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Histone deacetylase inhibitors: possible implications for neurodegenerative disorders

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During the past six years numerous studies identified HDAC inhibitors as candidate drugs for the treatment of neurodegenerative disorders. Two major neuroprotective mechanisms of HDAC inhibitors have been identified, namely the transcriptional activation of disease-modifying genes and the correction of perturbations in histone acetylation homeostasis, which have been shown to be intimately involved in the neurodegenerative pathomechanisms of Huntington's, Parkinson's and Kennedy disease, amyotropic lateral sclerosis, Rubinstein-Taybi syndrome as well as stroke. Based on the promising *in vitro* and *in vivo* analyses, clinical trials have been initiated to evaluate the safety and efficacy of HDAC inhibitors for the treatment of devastating diseases such as Huntington's disease, amyotropic lateral sclerosis and spinal muscular atrophy. Here, the authors summarize and discuss the findings on the emerging field of epigenetic therapy strategies in neurodegenerative disorders.

Keywords: adrenoleukodystrophy, Alzheimer's disease, amyotropic lateral sclerosis, dentatorubral-pallidoluysian atrophy, depsipeptide, histone acetyltransferase, histone deacetylase, histone deacetylase inhibitor, phenylbutyrate, romidepsin, sodium butyrate, spinal muscular atrophy, spinocerebellar ataxia, suberoylanilide hydroxamic acid, valproic acid

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1. Introduction

DNA within the eucaryotic nucleus is compacted through its association with two copies of the highly basic histone proteins H2A, H2B, H3 and H4 to form nucleosome core particles that, together with linker DNA and linker histone, assemble into a dynamic structure known as chromatin. Histones are subjected to extensive post-translational modifications that involve the addition or removal of acetyl, methyl or phosphate groups as well as the reversible transfer of the ubiquitin and sumo proteins. The acetylation and deacetylation of histones has shown to play an important role in transcription regulation of eukaryotic cells and is determined by histone deacetylases (HDACs) and histone acetyltransferases (HATs) which exert opposite activities. HATs add acetyl groups to lysine residues, whereas HDACs remove the acetyl groups. In general, histone acetylation promotes a more relaxed chromatin structure, allowing transcriptional activation. HDACs can act as transcription repressors, due to histone deacetylation, and consequently promote chromatin condensation. At present, there are no reports of small molecules that augment HAT function but numerous small-molecule HDAC inhibitors belonging to different chemical classes have been developed that allow the modulation of chromatin structure in a transient manner. HDAC inhibitors are by no means a brand new group of compounds, considering the fact that the first compound bearing an HDAC inhibitory function, sodium butyrate (SB), was identified in the late 1970s. However, the development of

potent and well-tolerated HDAC inhibitors gained a considerable momentum due to the observation that these drugs have a substantial antitumor activity. In 2006, the drug development efforts culminated in the FDA granting approval for the potent HDAC inhibitor vorinostat (suberoylanilide hydroxamic acid, SAHA), whereas numerous other compounds such as romidepsin (FK-228, depsipeptide) and SNDX-275 (MS-275) are under clinical investigation for the treatment of various types of cancer. However, intriguing preclinical evidence accumulated during the past six years indicating that HDAC inhibitors may be used for the treatment of numerous neurodegenerative disorders and based on these findings, clinical trials have been initiated to evaluate the safety and efficacy of HDAC inhibitors for the treatment of devastating disorders such as Huntington's disease (HD), amytropic lateral sclerosis and spinal muscular atrophy (SMA).

2. Histone deacetylases

The family of HDACs has already been extensively reviewed and is thus only briefly described [1]. In humans, 18 HDAC enzymes have been identified and classified dependent on cofactor dependency and sequence similarity. Generally, two HDAC families are discriminated: the classical HDACs which require Zn^{2+} for deacetylase activity and the sir2-related HDACs (sirtuins) which require NAD^+ as cofactor. Based on their homology to yeast HDACs, the classical HDACs have been further subdivided into class I, IIa, IIb and IV. The class I HDACs include HDAC1, HDAC2, HDAC3 and HDAC8, which are related to the yeast RPD3 deacetylase. Although HDAC3 is able to shuttle between the nucleus and the cytoplasm, the other three class I family members are found primarily in the nucleus [2,3]. Class II HDACs are related to yeast HDAC1 and include six HDAC isoenzymes. This class is further subdivided into class IIa, consisting of HDAC4, HDAC5, HDAC7 and HDAC9, and class IIb, consisting of HDAC6 and HDAC10 which contain two catalytic sites as unique feature. Class II HDACs can be primarily cytoplasmic and/or migrate between the cytoplasm and nucleus similar to the class I family member HDAC3. Several class II HDACs have been shown to interact with class I HDAC3 indicating a tight functional connection between class I and II isoenzymes. Class IV is represented by HDAC11 which contains conserved residues in the catalytic core regions shared by both class I and class II HDAC enzymes [4]. The NAD^+-dependent sirtuin family of HDACs (class III) is composed of seven members (SIRT1 to SIRT7) which are homologs of the yeast SIR2. Sirtuins have gained considerable attention as they may provide novel targets for diseases associated with ageing [5]. During recent years intriguing evidence accumulated that showed that predominantly the classical HDACs represent highly promising target proteins for the potential treatment of numerous neurodegenerative conditions. These insights are mainly driven by the finding that small-molecule inhibitors of classical HDACs exhibit definite neuroprotective properties *in vitro* and *in vivo*, although the specific role of each HDAC isoenzyme remains elusive in most cases.

3. Histone deacetylase inhibitors

With a single exception, as discussed later, small-molecule HDAC inhibitors investigated for the potential treatment of neurodegenerative disorders do not affect sirtuin (class III HDAC) activities. These compounds, which considerably differ in potency and HDAC isoenzyme selectivity, belong to four different classes, namely the short chain fatty acids, hydroxamic acids, benzamides and cyclic tetrapeptides. The fatty acid group of HDAC inhibitors comprises the compounds SB, phenylbutyrate (PB) and valproic acid (VPA). In 1977, Riggs and co-workers [6] linked the previously reported antiproliferative action of SB to histone hyperacetylation suggesting an HDAC inhibitory function. Today, the SB derivative PB is an orphan drug which achieved FDA approval for the treatment of urea cycle disorders. VPA, a commonly used anticonvulsant and mood stabilizer, achieved FDA approval in 1987 and its HDAC inhibitory function was discovered in 2001 [7,8]. Using HDACs isolated from rat liver, SB and VPA inhibit total HDAC activity incompletely and at comparatively high millimolar concentrations (**Figure 1A**), which is in line with a pronounced class I selectivity shown for VPA (**Table 1**). This is somewhat in contrast to the observation that VPA and SB alter histone acetylation levels already at submillimolar doses in single cell cultures [7,8], which suggests that mechanism(s) other than direct interference with the catalytic activity of HDAC isoenzymes are involved. Indeed, Kramer and co-workers [9] have shown that VPA and SB (but not TSA and SNDX-275, see below) reduce HDAC2 (class I) protein levels. For VPA, this effect has been shown to be based on proteosomal degradation of HDAC2 but not of other class I HDAC isoenzymes [9]. Thus, the fatty acids VPA and SB might be considered as class I-selective HDAC inhibitors with a pronounced activity against HDAC2 at submillimolar doses. Prominent members of the hydroxamic acid group of HDAC inhibitors are trichostatin A (TSA) and vorinostat. TSA, a natural product isolated from a *Streptomyces hygroscopicus* strain, was identified as a fungistatic antibiotic in 1976, and its activity was linked to HDAC inhibition by Yoshida and co-workers in 1990 [10,11]. TSA is a highly potent pan-HDAC inhibitor active already at low nanomolar doses (IC_{50}: 12 nM, [12]). Vorinostat (SAHA) [13] achieved FDA approval in 2006 for the treatment of cutaneous T-cell lymphoma. Like several other hydroxamic acids (such as scriptaid, oxamflatin, carboxycinnamic acid bishydroxamic acid and suberic bishydroxamic acid), vorinostat is a pan-HDAC inhibitor active at submicromolar concentrations (**Figure 1B**), whereas neither TSA nor vorinostat appear to show profound HDAC

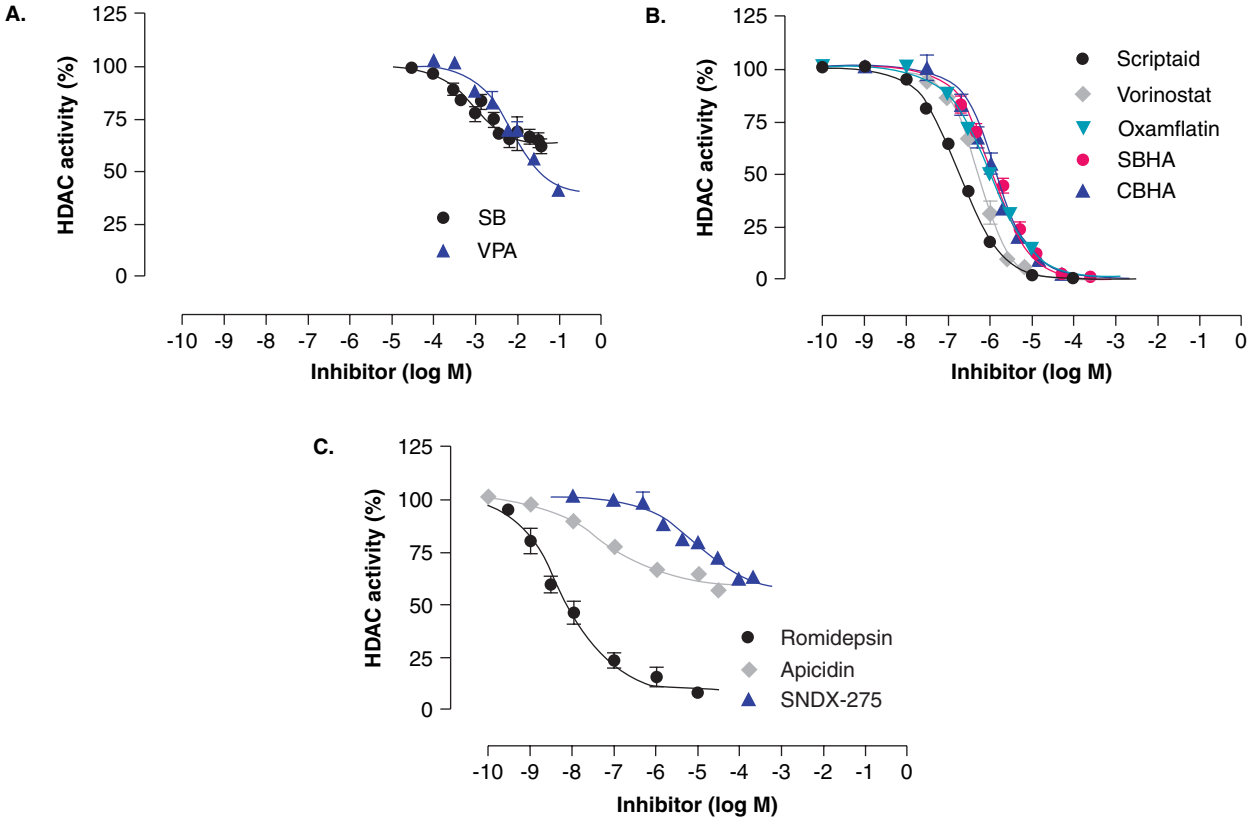

Figure 1. HDAC inhibition by the test compounds was investigated *in vitro* as described [84,118] using histone deacetylases purified from rat liver. Detailed quantitative analysis confirmed a concentration-dependent inhibition of total HDAC activity in all experiments, though with considerable variations in potency and efficacy. **Fattyacids (1A):** Inhibition of total HDAC activity by sodium butyrate (SB) and valproic acid (VPA) plateaued at 64 ± 2% and 40 ± 3% of control HDAC activity, respectively, suggesting a pronounced HDAC isoenzyme selectivity (means ± SE, n = 3 – 6 experiments performed in duplicate). For both compounds half maximum inhibition (IC_{50}) was observed at millimolar doses. Given are (-log) mean IC_{50} values ± SE throughout, the corresponding molar concentration is stated in brackets: SB: 3.10 ± 0.09 (0.80 mM); VPA: 2.14 ± 0.07 (7.24 mM). **Hydroxamic acids (1B):** The hydroxamic acids investigated inhibited HDAC activity completely at comparatively low concentrations, with a half maximum inhibition observed at µmolar doses (scriptaid: 6.73 ± 0.02 (0.19 µM); vorinostat (SAHA): 6.30 ± 0.02 (0.50 µM); oxamflatin: 6.01 ± 0.02 (0.98 µM); carboxycinnamic acid bishydroxamic acid: 5.94 ± 0.02 (1.15 µM) suberic bishydroxamic acid: 5.91 ± 0.03 (1.23 µM). **Cyclic tetrapeptides/benzamides (1C):** The cyclic tetrapeptide romidepsin (FK-228) inhibited HDAC activity almost completely (bottom: 9 ± 3%) with a half maximum inhibition observed 8.25 ± 0.09 (5.62 nM). In contrast, inhibition of total HDAC activity by apicidin (cyclic tetrapeptide) and SNDX-275 (MS-275, benzamide) plateaued at 57 ± 3% (apicidin) and 54 ± 5% (SNDX-275) of control HDAC activity, respectively, suggesting a pronounced HDAC isoenzyme selectivity. Half maximum inhibition of HDAC activity was observed at 7.26 ± 0.21 (0.06 µM) for apicidin and 5.03 ± 0.21 (9.33 µM) for SNDX-275. Romidepsin was kindly provided by Gloucester Pharmaceuticals, Cambridge, MA, USA.
HDAC: Histone deacetylases.

isoenzyme selectivities (**Table 1**). The cyclic tetrapeptide romidepsin (FK-228, depsipeptide), also a natural drug, is active at low nanomolar doses (**Figure 1C**) showing little activity against HDAC6 (**Table 1**), while the cyclic tetrapeptide apicidin appears to be highly selective with a pronounced activity against class I HDAC2, HDAC3 and HDAC8 (**Table 1**). Similar to apicidin, the benzamide SNDX-275 (MS-275) represents a highly selective HDAC inhibitor (**Figure 1C**) and does not affect HDAC8 (class I) or HDAC 4/6/7 (class II) activities (**Table 1**) [14]. These data demonstrate that the HDAC inhibitors frequently used in experimental settings differ in their potency and HDAC isoenzyme selectivity. Regarding the published patent literature of the last years, a plethora of new HDAC inhibitors has been developed and numerous HDAC inhibitors belonging to all four chemical classes (including PB, VPA, SAHA, FK-228, MS-275) are under clinical development for cancer treatment [1]. However, with respect to the potential treatment of neurodegenerative conditions, the panel of HDAC inhibitors investigated is limited and mainly focussed on the well-established experimental drug TSA and the clinically used HDAC inhibitors SB, PB, VPA and vorinostat, which are all known to penetrate the blood–brain barrier [15,16].

Table 1. HDAC inhibitors differ in potency and HDAC isoenzyme selectivity.

Inhibitor	Class I IC_{50}				Class IIa IC_{50}			Class IIb IC_{50}		Ref.
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	
TSA	0.002 µM	0.003 µM	0.004 µM	0.456 µM	0.006 µM	*	0.005 µM	0.006 µM	0.003 µM	*	[14]
Vorinostat (SAHA)	0.068 µM	0.164 µM	0.048 µM	1.524 µM	0.101 µM	*	0.104 µM	0.107 µM	0.090 µM	*	[14]
Romidepsin (FK-228)	0.036 µM	0.047 µM	*	*	0.510 µM	*	*	*	ǂ	*	[12]
redFK-228*	0.002 µM	0.004 µM	*	*	0.025 µM	*	*	*	0.790 µM	*	[12]
Apicidin	ǂ	0.120 µM	0.043 µM	0.575 µM	ǂ	*	ǂ	ǂ	ǂ	*	[14]
SNDX-275 (MS-275)	0.181 µM	1.155 µM	2.311 µM	ǂ	ǂ	*	ǂ	0.505 µM	ǂ	*	[14]
VPA	1.6 mM	3.1 mM	3.1 mM	7.4 mM	*	*	ǂ	ǂ	ǂ	*	[14]

*Not determined.

*On entering the cell, romidepsin (FK-228) is reduced to its active form, redFK-228.

ǂNo complete inhibition found at doses of 10 µM (romidepsin, apicidin, SNDX-275) or 100 mM (VPA).

HDAC: Histone deacetylases; IC_{50}: Half maximal inhibitory concentration; TSA: Trichostatin A; VPA: Valproic acid.

4. Histone deacetylase inhibition

Transcriptome-wide studies mainly performed using neoplastic cells have shown that HDAC inhibitors can affect the transcription levels of 7 – 10% of all genes [1]. However, there is increasing evidence that these changes are not solely based on histone hyperacetylation due to the observation that HATs and HDACs are not just for histones. Numerous non-histone protein targets of HDACs including transcription factors and regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators and viral proteins [1] have been identified which are likely to contribute to the hitherto reported effects induced by HDAC inhibitors. An additional mechanism of action is given by the finding that HDAC inhibitors counteract gene silencing by DNA methylation mediated by methyl-CpG-binding protein 2 (MeCP2). MeCP2 binds tightly to chromatin in a methylation-dependent manner and associates with a corepressor complex containing HDAC1 and HDAC2, suggesting that the fundamental mechanisms of epigenetic gene regulation, DNA methylation and histone acetylation, are linked by MeCP2 [17]. Jones and co-workers [18] demonstrated that gene silencing conferred by MeCP2 and methylated DNA can be relieved by HDAC inhibition using the pan-HDAC inhibitor TSA. A striking observation is that the consequences of HDAC inhibition are not limited to changes in protein acetylation but may also bring about changes in the state of DNA methylation. In single cell cultures, TSA and VPA have shown to trigger DNA-demethylation in a replication-independent manner [19-21] and DNA-demethylating activity of VPA, SNDX-275 and TSA has subsequently been confirmed *in vivo* [22,23]. However, Cameron and co-workers [24] reported that TSA alone is neither sufficient to modulate DNA methylation nor to transcriptionally reactivate heavily hypermethylated genes in neoplastic cells, indicating that CpG island methylation is the dominant mechanism of epigenetic gene silencing. Even though the exact mechanism by which HDAC inhibitors affect DNA methylation remains to be clarified, there is increasing evidence that HDAC inhibition may have manifold consequences, including the hyperacetylation of histone and non-histone proteins and the alleviation of DNA methylation.

5. Polyglutamine disorders

Polyglutamine (polyQ) expansion diseases [25] are a class of nine inherited neurodegenerative disorders that are known to be caused by mutations in polyglutamine-encoding CAG tracts in different genes that result in degeneration of different populations of neurons. These conditions include HD, Dentatorubral-Pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA) and six spinocerebellar ataxias (types 1, 2, 3, 6, 7 and 17). All these disorders are

caused by expansions of polymorphic (CAG)n repeats in the coding regions of the disease genes leading to long polyQ stretches, which confer a gain-of-function to the mutant proteins. As outlined subsequently, the polyQ disorders have many features in common and thus might be treatable by common pharmacologic interventions.

5.1 Huntington's disease

HD (MIM+143100) is caused by neuronal dysfunction and progressive neuronal cell death that is especially severe in the striatum. The disease is characterized by choreic movements, neuropsychiatric symptoms and severe cognitive deficits. HD follows an autosomal dominant mode of inheritance and is caused by expansion of a polymorphic (CAG)n repeat in the coding region of the *huntingtin* (*HD*) gene. Although the range of CAG repeat numbers in exon 1 of the *HD* gene is < 35 in unaffected individuals, the CAG repeat number is expanded to > 40 and unstable in HD patients, with repeat length inversely correlating with age of disease onset [15,26]. The polyQ expansion in the mutant huntingtin protein (htt) leads to its aberrant proteolytic cleavage, resulting in the release of N-terminal fragments that readily enter the nucleus and form aggregates in brain tissue from affected patients [27,28]. As loss of neuronal huntingtin function may also contribute to HD [29], the precise mechanisms leading to neurodegeneration in HD have not been fully elucidated. However, there is increasing evidence that transcriptional dysregulation plays a pivotal role in HD pathogenesis [30-32]. In line with these findings, mutant htt fragments bearing polyQ expansions have shown to directly interact and impair the function of several nuclear proteins involved in the transcription machinery [33], including the transcription factor Sp1, its co-activator $TAF_{II}130$ [34,35] as well as HATs [36,37]. *In vitro* studies revealed that mutant htt fragments inhibit the HAT activity of CREB-binding protein (CBP), its close homolog p300 and CBP/p300-associated factor (P/CAF) [37] and reduce overall H3/H4 acetylation levels *in vitro* and *in vivo* [37-39]. Concordant with these findings, histones associated with downregulated genes have been recently shown to be hypoacetylated in HD models [40]. *In vitro* studies gave the first evidence that HDAC inhibitors (SB, TSA, vorinostat) are able to counteract H3/H4 hypoacetylation induced by mutant htt [37]. Even though the involvement of p300 appears to be questionable [41,42], these data highlight changes in chromatin texture to be intimately involved in HD pathogenesis and identified HDAC inhibitors as candidate drugs for HD therapy. By employing a *Drosophila* model, Steffan and co-workers [37] showed that SB and vorinostat decelerated rhabdomere degeneration observed in transgenic flies expressing mutant htt fragments or polyQ peptides, whereas subsequent studies demonstrate neuroprotective effects of TSA in *Caenorhabditis elegans* models for HD [43,44]. The potential applicability of HDAC inhibitors for HD therapy was consecutively confirmed in transgenic mice which mimic HD-like features. R6/2 mice express an N-terminal portion of human huntingtin with a polyQ stretch of approximately 150. R6/2 mice show spatial learning deficits starting at 3 – 4 weeks of age, followed by abnormal performance in motor tests, tremor, gait disturbances and premature death at 13 – 15 weeks of age. Hockly and co-workers [45] demonstrated that oral application of vorinostat (100 mg/kg/day, starting at 4 weeks of age) improved motor impairment (rotarod performance) in R6/2 mice. Using the same mouse model, intraperitoneal (i.p.) injection of SB (400 – 1200 mg/kg/day, starting at 3 weeks of age) increased lifespan and rotarod performance in a dose-dependent manner [38]. In addition, high doses of SB (1200 mg/kg/day) mitigated brain atrophy and bilateral ventricular hypertrophy observed in untreated R6/2 animals. By using a different mouse model expressing an N-terminal htt fragment containing 82 CAG repeats (N171-82Q), Gardian and co-workers [39] confirmed that administration of PB (100 mg/kg, 6 days/week, i.p., starting at 11 weeks of age) improved survival and attenuated gross brain atrophy and ventricular enlargement. In contrast to the vorinostat and SB studies, effects of PB on rotarod performance have not been observed which might be due to the late drug application after onset of symptoms [39]. Interestingly, none of the HDAC inhibitors tested so far attenuate mutant htt aggregation [38,39,45]. In all, these studies may suggest that the administration of HDAC inhibitors corrects global histone hypoacetylation induced by mutant htt and ameliorates the HD phenotype *in vivo*. Based on these findings a clinical Phase II trial has been initiated to assess the safety and tolerability of PB for potential HD treatment (clinicaltrials.gov; identifier: NCT00212316). A pilot trial using VPA for the adjuvant therapy of HD patients suffering from myoclonic hyperkinesias revealed improved motor scores following treatment. Even though myoclonus is rare in HD, these findings may further emphasize the potential use of HDAC inhibitors for HD therapy [46].

5.2 Dentatorubral-Pallidoluysian atrophy

DRPLA (MIM#125370) is an autosomal dominant disease characterized by a number of symptoms that include ataxia, chorea, seizure, myoclonus, incoordination and dementia. DRPLA is caused by CAG expansion within the *Atropin-1* gene. The polyQ stretch in the Atropin-1 protein ranges from 6 to 35 in normal individuals and expands from 48 to 88 in DRPLA patients. Neuropathological analyses of DRPLA patients identified widespread nuclear inclusion bodies in affected neurons with concomitant loss in specific brain regions such as the dentate cerebellar nucleus, red nucleus, globus pallidus and subthalamic nucleus. Even though different brain regions are affected in HD and DRPLA, both disorders show striking similarities on a molecular level. Comparative microarray analyses of cerebellar gene expression in HD and DRPLA mouse models indicated that most of the mRNAs that changed in response to the mutant *htt* transgene (N171-82Q mice) changed

similarly in response to the mutant *atropin-1* transgene (At-65Q mice) [31]. In line with these findings, mutant atropin-1 interacts and impairs the function of nuclear proteins involved in the transcription machinery, including the transcriptional co-activator $TAF_{II}130$ and the HAT CBP, suggesting both proteins to be crucially involved also in DRPLA pathogenesis [42,47]. Mutant atropin-1 co-aggregates with CBP in human DRPLA postmortem brain tissue, inhibits CBP-mediated transcription and causes cell death *in vitro*. Interestingly, overexpression of CBP rescued cells from mutant atropin-1 as well as mutant htt toxicity, providing further evidence that the impairment of CBP activity represents a common pathomechanism in HD and DRPLA [31]. Ying and co-workers [48] reported that SB ameliorates the DRPLA phenotype *in vivo* using transgenic mice expressing expanded (118Q) full-length *atropin-1* under the control of a neuron-specific promoter. Atro-118Q14 mice developed a neurodegenerative phenotype including feet-clasping, tremor, ataxic gait and premature death with a mean lifespan of 22 weeks. Daily injection of SB (0.5 and 1.5 mg/kg/day, i.p., starting from 4 weeks of age) mitigated motor impairments (rotarod performance, hanging wire) and increased average lifespan, while the nuclear accumulation of mutant atropin-1 was not altered. Interestingly, drug application after disease onset (12 weeks of age) extended the average lifespan of Atro-118Q14 mice but did not improve motor performance. Together with the *in vivo* data presented by Gardian and co-workers using a HD mouse model (N171-82Q), these data indicate that HDAC inhibitors may be effective in antagonising polyQ toxicity even after disease onset. Similar to HD, histone H3 has shown to be hypoacetylated in brains of DRPLA mice, while H3 acetylation could be restored to control levels by SB treatment [48].

5.3 Spinal and bulbar muscular atrophy

SBMA (Kennedy disease, MIM#313200) is a slowly progressing polyQ disease that exclusively affects adult males. SBMA is characterized by proximal muscle weakness, atrophy and fasciculations of bulbar, facial and limb muscles mainly due to a loss of lower motor neurons in the anterior horn of the spinal cord. The disease is caused by CAG expansion in the first exon of the *androgen receptor* (*AR*) gene located on the X chromosome. In unaffected individuals, the CAG number ranges from 11 to 35, whereas repeat sizes of 40 – 62 cause SBMA. Very similar to HD and DRPLA, *in vitro* studies revealed that a truncated *AR* construct bearing an expanded polyQ tract associates with the HAT CBP and induces H3 hypoacetylation associated with polyQ toxicity [49]. The toxicity of mutant AR fragments could be mitigated by either HDAC inhibitor treatment (TSA, vorinostat, PB) or CBP overexpression, further supporting the hypothesis that transcriptional dysregulation plays a pivotal role also in SBMA pathology [49]. H3 hypoacetylation has subsequently been confirmed *in vivo* using spinal cord lysates of transgenic SBMA-like (AR-97Q) mice, although no differences were detected in H2A, H2B or H4 acetylation levels [50]. By employing the AR-97Q mouse model expressing a full-length AR containing 97 CAGs, Minamiyama and co-workers [50] demonstrated that oral application of SB (4 and 8 g/l in drinking water, starting from 5 weeks of age) ameliorated the neuromuscular phenotype (gait disturbances, rotarod performance) and survival rate of SBMA-like mice but did not inhibit nuclear aggregation of the mutant AR protein observed in the residual motor neurons and non-neuronal cells. Histologic analyses revealed that SB treatment restored H3 acetylation levels in nuclei of spinal cord motor neurons and increased the diameter of muscles, spinal roots and motor neurons as compared with non-treated SBMA mice [50].

5.4 Spinocerebellar ataxia 3

Spinocerebellar ataxia type 3 (SCA3, MIM#109150), also known as Machado–Joseph disease, is an autosomal dominant disorder characterized by progressive gait and limb ataxia and ocular movement abnormalities. SCA3 is caused by CAG repeat expansions in the *ataxin 3* (*ATXN3*) gene resulting in polyQ stretches in the C-terminus of the encoded protein ataxin-3 (AT3). Very similar to the disease mechanism proposed for HD, DRPLA and SBMA, AT3 binds to the HAT CBP as well as p300 and P/CAF and represses CBP, p300 and P/CAF-mediated transcription when overexpressed [51]. In contrast to HD, DRPLA and SBMA, these properties of AT3 appear to be not restricted to mutant AT3. Li and co-workers demonstrated that the C-terminus containing either a normal or pathological polyQ co-immunoprecipitated with CBP, p300 and P/CAF [51]. Interestingly, wild-type (Q28) and mutant (Q78) full-length AT3 were equally effective at inhibiting histone acetylation, a feature restricted to the respective pathogenic polyQ-expanded protein in HD, DRLPA and SBMA [51]. Evert and co-workers [52] provide *in vitro* evidence that ectopic expression or normal (Q23) but not mutant AT3 (Q70) induces histone H3 hypoacetylation, suggesting mechanistic differences in polyQ toxicity between HD, DRPLA and SBMA compared with SCA3 pathogenesis. Furthermore, the finding that histone H3 is hyperacetylated in pontine tissue of SCA3 patients may argue against HDAC inhibitors as potential drugs for SCA3 treatment [52].

5.5 Spinocerebellar ataxia 7

Autosomal dominant spinocerebellar ataxia 7 (SCA7, MIM#164500) is clinically characterized by progressive incoordination of gait and limb movements due to neuronal loss within the cerebellum and brainstem. A unique feature that distinguishes SCA7 from other polyQ disorders and hereditary ataxias is the degeneration of cone and rod photoreceptor neurons in the retina leading to blindness [53]. The hypothesis that also SCA7 is caused by transcriptional dysregulation was supported by the finding that the disease

gene *ataxin-7* is an integral component of GCN5-containing protein complexes such as TFTC and STAGA [54]. GCN5 is a well-characterized HAT closely related to P/CAF and consequently TFTC/STAGA complexes contain HAT activity [54]. Even though normal and mutant ataxin-7 are found in GCN5-containing protein complexes, *in vitro* studies demonstrated that incorporation of polyQ-expanded ataxin-7 (92Q) into STAGA dramatically reduced its ability to acetylate free histone H3 [55]. By employing a *sca7Δ* yeast strain, McMahon and co-workers [56] show that the loss of *sca7* reduces the HAT activity of the yeast protein complex homologuous to STAGA (SAGA) and demonstrate that normal (Q10) but not polyQ-expanded (Q60) human ataxin-7 is able to restore the yeast SAGA HAT activity *in vitro* [56]. Moreover, ataxin-7 interacts with the photoreceptor-specific transcription factor CRX (cone-rod homeobox protein) while polyQ-expanded ataxin-7 antagonised CRX transactiviation [57]. This finding and the observation that CRX mutations cause a cone–rod dystrophy phenotype suggest that retinal degeneration observed in SCA7 patients is mediated by CRX [57]. Concordantly, decreased transcript levels of CRX-regulated genes and genes with a retina-restricted expression have been observed in SCA7 mice expressing mutant ataxin-7 (Q92) [57,58]. In agreement with these findings, Palhan and co-workers [55] demonstrated a marked reduction of acetylated histone H3 in the promoter regions of the CRX-dependent photoreceptor genes in retinae of SCA7-92Q mice. Thus, an increasing body of evidence suggests that SCA7 is caused by transcriptional dysregulation due to reduced HAT activity and argues for HDAC inhibition as promising pharmacologic intervention. However, Helmlinger and co-workers [58] presented opposing results. Using mice expressing normal (Q10) or mutant (Q92) ataxin-7 in rod photoreceptors, TFTC/STAGA complexes immunopurified from SCA7 mouse retina showed normal levels of HAT activity. Unexpectedly, genes which were downregulated in the retinae of SCA7 mice have shown to be hyperacetylated, indicating that the exact causal relationship between histone acetylation and SCA7 pathogenesis and, consequently, the potential use of HDAC inhibitors require further investigation.

5.6 HDAC inhibitors in polyQ disorders: does isoenzyme selectivity matter?

The outlined studies provide preclinical *in vitro* and *in vivo* evidence for the hypothesis that HDAC inhibitors might be further exploited to treat polyQ disorders such as HD, DRPLA and SBMA. In numerous *in vitro* and *in vivo* paradigms, HDAC inhibition reduced polyQ toxicity and ameliorated neurodegeneration. However, inhibition of HDAC6 may have opposite effects. By employing a *Drosophila* SBMA model which ectopically expressed a mutated human *AR* in photoreceptor neurons, Pandey and co-workers demonstrated that overexpression of HDAC6 suppressed the degenerative phenotype in flies with polyQ-expanded AR, whereas knockdown of endogenous HDAC6 enhanced neurodegeneration [59]. Kawaguchi and co-workers [60] identified HDAC6 as a crucial player in the cellular management of misfolded protein-induced stress. In addition, Iwata and co-workers [61] have shown HDAC6 activity to be required for autophagic degradation of aggregated huntingtin, suggesting a role for endogenous HDAC6 activity in protecting cells from polyQ toxicity. Isoenzyme selectivity of HDAC inhibitors becomes thus a vitally important issue. HDAC6 has shown to be nearly resistant to VPA, PB, SNDX-275 and apicidin, and displays a comparatively low susceptibility against romidepsin [8,14,62,63]. However, it remains elusive whether HDAC inhibitors that do not affect HDAC6 activity show superior neuroprotective effects compared with neuroprotective pan-HDAC inhibitors such as vorinostat and TSA [14]. Dompierre and co-workers have shown that inhibition of HDAC6 activity by vorinostat and TSA rescues vesicular transport defects observed in HD [64], suggesting a two edged role of HDAC6 in neurodegenerative disorders which might be further clarified *in vivo* using selective HDAC6 inhibitors such as tubacin [65]. Bates and co-workers [43] confirmed a neuroprotective effect of TSA by employing a *C. elegans* model for HD. Interestingly, RNAi-mediated knockdown of most *C. elegans* HDACs enhanced mutant htt toxicity whereas knockdown specifically of hda-3 suppressed toxicity, supporting the idea that neuro-protective effects of HDAC inhibitors are mediated by specific HDAC isoenzymes only. In summary, these data suggest that further analysis of the specific role(s) of each HDAC isoenzyme in polyQ disorders and the decoding of the HDAC inhibitor selectivity represent important steps to identify and to develop optimized HDAC inhibitors for the potential treatment of polyQ disorders and other neurodegenerative conditions.

6. Amyotropic lateral sclerosis

Amyotropic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the death of motor neurons in the brain, brainstem and spinal cord, resulting in fatal paralysis. ALS usually begins with asymmetric involvement of the muscles in middle adult life. Approximately 10% of ALS cases are familial with an autosomal dominant mode of inheritance of most entities. About 20% of the familial cases are caused by gain of function mutations in the *SOD1* gene encoding the Cu/Zn superoxide dismutase 1. The mutant SOD1 forms intracellular aggregates in the brain and spinal cord of patients and mouse models showing ALS-like phenotypes. Even though the primary pathogenic event(s) are still a matter of debate, the toxic pathway has been linked to the secretion of mutant SOD1 indicating that the motor neuron death is not cell autonomous and may be caused by cell types in the vicinity of motor neurons [66,67]. Albeit most ALS cases occur sporadically, the phenotypes of mice overexpressing human SOD1 missense mutations

closely mimic human ALS and thus serve as valuable disease models. Interestingly, disturbance of histone acetylation homeostasis is associated with the ALS pathogenesis similar to polyQ disorders, suggesting that transcriptional dysregulation may play a central role in the pathogenesis of ALS. This hypothesis is supported by the finding that the immunoreactivities of acetylated histone H3 and the histone acetyltransferase CBP were severely reduced in motor neuron nuclei in the lumbar spinal cord of ALS-like mice [68]. Using SOD1/G93A mice, injection of PB (400 mg/kg/day, i.p.) starting before or at symptom onset significantly prolonged survival, improved motor performance and reduced motor neuron loss [69,70], and oral application of VPA (530 mg/kg/day) prolongs the lifespan of affected SOD1/G93A mice [71]. Consistent with the hypothesis that transcriptional dysregulation may play a role in the ALS pathogenesis, Ryu and co-workers [70] observed hypoacetylation of histones H2A, H2B, H3 and H4 in spinal cord of affected SOD1/G93A mice, while PB treatment restored histone acetylation to near-normal levels. Based on these promising findings, a clinical Phase I/II trial has been initiated to assess the safety of PB for potential ALS treatment (clinicaltrials.gov; identifier: NCT00107770). By employing a different ALS mouse model (G86R), Rouaux and co-workers [72] demonstrate that histone acetylation levels were dramatically decreased in motor neuron nuclei from affected mice, while proper histone acetylation and Cbp levels were maintained in mice treated with VPA (250 mg/kg/day, i.p.). Interestingly, VPA reactivated *Cbp* transcription *in vivo*, indicating that HDACis combat neurodegeneration at least in part through modulation of the *Cbp* transcriptional pathway. However, the data presented by Rouaux and co-workers partially contradict previous *in vivo* studies. Although VPA-treatment counteracts motor neuron death, delays disease onset and prevents from muscular atrophy at the early stage of the disease, the lifespan remained unaltered. Irrespective of its neuroprotective effects, the authors demonstrate that VPA does not mitigate neuromuscular denervation which might be the reason that motor neuron protection alone is not sufficient for ALS therapy [72].

7. Spinal muscular atrophy

SMA is an autosomal recessive inherited α-motor neuron disorder causing weakness and atrophy of voluntary muscles. The disease determining *survival motor neuron gene 1* (*SMN1*) is homozygously absent in 96% of all SMA patients and intragenic *SMN1* mutations are correspondingly rare [73]. Within the SMA region on chromosome 5q, the human *survival motor neuron gene* exists in two copies, *SMN1* and *SMN2*, which are ubiquitously expressed and encode identical proteins [74]. Even though all SMA patients lacking *SMN1* carry at least one *SMN2* gene copy, the amount of functional SMN protein produced by *SMN2* is not sufficient to prevent progressive α-motor neuron degeneration. This finding has been assigned to a single translationally silent mutation within exon 7, affecting the splicing of *SMN* transcripts [75]. As a consequence, the disease determining *SMN1* gene produces full-length transcripts only (FL-*SMN*), whereas the majority of *SMN2* transcripts lack exon 7 due to alternative splicing (Δ7-*SMN*). Truncated Δ7-SMN proteins are reduced in their ability to self-oligomerise which is essential for proper SMN function [76,77] and have been shown to ameliorate but not to prevent the SMA phenotype *in vivo* [78]. The disease-modifying property of the *SMN2* gene has been verified in transgenic mouse models, confirming *SMN2* as therapeutic target [79]. Consequently, transcriptional *SMN2* activation and/or modulation of the *SMN2* splicing pattern to increase FL-*SMN* levels may be an effective strategy for SMA treatment. Several HDAC inhibitors have shown to increase *SMN2*-derived FL-SMN protein levels *in vitro* by transcriptional activation and/or by modulation of the *SMN2* splicing pattern. These compounds include the fatty acids SB [80], PB [81] and VPA [82-84], the benzamide M344 [84,85] as well as the hydroxamic acids vorinostat and TSA [16,84,86]. The potential applicability of HDAC inhibitors for SMA therapy was confirmed in transgenic mice which mimic SMA-like features. By employing a knockout transgenic mouse model (*Smn*$^{-/-}$ *SMN2*) Chang and co-workers [80] demonstrated that oral application of SB (0.8 or 8 g/l in drinking water, starting after disease onset) increased lifespan and FL-SMN protein levels in motor neurons of affected mice. In agreement with these findings, Tsai and co-workers [87] demonstrated that oral application of VPA (0.2 g/l in drinking water) attenuates motor neuron death, increases spinal SMN protein levels and partially normalizes motor function in SMA-like mice. By employing a different mouse model (*Smn*$^{-/-}$ SMN2$^{+/+}$ SMNΔ7$^{+/+}$), Avila and co-workers [16] provide evidence that injection of the hydroxamic acid TSA (10 mg/kg/day, i.p., starting after disease onset) improved survival and motor function of SMA-like mice. Considering the apparent positive therapeutic responses in SMA model mice and the relatively few adverse effects, clinical Phase II trials have been initiated to evaluate the efficacy of PB and VPA for SMA therapy (clinicaltrials.gov; identifier: NCT00528268; NCT00481013; NCT00227266). Pilot trials with small numbers of patients treated with PB [88,89] or VPA [90,91] revealed increased quantitative muscle strength and subjective muscle function which might further emphasize the potential use of HDAC inhibitors for SMA treatment. These trials provide the first *in vivo* evidence that oral administration of PB and VPA increases *SMN2* expression in SMA patients. However, only 7 out of 20 patients displayed elevated *SMN2* transcript levels following VPA treatment, suggesting that some patients might be non-responders [91]. Similar to polyQ disorders, the selectivity of HDAC inhibitors represents an important issue for SMA therapy. The observed *SMN2* gene activation by the HDAC class I selective inhibitor

VPA initially identified class I HDACs (1, 2, 3 and 8) as potential therapeutic targets. The authors have shown that the HDAC1-specific inhibitor SNDX-275 [14] fails to increase *SMN2* expression levels, suggesting that *SMN2* activation by less selective drugs occurs independently of HDAC1 [84]. Kernochan and co-workers demonstrated that the human *SMN2* promoter is associated with HDAC1 and more pronounced with HDAC2 but not with HDAC3, -4 and -5 [86]. In all, these data point toward HDAC2 as a major target for epigenetic SMA therapy.

8. Adrenoleukodystrophy

X-linked adrenoleukodystrophy (X-ALD, MIM#300100) is an inherited disorder characterized by pathologic accumulation of very long chain fatty acids (VLCFA) in all tissues of the body, progressive demyelination of the CNS and adrenal insufficiency. The disease is due to mutations in the *ABCD1* gene encoding the adrenoleukodystrophy protein (ALDP). ALDP, a peroximal member of the ATP-binding cassette family, is thought to participate in the entry of VLCFA into the peroxisome where VLCFA are β-oxidized. ALDP is supposed to act in concert with three other peroxisomal ATP-binding cassette transporters encoded by *ABCD2*, *ABCD3* and *ABCD4* genes. Among those, *ABCD2* is the closest paralog of the disease gene *ABCD1* and appears to have a redundant function. Overexpression of the adrenoleukodystrophy-related protein (ALDRP), the *ABCD2* gene product, has been demonstrated to compensate for ALDP deficiency in *Abcd1*$^{-/-}$ mice by preventing VLCFA accumulation and delaying disease onset [92]. Thus, *ABCD2* appears to be a promising target for X-ALD therapy. Interestingly, PB substantially increases ALDPR expression and corrects VLCFA levels in cultured fibroblasts from X-ALD patients [93]. By employing X-ALD model mice (Abcd1$^{-/-}$), Kemp and co-workers [93] demonstrate that oral application of PB mitigates VLCFA accumulation in brain and adrenal glands, indicating that PB and related compounds may be relevant as a treatment option for patients with X-ALD. This hypothesis is further supported by the finding that SB as well as PB dose-dependently activate mouse *Abcd2* in cultured glial cells [94]. McGuiness and co-workers [95] demonstrated that although PB and TSA increase VLCFA beta-oxidation in cultured mouse ALD fibroblasts, the beneficial effect of TSA appears to be independent of an increase in *Abcd2*/ALDR expression. These data suggest that the correction of the biochemical abnormality in X-ALD by HDAC inhibitors might not be dependent on pharmacologic induction of a redundant gene. Even though the exact underlying mechanism(s) remain elusive, further studies using HDAC inhibitors might be worthwhile to explore how the *ABCD2* gene is epigenetically regulated and to evaluate the potential applicability of HDAC inhibitors for X-ALD therapy by either *ABCD2*-dependent or *ABCD2*-independent mechanisms.

9. Rubinstein-Taybi syndrome

Mental impairment syndromes are diagnosed based on below-average intellectual function originated during developmental periods. Intellectual abilities rely on the capability of the brain to obtain, process, store and retrieve information. One of these disorders is the autosomal dominant Rubinstein-Taybi syndrome (RSTS, MIM#180849) characterized by severe mental retardation, postnatal growth deficiency, microcephaly, facial characteristics, and broad thumbs and big toes [96]. RSTS can be caused by deletion or mutation of the histone acetyltransferase CBP or in rare cases by mutation of its close homolog p300 [97], suggesting that perturbation of histone acetylation is involved also in the pathogenesis of RSTS. RSTS mouse models heterozygous for either a CBP null allele or a mutation leading to the expression of a truncated CBP protein exhibit phenotypes similar to certain clinical aspects of RSTS, including growth retardation and skeletal abnormalities. Interestingly, behavioral studies of RSTS-like animals revealed long-term memory impairments (fear conditioning, object recognition) associated with histone hypoacetylation in the hippocampi of affected mice [98-101]. Alarćon and co-workers [101] show that application of the pan-HDAC inhibitor vorinostat before training (20 μg, intraventricular injection) augments histone H2B acetylation and most strikingly restores long-term memory in a fear conditioning paradigm. Simultaneously, Korzus and co-workers [102] generated a tetracycline-inducible mouse model expressing a dominant-negative CBP transgene that lacks HAT activity. The transgene expression was restricted to the hippocampal formation and induced 7 days before starting an experiment. Concordant with the data presented by Alarćon and co-workers, behavioral tests revealed that stabilization of short-term into long-term memory is impaired in these mice (recognition memory, spatial memory). Importantly, these defects were reversible either on termination of transgene expression or administration of TSA before training (2 mg/kg, i.p.). These studies highlight a pivotal role of CBP acetyltransferase activity in controlling memory consolidation, and based on these studies one might speculate that HDAC inhibitors may be useful to counteract the histone acetylation imbalance caused by CBP haploinsufficiency to alleviate some RSTS symptoms. Strikingly, HDAC inhibitors (SB, TSA) have subsequently been shown to enhance long-term memory formation also in non-diseased animals [103,104], suggesting that HDAC inhibition might represent a viable route also for the treatment of disturbances in memory formation such as age-related memory impairment.

10. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of progressive dementia in the elderly. It is a neurodegenerative

disorder characterized by the neuropathological findings of intracellular neurofibrillary tau aggregation and extracellular amyloid plaques accumulating in vulnerable brain regions. Several studies revealed also an accumulation of the protein fragment p25 in sporadic AD. The neurotoxic p25 derives from proteolysis of p35, and overactivates the tau kinase cyclin-dependent kinase 5. Transgenic mice expressing high levels of p25 exhibit hyperphosphorylation of tau as seen in AD, and neurodegeneration. By employing a tetracycline-inducible mouse model (CK-p25) expressing p25 under control of a neuron-specific promoter, Fischer and co-workers [105] demonstrate that recovery of learning and memory is associated with chromatin remodeling. In CK-p25 mice, p25 causes severe cognitive defects (fear conditioning, spatial learning) associated with neuronal loss and tangle formation (cortex, hippocampus) similar to AD pathology. Very similar to the RSTS mouse model, application of an HDAC inhibitor (SB, 1200 mg/kg/day, i.p.) mitigated learning and memory defects in CK-p25 mice. These effects were not restricted to diseased CK-p25 animals. Application of either SB (100 ng) or TSA (50 ng) into the brain ventricles of wild-type mice following fear conditioning significantly improved their long-term memory abilities when tested 24 h later. An intriguing observation of Fischer and co-workers is that environmental enrichment (large cages where exploratory activity is promoted by the presence of toys, tunnels and climbing devices) induces histone H3/H4 hyperacetylation in wild-type mice and normalizes memory performance in diseased CK-p25 mice. In line with these findings environmental enrichment has been demonstrated to be beneficial in reducing cognitive deficits and disease progression in several models of neurodegenerative pathologies such as HD [106] and AD [107], which might at least in part be triggered by increased histone acetylation. Even though the underlying mechanism(s) remain elusive, the link between environmental enrichment and chromatin remodeling might be exploited to develop a 'behavioral therapy' for human neurodegenerative conditions in which the histone acetylation homeostasis is disturbed.

11. Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD, affecting ~ 1% of the population > 50 years of age. PD is characterized by relatively selective depletion of dopaminergic neurons in the substantia nigra and nucleus coeruleus. Though the majority of PD cases are sporadic in origin, mutations in several proteins, including α-synuclein, have been linked to PD. Kontopoulos and co-workers [108] demonstrate that wild-type α-synuclein binds histone H3 *in vivo* and induces histone H3 hypoacetylation when overexpressed in SH-SY5Y cells. Even though no direct binding of α-synuclein to histone acetyltransferases was found, wild-type as well as mutant α-synuclein proteins (A30P, A53T) inhibit histone acetylation mediated by CBP, p300 and P/CAF *in vitro*. Based on these findings, the authors suggest that α-synuclein decreases acetylation by histone 'masking' and hypothesise that in case α-synuclein promotes neurotoxicity by inhibiting histone acetylation, increasing histone acetylation should ameliorate α-synuclein toxicity. Indeed, both SB and vorinostat diminished apoptotic cell death in response to α-synuclein expression *in vitro* and rescued dopaminergic neuron degeneration in α-synuclein transgenic flies. Very similar to the perturbation in histone acetylation homeostasis observed in spinocerebellar ataxia 3, these properties appear to be not restricted to mutant proteins. Kontopoulos and co-workers [108] demonstrate that mutant α-synuclein displays increased nuclear targeting, which underlines their finding that mutant α-synuclein toxicity is at least in part mediated by histone hypoacetylation. Most recently, Outeiro and co-workers [109] identified SIRT2, a member of the sirtuin HDAC family (class III), as a potential target for PD treatment. The authors demonstrate that pharmacologic SIRT2 inhibition rescues neuronal α-synuclein (A53T) toxicity *in vitro* and *in vivo* by employing a *Drosophila* model of PD. Interestingly, pharmacologic SIRT2 inhibition affects α-synuclein aggregation. *In vitro* studies revealed that SIRT2 inhibition results in accumulation of larger α-synuclein aggregates which appear to be protective, whereas smaller aggregates correlate with toxicity. Even though the underlying mechanisms remain elusive, these data suggest that, in addition to classical HDACs, SIRT2 targeting may be therapeutically beneficial in PD and other diseases where aggregation of misfolded proteins is central to disease pathogenesis.

12. Stroke

Stroke, also referred to as cerebral ischemia, is the third leading cause of death in the western world and a major cause of disability [110]. Besides polyQ disorders such as HD, DRPLA and SBMA, pertubation in histone acetylation homeostasis is considered as a central event also in the pathogenesis of stroke [111]. Faraco and co-workers [111] show that histone H3 acetylation is severely decreased in ischemic brain tissue in mice following transient middle cerebral artery occlusion (MCAO). Interestingly, injection of vorinostat (25 and 50 mg/kg, i.p., immediately and 6 h after MCAO) significantly decreased the infarct volume and blocked ischemia-induced H3 hypoacetylation. By employing a rat MCAO model, Ren and co-workers [112] demonstrated that subcutaneous injection of VPA (300 mg/kg; every 12 h starting immediately after MCAO) decreased infarct volume and reduced ischemia-induced neuronal deficit scores. In both transient MCAO studies, HDAC inhibitor treatment resulted in a 30 – 40% reduction of infarct volumes which implies that HDAC inhibitors efficiently counteract postischemic brain damage. By using a permanent rat MCAO model in which the cerebral blood flow in the ischemic brain area is permanently reduced (pMCAO), Kim and

co-workers [113] confirmed a histone H3 hypoacetylation in the ischemic tissue as well as the neuroprotective effects of pharmacologic HDAC inhibition. Subcutaneous injection of VPA (300 mg/kg), SB (300 mg/kg) or TSA (0.5 mg/kg) reduced infarct volumes ranging from 34% (TSA) to 50% (SB) and improves neurologic performance in ischemic rats. Strikingly, delayed administration of VPA or SB (3 or 6 h after ischemic onset) still reduced infarct volumes and improved neurologic scores suggesting a comparatively long therapeutic window for potential stroke treatment. The pathophysiology leading to cell death during cerebral ischemic injury is complex and includes fundamental mechanisms such as excitotoxicity and ionic imbalance, oxidative stress, apoptotic-like cell death and inflammation [110]. Using the rodent MCAO models, HDAC inhibition suppressed ischemia-induced p53 expression as well as neuronal caspase-3 activation and superinduced the expression of HSP70 and Bcl-2 which both have shown to protect from ischemic neuronal death [111-113]. In addition, HDAC inhibitors exhibit anti-inflammatory effects which might contribute to the beneficial effects observed in the MCAO paradigms [113]. Even though the HDAC isoenzymes involved in HDAC-inhibitor-induced neuroprotection against cerebral infarction remain to be identified, the finding that the class I selective HDAC inhibitor VPA [14] mitigated ischemia-induced brain damage suggests that class I HDACs (1, 2, 3, 8) represent promising future targets for pharmacologic intervention.

13. Other possible indications

Besides the above-mentioned diseases, other indications such as multiple sclerosis, Friedreich's ataxia (FRDA) and the fragile-X mental retardation syndrome may also be considered. TSA has shown to reduce inflammation, demyelination, neuronal and axonal loss in experimental autoimmune encephalomyelitis which is regarded as a model for multiple sclerosis [114]. Most recently, HDAC inhibition has shown to reverse *FXN* gene silencing that is causative for autosomal recessive FRDA (MIM#229300). FRDA is due to a defect in transcription resulting from expansion of GAA triplet repeats in the first intron of the essential mitochondrial protein frataxin, which results in progressive spinocerebellar neurodegeneration. Herman and co-workers [115] demonstrated transcriptional FXN downregulation to be associated with histone H3 and H4 hypoacetylation of the genomic region surrounding the expanded GAA repeat. Interestingly, although all HDAC inhibitors tested so far (including the pan-HDAC inhibitors TSA and vorinostat) induced global histone H3/H4 hyperacetylation *in vitro*, only the novel experimental HDAC inhibitor BML-210 has shown to activate *FXN* gene expression, which might suggest that BML-210 inhibits a yet unidentified HDAC enzyme associated with the *FXN* gene [115]. The fragile-X mental retardation syndrome (MIM#300624) is caused by a CGG expansion in the *FMR1* gene promoter. This alteration triggers DNA methylation of the repeat plus local changes in histone modifications leading to the clinical features of fragile X syndrome, the most common form of inherited mental retardation. *In vitro* studies revealed that TSA, SB and PB are able to transcriptionally activate the *FMR1* gene [116]. With respect to the observation that HDAC inhibitors promote DNA demethylation of specific genes *in vitro* and *in vivo* it would be interesting to evaluate whether HDAC inhibitors affect *FMR1* promoter methylation.

14. Conclusions

Despite the heterogeneous nature of neurodegenerative conditions presented here, HDAC inhibitor treatment appears to target two major neuroprotective mechanisms, namely: i) the transcriptional activation of disease-modifying genes in disorders such as spinal muscular atrophy or adrenoleukodystrophy; and ii) the correction of perturbations in histone acetylation homeostasis which have shown to be intimately involved in the neurodegenerative pathologies of Huntington's, Parkinson's and Kennedy disease, ALS, RSTS as well as stroke.

15. Expert opinion

Numerous neurodegenerative disorders have been identified as potential targets for HDAC inhibitor treatment and the number of diseases is likely to increase rapidly. Even though the neuroprotective effects of HDAC inhibitors are highly promising in preclinical experiments, the proof of their clinical efficacy for the treatment of neurodegenerative disorders is pending. Ongoing clinical trials for the potential treatment of HD, ALS and SMA are focussed on PB and VPA which are generally well-tolerated drugs and likely to be suitable for long-term treatment but comparatively weak HDAC inhibitors. In animal trials, high drug concentrations have been applied, ranging from 250 to 530 mg/kg for VPA and 100 to 1200 mg/kg for SB and PB. Despite the clear neuroprotective properties of the fatty acid class of HDAC inhibitors, one might argue whether neuroprotective drug concentrations are achievable in the patient's CNS. Highly potent HDAC inhibitors which penetrate the blood–brain barrier have been developed for cancer treatment but only little information is available concerning their efficacy in long-term therapy regimens. Thus, the results of presently ongoing clinical trials are eagerly awaited and will provide a significant impetus for future studies. Although considerable progress has been made in elucidating the disease-specific roles of HDACs and the effects of HDAC inhibitors, these areas are still in early stages of discovery. Thus, prospective efforts will very likely be focussed on the further decoding of the HDAC selectivity of known inhibitors and the further development of selective HDAC inhibitors. It remains elusive, however, whether selective HDAC inhibitors show superior neuroprotective effects compared with

neuroprotective pan-HDAC inhibitors such as vorinostat. Recently, novel mercaptoacetamide-based HDAC inhibitors have shown to possess superiour neuroprotective properties compared with pan-HDAC inhibitors such as TSA and scriptaid [117]. Besides the well-recognized inhibitors of classical HDACs, the finding that the inhibition of sirtuin activity rescues neuronal cell death in models of Parkinson's disease [109] might further boost the drug screening efforts to develop new or optimized sirtuin inhibitors for the treatment of neurodegenerative and age-related disorders. A remarkable observation by Fischer and co-workers [105] is that *in vivo* histone acetylation levels can either be modulated by drug treatment or environmental factors, which allows the assumption that the further examination of gene–environment relationships might open new avenues to ameliorate the progression of some neurodegenerative disorders. In respect of the highly complex chromatin 'language' consisting of DNA methylation and manifold post-translational histone modifications, the field of chromatin regulation in neurodegenerative disorders is still poorly understood. Thus, the further deciphering of chromatin modifications which correlate with either positive or negative transcriptional states might reveal novel therapeutic options to counteract transcriptional dysregulation observed in neurodegenerative disorders.

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