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BRAIN RESEARCH

Research Report

Low-potency glucocorticoid hydrocortisone has similar neurotoxic effects as high-potency glucocorticoid dexamethasone on neurons in the immature chicken cerebellum

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ABSTRACT

High-potency glucocorticoids (GC) are used in the prophylaxis and treatment of neonatal bronchopulmonal dysplasia, but there is concern about side effects on the developing brain. Recently, the low-potency GC hydrocortisone (HC) has been suggested as an alternative to high-potency GC. We compared the neurotoxic effects of HC with the high-potency GC dexamethasone (DEX) in chicken cerebellum. A single dose of GC was injected into the egg at embryonic day 16 and the death of granule neurons in histologic sections of the cerebellar cortex was examined 24 h later. DEX and HC showed a similar dose-dependent induction of morphological apoptosis and caspase-3 activation in the internal granular layer. A doubling of the apoptosis rate compared to the basal rate was seen for the highest dose of DEX (5 mg/kg) and medium dose of HC (1 mg/kg). In cultures of embryonic chicken cerebellar granule cells, DEX and HC increased cell death and induced rapid caspase-3 activation in a similar dose-dependent manner. Transfection of granule cells with a luciferase reporter gene revealed that the dose needed for the activation of gene transcription (classical signalling pathway) with DEX was much lower than for HC. In conclusion, HC does not present itself as a safer drug than DEX in this model. In addition, it appears that DEX and HC induce apoptosis in immature granule neurons via a non-genomic (non-classical) mechanism.

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Abbreviations: DEX, dexamethasone; EGL, external granular layer; GC, glucocorticoid; GR, glucocorticoid receptor; GRE-LUC, glucocorticoid response element—luciferase; HC, hydrocortisone; HPF, high power field; IGL, internal granular layer; MR, mineralocorticoid receptor

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1. Introduction

High-potency glucocorticoids (GC) (dexamethasone (DEX) or betamethasone) are routinely administered to women with threatening premature labor and have also been used postnatally to accelerate lung maturation and prevent respiratory distress syndrome and bronchopulmonal dysplasia in the premature infant. Lately, GC treatment has been shown to have adverse effects on brain motor and cognitive function, and a higher risk of cerebral palsy has been reported (O'Shea et al., 1999; Shinwell et al., 2000; Yeh et al., 2004). This has lead to a reduction in the postnatal use of high-potency GCs. Although prophylactic treatment of pregnant women (typically 6 mg i.m. DEX × 2 for 2 days) is considered safe, it is controversial at present whether repeat doses may be harmful to the immature brain (Crowther et al., 2007; Wapner et al., 2007). Hydrocortisone (HC) has recently been considered an alternative to DEX and betamethasone (Halliday et al., 2003), and one study investigating postnatal treatment for bronchopulmonal dysplasia with HC (starting dose 5 mg/kg/day, median duration, 27.5 days) reported no detectable reduction in motor and intellectual function and the same MRI findings at school age compared with the control group (Rademaker et al., 2007). HC is also used in the treatment of postnatal hypoglycemia and hypotension (typically 10 mg/kg daily). However, the possible side-effects of HC treatment have so far been insufficiently evaluated (Subhedar et al., 2007).

GCs primarily exert their effect by binding to the glucocorticoid receptor (GR) located in the cytosol. Some GCs also bind and activate the mineralocorticoid receptor (MR). Upon binding, GR translocates to the nucleus where it acts as a transcription factor. The various GCs bind GR with different affinity and have unequal effects on gene transcription. This generally correlates with their "relative steroid potency," a pharmacological estimate based on several parameters, including effect on hypothalamic–pituitary–adrenal axis, glycogen deposition, and anti-inflammatory and eosinopenic effect (Nakada et al., 1987; Mager et al., 2003). Traditional steroid potency can also be measured directly in transcription assays (Tanaka et al., 1994). However, our understanding of GC action has been complicated in later years by the demonstration of rapid non-genomic intracellular mechanisms (reviewed in Watson and Gametchu, 2003; Stahn et al., 2007). These involve GRs in non-nuclear locations such as the cell membrane, novel steroid binding proteins or the direct effect of GCs on membrane physical properties. The pathways may involve different binding affinity (K_d value) for GCs (Powell et al., 1999). Thus, although HC has a 25 times lower "steroid potency" than DEX, and is traditionally viewed as a safer drug with less side-effects, this may not be the case for effects mediated by non-genomic mechanisms.

In the present investigation, we have compared the effects of HC and DEX on cell death in cerebellar neurons in vivo and in vitro in a chicken embryo model. The chicken cerebellum shows rapid development corresponding to the stages in humans and presents itself as a useful model for the study of neurotoxic effects (Hamburger and Hamilton, 1992; Rakic and Sidman, 1970). Different doses of GCs were injected into the egg at embryonic day E16, and apoptotic cells were counted after 24 h in the internal granular layer (IGL) of the cerebellar cortex. In parallel experiments, the neurotoxic effect of HC and DEX were studied in cerebellar granule cells that had been isolated from chicken embryos and cultured in vitro (Jacobs et al., 2006a). These effects were compared to the capacity of HC and DEX to induce GR-mediated transcription in granule neurons. The concentrations needed to activate a luciferase reporter gene were much higher for HC than for DEX, in accordance with their steroid potency. In contrast,

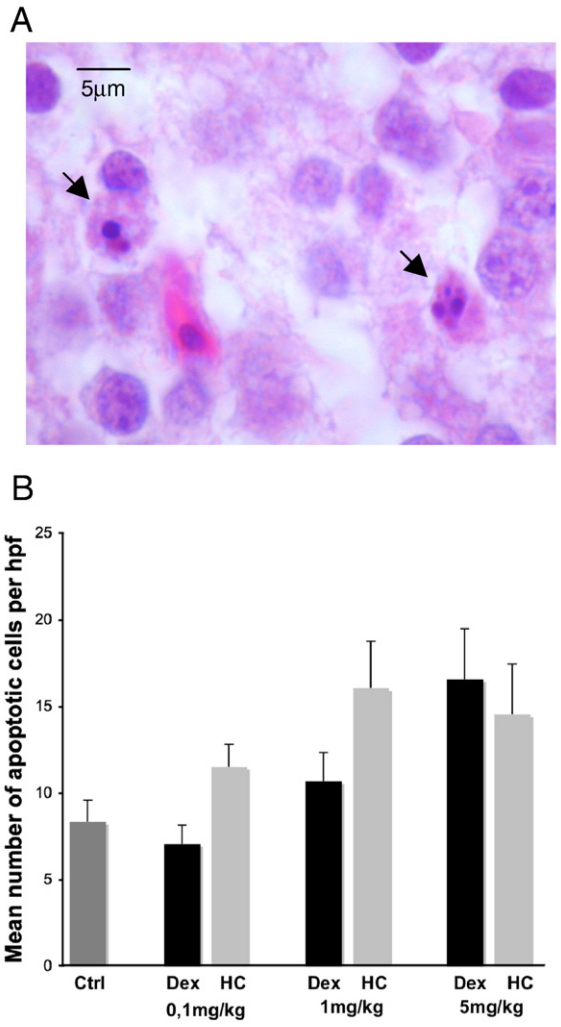

Fig. 1 – DEX and HC induce apoptosis in the internal granular layer in chicken cerebellum in a dose-dependent manner. (A) Sections show the IGL in the cerebellum of untreated chicken embryo at E17. Two granule cells (arrows) present the typical features of apoptosis, i.e., nuclear condensation and fragmentation, and eosinophilic cytoplasm (HE staining; ×400 magnification). The intensely red cell is a nucleus-bearing erythrocyte. The morphology of spontaneous and GC-induced apoptotic granule cells was the same. (B) Chicken embryos at E16 were treated with DEX and HC for 24 h in the given concentrations. Cells in the IGL showing the typical morphological signs of apoptosis were counted in H&E-stained sections. The mean number of apoptotic cells per HPF (×400 magnification) is shown with SEM ($n = 6$ experiments). There was a significant dose-dependent effect of DEX and HC ($p < 0.05$).