, 1998; Burke and Kholodilov, 1998; Ekshyyan and Aw, 2004). This type of cell death is characterized by a cascade of morphological and biochemical events resulting from the activation of a family of cysteine proteases, the caspases, irrespective of the death-inducing stimulus (Hengartner, 2000; Becker and Bonni, 2004). Activation of caspase-3 is a hallmark of neuronal apoptosis. It has been shown that caspase-3 is activated during brain development (Jeon et al., 1999; Sophou et al., 2006), in lesion-induced apoptotic cell death (Clark et al., 2000; Oo et al., 2002), as well as in neurodegenerative diseases (Hartmann et al., 2000). Fractin, a fragment produced by caspase-3 cleavage of actin during the late stages of apoptosis (Suurmeijer et al., 1999), has also been localized in apoptotic cells during developmental or experimentally-induced cell death (Jackson-Lewis et al., 2000; Oo et al., 2002).

The striatum is the principal input component of the basal ganglia, a set of subcortical structures that is connected to the cerebral cortex and thalamus by a closed neuronal loop (Levy et al., 1997; Mello and Villares, 1997). The striatum (or neostriatum) comprises the caudate nucleus and the putamen and is involved in the control of motor, cognitive and limbic functions. These functions are modulated by glutamatergic afferents from all areas of the cerebral cortex and the thalamus and catecholaminergic inputs from the midbrain (Parent and Hazrati, 1995; Graybiel, 2005). On the basis of differences in connectivity, neurochemistry, molecular identity and neuronal birthdates, the striatum is organized in compartments called patches and matrix (Gerfen, 1984; Gerfen et al., 1985; van der Kooy and Fishell, 1987). Medium spiny neurons (MSNs), which comprise approximately 90% of the striatal neurons, are the main projection neurons of the striatum and use γ-aminobutyric acid (GABA) as their main neurotransmitter and a variety of neuropeptides (for review see Tepper et al., 2007). These neurons are also the major targets of striatal afferents. MSNs receive asymmetrical synapses on the head of dendritic spines from fibers originating in nearly all areas of the neocortex, and symmetrical synapses on the neck of spines from dopaminergic nigrostriatal inputs (Somogyi et al., 1981; Bouyer et al., 1984). The remaining 10% of striatal neurons are interneurons, which comprise the GABAergic medium aspiny neurons and the cholinergic aspiny neurons (Tepper et al., 2004). Interneurons also receive inputs from the cerebral cortex and, in turn, their axons make synapses with MSNs (Bolam et al., 2000).

Neurogenesis in the rat striatum begins on embryonic day (E) 12 and continues throughout embryonic life (Lauder et al., 1986). Anatomical and electrophysiological investigations have suggested that striatal neurons, particularly the MSNs, undergo gradual maturation during the first postnatal month (Tepper et al., 1998). Axonal tracing studies have shown that cortical and midbrain catecholaminergic afferents are present in the striatum by birth, and become organized into mature networks during the following postnatal weeks (Graybiel, 1984; Kalsbeek et al., 1992; Voorn et al., 1988; Christensen et al., 1999). Brain-derived neurotrophic factor (BDNF) is anterogradely transported to the striatum by cortical and catecholaminergic afferents (Altar et al., 1997; Fawcett et al., 2000; von Bartheld et al., 2001). Neurotrophin-3 (NT-3) is transiently expressed in the cerebral cortex during development and is transported anterogradely by corticostriatal fibers (Friedman et al., 1991a). Factors controlling cell survival and maintenance in the striatum are of particular interest since primary cell loss occurs in this brain region in HD and PD (Hickey and Chesselet, 2003; Honig and Rosenberg, 2000; Nagatsu and Sawada, 2007).

In the present study we used the TUNEL method in conjunction with morphological analysis at the light and electron microscopic levels, and immunohistochemistry for active caspase-3 and fractin, aiming to investigate and quantify apoptotic cell death in the rat striatum during normal development. We also employed two models of induced cell death in the developing striatum to study the influence of lesions of afferent connections on the survival and phenotypic differentiation of striatal neurons. For this purpose we evaluated apoptotic cell death in the striatum at various developmental stages following i) unilateral ablations of the cerebral cortex at birth, P7 and P14 and ii) lesions of the catecholaminergic afferent systems of the striatum with 6-OHDA during the first days of postnatal development. In addition, by using immunohistochemistry for GABA we sought to examine the effect of these lesions on the survival and phenotype of the majority of striatal neurons.

2. Results

2.1. *Naturally occurring apoptosis in the striatum during development*

Dying cells, showing DNA fragmentation, were identified and quantified in paraffin sections, using the TUNEL method. A large number of TUNEL+ cells, first screened in semithin sections, were further examined with the electron microscope and their apoptotic morphology was confirmed. Moreover, the expression of the specific apoptotic markers active caspase-3 and fractin was examined at various time points.

TUNEL+ cells in the developing striatum, examined with the light microscope, were intensely and selectively labeled, among numerous toluidine blue-stained "live" cells (Figs. 1A, B). Dying cells, although sometimes appeared clustered, were distributed throughout the striatum at all ages examined, with no evidence of preferential localization. Labeled cells consistently displayed apoptotic morphological features (Clarke, 1990, 1999). Their pyknotic nuclei were intact or fragmented and often showed membrane blebbing. The reaction product formed a crescent or had a homogenous nuclear dispersion (Fig. 2). The cytoplasm was confined to a shrunken, thin rim