Histone Deacetylase Is a Direct Target of Valproic Acid, a Potent Anticonvulsant, Mood Stabilizer, and Teratogen*

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Valproic acid is widely used to treat epilepsy and bipolar disorder and is also a potent teratogen, but its mechanisms of action in any of these settings are unknown. We report that valproic acid activates Wnt-dependent gene expression, similar to lithium, the mainstay of therapy for bipolar disorder. Valproic acid, however, acts through a distinct pathway that involves direct inhibition of histone deacetylase (IC50 for HDAC1 = 0.4 mM). At therapeutic levels, valproic acid mimics the histone deacetylase inhibitor trichostatin A, causing hyperacetylation of histones in cultured cells. Valproic acid, like trichostatin A, also activates transcription from diverse exogenous and endogenous promoters. Furthermore, valproic acid and trichostatin A have remarkably similar teratogenic effects in vertebrate embryos, while non-teratogenic analogues of valproic acid do not inhibit histone deacetylase and do not activate transcription. Based on these observations, we propose that inhibition of histone deacetylase provides a mechanism for valproic acid-induced birth defects and could also explain the efficacy of valproic acid in the treatment of bipolar disorder.

Valproic acid (VPA) is a short-chained fatty acid widely used in humans as an anticonvulsant and as a mood stabilizer (1, 2). The effectiveness of VPA as an anticonvulsant was discovered serendipitously when other compounds were dissolved in VPA for administration to animals used in experimental models of epilepsy (1–3). Since then, VPA has been used to control a variety of seizures, including generalized and partial seizures (1). Several hypotheses have been put forth to explain the anticonvulsant activity of VPA, and, given the efficacy of VPA in diverse forms of epilepsy, it may act through more than one target (1). VPA increases the level of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), with acute administration causing a 15–45% increase in GABA in the brains of rodents (cited in Ref. 1). Because inhibition of GABAergic signaling can cause seizures and potentiation of GABA signaling can prevent seizures, this effect of VPA on GABA levels has been proposed as a mechanism for the anticonvulsant activity of VPA. However, the target(s) of VPA in this setting has not been definitively identified; VPA can stimulate GABA biosynthetic enzymes and inhibit enzymes involved in GABA degradation in vitro, but it is not clear whether these are important in vivo targets of VPA (1, 2, 4).

VPA is a potent teratogen in humans (5) and is widely studied as a model teratogen in rodents. Although the target of VPA in this setting is unknown, strict structural requirements have been defined for the teratogenic activity of VPA and VPA-related compounds. Thus, potently teratogenic analogues of VPA contain a tetrahedral α-carbon bound to a free carboxyl group, a hydrogen, and two alkyl groups (6, 7). In contrast, analogues such as valpromide (VPM), in which the carboxyl group is modified to an amide, and 2-methyl-2-propylpentenoic acid (2M2P), in which a methyl group is added to the α-carbon, do not cause neural tube defects in mouse embryos. These analogues can still protect against chemically induced seizures in mice (6, 7), suggesting that at least some of the clinically observed effects of VPA involve distinct molecular targets.

In the treatment of bipolar disorder, VPA is effective both in acute mania and as a prophylaxis for recurrent mania and depression, similar to lithium (1, 2). However, as with lithium, the mechanism of VPA action in bipolar disorder remains unknown. A number of interesting mechanisms have been proposed, but in each case, the direct target of VPA has not been defined. The characteristic delay in response to lithium or VPA has led to the proposal that both drugs act through modulation of gene expression, and this is supported by data from in vitro as well as in vivo systems (8–12). Furthermore, lithium and VPA can down-regulate expression of protein kinase C isofoms PKCa and PKCe, induce expression of the anti-apotopotic gene bcl-2, and activate AP-1-dependent transcription (through a direct effect on c-jun activity and by increasing expression of c-Jun (11, 12)). Both VPA and lithium also stimulate glutamate release and inositol 1,4,5-triphosphate accumulation in mouse cerebral cortex slices, although apparently through distinct mechanisms (14). Furthermore, both VPA and lithium have been shown to confer protection from neurotoxic agents (15–17). In each of these settings, there is a delay in the response, similar to that observed clinically; thus the direct targets of VPA in these settings have not been determined.

Several direct targets of lithium have been identified (reviewed in Ref. 18), including inositol monophosphatase (19, 20), a family of related phosphomonoesterases (21), and glycojen...
synthase kinase-3β (GSK-3β) (22). GSK-3β is a negative regulator of the Wnt signaling pathway, which regulates numerous processes, including axonal remodeling, cellular proliferation, embryonic patterning, and organogenesis (23–26). Because GSK-3β phosphorylates β-catenin, leading to its rapid degradation, inhibition of GSK-3β by either lithium or Wnt signaling leads to stabilization and accumulation of β-catenin protein (27, 28); β-catenin then translocates to the nucleus where it activates transcription of Wnt-dependent genes by binding to factors of the Tcf/Lef family. Activation of Wnt signaling by lithium has been proposed to explain the similarity between lithium and Wnts in a variety of settings (22), but a role for this pathway in bipolar disorder has not been demonstrated. Although VPA does not inhibit inositol monophosphatase (29, 30), it has also been reported to inhibit GSK-3β-mediated phosphorylation of a peptide derived from the CREB protein in vitro, and exposure of SH-SY5Y cells to VPA can also cause an increase in β-catenin protein levels (31), raising the interesting possibility that VPA and lithium both act through inhibition of GSK-3β. However, VPA has not yet been shown to inhibit GSK-3β in vivo nor to activate Wnt-dependent gene expression.

We have further investigated whether VPA activates Wnt signaling and find that VPA can indeed activate Wnt-dependent gene expression, similar to lithium, but through a distinct mechanism that involves direct activation of transcription. We show that VPA potently inhibits histone deacetylase (HDAC), a negative regulator of gene expression in multiple settings, at therapeutically relevant levels. Furthermore, the teratogenicity of VPA in vertebrate embryos is mimicked by the HDAC inhibitor trichostatin A, whereas non-teratogenic analogues of VPA do not inhibit HDAC. These findings lead us to propose that HDAC is an important target of VPA in the pathogenesis of birth defects. HDAC also offers a plausible novel target for VPA action in the treatment of bipolar disorder.

**EXPERIMENTAL PROCEDURES**

**Plasmids—**Luciferase constructs containing three wild-type (Lef-OT) or three mutated (Lef-OF) Lef binding sites were gifts from Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD). Mutated plasmids encoding secreted 2-methyl-2-propylpentenoic acid and 4-pentenoic acid were purchased from CLONTECH. Human HDAC1 in pRL-CMV were purchased from Promega. Plasmids encoding secreted 2-methyl-2-propylpentenoic acid and 4-pentenoic acid were purchased from Sigma (Sigma) were prepared in sterile water as concentrated stocks and added to the final concentrations as indicated in the figures. Valproic acid (sodium salt; Sigma Chemical Co.) and lithium chloride (Sigma) were prepared in 2 ml of 20 mM HEPES, 1 mM EDTA, 1 mM EGTA, followed by centrifugation through a Centrifuge 10 membrane to concentrate protein (final volume 150 μl). SDS sample buffer was added, and the proteins were separated by electrophoresis on 12.5% acrylamide gels (SDS-PAGE). Gels were either stained with Coomassie Blue or immunoblotted with acetyl-histone H4 antibody (1:1000; Upstate Biotechnology Inc.).

**In Vitro GSK-3β and HDAC1 Assays—**GSK-3β assay was performed as described previously (22), except that MgCl2 was 1 mM. VPA was added at concentrations indicated in Fig. 3B. For in vitro HDAC assays, myc epitope-tagged HDAC1 was transfected into HeLa cells and immunoprecipitated (36). Immunoprecipitates were washed, resuspended in HD buffer supplemented with 5 mM fluorodeoxyuridine, and stored as frozen aliquots. HDAC1 was then added to a tube containing 40,000 cpm ‘H-labeled acetylated histones (purified from HeLa cells) in 200 μl of HD buffer ± VPA, trichostatin A (Sigma), valproamide, or 2-methyl-2-propylpentenoic acid (at concentrations described in Figs. 3, 4, and 7). After rotation for 12 h at 37 °C, the reaction was stopped by the addition of 50 μl of stop solution (1 M HCl, 0.16 M acetic acid) and released ‘H-labeled acetic acid was extracted and analyzed by scintillation counting. To assay total nuclear HDAC activity, nuclear extracts from HeLa cells (30 μg) were used as a source of HDAC activity in place of immunoprecipitated HDAC1, as described (36). HDAC assay was otherwise as described above for HDAC1.

Embryos—*Xenopus* eggs and embryos were maintained in 0.1× MMR according to standard protocols (37). Stage 8 embryos were incubated in 0.1× MMR containing valproic acid or valproamide (1.0, 2.5, or 5.0 mM) or trichostatin A (25, 50, or 100 nM) for 24 h. Embryos were then transferred to fresh 0.1× MMR and cultured until tadpole stages. VPA stock (2 μM) was prepared in water, whereas valproamide (2 μM) and TSA (100 μM) stocks were prepared in MeSO. Control MeSO-treated embryos developed normally.

**RESULTS**

VPA Activates Tcf/Lef-dependent Transcription and Synergizes with Lithium—To test whether VPA can activate Wnt-dependent gene expression, we generated stable cell lines in human embryonic kidney cells (293T) transfected with firefly luciferase reporters containing either three wild-type Lef binding sites (Lef-OT) or three mutant Lef binding sites (Lef-OF) (32). These two stable cell lines were treated with VPA or lithium chloride (LiCl) for 24 h and then harvested to measure luciferase activity. Cells treated with lithium showed a dose-dependent increase in Lef-luciferase activity (over 70-fold) for the gene that contains wild-type, but not mutated, Lef sites (Fig. 1A), as reported for transiently transfected C57MG cells (38). Similarly, VPA also induces Lef-dependent luciferase activity over 20-fold (Fig. 1B). Interestingly, the addition of both drugs to the 293T stable lines resulted in marked synergistic activation of reporter activity (Fig. 1C), with up to 315-fold
activation, far exceeding additive effects. This synergy raises the possibility that lithium and VPA act through independent mechanisms in this assay. This could also explain the efficacy of combining lithium and valproate in bipolar disorder patients that are resistant to single drug therapy.

VPA Activates Transcription through Diverse Promoters—To test whether neuronal cells may respond to VPA in a similar manner, Neuro2A cells were transiently transfected with Lef-OT or Lef-OF, together with a control reporter (pRL-SV40) encoding Renilla luciferase driven by the SV-40 promoter, and firefly and Renilla luciferase activities were measured after 24 h. As in 293T cells, VPA activated OT-Lef up to 6-fold in Neuro2A cells (not shown). Surprisingly, VPA also consistently activated the control reporter up to 10-fold (Fig. 1D), with half-maximal activation at 0.8 mM VPA. Transfection efficiency, assessed by frequency of green fluorescence protein-positive cells or by expression of secreted alkaline phosphatase (SEAP), was similar in each group prior to addition of VPA. Lithium did not stimulate this or other control reporters (not shown).

VPA can activate AP-1-dependent transcription, and an increase in the activity of the SV-40 promoter has been proposed to be due to the presence of AP-1 sites within the SV-40 promoter (39). However, VPA also induces Renilla expression driven by the cytomegalovirus (CMV) promoter (pRL-CMV), which does not contain an AP-1 site (Fig. 4B), suggesting a more general mechanism of activation. VPA has also been reported to activate the Rous sarcoma virus promoter and peroxisomal proliferator-activated receptor-g-dependent transcription in F9 teratocarcinoma cells (6). The effect of VPA on diverse promoters suggests that VPA acts through a mechanism distinct from lithium and may involve direct activation of transcription.

VPA Increases β-Catenin Levels through a Novel Mechanism—Wnt signaling, or exposure to lithium, causes stabilization and accumulation of β-catenin protein (26). We therefore examined the effect of VPA on levels of β-catenin protein by Western blotting. In Neuro2A cells, both lithium (20 mM) and VPA (2 mM) caused accumulation of β-catenin protein, similar to published work on VPA in SY5Y cells (31). However, the rate of β-catenin accumulation differed with the two drugs. Lithium caused β-catenin accumulation within 30 min of treatment, whereas the effect of VPA was not evident until 10 h after treatment (Fig. 2A). Although this could reflect differences in the access of VPA and lithium, VPA has been shown to cross the blood-brain barrier within 1 min after intravenous injection.
and similarly is rapidly taken up by cells in culture (40). An alternative possibility to explain the delay in β-catenin accumulation after VPA exposure is that VPA acts by increasing the expression of β-catenin rather than stabilizing the protein. To distinguish between these two possibilities, Neuro2A cells were cultured in the presence of cycloheximide (CHX), an inhibitor of protein synthesis. Agents, such as lithium, that stabilize β-catenin should slow its degradation, but no new protein will accumulate. Agents that induce new transcription or translation of β-catenin should have no effect on β-catenin protein levels in the presence of CHX. Under these conditions, β-catenin is rapidly degraded and is almost undetectable after 30 min of CHX treatment (Fig. 2B, lane 2). In the presence of CHX, lithium stabilized existing β-catenin protein, slowing the rate of degradation so that β-catenin protein was readily detectable at 30 min, 5 h, and 10 h (Fig. 2B, lanes 3, 6, and 9). Conversely, VPA treatment did not stabilize β-catenin; the protein was rapidly degraded within 30 min and was barely detectable at 5 or 10 h (Fig. 2B, lanes 4, 7, and 10), as in cells treated with CHX alone. These observations suggest that VPA acts at the level of transcription or translation.

Northern blot analysis confirmed that VPA increases the level of β-catenin mRNA in Neuro2A cells in a dose- and time-dependent manner (Fig. 2C), with increased β-catenin mRNA detected as early as 4.5 h. These data strongly support that VPA induces β-catenin at the level of transcription (or message stability) rather than through post-translational regulation. VPA can inhibit GSK-3β-mediated phosphorylation of a CREB peptide in vitro, providing an intriguing potential mechanism for VPA action, but the effect of VPA on GSK-3β activity in vivo has not been studied. In vivo inhibition of GSK-3β can be followed by examining phosphorylation of the microtubule-associated protein tau, which is phosphorylated by GSK-3β in vivo at specific sites recognized by the PHF-1 antibody (34, 41). This phosphorylation is inhibited by lithium in vitro (22) and in vivo (27, 28), as well as by other GSK-3β inhibitors (18). We therefore examined the levels of tau phosphorylation in mouse Neuro2A cells treated with VPA. VPA from 0.5 to 20 mM did not inhibit tau phosphorylation even after 24 h of exposure (Fig. 3A). Rather, VPA caused a modest increase in the level of tau protein (phosphorylated and unphosphorylated forms). The non-teratogenic analogues of VPA, 2-methyl-2-propylpentenoic acid (2M2P) and 4-pentenoic acid (both at 2 mM) had no effect on levels of tau protein or tau phosphorylation. Furthermore, lithium inhibited tau phosphorylation in the presence of VPA (Fig. 3A), indicating that the tau phosphorylation under these conditions depends on GSK-3β activity. VPA also did not inhibit tau phosphorylation in Xenopus oocytes (data not shown).

Because VPA can inhibit phosphorylation of a CREB-derived peptide in vitro (31), we examined whether other GSK-3β substrates are also sensitive to VPA. VPA (0.125 to 10.0 mM) did not inhibit GSK-3β-dependent phosphorylation of GS-2, a peptide derived from glycogen synthase (Fig. 3B). The assay was performed at 1 mM MgCl₂, but was repeated over a wide range of magnesium concentrations (0.15–10 mM) with similar results. Inhibition of CREB phosphorylation by VPA may reflect substrate-specific inhibition of GSK-3β, as reported for other GSK-3β inhibitors (42). Taken together with the above data, these in vitro and in vivo results suggest that VPA does not act through the same mechanism as lithium in these settings.

VPA Inhibits Histone Deacetylase—The ability of VPA to activate transcription regulated by multiple promoters is reminiscent of molecules that inhibit histone deacetylases (HDACs). HDACs are recruited by a variety of transcription factor coactivator complexes and are believed to repress transcription by reducing the level of acetylation of core histones, thereby altering chromatin structure (43). We therefore assayed HDAC1 activity in the presence of VPA. HDAC1 was overexpressed in HeLa cells and immunoprecipitated, and the release of labeled acetyl groups from 3H-labeled, acetylated histones was measured (36). VPA inhibits HDAC1 in vitro in a dose-dependent manner, with an IC₅₀ of 0.4 mM (Fig. 4A), which is within the therapeutic range for VPA therapy in humans.
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FIG. 4. VPA inhibits HDAC activity. A, human HDAC1 activity was assayed in vitro as release of [3H]acetate from labeled histones (36) in the presence of 0–20 mM VPA. VPA inhibited HDAC1 with an IC_{50} of 0.4 mM, well within the therapeutic range of VPA in humans. Percent HDAC activity is shown with respect to the activity of HDAC alone (100%). B, VPA inhibits endogenous HDACs present in HeLa cell nuclear extracts. Nuclear extracts from untransfected HeLa cells were isolated and added to HDAC assay as described in A. Percent HDAC activity is shown with respect to the activity of HDAC alone (100%). Error bars represent standard deviation.

To test whether VPA inhibits HDACs other than HDAC1, we prepared nuclear extracts from HeLa cells, which express multiple HDACs, including HDAC1, 2, 3, 4, and 8 (36, 44, 45), and assayed HDAC activity as above in the presence of VPA (Fig. 4B). VPA inhibited nuclear HDAC activity, similar to inhibition of isolated HDAC1, with 50% inhibition between 0.5 and 2 mM.

These data show that VPA inhibits HDAC activity in vitro. If VPA also inhibits HDAC in cells, it should cause hyperacetylation of endogenous targets of HDACs, as seen with other HDAC inhibitors. Thus, Neuro2A cells were treated with VPA (0.5–5 mM) or with TSA (300 nM) for 24 h and then histones were isolated (Fig. 5A, lower panel). Histone acetylation was assessed by immunoblotting with an antibody specific to acetylated histone H4. Acetylation of H4 was detectable at 0.5 mM VPA. Coomassie Blue-stained gel (lower panel) shows loading of histones. Control lane (con) shows a mixture of purified, non-acetylated histones. H4 is the fastest migrating band in the lower panel, whereas the bracket indicates (in decreasing size) histones H3, H2B, and H2A.

FIG. 5. VPA causes hyperacetylation of endogenous histones in Neuro2A cells. Neuro2A cells were cultured for 24 h in 0–5 mM VPA or 300 nM TSA; nuclear proteins were isolated and immunoblotted with an antibody specific for acetylated histone-H4 (upper panel). Acetylation of H4 was detectable at 0.5 mM VPA. Coomassie Blue-stained gel (lower panel) shows loading of histones. Control lane (con) shows a mixture of purified, non-acetylated histones. H4 is the fastest migrating band in the lower panel, whereas the bracket indicates (in decreasing size) histones H3, H2B, and H2A.

FIG. 6. HDAC1 overexpression reverses VPA-mediated activation of transcription in vitro. 293T cells were transfected with CMV-Renilla and SV-40-SEAP, with or without an HDAC1 expression vector. Activities have been normalized to levels of SEAP in media prior to the addition of the VPA. Renilla activity without HDAC is shown in light bars, whereas activity in the presence of overexpressed HDAC1 is shown as dark bars. Experiments were performed in triplicate in three independent experiments. Error bars represent standard deviation.

tested whether overexpression of HDAC1 in tissue culture cells could reverse the VPA-induced activation of the Renilla reporter. 293T cells were transfected with CMV-Renilla, with or without HDAC1, and treated with VPA for 24 h. Overexpression of HDAC inhibited CMV-Renilla reporter activity 10-fold showing that this reporter can be regulated by HDAC (Fig. 6). Furthermore, HDAC prevented the transcriptional activation by VPA, suppressing Renilla activity 10-fold in the presence of VPA. These data are consistent with the proposal that HDAC is an in vivo target of VPA.

The HDAC Inhibitor Trichostatin A Mimics VPA Effects on Embryogenesis—VPA is highly teratogenic in humans, causing spina bifida aperta and other neural tube closure defects in 1–2% of offspring of women taking VPA during the first trimester of pregnancy (5). Similarly, VPA causes neural tube defects in rodents, and this has served as a commonly studied model of teratogenesis. However, the molecular target of VPA in vertebrate embryos is still unknown. Because HDAC is inhibited by therapeutically relevant concentrations of VPA, and plays important roles in embryonic development (47–49), it could be the target of VPA-induced teratogenesis. We have therefore investigated whether the structurally related VPA analogues, valpromide (VPM) and 2M2P, which function as

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2 M. Guenther and M. Lazar, unpublished data.
3 C. J. Phiel and P. S. Klein, unpublished data.
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anticonvulsants but are not teratogenic in mice (6, 7), are able to inhibit HDAC. Under conditions where VPA and trichostatin A (TSA, a well characterized HDAC inhibitor) potently inhibited HDAC, neither VPM nor 2M2P inhibited HDAC in doses ranging from 0.1 to 5 mM (Fig. 7A and data not shown). Similarly, TSA potently activated CMV and SV40 reporters, whereas VPM did not (data not shown). These observations are consistent with the failure of other non-teratogenic VPA analogues to activate Rous sarcoma virus reporter genes (6).

Because non-teratogenic VPA analogues do not inhibit HDAC, we then asked whether an established HDAC inhibitor causes defects in embryonic development similar to VPA. Exposure of *Xenopus* embryos to VPA (5 mM) after the midblastula transition (MBT) had a pronounced effect on development, with marked loss of anterior structures as well as shortening of the anterior-posterior axis in 88% of embryos (Fig. 7C), similar to previous work in *Xenopus* and other amphibians (50), whereas VPM did not (data not shown). These observations are consistent with the failure of other non-teratogenic VPA analogues to activate Rous sarcoma virus reporter genes (6).

DISCUSSION

The mechanisms of action for VPA as an anticonvulsant, mood stabilizer, and teratogen have not been defined. This work shows that VPA is an effective inhibitor of histone deacetylases, with an IC$_{50}$ (0.4 mM) well within the therapeutic range of VPA (0.35–0.7 mM in serum), and that VPA, like other HDAC inhibitors, activates transcription from diverse promoters. VPA, like lithium, activates Wnt-dependent gene expression, but unlike lithium, VPA does not inhibit GSK-3β in vitro. Rather, we propose that VPA activates Wnt-dependent gene expression through inhibition of HDAC, which in turn leads to both increased expression of β-catenin and de-repression of Tcf/Lef (as well as activation of other HDAC-regulated genes). The remarkable similarities in the effects of VPA and TSA in both mouse and *Xenopus* embryos indicate that inhibition of HDAC may be the mechanism of VPA-induced teratogenicity.

Several hypotheses have been put forth to explain the antiepileptic activity of VPA, and, given the diverse forms of epilepsy that respond to VPA, it may act through more than one target (1). Thus, VPA increases the level of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), selectively en-

Fig. 7. Inhibition of HDAC correlates with teratogenicity. A, non-teratogenic analogues valpromide (VPM; 5 mM) and 2-methyl-2-propylpentenoic acid (2M2P; 5 mM) do not inhibit HDAC1, whereas VPA (5 mM) and the established HDAC inhibitor TSA (300 nM) do inhibit HDAC1. Assay conditions are as in Fig. 4A. B–E, *Xenopus* embryos were treated from stage 8 until neurula stage with buffer, VPA, VPM, or TSA, and then scored at tadpole stages. B, control *Xenopus* tadpole. C, tadpole after exposure to VPA is shorter and lacks anterior structures. D, tadpole after exposure to VPM with normal anterior development. E, tadpole after exposure to TSA is shorter and lacks anterior structures, similar to VPA-treated tadpole.
hances GABA-mediated inhibition in the cerebral cortex, inhibits AMPA binding to its receptor, and antagonizes voltage-dependent sodium channels (1). In vitro, VPA can stimulate glutamic acid decarboxylase, which is involved in GABA biosynthesis, and inhibit GABA transaminase, succinic semialdehyde dehydrogenase, and α-ketoglutarate dehydrogenase, enzymes involved in GABA degradation. However, whether these in vitro effects of VPA are sufficient to explain its anticonvulsant activity in vivo remains unclear (1). Because analogues of VPA that do not inhibit HDAC (Fig. 7A) can still protect against chemically induced seizures in rodents (6, 7), inhibition of HDAC may not explain the anticonvulsant activity of VPA, at least in this experimental setting.

Strict structural requirements have been defined for the teratogenic activity of VPA and VPA-related compounds, and these features also appear to be important for inhibition of HDAC (Fig. 7A). Potently teratogenic analogues of VPA contain a tetrahedral α-carbon connected to a free carboxyl group, a hydrogen, and two alkyl groups (6, 7), features that are also found in the structure of butyrate, a well known inhibitor of HDAC. Thus the non-teratogenic analogues valpromide (VPM), found in the structure of butyrate, a well known inhibitor of HDAC, causes an increase in the replication of HIV (60). In addition, the HDAC inhibitors TSA and trapoxin induce the transcription of HIV in vivo and in vitro (61). The identification of VPA as an HDAC inhibitor thus offers a plausible explanation for the effect of VPA on HIV levels observed clinically.

Finally, the identification of VPA as an HDAC inhibitor suggests a potential therapeutic role in the treatment of malignant diseases (62). HDAC inhibitors can prevent proliferation and induce differentiation of numerous transformed cell types, including neuroblastoma, erythroleukemia, acute myelogenous leukemia, and carcinomas of the skin, breast, prostate, bladder, lung, colon, and cervix (62–67). Given the extensive clinical experience with VPA, it may provide a relatively safe, well tested alternative to the use of TSA and trapoxin in the therapy of malignant diseases. Indeed, VPA has been shown to inhibit proliferation and induce differentiation of cell lines derived from human malignant gliomas (10), and it may well find broader clinical use in the treatment of other types of cancer. Whether this occurs through hyperacetylation, and consequent activation of p53, or through regulation of other HDAC targets, is a subject for future study.

Acknowledgments—We thank Praveen Raju and Steve Liebhaber for comments on the manuscript and Tom Kadesch for helpful discussions. We also thank John Pehrsin for advice and for purified histones and Arpine Arzoumanian for excellent technical assistance.

Addendum—While this manuscript was being written, we became aware of another group who have also observed inhibition of HDAC by VPA (Martin Göttlicher and Thorsten Heinzell, personal communication.)

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J. Biol. Chem. 2001, 276:36734-36741.
doi: 10.1074/jbc.M101287200 originally published online July 25, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101287200

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