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Neurobiology of Aging 28 (2007) 112–121

NEUROBIOLOGY OF AGING

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Prenatal glucocorticoid exposure affects learning and vulnerability of cholinergic neurons

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Received 4 August 2005; received in revised form 10 October 2005; accepted 25 November 2005
Available online 6 January 2006

Abstract

Prenatal treatment with synthetic glucocorticoids is commonly used as a treatment for women at risk of preterm delivery. However, little is known about the life-long consequences of these treatments on the fetus. In the present study, we evaluated cognitive function as well as susceptibility of cholinergic neurons to ^{192}IgG-saporin immunolesion in adult rats after prenatal glucocorticoid treatment. Morris water maze results revealed a significant difference in learning and memory function in adult rats that were prenatally exposed to dexamethasone, and further cognitive deficits after ^{192}IgG-saporin exposure. Choline acetyl transferase activity was decreased in the cortex of dexamethasone-treated rats compared with controls. In addition, rats prenatally exposed to either dexa, or betamethasone revealed a dramatic decrease in choline acetyl transferase activity compared to control rats after ^{192}IgG-saporin lesion. We report behavioral and biochemical evidence for altered cognitive function and increased susceptibility of cholinergic neurons to ^{192}IgG-saporin in adult rats after prenatal glucocorticoid treatment. Taken together, these results suggest that prenatal treatment with dexamethasone could affect cognitive functions and render cholinergic neurons more vulnerable to challenges later in life.

Keywords: Dexamethasone; Betamethasone; Alzheimer's disease; ^{192}IgG-saporin; Glucocorticoid; Prenatal exposure; Cholinergic immunotoxin

1. Introduction

Prenatal treatment with synthetic glucocorticoids has been used in clinic for more than 20 years [18,20]. Two types of synthetic glucocorticoids have been developed, dexamethasone and betamethasone, which rapidly cross the placenta from mother to child [21]. It is known that elevated levels of glucocorticoids in the fetus can modify the development of several organs, including lung, gut, kidney, and heart [6,34]. However, it was recently discovered that the nervous system may also be affected, but relatively few studies have focused on the possible behavioral and learning alterations in children who were prenatally treated with synthetic glucocorticoids [1,20,48]. Interestingly, a recent clinical study of school age children who were postnatally treated with dexamethasone demonstrated decreased motor skills and coordination, and a significantly lower IQ score [52]. Despite the lack of clinical safety data and knowledge about long-term effects, many hospitals administrate repeated treatment courses of synthetic glucocorticoids. In fact, a survey of British obstetric units showed that 98% of the interviewed clinicians recommended repeated courses of prenatal corticoid therapy [4].

The fetal limbic system expresses high levels of corticoid receptors during development, and a number of studies have indicated that this system is particularly vulnerable to elevated levels of glucocorticoids, either due to chronic treatments or prolonged prenatal stress [20]. Such an elevation may induce hippocampal atrophy in animals and humans [21,32,41]. Increased levels of endogenous glucocorticoid/cortisol due to maternal stress can also induce permanent changes in the offspring's hormonal and

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0197-4580/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neurobiolaging.2005.11.015

behavioral response to stress [10,40,45,46], and also the hippocampal feedback regulation of the basal hypothalamo-pituitary-adrenal circuit (HPA) activity may be altered [20]. Disturbances of HPA-axis function have been predictably linked to changes in learning tasks that are dependent on the hippocampus. Prenatal stress retarded the acquisition of reversal discrimination learning, but did not affect simple discrimination in a non-spatial operant paradigm [14]. Spatial learning in water mazes was retarded in prenatally stressed male rats, but not in females. Moreover, the levels or function of monoamines in the brain were shown to be affected by mild to severe prenatal stress. However, very few data are available on cerebral acetylcholine and metabolism, and this is surprising since cholinergic neurons have a powerful impact on cognitive processes [24]. It is in fact known from both human and animal studies, that decreased activity of the cholinergic system in the forebrain can affect attention, learning, memory and contribute to cognitive decline in age related disorders like Alzheimer's disease (AD) [23]. Cholinergic neurons contain high levels of the low affinity p75 nerve growth factor (p75NGF) receptors and are rich in choline acetyl transferase (ChAT) [7]. The immunotoxin ^{192}IgG-saporin was specifically developed by Wiley and co-workers as a neurotoxin selective for cholinergic neurons [49], and has proven to be a powerful tool to study cholinergic degeneration in animal models of disorders like AD [2,23,30].

Starting from the fact that stress or chronically increased glucocorticoid levels have a major impact on hippocampal structure in young and adult animals and humans, which may cause a premature onset of diseases associated with aging [9,26,31,36], the aims of the present study were two-fold: First, the effects of prenatal treatment of synthetic glucocorticoids on spatial learning and memory using the Morris water maze test was investigated; and second, the effects of glucocorticoid prenatal treatment on the vulnerability of cholinergic neurons was evaluated using intracerebroventricular administration of the immunotoxin ^{192}IgG-saporin.

2. Materials and methods

2.1. Animals and prenatal treatment

Pregnant Sprague Dawley rats (Charles River, Calco, Italy) were injected i.p. from gestation day 8 until partus, with dexamethasone (Dexa; soldezam, 0.1 mg/kg), betamethasone (Beta; Bentelan, 0.1 mg/kg) or saline (Cont; 0.9% NaCl in sterile water). Male offspring were chosen for this study, and littermates from nine different litters were divided and hosted in the different treatment groups, i.e. control ($n = 11$), dexamethasone ($n = 11$) and betamethasone ($n = 10$; Table 1). The animals had access to food and water ad libitum and a 12 h light–dark cycle with light hours from 07:00 to 19:00. Body weight was assessed from the 1st day of the behavioral test until the rats were killed. Body weight was also monitored in a separate set of male rats prenatally treated with glucorticoids, from the age of 21 to 80 days.

Table 1
Number of rats included in the present study

	Cont		Beta		Dexa	
Water maze						
Prelesion	11		10		11	
	Cont		Beta		Dexa	
	IgG-S	S	IgG-S	S	IgG-S	S
Water maze						
Postlesion I	6	4	4	4	5	5
Postlesion II	6	3	4	3	5	5
ChAT analysis						
Cortex	6	4	4	3	5	5
Hippocampus	6	4	4	3	5	5
Basal forebrain	6	4	4	3	5	5

The rats were prenatally subjected to dexamethasone (Dexa), betamethasone (Beta), or vehicle (Cont). Morris water maze performance was tested in the adult offspring before (prelesion), and after intracerebroventricular injections with ^{192}IgG-saporin (IgG-S) or unconjugated saporin (S) (postlesion I and postlesion II). At the end of the experiment, ChAT levels were analyzed in the cortex, hippocampus and basal forebrain.

2.2. Surgery

Animals were anesthetized with 10 mg/kg Ketavet (Intervet, Gellini International s.r.l, Aprilia, LT, Italy) placed in a Kopf stereotactic apparatus and injected intracerebroventricular with either ^{192}IgG-saporin (2 μg/4.5 μl; Advanced Targeting System, San Diego, CA, USA) or the corresponding concentration of unconjugated saporin (Advanced Targeting Systems, lot # ATS4-1; 0.44 μg/4.5 μl, according to the manufacturer's recommendations). Injections were made at a speed of 1 μl/min using a 10 μl Hamilton syringe at stereotactic coordinates (in mm, with reference to bregma): rostral = −0.15; lateral = −1.2; ventral = −0.52, and tooth bar set at −0.5.

Animal care and treatments were in accordance with European Community Council Directives of 24 November 1986 (86/609/EEC) and approved by the intramural committee and Ministero Istruzione, Università e Ricerca at Bologna University, in compliance with the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Behavioral tests

Rats were 6 months of age at the beginning of the experiments and behavioral tests were performed 4 weeks prelesion, and at 7- and 12-weeks post-surgery (Fig. 1). Tasks were performed in a circular water maze pool (185 cm diameter) filled with water (22 °C) in a testing room with visual targets on the walls. A visible or hidden platform 2 cm under the water level was used as described below. The Morris water maze protocol used [50] was developed to analyze fine alterations in the