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Short communication

Insulin receptor sensitizer, dicholine succinate, prevents both Toll-like receptor 4 (TLR4) upregulation and affective changes induced by a high-cholesterol diet in mice

Tatyana Strekalova [a,*], João P. Costa-Nunes [a,b], Ekaterina Veniaminova [a,c], Aslan Kubatiev [c], Klaus-Peter Lesch [a,d], Vladimir P. Chekhonin [e], Matthew C. Evans [f], Harry W.M. Steinbusch [a]

[a] Department of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Universiteitssingel 40, 6200 MD Maastricht, The Netherlands
[b] CEDOC, NOVA Medical School, Faculdade de Ciências Médicas, FCM, Universidade Nova de Lisboa, Campo Mártires da Pátria, 130, 1169-056 Lisboa, Portugal
[c] Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Baltiyskaya 8, Moscow 125315, Russia
[d] Division of Molecular Psychiatry, Laboratory of Translational Neuroscience, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Fuechsleinstr. 15, 97080 Wuerzburg, Germany
[e] Serbsky National Research Center for Social and Forensic Psychiatry, Department of Fundamental and Applied Neurobiology, per. Kropotkin 23, Moscow 119034, Russian Federation
[f] Department of Pharmacology, Oxford University, Mansfield Road, OX1 3QT Oxford, UK

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ABSTRACT

Background: High cholesterol intake in mice induces hepatic lipid dystrophy and inflammation, signs of non-alcoholic fatty liver disease (NAFLD), depressive- and anxiety-like behaviors, and the up-regulation of brain and liver Toll-like receptor 4 (Tlr4). Here, we investigated whether dicholine succinate (DS), an insulin receptor sensitizer and mitochondrial complex II substrate would interact with these effects.
Methods: C57BL/6 J mice were given a 0.2%-cholesterol diet for 3 weeks, alone or along with oral DS administration, or a control feed. Outcomes included behavioral measures of anxiety/depression, and *Tlr4* and peroxisome-proliferator-activated-receptor-gamma coactivator-1b (*PPARGC1b*) expression.
Results: 50 mg/kg DS treatment for 3 weeks partially ameliorated the cholesterol-induced anxiety- and depressive-like changes. Mice were next treated at the higher dose (180 mg/kg), either for the 3-week period of dietary intervention, or for the last two weeks. Three-week DS administration normalized behaviors in the forced swim and O-maze tests and abolished the *Tlr4* up-regulation in the brain and liver. The delayed, 2-week DS treatment had similar effects on *Tlr4* expression and largely rescued the above-mentioned behaviors. Suppression of *PPARGC1b*, a master regulator of mitochondrial biogenesis, by the high cholesterol diet, was prevented with the 3-week administration, and markedly diminished by the a 2-week administration of DS. None of treatments prevented hepatic dystrophy and triglyceride accumulation.
Limitations: Other conditions have to be tested to define possible limitations of reported effects of DS.
Conclusions: DS treatment did not alter the patho-morphological substrates of NAFLD syndrome in mice, but ameliorated its molecular and behavioral consequences, likely by activating mitochondrial functions and anti-inflammatory mechanisms.

1. Introduction

An emerging body of human and animal experiments has shown that a “Western diet” (high in saturated fat and/or cholesterol) – with ensuing obesity, hypercholesterolemia and insulin resistance – is associated with higher rates of affective disorders, and decreased therapeutic efficacy of antidepressants (Pomytkin et al., 2015; Shelton and Miller, 2010; Sonawalla, 2002; de Wit et al., 2010). Whilst the peripheral mechanisms of the association between metabolic factors and affective disorders have received some attention in the literature (Castanon, et al., 2015; Kishi and Elmquist, 2005), very few studies have addressed the central molecular pathways involved. These require exploration as a potential source of targets for new pharmacotherapy (André et al., 2014; Lucas and Maes, 2013; Scheen, 2009).

* Correspondence to: Department of Neuroscience, Maastricht University, Universiteitssingel 40, NL 6229 ER Maastricht, The Netherlands.
E-mail address: t.strekalova@maastrichtuniversity.nl (T. Strekalova).

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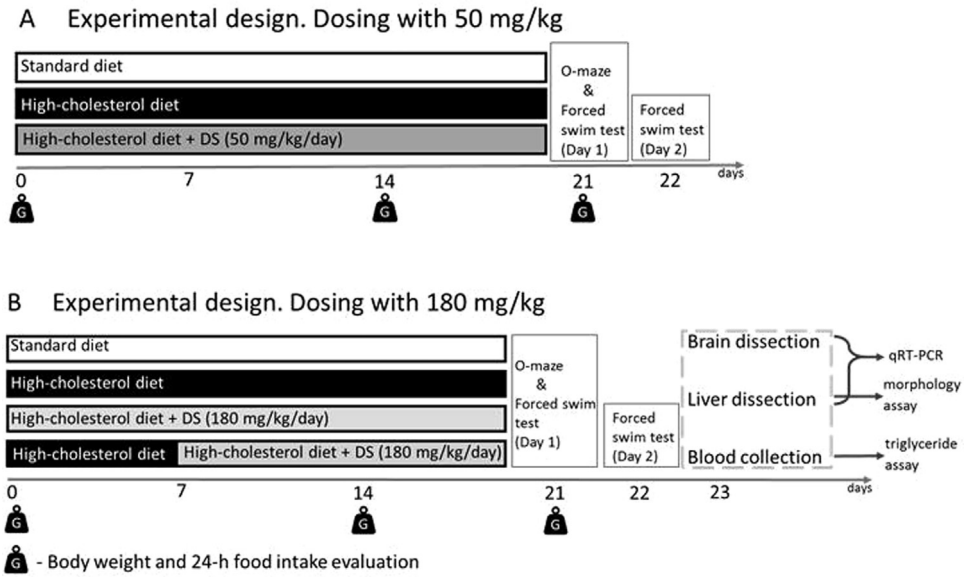

Fig. 1. Schematic of the study with administration of DS **(A)** at the dose 50 mg/kg and at the dose 180 mg/kg **(B)**, for 3-weeks or for 2 weeks, with late onset.

Insulin resistance, including deficient insulin receptor signalling, is regarded as a crucial pathogenic mechanism of affective changes associated with over-nutrition and metabolic syndrome (Kan et al., 2013; Pomytkin et al., 2015; Pyykkönen et al., 2011). Recent studies have shown that insulin itself and "neuronal insulin receptor sensitizers" have anti-depressant-like properties (Eissa Ahmed and Al-Rasheed, 2009; Gupta et al., 2014; Igarashi et al., 2008; Kemp et al., 2011; Mittal et al., 2009; Rasgon et al., 2010; Saubermann et al., 2002). These compounds increase neuronal mitochondrial biogenesis, are anti-inflammatory, and ameliorate neuronal damage (Ali et al., 2006; Asghar et al., 2007; De Nuccio et al., 2015; Salehi-Sadaghiani et al., 2012; Strum et al., 2007; Zhao et al., 2006). Given the crucial role of mitochondria in synaptic function (Maeder et al., 2014) and evidence of lowered mitochondrial adenosine triphosphate (ATP) and enzyme production, and inhibited respiratory chain and glucose metabolism during a depressive-like state (Gardner et al., 2003; Madrigal et al., 2001; Videbech, 2000), it is remarkable that insulin receptor sensitizers substantially ameliorate mitochondrial functions in the brain (De Nuccio et al., 2015; Dello Russo et al., 2002; Crenshaw et al., 2015). Notably, mitochondrial effects of insulin receptor sensitizers result in increased resting-state functional connectivity of hippocampal CA1 area to the hypothalamus and ventral thalamus (Crenshaw et al., 2015); currently, compromised brain connectivity is regarded as a biomarker of severe depression (Kaiser and Pizzagalli, 2015).

Antidepressant-like effects have also been reported for the non-esterified salt, dicholine succinate (DS), a mitochondrial complex II substrate, which enhances insulin-induced neuronal insulin receptor autophosphorylation in a dose-dependent manner (Persiyantseva et al., 2013; Storozheva et al., 2008; Storozhevykh et al., 2007), a mechanism for receptor activation (Wei et al., 1995). In a model of stress-induced anhedonia, DS, rescued normal contextual fear conditioning, increased hippocampal N-methyl-D-aspartate (NMDA) receptor subunit NR2 A expression (and increased the NR2 A/NR2B ratio), and increased rapid-eye-movement (REM) sleep rebound after acute predation (Cline et al., 2012, 2015). In a model of elderly depression, DS restored normal hedonic behaviors and increased the expression of neural plasticity factors in the hippocampus (Cline et al., 2015). Also, DS normalized hippocampal levels of the phosphorylated (inactive) form of glycogen synthase kinase three-beta (GSK3-β) during swim test in mice (Cline et al., 2015) and improved tail suspension behavior (Costa-Nunes et al., 2015).

Given the link between insulin receptor-meditated signalling and nutritional/metabolic factors (Kan et al., 2013; Pyykkönen et al., 2011), we hypothesized that DS can improve the conditions resulting from non-alcoholic fatty liver disease (NAFLD). To test this, we employed recently established NAFLD model, in which young female C57BL/6 J mice are exposed to a feed containing 0.2% cholesterol for 3 weeks (Comhair et al., 2011; Strekalova et al., 2015). These changes are accompanied by behavioral despair, anxiety-like and impulsive behaviors, and elevated brain and liver levels of Toll-like receptor 4 (Tlr4). The present study investigated whether DS could ameliorate the behavioral and molecular changes associated with NAFLD.

2. Methods

2.1. Animals

Three-month old female C57BL/6J mice were obtained from the Gulbenkian Institute of Science, Oeiras, Portugal were housed in groups of five under standard conditions and reversed lighting (*see* Supplementary material). All experiments were carried out in accordance with the European Communities Council Directive for the care and use of laboratory animals.

2.2. Study flow, dietary challenge and behavioral testing

Mice were exposed (n=23) for three weeks either to a regular diet (control group), with an energy content of 4.0 kcal/g (Mucedola Srl, Settimo Milanese, Italy), or a diet of similar composition with 0.2% (w/w) cholesterol and an energy content of 4.6 kcal/g (Research Diets Inc, New Brunswick, NJ, USA; *see* Supplemental Table 1), using an established protocol (Comhair et al., 2011; Strekalova et al., 2015). Body weight and 24-h food intake was evaluated on Days 0, 14 and 21 of dietary challenge. Eight mice were treated with DS via drinking water at 50 mg/kg/day (see Section 2.3) on days 1–21 of the dietary intervention. The DS-treated mice were tested using the O-maze on day 21, along with eight dietary challenged, but pharmacologically, naïve mice, and seven dietary control mice; lighting intensity was 5Lux (Fig. 1A;