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*Teaser Glucocorticoids modulate inflammation and apoptosis in the neonatal brain, providing potential therapeutic strategies that could be beneficial for the treatment of infants with HI brain injury.*



# Corticosteroids and perinatal hypoxic-ischemic brain injury

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**Perinatal hypoxic-ischemic (HI) brain injury is the major cause of neonatal mortality and severe long-term neurological morbidity. Yet, the effective therapeutic interventions currently available are extremely limited. Corticosteroids act on both mineralocorticoid (MR) and glucocorticoid (GR) receptors and modulate inflammation and apoptosis in the brain. Neuroinflammatory response to acute cerebral HI is a major contributor to the pathophysiology of perinatal brain injury. Here, we give an overview of current knowledge of corticosteroid-mediated modulations of inflammation and apoptosis in the neonatal brain, focusing on key regulatory cells of the innate and adaptive immune response. In addition, we provide new insights into targets of MR and GR in potential therapeutic strategies that could be beneficial for the treatment of infants with HI brain injury.**

## Introduction

Perinatal HI encephalopathy (HIE), a subgroup of neonatal encephalopathy, occurs in approximately three per 1000 live births and remains the leading cause of death in term infants [1,2]. HIE is most commonly caused by an intrapartum HI insult, such as placental abrupture, uterine rupture, and umbilical cord accidents. The morbidity and mortality of HIE is 23% with clinical consequences of seizures, cerebral palsy (CP), visual and hearing impairments, and mental abnormalities [1,3,4]. The clinical indications for HIE-induced CP are an umbilical cord pH less than 7.0, evidence of moderate–severe neonatal encephalopathy, and absence of other CP causes [1]. These associated comorbidities require significant, costly medical assistance throughout life and negatively affect the quality of life of the patient and their productivity as an adult [3]. Hypothermia, the standard of care for treatment of HIE, targets the cerebral metabolism and key injury mechanisms that occur in HIE [5]. Hypothermia, when started within 6 h of birth, significantly reduces the morbidity and mortality of newborns with HIE, yet many infants (>40%) die or experience severe neurological deficits after treatment [6]. In a multicenter, randomized trial of hypothermia treatment for patients with HIE, there was still an abrupt increase in proinflammatory cytokines despite hypothermia

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treatment [7]. It is clear that adjuvant therapies are needed to alleviate the severity of HIE outcomes and comorbidities.

The pathogenesis of HIE is complex, involving short-term neuronal damage that evolves into long-term chronic inflammation. The mechanisms responsible for the progression of HI brain injury include: excito-oxidative-, free-oxygen-radical-, caspase-, and cytokine-mediated cell damage, interrupted calcium and mitochondrial homeostasis, and inflammatory cell activation and recruitment [8,9]. Early events of HIE are dominated by damaged neuronal cells via the excito-oxidative cascade. During initial HI insult, ATP depletion occurs rapidly [10], leading to $Na^+/K^+$-ATPase pump failure and depolarization of the cells, resulting in calcium accumulation through reversal of the $Na^+/Ca^{2+}$-exchange carrier. Subsequent influx of calcium occurs, causing cellular swelling and irreversible neuronal energy failure, which ultimately leads to necrosis and a plethora of signaling cascades leading to more cellular death [11].

In HI brain injury, there is an early- and late-phase inflammatory process. The early inflammatory response lasts from hours to days. It is initiated by activation of microglial cells, the resident innate immune cells in the brain, by injured neuronal cells that release endogenous molecules and the proinflammatory cytokines TNF-α and IL-1β [12]. Activated microglial cells subsequently release proinflammatory cytokines and proteases, and activate NMDA-mediated toxicity, which leads to secondary neuronal injury [9]. Astrocytes, the largest population of glia cells in the neonatal brain, have a protective role through glutamate uptake metabolism, maintenance of the blood–brain barrier (BBB), and glial scar formation after injury [13]. In HI damage, astrocytes generate extracellular glutamate dysregulation, neuronal axonal injury, and release of TNF-α and IL-6 [9]. Neutrophils first appear in the cerebral vasculature 4 h after HI injury in the neonatal rat brain and extravasate into the brain parenchyma 42 h after initial injury [9]. Depletion of neutrophils confers neuroprotection only when depleted before HI brain injury [14]. Lastly, T and B cells are implicated in the delayed neuroinflammatory response to HI injury, and persist in the long-term inflammatory response, up to 35 days after HI injury [9].

Recent studies reported that direct glucocorticoid administration to the neonatal brain via intracerebroventricular (i.c.v.) injection as well as intranasal administration provided neuroprotection and ameliorated brain damage in neonatal HI injury [15,16]. Other studies have also proposed glucocorticoids to have therapeutic potential for the treatment of HI injury, yet evidence of both neuroprotective and neurotoxic effects of glucocorticoids exists [17,18]. Several lines of evidence indicate that the timing, dosing, duration of treatment, and severity of disease influences the effects of glucocorticoids [18]. The long-term consequences of dexamethasone are controversial, yet it might be the best adjuvant option with hypothermia treatment in devastating inflammatory diseases, such as HIE or neonatal stroke. Glucocorticoid agonists could have the potential to decrease inflammatory cytokines that negatively impact key neuronal cells.

## Regulation of corticosteroid receptors in the developing brain

### *Mineralocorticoid and glucocorticoid receptors in the developing brain*

GRs and MRs are crucial for fetal brain development and stimulation of the hypothalamic–pituitary–adrenal (HPA) axis for parturition, organ maturation, and fetal growth [19]. Their expression is region specific, occurring in the hippocampus and limbic system [19]. Throughout the fetal development, expression of GRs and MRs dynamically changes, giving insight into their function and control of the HPA axis (Fig. 1).

In the mouse hippocampus, MR mRNA expression is first detected embryonically, whereas that of GRs is first expressed after birth [20,21]. Conversely, rat and guinea pig show detectable mRNA levels of GRs and MRs before birth, with increased levels of GR mRNA closer to birth [22–24]. During hippocampal formation during midgestation, when fetal cortisol levels are low, hypothalamic MR mRNA expression is more abundant than is GR mRNA expression [20,23–25]. Closer to birth, the progression of MR and GR mRNA switches. MR mRNA gradually decreases in the CA1/2 hippocampal region from midgestation to birth, whereas GR mRNA increases in the CA1/2 hippocampal region [24]. Notably, the expression of both human hippocampal MRs and GRs is detectable between 24 and 34 weeks of gestation [20]. No other time points in the human hippocampus have been studied. The molecular modifications of MRs and GRs studied in the hippocampus of the mouse, rat, and guinea pig hippocampus are best contextualized when evaluating the control of the HPA axis.

In the fetus, negative feedback on the HPA axis is decreased to allow for a high output of cortisol, which is required for maturation of organs, including lung, kidney, and brain [19,23]. This is made possible by several separate mechanisms. First, MR expression is thought to be mainly involved in HPA axis negative feedback control, and decreased MR expression closer to birth might release the HPA axis from this inhibition [24]. At the same time, GR expression is decreased in the paraventricular nucleus (PVN) of the hypothalamus, allowing for a further decrease in the negative feedback control [23,26]. In addition, levels of the enzyme 11β- hydroxysteroid dehydrogenase type 2 (11β-HSD2) fall to allow for high levels of adrenocorticotropin hormone (ACTH), corticotropin-releasing hormone (CRH), and cortisol [27]. 11β-HSD2 converts cortisol to inactive cortisone to protect the fetus from glucocorticoid overexposure before the third trimester of gestation [27,28]. This results in high ACTH despite increased cortisol, allowing for a cortisol surge that is necessary for organ maturation.

### *Hypoxia and effects on MR and GR expression*

Glucocorticoids are steroid hormones secreted by the adrenal gland and have an important role in the regulation of metabolism and immune response regulation. Endogenous glucocorticoids, such as cortisol, bind to MRs and GRs [29]. MRs have a relatively high affinity and are highly bound at basal levels of glucocorticoids, whereas GRs bind corticosteroids relatively weakly at physiological levels. Synthetic glucocorticoids, such as betamethasone and dexamethasone, selectively bind GRs, but not MRs. GRs are retained in the cytoplasm through association with heat-shock protein 90 (HSP90) [30]. Once the ligand binds to the GR, HSP90 dissociates, and the activated GR–glucocorticoid complex is translocated to the nucleus.

Upon nuclear localization, the GR–glucocorticoid complex controls inflammation by several different mechanisms. The GR forms a homodimer at a consensus DNA site, called glucocorticoid response elements (GRE), where it recruits basal transcription





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