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Epilepsy Research 69 (2006) 165–169

Epilepsy Research

www.elsevier.com/locate/epilepsyres

Short communication

The antioxidant *N*-acetyl-L-cysteine does not prevent hippocampal glutathione loss or mitochondrial dysfunction associated with status epilepticus

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Received 9 December 2005; received in revised form 16 January 2006; accepted 17 January 2006
Available online 20 February 2006

Abstract

Hippocampal reduced glutathione (GSH) levels diminish after status epilepticus (SE), which precedes damage to mitochondrial enzymes, which is associated with cell death. The rat perforant pathway stimulation model was used to assess whether intraperitoneal administration of the GSH precursor *N*-acetyl-L-cysteine (NAC) protected against these changes. NAC (300 mg/kg) treated animals exhibited the same GSH decrease post SE as vehicle treated. Furthermore, NAC treatment had no protective effects on mitochondrial dysfunction.

Keywords: Status epilepticus; Glutathione; Neuroprotection; *N*-Acetyl-cysteine; Oxidative stress; In vivo

1. Introduction

Reduced glutathione (GSH) is an endogenous antioxidant found, within the brain, in astroglial cells. The antioxidant properties of GSH relate to its ability to scavenge reactive oxygen species. We have previously shown that GSH levels diminish significantly in the hippocampus after status epilepticus (SE), preceding later changes suggestive of oxidative damage to mitochondrial enzymes, which is associated with cell death (Sleven et al., 2006). SE is often prolonged, as first line treatment fails in about 40% of cases (Treiman et al., 1998), and has a high mortality rate and considerable morbidity, being associated with the development of learning difficulties, focal neurological deficits and chronic epilepsy in survivors (Shorvon, 1994; Walker, 1998). Both cell death and mitochondrial dysfunction are thought to be relevant to this seizure related brain damage (Cock, 2005), and possibly also to epileptogenesis (Kunz, 2002). Thus antioxidant/neuroprotective compounds are of therapeutic interest, both in the context of status epilepticus and potentially for patients with chronic intactable epilepsy. *N*-Acetyl-cysteine (NAC) is a thiol which can act as a precursor for glutathione synthesis

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doi:10.1016/j.eplepsyres.2006.01.006

as well as a stimulator of the cytosolic enzymes involved in glutathione regeneration (Banaclocha, 2001), and which when administered systemically has been shown to significantly increase brain GSH levels in rats (Pocernich et al., 2000). Furthermore, NAC can directly protect against oxidative damage by interacting with reactive oxygen species (Aruoma et al., 1989; Banaclocha, 2001). In vitro and in vivo evidence indicates that NAC offers some protection against age-related mitochondrial dysfunction (Martinez, 2000; Miquel et al., 1995). We set out to evaluate whether NAC protects against GSH depletion and mitochondrial dysfunction following SE in a rat model.

2. Methods

Unless specified all reagents were purchased from Sigma–Aldrich (Dorset, UK) or VWR international (Poole, UK).

2.1. Perforant pathway stimulation

All experimental procedures were within the guidelines of the Animals (Scientific Procedures) Act, 1986. Stimulating and recording electrodes (Plastics One, Bilaney Consultants Limited, Sevenoaks, UK) were surgically implanted in the perforant pathway and dentate granule cell layer respectively, of anaesthetised male Sprague-Dawley rats (Cock et al., 2002). Five to 7 days later, the perforant pathway was stimulated (2 mA, 50 μs monopolar pulses, 20 Hz) in freely moving animals for 2 h to induce self-sustaining status epilepticus (SSSE). Thirty minutes into SSSE, NAC (300 mg/kg; dissolved in phosphate buffered saline (PBS)) or vehicle (PBS, 3 ml/kg) was administered i.p. After a further 2.30 h, diazepam (20 mg/kg, i.p.) was administered to terminate seizures. A further three injections of NAC or PBS were administered 12 hourly until the rats were sacrificed 44 h post stimulation. Both hippocampi were extracted from the brain, and micro-dissected on ice into three discrete regions, CA1, CA3 and dentate gyrus/hilus (DG/H). The pooled left and right samples of each region were homogenised and stored at −70 °C (Cock et al., 2002). An aliquot from each sample was diluted 1:40 in 0.1% orthophosphoric acid for later GSH detection.

2.2. Biochemical studies

Samples were analysed for GSH levels using an electrochemical HPLC system, as previously described (Cock et al., 2002). Enzyme assays were performed within 7 days of sample collection. Assays for citrate synthase (CS), complex I, complex II/III, aconitase and alphaketoglutarate dehydrogenase (AKGDH) were performed on homogenised samples as described previously (Cock et al., 2002). Results presented are mean values of triplicate assays, which fell within 10% of one another. Samples from atleast one stimulated and one sham animal were assayed in parallel, with the experimenter blinded to the sample origin. The protein content of each sample was measured using the method of Lowry (Lowry et al., 1951).

2.3. Statistical analysis

Statistical comparisons were performed using multifactorial analysis of variance (ANOVA) followed by LSD post hoc analysis for significant main effects on SPSS for windows (Version 12.0). Statistical significance was taken as follows: not significant (ns) $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$ and $^{***}P < 0.005$.

3. Results

3.1. GSH levels

Levels of GSH in the hippocampi of sham and status rats are shown in Table 1. Compared to sham animals, status animals treated with vehicle had signif-

Table 1
Levels of GSH in hippocampal regions of sham-operated rats compared to stimulated rats treated with vehicle (PBS) or NAC

Hippocampal region	GSH levels (nmol/mg protein)		
	Sham ($n = 7$)	Status + PBS ($n = 6$)	Status + NAC ($n = 6$)
DG/H	12.4 ± 0.6	10.1 ± 0.8*	10.3 ± 1.1*
CA1	10.2 ± 0.6	9.5 ± 0.8	8.3 ± 0.5
CA3	14.0 ± 1.5	10.3 ± 0.9***	9.3 ± 0.4***

Values are mean ± S.E.M. GSH levels in stimulated rats treated with PBS (status + PBS) and NAC (status + NAC) were compared to levels in sham rats using one way ANOVA followed by post hoc LSD test.
* $P < 0.05$.
*** $P < 0.005$.