Neuroscience Letters 568 (2014) 67–71

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Changes in ascorbate, glutathione and α-tocopherol concentrations in the brain regions during normal development and moderate hypoglycemia in rats

Anirudh R. Rao [a], Hung Quach [b], Ed Smith [b], Govind T. Vatassery [b,1], Raghavendra Rao [a,c,*]

[a] Division of Neonatology, Department of Pediatrics, University of Minnesota, Minneapolis, MN 55455, USA
[b] Research Service and the Geriatric Research Education and Clinical Center (GRECC), Minneapolis VA Health Care System, Minneapolis, MN 55417, USA
[c] Center for Neurobehavioral Development, University of Minnesota, Minneapolis, MN 55455, USA

HIGHLIGHTS

- Postnatal changes in antioxidant concentrations in the brain regions are unknown.
- Ascorbate, glutathione and α-tocopherol were studied in 7, 14 and 60 day old rats.
- The effect of acute hypoglycemia on the three antioxidants also was determined.
- All three antioxidants were 100–600% higher during development than at adulthood.
- Hypoglycemia led to less antioxidant decrease in developing brain than adult brain.

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form 26 February 2014
Accepted 20 March 2014

Keywords:
α-Tocopherol
Ascorbate
Brain
Glutathione
Hypoglycemia
Rat

ABSTRACT

Ascorbate, glutathione and α-tocopherol are the major low molecular weight antioxidants in the brain. The simultaneous changes in these compounds during normal development, and under a pro-oxidant condition are poorly understood. Ascorbate, glutathione and α-tocopherol concentrations in the olfactory bulb, cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, cerebellum, pons and medulla oblongata were determined in postnatal day (P) 7, P14 and P60 male rats. A separate group of P14 and P60 rats were subjected to acute hypoglycemia, a pro-oxidant condition, prior to tissue collection. The concentrations of all three antioxidants were 100–600% higher in the brain regions at P7 and P14, relative to P60. The neuron-rich anterior brain regions (cerebral cortex and hippocampus) had higher concentrations of all three antioxidants than the myelin-rich posterior regions (pons and medulla oblongata) at P14 and P60. Hypoglycemia had a differential effect on the antioxidants. Glutathione was decreased at both P14 and P60. However, the decrease was localized at P14 and global at P60. Hypoglycemia had no effect on ascorbate and α-tocopherol at either age. Higher antioxidant concentrations in the developing brain may reflect the risk of oxidant stress during the early postnatal period and explain the relative resistance to oxidant-mediated injury at this age.

1. Introduction

Oxidants play a major role in acute brain injury and neurodegenerative disorders [1–3]. A system of antioxidant enzymes and low molecular compounds protects the brain against oxidant-mediated injury. Ascorbate, glutathione (GSH) and α-tocopherol are the major low molecular weight antioxidants in the brain. Ascorbate and GSH are water-soluble, while α-tocopherol is lipid-soluble [1]. Ascorbate and α-tocopherol are transported from plasma across the blood brain barrier *via* specialized transport systems, while GSH is locally synthesized from its constituents, glutamate, glycine and cysteine [4,5]. The three compounds differ in their cellular localization. Ascorbate is primarily localized in neurons and GSH in glia [6]. A primary localization site for α-tocopherol has yet to be determined, although its concentration is higher in the gray matter than the white matter [7]. Supplementation studies confirm that all three compounds independently protect against oxidant-mediated

Abbreviations: GSH, glutathione; P, postnatal day.

* Corresponding author at: Mayo Mail Code 391, 420 Delaware Street, SE, Minneapolis, MN 55455, USA. Tel.: +1 612 625 3260; fax: +1 612 624 8176.
E-mail addresses: raghurao@umn.edu, raghubkrao@gmail.com (R. Rao).

[1] Deceased.

http://dx.doi.org/10.1016/j.neulet.2014.03.035
0304-3940/

injury [8–10]. Additionally, they interact synergistically and help resynthesize those depleted during oxidant exposure [1,6,11]. The three compounds also have non-antioxidant properties and influence neuronal and glial maturation and function [6,12–14].

Ascorbate, GSH and α-tocopherol concentrations vary among the brain regions in adult rodents, with the concentrations being higher in the neuron-rich anterior regions (cerebral cortex and hippocampus) than the myelin-rich posterior regions (pons and medulla oblongata) [7,15–18]. Whether similar interregional variations exist during development is not well understood. In humans and rats, peak brain development occurs postnatally with the posterior brain regions maturing earlier than the anterior regions [19]. Energy-demanding processes, such as synaptogenesis and myelination are primarily postnatal events in both species. Free radicals produced during this metabolically active period could impair brain development by altering gene expression, DNA replication and cell division, suggesting the need for an effective antioxidant system during development.

Previous studies demonstrate that ascorbate concentrations are indeed higher in the developing brain than the mature brain [15,18,20]. A higher brain GSH concentration during development has also been demonstrated in some, but not all studies [15,21]. These studies have evaluated the changes in the whole brain or in a limited number of brain regions. To our knowledge, the developmental changes in α-tocopherol have never been evaluated. Similarly, no studies have compared the effects of a pro-oxidant condition on the three antioxidants in the developing and mature brains. The first aim of this study was to compare the ascorbate, GSH and α-tocopherol concentrations in nine brain regions in postnatal day (P) 7, P14 and P60 rats. The brain is still developing at P7 and P14, while at P60 it is mature [19]. The second aim was to compare the effect of acute hypoglycemia of equivalent severity and duration on the three antioxidants at P14 and P60. Free radicals initiate neuronal injury during hypoglycemia [2,3]. Previous studies have demonstrated that the developing brain is more resistant to injury than the mature brain during hypoglycemia [22,23], likely due to less severe oxidative stress [24]. Our purpose was to gain a better understanding of the role of antioxidants in the age-specific vulnerability, so that neuroprotective strategies can be developed using these compounds.

2. Materials and methods

2.1. Animal preparation

The Institutional Animal Care and Use Committee approved all procedures. Male P7 (body weight, 16 ± 1 g), P14 (39 ± 4 g) and P60 (311 ± 20 g) Sprague-Dawley rats were used. Pregnant rats were purchased (Harlan Laboratories, Indianapolis, IN, USA) and allowed to deliver spontaneously. The litter size was culled to eight on P3. Only males were studied, in order to avoid the confounding effects of sex on the antioxidants [16,25]. Animals in Aim 1 were killed after overnight fasting ($n = 6$ at P14 and P60, and $n = 14$ at P7). A separate set of P14 and P60 rats ($n = 6$) was subjected to overnight fasting and hypoglycemia before tissue collection in Aim 2. P7 rats were not subjected to hypoglycemia, as they do not sustain brain injury in this model [22].

2.2. Induction of hypoglycemia

Acute hypoglycemia was induced using previously published protocol [22,24]. In brief, after overnight fasting, rats were injected subcutaneously with regular insulin in a dose of 10 IU/kg. Fasting was continued and the rats were maintained at an ambient temperature of 34 ± 1 °C. Blood glucose was monitored every 30 min using a glucometer (Accu-Check®, Roche Laboratories, Indianapolis, IN, USA) and maintained between 20 mg/dL and 40 mg/dL as in our previous studies [22,24]. In this model, hypoglycemia (blood glucose <40 mg/dL) is achieved 30 min after the insulin administration and is maintained until 240 min [22,24,26]. The animals were killed 240 min after the insulin injection without correction of hypoglycemia and the brain was collected.

2.3. Tissue preparation

Animals were deeply anesthetized using pentobarbital (120 mg/kg ip) and perfused with ice-cold saline. The brain was removed and the following nine regions were dissected as previously described [7,16]: olfactory bulb, cerebral cortex, hippocampus, striatum, hypothalamus (anterior brain regions), cerebellum, pons, medulla oblongata (posterior brain regions) and midbrain. The tissue samples were manually homogenized and extractions for ascorbate and GSH analyses were performed immediately. The remainder of the homogenate was stored at −70 °C for protein, cholesterol and α-tocopherol analyses. Samples from individual rats were used at P14 and P60, resulting in $n = 6$. At P7, samples from two rats were combined to augment the tissue quantity, resulting in $n = 7$.

2.4. Determination of antioxidants

Tissue antioxidant concentrations were determined by HPLC using published methods described in **Supplementary Material**. Briefly, ascorbate was determined using the procedure of Margolis [27]. The modified method of Ubbink et al. [28,29] with minor revisions was used to determine free and total GSH. Cholesterol and α-tocopherol were determined in the hexane extracts of the tissue homogenate as previously described [30,31]. Total protein was determined using modified Lowry technique [32].

2.5. Statistical analysis

The effects of postnatal age and hypoglycemia on the regional antioxidant concentrations were determined using ANOVA. The intergroup differences were determined using Bonferroni-corrected unpaired t tests. Data are presented as mean ± SD. The ascorbate and GSH data are reported as nmol/mg protein. The α-tocopherol data are reported as nmol α-tocopherol/mmol cholesterol as is conventional [33]. A p value <0.05 was considered significant.

3. Results

3.1. Developmental changes in the antioxidant concentrations in the brain regions

There was a main effect of postnatal age on all three antioxidants in all the nine brain regions ($p < 0.001$; Fig. 1). Ascorbate concentrations were higher at P7, compared with those at P14 and P60 ($p < 0.01$; Fig. 1a). Between P7 and P14, ascorbate concentration decreased markedly in all the brain regions except the cerebellum ($p < 0.001$). The concentration decreased further, but less markedly, between P14 and P60 in all the brain regions, except the olfactory bulb ($p < 0.001$). There were inter-regional variations in the ascorbate concentrations at all the three ages. Overall, the concentrations were higher in the anterior regions than the posterior regions, particularly at P14 and P60 ($p < 0.001$; Fig. 1a).

Free GSH concentrations were also higher during development in all the brain regions ($p < 0.001$; Fig. 1b). Unlike ascorbate, GSH concentrations did not differ between P7 and P14; a P7 > P14