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**Is there a future for histone deacetylase (HDAC) inhibitors in the
pharmacotherapy of psychiatric disorders?**

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Abbreviations: AZA, 5-aza-2'-deoxycytidine; BDNF, brain derived neurotrophic factor; DNMT, DNA methyltransferase; GABA, gamma-aminobutyric acid; 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, Sirtuin (silent mating type information regulation 2 homolog); VPA, valproic acid; TSA, trichostatin A.

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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 upon our appreciation of transcription factor-based mechanisms of gene expression. While 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 (DNMTs) or histone deacetylases (HDACs) activate a variety of intracellular signaling pathways that ultimately impact 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, amongst 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 post-mitotic 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.

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Introduction

With the recent interest in epigenetic mechanisms regulating gene expression, there is a major effort to utilize drugs that impact 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 post-mitotic cells such as neurons. In this context, we are specifically referring to the use of drugs to correct epigenetic defects that impact brain function. Recently, this therapeutic approach has gained considerable interest as, 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 impact 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 et al., 2009). Epigenetic abnormalities, possibly introduced either during embryogenesis, puberty or adulthood, have also been noted in several psychiatric disorders, including schizophrenia, depression and drug addiction (Tsankova et al., 2007).

In eukaryotic cells, regulation of gene expression occurs on a complex and specialized structure called chromatin. The fundamental unit of chromatin is the nucleosome, which contains 147 bp of DNA wrapped in about 1.65 left-handed superhelical turns around an octamer of the core histones. The histone octamer is composed of two copies each of the histones H2A, H2B, H3 and H4. Histone proteins are subject to a large number of posttranslational modifications that

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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 et al., 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 (DNMT) 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. Importantly, the HDAC inhibitor SAHA (*N*-hydroxy-*N'*-phenyl-octanediamide) and two methylation inhibitors; 5-azacytidine and decitabine (5-aza-2'-deoxycytidine, AZA) have been approved by the FDA (the U.S. Food and Drug Administration) 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

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syndrome, and Huntington's disease, among others (Morrison et al, 2007; Kazantsev and Thompson, 2008; Abel and Zukin, 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 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 histone deacetylases (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 Zn^{2+} cation positioned at the bottom. An

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acetylated lysine fits into this pocket, whereas the Zn^{2+} 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, while 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. Since these have been recently reviewed (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 classical HDAC family; and 2) the silent information regulator 2 (Sir2)-related protein (sirtuin) family. The classical or Zn-dependent HDACs include: class I (HDAC1, 2, 3, and 8), class IIa (HDAC 4, 5, 7, and 9), class IIb (HDAC6 and 10), and class IV (HDAC11). These HDACs are structurally very similar and share a common Zn-dependent catalytic domain. Class I HDACs are primarily nuclear and are ubiquitously expressed. 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; Zn-independent) contains seven members (SIRT 1-7), which are structurally unrelated to classical HDACs. These enzymes have a unique mechanism of action requiring the co-factor NAD^+ for the enzyme activity (de Ruijter et al., 2003; Mottet and Castronovo, 2008).

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As expected, HDACs are generally associated with transcriptional repression. These enzymes are not able 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 co-repressor complexes, such as NuRD, SIN3A, and CoREST, have been shown to contain subunits with HDAC activity (Bhaumik et al., 2007; Kouzarides, 2007). It appears 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 co-activators. However, recent evidence 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-TFIIB) domain that recognizes unmodified and under acetylated histone tails (Shahbazian and Grunstein, 2007).

In addition, while 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. Non-histone HDAC targets have diverse biological functions, and include α -tubulin, β -catenin, p53 (protein 53), E2F, Hsp90 (heat shock protein 90), Rb (retinoblastoma), NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT (Signal transducers and activators of transcription) proteins, various transcription factors, and likely many others (Buchwald et al., 2009). It appears that HDAC inhibitors influence a variety of processes including cell cycle arrest, angiogenesis, immune modulation, and apoptosis by targeting non-histone proteins (reviewed in Bolden et al., 2006). Therefore, it is important to note that the effects of HDAC inhibitors are mediated by both transcription-dependent as well as transcription independent mechanisms (McLaughlin and La Thangue, 2004). The potential to

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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 classical 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 II).

Hydroxamates

Important members of the hydroxamate class include trichostatin A (TSA), suberoyl anilide bishydroxamide (SAHA, Vorinostat), cinnamic acid hydroxamates, PXD-101 (belinostat), and LBH589 (panobinostat). 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. In spite of 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 3 motifs (hydroxamic acid, a hydrophobic linker, and the bulky polar part of the molecule), each of which interacts with a

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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 the 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 (October of 2006). This drug is also undergoing clinical trials in combination with other drugs for multiple myeloma and metastatic renal cell carcinoma. Belinostat (PXD101) is also currently being administered in clinical trials for cutaneous T-cell lymphoma, peripheral T-cell lymphoma and also several other tumor types (Stimson et al., 2009; Prince et al., 2009). NVP-LAQ824 (Dacinostat) and NVP-LBH589 (Panobinostat) are cinnamic acid hydroxamates that were identified by Novartis scientists who were conducting a screen of chemical compounds with the intent of finding novel and efficacious HDAC inhibitors. These compounds are currently being evaluated and may prove efficacious in the treatment of advanced refractory solid tumors, hematologic 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, 3H-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 (milimolar as compared to nanomolar range).

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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 following cerebral ischemia (Kim et al., 2009). Valproic acid (VPA, 2-propylpentanoic acid, Divalproex) is an established central nervous system drug that has been used as an anticonvulsant, mood stabilizer, and adjuvant treatment for schizophrenia. While the fairly high therapeutic concentrations used preclude a single mode of action, VPA inhibits HDAC activity at therapeutic plasma concentrations (Phiel et al., 2001; Gottlicher et al., 2001). Both VPA and sodium butyrate show anti-inflammatory properties and are neuroprotective in rat ischemia models suggesting that they would be useful in suppressing ischemia-mediated cerebral inflammation (Kim et al., 2007; 2009). The action of VPA is confounded by numerous reports that it also activates genes in the extracellular-regulated kinase-AP-1 pathway, activates the peroxisome proliferator receptors alpha and delta, down regulates protein kinase C activity, and also negatively regulates Wnt signaling (see Blaheta and Cinatl, 2002 for review). Moreover, the drug is a potent teratogen and its use is often discontinued during pregnancies.

The beneficial action of VPA in the treatment of seizure disorders is thought to occur because of increased levels of γ -aminobutyric acid (GABA), presumably produced by increased levels of the corresponding biosynthetic enzymes, such as GAD67 (Phiel et al., 2001). This action may also be related to its efficacy in the treatment of bipolar disorder although this is less well understood. Randomized controlled trials have shown that VPA is effective in treating acute mania and as a maintenance therapy for bipolar disorder (Haddad et al., 2009). Interestingly, it was found that at therapeutic plasma levels, greater than 90% of VPA is bound to circulating plasma proteins and that the availability of free VPA increases with increasing dose (Bowden et

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al., 1996). This may explain why effective concentrations of the drug are generally quite high (50 to 100 µg/ml). The use of VPA in the context of psychiatry will be discussed in more detail (below).

Cyclic peptides. Apicidine and depsipeptide are representative examples of this class of HDAC inhibitor. Depsipeptide (FK228; romidepsin) is a bicyclic natural product, which 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. Because of its unique structure and ability to delay tumor growth in a National Cancer Institute screen 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. Amongst the benzamide HDAC inhibitors, both MS-275 (*N*-(2-aminophenyl)-4-[*N*-(pyridin-3-yl-methoxycarbonyl) aminomethyl]benzamide, SNDX-275®) and CI-994 (tacedinaline; 4-acetamido-*N*-(2-aminophenyl)benzamide) are currently in clinical trials for cancer treatment. In some studies, MS-275 is co-administered with an epidermal growth factor receptor inhibitor, a methylation inhibitor (AZA), while in others it is being used with aromatase inhibitors, growth factor inhibitors, or alone (relapsed or refractory Hodgkins lymphoma; see the Syndax clinical trials web site)). 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 acetylation of histone H3 tails in the frontal cortex, striatum, and

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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). Interestingly, 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 recently been replicated in neural progenitor cells in vitro and suggest that histone acetylation facilitates DNA demethylation (Kundakovic et al., 2009). This has led to speculation that the benzamide 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 III, 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. While 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 alpha motor neurons in the anterior horn of the spinal cord (Sumner, 2006). Recent studies have shown that survival motor neuron 2 (SMN2) 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 SMN2 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

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defect. Several conventional HDAC inhibitors are in various phases of testing for their utility in treating SMA (Sumner, 2006). The benzamide M344 (4-(diethylamino)-N-[7-(hydroxyamino)-7-oxoheptyl]benzamide) appears to promote an upregulation 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 appears that those HDAC inhibitors that facilitate DNA demethylation are likely more efficacious than those that do not (Hauke et al., 2009). MS-275 is also being used to battle various types of brain tumors (Furchert et al., 2007; Eyupoglu et al., 2006). 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 made for treating psychiatric disorders with HDAC inhibitors?

The ultimate utility of these drugs in the fight against various cancers still awaits, in many cases, the results of phase III trials and FDA approval. As recently pointed out (Bolden et al., 2006), while 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 likely a consequence of 1) the specificity of individual inhibitors for different HDACs, and 2) whether the targeted HDAC deacetylates histones and/or non-histone substrates. The various cellular responses induced by HDAC inhibitors include cell cycle arrest, apoptosis, angiogenesis, and immune modulation (see Bolden et al., 2006 for review).

In disorders of the brain, where the dysregulation of gene expression has been implicated in a wide variety of neurological and psychiatric diseases (Fig. 2), there is enormous potential to

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restore patterns of gene expression and restore 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, where 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 US (Wassef et al., 2000, 2001; Citrome et al., 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; Casey et al., 2009; reviewed in 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 say VPA is not of great benefit in randomly identified and unselected schizophrenia subjects already on 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 schizophrenia patients have an abundance of restrictive chromatin at baseline and that this chromatin is resistant to HDAC inhibitor treatment in-vivo and in-vitro (Sharma et al 2006, Gavin et al. 2008, 2009a, 2009b). If

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HDAC inhibitor treatment is to be effective in chronic degenerative conditions such as schizophrenia, then the objective must be two fold; 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 secondly, 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 those fraction of psychotropic medications acting on signaling pathways via surface receptors (Sharma 2005). Unfortunately, current clinical research in non-cancer 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; Grayson et al., 2009; Costa et al., 2009; Roth et al., 2009). It was noted earlier that sodium butyrate shows antidepressant properties either alone or when co-administered with fluoxetine (Schroeder et al., 2007). The tricyclic antidepressant imipramine was shown to reverse the downregulation of BDNF transcripts III and IV in the hippocampus of animals subjected to chronic social defeat stress (Tsankova et al., 2006). This is interesting because imipramine induced histone acetylation through inhibition of HDAC5. Similarly MS-275 when injected into the nucleus accumbens reverses the effects of chronic stress and reverses the effects of chronic 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

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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: 1) issues related to selectivity and drug permeability, 2) identification of biomarkers, 3) mono or combined therapy, and 4) rational patient choice.

1. Selectivity and brain permeability of HDAC inhibitors. As previously indicated, the majority of the currently available HDAC inhibitors block all classical HDACs and the recent focus in HDAC inhibitor development has been on improvement in specificity in order to overcome the nonspecific cytotoxicity of these drugs (see Johnstone, 2002 and Balasubramanian et al., 2009 for recent reviews). This is not an easy task as the structure of the HDAC active site is well conserved. However, there are compounds known to be class- or isoform-specific (Table II).

In the case of treating brain disorders, an additional challenge is the permeability of the inhibitors across the blood-brain barrier (see 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).

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Until the appropriate mRNA profiling experiments are carried out in various brain regions (cortex vs. striatum vs. hippocampus, vs. cerebellum, etc.) in response to systemic drug 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. Similarly, 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). While clearly defined genes of interest, including GAD67, reelin, and brain derived neurotrophic factor (Tsankova et al., 2007) have been identified, it would also 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 will also need to be assessed before and after drug treatment. While the mechanism linking these two epigenetic processes is still not clear, there is increasing evidence to support the concept that agents that mediate the opening of chromatin in the vicinity of promoters, such as HDAC inhibitors, also facilitate DNA demethylation (See Szyf, 2009 for review).

2) 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 cancer treatment field 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 that will likely respond to a particular

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pharmacotherapy and those that might not. Because of the enormous numbers of cancers that have been diagnosed and because each of these undergo 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 following HDAC inhibitor treatment is somewhere in the range of 2-5% (Stimson and La Thangue, 2009). While 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 surface when it comes to the identification of biomarkers to indicate therapeutic effectiveness of HDACs in the treatment of psychiatric disorders. In spite of 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 towards identifying measures of impaired inhibitory neurotransmission (Turetsky et al., 2007). Endophenotypes in psychiatry are measureable 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 non-affected family members. Recent data from twin studies has shown that the decreased amplitude of the evoked potential component (P300) might represent an endophenotype

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associated with schizophrenia (Bestelmayer et al., 2009). However, the P300 does not reliably distinguish between schizophrenia and bipolar disorder patients and might be a better marker for psychosis. There is evidence that these indices trend towards 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 four 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 schizophrenia patients showed that baseline levels of acetylated H3 (K9,K14) were reduced relative to the levels in non-psychiatric subjects (Gavin et al., 2008). Moreover, the lymphocytes from schizophrenia patients exhibited a blunted or reduced response to TSA treatment in vitro compared to controls. More recently, baseline levels of dimethyl H3 (K9me2) were shown to be increased in lymphocyte cultures from schizophrenia patients (Gavin et al., 2009b). The decreased acetylated H3 and increased dimethyl H3 content suggest that lymphocyte chromatin is more restricted in patients with schizophrenia than in controls. While larger sample sizes are needed to verify and extend these findings, the results

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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.

3) Mono 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; reviewed in Citrome, 2009). Based on data obtained thus far, it is unlikely that the use of any HDAC inhibitor alone may not 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 likely reside in their combinatorial efficacy when co-administered with other drugs.

4) Rational patient choice. Neuropsychiatric disorders manifest as a composite of symptoms, each very likely emerging from independent neuronal systems/networks. Consequently, 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 utilizing inhibitors targeted to a given HDAC enzyme. The required clinical trial would necessitate identifying treatment-

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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 co-administered 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 (reviewed in Tsankova et al., 2007; Abel and Zukin, 2008; Kazantsev and Thompson, 2008). While 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 efficacious. 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 will be needed before any of these compounds are ready for clinic. Relevant background information will need 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.

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Another important aspect of the design of any new HDAC inhibitor includes appropriate patient stratification, which would ideally be based on a measureable 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 as 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 impact or improve specific symptoms. One concern that also needs addressing is the potential cellular toxicity that is common amongst 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). While 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 likely underway, but results from these studies are not yet available.

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Legends for Figures.

Fig. 1. Acetylation of histone lysine residue and its consequences. Lysine acetylation is catalyzed by histone acetyltransferases (HATs) and reversed by the action of histone deacetylases (HDACs). Acetylation can promote transcription by two main mechanisms: 1) by causing direct structural changes to chromatin: acetyl groups neutralize the positive charge of the target lysine in the histone tails, which disrupts inter- and intranucleosomal interactions between the histones and the negatively charged phosphate groups in the DNA. This results in a more relaxed chromatin configuration that allows the transcriptional machinery to bind to the DNA. Acetylation of some residues, such as lysine 16 at histone H4, could also directly impact higher-order chromatin structure leading to chromatin relaxation; and 2) acetyl-lysines serve as recognition sites for transcriptional activators that contain protein motifs known as bromodomains, thus indirectly facilitating transcriptional initiation. Bromodomain-containing proteins include some transcription factors and components of chromatin remodeling complexes. Interestingly, certain proteins that possess HAT activity, such as PCAF and TAFII250, contain bromodomains themselves.

Fig. 2. Are epipharmaeaceuticals ready for prime time? Current research suggests that HDAC inhibitors could benefit a wide assortment of neurological and psychiatric disorders (for recent reviews see 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 impact gene expression? Clearly, these compounds have enormous potential for altering gene expression profiles in neurons and other cell types. HDAC inhibitors and drugs that impact DNA methyltransferases and additional histone modifications may together provide a level of specificity not attainable by more traditional pharmacotherapies.

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Table I. The Superfamily of Histone Deacetylases

I. The classical HDAC family		II. The sirtuin family	
Class I	HDAC1	Class III	SIRT1
	HDAC2		SIRT2
	HDAC3		SIRT3
	HDAC8		SIRT4
Class IIa	HDAC4		SIRT5
	HDAC5		SIRT6
	HDAC7		SIRT7
	HDAC9		
Class IIb	HDAC6		
	HDAC10		
Class IV	HDAC11		

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Table II. Representative Inhibitors of Classical HDAC Family Members

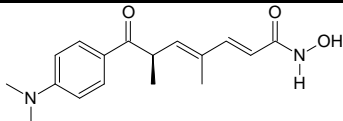
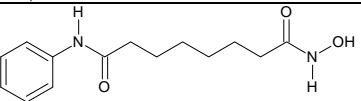
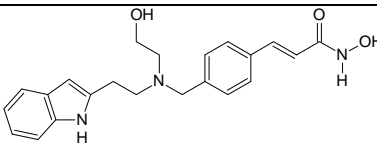
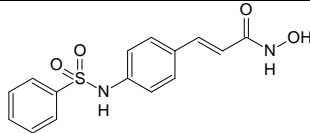
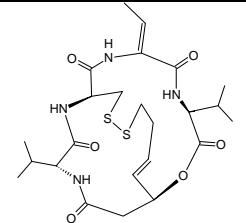
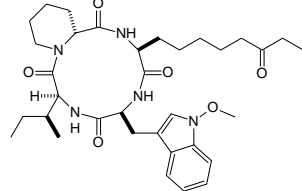
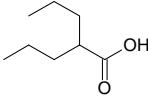
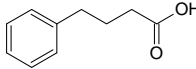
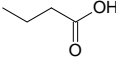
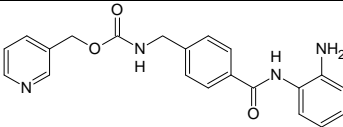
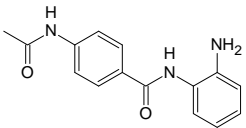
Class	Compound		HDAC target (<i>in vitro</i> potency)
Hydroxamate	Trichostatin A (TSA)		Class I and II (nM)
	SAHA (vorinostat)		Class I and II (μM)
	LAQ824		Class I and II (nM)
	PXD-101 (belinostat)		Class I and II (μM)
Cyclic peptides	Depsipeptide (FK-228; romidepsin)		Class I (nM)
	Apicidine		Class I and II (nM- μM)
Short-chain fatty acids	Valproic acid		Class I (mM)
	Phenyl butyrate		Class I and II (mM)
	Butyrate		Class I and II (mM)
Benzamides	MS-275		HDAC1, HDAC3 (μM)
	CI-994		HDAC1, HDAC3 (μM)

Fig. 1

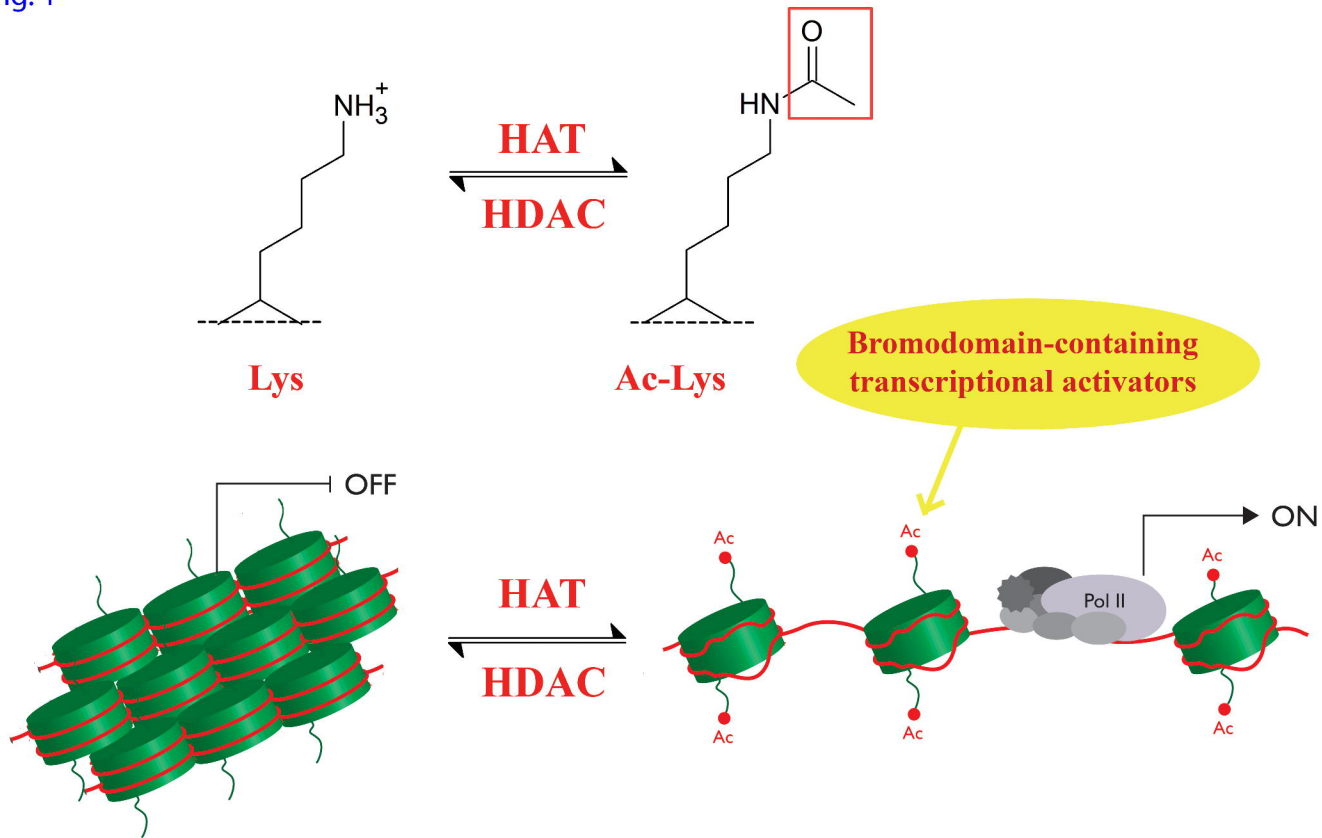


Fig. 2

