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Glucocorticoid receptor stimulation and the regulation of neonatal cerebellar neural progenitor cell apoptosis

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ABSTRACT

Glucocorticoids are used to treat respiratory dysfunction associated with premature birth but have been shown to cause neurodevelopmental deficits when used therapeutically. Recently, we established that acute glucocorticoid exposure at clinically relevant doses produces neural progenitor cell apoptosis in the external granule layer of the developing mouse cerebellum and permanent decreases in the number of cerebellar neurons. As the cerebellum naturally matures and neurogenesis is no longer needed, the external granule layer decreases proliferation and permanently disappears during the second week of life. At this same time, corticosterone (the endogenous rodent glucocorticoid) release increases and a glucocorticoid-metabolizing enzyme that protects the external granule layer against glucocorticoid receptor stimulation (11β-Hydroxysteroid-Dehydrogenase-Type 2; HSD2) naturally disappears. Here we show that HSD2 inhibition and raising corticosterone to adult physiological levels both can independently increase neural progenitor cell apoptosis in the neonatal mouse. Conversely, glucocorticoid receptor antagonism decreases natural physiological apoptosis in this same progenitor cell population suggesting that endogenous glucocorticoid stimulation may regulate apoptosis in the external granule layer. We also found that glucocorticoids which HSD2 can effectively metabolize generate less external granule layer apoptosis than glucocorticoids this enzyme is ineffective at breaking down. This finding may explain why glucocorticoids that this enzyme can metabolize are clinically effective at treating respiratory dysfunction yet seem to produce no neurodevelopmental deficits. Finally, we demonstrate that both acute and chronic glucocorticoid exposures produce external granule layer apoptosis but without appropriate control groups this effect becomes masked. These results are discussed in terms of their implications for glucocorticoid therapy and neurodevelopment during the perinatal period.

Introduction

Recent clinical research has found that the postnatal use of glucocorticoid (GC) therapy can cause permanent neuromotor and cognitive deficits (Yeh et al., 2004) leading to the recommendation that this drug regimen not be used outside of placebo controlled clinical trials (Committee on Fetus and Newborn, 2002). Despite these concerns, GC therapy continues to be used throughout the world. For example, approximately 10% of prematurely born infants in North America and Europe still receive this treatment (Onland et al., 2009). Additionally, while prenatal GC therapy (which is given to approximately 7–10% of all pregnant women in North America and Europe; Matthews et al., 2004) has generally been considered safe, more recent clinical research has shown that treatment regimens using multiple exposure produce decreased head circumference (a proxy measurement for brain size; Amaral et al., 2008), length, and weight in neonates at birth (Murphy et al., 2008). Unfortunately, despite the large percentage of humans exposed to this controversial therapy (Eventov-Friedman and Shinwell, 2008), surprisingly little is known about how GC exposure can iatrogenically produce the neurodevelopmental deficits seen clinically.

In previous research, we discovered that exposure to clinically relevant doses of dexamethasone (DEX) produced rapid neural progenitor cell (NPC) apoptosis (programmed cell death) in the external granule layer (EGL) of the immature mouse cerebellum (Noguchi et al., 2008). The window of vulnerability for this toxicity in the mouse corresponds to all time points during which perinatal GC therapy would occur in the human (Noguchi et al., 2008). One curious aspect of this toxicity is that it is selectively confined to the NPCs contained within the outer EGL, a transient proliferative region occupying the most superficial cerebellar layer. The EGL produces new neurons in its outermost layer that first congregate and mature in the inner EGL before migrating through the molecular and Purkinje cell layers to populate the internal granule layer as neurons (Fig. 1A). The

Abbreviations: DEX, dexamethasone; EGL, external granule layer; GC, glucocorticoid; GINA, glucocorticoid induced neural progenitor cell apoptosis; HSD2, 11β-Hydroxysteroid Dehydrogenase Type 2; NPC, neural progenitor cell; PND, postnatal day.

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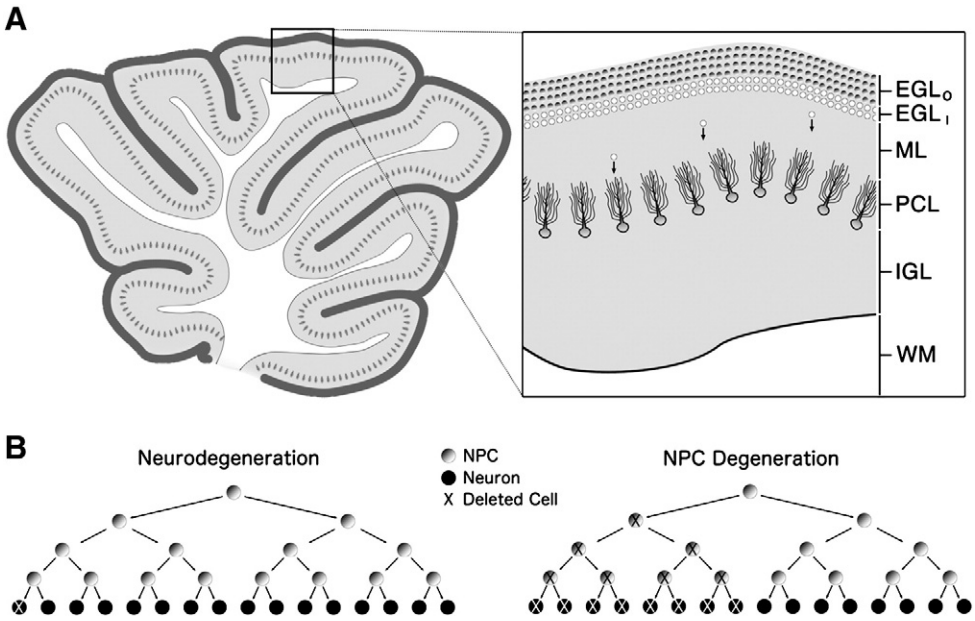

Fig. 1. Cerebellar development and apoptotic cell death. (A) The neonatal cerebellum is composed of white matter (WM) in its core surrounded by four cortical layers which include the internal granule layer (IGL) composed of granule cells, the Purkinje cell layer (PCL) which provides the sole output of the cerebellar cortex, the molecular layer (ML) which has few soma and mainly consists of synaptic connections between the Purkinje and granule cells, and the proliferative external granule layer (EGL). The EGL is further divided into the outer EGL (shaded circles; EGL_O) composed solely of NPCs and the inner EGL (white circles; EGL_I) composed of newly formed immature neurons that eventually migrate (cells with arrows) and populate the IGL. (B) The consequences of neural progenitor cell (NPC) apoptosis can be magnified due to the progenitor's ability to exponentially expand. Whereas the apoptotic death of a neuron leads to a net loss of only one cell (left), the apoptotic death of a single NPC not only eliminates that cell but also deletes any NPCs or neurons that cell would have produced in the future (right).

amount of EGL neurogenesis is quite extensive leading to the postnatal production of a homogenous population of internal granule layer cells so numerous that they represent over half the neurons in the brain (Andersen et al., 1992; Harvey and Napper, 1988). During the second week of normal rodent life, the EGL is rapidly removed from the cerebellum as proliferation in the EGL decreases and neurogenesis ends.

During normal cerebellar development, the GC system is designed such that GC stimulation in the EGL is low while this proliferative region is undergoing massive amounts of neurogenesis and growth but high when this same region decreases proliferation and disappears from the cerebellum (Dakine et al., 2000, Holmes et al., 2006, Pavlik & Buresova, 1984, Robson et al., 1998). Based on the ability of GCs to produce progenitor cell apoptosis, changes in endogenous GC stimulation may affect EGL apoptosis thereby contributing to its natural disappearance. In addition, the increased cell death in an exponentially expanding population of NPCs may have a magnified effect by decreasing proliferation as a secondary effect. (Altman and Bayer, 1997; de la Rosa and de Pablo, 2000; Depaepe et al., 2005; Haydar et al., 1999; Kuida et al., 1996; Fig. 1B). Based on this information, we examined how the GC system and its different regulating mechanisms might interact to effect NPC apoptosis in the developing cerebellum.

Materials and methods

Animals and drugs

Postnatal day (PND) 7 ICR mice (Harlan, IN, USA) were used unless otherwise indicated because previous research determined that this age was the middle of the window of vulnerability for this toxicity (Noguchi et al., 2008). Males and females were used, however, gender was not used as a factor during analysis since previous research found that it has no significant effect on this toxicity (Noguchi et al., 2008). Following final drug exposure for each drug regimen, all mouse pups were separated from the mother and maintained at 30 °C until perfusion 6 h later. All animal procedures used were in accordance with standards approved by the Washington University in Saint Louis Animal Studies Committee. DEX and betamethasone were administered using the preservative-free prodrug DEX sodium phosphate USP or betamethasone sodium phosphate USP respectively (Voigt Global Distribution LLC, Lawrence, KS, USA), the water soluble inorganic ester form of each drug typically administered clinically. DEX sodium phosphate and betamethasone sodium phosphate are expressed as molar equivalents to DEX and betamethasone respectively. Corticosterone, carbenoxolone, and mifepristone were obtained from Sigma-Aldrich (St. Louis, MO, USA). Doses of carbenoxolone and mifepristone were chosen based on previous research describing their ability to antagonize HSD2 (Heine and Rowitch, 2009) and the GC receptor (Mesripour et al., 2008) respectively. Corticosterone and mifepristone were dissolved in a sesame oil vehicle prior to injection whereas all other drugs were dissolved in sterile saline. The 100 mg/kg dose of mifepristone was administered in two 50 mg/kg doses. One was administered 15 min prior to GC or carbenoxolone injection and the other 2 h after. The 50 mg/kg mifepristone dose was administered as a single dose 15 min prior to GC exposure.

Activated caspase-3 immunohistochemistry

We previously established that activated caspase-3 immunohistochemistry is a sensitive marker for NPC apoptosis in the EGL by confirmation through the use of both electron microscopy (the gold standard for apoptotic detection) and the presence of pyknosis in plastic embedded semithin sections stained with methylene blue/Azure B (Noguchi et al., 2008). Animals in the current study were deeply anesthetized and transcardially perfused with 4% paraformaldehyde in 0.1 M Tris buffer 6 h after the last injection unless specified otherwise. Following postfixation, the cerebella were removed and sectioned at 75 μm in the sagittal plane using a vibratome. Immunohistochemistry for activated caspase-3 was performed as described previously (Noguchi et al., 2008). Based on results from pilot experiments in ICR