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Thiamine and benfotiamine improve cognition and ameliorate GSK-3β-associated stress-induced behaviours in mice

Nataliia Markova [a,b,c,d,1], Nataliia Bazhenova [a,c,d,1], Daniel C. Anthony [e,*], Julie Vignisse [a,f], Andrey Svistunov [d], Klaus-Peter Lesch [a,d,g], Lucien Bettendorff [a,f,1], Tatyana Strekalova [a,d,**,1]

[a] EURON - European Graduate School for Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Universiteitssingel 40, NL 6229 ER Maastricht, Netherlands
[b] Institute of Physiologically Active Compounds, Russian Academy of Sciences, Severnii proezd, 1, Chernogolovka 142432, Russia
[c] Institute of General Pathology and Pathophysiology, Baltiiskaya str, 8, Moscow 125315, Russia
[d] I.M.Sechenov First Moscow Medical University, Moscow, Russia
[e] Department of Pharmacology, Oxford University, Mansfield Road, OX1 3QT Oxford, UK
[f] GIGA-Neurosciences, University of Liege, 4000, Liege, Belgium
[g] Division of Molecular Psychiatry, Laboratory of Translational Neuroscience, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Fuechsleinstrasse 15, 97080 Wuerzburg, Germany

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ABSTRACT

Thiamine (vitamin B1) deficiency in the brain has been implicated in the development of dementia and symptoms of depression. Indirect evidence suggests that thiamine may contribute to these pathologies by controlling the activities of glycogen synthase kinase (GSK)-3β. While decreased GSK-3β activity appears to impair memory, increased GSK-3β activity is associated with the distressed/depressed state. However, hitherto direct evidence for the effects of thiamine on GSK-3β function has not been reported. Here, we administered thiamine or, the more bioavailable precursor, benfotiamine at 200 mg/kg/day for 2 weeks to C57BL/6J mice, to determine whether treatment might affect behaviours that are known to be sensitive to GSK-3β activity and whether such administration impacts on GSK-3β expression within the brain. The mice were tested in models of contextual conditioning and extinction, a 5-day rat exposure stress test, and a modified swim test with repeated testing. The tricyclic antidepressant imipramine (7.5 mg/kg/day), was administered as a positive control for the effects of thiamine or benfotiamine. As for imipramine, both compounds inhibited the upregulation of GSK-3β induced by predator stress or repeated swimming, and reduced floating scores and the predator stress-induced behavioural changes in anxiety and exploration. Coincident, thiamine and benfotiamine improved learning and extinction of contextual fear, and the acquisition of the step-down avoidance task. Our data indicate that thiamine and benfotiamine have antidepressant/anti-stress effects in naïve animals that are associated with reduced GSK-3β expression and conditioning of adverse memories. Thus thiamine and benfotiamine may modulate GSK-3β functions in a manner that is dependent on whether the contextual conditioning is adaptive or maladaptive.

1. Introduction

Thiamine (vitamin B1) is a pivotal regulator of mitochondrial function and metabolism. Thiamine is the precursor of thiamine diphosphate, and acts as a cofactor for several rate limiting enzymes in the Krebs cycle and the pentose phosphate pathway (Bettendorff et al., 2014). Compromised thiamine-dependent processes result in mitochondrial dysfunction and downstream oxidative stress, excitotoxicity, inflammatory changes, decreased neurogenesis and blood-brain barrier disruption (Abdou and Hazell, 2015). In addition, thiamine plays a role in the structural stabilization of neuronal membranes via non-enzymatic mechanisms (Mkrtchyan et al., 2015). Deficiencies in thiamine metabolism accompany chronic alcohol exposure and diabetes, and lead to neurodegenerative and depressive symptoms (Benton and Donohoe, 1999; Abdou and Hazell, 2015). Strikingly, the addition of a daily dose of 300 mg thiamine compared to 6 weeks of standard fluoxetine treatment was shown to improve Hamilton Depression Rating Scores in patients with major depression in a recent randomized double-blind placebo-controlled clinical trial (Ghaleiha et al., 2016).

* Correspondence to: D.C. Anthony, Department of Pharmacology, University of Oxford, University Mansfield Road, OX1 3QT, UK.
** Correspondence to: T. Strekalova, Department of Neuroscience, Maastricht, Universiteitssingel 40, NL 6229 ER Maastricht, Netherlands.
E-mail addresses: daniel.anthony@pharm.ox.ac.uk (D.C. Anthony), t.strekalova@maastrichtuniversity.nl (T. Strekalova).
[1] These authors contributed to this work equally.

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A number of thiamine precursors that exhibit improved bioavailability have been described, including benfotiamine, which has been investigated in animal models of Alzheimer's disease and other conditions to explore its efficacy (Balakumar et al., 2010; Portari et al., 2013). Both thiamine and benfotiamine suppress oxidative stress, inflammation and apoptosis (Abdou and Hazell, 2015). An 8-week treatment of benfotiamine reduced learning deficiencies (measured in the Morris Water maze), and decreased cortical amyloid plaque formation and phosphorylated tau levels in amyloid precursor protein/presenelin-1 overexpressing mice in a dose-dependent manner (Pan et al., 2010). Studies using supplementary thiamine in patients with Alzheimer's disease failed to reveal any clinical efficacy, but this was ascribed to limited intestinal absorption in these patients (Rodríguez-Martín et al., 2001).

The mode of action of thiamine in depressive illness and neurodegenerative disease is unclear. The anti-thiamine compound pyrithiamine and diet-induced thiamine deficiency decrease the phosphorylation rates of glycogen synthase kinase-3β (GSK-3β) and raise its enzymatic activity (Zhao et al., 2014). GSK-3β activation has become a well-recognised marker of distress and depression (Beurel et al., 2015). Benfotiamine decreases GSK-3β activity in vitro by increasing the phosphorylation of GSK-3β at serine 9 (p9SGSK-3β), which renders the enzyme inactive (Sun et al., 2012). Oral dosing of mice with thiamine or benfotiamine for 2 weeks increased brain thiamine levels and, for benfotiamine, an 8-week-dosing regimen reduced GSK-3β activities via increasing the proportion of p9SGSK3β in amyloid precursor protein/presenelin-1 overexpressing mice (Pan et al., 2010). Thus while downregulation of GSK-3β has been implicated as the mediator of thiamine-dependent mechanisms in the CNS, to date, there has been no direct in vivo evidence for its role under normal conditions.

Inactivation of GSK-3β results in learning and long-term potentiation deficiencies (Sintoni et al., 2013; Jurado-Arjona et al., 2016), while thiamine-mediated functions are important for brain plasticity (Bettendorff, 2014). Low GSK-3β activity has been linked to anti-depressant changes (Kaidanovich-Beilin and Woodgett, 2011; Li et al., 2014), which, as mentioned above, were recently reported to result from chronic thiamine administration (Ghaleiha et al., 2016). Given these seemingly opposing effects of GSK-3β inactivation on cognition and depression/stress-related mechanisms, we studied whether administration of thiamine or benfotiamine (200 mg/kg/day, p.o.) could alter GSK-3β-dependent tasks such as the acquisition of memory, depressive-like behaviour and the stress response. Thiamine- or benfotiamine-treated C57BL/6J mice were studied in the modified swim test, where depressive-like behaviour has previously been shown to correlate with over-expression of brain GSK-3β in the brain (Strekalova et al., 2016) and a 5-day rat exposure stress paradigm, which suppresses hippocampal neurogenesis and increases anxiety scores in mice (Strekalova et al., 2015b). Finally, contextual fear learning and its extinction, in which GSK-3β mechanisms are well known to have a pivotal role (Chew et al., 2015), are also investigated here (Vignisse et al., 2011, 2014).

2. Methods

2.1. Animals

3.5-month-old male C57BL/6J mice used in the study were obtained from the Gulbenkian Institute of Science, Oeiras, Portugal. 14 days before the start of the behavioural tests, mice were housed individually for acclimatization to a new facility, under a reversed 12-h light–dark cycle (lights on: 21:00 h) with food and water ad libitum, under constant controlled laboratory conditions (22 ± 1 °C, 55% humidity). All experiments were carried out in accordance with the European Committees Council Directives with the European Union's Directive 86/609/EEC and Council Directive 93/119/EC, and had been approved by the ethic committee for animal research of Maastricht University CPV and by General Directory of Ethical Committee of the New Lisbon University.

2.2. Study design

Mice were administered with thiamine, benfotiamine or imipramine via drinking water for two weeks and subjected to the modified swim test, predation stress, or a set of memory tests (Fig. 1A–C; group sizes are given in Figs. 2–4) (see below for details). For the modified swim test, sessions of 6 min in duration were performed on Days 1 and 2 and 5, and animals were culled 10 minute post-test, and simultaneously with naïve mice that were not exposed to the swim test or administration of substances. The hippocampus and prefrontal cortex were dissected and prepared for GSK-3β RT-PCR analysis and an ELISA assay was used to determine total and proportion of 9-Ser-phosphorylation in the hippocampus (Fig. 1A). All procedures were carried out after at least 1-hour acclimatization time to experimental room.

2.3. Behaviour

2.3.1. Swim test

In the modified swim test model, mice were exposed to a two-day swimming protocol (day 1 and day 2); they were then tested on again on day 5 (Markova et al., 2013, 2014; Strekalova et al., 2016). In each immersion, naïve mice or those dosed with imipramine, thiamine or benfotiamine were placed for 6 min in a transparent cylinder (Ø 17 cm) filled with water (+23 °C, water height 13 cm, height of cylinder 20 cm, under subdued lighting). Floating behaviour, defined by the absence of any directed movement of the head or body, was manually scored using previously established criteria using Noldus EthoVision XT 8.5 (Noldus Information Technology, Wageningen, The Netherlands) and CleverSys (CleverSys, Reston, VA, USA) as described elsewhere (Malatynska et al., 2012). The total time spent floating was calculated for the entire duration of the test.

2.3.2. Elevated O-maze

The apparatus (Technosmart, Rome, Italy) consisted of a circular path (runway width 5.5 cm, diameter 46 cm) that was placed 45 cm above the floor. The two opposing arms were protected by walls (closed area, height 10 cm), and the illumination strength was 25 lux. The apparatus was placed on a dark surface in order to maintain control over lighting conditions during testing. At the start, mice were placed in one of the closed-arm area of the apparatus, behaviour was video recorded and assessed for a 5-minute observation period as described elsewhere (Vignisse et al., 2011; Strekalova et al., 2015a). The latency to the first exit into the open compartments of the maze and the number of exits to the open arms were recorded.

2.3.3. Novel cage test

The novel cage test was performed to assess vertical exploratory activity in a new environment (Strekalova and Steinbusch, 2010; Couch et al., 2013). Mice were introduced into a standard plastic cage (21 × 21 × 15 cm) filled with fresh sawdust. The number of exploratory rears each minute was counted under red light for a 5-min period.

2.3.4. Step-down passive avoidance test

The step-down apparatus (Technosmart, Rome, Italy) consisted of a transparent plastic cubicle (25 cm × 25 cm × 50 cm) with a stainless-steel grid floor (33 rods 2 mm in diameter) onto which a square wooden platform (7 cm × 7 cm × 1.5 cm) was placed. A shocker was used to deliver an alternating electric current (AC, 50 Hz, Evolocus, Terrytown, NY, USA). In this paradigm, animals were trained not to step down from a platform onto a grid floor to avoid an electric shock. During the training session, mice were placed onto the platform inside a transparent cylinder for 30 s to prevent them from immediately stepping down. After removal of the cylinder, the time until the animal left the platform with all