

Tributylin May Have Practical Potential for Improving Cognition in Early Alzheimer's Disease Via Inhibition of HDAC2

Mark F. McCarty, Catalytic Longevity

Histone Deacetylase 2 Mediates Neuronal Dysfunction in Alzheimer's Disease

There is striking new evidence that dysregulation of histone acetylation is a key mediator of the failure of long term potentiation induced by amyloid beta, and that inhibitors of histone deacetylase 2 (HDAC2) may have potential for ameliorating cognitive function in patients with Alzheimer's disease.

Some years ago, Levenson and colleagues reported that acetylation of histone 3 in area CA1 of the hippocampus increases following stimulation of NMDA receptors in that area; inhibition of ERK blocked this effect, whereas alternative strategies which activated ERK mimicked it.¹ A reasonable interpretation of this finding is that appropriate histone acetylation promoted the new protein synthesis required for long term memory formation. Consistent with this view, preadministration of two histone deacetylase inhibitors, trichostatin A or sodium butyrate, enhanced late-phase long term potentiation in Shaffer collateral synapses of area C1; this effect was blocked by coadministration of a transcription inhibitor. Moreover, when rats were injected with sodium butyrate one hour before a session of one-shock contextual fear conditioning, significantly more freezing behavior (indicative of their remembering the prior shock) was observed in the rats when they were placed in the shock chamber again 24 hours later. More recently, other researchers have found that preadministration of these two histone deacetylase inhibitors aided learning and memory of young mice in two other learning paradigms, eyeblink classical conditioning and object recognition memory.² A similar effect was observed in SAMP-8-mutant mice, a model of premature senility associated with increased amyloid beta levels.

Now Graff and colleagues, working with CK-p25 mice, a model of neurodegeneration with many features similar to Alzheimer's disease, have demonstrated that neuronal expression of HDAC2 is increased in these mice, and that this is largely responsible for the cognitive dysfunction in these animals.³ mRNA expression of many genes required for neuronal plasticity was diminished in the hippocampus of these mice; the promoters of these genes showed increased binding of HDAC2 (relative to control mice), and showed a lower degree of acetylation. When HDAC2 expression was selectively knocked down by intracerebral administration of a short-hairpin RNA targeting HDAC2, expression of neuroplasticity genes was normalized, as was long term potentiation in area CA1 and performance in contextual fear conditioning and water maze tests, which assess hippocampus-dependent learning.

These researchers then demonstrated that exposure of primary hippocampal neurons to either amyloid beta oligomers or to hydrogen peroxide elicited increased expression of HDAC2. This was at least partially attributable to stimulation of the transactivational activity of glucocorticoid receptor 1– which binds to the HDAC2 promoter – via phosphorylation on S211. Increased phosphorylation of this receptor was also noted in the hippocampus of CK-p25 mice. Importantly, increased expression of HDAC2 was also seen in another mouse model of Alzheimer's disease, 5XFAD (which carry 5 different mutations linked to increased Alzheimer's risk in humans), and in hippocampal CA1 area of post-mortem brain samples from patients who had died with Alzheimer's. Hence, there is strong reason to suspect that

sustained hippocampal overexpression of HDAC2 is an important mediator of the loss of cognitive function typical of clinical Alzheimer's. The authors therefore suggest that selective inhibitors of HDAC2 may be of particular merit for managing this disorder.

Focus on HDAC2 was motivated by the previous work of Guan and colleagues, who reported that neuron-specific overexpression of HDAC2 (but not HDAC1) selectively blocks transcription of a number of proteins required for synaptic plasticity; HDAC2 was found to associate with the promoters of their genes.⁴ Memory formation is also impaired in these animals. Conversely, HDAC2-deficient mice have superior memory – and histone acetylase inhibitors fail to boost memory as they do in wild-type animals. Hence, this work clearly establishes that the memory-boosting impact of HDAC inhibitors reflects inhibition of HDAC2 specifically.

Why neuronal HDAC2 expression is amplified in AD remains to be clarified. As noted above, S211 phosphorylation of the glucocorticoid receptor may mediate HDAC2 induction in CK-p25 mice.³ This phosphorylation can be mediated by CDK5, which is chronically activated in CK-p25 mice, and which is also activated in neurons by amyloid beta oligomers.^{5,6} Hence, it is reasonable to suspect that CDK5 overactivation is responsible, at least in part, for neuronal overexpression of HDAC2 in AD.

Class I HDAC Inhibitors May Aid Cognitive Function in Alzheimer's Disease

These findings help to rationalize previous reports that inhibitors of class I HDACs – HDAC1, HDAC2, HDAC3, and HDAC8 – have favorable effects on cognition in various mouse models of Alzheimer's. Sodium butyrate – available as a nutraceutical – and 4-phenylbutyrate, an orphan drug approved for treatment of certain genetic disorders of the urea cycle, can inhibit HDACs with K_i s in the low micromolar range; valproic acid has a similar spectrum of activity, although slightly higher concentrations are required, and the cancer drug vorinostat inhibits HDACs 1,2, and 3 in the low nanomolar range.⁷ In 16-month-old Tg2576 mice, which show marked cognitive impairment and amyloid beta accumulation, 5 weeks of phenylbutyrate administration (200 mg/kg, i.p.) normalized their performance in the Morris water maze, without however reducing brain amyloid load; normalized hippocampal expression of certain brain neuroplasticity markers (GluR1, PSD95, MAP2), and decreased tau phosphorylation, was also observed.⁸ A subsequent study showed that commencing phenylbutyrate administration at age 6 months in these mice not only prevented cognitive dysfunction, but also sustained dendritic spine densities, reduced amyloid beta accumulation, and blunted astroglial activation.⁸ Another group administered phenylbutyrate orally (1 g/kg daily in drinking water) for 14 months in APPswePS1delta9 mice, and found that this normalized their performance in a contextual learning test and decreased amyloid plaque volume, even though amyloid beta production was not influenced.⁹ Another study with these mice found that daily injections of sodium valproate, sodium butyrate, or vorinostat for several weeks normalized their learning performance in contextual fear conditioning, and increased their ability to consolidate their learning over 2 weeks.⁷ Similarly, daily administration of sodium butyrate (1.2 g/kg i.p.) in APPS1-21 mice for 6 weeks markedly improved their learning performance in a fear conditioning paradigm, elevated acetylation of certain histone sites in the hippocampus but not cortex, and increased hippocampal expression of various neuronal plasticity markers.¹⁰ The same regimen of i.p. sodium butyrate was found to aid learning as well as recovery of long-term memories in the CK-p25 mouse model subsequently studied by Graff et al.¹¹

All of these findings are clearly consistent with the proposal that HDAC2 inhibition may improve cognitive performance in Alzheimer's – at least if the various rodent models of this disorder are highly pertinent to human disease. But which HDAC inhibitors have the greatest practical potential in this respect? Phenylbutyrate has the considerable advantage that it is already in clinical use. Phase 2 studies in Huntington's disease and ALS conclude that daily oral intakes of 9-15 g are well tolerated, and intakes as low as 9 g daily significantly enhance histone acetylation of blood cells.^{12, 13} Moreover, phenylbutyrate has the advantage that it can also act as a chaperone for protein folding, thereby lessening endoplasmic reticulum (ER) stress. Induction of ER stress is one mechanism whereby amyloid beta disrupts neuronal function, contributing to tau phosphorylation, and the ER stress inhibitory drug tauroursodeoxycholate has been shown to ameliorate cognitive deficits and suppress amyloid beta production in APP/PS1 mice.¹⁴ However, phenylbutyrate has the disadvantage that, as an orphan drug, it is extremely expensive; hence, its off-label use would not be feasible for most patients. Vorinostat evidently suffers from the same limitation, and valproate often produces an unacceptable level of drowsiness in therapeutic doses.

Butyrate and Tributyrin – A Nutraceutical Alternative

Sodium butyrate, on the other hand, is a relatively affordable nutraceutical, and is now even added to some animal feeds as an aid to intestinal health. Although it is not known to influence ER stress, it has the ancillary advantage of activating AMPK, as demonstrated in a recent study in which it was administered as 5% of diet in mice.¹⁵ In this study, inclusion of sodium butyrate in a high-fat diet substantially prevented the body fat accumulation and insulin resistance syndrome typically induced by such a diet. The sodium butyrate-fed mice displayed much greater brown fat thermogenesis, and increased mitochondrial biogenesis in skeletal muscle. It is tempting to speculate that the preservation of brown fat thermogenesis in sodium-butyrate-fed mice may have reflected a dampening of the hypothalamic inflammation which high-fat diets evoke in mice;^{16, 17} this inflammation may be driven by microglial activation, and there is some evidence that HDAC inhibitors such as sodium butyrate have the potential to blunt microglial activation.¹⁸⁻²¹ A blunting of microglial cytokine production might in itself have a favorable impact on progression of Alzheimer's.²² The activation of AMPK seen with sodium butyrate may reflect the generation of AMP entailed by conversion of butyrate to butyryl-coA.

Although most studies evaluating sodium butyrate's impact on cognition have administered it parenterally, the impact of oral sodium butyrate, in drinking water, has been studied in a transgenic mouse model of spinal and bulbar muscular atrophy.²³ At 4 g per liter, sodium butyrate had a markedly favorable impact on physical performance, survival, and maintenance of body weight; likely, this reflected an important impact on histone acetylation. Surprisingly, this agent had an adverse effect in these mice if the dose was further increased to 16 g/l. In the 4 g per liter group, the mice were ingesting approximately 16 mg of sodium butyrate daily; adjusted for relative body size by using the three-quarter power of relative weights, the corresponding human dose would be about 7-8 grams daily. Parenteral doses of sodium butyrate showing beneficial cognitive effects in various mouse models have ranged from 250-1,200 mg/kg daily, which in humans might correspond to 3-10 g daily. The fact that rather high doses of sodium butyrate would be required for modulation of histone acetylation reflects the fact that butyrate is avidly oxidized as a fuel substrate, and hence has quite a short half-life of about 6 minutes.

Fortunately, butyrate can also be administered in esterified form. The triglyceride tributyrin (glyceryl tributyrin) is an approved food additive that presumably could be administered as an emulsion;

effectively, it serves as delayed release source of butyrate, and hence achieves more sustained plasma levels.²⁴⁻²⁷ Its pharmacokinetics have already received a fair amount of study, as oncologists were hopeful that it could achieve the cancer-retardant benefits *in vivo* seen with butyrate *in vitro*; about 20% of cancer patients achieved long-term disease stabilization when receiving 200 mg/kg 3 times daily in a pilot trial.²⁴ Not surprisingly, tributyrin has also recently been reported to suppress the induction of obesity and insulin resistance in mice fed a high-fat diet.²¹ In light of these considerations, it would be intriguing to study the impact of tributyrin, administered orally in doses comparable to those employed in cancer trials, on the cognitive function of patients with early Alzheimer's disease. From the standpoint of practicality, however, it would be necessary to incorporate tributyrin into a functional food, as it would not be feasible to require the ingestion of many dozens of capsules daily.

Acetyl-L-Carnitine May Have Complementary Activity

There is emerging evidence that carnitine can function in neurons and other tissues to promote histone acetylation by shuttling acetyl groups from mitochondria to the nucleus; direct administration of acetyl-L-carnitine (ALC) should accomplish this effect more directly.²⁸⁻³² Hence, ALC has the potential to oppose the detrimental impact of HDAC2 on long-term potentiation, complementing the utility of HDAC inhibitors such as tributyrin in this regard. Plausibly, this mechanism could contribute to the favorable impact of ALC supplementation in mild cognitive dysfunction and early Alzheimer's, which has been confirmed by meta-analysis.³³ Carnitine's shuttle mechanism for acetyl groups may also boost the efficiency of acetylcholine synthesis.^{34, 35}

References

- (1) Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD. Regulation of histone acetylation during memory formation in the hippocampus. *J Biol Chem* 2004 September 24;279(39):40545-59.
- (2) Fontan-Lozano A, Romero-Granados R, Troncoso J, Munera A, Delgado-Garcia JM, Carrion AM. Histone deacetylase inhibitors improve learning consolidation in young and in KA-induced-neurodegeneration and SAMP-8-mutant mice. *Mol Cell Neurosci* 2008 October;39(2):193-201.
- (3) Graff J, Rei D, Guan JS et al. An epigenetic blockade of cognitive functions in the neurodegenerating brain. *Nature* 2012 March 8;483(7388):222-6.
- (4) Guan JS, Haggarty SJ, Giacometti E et al. HDAC2 negatively regulates memory formation and synaptic plasticity. *Nature* 2009 May 7;459(7243):55-60.
- (5) Kino T, Ichijo T, Amin ND et al. Cyclin-dependent kinase 5 differentially regulates the transcriptional activity of the glucocorticoid receptor through phosphorylation: clinical implications for the nervous system response to glucocorticoids and stress. *Mol Endocrinol* 2007 July;21(7):1552-68.

- (6) Town T, Zolton J, Shaffner R et al. p35/Cdk5 pathway mediates soluble amyloid-beta peptide-induced tau phosphorylation in vitro. *J Neurosci Res* 2002 August 1;69(3):362-72.
- (7) Kilgore M, Miller CA, Fass DM et al. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. *Neuropsychopharmacology* 2010 March;35(4):870-80.
- (8) Ricobaraza A, Cuadrado-Tejedor M, Perez-Mediavilla A, Frechilla D, Del RJ, Garcia-Osta A. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. *Neuropsychopharmacology* 2009 June;34(7):1721-32.
- (9) Wiley JC, Pettan-Brewer C, Ladiges WC. Phenylbutyric acid reduces amyloid plaques and rescues cognitive behavior in AD transgenic mice. *Aging Cell* 2011 June;10(3):418-28.
- (10) Govindarajan N, Agis-Balboa RC, Walter J, Sananbenesi F, Fischer A. Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. *J Alzheimers Dis* 2011;26(1):187-97.
- (11) Fischer A, Sananbenesi F, Wang X, Dobbin M, Tsai LH. Recovery of learning and memory is associated with chromatin remodelling. *Nature* 2007 May 10;447(7141):178-82.
- (12) Hogarth P, Lovrecic L, Krainc D. Sodium phenylbutyrate in Huntington's disease: a dose-finding study. *Mov Disord* 2007 October 15;22(13):1962-4.
- (13) Cudkowicz ME, Andres PL, Macdonald SA et al. Phase 2 study of sodium phenylbutyrate in ALS. *Amyotroph Lateral Scler* 2009 April;10(2):99-106.
- (14) Ramalho RM, Nunes AF, Dias RB et al. Tauroursodeoxycholic acid suppresses amyloid beta-induced synaptic toxicity in vitro and in APP/PS1 mice. *Neurobiol Aging* 2012 May 21.
- (15) Gao Z, Yin J, Zhang J et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009 July;58(7):1509-17.
- (16) Cai D, Liu T. Hypothalamic inflammation: a double-edged sword to nutritional diseases. *Ann N Y Acad Sci* 2011 December;1243:E1-39.
- (17) Arruda AP, Milanski M, Coope A et al. Low-grade hypothalamic inflammation leads to defective thermogenesis, insulin resistance, and impaired insulin secretion. *Endocrinology* 2011 April;152(4):1314-26.
- (18) Choi SJ, Kim F, Schwartz MW, Wisse BE. Cultured hypothalamic neurons are resistant to inflammation and insulin resistance induced by saturated fatty acids. *Am J Physiol Endocrinol Metab* 2010 June;298(6):E1122-E1130.
- (19) Suh HS, Choi S, Khattar P, Choi N, Lee SC. Histone deacetylase inhibitors suppress the expression of inflammatory and innate immune response genes in human microglia and astrocytes. *J Neuroimmune Pharmacol* 2010 December;5(4):521-32.
- (20) Huuskonen J, Suuronen T, Nuutinen T, Kyrylenko S, Salminen A. Regulation of microglial inflammatory response by sodium butyrate and short-chain fatty acids. *Br J Pharmacol* 2004 March;141(5):874-80.

- (21) Vinolo MA, Rodrigues H, Festuccia WT et al. Tributyrin attenuates obesity-associated inflammation and insulin resistance in high-fat fed mice. *Am J Physiol Endocrinol Metab* 2012 May 22.
- (22) Griffin WS, Barger SW. Neuroinflammatory Cytokines-The Common Thread in Alzheimer's Pathogenesis. *US Neurol* 2010;6(2):19-27.
- (23) Minamiyama M, Katsuno M, Adachi H et al. Sodium butyrate ameliorates phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. *Hum Mol Genet* 2004 June 1;13(11):1183-92.
- (24) Edelman MJ, Bauer K, Khanwani S et al. Clinical and pharmacologic study of tributyrin: an oral butyrate prodrug. *Cancer Chemother Pharmacol* 2003 May;51(5):439-44.
- (25) Egorin MJ, Yuan ZM, Sentz DL, Plaisance K, Eiseman JL. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemother Pharmacol* 1999;43(6):445-53.
- (26) Su J, He L, ZHANG N, Ho PC. Evaluation of tributyrin lipid emulsion with affinity to low-density lipoprotein: pharmacokinetics in adult male Wistar rats and cellular activity on Caco-2 and HepG2 cell lines. *J Pharmacol Exp Ther* 2006 January;316(1):62-70.
- (27) Li Y, Le MS, Xiao H, McClements DJ. Emulsion-based delivery systems for tributyrin, a potential colon cancer preventative agent. *J Agric Food Chem* 2009 October 14;57(19):9243-9.
- (28) Pomponi MG, Neri G. Butyrate and acetyl-carnitine inhibit the cytogenetic expression of the fragile X in vitro. *Am J Med Genet* 1994 July 15;51(4):447-50.
- (29) Pisano C, Pratesi G, Laccabue D et al. Paclitaxel and Cisplatin-induced neurotoxicity: a protective role of acetyl-L-carnitine. *Clin Cancer Res* 2003 November 15;9(15):5756-67.
- (30) Madiraju P, Pande SV, Prentki M, Madiraju SR. Mitochondrial acetylcarnitine provides acetyl groups for nuclear histone acetylation. *Epigenetics* 2009 August 16;4(6):399-403.
- (31) Chiechio S, Copani A, Zammataro M, Battaglia G, Gereau RW, Nicoletti F. Transcriptional regulation of type-2 metabotropic glutamate receptors: an epigenetic path to novel treatments for chronic pain. *Trends Pharmacol Sci* 2010 April;31(4):153-60.
- (32) Nasca C, Xenos D, Barone Y et al. L-acetylcarnitine causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors. *Proc Natl Acad Sci U S A* 2013 March 19;110(12):4804-9.
- (33) Montgomery SA, Thal LJ, Amrein R. Meta-analysis of double blind randomized controlled clinical trials of acetyl-L-carnitine versus placebo in the treatment of mild cognitive impairment and mild Alzheimer's disease. *Int Clin Psychopharmacol* 2003 March;18(2):61-71.
- (34) White HL, Scates PW. Acetyl-L-carnitine as a precursor of acetylcholine. *Neurochem Res* 1990 June;15(6):597-601.
- (35) Wawrzenczyk A, Nalecz KA, Nalecz MJ. Effect of externally added carnitine on the synthesis of acetylcholine in rat cerebral cortex cells. *Neurochem Int* 1995 June;26(6):635-41.

