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Research Report

Neonatal dexamethasone accelerates spreading depression in the rat, and antioxidant vitamins counteract this effect

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ABSTRACT

The use of dexamethasone (Dex) to treat chronic lung disease in preterm infants may produce adverse effects in the developing brain. Here, we evaluated the effects of neonatal Dex on the propagation of cortical spreading depression (CSD), and tested the action of vitamins C and E against the effect of Dex. Five groups of Wistar rats received, respectively: [1] no treatment (Naïve); [2] Vehicle (V); [3] tapering doses of Dex (Dex; 0.5 mg/kg, 0.3 mg/kg, and 0.1 mg/kg) on postnatal day (PND) 1–3; [4] Dex plus 200 mg/kg vitamin C and 100 mg/kg vitamin E (DexCE); [5] only vitamins C and E (CE). Vehicle and vitamins were administered on PND 1–6. CSD was recorded after the pups reached maturity (PND 60–70). The Dex-treated group presented with higher CSD velocities (mean values±SD, in mm/min: 4.14±0.22, $n=10$) compared with the control groups (Naïve: 3.52±0.13, $n=8$; V: 3.57±0.18, $n=10$; CE: 3.51±0.24, $n=10$; $p<0.05$ for all). Vitamins C and E antagonized this effect (DexCE group; CSD velocity: 3.43±0.12, $n=9$). No intergroup difference was observed concerning P-wave amplitude and duration. In all groups, after the cortex underwent CSD, the electrocorticogram (ECoG) amplitude increased approximately 50% compared with the baseline amplitude for the same animal (CSD-induced ECoG potentiation); however, no intergroup difference was observed. Data suggest that coadministration of antioxidant vitamins with Dex may be a helpful therapeutic strategy to reduce brain adverse effects of dexamethasone.

1. Introduction

Dexamethasone is a synthetic glucocorticoid hormone that is largely applied to preterm infants to prevent or treat chronic lung diseases (Choi et al., 2004; Doyle et al., 2014a, 2014b). However, neonatal treatment with glucocorticoids can produce unwanted side effects in the developing nervous system of children (Hitzert et al., 2014; Shinwell et al., 2000; Stark et al., 2001). Evidence from laboratory animals also indicates such adverse effects (Bhatt et al., 2013; Duksal et al., 2009;

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Ichinohashi et al., 2013; Kim et al., 2013; Menshanov et al., 2014; Neal et al., 2003; Sze et al., 2013; Zuloaga et al., 2011). These reports address the brain effects of dexamethasone on behavioral, biochemical, and morphological parameters. However, some other studies have demonstrated electrophysiological changes in animals previously treated with dexamethasone, including the appearance (Davidson et al., 2011) or modulation of epileptiform activity (Yılmaz et al., 2014). Electrophysiological alterations also include the modulation of synaptic plasticity-dependent phenomena, such as long-term potentiation (LTP) (Kamphuis et al., 2003; Lin et al., 2006; Wang et al., 2010). In this scenario, experimental investigation based on electrophysiological phenomena related to epilepsy and LTP is highly desirable. This is the case regarding the phenomenon known as cortical spreading depression (CSD), which has been studied by our group (Batista-de-Oliveira et al., 2012a; Torrente et al., 2014b; see Guedes, 2011 for a review) and by others (Footitt and Newberry, 1998; Dreier, 2011).

CSD has been experimentally described as a reversible and propagated wave of reduction of the spontaneous electrical activity of the cerebral cortex (Leão, 1944). This phenomenon occurs in response to electrical, chemical, or mechanical stimulation applied to one point of the cortical surface. Simultaneously with the depression of brain activity, a slow direct current (DC) potential change of the tissue has been described (Leão, 1947). CSD has been widely used to evaluate brain processes that depend on neural excitability (Batista-de-Oliveira et al., 2012b; Guedes et al., 2005; Lima et al., 2013), and a causal association between CSD and an LTP-like potentiation of spontaneous and evoked cortical electrical activity has been demonstrated both *in vitro* (Footitt and Newberry, 1998) and *in vivo* (Faraguna et al., 2010; Guedes et al., 2005; Souza et al., 2011).

Dexamethasone increases the presence of reactive oxygen species (ROS) in cultured hippocampus and cerebral cortex (McIntosh and Sapolsky, 1996), and reduces the basal activity of brain antioxidant enzymes (McIntosh et al., 1998). Considering the hypothesis that most of the adverse effects of dexamethasone are caused by oxidative stress, studies have employed the antioxidant vitamins C and E to counteract the adverse effects of this synthetic hormone (Camm et al., 2011; Herrera et al., 2010; Niu et al., 2013; Williams et al., 2012). These vitamins are able to minimize neuronal loss, as well as the oxidative stress produced in the brain by the dexamethasone treatment (Camm et al., 2011). However, no information is currently available regarding the effects of dexamethasone combined with antioxidant vitamins on brain electrical activity. In this context, the use of CSD has also been employed to study the actions of antioxidants on the brain (Abadie-Guedes et al., 2008; Guedes et al., 2012; Monte-Guedes et al., 2011).

The current *in vivo* study evaluated, in the albino rat, the long lasting effects of neonatal dexamethasone, combined or not with the administration of antioxidant vitamins C and E, on changes in CSD features and LTP-like electrocorticogram (ECoG)-potentiation associated with CSD.

2. Results

2.1. Body weight

The five experimental groups did not differ with respect to body weight on PND 1 and PND 2. From PND 3 to PND 6, rats previously treated intraperitoneally with dexamethasone and dexamethasone plus vitamins C and E (the Dex and DexCE groups, respectively) exhibited lower body weight than groups that were untreated (Naïve), vehicle-injected (V), and treated with both vitamin C and vitamin E (CE). However, no weight difference was observed when the pups reached PND 60 (Table 1).

2.2. CSD parameters

The 1-min application of a cotton ball (1–2 mm diameter) soaked with 2% KCl (approximately 270 mM) to a point of the occipital cortical surface was very effective in eliciting a single CSD episode that was propagated and sequentially recorded at two points on the parietal cortex. This is illustrated in the recordings shown in Fig. 1.

Measurements of the amplitude and duration of the CSD negative potential change revealed no intergroup difference; the mean amplitudes ranged from 7.50±1.45 mV to 8.22±2.24 mV, and the mean durations ranged from 72.18±3.98 s to 75.14±6.77 s (Table 2).

Table 1 – Body weights of the five experimental groups of rats, intraperitoneally injected with dexamethasone (Dex), antioxidant vitamins (CE), or both (DexCE). Two additional control groups were injected only with the vehicles in which these drugs were dissolved (V) or received no treatment (Naïve).

	Body weight (g)						
	PND1	PND2	PND3	PND4	PND5	PND6	PND60
Naïve	6.70±0.82	7.69±0.70	9.17±0.90	10.94±1.38	12.70±1.87	14.94±1.13	255.00±22.69
V	6.91±0.89	7.85±0.82	9.50±0.94	11.20±1.32	12.50±1.65	14.28±1,73	240.30±30.20
Dex	6.80±0.54	6.89±0.49	7.10±0.70*	8.10±0.84*	8.90±1.24*	10.25±1.60*	246.94±12.05
DexCE	6.95±0.76	7.20±0.79	7.80±1.06*	9.20±1.16*	10.35±1.13*	11.75±1.06*	244.44±24.96
CE	6.90±0.94	8.00±1.04	9.20±1.23	11.00±1.30	12.35±1.83	14.05±1.85	251.21±16.77

PND: postnatal day.
Data are reported as mean±S.D.
* Significantly different from the Naïve, V, and CE groups at the same age ($P<0.05$; one-way ANOVA plus the Holm–Sidak test).