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Vitamin B_{12} dependent changes in mouse spinal cord expression of vitamin B_{12} related proteins and the epidermal growth factor system

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ABSTRACT

Chronic vitamin B_{12} (cobalamin) deficiency in the mammalian central nervous system causes degenerative damage, especially in the spinal cord. Previous studies have shown that cobalamin status alters spinal cord expression of epidermal growth factor (EGF) and its receptor in rats. Employing a mouse model of cobalamin-depletion and loading, we have explored the influence of Cbl status on spinal cord expression of cobalamin related proteins, as well as all four known EGF receptors and their activating ligands. Following four weeks of osmotic minipump infusion ($n=7$ in each group) with cobinamide (4.25 nmol/h), saline or cobalamin (1.75 nmol/h) the spinal cords were analyzed for cobalamin and for the mRNA levels of cobalamin related proteins and members of the EGF system using quantitative reverse transcription PCR. The median spinal cord cobalamin content was 17, 32, and 52 pmol/gr of tissues in cobinamide, saline, and cobalamin treated animals, respectively. Both cobinamide and cobalamin induced a significant decrease in the expression of the lysosomal membrane cobalamin transporter. All four EGF receptors and their activating ligands, except for EGF, were expressed in the spinal cord. Notably, the expression of one of the EGF receptors, HER3, and the ligands heparin-binding EGF-like growth factor, transforming growth factor-α, and neuregulins 1α was increased in cobalamin treated mice. Our studies show that four weeks treatment of mice with cobinamide induces spinal cord cobalamin depletion and that cobalamin loading induces an altered expression pattern of the EGF system thus confirming a spinal cord cross talk between Cbl and the EGF system.

Abbreviations: Cbl, Cobalamin (Vitamin B_{12}); Cbi, Cobinamide; EGF, Epidermal growth factor; EGF-R, Epidermal growth factor receptor; HB-EGF, Heparin-binding EGF-like growth factor; LMBRD1, Lysosomal membrane Cbl transporter; MS, Methionine synthase; Mut, methylmalonylCoA mutase; NRGs, Neuregulins; q-rt-PCR, Quantitative reverse-transcription PCR; TC, Transcobalamin; TC-R, TC-receptor (CD320); TGF-α, Transforming growth factor-α

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1. Introduction

Vitamin B_{12} (cobalamin (Cbl))-deficient neuropathy is well established in humans, and has also been described in animal models (Scalabrino, 2001). The central nervous system and especially the spinal cord is severely damaged by Cbl deficiency (Scalabrino, 2009).

Cbl is transported in the blood bound to transcobalamin (TC) and internalized into cells by its receptor, (TC-R, also named CD320). In the cells, Cbl is transferred to the cytoplasm by the lysosomal membrane Cbl transporter (LMBRD1) and serves as cofactor for methyl malonylCoA mutase (Mut) and methionine synthase (MS).

Using a rat model, we previously demonstrated a link between Cbl-deficiency and the epidermal growth factor (EGF) system. We showed that Cbl-deficiency decreases rat spinal cord mRNA-levels of the EGF receptor (EGF-R) (Mutti et al., 2011).

EGF-R is part of a cross-talking family of receptors and ligands consisting of four receptor tyrosine kinases and more than ten ligands. The other receptors are HER2, HER3, and HER4 (Sharif and Prevot, 2010). The ligands binding only to EGF-R are EGF, transforming growth factor-α (TGF-α), and amphiregulin, whereas heparin-binding EGF-like growth factor (HB-EGF), betacellulin and epiregulin bind both EGF-R and HER4. The other ligands are the heregulins or neuregulins (NRGs) which consist of NRG1 and NRG2 (binding to both HER3 and HER4) as well as NRG3 and NRG4 (binding only to HER4).

HB-EGF and TGF-α are widely expressed in the neurons and glia cells (Nakagawa et al., 1998; Xian and Zhou, 1999). Transcripts of the HER2, HER3, HER4 and their specific ligands (NRG1, NRG2, NRG3) have also been identified in the neurons, and glia cells of the central and peripheral nervous system (Birchmeier, 2009; Carteron et al., 2006; Francoeur et al., 1995; Longart et al., 2004). Several lines of evidence suggest that these receptors and their ligands are important regulators during neural development and myelination (Birchmeier, 2009; Longart et al., 2004; Nave and Salzer, 2006; Newbern and Birchmeier, 2010).

Recently we showed that mouse TC binds not only Cbl but also the biological inert cobinamide (Cbi) (Hygum et al., 2011) allowing us to establish a mouse model of chronic Cbl load or Cbi induced Cbl depletion by administering Cbl or Cbi through osmotic minipumps. This treatment resulted in mean liver Cbl levels of 1.65 (Cbl treated) and 0.53 (Cbi treated) fold that observed in control mice (Lildballe et al., 2012).

Here we used the mouse model to characterize changes in spinal cord accumulation of Cbl and in transcript levels of genes involved in Cbl metabolism and genes of the EGF system including the receptors EGF-R and HER2-4 and some of the ligands involved in myelination or neurotrophism (EGF, HB-EGF, TGF-α, NRG1, NRG2, NRG3).

2. Results

We observed significant alterations in spinal cord content of Cbl in the two treated mice groups. In Cbi-treated animals, Cbl concentrations in spinal cord were reduced to 60% of the controls, with a median (range) of 17 (8–33) pmol/gr of tissue in vs. 32 (11–46) pmol/gr of tissue in control mice. In Cbl treated animals, the Cbl concentrations in spinal cord were 170% of the controls with a median (range) of 52 (41–66) pmol/gr of tissue.

The level of Cbi were below the detection limits (detection limit=2 pmol/g of tissue) in spinal cord of the control and Cbl-loaded mice compared to 36 (22–60) pmol/g of tissue in the Cbi-treated mice.

The mRNA levels of genes involved in the metabolism of Cbl as well as the four receptors of the EGF system together with their activating ligands are shown in Fig. 1. All examined genes related to the transport of Cbl (TC, TC-R and LMBRD1) as well as the three enzymes examined (MS, Mut and methylenetetrahydrofolate reductase) were expressed, only the expression of TC was very low (Fig. 1A). We also identified

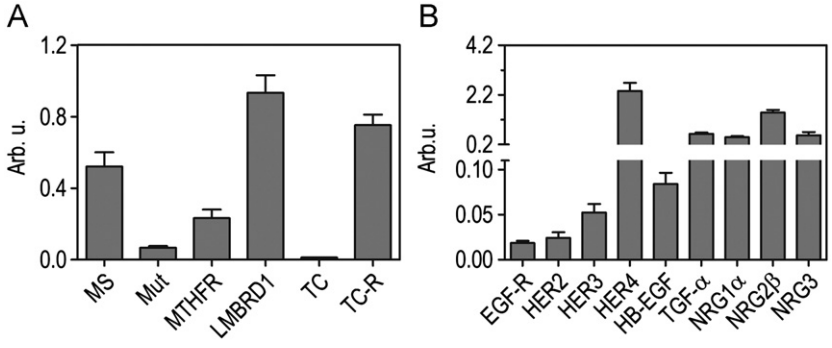

Fig. 1 – Transcript levels of Cbl-related genes (A) and selected EGF system genes (B) in the spinal cord of control mice. The columns represent mean (+SEM) mRNA levels determined by quantitative reverse-transcription (q-rt-)PCR and normalized against β-actin in the spinal cord of control mice (N=7 mice for each gene). The Y axis indicates the expression level relative to expression of β-actin. Note that expression level across the genes cannot be compared. Arb.u., arbitrary units; EGF-R, epidermal growth factor receptor; HB-EGF, heparin-binding EGF-like growth factor; LMBRD1, lysosomal membrane Cbl transporter; MS, methionine synthase; MTHFR, methylene tetrahydrofolate reductase; Mut, methylmalonyl-Coenzyme A mutase; NGR, neuregulin; TC, transcolabamin; TC-R, transcobalamin receptor (CD320); TGF-α, transforming growth factor-α.