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A differential impact of lithium on endothelium-dependent but not on endothelium-independent vessel relaxation

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ABSTRACT

Lithium is drug for bipolar disorders with a narrow therapeutic window. Lithium was recently reported to prevent stroke and protect vascular endothelium but tends to accumulate particularly in the brain and kidney. Here, adverse effects are common; however mechanisms are still vaguely understood. If lithium could also negatively influence the endothelium is unclear. We hypothesize that at higher lithium levels, the effects on endothelium reverses — that lithium also impairs endothelial-dependent relaxation of blood vessels. Vessel grafts from de-nerved murine aortas and porcine middle cerebral arteries were preconditioned using media supplemented with lithium chloride or acetate (0.4–100 mmol/L). Native or following phenylephrine-induced vasoconstriction, the relaxation capacity of preconditioned vessels was assessed by isometric myography, using acetylcholine to test the endothelium-dependent or sodium nitroprusside to test the endothelium-independent vasorelaxation, respectively. At the 0.4 mmol/L lithium concentration, acetylcholine-induced endothelium-dependent vessel relaxation was slightly increased, however, diminished in a concentration-dependent manner in vessel grafts preconditioned with lithium at higher therapeutic and supratherapeutic concentrations (0.8–100 mmol/L). In contrast, endothelium-independent vasorelaxation remained unaltered in preconditioned vessel grafts at any lithium concentration tested. Lithium elicits opposing effects on endothelial functions representing a differential impact on the endothelium within the narrow therapeutic window. Lithium accumulation or overdose reduces endothelium-dependent but not endothelium-independent vasorelaxation. The differentially modified endothelium-dependent vascular response represents an additional mechanism contributing to therapeutic or adverse effects of lithium.

1. Introduction

Lithium is a highly effective treatment for bipolar disorders (BD) and an adjuvant treatment for major depression with a narrow therapeutic window (Calkin and Alda, 2012; Geddes and Miklowitz, 2013; Mohammad and Osser, 2014). Recent studies have also identified several potential protective effects of lithium in many other neuropsychiatric and somatic diseases, including cerebrovascular disease (Gold et al., 2011; Lan et al., 2015), dementia (Gerhard et al., 2015), suicidality (Saunders and Hawton, 2013), diabetes mellitus (Svendal et al., 2012), and endothelial dysfunction (Bosche et al., 2013). In neuronal cells, lithium appears to primarily mediates its action, directly and indirectly, through inositol monophosphatase (IMPase), intracellular calcium concentration (Berridge, 1989, 1993; Wasserman et al., 2004; Li et al., 2012; Berridge, 2014) and the glycogen synthase kinase-3 beta (GSK-3β) enzyme, which in turn may control a variety of intracellular effector mechanisms (Gould and Manji, 2005; Trepiccione and Christensen, 2010; Rej et al., 2015b). Interestingly, emerging research suggests that lithium elicits similar intracellular signaling mechanisms in vascular endothelial

Abbreviations: ACH, acetylcholine; BD, bipolar disorders; ER, endothelial endoplasmic reticulum; GSK-3β, glycogen synthase kinase-3 beta; IMPase, inositol monophosphatase; IP_3, inositol trisphosphate; L-NMMA, L-N^G-monomethyl arginine; LiCl, lithium chloride; LiAc, lithium acetate; MCA, middle cerebral artery; NOS, nitric oxide synthase; SE, standard error of the mean; SNP, sodium nitroprusside; XeD, Xestospongin D.

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cells, too (Ryglewski et al., 2007; Munaron and Fiorio Pla, 2009). Thereby, the endothelium-protective effects of lithium may be important in cerebrovascular disease, traumatic brain injury with disturbed vascular regulation (Rajkowska, 2000; Bosche et al., 2003; Dohmen and Bosche et al., 2007; Gold et al., 2011; Halcomb et al., 2013; Leeds et al., 2014), and even bipolar disorder (Goldstein and Young, 2013). In endothelial cells, lithium prevents the discharge of calcium from endogenous stores by inhibition of the inositol trisphosphate (IP_3)-sensitive calcium channels of the endothelial endoplasmic reticulum (ER) (Schäfer et al., 2001), thus, counteracting cells stress-induced calcium overload and conferring lithium a cytoprotective potential (Bosche et al., 2013), possibly through inhibition of GSK-3β (Rej et al., 2015b) and/or IMPase (Chiu and Chuang, 2010; Dutta et al., 2014). Functionally, maintenance of intracellular calcium homeostasis together with other lithium effects manifest as modified endothelium-mediated vasodilation (Förstermann and Münzel, 2006), a potential marker of preserved or improved endothelial health (Yoo and Kim, 2009; Grove et al., 2015).

In long-term or at supratherapeutic levels, i.e. above the generally recommended concentrations in humans (Rej et al., 2015a), lithium can impair particularly kidney and brain function (Laliberté et al., 2015), where it tends to accumulate (Lichtinger et al., 2013; Johnson, 1998). Historically, lithium toxicity has been linked to inhibition of the GSK-3β or inositol monophosphate pathway leading to disturbed cellular metabolism (Rybakowski et al., 2013; Trepiccione and Christensen, 2010) On the other hand, lithium accumulation or toxicity may also be related to vascular hemodynamic abnormalities (Schou et al., 1968; Laliberté et al., 2015). One could postulate that the effect of lithium could be harmful to endothelium at high doses and impairs vasodilation, which may contribute to tissue specific toxicity, e.g. in the brain and kidney (Lichtinger et al., 2013; Johnson, 1998).

How can lithium be protective to blood vessel endothelium at low therapeutic doses, but be harmful to blood vessels at higher or supratherapeutic doses? We hypothesize that at high levels, the effect of lithium on endothelium *reverses* — that lithium impairs endothelium-dependent relaxation of blood vessels. To validate this hypothesis, an established approach was applied to study vascular function in vessel grafts (Ebner et al., 2011; Mulvany and Halpern, 1977; Kopaliani et al., 2014), which recently modified and translated also for human use (Wilbring et al., 2013).

Yet, to our knowledge, the data presented here uniquely suggested that supratherapeutic and higher therapeutic, but not lower therapeutic lithium levels impair particularly endothelium-dependent vascular relaxation underlining the concept of opposite effects of lithium at different therapeutic concentrations.

2. Material and methods

2.1. Ethics of the animal model

This experimental study was approved by the University Commission on Animal Experiments with respect to the animal welfare regulations of Germany, in accordance to the European Communities Council Directive and to the National Institutes of Health (NIH) Guidelines. For performing the study, written permission was obtained from local authorities.

2.2. Materials and drugs

All materials, reagents and drugs are described when mentioned within their respective method sub-sections (see below).

2.3. Cold storage solutions

The used cold storage Tiprotec™ solution (Dr. F. Köhler GmbH, Bensheim, Germany) contains the following substance concentrations (all are given in mmol/L): α-ketoglutarate 2, aspartate 5, *N*-acetyl-histidine 30, glycine 10, alanine 5, tryptophan 2, sucrose 20, glucose 10, Cl^-: 103.1, H_2PO_4: 1, Na^+: 16, K^+: 93, Mg^{2+}: 8, Ca^{2+}: 0.05, deferoxamine: 0.082 and LK 614: 0.017. The pH (at 20 °C) was 7.0 and the osmolarity was 305 mosmol/L. This standard solution served as the control preconditioning or was supplemented with the different lithium chloride (LiCl) or lithium acetate (LiAc) concentrations.

2.4. Murine and porcine vessel preparation

The vessel grafts were isolated from murine aortas or from porcine middle cerebral arteries (MCA). Vessel preparation was performed according to the slightly modified method for rodents as described in detail elsewhere (Ebner et al., 2011; Wilbring et al., 2013; Kopaliani et al., 2014). In brief, male CD57 mice 8 to 10 weeks of age (Charles River Laboratories, Sulzfeld, Germany) were sacrificed by cutting off the upper cervical spinal cord under deep anesthesia. Male swine (*Sus scrofa domesticus*, 24 to 26 weeks of age) were stunned by electroshock and sacrificed by exsanguination. All mice and swine were dissected immediately. The murine aortas (pars thoracalis without aortic arch) or the proximal part (M1 segment) of porcine middle cerebral arteries were recovered and directly placed into a storage solution containing either 1) Tiprotec™ solution only (Dr F. Köhler GmbH, Bensheim, Germany) or 2) modified Tiprotec™ solution supplemented with 0.4 to 100 mmol/L lithium (LiCl/LiAc, Sigma-Aldrich, Taufkirchen, Germany) and stored at 4 °C for ≥48 hours (h).

2.5. Post-mortem model of vascular tone control

The post-mortem model with de-nerved vessel grafts was performed to investigate the isolated vessel reaction in response to different lithium concentrations independently of the influence of lithium on the central and thereby also on the vegetative nerve system including its remote control of the vessel tone.

2.6. Type of vessel

The thoracic aorta (pars descendens) was chosen as the used vessel type, because a) it is an elastic type artery containing both the ordinary vascular smooth muscle cells (SMC) and the myointimal SMC in a relatively high number; moreover, because b) aortic endothelial cells were used in our previous vessel graft and cell culture studies regarding cytosolic [Ca^{2+}] measurements after long-term and immediate use of lithium and its influence on the specific type of endothelial cells taken from the aorta (Schäfer et al., 2001; Bosche et al., 2013). Vessel grafts of the MCA (M1 segment) from porcine brains were additionally used to investigate the influence of lithium on cerebral endothelium-dependent vasorelaxation.

2.7. Isometric force measurement

After cold storage aortic or cerebral vessel grafts (pipes or long rings, 2 mm in length, 500–600 μm or 1150–1400 μm internal width, respectively) were transferred to a 90%/10% (vol/vol) mixture of phosphate-buffered saline (PBS) solution and Hank's balanced salt solution (HBSS, Sigma-Aldrich, Taufkirchen, Germany) gently warmed to 37 °C/98.6 °F over 30 min. Then, the vessel grafts were studied, stretched with a resting tension equivalent to that obtained by exposure to an intraluminal pressure of 20 mm Hg for maximal responses. Vessel rings were equilibrated for 10 min prior to vasomotor analysis. Maximal contraction was induced by exposure of vessel rings to a potassium-enriched solution (123.7 mmol/L KCl) and/or to the application of 10 μmol/L phenylephrine (see below Vasoactive Agents). Vessel relaxation toward acetylcholine (ACH; $10^{-8.5}$ to $10^{-5.5}$ mol/L) or sodium nitroprusside (SNP; $10^{-8.5}$ to $10^{-5.5}$ mol/L) was tested after a plateau constriction induced by 10 μmol/L phenylephrine to assess