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POSTNATAL NEURONAL APOPTOSIS IN THE CEREBRAL CORTEX: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL MECHANISMS

M. NIKOLIĆ, [a] **H. A. R. GARDNER** [b] **AND K. L. TUCKER** [a]*

[a] *Interdisciplinary Center for Neurosciences, Department of Anatomy and Cell Biology, University of Heidelberg, 69120 Heidelberg, Germany*

[b] *AstraZeneca, 35 Gatehouse Drive, Waltham, MA 02451, USA*

Abstract—In the first week of postnatal life of all examined mammalian species, there is a wave of apoptosis in the cerebral cortex, accounting for a loss of up to 30% of neuronal content from birth to adulthood. In this review we examine recent advances in the understanding of this curious phenomenon. We survey the phenomenological literature and elaborate a putative relationship between the formation of active neuronal networks and selective apoptosis of non-participatory neurons. The underlying reason for this apoptotic wave remains unclear, but molecular mechanisms are starting to be elucidated that account for its mechanism, including a role for insulin-like growth factor I (IGF-1) and the Rho GTPases RhoA and RhoB. In addition, we discuss pathophysiological situations in which a variety of common drugs used either recreationally or for medical purposes, or pharmacological blockade of *N*-methyl-D-aspartate receptor (NMDAR) function, can also cause massive levels of apoptosis in this same developmental window. Experimentation linking molecular causes of developmental and pathophysiological apoptosis in postnatal cerebral cortex is discussed.

key words: apoptosis, cerebral cortex, NMDA, ethanol, RhoA, anesthetics.

Contents

INTRODUCTION: EMBRYONIC NEURONAL APOPTOSIS IN THE PERIPHERAL NERVOUS SYSTEM AND THE CENTRAL NERVOUS SYSTEM (CNS)

In contrast to pathological necrosis, naturally-occurring apoptosis lacks plasma membrane rupture, inflammatory reaction, or cytoplasmic swelling (Schweichel and Merker, 1973). Instead, apoptotic cells display plasma membrane blebbing, followed by nuclear condensation and degradation, and repackaging of cell organelles into vesicles that are shed and later degraded (Kroemer et al., 2009). Programmed neuronal cell death, or apoptosis, is essential for proper embryonic development of both the central and peripheral nervous systems (Hamburger and Levi-Montalcini, 1949), resulting in the refinement of nascent neuronal innervation and network formation (Purves, 1990). In the peripheral nervous system, maturing neurons compete for neurotrophic survival factors, and the failure to capture neurotrophin ligands results in an apoptotic response (Bibel and Barde, 2000). Evidence for the so-called ''neurotrophic hypothesis'' working in a similar fashion in the central nervous system has been noticeably scant, however (Nikoletopoulou et al., 2010).

Apoptosis is nevertheless very important for the prenatal development of the cerebral cortex. Cell degeneration was first recorded in the embryonic telencephalon many decades ago (e.g. (Graumann, 1950; Kallen, 1955)). Using modern molecular methods to specifically quantify apoptosis, widely varying estimates of its prevalence were reported, from practically nothing (Spreafico et al., 1995) to relatively low levels of about 1% (Thomaidou et al., 1997; van den Eijnde et al., 1999), either using the terminal deoxynucleotidyl transferase

*Corresponding author. Address: Department of Biomedical Sciences, College of Osteopathic Medicine, University of New England, 11 Hills Beach Rd, Biddeford, ME 04005, USA. Tel: +49-6221-54-8687; fax: +49-6221-54-4952.
E-mail addresses: Marina-Nikolic@gmx.de (M. Nikolić), Humphrey.Gardner@astrazeneca.com (H. A. R. Gardner), Kerry.Tucker@urz.uni-hd.de (K. L. Tucker).

Abbreviations: BDNF, brain-derived neurotrophic factor; IGFs, insulin-like growth factors (IGFs); GTP, guanosine triphosphate; NMDAR, *N*-methyl-D-aspartate receptor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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deoxy-uridine-5'-triphosphate (dUTP) nick end labeling (TUNEL) method (Spreafico et al., 1995; Thomaidou et al., 1997) or annexin V staining (van den Eijnde et al., 1999). Several reports even indicated levels as high as 70% (Blaschke et al., 1996, 1998), employing a modified TUNEL method with enhanced sensitivity, and modeling studies have supported the idea that such high levels of apoptosis are compatible with normal embryonic development (e.g. (McConnell et al., 2009)). What all of these studies agreed upon, however, was the notion that apoptosis tended to occur in proliferative zones of the cortex, at the ventricular zone where radial glial precursors are mitotically active (Malatesta et al., 2003), and even more so in the subventricular zone, where a mixture of basally-directed radial glial fibers, newborn neurons migrating toward the cortical plate upon these fibers, and the neuron-generating basal precursors are located (Haubensak et al., 2004).

The two central apoptosis-executing proteins caspase-3 (Kuida et al., 1996; Roth et al., 2000) and caspase-9 (Cysteinyl-Aspartate-Specific Protease, (Hakem et al., 1998; Kuida et al., 1998)), as well as the apoptosis-promoting proteins Apoptotic protease activating factor 1 (Apaf1) (Cecconi et al., 1998; Yoshida et al., 1998) and Bax (Shindler et al., 1997), have been shown with mouse knock-out studies to be crucial for the embryonic apoptosis program. In elegant epistasis analysis, the genetic pathways controlling apoptosis that had first been elucidated in *Caenorhabditis elegans* (Horvitz, 1999) were found to be substantially similar in the mouse brain (Shindler et al., 1998; Roth et al., 2000; Zaidi et al., 2001). A balance of competing pro- and anti-apoptotic proteins can be disturbed by a pro-apoptotic signal, leading to the release of cytochrome *c* from the mitochondria. Cytochrome *c* in turn binds to Apaf1 and promotes formation of the cytosolic apoptosome, in turn activating the key caspase-9, which activates caspase-3 and thus the full apoptotic program (Conradt, 2009). A characteristic phenotype in mouse mutants in which pro-apoptotic genes are disrupted is a massive overgrowth of cortical tissue, to the point where it is spilling out of the skull (Kuida et al., 1996, 1998; Cecconi et al., 1998; Hakem et al., 1998; Yoshida et al., 1998). This reflects a decrease in the levels of apoptosis of proliferating precursors localized at the ventricular and subventricular zone of the embryonic cortex and a subsequent expansion of the surviving cells. Conversely, targeted deletion of apoptosis-suppressing genes such as Bcl-X_L (Motoyama et al., 1995; Zaidi et al., 2001), survivin (Jiang et al., 2005) and Mcl-1 (Arbour et al., 2008) all led to decreases in cortical size.

The upstream activation of apoptotic machinery in the periphery is well investigated, but corresponding mechanisms in the embryonic and postnatal brain are poorly understood. In this review, we briefly survey phenomenological reports of postnatal cortical apoptosis and experimental analysis of its relation to neuronal activity, outline recent mechanistic advances in the understanding of these phenomena, describe acute insults that lead to neuronal cell death, and point to some connections between these developmental and pathophysiological processes.

POSTNATAL NEURONAL APOPTOSIS IN THE CEREBRAL CORTEX

Phenomenological surveys of postnatal apoptosis

In the postnatal mammalian cortex apoptosis becomes manifest as neuronal loss despite the fact that during early postnatal development both the cortical volume and the brain weight increase. How can this phenomenon be explained? Apoptosis is a key mechanism leading to neuronal loss, so how can the mammalian brain still grow? In order to find answers for these questions exhaustive analyses have been performed on the brains of mice, rats, cats, and hamsters, focusing on the first 30 days after birth. The results showed that approximately 32% of neuronal tissue is eliminated between postnatal day 5 (P5) and adult stages, with the consequence of relatively small loss of neurons during aging (reviewed in Naruse and Keino (1995)). In the mouse, examination of the period of time between P5 and P10 revealed a significant decrease of neuronal density in the mouse cortex (Heumann et al., 1978; Heumann and Leuba, 1983). In the six-layered isocortex, the loss of neuronal density manifested itself mostly in layers II, III and IV whereas layers I, V and VI showed fewer changes. The period from P10 to P30 showed a slighter decrease but was still more pronounced in layers II, III and IV. Layers I, V and VI again revealed higher neuronal density. Both in the first and the second time periods examined, the depth and cortical volume were shown to increase constantly (Heumann et al., 1978; Heumann and Leuba, 1983). Despite the loss of up to 30% of cortical neurons after birth, the brain volume still increases. This is due to the continued postnatal generation of glial cells and an increase in the amount of neuropil (Heumann et al., 1978; Heumann and Leuba, 1983). Similar conclusions were reached in analysis of postnatal cat (Price and Blakemore, 1985), hamster (Finlay and Slattery, 1983), and rat (Ferrer et al., 1990) brains. To summarize, these phenomenological studies showed that neuronal apoptosis in newborn mammals mostly takes place in the first 30 postnatal days, with the peak at P5 in the rodent cortex – mostly pronounced in layers II–IV. All told, the amount of neuronal loss in both rodent species averaged about 30% until the adult stage was achieved; afterward very small apoptotic rates had been observed. These observations led to many more questions, most importantly why such a high fraction of neurons were dying, specifically at this very short time period? And also, what were the extrinsic and intrinsic mechanisms controlling neuronal apoptosis? According to classical neurotrophin theory, the dying neurons were losing out in a competition for scarce sources of neurotrophins. If so, which neurotrophins could these be?