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Does growth impairment underlie the adverse effects of dexamethasone on development of noradrenergic systems?

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ABSTRACT

Glucocorticoids are given in preterm labor to prevent respiratory distress but these agents evoke neurobehavioral deficits in association with reduced brain region volumes. To determine whether the neurodevelopmental effects are distinct from growth impairment, we gave developing rats dexamethasone at doses below or within the therapeutic range (0.05, 0.2 or 0.8 mg/kg) at different stages: gestational days (GD) 17-19, postnatal days (PN) 1-3 or PN7-9. In adolescence and adulthood, we assessed the impact on noradrenergic systems in multiple brain regions, comparing the effects to those on somatic growth or on brain region growth. Somatic growth was reduced with exposure in all three stages, with greater sensitivity for the postnatal regimens; brain region growth was impaired to a lesser extent. Norepinephrine content and concentration were reduced depending on the treatment regimen, with a rank order of deficits of PN7-9 > PN1-3 > GD17-19. However, brain growth impairment did not parallel reduced norepinephrine content in magnitude, dose threshold, sex or regional selectivity, or temporal pattern, and even when corrected for reduced brain region weights (norepinephrine per g tissue), the dexamethasone-exposed animals showed subnormal values. Regression analysis showed that somatic growth impairment accounted for an insubstantial amount of the reduction in norepinephrine content, and brain growth impairment accounted for only 12%, whereas specific effects on norepinephrine accounted for most of the effect. The adverse effects of dexamethasone on noradrenergic system development are not simply related to impaired somatic or brain region growth, but rather include specific targeting of neurodifferentiation.

1. Introduction

Over two decades ago, the National Institutes of Health endorsed the use of antenatal glucocorticoids as the consensus treatment for preterm labor occurring between 24 and 34 weeks of gestation, a therapy designed to prevent neonatal respiratory distress syndrome (Gilstrap et al., 1995). It is estimated that this treatment saves up to 2000 lives annually in the U.S but as a consequence, 10% of all U.S. newborn children, roughly 400,000 per year, are exposed to glucocorticoids (Matthews et al., 2002). It is increasingly apparent that the promotional effect of this therapy on lung development in the small number of preterm infants who benefit, must be balanced against the potentially damaging impact of glucocorticoids on other processes, most notably brain development. It has long been known that excessive glucocorticoid exposure disrupts neuronal cell replication and differentiation, leading to synaptic deficiencies that culminate in a broad spectrum of neurobehavioral, endocrine and cardiovascular disorders (Cavalieri and Cohen, 2006; Drake et al., 2007; Meyer, 1985; Moritz et al., 2005; Pryce et al., 2011; Rokyta et al., 2008; Tegethoff et al., 2009), and these outcomes have now been verified in children exposed to glucocorticoids prenatally (Crowther et al., 2007; Hirvikoski et al., 2007; Needelman et al., 2008; Newnham, 2001; Peltoniemi et al., 2011).

The adverse impact of glucocorticoids on brain development are typically associated with somatic growth impairment and with reductions in brain regional volumes (Cheong et al., 2014; Parikh et al., 2007). This raises the important question of whether growth impairment itself provides a driving force for adverse effects of glucocorticoids on brain development, or, if not causatively related, whether growth impairment serves as an adequate predictor of neurodevelopmental effects. In the current study, we addressed these questions by administering dexamethasone to developing rats at stages corresponding to those of human brain development in which glucocorticoid therapy is typically given: gestational days (GD) 17-19, postnatal days (PN) 1-3, and PN7-9 (Rodier, 1988). At each stage, we studied doses below (0.05 mg/kg) or within (0.2–0.8 mg/kg) the therapeutic range (Gilstrap et al., 1995), which also span the threshold for growth retardation (Kreider et al., 2006; Slotkin et al., 2006). The three-day regimen

Abbreviations: ANOVA, analysis of variance; GD, gestational day; PN, postnatal day

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Table 1
Control values.

	PN30		PN45		PN75	
	male	female	male	female	male	female
Body weight (g)	106 ± 2	101 ± 3	235 ± 4	179 ± 3	451 ± 6	281 ± 6
Cerebral cortex weight (mg)	404 ± 4	384 ± 6	443 ± 5	428 ± 4	462 ± 5	435 ± 7
Midbrain weight (mg)	250 ± 4	241 ± 3	284 ± 4	273 ± 4	318 ± 3	306 ± 3
Brainstem weight (mg)	145 ± 2	137 ± 2	176 ± 3	172 ± 2	213 ± 3	202 ± 3
Cerebral cortex NE content (ng/region)	52 ± 3	51 ± 2	62 ± 4	59 ± 3	102 ± 4	88 ± 2
Midbrain NE content (ng/region)	82 ± 3	84 ± 3	90 ± 4	84 ± 3	152 ± 4	140 ± 3
Brainstem NE content (ng/region)	48 ± 2	45 ± 2	51 ± 3	46 ± 3	76 ± 2	70 ± 2
Cerebral cortex NE concentration (ng/g)	130 ± 7	133 ± 5	139 ± 7	138 ± 7	221 ± 8	202 ± 7
Midbrain NE concentration (ng/g)	328 ± 13	349 ± 12	317 ± 15	309 ± 12	480 ± 12	459 ± 11
Brainstem NE concentration (ng/g)	336 ± 12	331 ± 13	289 ± 17	269 ± 18	355 ± 9	345 ± 10

corresponds to the multiple glucocorticoid courses used in 85% of preterm labor cases (Dammann and Matthews, 2001).

We evaluated the relationship of dexamethasone's adverse effects on development of noradrenergic systems, to those on somatic growth impairment or on impairment of brain region growth. We chose norepinephrine as a target for several reasons. First, this transmitter system is widely distributed throughout early- and late-developing brain regions, enabling a dissection of critical periods of vulnerability. Second, norepinephrine plays critical roles in disorders of mood, attention, learning, memory and autonomic function (Ordway et al., 2007), all known targeted neurobehavioral outcomes for developmental exposure to glucocorticoids. Third, there is a paradoxical relationship between glucocorticoids and development of noradrenergic systems: dexamethasone directly promotes the emergence of the noradrenergic phenotype (Ciaranello et al., 1973; Ebert et al., 1997; Jameson et al., 2006), yet glucocorticoid treatments appear to impair development of noradrenergic projections, at least in the peripheral circuits that have been evaluated for such effects (Kallio et al., 1998; Kauffman et al., 1994; Lau and Slotkin, 1981). Fourth, although a number of studies from our and other laboratories have assessed norepinephrine levels after developmental glucocorticoid exposure (Muneoka et al., 1997; Slotkin et al., 1992, 2015; Slotkin and Seidler, 2011), there has been no systematic examination of the relationship to growth impairment, critical exposure periods, dose-response relationships, regional selectivity, or longitudinal developmental trajectories. Here, we addressed the following questions: (1) Does dexamethasone have a specific effect on noradrenergic system development, over and above somatic or brain growth impairment? (2) Do these effects occur at or below the doses used in the management of preterm labor? (3) Is there a critical developmental window in which adverse effects on noradrenergic development occur, and is that window separable from the window for growth impairment?

To answer these questions, we made use of the important relationships between norepinephrine content (total amount norepinephrine in a given brain region) and norepinephrine concentration (norepinephrine per gram tissue). Because the majority of each tissue does not consist of norepinephrine neurons, these two measures represent different parameters that can be used to identify both functional deficits and specificity toward norepinephrine. For example, a reduction in tissue weight that does not compromise norepinephrine neurons would produce no change in norepinephrine content but an increase in norepinephrine concentration. On the other hand, a specific deficit in norepinephrine would reduce both parameters in parallel.

2. Materials and methods

2.1. Animal treatments

All studies were performed in accordance with the *Declaration of Helsinki* and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. The tissues used in this report were archived from earlier studies (Kreider et al., 2006; Slotkin et al., 2006) and maintained frozen at -45 °C, so that no additional animals were actually used. Details of animal husbandry, and maternal and litter characteristics, have all been presented in earlier work from the original animal cohorts. Timed-pregnant Sprague-Dawley rats (Charles River, Raleigh, NC) were housed individually and given free access to food and water. For studies of gestational dexamethasone exposure, dams received daily subcutaneous injections of dexamethasone phosphate (Sigma-Aldrich, St. Louis, MO) at doses of 0.05, 0.2 or 0.8 mg/kg on GD17–19, whereas controls received equivalent volumes (1 ml/kg) of isotonic saline vehicle. On the day after birth, all pups were randomized within their respective treatment groups and redistributed to the nursing dams, maintaining a litter size of 10 to ensure standard nutrition. Randomization was repeated every 3–4 days and in addition, dams were rotated among litters to obviate any differences in maternal caretaking. Cross-fostering does not alter the developmental effects of dexamethasone, nor does fostering of normal pups by dexamethasone-treated dams produce apparent treatment effects in controls (Nyirenda et al., 2001). For studies of the effects of postnatal dexamethasone treatment, pups were given 0, 0.05, 0.2 or 0.8 mg/kg on PN1–3 or PN7-9 and the same randomization procedures were followed. Animals were weaned on PN21.

2.2. Tissues and assays

In adolescence and adulthood (PN30, PN45, PN75), animals were decapitated, the cerebellum was removed, and the forebrain was separated from the midbrain and brainstem by a cut rostral to the thalamus, after which the hippocampus and striatum were dissected away from the forebrain to isolate the cerebral cortex. For this study, we utilized the cerebral cortex, midbrain, brainstem and, at one age point, cerebellum. Brain regions were frozen in liquid nitrogen and stored at -45 °C. We utilized 6 males and 6 females for each treatment group at each age. Based on our earlier work with similar designs, these are adequate sample sizes to detect treatment effects and treatment interactions with the other factors across multiple ages and regions (Kreider et al., 2006; Slotkin et al., 2006).

For norepinephrine determinations, tissues were thawed on ice and deproteinized by homogenization in 0.1 N perchloric acid containing 3,4-dihydroxybenzylamine (Sigma-Aldrich) as an internal standard. Homogenates were sedimented at 26,000 × g for 20 min, the supernatant solutions were decanted, and norepinephrine was then trace-enriched by alumina adsorption, separated by reverse-phase high performance liquid chromatography and quantitated by electrochemical detection (Seidler and Slotkin, 1981); values were corrected for recovery of the internal standard.