MINIREVIEW

Is There a Future for Histone Deacetylase Inhibitors in the Pharmacotherapy of Psychiatric Disorders?

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ABSTRACT

In recent years, it has become widely recognized that a comprehensive understanding of chromatin biology is necessary to better appreciate its role in a wide range of diseases. The histone code has developed as a new layer of our appreciation of transcription factor-based mechanisms of gene expression. Although epigenetic regulation refers to a host of chromatin modifications that occur at the level of DNA, histones, and histone-associated proteins, how this regulation is orchestrated is still incompletely understood. Of those processes that comprise the epigenetic regulatory machinery, DNA methylation and histone acetylation/deacetylation have been the most thoroughly studied. Compounds that act as inhibitors of DNA methyltransferases or histone deacetylases (HDACs) activate a variety of intracellular signaling pathways that ultimately affect the coordinated expression of multiple genes. The altered patterns of mRNA and protein expression collectively converge on pathways linked to apoptosis and cell cycle arrest, among others. This has prompted a widespread search for epigenetic inhibitors that could be used as chemotherapeutic agents, and several are undergoing clinical evaluation. More recently, there has been interest in the use of HDAC inhibitors to activate the expression of mRNAs that are down-regulated in various neurological and psychiatric conditions. Considerably less is known regarding the effect these drugs have on postmitotic cells such as neurons. Before we consider the clinical use of additional HDAC inhibitors to treat schizophrenia or unipolar depression, there are a number of key issues that need to be resolved.

With the recent interest in epigenetic mechanisms regulating gene expression, there is a major effort to use drugs that affect these mechanisms in the treatment of various cancers (Acharya et al., 2005; Bolden et al., 2006; Kalin et al., 2009). A less compelling argument can be made for the use of epigenetic drugs to modify various regulatory cascades in postmitotic cells such as neurons. In this context, we are specifically referring to the use of drugs to correct epigenetic defects that affect brain function. There has been considerable recent interest in this therapeutic approach, because, in contrast to genetic alterations, changes in epigenetic marks are potentially reversible (Egger et al., 2004). Epigenetic mechanisms include a vast number of processes that affect chromatin structure and remodeling and that have both positive and negative transcriptional consequences (Li et al., 2007). In addition, increasing evidence suggests that adult neurons respond to various environmental signals via dynamic changes in DNA methylation and histone modifications. These processes are important to mechanisms of memory formation and cognition via modulation of genes involved in synaptic plasticity, such as brain-derived neurotrophic factor (BDNF) and reelin (Levenson and Sweatt, 2005; Szyf, 2009). Epigenetic abnormalities, possibly introduced during either embryogenesis, puberty, or adulthood, have also been noted in several psychiatric disorders, including schizophrenia, depression, and drug addiction (Tsankova et al., 2007).

ABBREVIATIONS: BDNF, brain-derived neurotrophic factor; SMA, spinal muscular atrophy; GAD67, glutamic acid decarboxylase 67; HAT, histone acetyltransferase; HDAC, histone deacetylase; MS-275, N-(2-aminophenyl)-4-[N-(pyridin-3-yl-methoxycarbonyl) aminomethyl]benzamide; M344, 4-(diethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl]benzamide; SIRT, siruin, silent mating-type information regulation 2 homolog; VPA, valproic acid; TSA, trichostatin A; CI-994, tacedinaline, 4-acetamido-N-(2-aminophenyl)benzamide; SAHA, N-hydroxy-N’-phenyl-octanediamide; FK228, romidepsin; PXD-101, belinostat; LBH589, panobostat; NVP-LAQ824, dacinostat.
In eukaryotic cells, the regulation of gene expression occurs on a complex and specialized structure called chromatin. The fundamental unit of chromatin is the nucleosome, which contains 147 base pairs of DNA wrapped in approximately 1.65 left-handed superhelical turns around an octamer of the core histones. The histone octamer is composed of two copies of each of the histones H2A, H2B, H3, and H4. Histone proteins are subject to a large number of post-translational modifications that include acetylation, methylation, ubiquitination, sumoylation, ADP ribosylation of lysine residues; methylation of arginine residues; and phosphorylation of serines and threonines. These modifications most likely act in a combinatorial or sequential fashion, defining the so-called “histone code.” The structural alterations to individual histones that result have been demonstrated to control the structure and function of chromatin and have a significant impact on the level of gene activity (Strahl and Allis 2000; Turner, 2002; Berger, 2007; Kouzarides, 2007; Li et al., 2007).

Histone acetylation and DNA methylation are two well-established epigenetic modifications that are targeted by currently available drugs known as histone deacetylase (HDAC) inhibitors and DNA methyltransferase inhibitors, respectively. The majority of clinical trials that have been conducted have been designed to evaluate the effects of these drugs on various types of cancers, and many have shown promising results, including favorable efficacy and safety profiles. It is noteworthy that the HDAC inhibitor N-hydroxy-N’-phenyl-octanediamide (SAHA) and two methyltransferase inhibitors, 5-azacytidine and decitabine (5-aza-2’-deoxycytidine) have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of refractory cutaneous T-cell lymphoma and myelodysplastic syndromes, respectively. However, the scope of epigenetic therapy is very likely to expand as more information becomes available regarding the role of epigenetic abnormalities and the etiology of a broad range of diseases, including diverse central nervous system disorders. Several pharmacological therapies using HDAC inhibitors have been beneficial in various experimental models of brain diseases. Evidence suggests that targeting HDACs and histone acetylation might prove advantageous for seizure disorders, amyotrophic lateral sclerosis, Alzheimer’s disease, Rubinstein-Taybi syndrome, spinal muscular atrophy, Rett syndrome, stroke, Fragile X syndrome, and Huntington’s disease, among others (Morrison et al., 2007; Abel and Zukin, 2008; Kazantsev and Thompson, 2008; Chuang et al., 2009). These drugs also hold promise for therapy relevant to several psychiatric disorders, including schizophrenia, depression, drug addiction, and anxiety disorders (Tsankova et al., 2007; Guidotti et al., 2009). For these reasons, HDACs represent attractive molecular targets for the treatment of several neurological and psychiatric diseases.

Regulation of Histone Acetylation

Acetylation is associated with gene activation and has been the most extensively studied histone modification. Acetylation of lysine residues imparts a negative charge to the affected amino acid, which tends to facilitate local chromatin relaxation. It can occur at various lysine residues on all four core histones, but the most often used acetylation sites include lysines 9, 14, 18, 23, and 56 of histone H3, as well as lysines 5, 8, 13, and 16 of histone H4. A high-resolution analysis of active yeast promoters showed that the levels of acetylated lysines in histones H3 and H4 are proportional to the transcription rate (Pokholok et al., 2005). Acetylation can promote transcription by at least two different mechanisms (Fig. 1): 1) by favoring a more open chromatin conformation that allows the transcriptional machinery to bind the DNA; and 2) by directly serving as recognition sites for factors that promote transcription (Shahbazian and Grunstein, 2007).

Histone acetylation levels are controlled by two antagonistic protein families: the histone acetyltransferases (HATs), and HDACs. A HAT catalyzes the transfer of an acetyl moiety from acetyl-coenzyme A to the ε-amino group of target lysine residues in histone proteins (Fig. 1). This reaction is reversed by HDACs via a catalytic site consisting of a narrow tubular pocket with hydrophobic walls and a Zn²⁺ cation positioned at the bottom. An acetylated lysine fits into this pocket, whereas the Zn²⁺ catalyzes hydrolysis of the acetyl group, thus regenerating the ε-amino group.

In general, the levels of gene expression can be modified by targeting either HAT or HDAC activities. However, although intensive effort has been focused on developing HDAC inhibitors, HAT inhibitors are less well known. There are numerous small-molecule HAT inhibitors that have been described, including several natural products such as garcinol and anacardic acid. Because these have been reviewed recently (Dekker and Haisma, 2009; Selvi and Kundu, 2009), we will focus further discussion on HDACs and the therapeutic potential of molecules acting as their inhibitors.

The Histone Deacetylase Family of Proteins

The superfamily of HDACs consists of at least 18 members that are divided into two main families (Table 1): 1) the classic HDAC family; and 2) the silent information regulator 2-related protein (sirtuin) family. The classic or zinc-dependent HDACs include class I (HDACs 1, 2, 3, and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), and class IV (HDAC11). These HDACs are structurally very similar and share a common zinc-dependent catalytic domain. Class I HDACs are primarily nuclear and are expressed ubiquitously. Class II HDAC enzymes generally shuttle between the nucleus and the cytoplasm and are expressed in a cell- and tissue-specific manner. HDAC11 is structurally different from class I and class II HDACs but has been shown to have some properties of both classes. The sirtuin family (class III; zinc-independent) contains seven members (SIRTs 1–7), which are structurally unrelated to classic HDACs. These enzymes have a unique mechanism of action requiring the cofactor NAD⁺ for the enzyme activity (de Ruijter et al., 2003; Mottet and Castronovo, 2008).

As expected, HDACs are generally associated with transcriptional repression. These enzymes are unable to bind to chromatin directly; their sequence-specificity is most likely determined by other proteins that associate with HDACs and target them to specific genomic regions. Accordingly, many transcriptional corepressor complexes, such as NuRD, SIN3A, and CoREST, have been shown to contain subunits with HDAC activity (Bhaumik et al., 2007; Kouzarides, 2007). It seems certain that deacetylation represses transcription by reversing the effects of acetylation (i.e., by restoring the positive charge on lysines and by reducing the affinity of acetyl-lysine-binding transcription factors and coactivators). However, recent evi-
ence suggests that the deacetylated state may also prevent transcription by promoting the binding of repressor proteins, such as those containing the SANT (Swi3-Ada2-NCoR-TFIIIB) domain that recognizes unmodified and underacetylated histone tails (Shahbazian and Grunstein, 2007).

In addition, although histone proteins were the first and the most important targets of HDACs to be discovered, an increasing body of evidence indicates that HDACs also deacetylate other proteins. Nonhistone HDAC targets have diverse biological functions and include α-tubulin, β-catenin, protein 53, E2F, 90-kDa heat shock protein, retinoblastoma, nuclear factor κ-light-chain-enhancer of activated B cells, signal transducers and activators of transcription proteins, various transcription factors, and probably many others (Buchwald et al., 2009). It seems that HDAC inhibitors influence a variety of processes, including cell cycle arrest, angiogenesis, immune modulation, and apoptosis by targeting nonhistone proteins (Bolden et al., 2006). Therefore, it is important to note that the effects of HDAC inhibitors are mediated by both transcription-dependent and -independent mechanisms (McLaughlin and La Thangue, 2004). The potential to disrupt multiple pathways using HDAC inhibitors adds an additional complication to rational drug design.

Inhibitors of HDACs

The majority of HDAC inhibitors that are currently either in clinical testing or that are on the market target multiple isoforms of the classic HDAC family (classes I, II, and IV) but do not inhibit SIRT family members. In recent years, there has been growing interest in drugs that activate (resveratrol) or inhibit (sirtinol) the NAD-dependent SIRT1 protein, and several have been identified (Kazantsev and Thompson, 2008). However, the activity of this subfamily of HDACs is beyond the scope of this minireview.

Currently available HDAC inhibitors can be divided into four classes based on their chemical structures. This classification includes hydroxamates, short-chain fatty acids, cyclic peptides, and benzamides (Table 2).

**Hydroxamates.** Important members of the hydroxamate class include trichostatin A (TSA), SAHA (vorinostat), cinnamic acid hydroxamates, PXD-101 (belinostat), and LBH589 (panobostat). TSA is a natural product that was originally isolated as an antifungal antibiotic and was one of the first and most potent HDAC inhibitors to be described. Despite its high toxicity and broad selectivity, it is still widely used as a reference compound in this research field. It has also been used as a core structure for the design of new HDAC inhibitors.

All hydroxamate-based inhibitors are considered pan-HDAC inhibitors, and generally, they show a high nanomolar potency. Their structure consists of three motifs (hydroxamic acid, a hydrophobic linker, and the bulky polar part of the molecule), each of which interacts with a distinct region of the HDAC catalytic pocket. It is assumed that all hydroxamate derivatives and probably some members of other HDAC inhibitor groups fit inside the catalytic pocket and bind zinc in a similar way, leading to the abolishment of HDAC activity (Villar-Garea and Esteller, 2004; Lin et al., 2006).

Among these compounds, SAHA is the most clinically advanced drug and the only HDAC inhibitor that has been FDA approved (in October 2006). This drug is also undergoing clinical trials in combination with other drugs for multiple myeloma and metastatic renal cell carcinoma. PXD101 (be-
linostat) is also currently being administered in clinical trials for cutaneous T-cell lymphoma, peripheral T-cell lymphoma, and several other tumor types (Stimson et al., 2009; Prince et al., 2009). LBH589 is a cinnamic acid hydroxamate identified by Novartis scientists who were conducting a screen of chemical compounds with the intent of finding novel and efficacious HDAC inhibitors. This compound is currently being evaluated and may prove efficacious in the treatment of advanced refractory solid tumors, hematological malignancies, and pancreatic cancers (Haefner et al., 2008). Other notable HDAC inhibitors of the hydroxamic acid class (Bolden et al., 2006) include scriptaid [6-(1,3-dioxo-1H-benzo[de]isoquinolin-2-yl)-hexanoic acid hydroxyamide] and oxamflatin [(2E)-5-(3-(phenylsulfonylamino)phenyl)pent-2-ene-4-ynohydroxamic acid].

**Short-Chain Fatty Acids.** These inhibitors include compounds with rather simple structures, such as valproic acid, phenyl butyrate, and butyrate. Short-chain fatty acid inhibitors all show similar profiles in terms of their action at class I and IIa HDACs with some differences detected in terms of individual potencies in vitro (Gurvich et al., 2004). They tend to be less potent in inhibiting HDAC activity than hydroxamic acids (millimolar compared with nanomolar range). Among these, sodium butyrate has diverse properties and has been shown to exert antidepressant properties in the mouse brain (Schroeder et al., 2007). Moreover, sodium butyrate has been shown to induce neurogenesis in the rat brain after cerebral ischemia (Kim et al., 2009). Valproic acid (VPI, 2-propyl pentanoic acid, divalproex) is an established product that is a potent HDAC inhibitor with a selectivity toward class I HDACs. It was originally isolated as a fermentation product that is a potent HDAC inhibitor with a selectivity toward class I HDACs. It was originally isolated as a fermentation product from *Chromobacterium violaceum* by the Fujisawa Pharmaceutical Company (Osaka, Japan). Because of its unique structure and ability to delay tumor growth in a National Institutes of Health National Cancer Institute screening of 59 human tumor cell lines, the Developmental Therapeutics Program selected it as a molecule of interest. Depsipeptide has undergone various clinical trials (phase I and II) for chronic lymphocytic leukemia and small cell lung cancers. It is a prodrug that is reduced to an active compound upon entering the cells through a mechanism that involves glutathione (Furumai et al., 2002). In addition to inhibiting class I HDACs, the compound activates a caspase 8-mediated apoptotic pathway (Aron et al., 2003).

**Benzamides.** Among the benzamide HDAC inhibitors, both MS-275 (also known as SNDX-275) and CI-994 are currently in clinical trials for the treatment of cancer. In some studies, MS-275 is coadministered with an epidermal growth factor receptor inhibitor, a methylation inhibitor (5-aza-2’-deoxycytidine), whereas in others, it is being used with aromatase inhibitors, growth factor inhibitors, or alone (relapsed or refractory Hodgkin’s lymphoma; see the Syndax clinical trials website available at http://www.syntaxscore.com/). MS-275 has garnered some attention recently because of its ability to inhibit HDACs in the brain (Simonini et al., 2006). Subcutaneous injection of MS-275 in mice was shown to increase the acetylation of histone H3 tails in the frontal cortex, striatum, and hippocampus. Moreover, chromatin immunoprecipitation shows that MS-275 increases Ac-H3-RELN and Ac-H3-GAD67 promoter association in the frontal cortex (Simonini et al., 2006). It is interesting that an examination of the levels of acetylated histone H3 associated with the GAD67 and reelin promoters showed that histone acetylation and DNA methylation are inversely correlated (Dong et al., 2007). These studies have been replicated recently in

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**TABLE 1**

<table>
<thead>
<tr>
<th>The superfamily of histone deacetylases</th>
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<tr>
<td><strong>I. The classic HDAC family</strong></td>
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<tr>
<td>Class I</td>
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<tr>
<td>HDAC1</td>
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<td>HDAC2</td>
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<td>HDAC3</td>
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<td>HDAC8</td>
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<tr>
<td>Class IIa</td>
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<td>HDAC9</td>
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<td>Class IIb</td>
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<td>Class IV</td>
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<td>HDAC11</td>
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<td><strong>II. The sirtuin family</strong></td>
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<td>Class III</td>
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<td>SIRT1</td>
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<td>SIRT7</td>
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TABLE 2
Representative inhibitors of classic HDAC family members

<table>
<thead>
<tr>
<th>Class &amp; Compound</th>
<th>HDAC Target</th>
<th>In Vitro Potency</th>
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<tr>
<td>Hydroxamate</td>
<td></td>
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</tr>
<tr>
<td>TSA</td>
<td>Classes I and II</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>SAHA (vorinostat)</td>
<td>Classes I and II</td>
<td>Micromolar</td>
</tr>
<tr>
<td>LAQ824</td>
<td>Classes I and II</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>PXD-101 (belinostat)</td>
<td>Classes I and II</td>
<td>Micromolar</td>
</tr>
<tr>
<td>Cyclic peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depsipeptide (FK-228; romidepsin)</td>
<td>Class I</td>
<td>Nanomolar</td>
</tr>
<tr>
<td>Apicidine</td>
<td>Classes I and II</td>
<td>Nanomolar to micromolar</td>
</tr>
<tr>
<td>Short-chain fatty acids</td>
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<td></td>
</tr>
<tr>
<td>Valproic acid</td>
<td>Class I</td>
<td>Millimolar</td>
</tr>
<tr>
<td>Phenyl butyrate</td>
<td>Classes I and II</td>
<td>Millimolar</td>
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<tr>
<td>Butyrate</td>
<td>Classes I and II</td>
<td>Millimolar</td>
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<tr>
<td>Benzamides</td>
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</tr>
<tr>
<td>MS-275</td>
<td>HDAC1, HDAC3</td>
<td>Micromolar</td>
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<tr>
<td>CI-994</td>
<td>HDAC1, HDAC3</td>
<td>Micromolar</td>
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neural progenitor cells in vitro and suggest that histone acetylation facilitates DNA demethylation (Kundakovic et al., 2009). This has led to speculation that the benzamid HDAC inhibitors may be useful in selectively activating promoters that may be insufficiently expressed in schizophrenia and other psychiatric conditions (Kundakovic et al., 2009). More recently, MS-275 was shown to alter mRNA expression profiles in the nucleus accumbens of treated mice similar to what has been observed with the antidepressant fluoxetine (Covington et al., 2009). Similar to the antidepressant properties of sodium butyrate (Schroeder et al., 2007), MS-275 also shows promise in the search for drugs that might be useful for treating depression. Although it may be premature to suggest that these drugs might be ready for psychiatric clinical trials, it is clearly a direction that warrants additional consideration.

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by the complete loss of survival motor neuron gene 1. The symptoms of SMA are caused by the degeneration of α motor neurons in the anterior horn of the spinal cord (Sumner, 2006). Recent studies have shown that survival motor neuron 2 mRNA is reduced due to differential promoter methylation (Hauke et al., 2009). Various studies have shown that disease and symptom severity correlate with the levels of survival motor neuron 2 protein, and this is also the case in various animal models in which the copy number of the gene is varied. The availability of a useful animal model of the disorder has spurred the in vivo testing of new drugs to treat the defect. Several conventional HDAC inhibitors are in various phases of testing for their usefulness in treating SMA (Sumner, 2006). The benzamide M344 seems to promote an up-regulation of SMN protein in fibroblasts from patients and is considered to be a promising new treatment for the disease (Riessland et al., 2006; Hauke et al., 2009). It seems that those HDAC inhibitors that facilitate DNA demethylation are probably more efficacious than those that do not (Hauke et al., 2009). MS-275 is also being used to battle various types of brain tumors (Eyüpoglu et al., 2006; Furchert et al., 2007). As the experimental neurobiology of HDAC inhibition expands, it may be possible to design compounds that will allow us to treat a variety of conditions in the brain, depending on the selectivity and potency of the particular inhibitor.

Is There a Case to Be Made for Treating Psychiatric Disorders with HDAC Inhibitors?

The ultimate usefulness of these drugs in the fight against various cancers still awaits, in many cases, the results of phase III trials and FDA approval. As pointed out recently (Bolden et al., 2006), although it seems clear that many HDAC inhibitors will be potent weapons in the battle against various cancers, the effects of these drugs are likely to be much broader and more complicated than originally envisioned. This is due in part to the observation that HDAC inhibitors have pleiotropic actions in different cell types. This is probably a consequence of the specificity of individual inhibitors for different HDACs and whether the targeted HDAC deacetylates histones and/or nonhistone substrates. The various cellular responses induced by HDAC inhibitors include cell cycle arrest, apoptosis, angiogenesis, and immune modulation (Bolden et al., 2006).

In disorders of the brain, in which the dysregulation of gene expression has been implicated in a wide variety of neurological and psychiatric diseases (Fig. 2), there is enormous potential to restore patterns of gene expression and neuronal function through the use of epigenetic drugs. VPA has a long and established history of efficacy in the treatment of seizures and bipolar disorder. In these conditions, often times it is effective as the primary medication. However, its use in the treatment of schizophrenia is less straightforward. Although there is no evidence to support a primary role in the treatment of psychosis, there is a sizeable body of clinical experience and published case reports in which the use of VPA, solely as an adjunctive medication and generally added to an ongoing primary regimen of antipsychotics, has been widespread with reported efficacy in seriously ill patients such as those in public hospitals in the United States (Wassef et al., 2000, 2001; Citrome, 2003). This initial line of evidence led to the design of randomized controlled trials with the objective of establishing efficacy in a large sample study of responsive schizophrenia subjects who were receiving active treatment with atypical antipsychotics (Casey et al., 2003, 2009; Citrome, 2009). These studies did not establish an augmentation effect of VPA above and beyond that observed with the primary antipsychotic. In summary, it is realistic to state that VPA is not of great benefit in randomly identified and unselected subjects with schizophrenia already receiving effective doses of antipsychotics. A more pertinent and unanswered question is whether an HDAC inhibitor might be effective in patients selected for treatment resistance or, as discussed below, based on data showing that certain patients have an excess of restrictive chromatin. Thus far, epigenetic parameters from individual patients have not been used to provide information for any of these earlier efforts.

In an effort to link HDAC inhibitor efficacy to epigenetic parameters, we have demonstrated that lymphocyte nuclear extracts prepared from patients with schizophrenia have an abundance of restrictive chromatin at baseline and that this

![Fig. 2. Are epipharmaecuticals ready for prime time? Current research suggests that HDAC inhibitors could benefit a wide assortment of neurological and psychiatric disorders (Bolden et al., 2006; Tsankova et al., 2007; Kazantsev and Thompson, 2008; Chuang et al., 2009; Thomas, 2009). Are HDAC inhibitors the new frontier in our search for drugs that affect gene expression? These compounds clearly have enormous potential for altering gene expression profiles in neurons and other cell types. HDAC inhibitors and drugs that affect DNA methyltransferases and additional histone modifications may together provide a level of specificity not attainable by more traditional pharmacotherapies. ALS, amyotrophic lateral sclerosis.](https://www.aspetjournals.org/content/molpharm/131/3/131.full.pdf)
chromatin is resistant to HDAC inhibitor treatment in vivo and in vitro (Sharma et al., 2006; Gavin et al., 2008, 2009a,b). If HDAC inhibitor treatment is to be effective in chronic degenerative conditions such as schizophrenia, then the objective must be 2-fold: first, to identify a priori and enrich the treatment sample with that subgroup of patients who are characterized by a pathological lesion (i.e., an excess of deacetylated histones); and second, to treat this patient sample with an HDAC inhibitor with sufficient specificity, potency, and a benign side effect profile. Conceptually, using an HDAC inhibitor to transition promoters embedded in restrictive chromatin to a more relaxed state may facilitate a response to the fraction of psychotrophic medications acting on signaling pathways via surface receptors (Sharma, 2005). Unfortunately, current clinical research in noncancer therapeutics is limited to VPA given the safety profile of various other medications targeting the epigenetic platform.

During the last several years, there has been increased interest in the epigenetic origins of psychiatric diseases (Tsankova et al., 2007; Deutsch et al., 2008; Oh and Petronis, 2008; Costa et al., 2009; Grayson et al., 2009; Roth et al., 2009). It was noted earlier that sodium butyrate shows antidepressant properties either alone or when coadministered with fluoxetine (Schoeder et al., 2007). The tricyclic antidepressant imipramine was shown to reverse the down-regulation of BDNF transcripts III and IV in the hippocampus of animals subjected to long-term social defeat stress (Tsankova et al., 2006). This is interesting because imipramine induced histone acetylation through the inhibition of HDAC5. Likewise, MS-275, when injected into the nucleus accumbens, reverses the effects of long-term stress and reverses the effects of long-term stress on various stress-regulated genes (Covington et al., 2009). VPA has also been shown to activate BDNF promoter IV in cultures of rat cortical neurons ( Yasuda et al., 2009). At the same time, the initial observations that VPA would prove useful in augmenting the efficacy of neuroleptics in the treatment of schizophrenia have not proven true. However, this does not preclude the possibility that more selective and more potent HDAC inhibitors might prove useful in the therapeutic arsenal of drugs that could be used in the treatment of psychosis associated with schizophrenia or might also prove to be beneficial in treating the mania associated with bipolar disorder. Before these drugs can be allowed in the clinics, several important areas need to be addressed experimentally. These include issues related to selectivity and drug permeability, identification of biomarkers, mono- or combined therapy, and rational patient choice.

Selectivity and Brain Permeability of HDAC Inhibitors. As indicated previously, the majority of the currently available HDAC inhibitors block all classic HDACs, and the recent focus in HDAC inhibitor development has been on improvement in specificity to overcome the nonspecific cytotoxicity of these drugs (Johnstone, 2002; Balasubramanian et al., 2009). This is not an easy task because the structure of the HDAC active site is well conserved. However, there are compounds known to be class- or isoform-specific (Table 2).

In the case of treating brain disorders, an additional challenge is the permeability of the inhibitors across the blood-brain barrier (Kazantsev and Thompson, 2008). So far, several drugs, including valproic acid, vorinostat (SAHA), MS-275, sodium butyrate, and phenyl butyrate have been shown to cross the blood-brain barrier. In addition, MS-275 has been demonstrated to be brain region-selective and is 30 to 100 times more potent than VPA in increasing histone acetylation in vivo (Simonini et al., 2006). This drug also shows selectivity for class I HDACs, with the highest affinity toward HDAC1. Therefore, MS-275 might be considered a second-generation HDAC inhibitor with improved specificity, which holds promise not only for cancer treatment (Hess-Stumpp et al., 2007) but also for various psychiatric disorders (Kazantsev and Thompson, 2008).

Until the appropriate mRNA profiling experiments are carried out in various brain regions (cortex versus striatum, versus hippocampus, versus cerebellum, etc.) in response to systemic brain administration and these data are compared with similar profiles obtained from livers, kidneys, and pancreata of these same treated animals, it may be difficult to assess the full therapeutic potential of any candidate HDAC inhibitor. Likewise, it would be necessary to obtain this information as a function of dose, time, and route of administration. Some data are available regarding the peripheral administration of HDAC inhibitors and regional brain acetylated histone content (Simonini et al., 2006; Costa et al., 2009). Although clearly defined genes of interest, including GAD67, reelin, and brain-derived neurotrophic factor (Tsankova et al., 2007) have been identified, it would be important to assess mRNA profiles in peripheral tissues as well. Because of the parallels between DNA methylation and histone acetylation, the methylation status of many of these promoters also needs to be assessed before and after drug treatment. Although the mechanism linking these two epigenetic processes is still not clear, there is increasing evidence to support the concept that agents mediating the opening of chromatin in the vicinity of promoters, such as HDAC inhibitors, also facilitate DNA demethylation (Szyf, 2009).

Identification of Biomarkers. A key question is whether biomarkers can be identified that may represent positive outcome predictors. Is it possible to identify mRNAs and/or proteins that increase in response to a particular HDAC inhibitor and that also might be used as indicators of drug efficacy? This is an ongoing discussion in the field of cancer treatment, and the answer may help to provide insight into patient stratification and possible effectiveness of response (Stimson and La Thangue, 2009). That is, if suitable biomarkers can be identified, it might permit the classification of patients into groups based on those who will probably respond to a particular pharmacotherapy and those who might not. Because of the enormous numbers of cancers that have been diagnosed and because each of these undergoes a series of biological stages, this becomes a huge task. Nevertheless, it is remarkable that genome-wide mRNA profiling studies indicate that the percentage of genes that are induced after HDAC inhibitor treatment is somewhere in the range of 2 to 5% (Stimson and La Thangue, 2009). Although there is still a long way to go before we have predictive biomarkers in hand, this is certainly attainable in the very near future. As mentioned above, measurements of SMN protein in fibroblasts from patients with SMA is being carried out to test the efficacy of M344 in the treatment of this disorder (Riessland et al., 2006; Hauke et al., 2009).

A different set of problems surfaces when it comes to the identification of biomarkers to indicate therapeutic effectiveness of HDACs in the treatment of psychiatric disorders.
Despite the vast amount of money and research effort put into studying the genetics of these diseases, there are still no reliable tests for schizophrenia, bipolar disorder, or unipolar depression. The search for measurable endophenotypes associated with schizophrenia, for example, has been exhaustive, and progress has been made toward identifying measures of impaired inhibitory neurotransmission (Turetsky et al., 2007). Endophenotypes in psychiatry are measurable and include indices of auditory processing and/or measures of cognitive deficits. Endophenotypes reflect the action of sets of predisposing genes that might also be used in measuring the therapeutic efficacy of various drugs (Gottesman and Gould, 2003). Neurophysiological endophenotypes, which include prepulse inhibition of startle, P50 auditory-evoked potential suppression, and antisaccade eye movements, are not definitively diagnostic and are evident in nonaffectted family members. Recent data from twin studies have shown that the decreased amplitude of the evoked potential component (P300) might represent an endophenotype associated with schizophrenia (Bestelmeyer et al., 2009). However, the P300 does not reliably distinguish between patients with schizophrenia and those with bipolar disorder and might be a better marker for psychosis. There is evidence that these indices trend toward normalization with antipsychotic medications; however, a reliable biomarker would provide supportive data for pharmacological studies. Having stated this, it should be noted that many factors contribute to these complex measures, and the use of endophenotypes will not be considered reliable until we better appreciate the underlying genes that contribute to these macromolecular processes.

In the context of identifying therapeutic biomarkers, the only readily accessible material from psychiatric subjects is blood. The arguments against using biomarkers in lymphocytes to monitor changes that are presumably occurring in the brain are clear. However, it may be possible to measure various responses to HDAC inhibitors using lymphocytes both before and during treatment as an indirect measure of therapeutic efficacy. Preliminary studies have shown that when VPA is administered over a 4-week period either with or without an antipsychotic, the acetylated histone H3 content increased significantly (Sharma et al., 2006). The increase was more pronounced in patients with bipolar disorder who also showed higher baseline levels. In vitro studies with lymphocytes from patients with schizophrenia showed that baseline levels of acetylated H3 (Lys9, Lys14) were reduced relative to the levels found in nonpsychiatric subjects (Gavin et al., 2008). Moreover, the lymphocytes from patients with schizophrenia exhibited a blunted or reduced response to TSA treatment in vitro compared with controls. More recently, baseline levels of dimethyl histone H3 (Lys9Me2) were shown to be increased in lymphocyte cultures from schizophrenia patients (Gavin et al., 2009b). The decreased acetylated histone H3 and increased dimethyl histone H3 content suggest that lymphocyte chromatin is more restricted in patients with schizophrenia than in controls. Although larger sample sizes are needed to verify and extend these findings, the results suggest a possible means of monitoring HDAC inhibitor treatment in patients. It remains plausible that the measurement of restrictive chromatin marks and the monitoring of a neurophysiological endophenotype could be a more stringent guide to the therapeutic efficacy of new HDAC inhibitors.

Monotherapy or Combined Therapy. As discussed above, it is evident that the use of VPA together with an antipsychotic does not provide a therapeutic advantage over the antipsychotic alone (Casey et al., 2009; Citrome, 2009). Based on data obtained thus far, it is unlikely that the use of any single HDAC inhibitor alone would be sufficient to treat the observed spectrum of schizophrenia symptoms that is typical of most patients. Instead, it is hoped that better and more selective drugs may be beneficial in treating psychosis-related symptoms and that patient management may also require the use of antipsychotic medications. Similar to what has been suggested for the treatment of various cancers (Bots and Johnstone, 2009), the true potential for the use of HDAC inhibitors in treating psychiatric diseases will probably reside in their combinatorial efficacy when coadministered with other drugs.

Rational Patient Choice. Neuropsychiatric disorders manifest as a composite of symptoms, each very likely emerging from independent neuronal systems/networks. As a consequence, the type of histone modifications, the intensity of the underlying DNA methylation, and the resistance to modifications may depend on the chronicity or the recalcitrant nature of the targeted clinical symptoms and would require independent considerations when evaluating a novel therapy. Therefore, future HDAC inhibitor interventions could include knowledge of the regional brain distribution of individual HDAC enzymes, similar to what has been done in the rat brain (Broide et al., 2007), and the possibility of using inhibitors targeted to a given HDAC enzyme. The required clinical trial would necessitate identifying treatment-resistant patients whose partially effective primary therapy has been optimized and then supplementing this regimen with an HDAC inhibitor to elicit or coax the required gene expression associated with clinical response. In other words, treatment-resistant patients might benefit from a strategy designed first to relax regional chromatin which would then be coadministered with the optimal antipsychotic medication. Perhaps with additional information based in part on chromatin plasticity assays as determined via lymphocyte H3 acetylation measurements, it may be possible to select an appropriate inhibitor to use as a starting point.

Conclusions

With the increased interest in the epigenetic origins of psychiatric disorders (Costa et al., 2007, 2009; Grayson et al., 2009), there has been a corresponding increase in the number of studies examining HDAC inhibitors and gene expression in the central nervous system (Tsankova et al., 2007; Abel and Zukin, 2008; Kazantsev and Thompson, 2008). Although initial results examining VPA administered either alone or with an antipsychotic have been disappointing, attention has now turned to more potent and selective compounds that may prove to be more effective. There is increasing evidence that chronic depression and schizophrenia may be associated with neuroinflammation, and several studies have shown the robust anti-inflammatory properties of VPA and other type I and II HDAC inhibitors (Peng et al., 2005; Chen et al., 2007; Kim et al., 2007; Sinn et al., 2007). However, additional information is needed before any of these compounds are ready for clinic. Relevant background information needs to include brain region-specific mRNA and protein profiling in
rodents and primates as a function of both dose and time. Because the target tissue/cell population is not readily accessible, it would be preferable to also identify appropriate biomarkers in lymphocytes that could be used to assess the effectiveness of each compound in terms of HDAC inhibition. Another important aspect of the design of any new HDAC inhibitor includes appropriate patient stratification, which would ideally be based on a measurable parameter designed to predict efficacy. For example, chromatin plasticity in vitro in response to the drug of interest could help to establish the extent to which a patient might benefit from an HDAC inhibitor (along with an antipsychotic). Whether this experimental measure actually represents a beneficial therapeutic endpoint is secondary because it primarily serves the purpose of identifying suitable candidate patients. Whether HDAC inhibitors and/or other epigenetic drugs may be of use in treating psychiatric disorders still awaits a great deal of biological testing. Initial results suggest that these drugs have great potential to activate the expression of selected target genes that are also down-regulated in these disorders. However, not enough is known regarding regional expression profiles and the effects of long-term activation of these mRNAs and corresponding proteins and how these changes will affect or improve specific symptoms. One concern that also needs addressing is the potential cellular toxicity that is common among the broad spectrum inhibitors, such as TSA. Apoptotic cell death as a consequence of high doses of HDAC inhibitors is evident in vivo and in vitro and increases with time of exposure (Bolden et al., 2006). Although this is clearly a desired outcome when these drugs are used to combat various cancers, the nature of psychiatric disorders would most likely necessitate long-term or even lifetime use. At present, longitudinal studies of the effects of HDAC inhibitors are probably underway, but results from these studies are not yet available.

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References


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