UC San Diego
UC San Diego Previously Published Works

Title
Ciliary neurotrophic factor reverses aberrant mitochondrial bioenergetics through the JAK/STAT pathway in cultured sensory neurons derived from streptozotocin-induced diabetic rodents.

Permalink
https://escholarship.org/uc/item/3gh6b024

Journal
Cellular and Molecular Neurobiology, 34(5)

Authors
Chowdhury, Subir
Saleh, Ali
Akude, Eli
et al.

Publication Date
2014-07-01

DOI
10.1007/s10571-014-0054-9

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Cell Mol Neurobiol (2014) 34:643–649
DOI 10.1007/s10571-014-0054-9

SHORT COMMUNICATION

Ciliary Neurotrophic Factor Reverses Aberrant Mitochondrial Bioenergetics Through the JAK/STAT Pathway in Cultured Sensory Neurons Derived from Streptozotocin-Induced Diabetic Rodents

Subir Roy Chowdhury · Ali Saleh · Eli Akude · Darrell R. Smith · Dwane Morrow · Lori Tessler · Nigel A. Calcutt · Paul Fernyhough

Received: 10 February 2014 / Accepted: 21 March 2014 / Published online: 30 March 2014
© Springer Science+Business Media New York 2014

Abstract Mitochondrial dysfunction occurs in sensory neurons and contributes to diabetic neuropathy. Ciliary neurotrophic factor (CNTF) stimulates axon regeneration in type 1 diabetic rodents and prevents deficits in axonal caliber, nerve conduction, and thermal sensation. We tested the hypothesis that CNTF enhances sensory neuron function in diabetes through JAK/STAT (Janus kinase/signal transducers and activators of transcription) signaling to normalize impaired mitochondrial bioenergetics. The effect of CNTF on gene expression and neurite outgrowth of cultured adult dorsal root ganglia (DRG) sensory neurons derived from control and streptozotocin (STZ)-induced diabetic rodents was quantified. Polarization status and bioenergetics profile of mitochondria from cultured sensory neurons were determined. CNTF treatment prevented reduced STAT3 phosphorylation (Tyr 705) in DRG of STZ-diabetic mice and also enhanced STAT3 phosphorylation in rat DRG cultures. CNTF normalized polarization status of the mitochondrial inner membrane and corrected the aberrant oligomycin-induced mitochondrial hyperpolarization in axons of diabetic neurons. The mitochondrial bioenergetics profile demonstrated that spare respiratory capacity and respiratory control ratio were significantly depressed in sensory neurons cultured from STZ-diabetic rats and were corrected by acute CNTF treatment. The positive effects of CNTF on neuronal mitochondrial function were significantly inhibited by the specific JAK inhibitor, AG490. Neurite outgrowth of sensory neurons from age-matched control and STZ-induced diabetic rats was elevated by CNTF and blocked by AG490. We propose that CNTF's ability to enhance axon regeneration and protect from fiber degeneration in diabetes is associated with its targeting of mitochondrial function and improvement of cellular bioenergetics, in part, through JAK/STAT signaling.

Keywords Bioenergetics profile · Dorsal root ganglia · CNTF · Diabetic sensory neuropathy · Mitochondrial membrane potential

Subir Roy Chowdhury and Ali Saleh have contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10571-014-0054-9) contains supplementary material, which is available to authorized users.

S. R. Chowdhury · A. Saleh · E. Akude · D. R. Smith · D. Morrow · L. Tessler · P. Fernyhough (✉)
Division of Neurodegenerative Disorders, St Boniface Hospital Research Centre, R4046 – 351 Taché Ave, Winnipeg, MB R2H 2A6, Canada
e-mail: pfernyhough@sbrc.ca

N. A. Calcutt
Department of Pathology, UCSD, La Jolla, CA, USA

P. Fernyhough
Department of Pharmacology & Therapeutics, University of Manitoba, Winnipeg, MB, Canada

Introduction

Ciliary neurotrophic factor (CNTF) is expressed by Schwann cells and promotes survival and axonal regeneration of sensory and motor neurons (Sleeman et al. 2000). CNTF also has anti-obesity effects mediated through modulation of hypothalamic physiology, reduction of food intake (Allen et al. 2011) and the ability to reduce fat deposition via promotion of mitochondrial biogenesis, and enhanced oxidative capacity in adipose tissue (Crowe et al. 2008).

Mitochondrial abnormalities in sensory neurons contribute to the etiology of diabetic neuropathy (Akude et al. 2011;

Roy Chowdhury et al. 2012). Inner mitochondrial membrane potential is depolarized in sensory neurons derived from streptozotocin (STZ)-induced diabetic rats and lumbar dorsal root ganglia (DRG) from diabetic rats exhibit reduced respiration that correlates with down-regulation of proteins of respiratory chain complexes (Akude et al. 2011). Diabetes also impairs CNTF expression in sciatic nerve and CNTF treatment prevented indices of peripheral neuropathy in diabetic rodents (Calcutt et al. 2004; Mizisin et al. 2004; Saleh et al. 2013). Our recent work showed that CNTF signals through NF-κB to optimize mitochondrial function in STZ-diabetic mice (Saleh et al. 2013). To extend our investigation of the regulation of mitochondrial bioenergetics by CNTF, we evaluated the contribution of the Janus kinase signal transducers and activators of transcription (JAK/STAT) pathway (Zigmond 2011).

Methods

Induction of Diabetes

Diabetes was induced by a single intraperitoneal injection of 75–85 mg/kg (Sprague Dawley rats) or two injections of 90 mg/kg (C57Bl6/J mice) of STZ (Sigma, St Louis, MO). A cohort of diabetic mice was treated with CNTF as described (Saleh et al. 2013). CNTF-treated control mice were not included, as our previous studies demonstrated that systemically delivered CNTF had no impact on diverse indices of peripheral nerve structure and function (Calcutt et al. 2004; Mizisin et al. 2004). Tissue was collected after 3–5 months (rats) or 7–8 months (mice) of diabetes. All control or diabetic animals were male. Animal procedures were approved by the University of Manitoba or UCSD Animal Care Committees.

Adult Rat DRG Sensory Neuron Culture

Dissociated DRG neurons from adult rats were cultured under defined conditions (Akude et al. 2011). Cultures were prepared for Western blotting (Akude et al. 2011; Roy Chowdhury et al. 2012) or fixed and stained for neuron-specific β-tubulin III. The mean pixel area of fluorescent signal (minus cell body signal) was adjusted for total cell number and used as a measure of total neurite outgrowth. In each well up to 10 fields were imaged at 20× with 50–100 neurons counted per well. Cultures were treated with 10 ng/ml CNTF and/or 10 μM AG490, a specific inhibitor of JAK (EMD-Calbiochem, San Diego, CA).

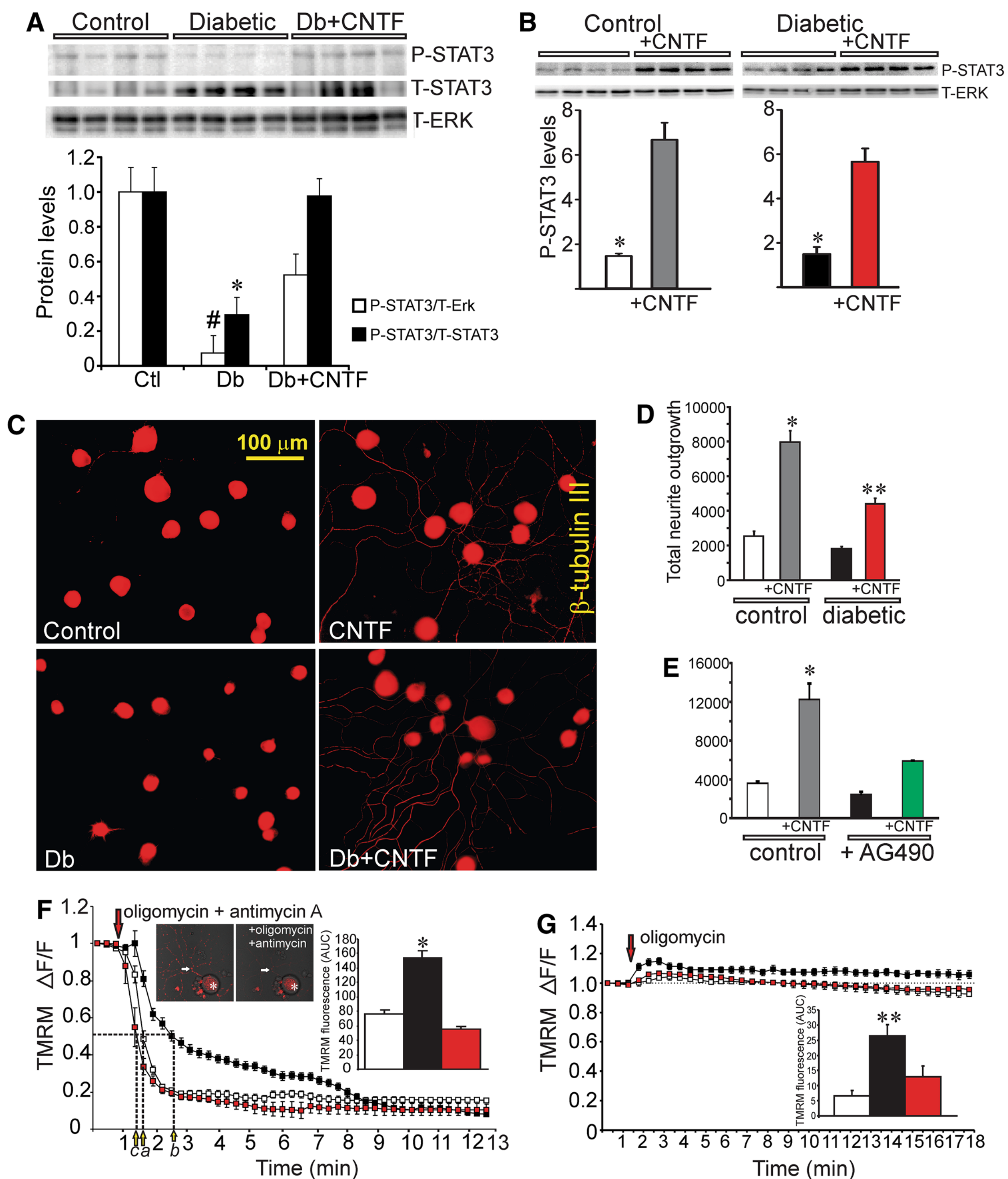
Western Blotting for STAT3

Blots were probed with antibodies for phosphorylated (Tyr705) and total signal transducer and activator of transcription 3 (STAT3, both Cell Signaling Technology, Danvers, MA) and total extracellular signal-related kinases (ERK, Santa Cruz Biotechnology, Santa Cruz, CA). The latter antibody was used to control for loading on blots since its expression never changes in DRG neurons under a variety of treatments or stresses.

Mitochondrial Membrane Potential in Cultured Neurons

Rat neurons were loaded with 3.0 nM tetramethylrhodamine methyl ester (TMRM; Molecular probes, Eugene, OR) for 1 h and the fluorescence signal in axons detected with a Carl Zeiss

Fig. 1 CNTF prevented reduced P-STAT3 in DRG in STZ-diabetic ▶ mice, enhanced neurite outgrowth through JAK/STAT pathway and normalized mitochondrial polarization status in axons of neurons derived from STZ-diabetic rats. In **a** DRG from control, STZ-induced diabetic mice and mice treated with CNTF [as described (Saleh et al. 2013)] were probed for P-STAT3, T-STAT3, and T-ERK. Values are means ± SEM ($n = 4$); *$p < 0.001$ vs all others relative to T-STAT3 and $^{\#}p < 0.001$ vs all others relative to T-ERK (one-way ANOVA with Tukey's posthoc analysis). In **b** normal or diabetic rat cultures were treated with 10 ng/ml CNTF for 30 min and levels of P-STAT3 (relative to T-ERK) expression quantified (total STAT3 expression did not change). Values are means ± SEM ($n = 4$); *$p < 0.001$ vs CNTF-treated (Student's *t* test). Effect of CNTF on neurite outgrowth; **c** shows images of neurons stained with neuron-specific β-tubulin III, **d** *bar chart* detailing effect of 24-h treatment with 10 ng/ml CNTF on total neurite outgrowth of age-matched control and diabetic neurons of rats and **e** effect of JAK blockade with AG490 (the inactive congener AG9 had no effect—not shown). Values are means ± SEM, $n = 3$–4 replicate cultures. *$p < 0.001$ vs all other groups; **$p < 0.05$ vs all other groups (one-way ANOVA with Tukey's posthoc analysis). In **f** are shown typical TMRM signals from axons of cultured sensory neurons showing loss of fluorescence intensity upon induction of mitochondrial depolarization. *White arrows* indicate axonal signal and *asterisk* is a cell body. In **f** are shown quantified TMRM signals in axons of cultured DRG neurons isolated from control (*open square*), 3–5 month STZ-induced diabetic rats (*black square*) and diabetic cultures treated with 10 ng/ml CNTF for 24 h (*red square*). *Inset* in **f** shows the area under the TMRM fluorescence trace (AUC) for control (*open bar*), diabetic (*black bar*), and diabetic with CNTF (*red bar*). The TMRM trace was characterized by non-linear regression (one phase exponential decay). The rate constant of decay $(K) = 0.01217 \pm 0.0006$ (*a*; control), 0.0054 ± 0.0001 (*b*; diabetic), and 0.01516 ± 0.0008 (*c*; diabetic + CNTF) (indicated by *yellow arrows*). Half-life of decay = 33 s (control), 96 s (diabetic), and 25 s (diabetic + CNTF). The F (Fisher parameter)-ratio = 319.2, $P < 0.0001$, diabetic vs diabetic + CNTF. The AUC was estimated from the baseline (at the point of injection) to a fluorescence level of 0.1 and between time points of 1.0 and 9 min using sums-of-squares. Values are the means ± SEM, $n = 45$–65 axons; *$p < 0.001$ diabetic vs diabetic + CNTF, Students *t* test. **g** TMRM fluorescence trace showing the effect of oligomycin. TMRM fluorescence trace was characterized by one-exponential association. The rate constant/slope, $(K) = 0.057 \pm 0.012$ (control), 0.039 ± 0.007 (diabetic), and 0.050 ± 0.008 (diabetic + CNTF). The F (Fisher parameter)-ratio = 23.83, $P < 0.0001$, control vs diabetic. The AUC was estimated from the baseline (at the point of injection), and between time points of 1.0 and 6 min using sums-of-squares. Values are the means ± SEM, $n = 44$–53 axons, **$P < 0.01$ compared to control, Students *t* test (Color figure online)



LSM510 confocal inverted microscope (Akude et al. 2011). Antimycin A and/or oligomycin (Sigma) was added to culture media at final concentrations of 10 and 1 μM, respectively, at 1 min following baseline fluorescence measurements. Upon drug treatment, the rate and extent of loss of fluorescence signal is a relative inverse measure of the polarization state of mitochondria prior to drug addition, such that the quicker and greater the loss of signal, the greater the degree of polarization prior to drug. Axon signals in each field were assessed as average fluorescence pixel intensity per axon length using the Carl Zeiss software package and are presented as relative change in fluorescent intensity ($\Delta F/F$).

Mitochondrial Respiration in Cultured DRG from Rats

An XF24 Analyzer (Seahorse Biosciences, Billerica, MA) measured mitochondrial bioenergetics in rat neurons cultured under defined conditions as described (Roy Chowdhury et al. 2012). Media were changed to DMEM (Dulbecco's modified Eagle's medium, pH 7.4) supplemented with 1 mM pyruvate

(Gibco) and 10 mM D-glucose 1 h before the assay. Oligomycin (1.0 µM), FCCP (1.0 µM, unless stated otherwise) and rotenone (1.0 µM) + antimycin A (1.0 µM) were injected sequentially. Basal oxygen consumption rate (OCR), oxygen consumption linked to ATP production, spare respiratory capacity, and respiratory control ratio were determined (Brand and Nicholls 2011). Cultures were treated for 24 h with 10 ng/ml CNTF with or without 10 µM AG490 or its inactive congener, AG9. Following OCR measurement, the cells were fixed and stained for neuron-specific β-tubulin III. Plates were then inserted into a Cellomics Arrayscan-VTI HCS Reader (Thermo Scientific, Pittsburgh, PA) equipped with Cellomics Arrayscan-VTI software to determine total neuronal number per well.

Statistical Analysis

Where appropriate, data were subjected to one-way ANOVA with post hoc comparison using Tukey's test or regression analysis with Fisher's non-parametric test (GraphPad Prism 4, GraphPad Software Inc., San Diego, CA). In all other cases, two-tailed Student's *t* tests were performed.

Results

STAT3 Phosphorylation in DRG is Reduced by Diabetes and Augmented by CNTF

DRG taken directly from STZ-diabetic mice had a 60 % or greater deficit in P-STAT3 relative to either T-ERK or T-STAT3 (Fig. 1a) that was prevented by systemic CNTF at a dose that also prevented impaired NF-κB signaling and indices of neuropathy in the same animals (Saleh et al. 2013). Diabetes augmented T-STAT3 levels and this effect was partially prevented by CNTF treatment. This unexpected finding was possibly the result of loss of P-STAT3 and associated transcriptional activity positively feeding back on gene expression to enhance endogenous levels of total STAT3 to overcome loss of STAT3 signaling. Sensory neurons isolated from DRG of age-matched control or 3–5 month STZ-diabetic rats were cultured under defined conditions and treated with CNTF (10 ng/ml) for 30 min. Basal levels of P-STAT3 were not different between control and diabetic DRG (Fig. 1b), possibly reflecting tissue manipulations involving axotomy of DRG neurons prior to the cell culture assay (Qiu et al. 2005). The Qui et al. (2005) study revealed that axotomy elevated P-STAT3 levels within 6 h in DRG neurons, and thus the basal level of P-STAT3 expression in Fig. 1b cannot be compared with that in Fig. 1a. CNTF caused a 2–3-fold increase in P-STAT3 (Fig. 1b) that was prevented by the JAK inhibitor, AG490 (Supplementary Fig. 1a–c).

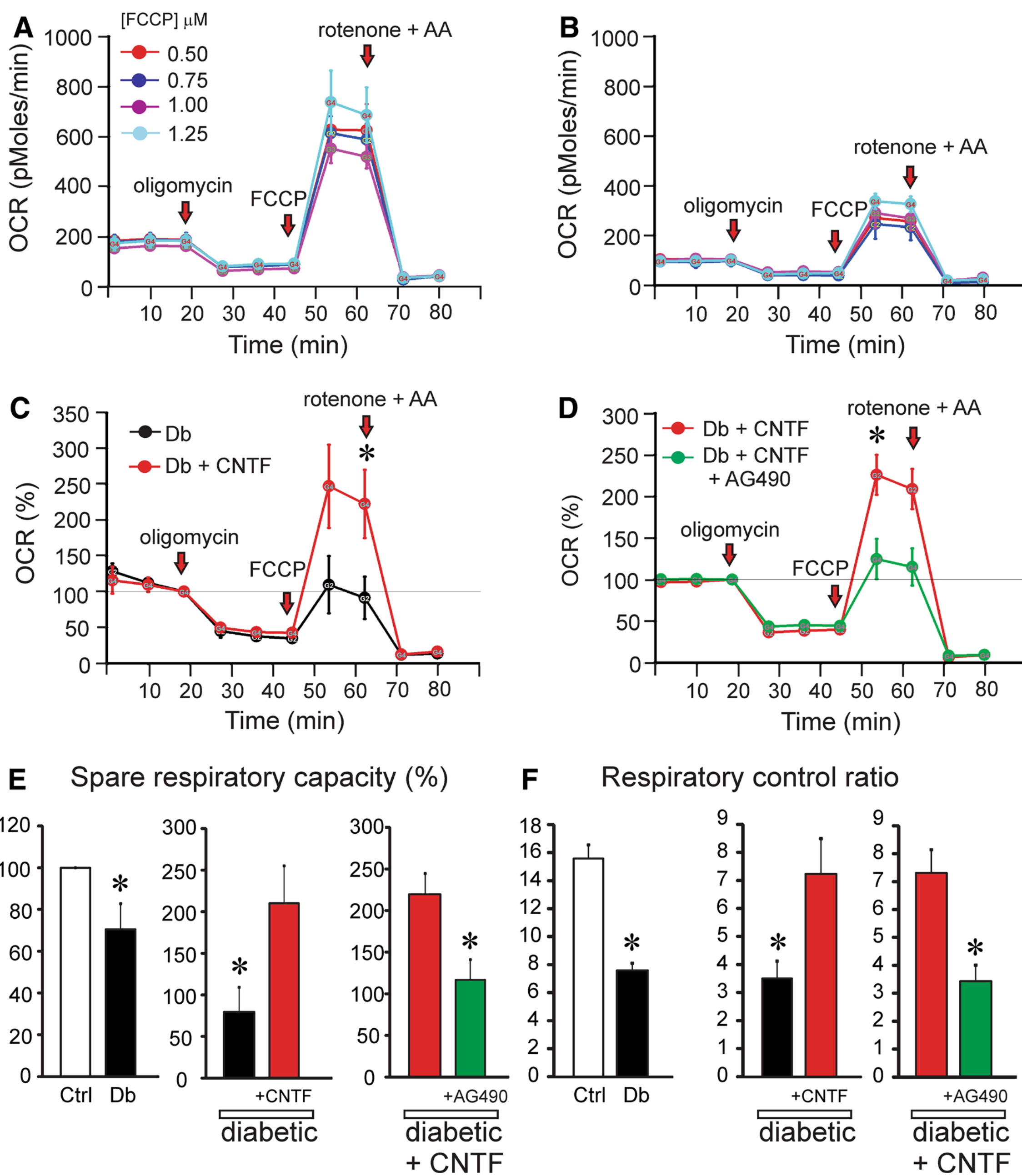
Fig. 2 Mitochondrial bioenergetics is abnormal in cultured neurons ▶ from diabetic rats and is corrected by CNTF. OCR was measured at basal level with subsequent and sequential addition of oligomycin (1 µM), FCCP (0.50–1.25 µM), and rotenone (1 µM) + antimycin A (AA; 1 µM) to DRG neurons cultured from age-matched control and 3–5 month STZ-induced diabetic rats. **a** Age-matched control and **b** diabetic animals. 1 µM of FCCP was used for further measurements of OCR due to good uncoupling effect and lack of toxicity. Oligomycin inhibits the ATP synthase leading to a build-up of the proton gradient and dearth of ATP that inhibits electron flux and reveals the state of coupling efficiency. Uncoupling of the respiratory chain by FCCP injection reveals the maximal capacity to reduce oxygen. Finally, rotenone + antimycin A were injected to inhibit the flux of electrons through complexes I and III, and thus no oxygen was further consumed at cytochrome *c* oxidase. The remaining OCR determined after this intervention is primarily non-mitochondrial. Non-mitochondrial respiration was subtracted for the calculation of all bioenergetics parameters as previously described (Roy Chowdhury et al. 2012; Saleh et al. 2013; Brand and Nicholls 2011). Data are expressed as OCR in pmoles/min for 1,000 cells (there were approximately 2,500–5,000 cells per well). Cultures derived from diabetic rats were treated for 24 h with 10 ng/ml CNTF and OCR determined and presented as % in **c**. In **d** diabetic cultures were treated with 10 ng/ml CNTF with or without 10 µM AG490 (the inactive congener AG9 had no effect—not shown). Spare respiratory capacity **e** and respiratory control ratio **f** values are shown in control (*open bar*), diabetic (*black*), diabetic + CNTF (*red*), and diabetic + CNTF + AG490 (*green*). Values are mean ± SEM of $n = 4$–5 replicate cultures; $*p < 0.05$ by Student's *t* test. Proton leak was elevated twofold in diabetic cultures and normalized by CNTF treatment; AG490 prevented the effect of CNTF (data not shown) (Colour Figure online)

CNTF Enhances Neurite Outgrowth Through JAK/STAT in Cultured Neurons

CNTF increased neurite outgrowth in neurons from control and diabetic rats (Fig. 1c, d). Co-treatment with the JAK inhibitor, AG490, significantly attenuated the positive effect of CNTF on neurite outgrowth (Fig. 1e).Colour Figure online

CNTF Normalizes Mitochondrial Membrane Polarization in Axons from Diabetic Rats

Oligomycin/antimycin A-induced mitochondrial membrane depolarization occurred at a greater rate in control neurons (Fig. 1f) because neurons from diabetic animals were already partially depolarized prior to drug treatment (Akude et al. 2011). Neurons from diabetic animals treated with CNTF for 24 h had TMRM decay kinetics that mimicked those of control animals, consistent with CNTF raising their mitochondrial polarization status. When neurons from control animals were treated with oligomycin alone there was a small hyperpolarization of the mitochondrial membrane, consistent with ATPase blockade and attenuated decline of the proton gradient through ATP synthesis (Fig. 1g). This hyperpolarization is transient, since adaptive systems within mitochondria prevent extensive hyperpolarization (Echtay et al. 2003). Neurons



from diabetic animals displayed a greater and longer lasting hyperpolarization upon oligomycin treatment, suggesting that protective mechanisms were dysfunctional. CNTF normalized the aberrant oligomycin response.

Abnormal Bioenergetics Profile of Neurons Cultured from Diabetic Rats is Corrected by CNTF

Maximal OCR induced by the uncoupler FCCP (0.50–1.25 μM) in neurons cultured from diabetic rats was decreased compared to neurons from age-matched control rats (Fig. 2a, b). This impairment of maximal electron transport activity in diabetic neurons indicates suboptimal spare respiratory capacity (Fig. 2e). CNTF (10 ng/ml, 24 h) significantly improved maximal OCR (Fig. 2c), spare respiratory capacity (Fig. 2e), and respiratory control ratio (Fig. 2f) of neurons from diabetic rats. The effect of CNTF was blocked by treatment with AG490 (Fig. 2d, e, f).

Discussion

CNTF prevents indices of diabetic neuropathy in rats (Calcutt et al. 2004; Mizisin et al. 2004) and mice (Saleh

et al. 2013). In diabetic mice, efficacy of CNTF involves overcoming impairment of NF-κB signaling (Saleh et al. 2013). We now demonstrate that DRG extracts from the same diabetic mice exhibit a concurrent defect in JAK/STAT signaling that was corrected by CNTF and that CNTF increased JAK/STAT signaling in rat neurons in vitro. These data prompted us to investigate whether enhancement of JAK/STAT signaling contributes to the ability of CNTF to enhance neuronal mitochondrial function and neurite outgrowth, both of which are impaired by diabetes.

Our finding that sensory neurons from diabetic rats are energetically stressed with increased mitochondrial workload and reduced spare respiratory capacity, in the absence of loss of mitochondrial number or mass, is consistent with our recent studies (Akude et al. 2011; Roy Chowdhury et al. 2012), as is the capacity of CNTF to overcome diabetes-induced mitochondrial dysfunction and promote neurite outgrowth in neurons from diabetic rats (Saleh et al. 2013). The novel finding that the ability of CNTF to restore the bioenergetics profile in neurons from diabetic rats and promote neurite outgrowth involves JAK/STAT signaling suggests that CNTF activates parallel pro-growth pathways involving both NF-κB and JAK/STAT. Recent studies have revealed that CNTF can reduce food intake, improve insulin sensitivity and lower fat deposition (Allen et al. 2011; Crowe et al. 2008) via activation of the AMP-activated protein kinase (AMPK)/peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) pathway in muscle and adipocytes (Crowe et al. 2008; Steinberg et al. 2009). Interestingly, diabetes impairs AMPK activation and PGC-1α expression in sensory neurons (Roy Chowdhury et al. 2012), just as it impedes signaling through NF-κB and JAK/STAT. AMPK may, therefore, join NF-κB and JAK/STAT as complementary targets for CNTF actions. Isolating the cellular and physiological consequences of CNTF signaling though each of these pathways may identify sites for intervention with discrete therapeutic effects.

Reduced spare respiratory capacity limits the ability of neurons to meet energetic demands and renders cells susceptible to secondary stressors (Brand and Nicholls 2011). CNTF augmentation of mitochondrial performance allows increased ATP synthesis under conditions of stress or energy demand. This CNTF-driven enhanced mitochondrial oxidative capacity could increase axonal plasticity as ATP demand for growth cone motility is extensive (Bernstein and Bamburg 2003). The ability of CNTF to enhance mitochondrial-dependent processes such as axonal plasticity may underlie its ability to enhance axon regeneration and protect against axonal degeneration in diabetic neuropathy.

Acknowledgments Drs. S. Roy Chowdhury and A. Saleh were supported by grants to P. Fernyhough from Canadian Institutes for Health Research (CIHR; Grant # MOP-84214) and Juvenile Diabetes Research Foundation (Grant # 1-2008-193). Dr. E. Akude was supported by a Grant from the National Science and Engineering Research Council (Grant # 3311686-06) to P. Fernyhough and subsequently a postgraduate scholarship from the Manitoba Health Research Council. Dr. Calcutt was supported by NIH Grant DK057629. The authors thank St. Boniface Research for funding support, Dr. G. Glazner, University of Manitoba and St. Boniface Hospital Research Centre, for permitting access to the Carl Zeiss LSM 510 microscope and Ms. Katie Frizzi for expert technical assistance.

Conflict of interest The authors, Subir Roy Chowdhury, Ali Saleh, Eli Akude, Darrell Smith, Dwane Morrow, Lori Tessler, Nigel Calcutt and Paul Fernyhough state that they have no financial relationships with the organizations that sponsored this research.

References

Akude E, Zherebitskaya E, Chowdhury SK, Smith DR, Dobrowsky RT, Fernyhough P (2011) Diminished superoxide generation is associated with respiratory chain dysfunction and changes in the mitochondrial proteome of sensory neurons from diabetic rats. Diabetes 60:288–297

Allen TL, Matthews VB, Febbraio MA (2011) Overcoming insulin resistance with ciliary neurotrophic factor. Handb Exp Pharmacol 203:179–199

Bernstein BW, Bamburg JR (2003) Actin-ATP hydrolysis is a major energy drain for neurons. J Neurosci 23:1–6

Brand MD, Nicholls DG (2011) Assessing mitochondrial dysfunction in cells. Biochem J 435:297–312

Calcutt NA, Freshwater JD, Mizisin AP (2004) Prevention of sensory disorders in diabetic Sprague−Dawley rats by aldose reductase inhibition or treatment with ciliary neurotrophic factor. Diabetologia 47:718–724

Crowe S, Turpin SM, Ke F, Kemp BE, Watt MJ (2008) Metabolic remodeling in adipocytes promotes ciliary neurotrophic factor-mediated fat loss in obesity. Endocrinology 149:2546–2556

Echtay KS, Esteves TC, Pakay JL, Jekabsons MB, Lambert AJ, Portero-Otin M, Pamplona R, Vidal-Puig AJ, Wang S, Roebuck SJ, Brand MD (2003) A signalling role for 4-hydroxy-2-nonenal in regulation of mitochondrial uncoupling. EMBO J 22:4103–4110

Mizisin AP, Vu Y, Shuff M, Calcutt NA (2004) Ciliary neurotrophic factor improves nerve conduction and ameliorates regeneration deficits in diabetic rats. Diabetes 53:1807–1812

Qiu J, Cafferty WB, McMahon SB, Thompson SW (2005) Conditioning injury-induced spinal axon regeneration requires signal transducer and activator of transcription 3 activation. J Neurosci 25:1645–1653

Roy Chowdhury SK, Smith DR, Saleh A, Schapansky J, Marquez A, Gomes S, Akude E, Morrow D, Calcutt NA, Fernyhough P (2012) Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. Brain 135:1751–1766

Saleh A, Roy Chowdhury SK, Smith DR, Balakrishnan S, Tessler L, Martens C, Morrow D, Schartner E, Frizzi KE, Calcutt NA, Fernyhough P (2013) Ciliary neurotrophic factor activates NF-kappaB to enhance mitochondrial bioenergetics and prevent

neuropathy in sensory neurons of streptozotocin-induced diabetic rodents. Neuropharmacology 65:65–73

Sleeman MW, Anderson KD, Lambert PD, Yancopoulos GD, Wiegand SJ (2000) The ciliary neurotrophic factor and its receptor, CNTFR alpha. Pharm Acta Helv 74:265–272

Steinberg GR, Watt MJ, Ernst M, Birnbaum MJ, Kemp BE, Jorgensen SB (2009) Ciliary neurotrophic factor stimulates muscle glucose uptake by a PI3-kinase-dependent pathway that is impaired with obesity. Diabetes 58:829–839

Zigmond RE (2011) gp130 cytokines are positive signals triggering changes in gene expression and axon outgrowth in peripheral neurons following injury. Front Mol Neurosci 4:62