CORA Cork Open Research Archive
Cartlann Taighde Oscailte Chorcaí

Title	A small molecule activator of p300/CBP histone acetyltransferase promotes survival and neurite growth in a cellular model of Parkinson's disease
Authors	Hegarty, Shane V.;O'Leary, Eimear;Solger, Franziska;Stanicka, Joanna;Sullivan, Aideen M.;O'Keeffe, Gerard W.
Publication date	2016-06-02
Original Citation	Hegarty, S.V., O'Leary, E., Solger, F., Stanicka, J., Sullivan, A.M. and O'Keeffe, G.W. (2016) 'A small molecule activator of p300/CBP histone acetyltransferase promotes survival and neurite growth in a cellular model of Parkinson's disease', Neurotoxicity Research, 30(3) pp.510-520. doi:10.1007/s12640-016-9636-2
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1007/s12640-016-9636-2
Rights	© 2016, Springer Science+Business Media, New York. The final publication is available at Springer via http://dx.doi.org/10.1007/s12640-016-9636-2
Download date	2026-11-09 16:57:28
Item downloaded from	https://hdl.handle.net/10468/3180

UCC
University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

A small molecule activator of p300/CBP histone acetyltransferase promotes survival and neurite growth in a cellular model of Parkinson's disease.

Shane V. Hegarty[1], Eimear O'Leary[1], Franziska Solger[1], Joanna Stanicka[1], Aideen M. Sullivan[1]*, Gerard W. O'Keeffe[1, 2]*.

[1] Department of Anatomy and Neuroscience, Biosciences Institute, University College Cork, Cork, Ireland.

[2] The Irish Centre for Fetal and Neonatal Translational Research (INFANT), Cork University Maternity Hospital, Cork, Ireland.

Address correspondence to
Dr. Shane V. Hegarty
Phone (+353) 21 490 1348
Fax (+353) 21 427 3518
Email shane.hegarty@ucc.ie

*These authors contributed equally to this work.

Acknowledgements

Studies in the authors' laboratories are supported by grants from the Irish Research Council (R15897; SVH/AS/G'OK) and the National University of Ireland (R16189; SVH/AS/G'OK).

Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disease characterised by motor and non-motor symptoms, resulting from the degeneration of nigrostriatal dopaminergic neurons and peripheral autonomic neurons. Given the limited success of neurotrophic factors in clinical trials, there is a need to identify new small molecule drugs and drug targets to develop novel therapeutic strategies to protect all neurons that degenerate in PD.

Epigenetic dysregulation has been implicated in neurodegenerative disorders, while targeting histone acetylation is a promising therapeutic avenue for PD. We and others have demonstrated that histone deacetylase inhibitors have neurotrophic effects in experimental models of PD. Activators of histone acetyltransferases (HAT) provide an alternative approach for the selective activation of gene expression, however little is known about the potential of HAT activators as drug therapies for PD.

To explore this potential, the present study investigated the neurotrophic effects of CTPB (N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide), which is a potent small molecule activator of the histone acetyltransferase p300/CBP, in the SH-SY5Y neuronal cell line. We report that CTPB promoted the survival and neurite growth of the SH-SY5Y cells, and also protected these cells from cell death induced by the neurotoxin 6-hydroxydopamine. This study is the first to investigate the phenotypic effects of the HAT activator CTPB, and to demonstrate that p300/CBP HAT activation has neurotrophic effects in a cellular model of PD.

Key words:

Parkinson's disease; neurotrophic therapy; epigenetic regulation; p300/CBP histone acetyltransferase; CTPB; neuronal survival and growth.

Abbreviations

6-OHDA - 6-hydroxydopamine

BMP - bone morphogenetic protein(s)

CTPB - N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide

DA – dopaminergic/dopamine

DAPI - 4'-6-diamidino-2-phenylindole

DMSO - dimethyl sulfoxide

DIV – day(s) *in vitro*

H3- total histone H3

HAT - histone acetyltransferase(s)

HDAC - histone deacetylase(s)

LDH - lactate dehydrogenase

MTT - Thiazolyl Blue Tetrazolium Bromide

N - number of repetitions

pAcH3 - p-acetylated-histone H3

PBS-T - 10mM PBS containing 0.02% Triton X-100

PD - Parkinson's disease

SNpc - substantia nigra pars compacta

Introduction

Parkinson's disease (PD) is a chronic neurodegenerative disease that is second only to Alzheimer's disease in its worldwide prevalence. It affects 1-2% of people over the age of 65, and this incidence significantly increases with age (Dorsey et al. 2013). PD is a motor disorder, with many accompanying non-motor symptoms (Jankovic 2008; Lees et al. 2009). PD is characterised by the progressive loss of midbrain dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) resulting in reduced DA neurotransmission in the striatum, leading to motor dysfunction. A second defining feature of PD is the presence of intraneuronal Lewy body inclusions, consisting of predominantly α-synuclein that are found throughout the central, peripheral, and autonomic nervous systems (CNS, PNS and ANS) (Bethlem and Den Hartog Jager 1960; Jellinger 1991; Jellinger 2012). In parallel to the striatal DA denervation (Bedard et al. 2011), there is also heterogeneous peripheral denervation (Goldstein et al. 1997; Goldstein et al. 2000; Kaufmann and Goldstein 2013), with the most common being sympathetic denervation of the left ventricular myocardium leading to orthostatic hypotension (Lucio et al. 2013). One of the most promising disease-modifying therapies for PD involves the delivery of neurotrophic factors to the striatum and/or SNpc to protect DA neurons (Hegarty et al. 2014b). While this is a promising approach, it has had limited success to date in clinical trials (Olanow et al. 2015). In addition, such localised administration of neurotrophic factor ligands is unlikely to protect other neurons that are affected by the disease process. Therefore, while neurotrophic factors remain a promising therapeutic approach for protecting DA neurons, it is important to identify new drugs, and drug targets, that may be useful in protecting all neurons affected by PD.

It is increasingly recognized that epigenetic dysregulation plays a key role in disorders of the nervous system (Abel and Zukin 2008; Chuang et al. 2009; Hahnen et al. 2008; Hodges et al. 2006; Jiang et al. 2008; Kazantsev and Thompson 2008; Saha and Pahan 2006). One key epigenetic process is histone acetylation which controls the ease with which transcription factors can access DNA to regulate gene expression (Allfrey et al. 1964; Serrano et al. 2013). Histone acetylation involves a dynamic interplay between two classes of enzymes, known as histone acetyltransferases (HAT) and histone deacetylases (HDAC), that respectively control histone acetylation and deacetylation (Yang and Seto 2007). We and others have shown that pan- and class-specific HDAC inhibitors can protect DA neurons (Chen et al. 2006; Chen et al. 2007; Collins et al. 2015; Gardian et al. 2004; Kidd and Schneider 2010; Kidd and Schneider 2011; Kontopoulos et al. 2006; Outeiro et al. 2007; St Laurent et al. 2013; Wu et

al. 2008; Zhu et al. 2014) and sympathetic neurons (Collins et al. 2015) in experimental models of PD. The potential of this approach for clinical translation is highlighted by an on-going Phase I clinical trial of the FDA-approved drug Glycerol Phenylbutyrate (a HDAC inhibitor) which is exploring the potential of this drug to increase the removal of α-synuclein from the brain (NCT02046434) (for recent reviews see Harrison and Dexter 2013; Schneider et al. 2013; Valor et al. 2013). An alternative epigenetic approach for the broad, yet selective, activation of gene expression is the induction of HAT activity, instead of HDAC inhibition. However, there is little known about the potential of HAT activators as potential drug therapies for PD.

The global transcriptional co-activator p300/CBP is a potent HAT that plays essential roles in nervous system development (Lopez-Atalaya et al. 2014; Sheikh 2014; Tanaka et al. 2000; Yao et al. 1998). p300/CBP HAT is recruited to DNA by direct interaction with specific transcription factors, which enhances transcriptional activity by acetylation of histones proximal to the promoter region. Recent reports have shown that peripheral administration of carbon nanosphere-conjugated small molecular activators of p300/CBP increases histone acetylation in the brain (Chatterjee et al. 2013; Selvi et al. 2008). Furthermore, p300/CBP has also been shown to activate bone morphogenetic protein (BMP)-Smad-mediated transcription (Pearson et al. 1999), which is known to promote the survival and growth of midbrain DA (Hegarty et al. 2014a; Hegarty et al. 2013) and sympathetic neurons (O'Keeffe et al. 2016). This highlights the potential for targeting p300/CBP as a therapeutic approach (Valor et al. 2013) to protect both DA and sympathetic neurons in PD.

To begin to address this potential, we employed a selective and potent small molecular activator of p300/CBP known as CTPB (N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide) (Balasubramanyam et al. 2003). CTPB is a benzamide that activates p300/CBP HAT activity but not that of p300/CBP-associated factor (PCAF). CTPB enhances p300/CBP HAT-dependent transcriptional activation, and has no effect on histone deacetylase activity (Balasubramanyam et al. 2003). The ability of CTPB to selectively modulate p300 HAT activity has been further characterised (Devipriya and Kumaradhas 2013; Devipriya et al. 2010; Mantelingu et al. 2007), exploited to synthesize novel p300/CBP-targeting compounds (Souto et al. 2010; Souto et al. 2008), and demonstrated *in vivo* (Selvi et al. 2008). However, there have been no studies investigating the phenotypic effects of CTPB-mediated p300/CBP HAT activation in cellular or animal models of PD. Herein we provide the first evidence showing that CTPB exerts neurotrophic effects in the SH-SY5Y cell line, which is a widely used model of human DA and sympathetic neurons.

Materials and Methods

Cell Culture

SH-SY5Y cells were cultured in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (Sigma), supplemented with 10% foetal calf serum (Sigma), 100nM L-Glutamine (Sigma), 100U/ml Penicillin/Streptomycin (Sigma), in a humidified atmosphere containing 5% CO_2 at 37˚C. For neurite growth assays, a plating density of 5×10^4 cells/well of a 24-well plate was used, while viability and death assays had a plating density of 1×10^5 cells/well in a 24-well plate. For western blotting, 2×10^6 cells were plated per well of a 6-well plate. Where indicated, cells were treated with 0.5-200μM of CTPB (Enzo Life Sciences) or a vehicle (equivalent % of Dimethyl Sulfoxide (DMSO); Sigma) for 24h or daily for 4 days *in vitro* (DIV). For DMSO controls, cells were treated with % of DMSO equal to highest concentration of CTPB within the experiment. For the neurotoxin assays, cells were treated with 5-100μM of 6-hydroxydopamine (6-OHDA; Sigma) for 24h.

Immunocytochemistry

Cultures were fixed in 4% paraformaldehyde in 10mM PBS for 10 min at -20˚C. Following three washes in 10mM PBS containing 0.02% Triton X-100 (PBS-T in 10mM PBS) for permeabilization, cultures were incubated in blocking solution (5% bovine serum albumin (Sigma) in 10mM PBS) for 1h at room temperature. Cultures were subsequently incubated in the following primary antibodies: p-acetylated-histone H3 (Ser 11/Lys 15) (pAcH3; 1:200; rabbit polyclonal IgG; Santa Cruz) and β-actin (1:200; mouse polyclonal IgG; Sigma) diluted in 1% bovine serum albumin in 10mM PBS at 4˚C overnight. Following 3 x 5min washes in PBS-T, cells were incubated in Alexa Fluor 488- or 594-conjugated secondary antibodies (1:500; Invitrogen) reactive to the species of the primary antibodies and diluted in 1% bovine serum albumin in 10mM PBS, at room temperature for 2h in the dark. Cultures were counterstained with 4'-6-diamidino-2-phenylindole (DAPI; 1:1000 in 10mM PBS; Sigma). Cells were imaged under an Olympus IX70 inverted microscope fitted with an Olympus DP70 camera and AnalysisD™ software. The fluorescence intensity of individual cells stained for pAcH3 was measured using Image J analysis software (Rasband, WJ, http://rsb.info.nih.gov/ij/). The relative fluorescence intensity was calculated as the intensity of each individual cell after subtraction of the background noise.

Cell Viability and Cell Death Assessment

Thiazolyl Blue Tetrazolium Bromide (MTT) assays were performed to assess cell viability as previously described (Hegarty et al. 2013). MTT was added to cells at a concentration of 0.5mg/ml. To assess cellular toxicity/death, lactate dehydrogenase (LDH) activity was measured in 100µl of the cell culture medium of each treatment group using an LDH Activity Assay Kit (Sigma), according to the provider's instructions. Fresh cell culture medium, which had no contact with cultured cells, was used as the negative control.

Measurement of Cellular Morphology

5 microscopic fields were randomly selected for each experiment, and photographed using an Olympus IX70 inverted microscope. All cells in each photograph were measured. The length of the neuritic arborisation was estimated using standard stereological procedures (Mayhew 1992). A line grid was superimposed on the microscopic images and the number of times each neurite intersected the grid was recorded using Image J analysis software. The neurite length was calculated using the following formula: $NL = \alpha \times T \times (\pi/2)$, where α is the number of times the neurite intersects the grid lines, and T is the distance between the gridlines on the magnified image (taking into account the magnification factor).

Western Blotting

Western blotting was carried out as described (Crampton et al. 2012). Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (50µl RIPA per 1×10^6 cells; 50mM Tris-HCL; 150mM NaCl, 1% Triton X-100, 1mM EDTA, 1mM NaF, 1mM Na_3VO_4, 1µg/ml leupeptin and 1µg/ml pepstatin) for 1h on ice, and insoluble debris was removed by centrifugation. 15µg of protein was run by SDS-PAGE and transferred to nitrocellulose membranes using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad, CA, USA). The membranes were incubated with primary antibodies against pAcH3 (1:1000), total histone H3 (H3; rabbit polyclonal IgG; Abcam) or β-actin (1:2000) overnight at 4°C, washed, incubated with the appropriate goat IR700/800-labelled secondary antibodies (1:10000; Licor), washed and visualised with Odyssey (Licor). Protein levels were normalised to β-actin by densitometry using Image Studio Lite software (Licor).

Statistical Analysis

Unpaired Student's t-test or one-way ANOVA with a *post hoc* Tukey's test was performed, as appropriate, to determine significant differences between groups. Results were expressed as means with SEM and deemed significant when $p<0.05$.

Results

A dose-response analysis of CTPB in SH-SY5Y cells.

To determine a viable working concentration of the small molecular activator of p300/CBP HAT, CTPB, in the SH-SY5Y neuronal cell line, these cells were treated with increasing concentrations of CTPB ranging from 0.5-200μM. An MTT assay, which is often used to measure cell viability by measuring cellular respiration through mitochondrial activity, showed that CTPB concentrations above 100μM caused non-selective SH-SY5Y cell death after 1DIV (data not shown). At 4DIV, daily treatments with CTPB concentrations of 50μM, 100μM or 200μM significantly decreased MTT absorbance compared to control (Fig. 1A). Examination of phase contrast images indicated that there was cell death at these higher doses (Fig. 1B), which accounted for the reduction in cellular viability (Fig. 1A). To confirm that DMSO was not toxic, SH-SY5Y cells were cultured in 0.2% or 0.4% DMSO (equivalent concentration of DMSO to that in 100μM or 200μM of CTPB, respectively) for 4DIV. DMSO at any concentrations tested had no significant effect on cell viability (Fig. 1A). At CTPB concentrations of 10μM and below, SH-SY5Y cell viability was not adversely affected by daily CTPB treatments for up to 4DIV (Fig. 1A-B). The MTT assay also showed that daily treatment with 5μM and 10μM of CTPB significantly increased the MTT absorbance of SH-SY5Y cells compared to control at 4DIV (Fig. 1A). These data suggest that there is a therapeutic range in which CTPB alters the basal state of SH-SY5Y cells.

CTPB promotes neurite growth in SH-SY5Y cells.

As an increase in MTT absorbance (mitochondrial activity) could indicate increased proliferation, decreased cell death or differentiation and neurite growth (Habash et al. 2015; Steketee et al. 2012; Tao et al. 2014), we next examined whether targeted activation of p300/CBP HAT using CTPB altered the total number of cells, the numbers of pyknotic nuclei or neurite growth in SH-SY5Y cells. We used neurite growth as a measure of differentiation as class-specific HDAC inhibitors have previously been shown to induce neurite growth of SH-SY5Y cells (Collins et al. 2015). Having identified working concentrations of CTPB, SH-SY5Y cells were treated daily with either control (HBSS or 0.01% DMSO) or 0.5μM, 1.0μM, 2.5μM or 5μM of CTPB before being immunostained for β-actin at 4DIV, to allow visualisation of the cellular cytoskeleton, and counterstained with DAPI. Daily treatment with either 2.5μM or 5μM of CTPB resulted in a significant increase in the total neurite length/cell of SH-SY5Y cells compared to vehicle-treated control cultures (Fig. 2A-B). However, SH-

SY5Y cells treated with the lower concentrations of 0.5μM or 1.0μM did not have significantly longer neurites when compared to controls (Fig. 2A). In contrast, there were no significant differences in the number of pyknotic cells per field (DMSO: 2.1 ± 0.3; CTBP 2.0 ± 0.3) or total cell number per field (DMSO: 149.1 ± 32.0; CTBP 114.7 ± 26.0) in SH-SY5Y cells treated with 0.01% DMSO or 5μM of CTPB for 4DIV, suggesting that 5μM of CTPB does not affect the numbers of viable or dying SH-SY5Y cells. To explore this further, we next determined whether CTBP increased the numbers of neurites and/or the total neurite length in individual cells. Daily treatment of SH-SY5Y cells with 5μM of CTPB resulted in a significant increase in neurite length of individual SH-SY5Y cells compared to 0.01% DMSO-treated controls at 4DIV, but had no effect on the number of primary neurites (Fig. 2C-D). These findings indicate that CTPB-induced neurite growth in SH-SY5Y cells results from increasing the length of existing neurites rather than their number.

6-OHDA neurotoxin dose-dependently induces cell death in SH-SY5Y cells.

To further examine the neurotrophic potential of CTPB, the ability of CTPB to protect SH-SY5Y cells against the cell death induced by DA neurotoxin 6-OHDA was next assessed. To facilitate such an investigation, a dose-response experiment was initially performed to identify a concentration of 6-OHDA which was submaximal (~50%) in its neurotoxic effect. SH-SY5Y cells were cultured in increasing concentrations of 6-OHDA (5-100μM) for 24h, after which cell viability and cell death were measured by MTT assay and LDH assay, respectively. Increasing concentrations of 6-OHDA resulted in a concentration-dependent reduction in cell viability, and a corresponding concentration-dependent increase in cell death (Fig. 3A-C). Maximal cell death was observed with 6-OHDA concentrations of 25μM and above after 24h, while 5μM of 6-OHDA had no significant effect on cell viability or death (Fig. 3A-B). A 15μM concentration of 6-OHDA was selected for subsequent experimentation, as this concentration induced a ~50% reduction in MTT absorbance (Fig. 3A) and a ~50% increase in LDH release (Fig. 3B), thus facilitating the examination of any neuroprotective effects of CTPB.

CTPB increases histone acetylation in SH-SY5Y cells.

CTPB is a selective and potent small molecular activator of p300/CBP that has been shown to enhance p300/CBP HAT activity *in vitro* (Balasubramanyam et al. 2003; Devipriya and Kumaradhas 2013; Devipriya et al. 2010; Mantelingu et al. 2007) and *in vivo* when coupled to a CSP carrier (Selvi et al. 2008). To determine if CTPB significantly increases p300/CBP

HAT activity in this cell line, the levels of acetylated histones were first measured by immunocytochemical staining of SH-SY5Y cells for pAcH3 following CTPB treatment. Treatment of SH-SY5Y cells with 2.5μM or 5μM of CTPB for 24h induced a significant increase in the levels of pAcH3 relative to vehicle-treated counterparts, which was quantified by densitometry (Fig. 4A-B). This finding was confirmed by western blot analysis of cells treated with 5μM CTPB for 24h. CTPB treatment significantly increased the levels of pAcH3 relative to total histone H3 protein levels, when compared to control treated SH-SY5Y cells (Fig. 4C). This was measured by densitometric quantification of the ratio of pAcH3 to total histone H3 protein levels, relative to the β-actin loading control. In addition to this, the effect of the 6-OHDA on the levels of acetylated histones was also assessed. Treatment of SH-SY5Y cells with the submaximal (~50%) neurotoxic dose of 6-OHDA, 15μM, for 24h had no effect on the levels of pAcH3/H3 compared to control (Fig. 4C). Furthermore, CTPB or 6-OHDA treatment did not significantly increase the levels of total histone H3 compared to control, despite an increase in total histone H3 levels following CTPB treatment. These data indicate that CTPB-induced p300/CBP HAT activation induces hyperacetylation of histone H3 in SH-SY5Y cells.

CTPB significantly protects against the cell death induced by neurotoxin 6-OHDA in SH-SY5Y cells.

To further examine the neurotrophic potential of CTPB-induced activation of p300/CBP HAT, the ability of CTPB to protect SH-SY5Y cells against the cell death induced by DA neurotoxin 6-OHDA was assessed. SH-SY5Y cells were cultured in the presence of 15μM of 6-OHDA with or without 5μM of CTPB for 24h, at which time MTT and LDH assays were performed to measure cell viability and cell death respectively. SH-SY5Y cells treated with 6-OHDA or 6-OHDA and CTPB, had significantly reduced cell viability and significantly increased cell death compared to vehicle-treated controls, as assessed by MTT assay and LDH assay (Fig. 5A-C). However, CTPB significantly protected against the cell death induced by 6-OHDA in SH-SY5Y cells. CTPB- and 6-OHDA-treated SH-SY5Y cells had significantly increased cell viability (Fig. 5A) and significantly decreased cell death (Fig. 5B) compared to those treated with 6-OHDA alone. These data have demonstrated that CTPB protects SH-SY5Y cells against neurotoxic insult. Taken together with the findings above, these results suggest that CTPB-induced p300/CBP HAT activity promotes survival and neurite growth in this neuronal cell line.

Discussion

A number of reports have documented neurotrophic effects of HDAC inhibitors in SH-SY5Y cells and other cellular models of midbrain DA neurons *in vitro* (Chen et al. 2006; Chen et al. 2007; Collins et al. 2015; Gardian et al. 2004; Kidd and Schneider 2010; Kidd and Schneider 2011; Kontopoulos et al. 2006; Outeiro et al. 2007; St Laurent et al. 2013; Wu et al. 2008; Zhu et al. 2014). However, to our knowledge, the present study is the first to show that a selective HAT activator can induce similar neurotrophic effects in the SH-SY5Y neuronal cell line. SH-SY5Y cells have been used extensively as models of human DA and sympathetic neurons (Collins et al. 2015; Hegarty et al. 2013; Toulouse et al. 2012; Xie et al. 2010) and have been shown to be capable of differentiation into DA-synthesising neurons (Gomez-Santos et al. 2002; McMillan et al. 2007; Presgraves et al. 2004; Xie et al. 2010). In the present study, we found that CTPB-induced p300/CBP HAT activation promotes the survival and neurite growth of SH-SY5Y cells. We also found that CTPB significantly increases histone acetylation in these cells, most likely through induction of p300/CBP HAT activity. Finally, this study found that CTPB is capable of protecting SH-SY5Y cells from the cell death induced by the DA neurotoxin 6-OHDA. This study is the first to investigate the phenotypic effects of p300/CBP HAT activation by CTPB, and to demonstrate that small molecule-mediated p300/CBP HAT activation has neurotrophic effects in a cellular model of PD.

In support of our findings, p300/CBP has been shown to contribute to the survival, differentiation and neurite growth of a variety of neuronal cell types in the nervous system *in vitro* and *in vivo* (Chatterjee et al. 2013; Chen et al. 2010; Cong et al. 2005; Culmsee et al. 2003; Gaub et al. 2011; Gaub et al. 2010; Koyano-Nakagawa et al. 1999; Lee et al. 2009; Morikawa et al. 2005; Rouaux et al. 2003; Seo et al. 2012; Sun et al. 2001; Tsui et al. 2014; Wang et al. 2010; Wong et al. 2005). In terms of the development of midbrain DA neurons, which progressively degenerate in PD, there is evidence to suggest that p300/CBP contributes to the acquisition of a DA phenotype in differentiating neurons (Seo et al. 2012). Furthermore, in relation to PD-affected peripheral sympathetic neurons, p300/CBP has been shown to be an important transcriptional co-activator in the neuronal survival- and differentiation-inducing effects of NGF/NF-kappaB signalling (Chen et al. 2010; Culmsee et al. 2003; Wong et al. 2005), which are essential regulators of neuronal development in the PNS (Davies 2009; Glebova and Ginty 2005; Gutierrez et al. 2005; Gutierrez et al. 2008). In addition to this, Hand2 is an important transcription factor for sympathetic nervous system

development whose function is dependent on its interaction with p300/CBP (Morikawa et al. 2005). p300/CBP is also a transcriptional co-activator for BMP-Smad signalling, which is known to promote the survival and growth of midbrain DA neurons and sympathetic neurons (Hegarty et al. 2014a; Hegarty et al. 2013; O'Keeffe et al. 2016; Pearson et al. 1999). Taken these findings together, p300/CBP may act as an important transcriptional co-activator in the development, growth and survival of DA and sympathetic neurons, which would make p300/CBP HAT a potential therapeutic target for PD.

In the present study, following the identification of viable working concentrations of CTPB, we initially found that CTPB induced the neurite growth of SH-SY5Y cells. While HDAC inhibitors have previously been shown to induce neurite growth in these cells (Collins et al. 2015; Yuan et al. 2001), this is the first report documenting the ability of a HAT activator to promote SH-SY5Y neurite growth. This is an important finding considering the limited number of HAT activators which have been produced (Schneider et al. 2013), and the corresponding paucity of studies investigating their neurotrophic potential. The ability of p300/CBP activity to promote neurite growth has been reported in other neuronal populations (Gaub et al. 2011; Gaub et al. 2010; Lee et al. 2009), while small molecule-mediated activation of p300/CBP promotes the growth of neurons in the adult mouse dentate gyrus *in vivo* (Chatterjee et al. 2013).

We subsequently confirmed the ability of CTPB to induce hyperacetylation of histone H3 in the SH-SY5Y cells, which was most likely due to selective activation of p300/CBP HAT activity. Indeed, CTPB has been consistently demonstrated to induce p300/CBP HAT activity, in which histone acetylation levels were typically assessed *in vitro* (Balasubramanyam et al. 2003; Devipriya and Kumaradhas 2013; Devipriya et al. 2010; Mantelingu et al. 2007) and *in vivo* when coupled to a CSP carrier (Selvi et al. 2008). This suggests that the CTPB-induced neurotrophic effects observed in this study may be mediated by increased p300/CBP HAT activity. Finally, we demonstrated that CTPB significantly protects SH-SY5Y neuronal cells against 6-OHDA-induced neurodegeneration. p300/CBP HAT activity has previously been shown to promote the survival of a variety of neuronal cell types (Culmsee et al. 2003; Rouaux et al. 2003; Wong et al. 2005); however, this is the first demonstration that p300/CBP HAT activity protects against cell death induced by neurotoxin 6-OHDA. Thus, this study has identified p300/CBP HAT activator CTPB as a promising neuroprotective agent with relevance to PD.

Given the neurotrophic effects of small molecule HAT activator CTPB reported in this study, targeting p300/CBP HAT activity may be a viable neurotrophic strategy.

Interestingly, α-synuclein has been reported to reduce p300/CBP expression and HAT activity in DA neurons (Jin et al. 2011). Therefore, targeting p300/CBP may be beneficial in two ways; firstly through the promotion of neuronal survival and growth, and secondly by counteracting the α-synuclein-induced reduction of p300/CBP HAT activity. CTPB is potentially a promising molecule for targeted induction of p300/CBP activity in this regard (Balasubramanyam et al. 2003), however much more work is needed in translational animals models of PD, including exploring strategies to deliever it to the brain. Interestingly, carbon nanosphere-conjugated CTPB has the ability to cross the blood-brain-barrier, localize to specific nuclei in the brain and induce hyperacetlyation *in vivo* (Selvi et al. 2008). As such these non-toxic, carbon nanospheres-conjugated CTPB small molecules should be investigated for their ability to induce neuroprotective effects in 6-OHDA and in particular α-synuclein animal models of PD. Also the ability of CTPB to act both centrally and peripherally in the nervous system also provides a rationale for studying its effects on sympathetic axons *in vivo.* This is important as the majority of proposed disease-modifying therapies for PD to date have focused on midbrain DA neurons, while PNS neurons which contribute to debilitating non-motor symptoms are overlooked.

Collectively, the above reports in conjunction with our present study suggest that HAT activators, such as the small molecule CTPB, provide a promising and exciting area of research for neurological disorders. The *in vitro* findings reported in this study suggest that CTPB may be beneficial in PD. CTPB should be examined in animal models of PD as the next stage in rationalizing its use as a potential therapy for this disorder.

References

Abel T, Zukin RS (2008) Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders Curr Opin Pharmacol 8:57-64 doi:S1471-4892(07)00213-5 [pii]

10.1016/j.coph.2007.12.002 [doi]

Allfrey VG, Faulkner R, Mirsky AE (1964) ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS Proc Natl Acad Sci U S A 51:786-794

Balasubramanyam K, Swaminathan V, Ranganathan A, Kundu TK (2003) Small molecule modulators of histone acetyltransferase p300 J Biol Chem 278:19134-19140 doi:10.1074/jbc.M301580200 [doi]

M301580200 [pii]

Bedard C, Wallman MJ, Pourcher E, Gould PV, Parent A, Parent M (2011) Serotonin and dopamine striatal innervation in Parkinson's disease and Huntington's chorea Parkinsonism Relat Disord 17:593-598 doi:S1353-8020(11)00144-1 [pii]

10.1016/j.parkreldis.2011.05.012 [doi]

Bethlem J, Den Hartog Jager WA (1960) The incidence and characteristics of Lewy bodies in idiopathic paralysis agitans (Parkinson's disease) J Neurol Neurosurg Psychiatry 23:74-80

Chatterjee S et al. (2013) A novel activator of CBP/p300 acetyltransferases promotes neurogenesis and extends memory duration in adult mice J Neurosci 33:10698-10712 doi:33/26/10698 [pii]

10.1523/JNEUROSCI.5772-12.2013 [doi]

Chen PS et al. (2006) Valproate protects dopaminergic neurons in midbrain neuron/glia cultures by stimulating the release of neurotrophic factors from astrocytes Mol Psychiatry 11:1116-1125 doi:4001893 [pii]

10.1038/sj.mp.4001893 [doi]

Chen PS et al. (2007) Valproic acid and other histone deacetylase inhibitors induce microglial apoptosis and attenuate lipopolysaccharide-induced dopaminergic neurotoxicity Neuroscience 149:203-212 doi:S0306-4522(07)00809-3 [pii]

10.1016/j.neuroscience.2007.06.053 [doi]

Chen YL, Monteith N, Law PY, Loh HH (2010) Dynamic association of p300 with the promoter of the G protein-coupled rat delta opioid receptor gene during NGF-induced

neuronal differentiation Biochem Biophys Res Commun 396:294-298 doi:S0006-291X(10)00752-7 [pii]

10.1016/j.bbrc.2010.04.083 [doi]

Chuang DM, Leng Y, Marinova Z, Kim HJ, Chiu CT (2009) Multiple roles of HDAC inhibition in neurodegenerative conditions Trends Neurosci 32:591-601 doi:S0166-2236(09)00133-7 [pii]

10.1016/j.tins.2009.06.002 [doi]

Collins LM, Adriaanse LJ, Theratile SD, Hegarty SV, Sullivan AM, O'Keeffe GW (2015) Class-IIa Histone Deacetylase Inhibition Promotes the Growth of Neural Processes and Protects Them Against Neurotoxic Insult Mol Neurobiol 51:1432-1442 doi:10.1007/s12035-014-8820-8 [doi]

Cong SY, Pepers BA, Evert BO, Rubinsztein DC, Roos RA, van Ommen GJ, Dorsman JC (2005) Mutant huntingtin represses CBP, but not p300, by binding and protein degradation Mol Cell Neurosci 30:560-571

Crampton SJ, Collins LM, Toulouse A, Nolan YM, O'Keeffe GW (2012) Exposure of foetal neural progenitor cells to IL-1beta impairs their proliferation and alters their differentiation - a role for maternal inflammation? J Neurochem 120:964-973 doi:10.1111/j.1471-4159.2011.07634.x [doi]

Culmsee C et al. (2003) Reciprocal inhibition of p53 and nuclear factor-kappaB transcriptional activities determines cell survival or death in neurons J Neurosci 23:8586-8595 doi:23/24/8586 [pii]

Davies AM (2009) Extracellular signals regulating sympathetic neuron survival and target innervation during development Auton Neurosci 151:39-45 doi:S1566-0702(09)00413-5 [pii]

10.1016/j.autneu.2009.07.011 [doi]

Devipriya B, Kumaradhas P (2013) Charge density distribution and the electrostatic moments of CTPB in the active site of p300 enzyme: a DFT and charge density study J Theor Biol 335:119-129 doi:S0022-5193(13)00257-9 [pii]

10.1016/j.jtbi.2013.06.001 [doi]

Devipriya B, Parameswari AR, Rajalakshmi G, Palvannan T, Kumaradhas P (2010) Exploring the binding affinities of p300 enzyme activators CTPB and CTB using docking method Indian J Biochem Biophys 47:364-369

Dorsey ER, George BP, Leff B, Willis AW (2013) The coming crisis: obtaining care for the growing burden of neurodegenerative conditions Neurology 80:1989-1996 doi:WNL.0b013e318293e2ce [pii]

10.1212/WNL.0b013e318293e2ce [doi]

Gardian G, Yang L, Cleren C, Calingasan NY, Klivenyi P, Beal MF (2004) Neuroprotective effects of phenylbutyrate against MPTP neurotoxicity Neuromolecular Med 5:235-241 doi:NMM:5:3:235 [pii]

10.1385/NMM:5:3:235 [doi]

Gaub P, Joshi Y, Wuttke A, Naumann U, Schnichels S, Heiduschka P, Di Giovanni S (2011) The histone acetyltransferase p300 promotes intrinsic axonal regeneration Brain 134:2134-2148 doi:awr142 [pii]

10.1093/brain/awr142 [doi]

Gaub P, Tedeschi A, Puttagunta R, Nguyen T, Schmandke A, Di Giovanni S (2010) HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation Cell Death Differ 17:1392-1408 doi:cdd2009216 [pii]

10.1038/cdd.2009.216 [doi]

Glebova NO, Ginty DD (2005) Growth and survival signals controlling sympathetic nervous system development Annu Rev Neurosci 28:191-222 doi:10.1146/annurev.neuro.28.061604.135659 [doi]

Goldstein DS, Holmes C, Cannon RO, 3rd, Eisenhofer G, Kopin IJ (1997) Sympathetic cardioneuropathy in dysautonomias N Engl J Med 336:696-702 doi:10.1056/NEJM199703063361004 [doi]

Goldstein DS, Holmes C, Li ST, Bruce S, Metman LV, Cannon RO, 3rd (2000) Cardiac sympathetic denervation in Parkinson disease Ann Intern Med 133:338-347 doi:200009050-00009 [pii]

Gomez-Santos C, Ambrosio S, Ventura F, Ferrer I, Reiriz J (2002) TGF-beta1 increases tyrosine hydroxylase expression by a mechanism blocked by BMP-2 in human neuroblastoma SH-SY5Y cells Brain Res 958:152-160 doi:S0006899302036806 [pii]

Gui CY, Ngo L, Xu WS, Richon VM, Marks PA (2004) Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1 Proc Natl Acad Sci U S A 101:1241-1246 doi:10.1073/pnas.0307708100 [doi]

0307708100 [pii]

Gutierrez H, Hale VA, Dolcet X, Davies A (2005) NF-kappaB signalling regulates the growth of neural processes in the developing PNS and CNS Development 132:1713-1726 doi:dev.01702 [pii]

10.1242/dev.01702 [doi]

Gutierrez H, O'Keeffe GW, Gavalda N, Gallagher D, Davies AM (2008) Nuclear factor kappa B signaling either stimulates or inhibits neurite growth depending on the phosphorylation status of p65/RelA J Neurosci 28:8246-8256 doi:28/33/8246 [pii]

10.1523/JNEUROSCI.1941-08.2008 [doi]

Habash T, Saleh A, Roy Chowdhury SK, Smith DR, Fernyhough P (2015) The proinflammatory cytokine, interleukin-17A, augments mitochondrial function and neurite outgrowth of cultured adult sensory neurons derived from normal and diabetic rats Exp Neurol 273:177-189 doi:S0014-4886(15)30077-7 [pii]

10.1016/j.expneurol.2015.08.016 [doi]

Hahnen E, Hauke J, Trankle C, Eyupoglu IY, Wirth B, Blumcke I (2008) Histone deacetylase inhibitors: possible implications for neurodegenerative disorders Expert Opin Investig Drugs 17:169-184 doi:10.1517/13543784.17.2.169 [doi]

Harrison IF, Dexter DT (2013) Epigenetic targeting of histone deacetylase: therapeutic potential in Parkinson's disease? Pharmacol Ther 140:34-52 doi:S0163-7258(13)00125-3 [pii]

10.1016/j.pharmthera.2013.05.010 [doi]

Hegarty SV, Collins LM, Gavin AM, Roche SL, Wyatt SL, Sullivan AM, O'Keeffe GW (2014a) Canonical BMP-Smad signalling promotes neurite growth in rat midbrain dopaminergic neurons Neuromolecular Med 16:473-489 doi:10.1007/s12017-014-8299-5 [doi]

Hegarty SV, O'Keeffe GW, Sullivan AM (2014b) Neurotrophic factors: from neurodevelopmental regulators to novel therapies for Parkinson's disease Neural Regen Res 9:1708-1711 doi:10.4103/1673-5374.143410 [doi]

NRR-9-1708 [pii]

Hegarty SV, Sullivan AM, O'Keeffe GW (2013) BMP2 and GDF5 induce neuronal differentiation through a Smad dependant pathway in a model of human midbrain dopaminergic neurons Mol Cell Neurosci 56C:263-271 doi:S1044-7431(13)00066-3 [pii]

10.1016/j.mcn.2013.06.006 [doi]

Hodges A et al. (2006) Regional and cellular gene expression changes in human Huntington's disease brain Hum Mol Genet 15:965-977 doi:ddl013 [pii]

10.1093/hmg/ddl013 [doi]

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis J Neurol Neurosurg Psychiatry 79:368-376 doi:79/4/368 [pii]

10.1136/jnnp.2007.131045 [doi]

Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway Mol Chem Neuropathol 14:153-197

Jellinger KA (2012) Neuropathology of sporadic Parkinson's disease: evaluation and changes of concepts Mov Disord 27:8-30 doi:10.1002/mds.23795 [doi]

Jiang Y et al. (2008) Epigenetics in the nervous system J Neurosci 28:11753-11759 doi:28/46/11753 [pii]

10.1523/JNEUROSCI.3797-08.2008 [doi]

Jin H, Kanthasamy A, Ghosh A, Yang Y, Anantharam V, Kanthasamy AG (2011) alpha-Synuclein negatively regulates protein kinase Cdelta expression to suppress apoptosis in dopaminergic neurons by reducing p300 histone acetyltransferase activity J Neurosci 31:2035-2051 doi:31/6/2035 [pii]

10.1523/JNEUROSCI.5634-10.2011 [doi]

Kaufmann H, Goldstein DS (2013) Autonomic dysfunction in Parkinson disease Handb Clin Neurol 117:259-278 doi:B978-0-444-53491-0.00021-3 [pii]

10.1016/B978-0-444-53491-0.00021-3 [doi]

Kazantsev AG, Thompson LM (2008) Therapeutic application of histone deacetylase inhibitors for central nervous system disorders Nat Rev Drug Discov 7:854-868 doi:nrd2681 [pii]

10.1038/nrd2681 [doi]

Kidd SK, Schneider JS (2010) Protection of dopaminergic cells from MPP+-mediated toxicity by histone deacetylase inhibition Brain Res 1354:172-178 doi:S0006-8993(10)01631-8 [pii]

10.1016/j.brainres.2010.07.041 [doi]

Kidd SK, Schneider JS (2011) Protective effects of valproic acid on the nigrostriatal dopamine system in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease Neuroscience 194:189-194 doi:S0306-4522(11)00933-X [pii]

10.1016/j.neuroscience.2011.08.010 [doi]

Kontopoulos E, Parvin JD, Feany MB (2006) Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity Hum Mol Genet 15:3012-3023 doi:ddl243 [pii]

10.1093/hmg/ddl243 [doi]

Koyano-Nakagawa N, Wettstein D, Kintner C (1999) Activation of Xenopus genes required for lateral inhibition and neuronal differentiation during primary neurogenesis Mol Cell Neurosci 14:327-339 doi:S1044-7431(99)90783-2 [pii]

10.1006/mcne.1999.0783 [doi]

Lee S, Lee B, Lee JW, Lee SK (2009) Retinoid signaling and neurogenin2 function are coupled for the specification of spinal motor neurons through a chromatin modifier CBP Neuron 62:641-654 doi:S0896-6273(09)00349-3 [pii]

10.1016/j.neuron.2009.04.025 [doi]

Lees AJ, Hardy J, Revesz T (2009) Parkinson's disease Lancet 373:2055-2066 doi:S0140-6736(09)60492-X [pii]

10.1016/S0140-6736(09)60492-X [doi]

Lopez-Atalaya JP, Valor LM, Barco A (2014) Epigenetic factors in intellectual disability: the Rubinstein-Taybi syndrome as a paradigm of neurodevelopmental disorder with epigenetic origin Prog Mol Biol Transl Sci 128:139-176 doi:B978-0-12-800977-2.00006-1 [pii]

10.1016/B978-0-12-800977-2.00006-1 [doi]

Lucio CG, Vincenzo C, Antonio R, Oscar T, Luigi M (2013) Neurological applications for myocardial MIBG scintigraphy Nucl Med Rev Cent East Eur 16:35-41 doi:VM/OJS/J/23515 [pii]

10.5603/NMR.2013.0007 [doi]

Mantelingu K et al. (2007) Activation of p300 histone acetyltransferase by small molecules altering enzyme structure: probed by surface-enhanced Raman spectroscopy J Phys Chem B 111:4527-4534 doi:10.1021/jp067655s [doi]

Mayhew TM (1992) A review of recent advances in stereology for quantifying neural structure J Neurocytol 21:313-328

McMillan CR, Sharma R, Ottenhof T, Niles LP (2007) Modulation of tyrosine hydroxylase expression by melatonin in human SH-SY5Y neuroblastoma cells Neurosci Lett 419:202-206 doi:S0304-3940(07)00437-5 [pii]

10.1016/j.neulet.2007.04.029 [doi]

Morikawa Y, Dai YS, Hao J, Bonin C, Hwang S, Cserjesi P (2005) The basic helix-loop-helix factor Hand 2 regulates autonomic nervous system development Dev Dyn 234:613-621 doi:10.1002/dvdy.20544 [doi]

O'Keeffe GW et al. (2016) Region-specific role of growth differentiation factor-5 in the establishment of sympathetic innervation Neural Dev 11:4 doi:10.1186/s13064-016-0060-3 [doi]

10.1186/s13064-016-0060-3 [pii]

Outeiro TF et al. (2007) Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease Science 317:516-519 doi:1143780 [pii]

10.1126/science.1143780 [doi]

Pearson KL, Hunter T, Janknecht R (1999) Activation of Smad1-mediated transcription by p300/CBP Biochim Biophys Acta 1489:354-364 doi:S0167-4781(99)00166-9 [pii]

Presgraves SP, Ahmed T, Borwege S, Joyce JN (2004) Terminally differentiated SH-SY5Y cells provide a model system for studying neuroprotective effects of dopamine agonists Neurotox Res 5:579-598

Rouaux C, Jokic N, Mbebi C, Boutillier S, Loeffler JP, Boutillier AL (2003) Critical loss of CBP/p300 histone acetylase activity by caspase-6 during neurodegeneration EMBO J 22:6537-6549 doi:10.1093/emboj/cdg615 [doi]

Saha RN, Pahan K (2006) HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis Cell Death Differ 13:539-550 doi:4401769 [pii]

10.1038/sj.cdd.4401769 [doi]

Schneider A et al. (2013) Acetyltransferases (HATs) as targets for neurological therapeutics Neurotherapeutics 10:568-588 doi:10.1007/s13311-013-0204-7 [doi]

Selvi BR et al. (2008) Intrinsically fluorescent carbon nanospheres as a nuclear targeting vector: delivery of membrane-impermeable molecule to modulate gene expression in vivo Nano Lett 8:3182-3188 doi:10.1021/nl801503m [doi]

Seo S et al. (2012) Kruppel-like factor 11 differentially couples to histone acetyltransferase and histone methyltransferase chromatin remodeling pathways to transcriptionally regulate dopamine D2 receptor in neuronal cells J Biol Chem 287:12723-12735 doi:M112.351395 [pii]

10.1074/jbc.M112.351395 [doi]

Serrano L, Vazquez BN, Tischfield J (2013) Chromatin structure, pluripotency and differentiation Exp Biol Med (Maywood) 238:259-270 doi:238/3/259 [pii]

10.1177/1535370213480718 [doi]

Sheikh BN (2014) Crafting the brain - role of histone acetyltransferases in neural development and disease Cell Tissue Res 356:553-573 doi:10.1007/s00441-014-1835-7 [doi]

Souto JA, Benedetti R, Otto K, Miceli M, Alvarez R, Altucci L, de Lera AR (2010) New anacardic acid-inspired benzamides: histone lysine acetyltransferase activators ChemMedChem 5:1530-1540 doi:10.1002/cmdc.201000158 [doi]

Souto JA, Conte M, Alvarez R, Nebbioso A, Carafa V, Altucci L, de Lera AR (2008) Synthesis of benzamides related to anacardic acid and their histone acetyltransferase (HAT) inhibitory activities ChemMedChem 3:1435-1442 doi:10.1002/cmdc.200800096 [doi]

St Laurent R, O'Brien LM, Ahmad ST (2013) Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of Parkinson's disease Neuroscience 246:382-390 doi:S0306-4522(13)00362-X [pii]

10.1016/j.neuroscience.2013.04.037 [doi]

Steketee MB, Moysidis SN, Weinstein JE, Kreymerman A, Silva JP, Iqbal S, Goldberg JL (2012) Mitochondrial dynamics regulate growth cone motility, guidance, and neurite growth rate in perinatal retinal ganglion cells in vitro Invest Ophthalmol Vis Sci 53:7402-7411 doi:iovs.12-10298 [pii]

10.1167/iovs.12-10298 [doi]

Sun Y et al. (2001) Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms Cell 104:365-376 doi:S0092-8674(01)00224-0 [pii]

Tanaka Y, Naruse I, Hongo T, Xu M, Nakahata T, Maekawa T, Ishii S (2000) Extensive brain hemorrhage and embryonic lethality in a mouse null mutant of CREB-binding protein Mech Dev 95:133-145 doi:S0925477300003609 [pii]

To KK, Polgar O, Huff LM, Morisaki K, Bates SE (2008) Histone modifications at the ABCG2 promoter following treatment with histone deacetylase inhibitor mirror those in multidrug-resistant cells Mol Cancer Res 6:151-164 doi:6/1/151 [pii]

10.1158/1541-7786.MCR-07-0175 [doi]

Toulouse A, Collins GC, Sullivan AM (2012) Neurotrophic effects of growth/differentiation factor 5 in a neuronal cell line Neurotox Res 21:256-265 doi:10.1007/s12640-011-9266-7 [doi]

Tsui D, Voronova A, Gallagher D, Kaplan DR, Miller FD, Wang J (2014) CBP regulates the differentiation of interneurons from ventral forebrain neural precursors during murine development Dev Biol 385:230-241 doi:S0012-1606(13)00605-2 [pii]

10.1016/j.ydbio.2013.11.005 [doi]

Valor LM, Viosca J, Lopez-Atalaya JP, Barco A (2013) Lysine acetyltransferases CBP and p300 as therapeutic targets in cognitive and neurodegenerative disorders Curr Pharm Des 19:5051-5064 doi:CPD-EPUB-20130219-24 [pii]

Wang J et al. (2010) CBP histone acetyltransferase activity regulates embryonic neural differentiation in the normal and Rubinstein-Taybi syndrome brain Dev Cell 18:114-125 doi:S1534-5807(09)00493-6 [pii]

10.1016/j.devcel.2009.10.023 [doi]

Olanow CW et al. (2015) Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: A double-blind, randomized, controlled trial Ann Neurol 78:248-257 doi:10.1002/ana.24436 [doi]

Tao K, Matsuki N, Koyama R (2014) AMP-activated protein kinase mediates activity-dependent axon branching by recruiting mitochondria to axon Dev Neurobiol 74:557-573 doi:10.1002/dneu.22149 [doi]

Wong K et al. (2005) HIV-1 Tat interactions with p300 and PCAF transcriptional coactivators inhibit histone acetylation and neurotrophin signaling through CREB J Biol Chem 280:9390-9399 doi:M408643200 [pii]

10.1074/jbc.M408643200 [doi]

Wu X et al. (2008) Histone deacetylase inhibitors up-regulate astrocyte GDNF and BDNF gene transcription and protect dopaminergic neurons Int J Neuropsychopharmacol 11:1123-1134 doi:S1461145708009024 [pii]

10.1017/S1461145708009024 [doi]

Xie HR, Hu LS, Li GY (2010) SH-SY5Y human neuroblastoma cell line: in vitro cell model of dopaminergic neurons in Parkinson's disease Chin Med J (Engl) 123:1086-1092

Yang XJ, Seto E (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention Oncogene 26:5310-5318 doi:1210599 [pii]

10.1038/sj.onc.1210599 [doi]

Yao TP et al. (1998) Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300 Cell 93:361-372 doi:S0092-8674(00)81165-4 [pii]

Yuan PX, Huang LD, Jiang YM, Gutkind JS, Manji HK, Chen G (2001) The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth J Biol Chem 276:31674-31683 doi:10.1074/jbc.M104309200 [doi]

M104309200 [pii]

Zhu M, Li WW, Lu CZ (2014) Histone decacetylase inhibitors prevent mitochondrial fragmentation and elicit early neuroprotection against MPP+ CNS Neurosci Ther 20:308-316 doi:10.1111/cns.12217 [doi]

Figures and Figure Legends

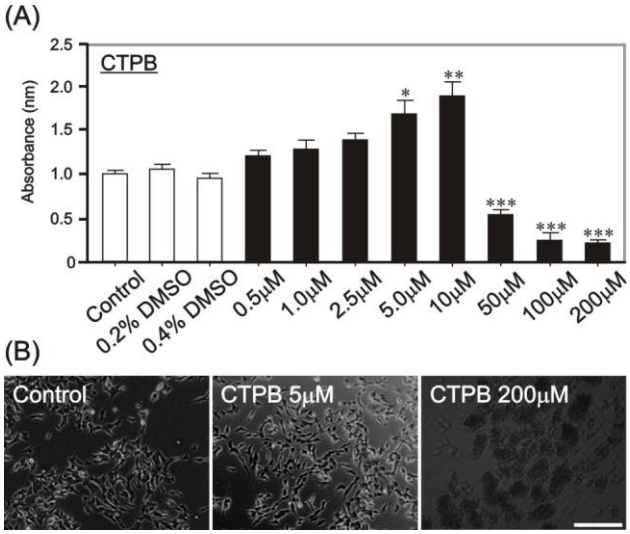


Figure 1: A dose-response analysis of CTPB in SH-SY5Y cells.

(A) Standardised MTT assay of CTPB-treated SH-SY5Y cells when treated daily for 4DIV with either control, DMSO (0.2% or 0.4%) or CTPB (0.5-200μM), as indicated (*p<0.05, **p<0.01, ***p<0.001 v control; ANOVA with post-hoc Tukey's test. Number of repetitions (N) = 7). (B) Representative photomicrograph of control and CTPB-treated (5μM and 200μM) SH-SY5Y cells. Scale bar = 100μm.

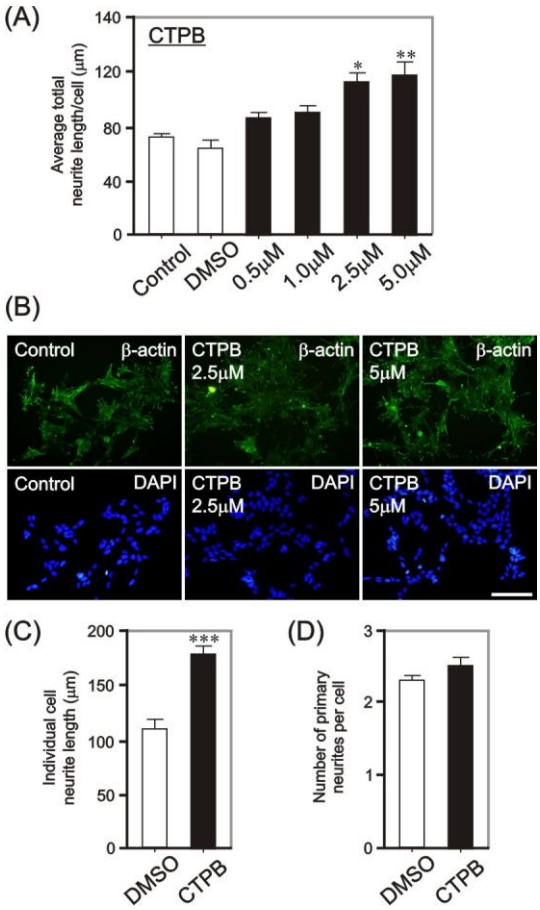


Figure 2: CTPB promotes neurite growth in SH-SY5Y cells.

(A) Graph showing the total neurite length per cell when treated daily for 4DIV with either control, DMSO (0.01%) or CTPB (0.5-5µM), as indicated (*p<0.05, **p<0.01 v control; One-way ANOVA with post-hoc Tukey's test; 20 images analysed per group per experiment. N = 3). (B) Representative photomicrographs of SH-SY5Y cells grown in control or CTPB (2.5µM and 5µM) and immuocytochemically stained for β-actin and counterstained with DAPI at 4DIV. Scale bar = 100µm. Graph showing the individual cell neurite length (C) or number of primary neurites (D) of SH-SY5Y cells treated daily for 4DIV with either DMSO (0.01%) or CTPB (5µM), as indicated (***p<0.001 v control; Unpaired student t-test; 20 cells per group. N = 3).

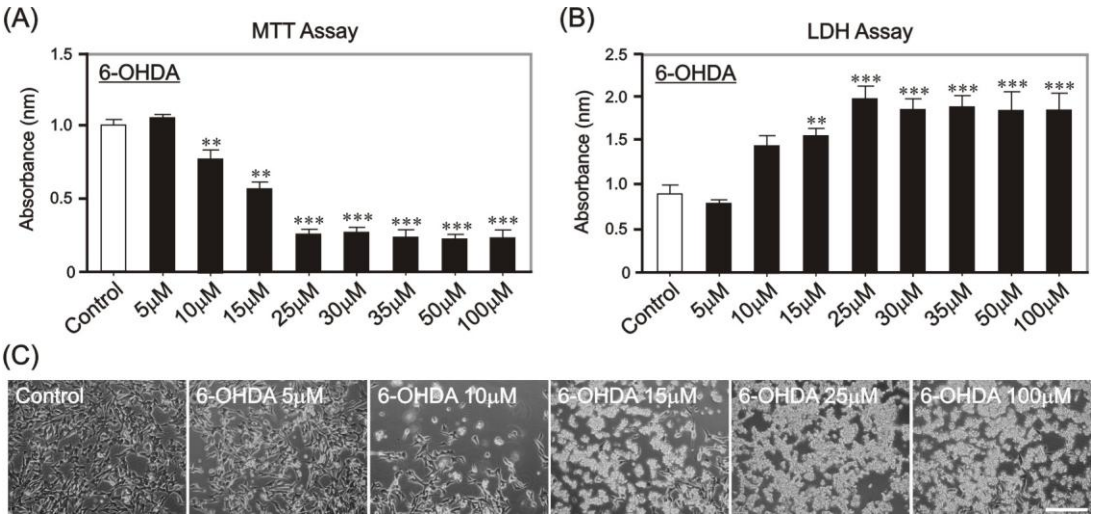


Figure 3: Concentration-dependent effect of 6-OHDA on cell death in SHSY5Y cells.

(A) Standardised MTT assay and (B) LDH assay of SH-SY5Y cells treated with increasing concentrations of 6-OHDA (5-100μM) for 24h (**p<0.01, ***p<0.001 v control; One-way ANOVA with post-hoc Tukey's test; N = 6). (C) Representative photomicrographs of control and CTPB-treated (10μM and 200μM) SH-SY5Y cells after 24h. Scale bar = 100μm.

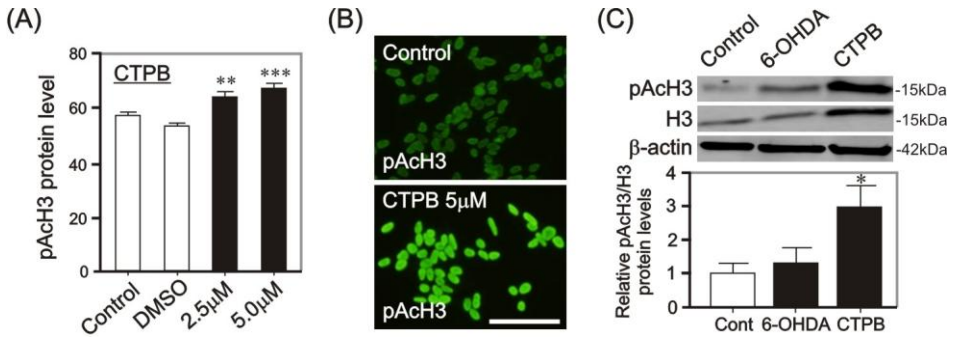


Figure 4: CTPB increases histone acetylation in SH-SY5Y cells.

(A) Graph showing the relative immunofluorescence intensity of acetylated histones (pAcH3) in SH-SY5Y cells treated for 24h with either control, DMSO (0.01%) or CTPB (2.5-5μM), as indicated (**p<0.01, ***p<0.001 v control; One-way ANOVA with post-hoc Tukey's test; 50 cells analysed per group per experiment. N = 3). (B) Representative photomicrographs of control and CTPB (5μM)-treated SH-SY5Y cells and immunocytochemically stained for pAcH3 after 24h. Scale bar = 100μm. (C) Western blotting showing pAcH3 and total histone H3 protein levels in SH-SY5Y cells treated for 24h with control, CTPB (5μM) or 6-OHDA (15μM). β-actin was used as a loading control. Graph showing densitometric quantification of the ratio of pAcH3 to total histone H3 protein levels, relative to β-actin levels (*p<0.05 v control; Unpaired student t-test; N = 3).

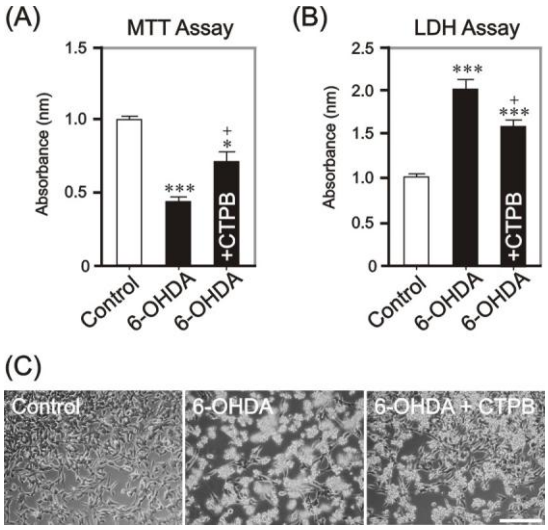


Figure 5: CTPB significantly protects against the cell death induced by neurotoxin 6-OHDA in SH-SY5Y cells.

(A) Standardised MTT assay and (B) LDH assay on SH-SY5Y cells treated with 15μM 6-OHDA in the presence or absence of 5μM CTPB for 24h (*p<0.01, ***p<0.001 v control; + p<0.05 v 6-OHDA alone; One-way ANOVA with post-hoc Tukey's test; N = 6). (C) Representative photomicrographs of control, 6-OHDA (15μM) and/or CTPB (5μM)-treated SH-SY5Y cells at 24h. Scale bar = 100μm.