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Research Report

Lithium protects against glucocorticoid induced neural progenitor cell apoptosis in the developing cerebellum

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ABSTRACT

Respiratory dysfunction is one of the most common causes of death associated with premature birth (Barton et al., 1999). In the United States, 7–10% of pregnant women receive antenatal glucocorticoid (GC) therapy (Matthews et al., 2004), while approximately 19% of very low birth weight infants receive postnatal GC therapy (Jobe, 2009). Clinical research suggests that GC treatment causes permanent neuromotor and cognitive deficits (Yeh et al., 2004) and stunts cerebellar growth (Parikh et al., 2007; Tam et al., 2011). We previously reported that GC-mediated neural progenitor cell (NPC) apoptosis may be responsible for cerebellar neuropathology (Maloney et al., 2011; Noguchi et al., 2008, 2011). The goal of the current study was to determine whether lithium protects NPCs from GC neuroapoptosis *in vivo* and *in vitro*. Given that it protects against a range of brain insults, we hypothesized that lithium would significantly attenuate GC induced NPC toxicity. We report that acute lithium pretreatment provides potent, cell-intrinsic neuroprotection against GC induced NPC toxicity *in vivo* and *in vitro*.

1. Introduction

Respiratory dysfunction is one of the most common causes of death associated with premature birth (Barton et al., 1999). As a result, perinatal glucocorticoid (GC) therapy is widely used to mature the lungs and improve respiratory function in prematurely born infants. Opinions on the safety of GC therapy vary dependent on when (antenatally or postnatally) or how long (acutely or chronically) it is administered. In the United States, approximately 7–10% of pregnant women receive antenatal GC therapy (Matthews et al., 2004), and there is a broad consensus that the benefits of a single treatment greatly outweigh the risks (National Institutes of Health Consensus Development Panel [NIHCDP] 1994). However, there are concerns that GC therapy stunts growth when multiple antenatal treatments are given (Murphy et al., 2008; NIHCDP, 1994). Similarly, approximately 19% of very low birth weight infants receive postnatal GC therapy (Jobe, 2009). Unfortunately, clinical research suggests that this treatment produces permanent neuromotor and cognitive deficits

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(Yeh et al., 2004) and stunts cerebellar growth (Parikh et al., 2007; Tam et al., 2011). Initially, the American Academy of Pediatrics (2002) recommended that postnatal GC therapy not be used outside of randomized controlled clinical trials. However, in 2010, a revised policy recommended that the clinician balance potential adverse and beneficial effects when deciding whether to administer postnatal GC therapy (Watterberg, 2010). Currently, this treatment remains highly individual/institutional specific (Tin and Wiswell, 2009), suggesting that there is no clear consensus for its use.

Despite these concerns, surprisingly little is known about how GC stimulation disrupts cerebellar development. Recently, we reported that neonatal GC exposure in mouse pups produces rapid (within 4 h) and selective apoptosis (programmed cell death) in the neural progenitor cells (NPC) of the external granule layer (EGL) (Noguchi et al., 2008). The EGL is a transient proliferative region occupying the outermost layer of the developing neonatal cerebellum. During ontogenesis, the EGL produces granule cell neurons in the outer EGL that mature in the inner EGL before migrating past the molecular and Purkinje cells layers to populate the internal granule cell layer (Fig. 1A). While almost all other neural proliferative layers disappear prenatally, the human and rodent EGL continues proliferating during the postnatal period. In rodents, the EGL proliferates until around two weeks of age at which point it rapidly disappears once neurogenesis is no longer needed (Carletti and Rossi, 2008). The importance of the EGL in normal brain development and function is underscored by the fact that it produces 90% of the neurons in the cerebellum which represent over half the neurons in the brain (Andersen et al., 1992; Harvey and Napper, 1988; Herculano-Houzel, 2009). Due to the large number of neurons a single NPC can produce, NPC apoptosis can magnify cerebellar pathology when compared to neuronal apoptosis (Noguchi et al., 2011). Consistent with this concept, a single neonatal injection of GCs permanently reduces the number of granule cell neurons in the cerebellum by 18% in rodents (Noguchi et al., 2008).

Interestingly, the ability of GCs to cause NPC apoptosis may be related to their role in normal neurodevelopment. For instance, we reported endogenous GC release, GC metabolism, and GC receptor expression are carefully orchestrated to increase GC stimulation *selectively* in the EGL as this proliferative region is naturally eliminated from the cerebellum (Noguchi et al., 2011). As a result, this selective toxicity may be a byproduct of the natural role of GC stimulation in apoptotically eliminating the EGL once neurogenesis is no longer needed. This concept is supported by research showing adrenalectomy (which eliminates endogenous GC release) dramatically delays the natural disappearance of the EGL (Meyer, 1983; Yehuda et al., 1989; Yehuda and Meyer, 1991).

Since the cerebellum is involved in a variety of neuromotor and cognitive functions (Schmahmann, 2004), GC induced EGL apoptosis may be responsible for cerebellar stunting and behavioral deficits reported clinically in humans exposed to GCs (Parikh et al., 2007; Tam et al., 2011; Yeh et al., 2004). Indeed, in a recent study, we found postnatal GC exposure led to permanent neuromotor deficits and selective cerebellar stunting in mice (Maloney et al., 2011). If GC induced EGL apoptosis is responsible for these deficits, the use of neuroprotective agents may prevent the iatrogenic effects of GC therapy, while retaining beneficial effects on lung maturation. Interestingly, lithium protects against neuronal apoptosis produced by a wide variety of insults (Jordà et al., 2004; Straiko et al., 2009; Young et al., 2008). In addition, it has been shown to delay NPC apoptosis in the EGL following radiation exposure (Inouye et al., 1995). Based on these findings, the neuroprotective effect of lithium on GC induced apoptosis was examined.

2. Results

2.1. *Lithium carbonate protects against GC induced EGL apoptosis*

In previous research, we established that DEX exposure produces rapid apoptosis (as measured by activated caspase-3 immunolabeling) in the EGL of ICR mice at 6 h postinjection (Noguchi et al., 2011). In order to examine the neuroprotective abilities of lithium, a 6 milliEquivalent (mEq)/kg (*i.e.*, 221.67 mg/kg) intraperitoneal injection of lithium carbonate or saline was administered 15 min prior to 3.0 mg/kg DEX or saline. Animals were isolated from the mother at 30 °C until perfusion 6 h later. Following semiquantitative analysis, one-way ANOVA revealed a statistically significant effect of drug treatment on EGL apoptosis, $F(3,18)=77.87$, $p<0.0001$. A Bonferroni planned comparison *versus* control group revealed DEX alone significantly increased EGL apoptosis but this effect was prevented by lithium pretreatment (Fig. 2A). Lithium carbonate exposure alone had no significant effect on degeneration scores.

2.2. *Lithium chloride is as neuroprotective as lithium carbonate*

In the clinical setting, lithium is typically taken orally in the form of lithium carbonate. Once ingested, hydrochloric acid in the stomach converts lithium carbonate to lithium chloride ($2HCl+Li_2CO_3=2LiCl+H_2+CO_2$). Since an intraperitoneal injection of lithium carbonate would bypass stomach acid exposure, we also tested the neuroprotective effect of a 6 mEq/kg dose of lithium chloride (253.8 mg/kg) and compared it to the same mEq/kg dose of lithium carbonate. Lithium carbonate, lithium chloride, or saline was administered 15 min prior to a 3.0 mg/kg DEX or saline injection. Animals were perfused 6 h later for activated caspase-3 immunohistochemistry. Following semiquantitative analysis, one-way ANOVA revealed a statistically significant effect of drug treatment, $F(5,31)=12.04$, $p<0.0001$ (Fig. 2B). A Bonferroni planned comparison *versus* control group revealed DEX alone significantly increased EGL apoptosis which was prevented by both lithium chloride and lithium carbonate (Figs. 1B–D). Administration of either form of lithium alone had no significant effect on degeneration scores. These results reveal that lithium carbonate is as neuroprotective as lithium chloride. Since lithium carbonate needs acidification prior to injection and would necessitate the addition of hydrochloric acid in our cell culture work, lithium chloride was used in all subsequent experiments.