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Short report

Regulation of growth hormone induced JAK2 and mTOR signalling by hepatic protein tyrosine phosphatase 1B

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Abstract

Protein tyrosine phosphatase 1B (PTP1B) regulates various signalling pathways including insulin, leptin, IGF-1 and growth hormone (GH) signalling. Transmission of the GH signal depends on Janus kinase 2 (JAK2), which is how PTP1B is thought to modulate GH signalling in the liver, based on studies utilising global PTP1B knockout mice (*Ptp1b*$^{-/-}$). Here, we investigated the liver-specific role of PTP1B in GH signalling, using liver-specific *Ptp1b*$^{-/-}$ mice (*alb-crePtp1b*$^{-/-}$), under physiological (chow) or insulin resistant (high-fat diet [HFD]) feeding conditions. Body weight and adiposity were comparable between female *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ control mice. On chow diet, under 48-hour fasting GH-resistant conditions, GH stimulation *in vivo* led to a robust stimulation of the JAK-STAT signalling pathway. *Alb-crePtp1b*$^{-/-}$ mice exhibited significantly higher GH-induced JAK2 phosphorylation and *SOCS3* gene expression post-GH stimulation. However, STAT3, STAT5 and ERK1/2 phosphorylation and *SOCS2* gene expression were similar between groups. Interestingly, GH-induced mTOR phosphorylation was significantly higher in *alb-crePtp1b*$^{-/-}$ mice 5-min post-GH stimulation compared to controls, revealing this part of the pathway under direct control of PTP1B. Under *ad lib* HFD-fed conditions, GH-induced STAT5 phosphorylation significantly increased in *alb-crePtp1b*$^{-/-}$ mice only, with no alterations in the controls. Overall, our data demonstrate that liver-specific PTP1B deletion leads to significant alterations in GH signalling with increased JAK2, STAT5 and mTOR phosphorylation and *SOCS3* gene expression.

Keywords: Liver; PTP1B; JAK2; STAT; Growth hormone

1. Introduction

Human growth hormone (hGH) is an important metabolic hormone which has effects on glucose, protein and lipid metabolism [1,2]. Secondary consequences such as increased adiposity and insulin resistance can occur in individuals suffering with abnormal hGH secretion [1,3]. Thus, it is important to understand the regulators of growth hormone (GH) signalling *in vivo*.

Initial events in GH signalling include homodimerization of the GH receptor (GHR), recruitment of Janus kinase 2 (JAK2) to the cytoplasmic domain of the receptor, and activation of JAK2 via autophosphorylation [4,5]. Various signalling proteins are then recruited to high affinity binding sites on tyrosine phosphorylated JAK2 and GHR which leads to the activation of signal transducers and activators of transcription (STATs) 1, 3, and 5b, and several other pathways including Ras-MAP and IRS-1/PI3K/Akt [2,5].

The mechanisms terminating GHR signalling are less well understood. A study in 2003 identified protein tyrosine phosphatase 1B (PTP1B) as a negative regulator of GH signalling, using livers from globally-deficient PTP1B knockout mice [6]. An absence of PTP1B resulted in GH-dependent hyperphosphorylation of JAK2 and enhanced activation of STAT3 and STAT5 [6]. However, since this study examined PTP1B deletion in globally-deficient mice, which exhibit decreased body weight under high-fat diet (HFD) feeding conditions, it is unclear whether the effects on GH signalling were specifically caused by PTP1B deletion in the liver.

In order to determine the specific role of liver-PTP1B in GH signalling [6], phosphorylation of the JAK-STAT pathway and downstream signalling targets were assessed in adult, female chow- and HFD-fed liver-specific knockout mice (*alb-crePtp1b*$^{-/-}$), following a hGH stimulation time course under

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ad lib-fed and 48-hour fasted states (to induce GH resistance).

2. Materials and methods

2.1. Ethics statement

All animal procedures were approved by the University of Aberdeen Ethics Review Committee Board and performed under a project license approved by the Home Office under the animals (Scientific Procedures) Act 1986 (PPL60/3951).

2.2. Animal studies

Female *Ptp1b*$^{fl/fl}$ and *alb-crePtp1b*$^{-/-}$ mice expressing *Cre* recombinase under the control of the serum albumin promoter were described previously [7]. DNA extraction and genotyping for the *Ptp1b* floxed allele and the presence of *Cre* by PCR were performed as described previously [8]. Mice studied were age-matched littermates, which were generated on a C57BL/6 background. Mice were housed in groups and maintained at 22–24 °C on a 12-h light/dark cycle with free access to food and water. At weaning (~21 days), mice were placed on standard 3.4% fat chow pellet diet (rat and mouse breeder and grower, special diets services, DBM, Scotland) or HFD (adjusted calories diet, 55% fat, Harlan Teklad, USA) and weight was recorded every two weeks. The approximate fatty acid profile of adjusted calories diet (% total fat) was 28% saturated, 30% trans, 28% monounsaturated (*cis*) and 14% polyunsaturated (*cis*), as described previously [9]. For signalling studies, chow- or HFD-fed female *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ mice were injected with saline or 1 μg/g body weight hGH. Mice were either fasted for 48-hours (to induce GH resistance) or used *ad lib* fed. Mice were sacrificed by cervical dislocation at 0, 5, 10, 30 and 90 min. Mice in the 0 min group were injected with saline. Tissues were dissected immediately and frozen in liquid nitrogen.

2.3. Body composition using dual-energy X-ray absorptiometry (DXA)

The DXA instrument used was the Lunar PIXIMUS-Densitometer (GE Medical Systems, USA). DXA scans and analyses were carried out as instructed by the manufacturer. A quality control phantom mouse was scanned daily before samples were scanned. The phantom and samples were mounted on an adhesive disposable plastic tray placed on the imaging surface. Mice were anesthetised with isoflurane (1.5–2.0% with 2 L/min O_2). Mice were then spread out on the adhesive trays in the prostrate position with limbs extended away from the body. Mouse tails were not measured. As recommended by the manufacturer, a region of interest (ROI) was created to exclude the head from analyses.

2.4. Immunoblotting

Tissue lysates were prepared in RIPA buffer containing fresh sodium orthovanadate and protease inhibitors, as described previously [10]. Proteins were separated by 4–12% SDS-PAGE and transferred to nitrocellulose membranes. Immunoblots were performed using antibodies from cell signaling (cell signaling by NEB, Hitchin, UK) (unless stated otherwise) against PTP1B (Millipore), SHP2 (Santa Cruz), pJAK2 Y1007/1008 (Invitrogen), JAK2, pSTAT3 Y705, STAT3, pSTAT5 Y694/699, STAT5, pERK1/2 MAPK T202/Y204, p-FRAP/p-mTOR S2448 (Santa Cruz), mTOR, pS6 ribosomal protein S235/236, S6 ribosomal protein, Akt/PKB (Santa Cruz) and IRβ (Santa Cruz). Immunoblots were developed using horseradish peroxidase-conjugated secondary antibodies, visualised using enhanced chemiluminescence, and quantified by densitometry scanning with Image J or Bio1D software (PeqLab, Fareham, UK).

2.5. Gene expression analysis

Total RNA was isolated from mouse liver using TRI reagent (Ambion, Warrington UK), according to the manufacturer's protocol. First strand cDNA was synthesized from 1 μg of total RNA employing the Bioline Bioscript™ Pre-amplification System and oligo (dT) [12–18]. Two μL of diluted cDNA (1:10) was used to amplify target genes by real-time RT-PCR (10 μL), using GoTaq qPCR Master Mix (Promega, Southampton, UK). The Roche LightCycler® 480 System (Roche Diagnostics, Burgess Hill, UK) was used for analysis. Relative gene expression was calculated using the comparative Ct ($2-\delta\delta$Ct) method. A geometric mean of three commonly used reference genes; hypoxanthine-guanine phosphoribosyltransferase (Hprt), 18S ribosomal RNA (18S) and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) were used to normalise data. A geometric mean of the relative copy numbers of mouse PCRs were followed by melting curves (70–95 °C).

2.6. Data analysis

Data are expressed as mean ± SEM and n represents the number of mice or biological replicates. Statistical analyses were performed using one-way Anova with Tukey's multiple comparison post-tests, repeated measures two-way Anova with Bonferroni multiple comparison post-tests or two-tailed Student's t-tests, as appropriate. The critical alpha level (P) was set at 0.05. $P < 0.05$ was considered statistically significant. GraphPad Prism 5 statistical software was used for analyses.

3. Results

3.1. Liver-PTP1B deletion has no effect on body weight or adiposity

Body weight was comparable between HFD-fed *alb-crePtp1b*$^{-/-}$ and HFD-fed *Ptp1b*$^{fl/fl}$ control female mice throughout the study (Fig. 1A), as previously reported in male mice [7]. There was ~50% decrease in PTP1B protein levels in whole liver lysates (Fig. 1B). Furthermore, body weight, adiposity and bone mineral density were comparable between chow-fed