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Thiamine and benfotiamine prevent stress-induced suppression of hippocampal neurogenesis in mice exposed to predation without affecting brain thiamine diphosphate levels

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ABSTRACT

Thiamine is essential for normal brain function and its deficiency causes metabolic impairment, specific lesions, oxidative damage and reduced adult hippocampal neurogenesis (AHN). Thiamine precursors with increased bioavailability, especially benfotiamine, exert neuroprotective effects not only for thiamine deficiency (TD), but also in mouse models of neurodegeneration. As it is known that AHN is impaired by stress in rodents, we exposed C57BL6/J mice to predator stress for 5 consecutive nights and studied the proliferation (number of Ki67-positive cells) and survival (number of BrdU-positive cells) of newborn immature neurons in the subgranular zone of the dentate gyrus. In stressed mice, the number of Ki67- and BrdU-positive cells was reduced compared to non-stressed animals. This reduction was prevented when the mice were treated (200 mg/kg/day in drinking water for 20 days) with thiamine or benfotiamine, that were recently found to prevent stress-induced behavioral changes and glycogen synthase kinase-3β (GSK-3β) upregulation in the CNS. Moreover, we show that thiamine and benfotiamine counteract stress-induced bodyweight loss and suppress stress-induced anxiety-like behavior. Both treatments induced a modest increase in the brain content of free thiamine while the level of thiamine diphosphate (ThDP) remained unchanged, suggesting that the beneficial effects observed are not linked to the role of this coenzyme in energy metabolism. Predator stress increased hippocampal protein carbonylation, an indicator of oxidative stress. This effect was antagonized by both thiamine and benfotiamine. Moreover, using cultured mouse neuroblastoma cells, we show that in particular benfotiamine protects against paraquat-induced oxidative stress. We therefore hypothesize that thiamine compounds may act by boosting anti-oxidant cellular defenses, by a mechanism that still remains to be unveiled. Our study demonstrates, for the first time, that thiamine and benfotiamine prevent stress-induced inhibition of hippocampal neurogenesis and accompanying physiological changes. The present data suggest that thiamine precursors with high bioavailability might be useful as a complementary therapy in several neuropsychiatric disorders.

1. Introduction

Increasing evidence suggests that thiamine (vitamin B1) and precursors with higher bioavailability can exert prominent neuroprotective effects in the mammalian brain (Gibson et al., 2016; Pan et al., 2016). It is well known that the principal phosphorylated derivative of thiamine, thiamine diphosphate (ThDP), is an essential cofactor for glucose metabolism, being required for the activity of transketolase and the mitochondrial pyruvate and oxoglutarate dehydrogenase complexes.

Abbreviations: AD, antidepressant; AHN, adult hippocampal neurogenesis; BrdU, bromodeoxyuridine; GCL, granule cell layer; GSK-3β, glycogen synthase kinase-3β; NS-NT, non stressed-non treated; S-BFT, stressed-treated with benfotiamine; S-NT, stressed-not treated; S-Thia, stressed-treated with thiamine; TD, thiamine deficiency; ThDP, thiamine diphosphate; ThMP, thiamine monophosphate.

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Therefore, it is not surprising that thiamine deficiency has deleterious effects on brain activity, which heavily relies on oxidative glucose metabolism (Gibson and Blass, 2007). However, it has long been thought that a general impairment of brain energy metabolism does not adequately account for the selective vulnerability of diencephalic structures in thiamine deficiency (TD). This has led to the idea that thiamine may exert neuromodulatory or neuroprotective actions through mechanisms unrelated to its coenzyme role (Bettendorff, 1994; Bettendorff, 2013; Mkrtchyan et al., 2015).

The vulnerability of the brain to TD is thought to be owing to the slow absorption of thiamine through the intestinal epithelium and through the blood-brain barrier (Greenwood et al., 1982). Therefore, lipophilic precursors with higher bioavailability have been developed in order to increase the absorption of the vitamin. The widely used precursor benfotiamine (S-benzoylthiamine-O-monophosphate) is not lipophilic, but, after oral administration, it is dephosphorylated by intestinal ecto-alkaline phosphatases to the liposoluble S-benzoylthiamine, which in turn is converted to thiamine in liver and blood (Volvert et al., 2008). Benfotiamine had first been used as a possible treatment for microvascular complications of type-2 diabetes (Hammes et al., 2003; Marchetti et al., 2006; Beltramo et al., 2008). Further studies on various pathological conditions, have shown the beneficial effects of benfotiamine treatment in several animal models (Balakumar et al., 2010; Sanchez-Ramirez et al., 2006), including a mouse model of Alzheimer's disease (Pan et al., 2010), as well as an improvement of cognitive function in Alzheimer's patients (Pan et al., 2016).

As TD is associated with memory loss, even before the appearance of diencephalic lesions (Vetreno et al., 2011), and since cognitive impairment is generally associated with hippocampal dysfunction, such possible alterations were investigated in mice undergoing TD at a pre-pathological lesion stage (Zhao et al., 2008). In these TD mice, learning abilities were markedly decreased and this was concomitant with an impairment of progenitor cell proliferation and neurogenesis in the dentate gyrus. Our own recent studies revealed memory enhancing effects of thiamine and benfotiamine when orally administered to mice for twenty days, on two hippocampus-dependent forms of memory, such as fear conditioning and step down avoidance tests (Markova et al., 2017).

Many studies have shown that, in rodents and other mammals, exposure to stress causes a marked impairment of adult hippocampal neurogenesis (AHN) (Gould et al., 1998; Malberg and Duman, 2003) and antidepressants protect against this decreased AHN (Warner-Schmidt and Duman, 2006; David et al., 2009; Miller and Hen, 2015). Environmental enrichment and physical exercise also protect against impairment of AHN (Snyder et al., 2009). Thus, it appears that AHN is controlled by a number of different factors and it can be anticipated that several kinds of drugs (in addition to antidepressants) could be used to protect and boost neurogenesis when impaired by stressful events.

We therefore considered the possibility that thiamine and/or benfotiamine might exert protective effects on AHN when mice are exposed to stressful events. Indeed, the involvement of thiamine-dependent protective mechanisms in stress response has previously been reported in forced swim and immobilization stress models (Dief et al., 2015). Recently, we have shown that thiamine as well as benfotiamine have antidepressant effects, that are associated with reduced glycogen synthase kinase-3β (GSK-3β) expression (Markova et al., 2017). Therefore, we hypothesized that thiamine or benfotiamine could improve hippocampal neurogenesis and we investigated possible protective effects of thiamine and benfotiamine treatment on mice subjected to predator stress. As stress, including predation stress, is known to inhibit hippocampal neurogenesis (Tanapat et al., 2001; Hanson et al., 2011a; Hanson et al., 2011b), we chose a previously validated 5-day stress paradigm (Strekalova et al., 2015; Markova et al., 2017) in which stressed mice display reduced proliferation of progenitor cells and decreased survival of newborn neurons in the subgranular zone of the dentate gyrus. In the present study, we show that both thiamine and benfotiamine efficiently prevent stress-induced impairment of hippocampal neurogenesis. They also protect against weight loss, anxiety-like behavior and oxidative stress. These protective effects were not accompanied by any increase in brain content of the essential coenzyme ThDP, strongly suggesting that the beneficial effects of thiamine and benfotiamine are not due to boosting of brain energy metabolism and implicate non-cofactor roles of thiamine in the brain.

2. Methods

2.1. Animals

Three-month-old male C57BL/6 J mice were supplied by Instituto Gulbenkian de Ciência, Oeiras, Portugal). Two to five-months-old Wistar rats (Medical Faculty of New Lisbon University, Lisbon, Portugal) were used for predator stress. The animals for experiment 4 (cohort 4) were from Pushchino Research Center of Russian Academy of Sciences, Moscow Region by a provider licensed by Charles River (http://www.spf-animals.ru/about/providers/animals, accessed 3.04.2017). Mice and rats were single housed under a reversed 12-h light–dark cycle (lights on: 21:00 h) with food and water ad libitum, under controllable laboratory conditions (22 ± 1 °C, 55% humidity). Experiments were carried out in accordance with the European Communities Council Directive for the care and use of laboratory animals (2010/63/EU) and were approved by respective local governmental bodies.

2.2. Reagents

Thiamine and benfotiamine were from Sigma-Aldrich NV/SA (Diegem, Belgium). Thiamine hydrochloride (1.7 g/l or 5 mM) was dissolved in water and pH was adjusted to 7 with NaOH. Benfotiamine (1.7 g/l or 3.7 mM) was dissolved in alkalinized water and pH was adjusted to 7 with HCl. Bromodeoxyuridine (BrdU, Sigma-Aldrich) was dissolved in 0.9% NaCl and 0.007 M NaOH. Primary antibody rat anti-BrdU (1:500, AbD Serotec, Raleigh, NC, USA), primary antibody mouse anti-Ki67 (1:500, BD Biosciences, San Jose, CA, USA) anti-rat and anti-mouse secondary antibodies (1:500, Jackson ImmunoResearch, Europe Ltd., Suffolk, U.K.) were used.

2.3. Experimental design

In a first experiment, 3.5-month-old male C57Bl/6 J mice ($n = 40$) were randomly divided into 4 experimental groups ($n = 10$ for each group): not stressed-not treated (NS-NT), stressed-not treated (S-NT), stressed-treated with thiamine (S-Thia) and stressed-treated with benfotiamine (S-BFT). In each group of 10 mice, 5 animals belonged to the first cohort (that would receive BrdU injections, see below) and the other 5 belonged to the second cohort. Mice were single housed and received either vehicle (tap water), thiamine (200 mg/kg/day) or benfotiamine (200 mg/kg/day) in tap water ad libitum for the 20 days of experiment. Thiamine and benfotiamine solutions were replaced every 3 days. At day 14, before the first stress session, mice of the first cohort received four BrdU (50 mg/kg) intraperitoneal injections, spaced one from the others by 2 h. Between day 15 and 20, mice from 3 experimental groups (S-NT, S-Thia, S-BFT) underwent predator stress, i.e. rat exposure while in a small container (Strekalova et al., 2015): mice were introduced into transparent glass cylinder (15 cm high × 8 cm diameter) and placed into the rat cage. 15-h exposure was performed between 18:00 and 9:00 for 5 consecutive nights. Mice only had access to food and water between the stress sessions.

To perform Ki67 and BrdU detection (Fig. 1, Exp 1), mice of the first cohort (4 × 5 animals) were sacrificed 24 h after the last stress session. They were deeply anaesthetized with Nembutal (CEVA, Santé Animale, Brussels, Belgium, 0.01 ml/g body weight) and transcardially perfused