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Epigenetic targeting of histone deacetylase: Therapeutic potential in Parkinson's disease?

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ABSTRACT

Parkinson's disease (PD) is the most common movement disorder affecting more than 4 million people worldwide. The primary motor symptoms of the disease are due to degeneration of dopaminergic nigrostriatal neurons. Dopamine replacement therapies have therefore revolutionised disease management by partially controlling these symptoms. However these drugs can produce debilitating side effects when used long term and do not protect degenerating neurons against death. Recent evidence has highlighted a pathological imbalance in PD between the acetylation and deacetylation of the histone proteins around which deoxyribonucleic acid (DNA) is coiled, in favour of excessive histone deacetylation. This mechanism of adding/removing acetyl groups to histone lysine residues is one of many epigenetic regulatory processes which control the expression of genes, many of which will be essential for neuronal survival. Hence, such epigenetic modifications may have a pathogenic role in PD. It has therefore been hypothesised that if this pathological imbalance can be corrected with the use of histone deacetylase inhibiting agents then neurodegeneration observed in PD can be ameliorated. This article will review the current literature with regard to epigenetic changes in PD and the use of histone deacetylase inhibitors (HDACIs) in PD: examining the evidence of the neuroprotective effects of numerous HDACIs in cellular and animal models of Parkinsonian cell death. Ultimately answering the question: does epigenetic targeting of histone deacetylases hold therapeutic potential in PD?

Contents

Abbreviations: 6-OHDA, 6-hydroxydopamine; Ac, acetyl; Ac-Lys, acetyl-lysine; AD, Alzheimer's disease; ALS, Amylotrophic Lateral Sclerosis; BBB, blood brain barrier; BDNF, brain derived neurotrophic factor; CBP, p300/CREB binding protein; DA, dopamine; DNA, deoxyribonucleic acid; DNMT, DNA methyltranferases; DOPAC, 3,4-Dihydroxyphenylacetic acid; FDA, Food and Drug Administration; GABA, γ-Aminobutyric acid; GDNF, glial derived neurotrophic factor; GNAT, Gcn5-related acetyltransferases; GSK-3, glycogen synthase kinase 3; HAT, histone acetyltransferases; HD, Huntington's disease; HDAC, histone deacetylase; HDACI, histone deacetylase inhibitor; HDM, histone demethylases; HMT, histone methyl-transferases; HPLC, high performance liquid chromatography; HSP, heat shock protein; HVA, homovanillic acid; LPS, lipopolysaccharide; Lys, lysine; LSD, lysine-specific histone demethylase; MAO, monoamine oxidase; Me, methyl; MeCP2, methyl-CpG binding protein 2; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenylpyridinium; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MYST, MOZ Ybf2/Sas3 Sas2 and Tip60; NAD+, nicotinamide adenine dinucleotide; PD, Parkinson's disease; PE, phycoerythrin; qRT-PCR, quantitative real time polymerase chain reaction; SAH, S-adenosyl-homocysteine; SAHA, suberoylanilide hydroxamic acid; SAM, S-adenosyl-methionine; SCFA, short chain fatty acid; SIR2, silent information regulator 2; SNpc, Substantia Nigra pars compacta; SOD, superoxide dismutase; TH, tyrosine hydroxylase; TSA, trichostatin A; αSyn, α-synuclein.

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1. Introduction

Parkinson's disease (PD) is the most prevalent movement disorder and the second most common neurodegenerative disease, affecting >4 million people worldwide (de Lau & Breteler, 2006). It is characterised by progressive hypokinesia; exhibited as slowness of movement, rigidity, development of a resting tremor, and loss of postural reflex (Thomas & Beal, 2007). The prevalence of PD in industrialised populations is estimated to be ~0.3%:1% in those aged >50 years and even greater in aged populations (de Lau & Breteler, 2006). Despite its abundance in today's society, current pharmacotherapy for PD is lacking, only able to partially tackle symptoms of the disorder yet unable to stop the progressive degeneration of neurons.

Epigenetics, the process by which gene activity is altered without altering genetic information, has long attracted interest in neurodegenerative disease, due to the multifactorial origins of pathology. Epigenetic factors are thought to contribute to neuronal cell death in PD (Migliore & Coppedè, 2009; Marques et al., 2011), and it is suggested that alteration in epigenetic regulation could hold therapeutic promise against neurodegeneration (Abel & Zukin, 2008; Hahnen et al., 2008; Kazantsev & Thompson, 2008; Chuang et al., 2009; Dietz & Casaccia, 2010; Konsoula & Barile, 2012). One way by which this can be achieved is through histone remodelling; via acetylation and deacetylation of the histone proteins around which deoxyribonucleic acid (DNA) is coiled. This article will review the primary literature on the pharmacological targeting of histone acetylation mechanisms for treatment of PD, ultimately attempting to answer the question: does the epigenetic targeting of histone deacetylase hold therapeutic potential in PD?

2. Parkinson's disease

2.1. Symptomology

The cardinal clinical symptoms of PD (bradykinesia, rigidity, loss of postural reflex and resting tremor), are primarily the result of neurodegeneration of dopaminergic nigrostriatal pathways within the central nervous system of sufferers (Goetz & Kompoliti, 2005; Thomas & Beal, 2007). Degeneration is most notable within the Substantia Nigra pars compacta (SNpc), the mesencephalic brain nucleus responsible for the synthesis of the neurotransmitter, dopamine (DA) (Moore et al., 2005). Dopaminergic nigrostriatal pathways project from the SNpc to the striatum, the subcortical brain nucleus composed of the caudate and putamen, which is responsible for planning and modulation of movement. Therefore degeneration of these pathways in PD results in clinical presentation of disruption of movement and motor based symptoms. Additionally, during disease development secondary non-motor symptoms also manifest, such as dementia, cognitive decline, depression, apathy, sleep disorders, constipation and erectile dysfunction (Chaudhuri & Schapira, 2009). The combination of these motor and non-motor symptoms results in a declining quality of life for PD patients and a subsequent ever increasing cost of care.

2.2. Pharmacotherapy and current approaches to treatment

The first line of PD therapy consists of DA replacement strategies through augmenting the activity of those dopaminergic neurons remaining within the nigrostriatal system. L-Dopa, a precursor in the DA synthetic pathway has therefore been given therapeutically for over 40 years and is thought by many to be the 'gold standard' in PD treatment, despite the development of the well characterised 'L-Dopa induced dyskinesia' upon its long term use (Poewe et al., 2010; Buck & Ferger, 2010). Multiple other DA replacement therapies have also been developed and are routinely used in the clinic e.g. DA receptor agonists such as Apomorphine and Pergolide, and DA degradation enzyme inhibitors such as Catacholamine-o-methyl transferase inhibitor Entacapone and monoamine oxidase (MAO) inhibitor Selegiline. Many of these drugs are often used in combination with L-Dopa, hence lowering the effective dose of L-Dopa and limiting the development of drug induced dyskinesia (Buck & Ferger, 2010). Recent advances in deep brain stimulation have led to even greater use of surgical treatment of PD patients, in direct electrical stimulation of areas such as the subthalamic nucleus and the globus pallidus interna (Foltynie & Hariz, 2010). This plethora of therapeutic options available for the treatment of PD has therefore revolutionised its management, allowing for therapeutic control of many motor symptoms. However none of these drugs provide long term protection against continual dopaminergic neuronal cell death. Additionally, none of the current drug therapies treat the non-motor symptoms which are the main determinants for deteriorating quality of life and patient care costs. Hence greater understanding of the pathogenesis and aetiology of PD is sought to aid development of better therapeutics.

2.3. Neuropathology and aetiology

PD is characterised by the loss of dopaminergic neurons within the SNpc and subsequent loss of striatal dopamine content. The primary pathological hallmark of these degenerating neurons in PD are concentric hyaline intracytoplasmic inclusions composed predominantly of a synaptic protein called α-synuclein (αSyn) (Spillantini et al., 1997; Moore et al., 2005). These ubiquitinated aggregates form intraneuronal structures known as Lewy bodies and dystrophic neurites known as Lewy neurites, which cause numerous detrimental consequences for their inhabiting neurons (Lee & Trojanowski, 2006). The cellular consequences of αSyn are yet to be fully elucidated, however associated events such as oxidative stress, inflammation and microglial activation, disruption of axonal transport, synaptic dysfunction, inhibition of the ubiquitin proteosome system and multiple organelle dysfunction, are thought to lead rapidly to neuronal death (Lee & Trojanowski, 2006). In addition, during disease development this αSyn pathology is thought to spread to other non-dopaminergic nuclei, for example the locus coeruleus, reticular formation of the brain stem, raphe nucleus, dorsal motor nucleus of vagus, basal nucleus of Meynert, the amygdala and the hippocampus (Jellinger, 2012). It is thought that this spread of αSyn pathology occurs in a rostral direction resulting in the gradual onset of non-motor based symptoms during the course of disease development (Braak et al., 2003).

An effective neuroprotective therapeutic for PD has still proved elusive, primarily due to the multifactorial origins of disease development and progression. In order to modify disease processes, an effective treatment strategy would need to not only aid the protection of neurons but also limit neuroinflammation, inhibit overactivation of microglial, while at the same time maintain a parenchymal environment conducive to heath and repair (Dietz & Casaccia, 2010). The multi-targeted demands of a PD therapeutic have hence led to potential manipulation of gene expression underlying several different cellular processes, through epigenetic approaches (Dietz & Casaccia, 2010).

3. Epigenetic regulation of gene expression

All cells inherit the same genetic material and code. However, the regulation of these genes which enables cells to retain their unique physical and biological phenotype in line with the specific tissue or organ to which they reside varies from cell to cell. This collection of regulatory mechanisms is hence named the epigenome (literally meaning 'above the genome'): the combination of DNA and histone post-translational modifications and related interacting proteins that together package the genome and help define the transcriptional

programme of a given cell (Bernstein et al., 2007; Arrowsmith et al., 2012). Dynamic changes in epigenetic regulation therefore underlie the physiological basis of cell function and crucially enable its malleability in response to environment (Meaney, 2010). Likewise by extension, perturbations of these epigenetic mechanisms are now known to play a pivotal role in a number of disease states, from cancer to metabolic disease and from neuropsychiatric to neurodegenerative disorders (Portela & Esteller, 2010).

To enable the highly compact packaging of the eukaryotic genome, DNA is tightly coiled around histone proteins forming nucleosome structures which make up chromatin (see Fig. 1). Electrostatic interactions between the negatively charged DNA and positively charged octamers of four highly basic histone proteins (H3, H4, H2A and H2B) as well as a linker histone (H1) enable this wrapping. Each 147 base pairs of DNA are tightly coiled around each histone octamer. However, in order for promoter and transcriptional factors to have access to the DNA, its packaging needs to be highly dynamic and changeable. Hence molecular masking and unveiling of promoter sequences along with local interactions between histone molecules with one another and with their surrounding DNA enables the changeability in chromatin structure vital for transcriptional activation and repression. A large number of molecular mechanisms contribute to this epigenetic regulation of genes. In order for us to understand how any of these mechanisms can be advantageously manipulated for the treatment of PD, we must first understand the relevance of their dysregulation in PD and the physiological impact their manipulation has upon disease state. Below the two most abundant and important mechanisms will be introduced and a brief explanation of how they are thought to be relevant in PD.

3.1. DNA methylation

The brain as an organ displays the highest level of DNA methylation in the body, however this equates to just ~1% of nucleic acid bases being methylated (Ehrlich et al., 1982). DNA methylation, unlike histone post-translational modification, is less transiently dynamic, playing a vital role in longer term repression of gene expression (for reviews see Miranda & Jones, 2007; Rottach et al., 2009, and Moore et al., 2012). The covalent methylation of the 5′ position of cytosine residues in CpG dinucleotides in the DNA sequence is catalysed by DNA methyltranferases (DNMTs) (Miranda & Jones, 2007; Rottach et al., 2009). There are three DNMTs known to be active in mammals: DNMT1, the maintenance DNMT which maintains the methylation pattern of CpG sites which have been previously established by DNMT3a and DNMT3b, the de novo DNMTs, which are active mainly during development (Miranda & Jones, 2007; Rottach et al., 2009). The main methyl (CH_3) donor for DNMT mediated methylation is S-adenosyl-methionine (SAM), a compound which results from one-carbon metabolism of numerous B vitamins, such as folic acid, B_6 and B_{12} (Miranda & Jones, 2007; Rottach et al., 2009). Therefore the potential of CpG methylation is partially dependent on the ratio between SAM and its unmethylated counterpart: S-adenosyl-homocysteine (SAH). The large majority of CpG dinucelotides in the mammalian genome are richly methylated resulting in gene repression through both physical blocking of DNA to transcription factors (Miranda & Jones, 2007; Rottach et al., 2009) and via recruitment of methyl binding proteins such as methyl-CpG binding protein 2 (MeCP2), which further exacerbate this blockade and are thus associated with gene silencing (Fuks et al., 2003; Fuks, 2005).

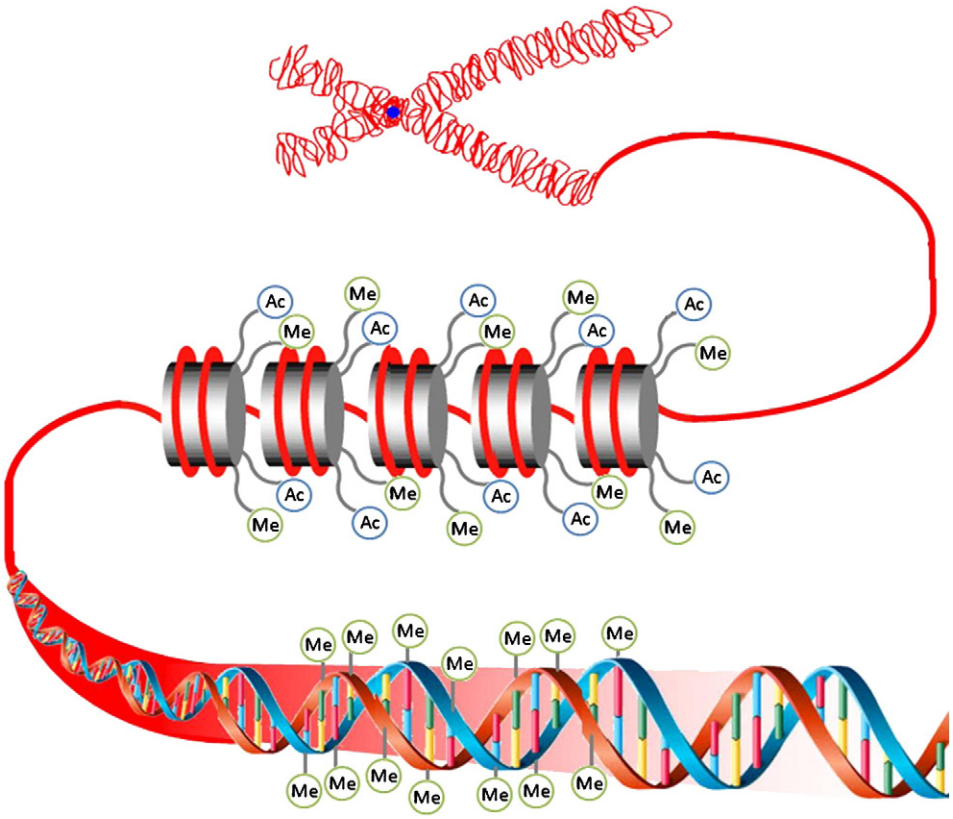

Fig. 1. Packaging of DNA into chromatin and the epigenome. DNA is tightly coiled around cores of eight histone proteins to form nucleosomes. Post translational modifications of these histone proteins (acetylation and methylation) as well as direct modification of DNA (methylation) help control the compression of this structure and enable transctiptional factor access to DNA. Abbreviations: Ac, acetyl; Me, methyl.

In sporadic PD patients it has been noted that there is impaired one-carbon metabolism in areas such as the SNpc, putamen and cortex: reducing DNA methylation of intron 1 of the αSyn gene, *SNCA*, linking DNA demethylation to αSyn expression (Jowaed et al., 2010). Furthermore demethylation of a CpG rich island in *SNCA* was identified in PD patients (Matsumoto et al., 2010). It was shown using cell culture that demethylation of this region of the *SNCA* gene resulted in increased expression of αSyn (Matsumoto et al., 2010). Similarly, a recent collaborative project identified methylation and expression changes in a further three PD risk gene variants (*PARK16/1q32, GPNMB* and *STX1B*) in PD patients, indicating that *SNCA* is not the only hypomethylated gene subjected to altered epigenetic regulation in PD (IPDGC & WTCCC, 2011). Lastly, for obvious reasons, the maintenance DNMT, DNMT1, is most abundantly located in the nuclear compartment. However it was discovered recently that there is a reduction in nuclear DNMT1, combined with a translation to the cytoplasm, in post-mortem PD brains as well as in the brains of αSyn transgenic mice (Desplats et al., 2011). This sequestration of DNMT1 to the cytoplasm was shown to result in global DNA hypomethylation in both human and transgenic αSyn mice brains, including CpG islands in the *SNCA* gene (Desplats et al., 2011). These effects were partially reversed in cell culture and transgenic animal experiments by overexpressing DNMT1, indicating that αSyn might mediate aberrant subcellular localisation of DNMT1 in PD (Desplats et al., 2011).

3.2. Post-translational histone modifications

As well as direct methylation of promoter sequences, gene expression profiles are also modulated by the extent of chromatin packaging, which determines how accessible the genome is to transcriptional factors. Appropriately, chromatin can be condensed, hence becoming transcriptionally inactive (heterochromatin) or relaxed, becoming transcriptionally active (euchromatin). This process of condensing and relaxing the genome is controlled principally through histone post-translational modification (for review see Maze et al., 2012). Histones are subjected to a number of covalent modifications including but not limited to phosphorylation, ubiquitination, sumoylation, acetylation and methylation (Maze et al., 2012). In fact proteins recruited to the sites of CpG methylation induce gene silencing not only through blockade of DNA but also through recruitment of histone modifying enzymes such as histone methyl transferases and histone deacetylases (Fuks et al., 2003; Fuks, 2005). This crosstalk between the epigenetic mechanisms is only just beginning to be understood (Fuks, 2005). Below, the two most abundant mechanisms of histone modification will be introduced: histone methylation and histone acetylation.

3.2.1. Histone methylation/demethylation

First described in 1964 (Allfrey et al., 1964), histone methylation is a dynamic process which involves the methylation of either arginine or lysine residues on the N-terminal tails of either H3 or H4 histone proteins to bring about a transcriptional change (Kouzarides, 2007). This methylation itself is facilitated, on arginine residues, by arginine specific histone methyl-transferases (HMTs) and accordingly on lysine residues by lysine specific HMTs (Habibi et al., 2011). As is the case with DMNTs and DNA methylation, both arginine and lysine specific HMTs use SAM as a cofactor and methyl donor (Arrowsmith et al., 2012). Correspondingly this methyl group is then returned to SAH after cleavage from either the histone lysine or arginine residue by histone demethylases (HDMs): either the Jumonji C family of 2-oxoglutarate-dependent demethylases (Tsukada et al., 2006) or the flavin-dependent enzymes lysine-specific histone demethylase 1 (LSD1) and LSD2 (Shi et al., 2004).

To add further complexity, multiple methylation valences of lysine and arginine residues on histone proteins H3 and H4 are also possible: lysine residues can be either monomethylated, dimethylated or trimethylated and similarly arginine residues can be either monomethylated or dimethylated (Habibi et al., 2011). In addition, arginine residues can be methylated either symmetrically or asymmetrically, an intricacy which has been shown to be associated with differing functional consequences (Habibi et al., 2011). Accordingly, methylation does not affect the structure of chromatin directly as the addition of a methyl group does not change the charge of the lysine or arginine residue. Instead the methylated sites act as recognition sites for other proteins that either aid condensation of chromatin or recruit other transcriptionally regulating proteins (Arrowsmith et al., 2012). The functional effect of histone methylation on transcriptional activation is therefore dependent on the site of the residue within the histone tail and the degree to which it is methylated (Kouzarides, 2007). For example both positive transcriptional methylation marks (histone protein H3 lysine 4 (H3-Lys4), H3-Lys36, H3-Lys79; H3-Arg2, H3-Arg17, H3-Arg26 and H4-Arg3) and negative transcriptional methylation marks (H3-Lys9, H3-Lys7, H3-Lys36, and H4-Lys20; H4-Arg8 and H4-Arg3) exist (Habibi et al., 2011; Maze et al., 2012).

In 2008, Nicholas and colleagues measured the level of the positive histone methylation mark H3-Lys4-me3 in striatal neurons (Nicholas et al., 2008). It was shown in both the murine and primate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD that a reduction of this methylation mark was associated with a depletion of DA in these neurons (Nicholas et al., 2008). A number of MAO inhibitors are used in the treatment of PD due to their ability to prevent the degradation of DA. Moreover the active site structure of MAO-A and B has significant sequence homology to LSD1 (Shi et al., 2004). Therefore it has been shown that the classical MAO inhibitor tranylcypromine inhibits demethylation by LSD1 (Mimasu et al., 2008; Lee et al., 2006; Schmidt & McCafferty, 2007). This was accompanied by a global increase in H3-Lys4-me2 in the P19 embryonic carcinoma cell line (Lee et al., 2006; Schmidt & McCafferty, 2007). This has great implications in PD in that the treatment of patients with MAO inhibitors such as Selegiline and Rasagaline could contribute to the rectification of the reduced H3-Lys4 levels previously described in PD models by Nicholas et al. (2008). Nevertheless, far greater understanding of the role of LSD1 in PD is required to understand how this could contribute to disease aetiology and pathophysiology.

3.2.2. Histone acetylation/demethylation

Histone acetylation, like histone methylation, was first described in 1964 (Allfrey et al., 1964), and is a highly dynamic process regulated by two classes of enzyme: histone acetyltransferases (HATs) and histone deacetylase (HDACs). HATs are categorised into three families: the Gcn5-related acetyltransferases (GNATs); the MOZ, Ybf2/Sas3, Sas2 and Tip60 (MYST)-related HATs; and p300/CREB binding proteins (CBP) HATs. All of these use acetyl-coenzyme A as an acetyl group donor to transfer an acetyl group to the ε-amino of lysine residues on the N-terminal tails of the four core histones, H2A, H2B, H3 and H4 (Roth et al., 2001). Addition of an acetyl group to lysine neutralises the

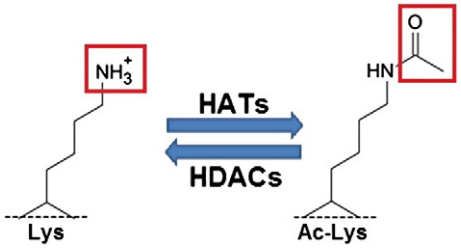

Fig. 2. Acetylation of histone lysine residues. Addition of an acetyl group to a histone lysine residue neutralises its positive charge, reducing the electrostatic interaction between histone tail lysine residues and negatively charged phosphate groups on DNA. Abbreviations: Ac-Lys, acetyl-lysine; Lys, lysine; HATs, histone acetyl transferases; HDACs, histone deacetylase.

positive charge of the residue (see Fig. 2), hence reducing the electrostatic interaction between the lysine in the histone tail and the negatively charged phosphate group on DNA which disrupts the inter- and intra-nucleosomal interactions between the histone and DNA (Grayson et al., 2010). This causes a relaxation in the structure of chromatin, otherwise referred to as euchromatin, and allows transcriptional factor access to the DNA. Deacetylation of lysine residues is facilitated by HDACs, which remove the acetyl groups from the ε-amino of lysine restoring the positive charge and causing a condensation of chromatin, known as heterochromatin, thus repressing transcription. In addition to enabling transcription through remodelling of chromatin, acetyl-lysine residues serve as recognition sites for transcriptional activators that contain protein motifs known as bromodomains, thus indirectly facilitating transcriptional initiation (Grayson et al., 2010). Crosstalk between acetylation and methylation at specific residues further influences transcriptional activation, for example, simultaneous methylation and deacetylation of H3-Lys9 has been shown to cause stringent silencing of surrounding genes (Fuks, 2005). To this end, of the numerous histone lysine acetylation sites, some appear to be more crucial in the switching from heterochromatin to euchromatin, for example this methylated and acetylated residue H3-Lys9 and also another residue in histone protein H4: H4-Lys16 (Shahbazian & Grunstein, 2007).

In health, a tightly controlled equilibrium exists between HAT and HDAC activity enabling the dynamic control of transcription (see Fig. 3) (Dietz & Casaccia, 2010; Saha & Pahan, 2006). In neurons, such a harmonised balance is therefore conducive to appropriate regulation of gene expression and subsequently facilitates appropriate neuronal homeostasis (Saha & Pahan, 2006). Therefore it is thought that if an imbalance of HAT/HDAC was to occur then neuronal cell death would likely follow, implicating the possibility of HAT/HDAC misbalance in disease (Dietz & Casaccia, 2010; Saha & Pahan, 2006). Rouaux and colleagues in 2003 were the first researchers to identify alterations of histone acetylation levels in neurodegeneration, by demonstrating that histone acetylation levels were decreased globally in neurons accompanied by a decrease in HAT CBP/p300. Authors observed levels of histone deacetylation in both an in vitro model of cortical neuronal cell death induced by activation of amyloid precursor protein signalling, a hallmark of Alzheimer's disease (AD), and in an in vivo model of Amylotrophic Lateral Sclerosis (ALS): the G86R mutant Superoxide Dismutase 1 (SOD-1) mice displaying motor neuron degeneration (Rouaux et al., 2003). Since then, histone hypoacetylation has become heavily implicated in numerous neurodegenerative diseases, especially PD. For example, in PD it has also been shown that αSyn accumulation promotes histone H3 hypoacetylation as ascertained from overexpression studies in SH-Sy5Y cells as well as in an in vivo αSyn transgenic Drosophila model (Kontopoulos et al., 2006). No direct binding was observed between αSyn and H3 therefore the effect of reduced acetylation of H3 is said to likely be through histone 'masking': the mechanism through which transcription is inhibited by ataxin-3, a polyglutamine containing protein involved in Huntington's disease (HD) (Li et al., 2002). Therefore it is theorised that the accumulation of misfolded αSyn promotes neurotoxicity in PD by 'masking' histone proteins: preventing histone acetylation, condensing chromatin, repressing

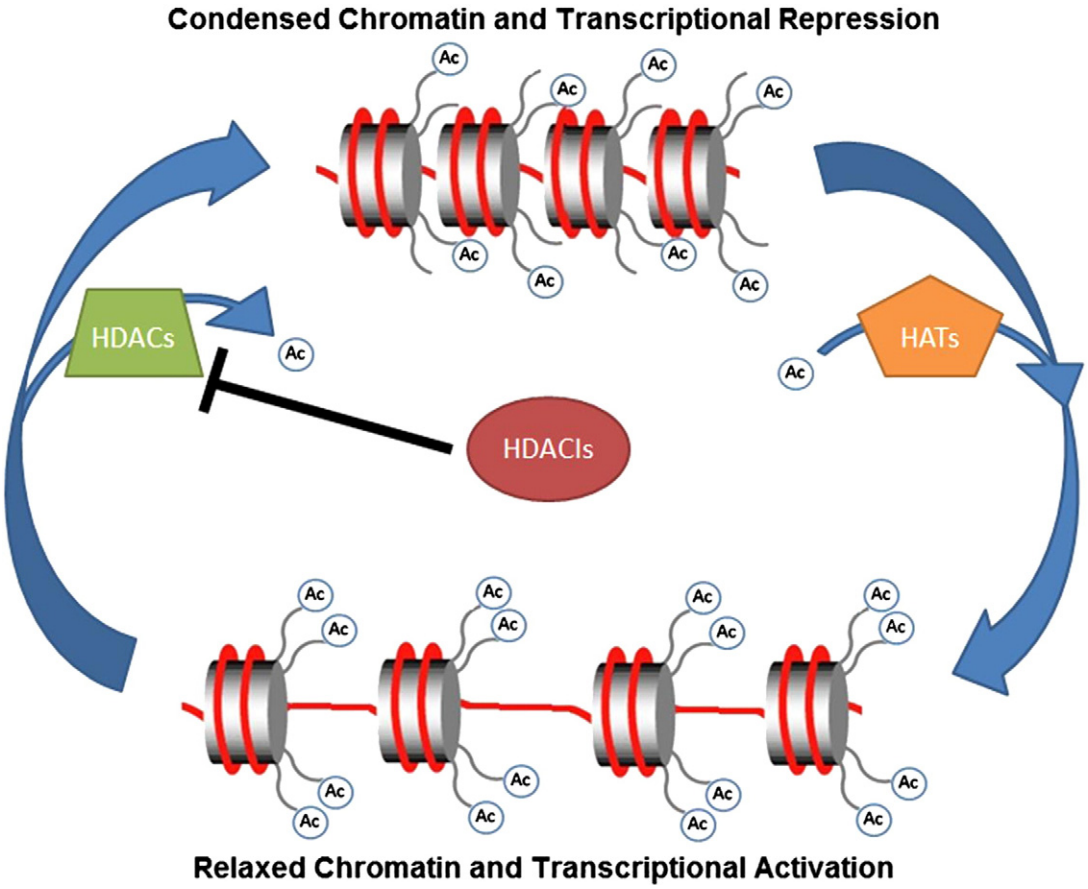

Fig. 3. Relaxation and condensation, facilitated by acetylation and deacetylation of histone proteins respectively. The level of histone acetylation depends on the interplay between HATs and HDACs. Inhibition of HDACs results in a net increase of histone acetylation, relaxation of chromatin and transcriptional activation. Abbreviations: Ac, acetyl; HATs, histone acetyl transferases; HDACs, histone deacetylases; HDACIs, histone deacetylase inhibitors.

gene expression and ultimately leading to cell death. Accumulates of misfolded proteins such as αSyn are a commonality in a number of neurodegenerative diseases and it is therefore thought this histone hypoacetylation could be at least partly responsible for the induction of cell death in these disorders. The use of HDAC inhibitors (HDACIs) to restore the imbalance between HAT/HDACs is therefore becoming a popular area of neurodegenerative research (for reviews see Chuang et al., 2009; Dietz & Casaccia, 2010; Hahnen et al., 2008; Kazantsev & Thompson, 2008; Saha & Pahan, 2006), in AD (Xu et al., 2011), in motor neuron diseases such as ALS (Echaniz-Laguna et al., 2008; Schmalbach & Petri, 2010), HD (Sadri-Vakili & Cha, 2006; Butler & Bates, 2006), Multiple Sclerosis (Faraco et al., 2011), and most relevant to this review, PD. However to understand the relevance of inhibiting HDACs, we must first understand the functionality of HDACs and more importantly their expression patterns both within the cell and the brain.

4. Histone deacetylase

HDACs are conserved between yeast and man. To date 18 human HDAC isoforms have been characterised, and based on their sequence homologies and co-factor dependencies they have been phylogenetically categorised into 4 main classes: classes I, II (a and b), III and IV (see Table 1) (Xu et al., 2007). Classes I, II and IV are all zinc dependent enzymes containing zinc-dependent catalytic domains. Class III on the other hand work independently of zinc, however require nicotinamide adenine dinucleotide (NAD+) for their enzymatic activity (Blander & Guarente, 2004; Saunders & Verdin, 2007). Class I HDACs include HDACs 1, 2, 3, and 8, and share high sequence homology in their catalytic sites with yeast RPD3 deacetylase (Xu et al., 2007). Class II HDACs are closely related to yeast Hda1 and include HDACs 4, 5, 6, 7, 9, 10. This class is further subcategorised into class IIa, consisting of HDACs 4, 5, 7 and 9, which contain only one catalytic site, and class IIb consisting of HDACs 6 and 10, which contain two catalytic sites (Xu et al., 2007). Class III, the NAD+ dependent class, are structurally and enzymatically distinct from other HDAC classes and share homology with yeast silent information regulator 2 (SIR2) (Blander & Guarente, 2004; Saunders & Verdin, 2007,). This class comprises of Sirtuin 1, 2, 3, 4, 5, 6, and 7 (Blander & Guarente, 2004; Saunders & Verdin, 2007; Haigis & Sinclair, 2010; Outeiro et al., 2008). Lastly, class IV consists of HDAC 11 alone, due to conserved residues within its catalytic core region shared by both classes I and II (Xu et al., 2007).

Despite their principle role in the cell being to deacetylate histone proteins in the nucleus, the different classes and isoforms of HDACs vary in their sub-cellular localisation (see Table 1) (Konsoula & Barile, 2012; Salminen et al., 1998; Haigis & Sinclair, 2010; Outeiro et al., 2008). Similarly in the brain, expression of the different classes of HDACs varies between cell types: expression is restricted mainly to neurons however numerous classes are known to be present in glia (Broide et al., 2007). Likewise in the brain, the expression level of HDAC isoforms varies from nucleus to nucleus (see Table 2). To our knowledge there has been no distribution study of the expression of class III HDACs (the Sirtuins) in the brain, however the expression levels of the 11 'classical' HDAC isoforms, i.e. classes I, II and IV, were studied and the relative expression patterns mapped in the rat brain in 2007 using high-resolution in situ hybridisation study of 56 brain regions in the rat brain (Broide et al., 2007). Related to PD it is of interest that the majority of HDAC expression in the SNpc is limited mainly to HDAC 2, 3, 4, 5, and 11, however lower levels of HDAC 1, 6, 7, 8 and 9 also exist here (see Table 2) (Broide et al., 2007). Moreover in this study, HDAC 10 was not detected in the SNpc at all (Broide et al., 2007).

The role of HDACs in epigenetic regulation is to reverse the effects of HATs, by catalysing the deacetylation of N-terminal tails of histone proteins. This facilitates a condensation of chromatin structure which prevents transcription factor access to DNA, thus leading to transcriptional repression. HDACs are therefore effective deacetylating enzymes; however their activity is not restricted to histone proteins. A recent phylogenetic study of bacterial HDACs revealed that all four classes of HDACs preceded the evolution of histone proteins, suggesting that the primary function of HDACs may have been towards non-histone proteins (Gregoretti et al., 2004). For example at least 50 acetylated non-histone proteins of known biological function are known to act as substrates for HDACs (Glozak et al., 2005; Spange et al., 2009). Notably non-histone targets include transcription factors and co-regulators, signalling mediators, nuclear hormone receptors and cytoskeletal elements (Glozak et al., 2005; Spange et al., 2009).

It is evident that HDACs represent a vast and diverse class of deacetylating enzyme and given their distinct expression patterns in the brain and in the cell they represent an ideal target for therapeutic inhibition. To that end recent years have witnessed increasing interest in and vast acceleration of the development of both broad isoform non-selective inhibitors as well as isoform specific inhibitors of HDACs (Carey & La Thangue, 2006; Burridge, 2013). Hence the functional roles of HDACs have largely been inferred from their inhibition using either broad-spectrum class inhibitors or more recently designed isoform selective inhibitors.

Table 1
Sub-cellular localisations of histone deacetylases.
Abbreviations: HDAC, histone deacetylase; NAD+, nicotinamide; Zn^{2+}, zinc.

Histone deacetylase class	Protein(s)	Sub-cellular location
Class I *(Zn^{2+} dependent)*	HDAC 1, 2, 8	Nucleus
	HDAC 3	Shuttles between nucleus and cytoplasm
Class IIa *(Zn^{2+} dependent)*	HDAC 4, 5, 7	Shuttles between nucleus and cytoplasm
	HDAC 9	Nucleus-cytoplasm
Class IIb *(Zn^{2+} dependent)*	HDAC 6, 10	Cytoplasm
Class III *(NAD+ dependent)*	Sirtuin 1 & 6	Nucleus
	Sirtuin 2	Cytosol
	Sirtuin 3, 4, 5	Mitochondria
	Sirtuin 7	Nucleolus
Class IV *(Zn^{2+} dependentn*	HDAC 11	Nucleus

5. Histone deacetylase inhibitors

HDACIs have shown to be efficacious in numerous disorders: for example sickle-cell anaemia, diabetes and immune disorders (for review see Lawless et al., 2009). However they are most commonly therapeutically used and studied as anti-cancer agents. HDAC inhibition emerged as a potential therapeutic strategy to reverse aberrant epigenetic changes associated with cancer a number of years ago (Marks et al., 2001a; Bolden et al., 2006) and hence HDACIs have since been shown to cause growth arrest, differentiation and/or apoptosis of many tumours cells by altering the transcription of a small number of genes (Marks et al., 2001b). Subsequently clinical trials are on-going of the use of HDACIs in various cancers: non-small cell cancers and hepatocellular carcinomas, leukaemia and t-cell lymphoma (Minucci & Pelicci, 2006; Wagner et al., 2010).

It seems counter intuitive that a drug class studied for therapy in cancer, where cells refuse to die, could be efficacious for a neurodegenerative disease such as PD, where cells prematurely die in the midbrain. However a number of cell systems have emerged in recent years which represent a substantial pathological convergence between cancer and PD (Devine et al., 2011). For example alterations in protein folding and degeneration, cell cycle and DNA repair, mitochondria and oxidative stress, and chronic inflammation are all implicated in both cancer and PD (for review see Devine et al., 2011). It is therefore not unreasonable to say that a drug class with such a varied

Table 2
Brain regional expression of histone deacetylases. Summary of the regional expression of histone deacetylase in the rat brain, as ascertained through high resolution in situ hybridisation analysis performed by Broide et al. (2007). Expression is listed and scored from low to high (0–5).
Abbreviations: HDAC, histone deacetylase.

Brain region	HDAC 1	HDAC 2	HDAC 3	HDAC 4	HDAC 5	HDAC 6	HDAC 7	HDAC 8	HDAC 9	HDAC 10	HDAC 11
Olfactory bulb	2.5	3.5	4	4	4	1.5	1	1	0.5	0.5	4.5
Cortex	2	3	3.5	3.5	3.5	1	0.5	0.5	0.5	0.5	4
Caudate putamen	0.5	2	3	1	3	0.5	0.5	0	0	0	4
Nucleus accumbens	0.5	2	3	1	3	0.5	0.5	0	0	0	4
Globus palidus	0	0	1	0.5	1	0	0	0	0	0	2
Amygdala	2.5	3	3.5	3	3	1	1	1	0.5	0.5	4.5
Hippocampus	2.5	4.5	5	4.5	5	2	1	2	1	1	4
Choroid plexus	2	0	3	0	2	0	0	0	0	0	0
Substantia nigra—compacta	1	1.5	3.5	2.5	3.5	1	1	1	1	0	4
Substantia nigra—reticulata	0.5	1	1	1	1.5	0	0	0	0	0	3
Hypothalamus	1	1.5	3.5	2.5	3.5	1	0.5	0.5	0	0	4.5
Pons	1.5	2	2.5	2.5	3	1	0.5	0.5	0.5	0	4.5
Cerebellum	4	4	5	5	5	1.5	1.5	2	0.5	0	4
Medulla	0.5	1.5	3	2	2.5	0.5	0.5	0.5	0	0	3
Spinal cord	1	2	2.5	2	2.5	0.5	0.5	0.5	0	0	3.5

scope of action such as HDACIs would represent a viable therapeutic option for both cancer as well as PD.

Small molecule HDACIs which inhibit the zinc dependent classes of HDACs, fall into 4 main classes according to their chemical structure: hydroxamates, cyclic peptides, short chain fatty acids and benzamides (see Figs. 4 and 5). It is important to note that these small-molecule HDACIs do not affect class III HDACs, the Sirtuins, due to the structural and functional dissimilarity between this class of HDACs and classes I,

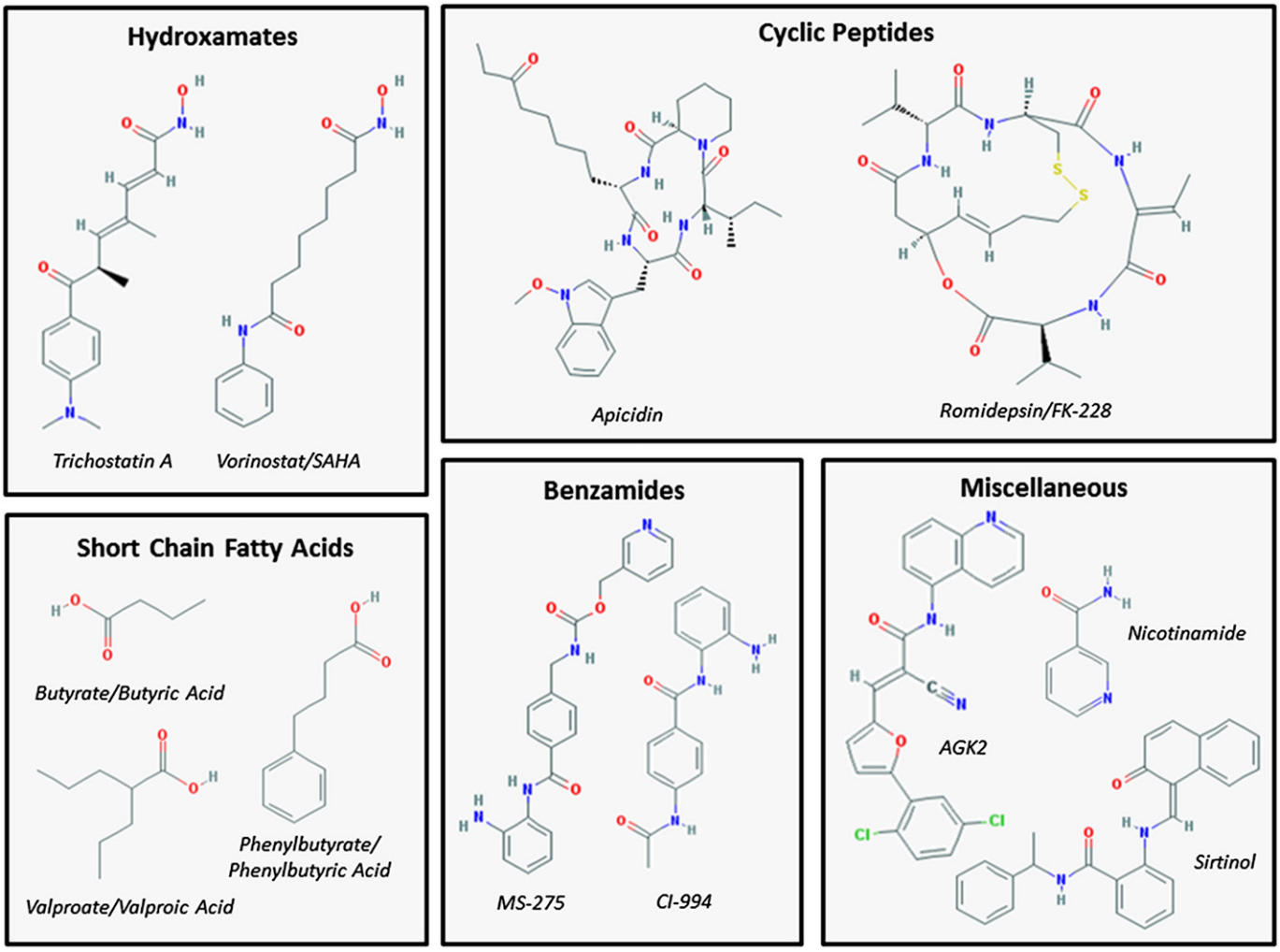

Fig. 4. Chemical classes of histone deacetylase inhibitors. Inhibitors of the zinc dependent HDACs can be divided into 4 classes dependent on their chemical structure: hydroxamates, short chain fatty acids, cyclic peptides and benzamides. Inhibitors of the NAD+ dependent HDACs however vary greatly in their structure, see miscellaneous. Abbreviations: SAHA, suberoylanilide hydroxamic acid.

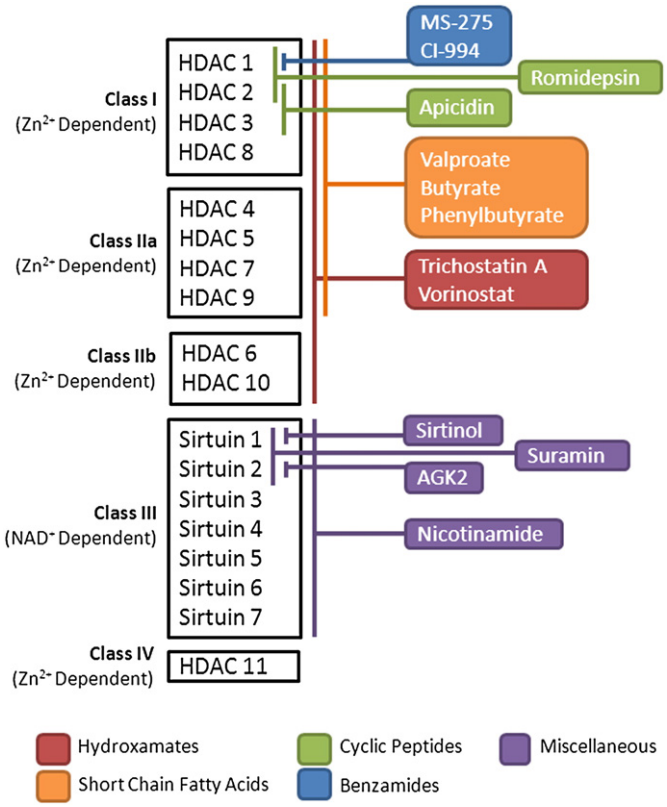

Fig. 5. Target selectivity of isoform specific and isoform non-specific histone deacetylase inhibitors. Abbreviations: HDAC, histone deacetylase; NAD+, nicotinamide; Zn^{2+}, zinc.

II and IV. However a number of Sirtuin inhibitors have now been developed both isoform selective and isoform non-selective (see Figs. 4 and 5). Below the currently available chemical classes of HDACIs will be introduced in terms of their chemical structure and pharmacological mechanisms of action, and the isoforms they are known to have most potency for.

5.1. Hydroxamates

Hydroxamate-based inhibitors are composed of three main elements: the hydroxamic acid (–CO–NH–OH), a hydrophobic linker, and a polar tail (see Fig. 4). Each of the elements of the inhibitor is thought to interact with a different part of the catalytic site of the HDAC (Marks et al., 2004; Villar-Garea & Esteller, 2004). The hydroxamate moiety is thought to bind to the zinc ion in the catalytic domain of HDACs thus inactivating the enzyme (Marks et al., 2004; Villar-Garea & Esteller, 2004). It is for this reason that hydroxamates are known to only inhibit classes of HDACs which share the same zinc dependent catalytic site: classes I and II HDACs only (see Fig. 5). The large majority of hydroxamate HDACIs act as pan-HDACIs meaning that they possess no profound isoform selectivity within the zinc dependent classes (Hahnen et al., 2008). In general drugs within this class of HDACIs are known to have relatively short half-lives, however possess long last effects (Plumb et al., 2003).

A large number of hydroxamate containing HDACIs have now been engineered, for example Scriptaid, Oxamflatin, Belinostat, Dacinostat, Panobinostat, Givinostat and Abexinostat (Xu et al., 2007; Grayson et al., 2010; Wagner et al., 2010). A number of these compounds are now in various stages of clinical development for cancers (for review see Wagner et al., 2010). However the two most clinically advanced and most prominent hydroxamate HDACIs are trichostatin A (TSA) and vorinostat (suberoylanilide hydroxamic acid (SAHA)), both of which cross the blood brain barrier (BBB) (Chuang et al., 2009), hence highlighting them as targets from neurological conditions such as PD.

5.2. Cyclic peptides

A number of cyclic peptides act as HDACIs as they too are thought to interact with the catalytic zinc ion site of HDACs to cause inhibition (Furumai et al., 2002). However due to the large variation in chemical structure among peptides (see Fig. 4), peptide HDACIs exert inhibition of various different specific HDAC isoforms. Nevertheless, the development and research into the use of cyclic peptide drugs has been somewhat lacking, there being only two notable examples of drugs of this class to date: apicidin and romidepsin (FK-228, desipeptide). Apicidin displays selectivity for HDAC2 and HDAC3 (and HDAC8) while romidepsin shows potent efficacy for inhibition of HDAC1 and HDAC2 (and HDAC4) (see Fig. 5) (Khan et al., 2008). Due to their structurally dependent selectivity for HDAC isoforms, cyclic peptide HDACIs remain an encouraging template for development of HDACIs with selectivity for differing combinations of HDAC isoforms, limiting the likely side effects with their treatment.

5.3. Short chain fatty acids

Short chain fatty acids (SCFAs), compared with other HDACIs, are relatively small, simple structured compounds, the molecular weights of which do not much exceed 150 g mol^{-1}. The three most notable drugs within this class being valproate, butyrate and phenylbutyrate. Most SCFAs share HDAC isoform inhibition profiles: inhibiting the action of classes I and IIb with most efficacy (see Fig. 5) (Grayson et al., 2010). Compared with the other HDACIs described thus far, SCFAs are relatively less potent, working in the range of millimoles rather than nanomoles (Grayson et al., 2010; Hahnen et al., 2008). It is thought that this weak potency is attributable to their inability to access the zinc cation in the HDAC active-site pocket, which appears to be pivotal to the deacetylation catalysis (Lu et al., 2003). It therefore appears likely that SCFAs use mechanisms other than direct interface with the catalytic site of HDACs to bring about inhibition. SCFAs do however have hugely diverse properties, and due to their small molecular weights are able to cross the BBB with ease (Xu et al., 2007). SCFAs hence remain an encouraging class of HDACIs for neuroscience research.

5.4. Benzamides

As opposed to SCFAs, cyclic peptides and the hydroxamates, benzamides represent a new relatively selective class of HDACI which exhibit a relatively long half-life as compared with other potent HDACIs (Glozak et al., 2005). Two notable drugs within this class are currently in clinical trial for cancers: MS-275 and CI-994 (see Fig. 4) (Grayson et al., 2010). Both MS-275 and CI-994 selectively inhibit HDAC1 (and HDAC3 to a lesser extent) over other HDAC isoforms within class I (see Fig. 5). They therefore represent an exciting new population of HDAC inhibiting agents being designed selectively against individual HDAC isoforms. The vast majority of pan-HDACIs described above exhibit some toxic effects due to their wide selectivity for numerous HDACs and other off target effects. Design of more isoform specific HDACIs will lead to an even greater potential of the use of HDACIs in diseases other than cancers where HAT/HDAC activity is known to be perturbed, such as PD.

5.5. Miscellaneous

Thus far all of the classes of HDACIs mentioned exhibit selectivity for the classical zinc dependent HDAC classes. However, as has already been alluded to, the NAD+-dependent class of HDACs represents a large functional sub-group of HDACs. Class III HDACs are

structurally and enzymatically distinct from classes I, II and IV, therefore HDACIs which interact with this class do not fit into any of the previous mentioned chemical structural groups of classical HDACIs. Class III HDACs are NAD+-dependent and therefore they require the binding of an NAD+ molecule in their active site to enable deacetylation (Spange et al., 2009). Nicotinamide (Niacinamide) is a competitive inhibitor of all 7 Sirtuin HDACs due to its ability to competitively bind to the NAD+ binding site of Sirtuins, preventing NAD+ from binding and thus inhibiting deacetylation of acetylated substrates such as histone proteins. Nicotinamide represents an encouraging HDACI in neuroscience due to its ability to cross the BBB when given orally, a feature that few HDACIs possess (Broide et al., 2007). Another more specific inhibitor of this class of HDACs is Suramin, a symmetric polyanionic nephthylurea, which itself and its structural homologs has been shown to inhibit both Sirtuin1 and Sirtuin2 isoforms within the class (Gregoretti et al., 2004). Moreover, two further more specific compounds, Sirtinol and AGK2 have been identified to inhibit Sirtuin1 (Trapp et al., 2007) and Sirtuin2 (Outeiro et al., 2007), respectively (see Fig. 5).

6. Histone deacetylase inhibitors in Parkinson's disease

HDACIs have been theorised to be efficacious in neurodegenerative disease. Yet the mechanism of action in how histone acetylation is transferred to neuroprotection still remains elusive. Neuroprotection and neurotrophicity are thought to be maintained through the combined transcriptional and non-transcriptional effects of HDACIs. Inhibition of HDACs reduces the deacetylation of histones which is therefore thought to lead chromatin relaxation and activation of multiple gene products conducive to neurotrophicity, anti-inflammation and subsequently neuroprotection. For example, brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), heat shock protein 70 (HSP70), αSyn, Blc-2, Bcl-XL, p21 and gelosolin have all been shown to be induced upon HDACI treatment (de Ruijter et al., 2003; Chuang et al., 2009). Similarly, non-transcriptional effects of HDACs i.e. the non-histone targets of HDAC catalysed deacetylation, are also thought to be involved in aiding neuroprotection (Glozak et al., 2005). For example inhibition of HDACs increases the acetylation of α-tubulin, a non-histone target, which increases microtubule stabilisation and axonal transportation aiding the release of BDNF and neuroprotection (Zhang et al., 2003). Additionally, gathering evidence highlights the multicellular involvement of HDAC inhibitors with other brain cells: immuno-modulatory effects in microglia, and reducing astrocyte and T-cell mediated inflammation (Dietz & Casaccia, 2010). It is apparent that the mechanism of neuroprotection of HDACIs is likely to be multi-targeted. Given the multi-faceted origins of PD pathogenesis, HDACIs seem like an optimistic candidate for therapy.

The distinct patterns of expression of HDACs in the brain crossed with the large number of compounds now available designed to selectively target specific isoforms and combinations of isoforms of HDACs, position HDACIs as an excellent therapeutic target in neuroscience. However current research of the use of HDACIs in neurodegeneration is limited to those that have been previously shown to cross the BBB (for reviews see Morrison et al., 2007; Kazantsev & Thompson, 2008). Below the evidence surrounding the potential therapeutic use of the most abundantly researched HDACIs will be reviewed with reference to PD: from the effects of drug treatment in cell culture models of PD cytotoxicity, to the effects of HDACIs in the well-established animal models of the disease.

6.1. Trichostatin A

Trichostatin A (TSA) is an orphan drug, a natural product isolated from *Streptomyces hygroscopicus*, which was originally developed as an anti-fungal antibiotic (Tsuji et al., 1976). Its HDACI function was first described in 1990 (Yoshida et al., 1990) and TSA is now known to be a broad spectrum pan-HDACI. The drug itself is known to have high levels of basal toxicity possibly due to its potent non-selective inhibition of both classes I and II, however it is often used as a reference compound for HDAC inhibition in research. Similarly it has been used extensively as a template for the design and development of new HDACI compounds. However in cerebral cortical neurons, TSA has been shown to increase the lifespan of cultures (Jeong et al., 2003) and increase expression of the neuroprotective and anti-inflammatory molecular chaperone HSP70 (Marinova et al., 2009). Similarly a number of studies have noted the anti-inflammatory and neuroprotective effects of TSA in in vitro and in vivo models of ischemic stroke (Fleiss et al., 2012; Wang et al., 2012; Kim et al., 2007; Meisel et al., 2006). There is also compelling evidence to suggest its neuroprotective effects in numerous models of Parkinsonian cell death.

Activated microglia are thought to contribute significantly to dopaminergic neuronal cell death in PD through their release of numerous inflammatory cytokines (IL-1β, TNF-α, IL-6), nitric oxide, prostaglandins, and superoxides which are toxic to neurons, as well as the discovery that they phagocytose not only damaged cell debris but also neighbouring intact dopaminergic neurons (Kim & Joh, 2006). It was previously shown that in primary human fetal microglial cultures activated by lipopolysaccharide (LPS), TSA reduces the expression of numerous cytokines and chemokines (Suh et al., 2010). However, Suh et al. (2010) fail to confirm microglial viability upon treatment with TSA and given previous evidence from Chen et al. (2007) it may be likely that the reductions seen of inflammatory cytokines are a result of microglial cell death rather than an active HDAC dependent mechanism. In 2007 it was shown by Chen and colleagues that TSA induces apoptosis of microglial cells in culture: when treated with TSA, the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed a significantly reduced number of viable cells in primary rat microglial cultures than controls. Correspondingly a significantly greater percentage of apoptotic microglia were observed upon treatment with TSA than vehicle, as ascertained through flow cytometry using phycoerythrin (PE)-conjugated annexin V to detect the externalisation of phosphatidylserine on apoptotic cells (Chen et al., 2007). This induction of apoptosis by TSA was also accompanied by a robust increase in histone H3 acetylation, and reduction in mitochondrial transmembrane potential as ascertained through rhodamine 123 staining. These changes in microglial viability induced by HDAC inhibition translated to attenuation by TSA of LPS-induced production of microglial pro-inflammatory factors in mesencephalic neuron–glia cultures and subsequently a reduction in microglial dopaminergic neurotoxicity. These results highlight a novel mechanism whereby this HDACI induces neuroprotection and underscores the potential use of HDACIs such as TSA in preventing inflammation-related neurodegenerative disorders such as PD (Chen et al., 2007).

MPTP is a neurotoxin routinely used to selectively destroy dopaminergic neurons in the nigrostriatal system by causing mitochondrial dysfunction and production of reactive oxygen species in dopaminergic neurons (Przedborski et al., 2000). MPTP is routinely used to model PD in mice and primates, causing progressive nigrostriatal neurodegeneration when injected peripherally, depending on the extent and duration of injection. 1-methyl-4-phenylpyridinium (MPP+) is the active metabolite of MPTP and is therefore similarly used to model dopaminergic cell degeneration in vitro. It was shown in 2008 (Wu et al., 2008b) that TSA dose dependently protects dopaminergic neurons from MPP+ toxicity in primary neuron–glia co-cultures: immunocytochemical staining of dopaminergic cells revealing a significant protection of tyrosine hydroxylase (TH)-immuno-reactive cells in co-cultures when treated with TSA and MPP+ over treatment with MPP+ alone. Similarly, Wu and colleagues went on to show that TSA produces a significant time dependent increase in the expression of the neurotrophic factors BDNF and GDNF through quantitative real-time polymerase chain reaction (qRT-PCR) from primary cortical astrocyte cultures. Furthermore previous findings demonstrate that TSA also prevents

elevation of glutamate in the medium of MPP+ treated primary cultured astrocytes (Wu et al., 2008a). This promotion of the uptake of glutamate by astrocytes upon TSA treatment was accompanied by a prevention of the downregulation of glutamate transporter 1 and glutamate/aspartate transporter induced by MPP+. Taken together these findings indicate that astrocytes may also be critical in the neuroprotective mechanism of this HDACI.

In contrast, in 2009 it was observed that acute dosing with TSA causes apoptosis and is toxic to three dopaminergic cell lines: rat N27 cells, mouse MN9D cells, and human SH-SY5Y cells (Wang et al., 2009). Similarly authors showed that this acute TSA dose exacerbates cell death induced by treatment of dopaminergic cells with MPP+ and the mitochondrial toxin Rotenone. These findings are difficult to interpret considering that TSA was shown to induce cell death in cell cultures independent of toxin administration, a finding that is in direct conflict to those of others (Wu et al., 2008b). Also the concentrations of MPP+ used to cause cell death by Wang et al. are far greater than those used by others to induce cell death (Kidd & Schneider, 2010; Wu et al., 2008a). Given evidence from Langley et al. (2008) that TSA protects primary cortical neurons from cell death when pulse treated with TSA as opposed to acutely treated, it is clear that evidence surrounding these neuroprotective/toxic effects of these HDACIs need to be carefully interpreted.

In PD native unfolded αSyn self-aggregates to form oligomers and fibrillar structures that accumulate to form Lewy bodies (Norris et al., 2004). αSyn is therefore generally considered to have a neurotoxic role in the pathogenesis of PD. However there is gathering evidence to say that native endogenous unfolded αSyn can have neuroprotective effects, its protective effect being evident in cultured neurons against numerous insults (da Costa et al., 2000, 2003; Kaul et al., 2005). It was shown in 2006 that TSA treatment of cerebellar granule cells in culture dose dependently increased the protein expression of αSyn, an effect which was accompanied by dose dependent increase of acetylated histone protein H3 in the αSyn promoter and increased αSyn promoter activity (Leng & Chuang, 2006). Crucially however these increases in αSyn and acetylated H3 were also accompanied by a dose dependent protection of the cells from glutamate induced excitotoxicity. Although the cause–effect relationship between these factors and neuroprotection is not unequivocally established, these results suggest that increase of histone acetylation via treatment with the HDACI, TSA, causes induction of αSyn which in part could be responsible for its neuroprotective effects in cerebral granule cells. The cerebellum as an anatomical brain area is not typically affected in PD. However in the context of Leng and Chuang's study (2006) cerebellar granule cells represent a powerful tool for study of the effects of HDACIs upon neurons, due to the abundant and homogenous nature of this cell population when used in culture. The authors of this paper also go on to show that upon peripheral daily administration of TSA to rats for four days, greater expression of αSyn is observed from western blot analysis of tissue homogenates from both the frontal cortex and the cerebellum compared with vehicles injected controls (Leng & Chuang, 2006). These findings add further weight to those previous described in that TSA crosses the BBB with ease to produce a measurable change in neuronal αSyn in vivo.

Evidence underlining the neuroprotective effects of TSA is encouraging, highlighting the multi-targeted mechanism of the neuroprotective function of its HDAC inhibition as a candidate for PD. Similarly pharmacokinetic studies suggest that when TSA is given peripherally it is quickly absorbed and rapidly metabolised with no compromise of its inhibition of HDAC activity (Sanderson et al., 2004). However, due to the broad, non-selective nature of TSA HDAC class inhibition, evidence is mixed as to its effect upon neuronal survival: it appearing to have relatively high levels of basal toxicity. Hence it is generally used only as an experimental reference compound. Despite this, the above mentioned evidence is compelling for proof of concept of the neuroprotective potential of HDACIs for the treatment of PD.

6.2. Vorinostat

Vorinostat (suberoylanilide hydroxamic acid (SAHA)), another notable hydroxamate based compound is the most clinically advanced HDACI: Food and Drug Administration (FDA) approved for advanced primary cutaneous T-cell lymphoma in October 2006 (Mann et al., 2007). Phase I pharmacokinetic studies indicate that vorinostat is well tolerated, quickly absorbed and metabolised efficiently resulting in two main non-pharmacologically active compounds (Ramalingam et al., 2010). Like TSA, vorinostat has previously been demonstrated to exert neuroprotection in an in vivo model of stroke: the middle cerebral artery occlusion model (Faraco et al., 2011). Along with reducing the infarct size in this model, histone acetylation was accompanied by increased expression of the neuroprotective proteins HSP70 and Bcl-2 (Faraco et al., 2011). In contrast to TSA however, studies of the neuroprotective effects of vorinostat in models of PD are lacking.

In 2010, Kidd and Schneider demonstrated that vorinostat protected two different dopaminergic neuronal cell lines (human neuroblastoma-derived SK-N-SH and rat mesencephalic-derived MES 23.5 cells) from apoptotic cell death induced by MPP+ (Kidd & Schneider, 2010). Authors confirmed HDAC inhibition by quantifying the acetylation of numerous histone acetylation marks (AcH2A-Lys5, AcH2B-Lys5, AcH3-Lys9 and AcH4-Lys8) and subsequently demonstrated that neuroprotection of TH+ cells was observed in only those cells in which histone hyperacetylation was observed. Moreover it was also shown that vorinostat completely inhibited the caspase-3 activation induced by MPP+ in MES23.5 cells indicating the inhibition of apoptosis in these dopaminergic cells. The fact that only neurons, and not microglia or astrocytes, were present in the cultures of the above mentioned study suggests that the neuroprotective mechanism observed by vorinostat, unlike TSA, is glial-independent.

Chen et al. (2012) however tested the neuroprotective profile of vorinostat in a number of cell culture systems with differing compositions of brain cells: neurons, microglia and astrocytes inclusive. Three different cell culture systems were then treated with vorinostat: neuron-enriched (>98% purity), neuron–astrocyte (~54% astrocytes, <1% microglia and 45% neurons) and neuron–microglia (80% neurons and 20% microglia), all isolated from rat (Chen et al., 2012). Vorinostat failed to produce neuroprotection in neuron-enriches cultures and neuron–microglia cultures however a dose-dependent increase in [^{3}H] DA uptake was observed in neuron–astrocyte cultures indicative of an astrocyte mediated mechanism of neuroprotection exerted by vorinostat. Authors went on to confirm this using conditioned media experiments whereby medium from neurons is replaced with the conditioned media from vorinostat treated astrocytes, resulting in promotion of dopaminergic neuron survival. Further experiments identified the release of BDNF and GDNF from astrocytes, via induction of histone hyperacetylation, to be responsible for this neuroprotection (Chen et al., 2012). Finally authors conclude by demonstrating that vorinostat treatment of neuron–glia cultures resulted in significant dopaminergic neuroprotection when treated with either MPP+ or LPS.

These two studies both suggest a dopaminergic neuroprotective phenotype of vorinostat however they differ in terms of the brain cell type they propose to be responsible for such a phenotype. It is most likely that both neurons and glia are at least partly responsible for bringing about the neuroprotective effects of the drug, be it by different mechanisms. Glial cells maintain a parenchymal environment that is conducive to neuronal health. However glia derived neurotrophic factors are not solely responsible for induction and maintenance of neuronal health. Numerous neuronal factors such as free radical scavengers (MnSOD, SOD1 and catalase) and pro-survival pathways such as the $p21^{waf/cip}$ pathway are vital in the conservation of neuronal survival and are all known to be modulated by HDAC inhibition (Ryu et al., 2003, and Langley et al., 2008 respectively). It is therefore likely that the neuroprotective effects of HDACIs is a composite of the contributions of the different brain cells, highlighting

once again the multi-model mechanism of action of this class of drugs and hence the desirability as a candidate in PD.

6.3. Apicidin

Apicidin is a cyclic tetra peptide first described as a fungal metabolite, which exhibits potent, broad spectrum antiprotozoal activity (Darkin-Rattray et al., 1996). It is now commonly known as a potent inhibitor of HDAC2, HDAC3, and to a lesser degree HDAC8 (Khan et al., 2008). Unlike the HDACIs previously mentioned (TSA and vorinostat), apicidin is one of the more selective HDACIs, inhibiting the actions of distinct subclasses of HDACs with relatively high potency as opposed to pan-inhibition of whole classes. Despite a recent pharmacokinetic study proving its ability to cross the BBB (Shin et al., 2011), studies detailing the neuroprotective potential of apicidin are extremely lacking.

The only study associating apicidin treatment to a neuroprotective phenotype came in 2009 (Marinova et al.). HSP70 is a molecular chaperone, neuroprotective and anti-inflammatory agent triggered by the heat-shock response (Pirkkala et al., 2001). HSPs play a vital role in facilitation of the folding of proteins acting as molecular chaperones. They are also known to play a large role in the degradation of misfolded proteins and have been shown to be cytoprotective (for review see Morimoto & Santoro, 1998). As part of a larger study, Marinova and colleagues (2009) showed that treatment of rat cortical neurons in culture with apicidin induced a dose dependent increase in protein levels of HSP70, accompanied by a similar increase in histone acetylation. It is therefore apparent that apicidin's inhibition of subclasses HDAC2, HDAC3 (and HDAC8) is sufficient enough to produce an increase of histone acetylation leading to induction of the neuroprotective HSP70. Authors do not show if this increase resulted in neuroprotection or induction of neuronal survival yet this result proves the concept that neuroprotective pathways are still able to be produced by specific inhibition of minimal HDAC subclasses at relatively high potency as opposed to pan-inhibition of many.

According to the comprehensive expression mapping of HDACs in the rat brain using high resolution in situ hybridisation (Broide et al., 2007), HDAC2 and 3, the targets of apicidin, are two of the most highly expressed HDAC classes within the SNpc (see Table 2). Combined with the specific nature of HDAC subclass inhibition by apicidin, this drug target represents a highly attractive therapeutic avenue for exploration in PD, bypassing the unnecessary risk of side effects caused by inhibition of non-essential HDAC classes. Further investigation of apicidin in in vitro models of Parkinsonian cell death and animal models of PD is therefore warranted.

6.4. Romidepsin

Like apicidin, romidepsin (FK-228) is a cyclic tetra peptide. Originally isolated as a fermentation product from *Chromobacterium violaceum*, a Gram-negative bacterium isolated from a Japanese soil sample (Nakajima et al., 1998), it has now been approved for the treatment of cutaneous T-cell lymphoma in November 2009 by the FDA (VanderMolen et al., 2011). It presents a linear pharmacokinetic profile and is generally well tolerated (VanderMolen et al., 2011). Romidepsin itself is a prodrug, the disulphide bridge (see Fig. 4) of which is reduced via a glutathione dependent mechanism to an active compound upon entering the cell (Furumai et al., 2002). It is thought that the sulfhydryl groups in this active component, 'redFK-228' is responsible for the interaction with the catalytic zinc ion thus causing inhibition of HDACs (Furumai et al., 2002). Nevertheless, like apicidin, romidepsin displays potent inhibition of distinct subclasses of HDACs with relatively high potency as opposed to pan-inhibition of whole classes. Romidepsin inhibits the action of HDAC1, HDAC2 and to a lesser extent HDAC4. Given its recent FDA approval, substantial evidence exists as to the therapeutic use of romidepsin in cancer; however none exists as to its repositioning in neurodegenerative disease as a neuroprotective agent for PD.

Dementia and cognitive impairment have for a long time known to become prevalent towards the later stages of PD development (Pagonabarraga & Kulisevsky, 2012; Svenningsson et al., 2012; Chaudhuri & Schapira, 2009). Moreover, a study in 2004 was first to discover that regulation of histone acetylation is associated with memory formation (Levenson et al., 2004). This finding has since been confirmed by numerous follow up studies, inferring that increased histone acetylation accompanies memory formation in the brain (Gräff et al., 2012). Correspondingly histone hypoacetylation is evident in numerous disease states which exhibit cognitive decline as a common feature (for review see Gräff & Mansuy, 2009). More recently the question has been raised as to whether HDACs are involved in this process and more importantly if they can be targeted using HDACIs in the treatment of cognitive impairment (for reviews see Fischer et al., 2010, and Gräff & Tsai, 2013). Answering these questions is not within the scope of this review; however recent findings have highlighted HDAC2 to be key in mediating cognitive impairment in the degenerating brain (Gräff et al., 2012). Romidepsin and the other cyclic tetra peptide HDACI detailed in this review, apicidin, share potency for HDAC2 with relative selectivity over other HDACs. Therefore given the recent implication of HDAC2 being shown to mediate cognitive impairment, the investigation of the role of these HDACIs in neurodegenerative diseases is of great interest. It has been shown abundantly in this review that HDACIs exhibit neuroprotection in numerous models of dopaminergic cell death. Similarly it has been shown that apicidin itself induces HSP70, a neuroprotective agent. If drugs within this class such as apicidin and romidepsin can exert a similar neuroprotective profile as other less specific inhibitors, their inhibition of HDAC2 may give them the added advantage to also treat the cognitive impairment observed by so many PD patients. Likewise, as mentioned previously, the specific nature of HDAC subclass inhibition by these cyclic peptides, adds yet further interest in bypassing of the unnecessary risk of side effects caused by inhibition of non-essentially inhibited HDAC classes.

6.5. Valproate

Valproate is by far the most investigated HDACI in terms of its neuroprotective action in neurodegenerative diseases such as PD. Notably, The Committee on Research of the American Neuropsychiatric Association in 2011 deemed valproate one of the 'most promising investigative priorities' in PD. Based on preclinical evidence and clinical criteria, authors identify FDA-approved, first-line psychotropic drugs which affect intracellular mechanisms and merit disease-modifying clinical trials in neurodegenerative disease. Authors identify valproate for the treatment of PD (Lauterbach & Mendez, 2011).

Valproate (2-propylpentanoic acid), or Valproic Acid to which it is often referred to, was first marketed as an anti-epileptic drug in France over 45 years ago (Löscher, 2002) and is now the most commonly prescribed anti-epileptic for both generalised and partial seizures in adults and children (Perucca, 2002). In 2001 valproate was discovered to be a powerful HDACI due to its ability to relieve HDAC-dependent transcriptional repression and cause histone hyperacetylation both in vitro and in vivo (Gottlicher et al., 2001; Phiel et al., 2001). Valproate is now known as a pan-inhibitor of HDAC classes I and IIa (Gurvich et al., 2004).

Due to its use in psychiatric medicine, valproate is known to readily cross the BBB. Pharmacokinetically it is characterised by nonlinear plasma protein binding and multiple metabolic pathways of elimination (DeVane, 2003). It has an extensive record of use across its lifespan in the clinic and a good record of tolerability (DeVane, 2003). Rationally for its treatment of epilepsy, valproate has numerous effects on the brain, HDAC inhibition being just one. It's molecular targets involved in neuroprotection are therefore numerous: including effects on

Glycogen Synthase Kinase 3 (GSK-3), Akt/ERK pathways, γ-aminobutyric acid (GABA)/Glutamate neurotransmission, Na^+ and Ca^{2+} voltage-dependent channels, phosphoinositol/TCA pathways and the oxidative phosphorylation pathway (Ximenes et al., 2012). For this reason a number of studies have now been conducted in investigation of valproate's neuroprotective potential both in vitro and in vivo (for reviews see Monti et al., 2009; Ximenes et al., 2012; Vajda, 2002). Valproate has shown to be neuroprotective in models of traumatic brain injury (Dash et al., 2010), spinal cord injury (Lee et al., 2012, Song-Hee Yu et al., 2012) and stroke (Xuan et al., 2012; Kim et al., 2007). However, whether valproate is able to overcome the numerous cellular pathologies involved with neurodegenerative diseases such as PD remains to be answered. Two studies from the 1970s examined the effects of valproate on disease progression in PD patients clinically prior to its discovery as a HDACI (Price et al., 1978; Nutt et al., 1979). Neither of these studies claims to notice any significant amelioration of symptoms. Hence given the age of these publications and the wealth of evidence in favour of the neuroprotective potential of HDACIs, currently the use of valproate in PD is being re-challenged.

The large majority of the in vitro evidence for valproate's neuroprotective action comes from the lab of Jau-Shyong Hong at the National Institutes of Health. In 2005 it was shown that valproate pretreatment of rat primary mesencephalic neuron–glia cultures was able to protect dopaminergic neurons from LPS-induced neurotoxicity in a dose-dependent manner as ascertained through DA uptake experiments and TH+ cell quantification (Peng et al., 2005). Similarly it was also shown that this pretreatment suppressed LPS-induced activation of microglia, ascertained through morphological analysis of OX-42 staining, a microglial cell surface marker. Authors went on to demonstrate that this suppression of microglial activation translates to valproate decreasing the release of pro-inflammatory factors such as TNFα, nitrite and intracellular reactive oxygen species. Similarly they show that microglial cell number decrease upon valproate treatment both time- and dose-dependently (Peng et al., 2005). It was also shown that this previously described reduction in microglial cell number is a result of microglial apoptosis. When treated with valproate, the MTT assay revealed a significantly reduced number of viable cells in primary rat microglial cultures than controls, and correspondingly flow cytometry using PE-conjugated annexin V showed a significantly greater percentage of apoptotic microglia when treated with valproate than controls (Chen et al., 2007). This induction of apoptosis in microglia by valproate was also accompanied by a robust reduction in the microglial mitochondrial transmembrane potential as ascertained through rhodamine 123 staining, and increased histone H3 acetylation upon valproate treatment. These results strongly suggest the involvement of microglia in the production of the neuroprotective phenotype of valproate and the fact this correlates with the extent of histone H3 acetylation indicates the mechanism of this effect is in part due to valproate's inhibition of HDAC activity.

The same group have also since studied the contribution of astrocytes to the neurodegenerative effects of valproate (Chen et al., 2006; Wu et al., 2008b). Firstly it was shown that valproate protects against spontaneous dopaminergic neuronal cell death in microglia-depleted and non-microglia-depleted rat primary mesencephalic neuronal–glia cultures: the [^{3}H]dopamine uptake assay and immunocytochemical staining of TH-immuno reactive cells revealing a significant protection of dopaminergic cells (Chen et al., 2006). To this same end, valproate was shown to dose-dependently protect against both LPS and MPP + induced toxicity of dopaminergic neurons in rat primary mesencephalic neuron–glia cultures (Chen et al., 2006; Wu et al., 2008b). Furthermore, applying the conditioned medium from astrocytic cultures onto midbrain neuron-enriched cultures, proved that a time-dependent increase in expression of GDNF and BDNF in astrocytes treated with valproate transfers to neuroprotection in neuronal cultures treated with this medium (Chen et al., 2006; Wu et al., 2008b). Additionally, chromatin immunoprecipitation experiments revealed that the GDNF promoter-associated histone H3 is significantly hyperacetylated in cultured astrocytic cells when treated with valproate (Wu et al., 2008b). These experiments suggest the involvement of astrocytes in valproate's neuroprotective phenotype. More specifically, given the changes observed in astrocytic histone acetylation, it is likely that valproate's HDAC inhibition and transcriptional induction of neurotrophic factors such as GDNF are responsible.

It therefore appears evident that glial cells are crucially involved in mediating the neuroprotective effects of valproate; however it is also important to consider the contribution of neurons. As previous mentioned, a study by Leng and Chuang (2006) showed that HDACI treatment of cerebellar granule cells, in this case valproate, dose dependently increased the protein expression of endogenous αSyn, an effect which was accompanied by dose dependent increase of acetylated histone protein H3 in the αSyn promoter and increased αSyn promoter activity (Leng & Chuang, 2006). Moreover this increase in αSyn was accompanied by dose-dependent protection of these cerebral granule cells from glutamate induced excitotoxicity. Authors go on to show this is also the case in vivo: peripheral daily administration of valproate to rats for four days showing greater expression of αSyn as observed though western blotting analysis of tissue homogenates from both the frontal cortex and the cerebellum compared with vehicles injected controls (Leng & Chuang, 2006). Similar findings by another group add weight to these findings in relation to αSyn in valproate treated cells. Monti et al. (2007) demonstrated that not only does valproate protect against 6-hydroxydopamine (6-OHDA) toxicity in cerebral granule cells by increasing αSyn expression but it also prevents the mono-ubiquitination of αSyn and its nuclear translocation. It therefore appears evident that αSyn plays a vital role in the neuroprotective mechanism of valproate. It is not clear however if these effects are solely the consequences of HDAC inhibition or whether valproate works through another mechanism to provide such alterations.

Further to its affects upon αSyn expression and cellular handling in neurons, valproate has also been shown to induce a dose dependent increase in HSP70 protein in rat cortical neurons, accompanied by a similar increase in histone acetylation (Marinova et al., 2009). Similar results have previously been noted in neuroblastoma cells, in that Rotenone induced apoptosis was avoided by the induction of HSP70 (Pan et al., 2005). Also, in 2010 it was shown that valproate protects both human dopaminergic neuroblastoma-derived SK-N-SH cells and rat dopaminergic mesencephalic-derived MES 23.5 cells against MPP + induced neurotoxicity (Kidd & Schneider, 2010). Authors showed that in both of these cell types, valproate causes histone hyperacetylation via increases in four different histone acetylation marks (AcH2A-Lys5, AcH2B-Lys5, AcH3-Lys9 and AcH4-Lys8). Furthermore valproate treatment was shown to significantly reduce caspase-3 activation induced by MPP+ in MES23.5 cells indicating the inhibition of apoptosis in these dopaminergic cells. Taken together these studies by Leng and Chuang (2006), Marinova et al. (2009), Pan et al. (2005) and Kidd and Schneider (2010) indicate that valproate induced neuroprotection is not only mediated by glia, but also, at least in part, by the neurons themselves. Histone hyperacetylation by valproate has hence been seen to increase transcription of endogenous αSyn and the neuroprotective agent HSP70, as well as downregulate caspase-3 and hence reduce apoptosis. Whether or not these events are a cause or an effect of the changes observed in glia however remain to be shown. Given the global epigenetic mechanism of action of valproate it is most likely that these are all a direct effect of drug treatment, returning us to the attractive multi-target rationale for the use of this drug class within neurodegenerative disorders such as PD.

Drug challenge studies using in vivo models of PD with valproate are the most prevalent of all of the HDACIs detailed in this review. The first case of which came in 2010 where the neuroprotective effects of valproate were studies in vivo, in the Rotenone rat model of

PD (Monti et al., 2010). Rotenone is an inhibitor of mitochondrial complex 1 and like MPTP when infused periphery causes degeneration of the rat dopaminergic nigral neurons projecting to the striatum (Sherer et al., 2003). Monti et al. (2010) used dietary administration of valproate in rats implanted with osmotic mini-pumps delivering a sub-clinical administration of Rotenone. In this model, valproate significantly prevented the loss of TH+ dopaminergic cells observed with 7 days of Rotenone administration. The reduction in striatal DA observed with Rotenone was also avoided with valproate feeding. Consistent with in vitro findings from the group (Monti et al., 2007) it was also shown that native αSyn expression was increased in the SNpc and striatum upon valproate treatment in vivo, mono-ubiquitinated αSyn was reduced and as was its nuclear translocation. Monti et al. have since also demonstrated the neuroprotective effect of valproate in the 6-OHDA rat model of PD (Monti et al., 2012). Following multiple striatal toxin injections, immunohistochemical analysis revealed that dietary valproate treatment significantly reduced the degeneration of dopaminergic neurons in the SNpc and protected dopaminergic neuronal terminals in the striatum. Consistent with their previous work using Rotenone, dietary valproate treatment was also observed to increase endogenous αSyn expression in the SNpc and also in the striatum (Monti et al., 2012). Taken together these are extremely encouraging findings given the dietary administration of the drug in these studies, highlighting the translatability of valproate therapy into the clinic. However care must be taken when interpreting these data as valproate treatment was commenced prior to toxin administration: 4 weeks in the case of both of these studies, questioning the clinical relevance of such a study design. At time of symptom onset and presentation to the clinic, a large majority of cell loss is already apparent within the SNpc of PD patients. Therefore for greater clinical relevance, preclinical studies seeking to determine the neuroprotective effects of a drug in PD need to be designed such that the drug treatment is administered after the establishment of a preclinical model of Parkinsonian cell death, i.e. after toxin administration. Only then will the clinical potential of a drug to delay/avoid further dopaminergic cell death be determined.

Kidd and Schneider demonstrated that valproate protected dopaminergic neurons from MPP+ induced degeneration in vitro (Kidd & Schneider, 2010), and hence this group also went on to verify these findings in vivo. MPTP was administered to mice with/without valproate for five days (Kidd & Schneider, 2011). Analysis of the brains confirmed that valproate mimicked results shown in vitro, in that it partially protected dopaminergic neurons from MPTP-induced cell death. Similarly the reduction of striatal DA induced by MPTP was also prevented. These changes were accompanied by robust increase of the histone acetylation mark AcH3-Lys9 (Kidd & Schneider, 2011). MPTP was also used in a very recent study by Castro et al. (2012) where the neuroprotective potential of valproate was investigated in an elegant animal model used to recapitulate common non-motor aspects of PD i.e. olfaction, emotional, cognitive functions (for review see Prediger et al., 2011). Castro and colleagues administered MPTP intranasally to rats and using a combination of behavioural tests for olfactory disturbances, short-term-memory impairment and depression. It was shown that valproate prevented the development of all of these symptoms as compared with MPTP treatment alone (Castro et al., 2012). On a molecular and cellular level, the peripheral valproate treatment of these animals significantly prevented against the reduction observed in olfactory bulb and striatal DA levels. However, again great care must be taken when interpreting the significance of these findings with relation to their clinical relevance, since similar to the Monti et al. studies (2007, 2012) mentioned above, Castro and colleagues pretreated the animals with valproate for 7 consecutive days prior to toxin administration.

Of all of the HDACIs reviewed here, valproate represents the most promising for treatment of PD. It has been shown to be efficacious in numerous cell based models of dopaminergic neuronal cell death, giving clues as to the mechanism of neuroprotection induced by inhibition of HDACs. Similarly, its efficacy has also been extensively seen in animal models of both motor and non-motor symptoms of the disease, albeit some using valproate pretreatment. Clinical investigation of this drug in PD patients is therefore timely.

6.6. Butyrate

Sodium butyrate was first noted to have HDAC inhibitor activity in 1977 where its effects as an anti-proliferation agent were first linked to HDAC inhibition (Saunders & Verdin, 2007). Butyrate (or sodium butyrate) is also a short chain fatty acid and therefore shares inhibition profiles of HDACs with valproate: inhibiting classes I and IIa with most efficacy. However, like other SCFAs this is achieved at a relatively low potency (Chuang et al., 2009; Grayson et al., 2010). Despite this, sodium butyrate has shown efficacy for neuroprotection in a number of studies, two notable examples being in animal models of stroke (Kim et al., 2007) and HD (Ferrante et al., 2003). In addition, a large body of evidence is beginning to emerge implicating the therapeutic potential of sodium butyrate in PD.

As part of a larger study it was shown that sodium butyrate treatment of cultured rat cortical neurons dose-dependently increases the expression of the neuroprotective and anti-inflammatory molecular chaperone HSP70 (Marinova et al., 2009). It was also shown that sodium butyrate increased the acetylated form of histone protein H3 dose-dependently in accordance with this increase in HSP70. Similarly, Kidd and Schneider also showed that sodium butyrate increased acetylation of various acetylation marks (AcH2A-Lys5, AcH2B-Lys5, AcH3-Lys9 and AcH4-Lys8) in both human dopaminergic neuroblastoma-derived SK-N-SH cells and rat dopaminergic mesencephalic-derived MES 23.5 cells (Kidd & Schneider, 2010). These findings were followed by demonstrating that sodium butyrate protects against MPP+ induced neurotoxicity as shown by quantification of TH+ cells, and that sodium butyrate significantly reduces caspase-3 activation induced by MPP+ in MES23.5 cells indicating the inhibition of apoptosis (Kidd & Schneider, 2010). These are both compelling data implicating sodium butyrate in the direct neuroprotection of dopaminergic neurons. However, it is also important to consider the contribution of glia in butyrate's neuroprotective phenotype.

In 2007 it was shown by Chen and colleagues that sodium butyrate induced apoptosis of microglial cells in culture (Chen et al., 2007): when treated with sodium butyrate, the MTT assay revealed a significantly reduced number of viable cells in primary rat microglial cultures than controls. Correspondingly flow cytometry using PE-conjugated annexin V showed a significantly greater percentage of apoptotic microglia when treated with sodium butyrate than controls. This induction of apoptosis by sodium butyrate was also accompanied by a robust reduction in the mitochondrial transmembrane potential as ascertained through rhodamine 123 staining and increased histone H3 acetylation upon sodium butyrate treatment. These changes in microglial viability translated to an attenuation by sodium butyrate of LPS-induced production of microglial pro-inflammatory factors in mesencephalic neuron–glia cultures and subsequently a reduction in dopaminergic neurotoxicity.

Similarly, it was shown in 2008 (Wu et al.), that sodium butyrate protects dopaminergic neurons from MPP+ toxicity in primary neuron–glia co-cultures: immunocytochemical staining of dopaminergic cells revealing a significant protection of TH-immuno reactive cells in co-cultures when treated with sodium butyrate and MPP+ over treatment with MPP+ alone. This neuroprotective effect of sodium butyrate was accompanied by a dose dependent increase in DA uptake. Wu and colleagues went on to shown that sodium butyrate produces a significant time dependent increase in the expression of BDNF and GDNF quantified through qRT-PCR from primary cortical astrocyte cultures.

To our knowledge, no neuroprotection studies have been conducted using sodium butyrate in an animal model of PD. However,

in 2012 a study was published in which the potential of sodium butyrate to alleviate cognitive deficits in the pre-motor stages of the 6-OHDA model. Given the implication of histone acetylation in memory (Gräff et al., 2012; Levenson et al., 2004) and the recent interest in HDACIs as cognitive enhancers (Gräff & Tsai, 2013), it seems fitting to study the effects of sodium butyrate in this very underplayed stage of PD development. Rane et al. (2012) tested executive function in striatal 6-OHDA lesioned rats using a rat analogue of the Wisconsin Card Sorting Test called the Extra Dimentional/Intra Dimentional Set Shifting Task (Birrell & Brown, 2000). Authors were able to show that treating the rats for 5 days with sodium butyrate, starting 2 weeks post-surgery, the set-shifting deficits observed in control animals were alleviated. Similarly the drug was also able to improve attentional set formation in the animals. Although no cellular or molecular follow up work was performed in this study to confirm these finding ex vivo, the findings in conjunction with those previously described from in vitro studies place sodium butyrate as a promising HDACI therapeutic for study. However, much further work is required to ascertain the ability of sodium butyrate to cause neuroprotection in the number of commonly used animal models of PD.

6.7. Phenylbutyrate

Phenylbutyrate is an orphan drug which was previously FDA approved for the treatment of urea cycle disorders in its ability to reduce plasma ammonia and glutamine (Maestri et al., 1996). Moreover, pharmacokinetic study detailing the cerebrospinal fluid penetration of phenylbutyrate confirmed that it readily crosses the BBB when given systemically highlighting its viability for treatment in the clinic (Berg et al., 2001). Phenylbutyrate, as the name suggests is a structural derivative of sodium butyrate, exhibiting the addition of a phenyl group at carbon-4 (see Fig. 4). For this reason phenylbutyrate is known to share the same HDAC inhibition profile as sodium butyrate, inhibiting classes I and IIa with most efficiency. Like many of the HDACIs reviewed above, phenylbutyrate has displayed encouraging efficacy in numerous models of neurodegenerative conditions, including HD (Gardian et al., 2005), AD (Wiley et al., 2011), ALS (Petri et al., 2006) and ischaemic stroke (Qi et al., 2004). In recent years however it has gained much attention as a potential neuroprotective agent in PD, showing efficacy against dopaminergic cell death in a number of cell based and animal models of the disease.

First, the notable study by Leng and Chuang (2006) demonstrated that phenylbutyrate was able to dose dependently increase the expression of endogenous αSyn in cerebral granule cells. It was also shown that this effect was accompanied by a similar dose-dependent increase in histone acetylation. More importantly it was shown that phenylbutyrate was able to protect against glutamate induced excitotoxicity in these cells. To prove the concept of this drug mechanism in vivo, phenylbutyrate was also given subcutaneously to rats for four days: greater expression of αSyn was observed from western blot analysis of tissue homogenates from both the frontal cortex and the cerebellum compared with vehicles injected controls (Leng & Chuang, 2006). Glia are also known to contribute significantly to the neuroprotective effects phenylbutyrate: suppressing the production of both proinflammatory cytokines and reactive oxygen species in activated mouse and human microglial cell lines (Roy et al., 2012). Given the notable efficacy of this drug in vitro, a number of studies now exist as to the neuroprotective effects of phenylbutyrate, in vivo.

As early as 2004 (Gardian et al., 2004) it was shown that phenylbutyrate exerts neuroprotective efficacy in the MPTP mouse model of PD. In this relatively simple study it was shown by high performance liquid chromatography (HPLC) analysis that pretreatment of MPTP mice with phenylbutyrate significantly attenuated the loss of DA, and its metabolites 3,4-Dihydroxyphenylacetic acid (DOPAC) and Homovanillic acid (HVA) in the striatum observed with MPTP treatment. Additionally immunohistochemical staining and cell quantification in the SNpc revealed that the drug was able to protect TH+ neurons from MPTP induced toxicity. By refining and repeating these experiments to eliminate the use of drug pretreatment, Roy et al. (2012) confirmed phenylbutyrate to be neuroprotective in the MPTP mouse model of PD. MPTP induced dopaminergic cell death was avoided in the SNpc of mice, as was the alteration observed in striatal neurotransmitter concentrations. These changes translated to a significant improvement of motor behaviour scores in MPTP and drug treated animals as compared to MPTP treated animals. Further investigation also showed that the drug induced changes were accompanied by inhibition of proinflammatory molecules and suppression of glial cells in the SNpc.

In addition, it was shown recently that phenylbutyrate stringently up-regulates the DJ-1 protein and protects dopaminergic neurons from death (Zhou et al., 2011). Mutations in the DJ-1 gene (*PARK7*) lead to early-onset autosomal recessive PD (for review see Lev et al., 2006). The function of DJ-1 is still largely unknown however it is thought to be neuroprotective by a number of mechanisms, such as mitigating oxidative stress, apoptosis and ubiquitination (Zhou et al., 2011). It was shown that phenylbutyrate increases the expression of DJ-1 in rat mesencephalic dopaminergic cells in culture by around 300% of control, rescuing them from oxidative stress and mutant αSyn toxicity (Zhou et al., 2011). Furthermore it was also shown that this drug increases DJ-1 in the brains of MPTP treated mice and similarly protects nigral dopaminergic cells from death. In a transgenic animal model of αSyn accumulation (αSyn Y39C), phenylbutyrate was able to reduce aggregation of αSyn and prevent deterioration of motor symptoms and cognitive decline (Zhou et al., 2011). These are very encouraging results given the efficacy of phenylbutyrate to prevent against PD relevant pathology even in a transgenic model.

In addition, phenybutyrate was shown to be effective at attenuating the pathogenic potency of αSyn in the A30P + A53T double mutation transgenic mouse model of PD (Ono et al., 2009). Behavioural testing was carried out on this transgenic model using the pole test and a rotarod treadmill task to test for bradykinesia and motor coordination respectively. Over the study period (12 months) the motor deteriorations observed by these two tests gradually improved over the 9 months of oral phenylbutyrate treatment (Ono et al., 2009). Similarly immunohistochemical analysis revealed that phenylbutyrate had a neuroprotective effect at the cellular level, protecting against the loss in dopaminergic cells and DA concentration in the SNpc of transgenic mice. Phosphorylated αSyn is thought to be important in the pathogenesis of PD, the majority of αSyn deposits in PD being phosphorylated (Fujiwara et al., 2002). Correspondingly staining of αSyn in the brains of these animals revealed that phenylbutyrate significantly reduced the level of phosphorylated αSyn but not non-phosphorylated αSyn (Ono et al., 2009), adding further weight to the neuroprotective effects observed by phenylbutyrate in this model. Finally, a variation of the classic Rotenone model was used to test the neuroprotective efficacy of phenylbutyrate by Inden et al. (2007). Authors administered Rotenone orally to C57BL/6 mice 30 min after i.p. administration of phenylbutyrate for 28 days. It was shown that phenylbutyrate dose-dependently protected nigral dopaminergic neurons from Rotenone-induced cell death. In addition, drug treatment avoided the development of motor deficits induced by Rotenone as well as the increase in αSyn in remaining nigral TH+ neurons.

To conclude, it is extremely encouraging that neuroprotection studies using phenylbutyrate are so abundant, particularly that the drug has shown efficacy in models such as the A30P + A53T double mutation transgenic mouse model. However very few studies have attempted to elucidate the mechanism of neuroprotection through which phenylbutyrate acts. Similarly none of the above studies correlate the acetylation of histone proteins to the neuroprotective effects therefore we are unable to tell whether the HDAC inhibition of this drug is at all related to its neuroprotection. Much further work is therefore required to elucidate just how phenylbutyrate causes neuroprotection and if its inhibition of HDACs is inclusive in this.

6.8. Nicotinamide

Nicotinamide (niacinamide), otherwise known as Vitamin B_3 is found in numerous food sources including liver, chicken, beef, fish, cereals, peanuts and legumes and has a wide range of biological functions in the body. These include energy production, synthesis of fatty acids, cholesterol and steroids, signal transduction and the maintenance of genomic integrity (Maiese & Chong, 2003). Nicotinamide is a precursor for NAD+ and for that reason it is known to inhibit class III HDACs through competitive binding to the NAD+ binding site of the Sirtuin HDACs (Avalos et al., 2005). Due to the dietary requirement of nicotinamide it is extremely well tolerated when given orally and known to cross the BBB with ease. It displays a linear pharmacokinetic profile exhibiting maximal plasma drug levels as little as 30 min after ingestion, with higher doses maintaining high plasma levels for up to 4 h (Dragovic et al., 1995). Therefore recent years have seen increased interest in this compound as a neuroprotective agent in neurodegenerative conditions. For example nicotinamide has been shown to improve neurological outcome and reduce infarct volume in models of stroke (Mokudai et al., 2000; Liu et al., 2009; Ayoub et al., 1999), restore cognitive function in transgenic models of AD (Green et al., 2008), and improve motor deficits and upregulate neurotrophic factors in models of HD (Hathorn et al., 2011). Furthermore greater focus has been placed upon the potential use of nicotinamide in PD due to its modulatory effects of cellular energy metabolism and the implications these have upon dopaminergic cell death within the disease (for review see Beal, 2003).

The two most notable studies came from the work of Anderson et al. (2006, 2008). The neuroprotective efficacy of nicotinamide was assessed in different mouse MPTP models taking advantage of differing dose regimes of the toxin to induce dopaminergic neuronal cell death. By analysing striatal DA levels and changes in number of TH+ and cresyl violet stained neurons in the SNpc at differing time point authors were able determine the neuroprotective profile of nicotinamide. Administration of nicotinamide peripherally prior to MPTP injection, induced a dose dependent neuroprotective profile in the 'acute' MPTP (four injections in 1 day at 2 h intervals) but not in the 'sub-acute' model (two injections per day at 4 h intervals for 5 days). The highest dose of nicotinamide was shown to be neuroprotective in this latter 'sub-acute' model however not to the same degree as was seen in the 'acute' model. Authors suggest that this is a result of nicotinamide interacting directly with the specific mechanism of cell death operating in the two different models (Anderson et al., 2008). However, Anderson et al. fail to elucidate the mechanism of cell death in each of the models. In addition, the focus of their paper is not on the HDAC inhibition by nicotinamide hence they do not correlate the extent of neuroprotection with histone acetylation in any way. Therefore it is impossible to say whether the neuroprotective effects observed are in any way a result of HDAC inhibition. Regardless, nicotinamide at higher doses appears to be neuroprotective towards dopaminergic neurons in this model of Parkinsonian cell death.

Class III HDACs are structurally and enzymatically dissimilar from the remaining three classes and hence their ease of specific inhibition represents them as an ideal target, avoiding the possible negative implications of global non-specific HDAC inhibition. However much further work needs to be carried out to understand the true effects nicotinamide has on the activity of Sirtuins 1–7 and indeed its other non-acetylation effects within the cell. It is thought to be largely beneficially in terms of the bioenergetics of the cell but with such a ubiquitous compound much care must be taken as to understanding its other cellular physiological effects.

6.9. AGK2

In 2006, a small compound (named 'compound B2') was discovered to increase the size of αSyn inclusions in cells transfected with a tagged αSyn construct (Bodner et al., 2006). It is thought that larger size accumulates of αSyn have a reduced toxicity compared with their smaller size counterparts (Tanaka et al., 2004), and hence this 'compound B2' received much attention. Following the testing of the inhibition profile of 'compound B2' of various targets, Outeiro et al. (2007) demonstrated its weak but selective inhibition of Sirtuin2, one of the NAD+ dependent class III HDACs. Subsequent library screenings identified a novel compound, AGK2 as a potent inhibitor of Sirtuin2, 10-fold greater potency over 'compound B2'. As previously mentioned, one of the non-histone targets of HDACs is α-tubulin and subsequently authors demonstrated that AGK2 dose-dependently increased the acetylated state of α-tubulin in HeLa cells. Molecular docking experiments and virtual ligand modelling studies then led the authors to propose that AGK2 inhibits Sirtuin2 by competitively binding to the nicotinamide-binding site for the NAD+ co-substrate.

Following on from these mechanistic studies, Outeiro et al. showed that human neuroglioma H4 cells transfected with αSyn can be rescued from αSyn mediated toxicity dose dependently upon treatment with AGK2 (Outeiro et al., 2007). Furthermore, treatment with AGK2 and transfection with both αSyn and synphillin-1 (to promote inclusion formation) increased the size of αSyn inclusions, highlighting the originally proposed mechanism of neuroprotection of this drug target. Finally in order to demonstrate these findings in vivo, authors used a transgenic Drosophila model of PD in which αSyn is over expressed in the fly brain (Outeiro et al., 2007). AGK2/vehicle was fed to flies at various concentrations for the first 20 days of life. As expected vehicle treatment resulted in a loss in TH+ dorsomedial neurons, the area which is sensitive to αSyn in this model. Most importantly authors showed that AGK2 dose-dependently protected these TH+ dorsomedial neurons from death (Outeiro et al., 2007). No difference was observed in the level of αSyn in the brains of flies fed with AGK2 compared with vehicle. This suggests that AGK2 rescues neurons via αSyn inclusion enlargement: the reduction in total surface area correlating with the cytoprotective role of aggregates (Tanaka et al., 2004). These findings are extremely encouraging given the distinct isoform specificity of AGK2 towards Sirtuin2 alone, providing proof of concept that single HDAC isoform inhibition is sufficient in producing a neuroprotective phenotype.

7. Future prospects

Historically success treating the classical animal models of PD has translated little to success in clinical trials. Therefore although the evidence in favour of the use of HDACIs for the treatment of PD is compelling, much further work is required to validate the findings detailed above before they can be advanced to the clinic. In order to bridge this gap between bench and bedside the neuroprotective effects of HDACIs must be validated appropriately in more clinically translatable animal experiments. For example as has already been discussed, at time of symptom onset and presentation to the clinic, a large majority of cell loss is already apparent within the SNpc of PD patients. Therefore for greater clinical relevance and translatability, preclinical studies seeking to determine the neuroprotective effects of HDACIs in PD need to be designed such that the drug treatment is administered after neurodegeneration is induced in the animal model, i.e. after toxin administration. This is not the case with a number of the studies detailed above. Similarly, PD is a progressive neurodegenerative disease and therefore it is important that the neuroprotective effects of HDACIs are validated in a preclinical model of PD which recapitulates the progressive aspect of the disorder. For example, the Lactacystin rat model of PD is a relatively recently developed model which has been suggested to be a novel, pathologically relevant model of PD (McNaught et al., 2002). Unlike many of the 'acute' model mentioned previously, when injected into the SNpc, Lactacystin, an irreversible proteosome inhibitor, causes progressive development of inclusion bodies mimicking the progressive

degenerative nature of neuronal cell death in PD. Therefore if successful, validation of the neuroprotective effects of HDACIs in a progressive model will add greatly to the clinical translatability of the HDACI and likelihood of success in the clinic.

Regardless, inhibition of HDAC classes has been observed to normalise the deficiency of histone acetylation in numerous in vitro and in vivo models of PD leading to stringent neuroprotection. However, thus far there is no consensus as to how this HDAC inhibition and normalisation of histone acetylation translates to neuroprotection. As detailed above, multiple neuroprotective and neurotrophic factors have been identified to be upregulated as a result of HDACI mediated histone hyperacetylation, not only in neurons but also in microglia and astrocytes. Similarly non-transcriptional consequences of HDAC inhibition have also been noted, such as improvement of microtubule stability due to acetylation. HDACI mediated neuroprotection is therefore likely achieved through an amalgamation of these effects: microtubule stabilisation in conjunction with numerous neurotrophic and neuroprotective agents being upregulated simultaneously to evoke a neuroprotective parenchymal environment inauspicious to neuronal cell death.

It remains unknown as to whether isoform-specific or pan-HDAC inhibitors present most efficacy for neuroprotection in models of Parkinsonian neurodegeneration. Similarly, it is unknown which of the numerous HDAC subclasses presents greatest neuroprotective efficacy: mainly due to previous unavailability of specific subtype inhibitors. However, in recent years the development of HDACIs have advanced hugely: a large variety of both isoform-specific and non-specific inhibitors now being available. Nevertheless at present, the PD field is not keeping up with the recent flourish in HDACI development: notable examples of isoform-specific drugs such as MS-275 and CI-994 have yet to be tested in models of PD. Only with investigation of these novel compounds will we be able to better understand the intricacies of the HDAC subclasses and comprehend their neuroprotective potential when inhibited.

Similarly it is still unclear as to what dosing regime is most optimal for HDACI induced neuroprotection i.e. long term treatment vs. pulse treatment vs. acute treatment. It has previously been shown that pulse treatment with TSA is able to produce histone hyperacetylation and neuroprotection (Langley et al., 2008). Perhaps treatment strategies such as this could avoid some of the toxicity and side effects of long term HDACI drug treatment. Additionally, HDACIs represent an ideal target for combination therapy with other neuroprotective drugs: further study to ascertain the possible synergistic and/or additive effects of HDACIs in combination with other neuroprotectants should therefore be encouraged.

Lastly, as has been detailed above, accumulating evidence implicates histone hypoacetylation and transcriptional dysfunction in the pathogenesis of neurodegenerative diseases such as PD hence investigation of the neuroprotective potential of HDACIs. However, these findings of histone hypoacetylation and transcriptional dysfunction have yet to be confirmed in the brains of PD patients: all work previously being described in animal and cellular models of neurodegeneration. The acetylation level of histone proteins within degenerating regions of the Parkinsonian brain must therefore be quantified and compared with aged matched control subjects to confirm this hypothesis in the human disease. This represents a vital stage in the drug developmental process as without confirming the dysregulation of histone acetylation and transcriptional dysfunction in primary diseased tissue, the use of HDACIs for the treatment of PD cannot truly be rationalised.

8. In conclusion

Given the small molecular nature of the vast majority of HDACIs, they embody an exciting target for therapy in neuroscience due to their ability to traverse the BBB with ease. The multi-targeted and multi-cellular neuroprotective and neurotrophic affects induced upon HDACI treatment make HDACIs the most disease relevant drug class being investigated in PD today. Further study to facilitate our understanding of the consequences of HDAC isoform inhibition and their effects in producing neuroprotection are now required to further refine the prospective use of HDACIs in this complex disorder and translate their use to the clinic. Once this is achieved, we must learn how best to wield the power of these drugs in PD: how best to use them in the clinic, what drugs to combine them with, and how they can be translated to the treatment of other neurodegenerative disease.

Conflict of interest statement

The authors declare that there are no conflicts of interests.

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