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Taurine deficiency damages photoreceptors and retinal ganglion cells in vigabatrin-treated neonatal rats

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ABSTRACT

The anti-epileptic drug vigabatrin induces an irreversible constriction of the visual field, but is still widely used to treat infantile spasms and some forms of epilepsy. We recently reported that vigabatrin-induced cone damage is due to a taurine deficiency. However, optic atrophy and thus retinal ganglion cell degeneration was also reported in children treated for infantile spasms. We here show in neonatal rats treated from postnatal days 4 to 29 that the vigabatrin treatment triggers not only cone photoreceptor damage, disorganisation of the photoreceptor layer and gliosis but also retinal ganglion cell loss. Furthermore, we demonstrate in these neonatal rats that taurine supplementation partially prevents these retinal lesions and in particular the retinal ganglion cell loss. These results provide the first evidence of retinal ganglion cell neuroprotection by taurine. They further confirm that taurine supplementation should be administered with the vigabatrin treatment for infantile spasms or epilepsy.

Introduction

Infantile spasms are severe epileptic encephalopathies with heterogeneous aetiologies that develop in infancy and early childhood. Vigabatrin (VGB) has been found to be effective for treating infantile spasms and complex partial seizures in adults (Ben-Menachem et al., 2008; Curatolo et al., 2006). VGB or gamma-vinyl GABA is an irreversible and highly selective inhibitor of γ-aminobutyric acid (GABA) transaminase, resulting in an increase in tissue GABA concentrations. Unfortunately, long-term VGB treatment generates irreversible adverse secondary events leading to a bilateral concentric constriction of the visual field in 10 to 40% of adult patients depending on which study was considered (Eke et al., 1997; Krauss et al., 1998; Ruether et al., 1998). This loss in visual field is associated with amplitude decreases of both the photopic electroretinogram (ERG) b-wave and the 30 Hz flicker response (Krauss et al., 1998; Miller et al., 1999; van der Torren et al., 2002). In infants, visual losses were indicated by a decrease of both the photopic ERG b-wave amplitude and the flicker responses (Westall et al., 2002). Furthermore, atrophy of the optic nerve was detected in some VGB-treated infants using in vivo imaging technique (Buncic et al., 2004; Frisen and Malmgren, 2003). Such optic nerve atrophy was subsequently also reported in VGB-treated adult patients with visual field loss (Wild et al., 2006). Optic nerve atrophy was confirmed by histological analysis of post-mortem retinal tissues (Ravindran et al., 2001). In this case, not only had the photoreceptors been damaged, but there was also evidence for the loss of retinal ganglion cell (RGC) bodies and the loss of their axons in the retinal fibre layer. Despite these damaging secondary effects, VGB remains the first line treatment for infantile spasms in Europe and was recently approved in the US for this application (Ben-Menachem et al., 2008; Curatolo et al., 2006).

Retinal toxicity of VGB was first described in albino rats (Butler et al., 1987) and has subsequently been investigated in rabbits (Ponjavic et al., 2004) and mice (Wang et al., 2008). The most obvious retinal damage reported was the disorganisation of the peripheral retinal photoreceptor layer in VGB-treated animals (Butler et al., 1987; Duboc et al., 2004; Wang et al., 2008). Cone photoreceptors

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were later found to degenerate (Duboc et al., 2004; Wang et al., 2008); glial cells significantly increased their expression of the glial fibrillary acidic protein (GFAP), a classic marker for retinal lesions (Duboc et al., 2004; Ponjavic et al., 2004) and a major synaptic plasticity was observed at rod terminals with the formation of ectopic synapses (Wang et al., 2008). Indeed, rod bipolar and horizontal cells were found to grow long dendrites into the photoreceptor nuclear layer contacting displaced rod terminals. Finally, ERG measurements showed changes such as amplitude decreases of both the photopic ERG and the flicker response (Duboc et al., 2004; Ponjavic et al., 2004). Considering the mechanisms of retinal toxicity, it was first suggested that VGB mediates phototoxicity, as albino animals alone are susceptible to the toxic effects of VGB (Butler et al., 1987). This suggestion was further supported by photoreceptor degeneration observed in retinal explants exposed to light of strong intensity in the presence of VGB; however, this degeneration was not observed in the presence of GABA (Izumi et al., 2004). Recently, we confirmed that VGB mediates phototoxicity by showing that albino animals maintained in darkness experienced no features of retinal toxicity (Jammoul et al., 2009). Furthermore, we found that VGB-treated animals had lower taurine plasma levels and that taurine supplementation prevented the development of retinal lesions (Jammoul et al., 2009). The clinical relevance of these findings was observed in five infants with undetectable taurine plasma levels or at least levels below normal ranges (Jammoul et al., 2009). These effects of taurine deficiency are consistent with mechanisms of phototoxicity because retinal lesions are known to be exacerbated by light in taurine-deficient animals (Rapp et al., 1988). However, no RGC degeneration was demonstrated in VGB-treated animals or in taurine-deficient animals.

As all previous preclinical studies involved adult animals, we investigated the nature of retinal lesions in VGB-treated neonatal rats, providing an animal model that more closely resembled infants treated for infantile spasms. An important aim was to examine whether RGCs degenerate in VGB-treated neonatal rats, as found in infants. Furthermore, we wanted to define whether this eventual cell loss and other retinal damage were also caused by taurine deficiency in these neonatal animals. This work was previously presented as an abstract (Jammoul., ARVO abstract [3605], 2009).

Results

We had demonstrated previously that VGB leads to taurine deficiency in adult animals (Jammoul et al., 2009). In the current study, VGB (0.6 mg/day, representing 50 mg/kg for the smallest animal sizes) was injected into 4-day-old rats for 25 days (age of retinal maturation) to model retinal lesions observed in VGB-treated infants and a group of VGB-treated neonatal rats was supplemented with taurine by intraperitoneal injections (5 mg/day representing 420 mg/kg for the smallest animal size) (10 animals per group). To examine whether VGB treatment produced similar taurine deficiency during the developmental period, rat blood was collected at the end of the injection period. Table 1 provides the amino acid measurements in three groups: control rats (group I), VGB-treated animals (group II) and VGB-treated animals receiving taurine supplementation (group III). For all tested amino acids, a statistically significant decrease was observed only for taurine in the plasma of VGB-treated animals (group II) (Fig. 1). Taurine levels were 26.8% lower in VGB-treated neonatal rats (group II: 178.7 ± 23.6 µM, $n = 10$, s.e.m., $p < 0.05$) than in the control group (group I: 244.9 ± 54.3 µM, s.e.m., $n = 10$). Taurine supplementation restored taurine plasma concentrations to normal (group III: 243.1 ± 43.1 µM, $n = 10$, s.e.m.) (Fig. 1). These measurements indicated that VGB also triggers a taurine decrease in neonatal rats.

To determine if VGB treatment and subsequent taurine plasma level decreases induced retinal dysfunction, photopic ERGs were measured in the three animal groups. Fig. 2A illustrates representative photopic ERGs in these groups. VGB-treated rats (group II) exhibited significantly lower ERG amplitudes than the control group (Fig. 2B, $p < 0.001$). Taurine supplementation (group III) restored 77.2% of the decrease (51.7%) in photopic ERG amplitude resulting from the VGB treatment (Fig. 2); the difference with the group of VGB-treated animals (group II) was statistically significant ($p < 0.001$) but that with the control group (group I) was no longer statistically significant. Thus, VGB induces retinal dysfunction in neonatal rats that could be restored by taurine supplementation.

To examine whether retinal dysfunction was related to cellular damage, a histological examination of retinal vertical sections was performed (Fig. 3). As originally described in adult animals (Butler et al., 1987; Duboc et al., 2004; Jammoul et al., 2009), VGB induced a disorganisation of the photoreceptor nuclear layer with nuclei displaced toward the retinal pigment epithelium in neonatal rats (Figs. 3A–B). If VGB-treated animals were administered taurine, the disorganized retinal layer persisted (Fig. 3C), but its length was reduced by 67.5% (Fig. 3J, $p < 0.05$). To further analyse the extent of retinal lesions, retinal sections were immuno-labelled against the glial fibrillary acidic protein (GFAP), a classic marker for retinal lesions (Picaud et al., 1993). In control animals, few glial proteins were

Table 1
Amino acid levels in the plasma of neonatal rats untreated (control), treated with vigabatrin (VGB) or treated with both vigabatrin and taurine (VGB + taurine). The differences were statistically significant for taurine level between VGB-treated animals and either the control group or VGB-treated animals administered with taurine. Measurements are provided in µM ($n = 10$, s.e.m. $^*p < 0.05$).

Amino acids	Control	VGB	VGB + taurine
Taurine	244.9 ± 54.3	178.7 ± 23.6	244.3 ± 42.5*
Threonine	153.2 ± 32.7	158.3 ± 30.3	169.3 ± 35.1
Serine	198.7 ± 40.6	206.4 ± 40.2	224.3 ± 32
Glutamic acid	110.5 ± 34.2	104.8 ± 37.7	113.8 ± 25.5
Valine	189.1 ± 27.4	184.5 ± 33	176.8 ± 28.1
Citrulline	135.5 ± 18.3	126.7 ± 16	142.8 ± 18
Alanine	521.2 ± 99.6	516.3 ± 115.1	531 ± 77.3
Glycine	225.4 ± 46.3	222.6 ± 36.6	254.2 ± 52
Proline	318.4 ± 62.2	310 ± 50.3	313.6 ± 62.5
Glutamine	694.2 ± 71	621 ± 88.5	614.1 ± 76.4
Methionine	54.9 ± 10.5	57.3 ± 8	58.2 ± 9.8
Isoleucine	93.6 ± 12.1	92.7 ± 15.3	88.8 ± 13.6
Leucine	169.1 ± 20.5	193.3 ± 36.7	191.2 ± 46.2
Tyrosine	64.3 ± 12.2	68.5 ± 16.4	67.6 ± 18.1
Phenylalanine	62.4 ± 6.4	62.5 ± 8.3	64.7 ± 7.3
Arginine	135.7 ± 19.5	125.1 ± 30.9	128.4 ± 22.5
Histidine	46.3 ± 5.5	51.8 ± 10.5	48.7 ± 4.4
Lysine	265 ± 47.7	223.8 ± 89.1	241.6 ± 24.5
Ornithine	85.8 ± 13.9	84 ± 16.7	90.1 ± 14.6

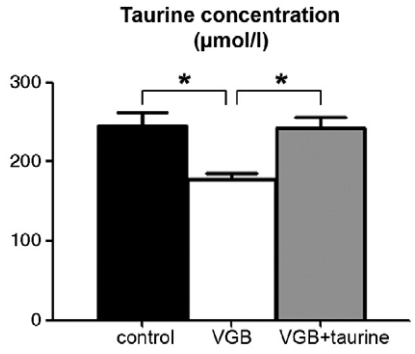

Fig. 1. Taurine deficiency in VGB-treated neonatal rats. Plasma taurine levels were measured in control animals ($n = 10$) and in rats treated with VGB from postnatal day 4 to postnatal day 29 with (VGB + taurine, $n = 10$) or without (VGB, $n = 10$) taurine administration. (s.e.m., $^*p < 0.05$).