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2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP–dependent metabolic regulation of chromatin structure

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Temporal lobe epilepsy is a common form of drug-resistant epilepsy that sometimes responds to dietary manipulation such as the 'ketogenic diet'. Here we have investigated the effects of the glycolytic inhibitor 2-deoxy-D-glucose (2DG) in the rat kindling model of temporal lobe epilepsy. We show that 2DG potently reduces the progression of kindling and blocks seizure-induced increases in the expression of brain-derived neurotrophic factor and its receptor, TrkB. This reduced expression is mediated by the transcription factor NRSF, which recruits the NADH-binding co-repressor CtBP to generate a repressive chromatin environment around the BDNF promoter. Our results show that 2DG has anticonvulsant and antiepileptic properties, suggesting that anti-glycolytic compounds may represent a new class of drugs for treating epilepsy. The metabolic regulation of neuronal genes by CtBP will open avenues of therapy for neurological disorders and cancer.

Epilepsy afflicts more than 50 million people worldwide, and more than 20 million of those affected continue to have seizures despite treatment with current antiepileptic drugs or surgery. Recurring behavioral seizures are not merely disruptive; they are accompanied by long-term co-morbidities such as memory, cognitive and affective dysfunction. Given that half of all drug-resistant individuals experience seizure control with dietary manipulation, such as isocaloric substitution of carbohydrates with fats and protein referred to as the 'ketogenic diet'[1], we considered that the pathways involved in energy metabolism might represent targets for pharmacological intervention to treat epilepsy.

It was noticed over 100 years ago that some individuals with epilepsy showed a progressive worsening of their condition, suggesting that "seizures beget seizures"[2]. The phenomenon of 'kindling' recapitulates many of the features of this progression[3] and has been used extensively to investigate the most common form of epilepsy—temporal lobe epilepsy. In the kindling model, repeated application of electrical stimuli generates brief electrographic seizures or afterdischarges, which are accompanied by initially focal behavioral seizures that gradually evolve into generalized tonic-clonic seizures and cause permanent alterations in neuronal circuitry. The mechanisms underlying this progression are not fully understood, but studies in mice have shown that the progression of kindling is impeded by deletion of the gene encoding BDNF and is blocked by deletion of that encoding its principal receptor TrkB, suggesting that these genes contribute to epileptogenesis[4]. The genes encoding BDNF and TrkB are among 1,800 in the mouse and human genome that possess a 23-base-pair (bp) sequence in their promoter regions known as a 'neuron restrictive silencing element' (NRSE)[5–7]. Genes with NRSEs are repressed in both neural and non-neural tissue by the transcription factor NRSF (neural restrictive silencing factor; also referred to as repressor of expression of sodium type II or REST; see ref. 8 and references therein). NRSF functions by recruiting co-repressor complexes to generate a chromatin environment that is repressive to transcription[9–14].

Chromatin is a complex of nucleic acid and protein comprising nucleosome repeats of 147 bp of DNA wrapped around two copies each of histones H2A, H2B, H3 and H4 (ref. 15). The amino (N)-terminal tails of core histones are subject to many modifications including acetylation and methylation that control the expression of genes on the associated DNA. Some histone modifications, such as acetylation of histone H3 on lysine 9 (H3-K9), are associated with active gene expression. Other marks, such as methylation of H3-K9, are associated with repressed or silent genes and are generated by the recruitment of co-repressor complexes[16]. Various metabolic intermediates act as small-molecule regulators of co-repressors and co-activators, thereby linking energy availability to chromatin structure and transcriptional output[17,18]. For example, glycolysis-derived NADH is known to be an allosteric regulator of the transcriptional co-repressor CtBP, which suggests that CtBP could function as a redox sensor that directly integrates metabolic demands with gene expression[19].

Here we show that administration of the glycolytic-inhibitor 2DG increases the afterdischarge threshold and potently reduces the progression of epileptogenesis by kindling in rats. Impaired epileptogenesis

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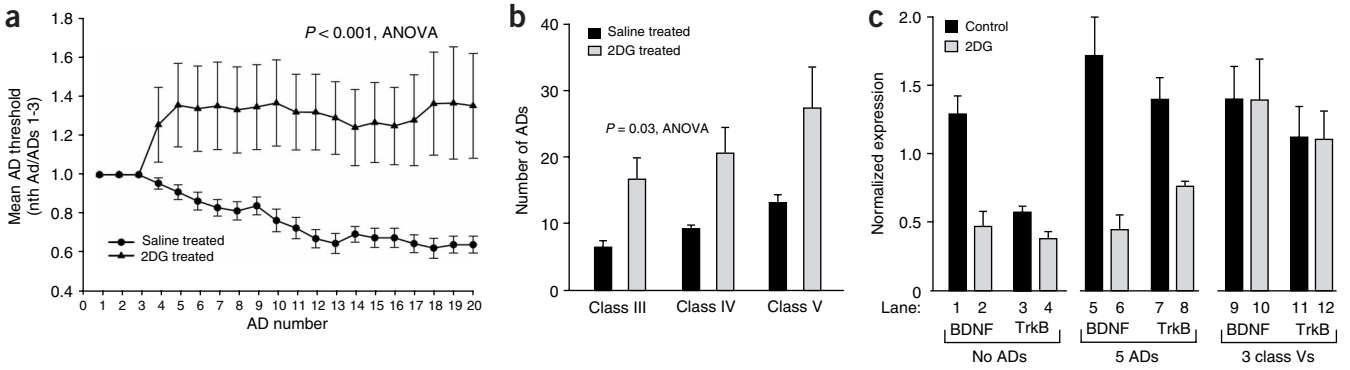

Figure 1 Glycolytic inhibition abrogates epileptogenesis *in vivo*. (**a**) Rats treated with saline 30 min before being subjected to 1-s 60-Hz stimulation trains showed a gradual reduction in afterdischarge (AD) threshold, expressed as a fraction of the mean stimulation current required to evoke first three afterdischarges before saline injections. By contrast, the afterdischarge threshold increased in rats treated with 2DG (250 mg/kg). (**b**) Rats treated with 2DG required more afterdischarges to evoke class III, IV or V behavioral seizures. (**c**) Rats administered 2DG or saline for either 2 weeks or until the fifth afterdischarge or third class V seizure were killed and QRT-PCR was done on hippocampal tissue with primers to BDNF, TrkB and actin. Values plotted are BDNF or TrkB expression over actin expression. Error bars represent s.e.m.

is associated with reduced upregulation of BDNF and TrkB expression in the hippocampus during seizures. Glycolytic inhibition results in an increase in recruitment of the CtBP co-repressor to NRSF-binding sites, together with localized inhibitory histone modifications (a hallmark of enhanced NRSF repression) both *in vitro* and *in vivo*. We also show that CtBP is a co-repressor of neuronal genes and that the interaction of NRSF with CtBP is NADH sensitive, a property that contributes to the metabolic regulation of neuronal gene expression involved in epilepsy.

To our knowledge, this is the first report to show that small-molecule regulation of energy metabolism *in vivo* has a direct impact on both chromatin structure and the expression of genes that are key to disease *in vivo*. We propose that 2DG may represent the founding member of a class of antiepileptic drugs that work by targeting energy metabolism to modulate gene expression through alterations in chromatin structure.

RESULTS

Glycolytic inhibition *in vivo* impairs epileptogenesis

Many individuals who do not achieve seizure suppression with conventional anticonvulsants experience seizure control with dietary manipulation such as the ketogenic diet. Given that seizure protection achieved by the diet is rapidly lost by ingestion of carbohydrates, we reasoned that glycolytic inhibition might be involved in the therapeutic action of ketogenic diets. We therefore investigated whether administration of a glycolytic inhibitor (2DG) afforded seizure protection in rats subjected to electrical kindling.

Treatment with the glycolytic inhibitor 2DG elicited the transcriptional hallmarks of reduced glycolysis in the hippocampus (**Supplementary Fig. 1** online). Saline-treated rats showed a gradual reduction in afterdischarge threshold from 866.7 ± 88.2 μA at the initiation of kindling stimulation to 589.9 ± 80.7 μA after repeated stimulations to the stage of class V seizures, consistent with the progressive features of kindling. By contrast, rats treated with 2DG (250 mg per kg, body weight) 30 min before kindling stimulation showed an increase in the stimulation current intensity required to evoke afterdischarges from 693.3 ± 85.9 to 750 ±116.5 μA during the progression to class V seizures. The effects of 2DG on afterdischarge threshold normalized to the initial afterdischarges before treatment with saline or 2DG were plotted as a function of afterdischarge number (**Fig. 1a**). The increase in afterdischarge threshold, a measure of the current required to evoke network synchronization and behavioral seizures, indicates an anticonvulsant effect of treatment with 2DG.

Prevention of the expected reduction in afterdischarge threshold in kindled rats treated with 2DG suggested that 2DG impairs kindling progression. This idea was confirmed by the observation that rats treated with 2DG required more evoked afterdischarges to reach the stage of class III, IV and V seizures (**Fig. 1b**). There were no differences in the mean afterdischarge duration in treated and control rats (40.1 ± 2.76 s for saline-treated rats and 43.15 ± 5.28 s for 2DG-treated rats), but the total cumulative seizure duration required to reach the stage of a class V seizure in the 2DG-treated group was correspondingly increased with the greater number of evoked afterdischarges (1,350 ± 391 s in rats treated with 2DG versus 637 ± 144 s in saline-treated controls). The increase in the number of afterdischarges required to reach this stage shows that 2DG reduces the progression of seizure-

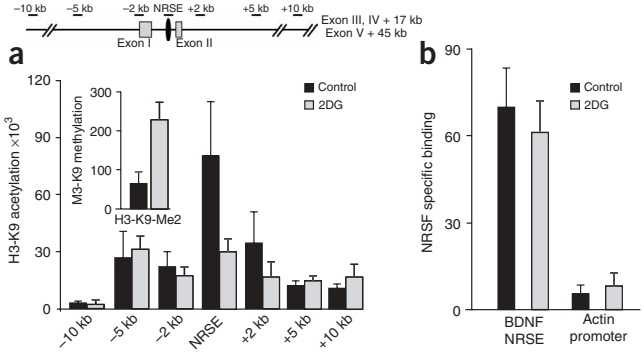

Figure 2 Glycolytic inhibition generates repressive chromatin over the NRSE *in vivo*. (**a**) Hippocampi from rats administered 2DG or saline for 2 weeks were subjected to ChIP with anti–H3-K9-acetyl or sham antibody and primers targeted to sequences flanking the NRSE of BDNF or a region 2-, 5- or 10-kb up- or downstream (top). Data are plotted as the quantity of DNA (in picograms) specifically precipitated (bottom). Inset, chromatin was precipitated with anti–H3-K9-dimethyl or sham antibody and interrogated with primers to sequences flanking the NRSE of BDNF. (**b**) Chromatin from **a** was precipitated with anti-NRSF or sham antibody and assayed with primers targeted to sequences flanking the NRSE of BDNF or the actin promoter. Data are plotted as in **a**. Error bars represent s.e.m.

induced plasticity and kindling. Notably, no adverse systemic effects or differences in body weight were observed between kindled rats treated for 6 months with 2DG and normal rats treated with 2DG at 500 mg per kg (twice the dose that produced anticonvulsant and antiepileptic effects). In addition, no performance differences in the Morris water maze were detected after doses as high as 2 mg per kg for 2 weeks (data not shown).

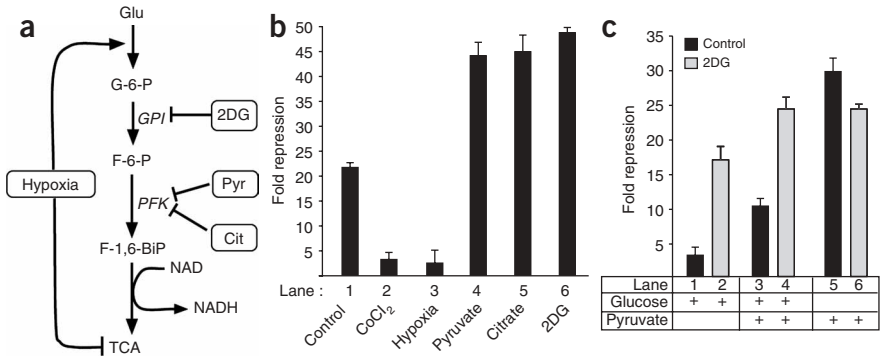

Figure 3 NRSF repression is regulated by metabolism. (**a**) Glycolytic pathway. Glu, glucose; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-1,6-BiP, fructose-1,6-bisphosphate; TCA, tricarboxylic acid cycle; GPI, glucose-phosphate isomerase; PFK, phospho-fructo-kinase; 2DG, 2-deoxy-glucose; Pyr, pyruvate; Cit, citrate. (**b**) JTC-19 cells were transfected with a G5-TATA-Luc reporter plasmid and plasmids expressing the Gal4 DBD or Gal4 fused to NRSF and incubated under the stated conditions for 16 h. Firefly luciferase measurements were normalized to co-transfected pRL-TK and are expressed as fold repression (Gal4 DBD/Gal4-NRSF). Data are the mean ± s.e.m. of at least three experiments performed in triplicate. (**c**) JTC-19 cells were transfected as in **b** but with one-fifth of the expression plasmid and incubated under the stated conditions. Data are the mean ± s.e.m. of at least three experiments performed in triplicate.

Glycolytic inhibition represses NRSF targets

Conditional deletion of the gene encoding BDNF or its main receptor TrkB in the hippocampus impairs or blocks kindling respectively[4]. We therefore examined whether BDNF and TrkB expression was altered in the hippocampus of rats treated with 2DG. Quantitative real-time PCR of reverse-transcribed RNA (QRT-PCR) showed that 2DG-treated rats had appreciably less hippocampal BDNF and TrkB expression than control rats (**Fig. 1c**, lane 1 versus 2 and lane 3 versus 4; see **Supplementary Fig. 2** online for western blotting). These differences in expression of BDNF or TrkB in the absence or presence of 2DG were present after five afterdischarges, in association with the difference in afterdischarge threshold between 2DG-treated and control rats (**Fig. 1c**, lane 5 versus 6 and lane 7 versus 8). There was no difference in expression between 2DG-treated and control rats that experienced three class V seizures (**Fig. 1c**, lane 9 versus 10 and lane 11 versus 12), however, suggesting that 2DG blocks the seizure-induced increases in BDNF and TrkB expression that underlie progression to the stage of class V seizures.

Glycolytic inhibition alters chromatin structure around NRSEs

The genes encoding BDNF[20] and TrkB (**Supplementary Fig. 3** online) are under the control of NRSF, which represses transcription, in part, by recruiting histone deacetylases and methylases to genes to generate localized regions of unacetylated and methylated histones)[14]. To ascertain whether NRSF contributes to the differential expression of epilepsy genes in the presence or absence of 2DG, we carried out a scanning chromatin immunoprecipitation (ChIP)[14] assay over 20 kb spanning the BDNF NRSE for diacetylated H3-K9 using hippocampal tissue extracted from 2DG-treated rats. The NRSE was the only amplicon that showed a marked reduction in H3-K9 acetylation in 2DG-treated rats as compared with controls (**Fig. 2a**). At the NRSE of BDNF, there was also a robust increase in H3-K9 methylation on 2DG treatment (**Fig. 2a**), consistent with a decrease in BDNF expression and the known interaction between NRSF and the G9a histone methylase[14,21]. We found that NRSF itself was bound to the NRSE of BDNF *in vivo*, but not to the actin promoter, and its binding was not effected by 2DG administration (**Fig. 2b**). These results indicate that the transcription factor NRSF may mediate the downregulation of transcription and contribute to the antiepileptic effects of 2DG *in vivo* through the regulation of BDNF and TrkB.

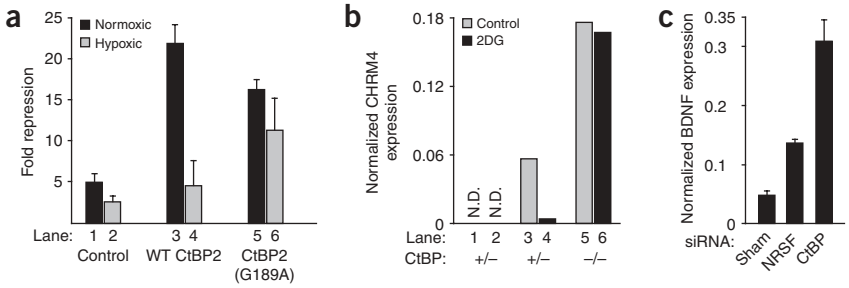

Figure 4 CtBP confers metabolic regulation on NRSF. (**a**) JTC-19 cells were transfected with a G5-TATA-Luc reporter plasmid, limiting amounts of Gal4 DBD or Gal4 fused to NRSF (hence the lower repression in lanes 1 and 2 as compared with **Figure 1**) and plasmid expressing wild-type or mutant CtBP2. Cells were placed under normoxic or hypoxic conditions for 16 h and assayed as in **Figure 1b**. (**b**) Wild-type MEFs, or MEFs heterozygous for both CtBP1 and CtBP2 ($CtBP1^{-/+}$ $CtBP2^{-/+}$), or homozygous for both CtBP1 and CtBP2 ($CtBP1^{-/-}CtBP2^{-/-}$) grown in the absence or presence of 1 mM 2DG were analyzed for CHRM4 and HPRT expression by QRT-PCR. RNA was analyzed as in **Figure 1c** and is plotted as CHRM4 over HPRT1 expression. N.D., none detectable. (**c**) Normal murine mammary gland cells transfected with sham siRNA or siRNA targeted to NRSF or CtBP1 and CtBP2 were analyzed for BDNF and actin expression by QRT-PCR. RNA was analyzed as in **b**. Error bars represent s.e.m.

Metabolism regulates repression of NRSF

The localization of chromatin changes to the NRSF-binding site on 2DG treatment led to examine whether the ability of NRSF to repress transcription is itself controlled by glycolytic rate. To address this issue, we used the fibroblastic cell line JTC-19, a well-characterized model for studying NRSF repression[7,14,22,23]. We transfected JTC-19 rat lung fibroblasts with a plasmid expressing NRSF fused to the Gal4 DNA-binding domain (DBD) and a Gal4-responsive reporter, and incubated cells under conditions of increased or reduced glycolysis (**Fig. 3a**). Under increased glycolytic flux and reduced oxidative respiration (such as in $CoCl_2$ or hypoxia), NRSF-mediated repression was abrogated (**Fig. 3b**, lanes 2 and 3 versus lane 1). Conversely, addition of the glycolytic inhibitors pyruvate, citrate or 2DG increased repression

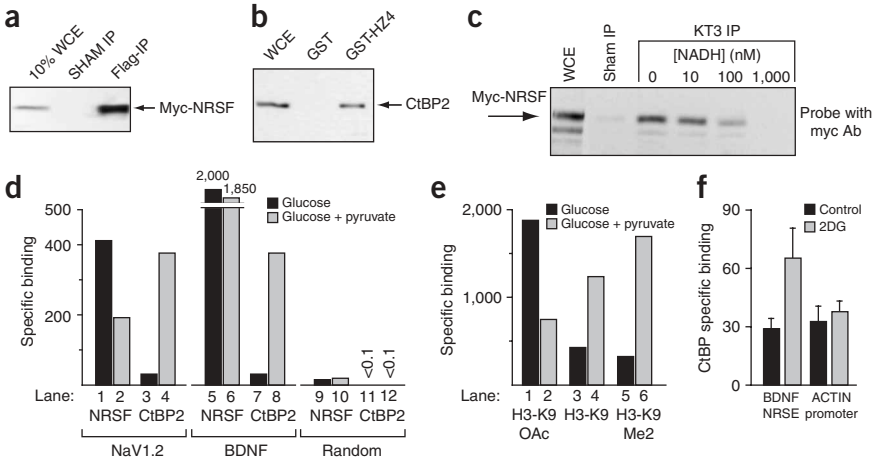

Figure 5 NRSF and CtBP interact *in vivo*. (**a**) HEK cells were co-transfected with plasmids expressing Myc-tagged NRSF or Flag-tagged CtBP2. Protein was collected and immunoprecipitated with anti-Flag or sham antibody (anti–goat IgG). Immune complexes were washed with RIPA buffer and subjected to western blot analysis with antibody to Myc. (**b**) Either GST fused to the HZ4 fragment of NRSF or GST alone was incubated with purified recombinant His_6-tagged CtBP2, washed and subjected to western blot with antibody to CtBP2. WCE, whole-cell extract (1% of the amount used in the pull-down assay). (**c**) HEK cells were co-transfected with plasmids expressing Myc-tagged NRSF or KT3-tagged CtBP2. Protein was collected, incubated with the indicated concentrations of NADH and immunoprecipitated with anti-KT3 or sham antibody (anti-Jun) . Immune complexes were analyzed as in **a**. (**d**) ChIP was done on JTC-19 cells grown in the presence or absence of pyruvate with antibodies to NRSF or CtBP2. Precipitated DNA was subjected to QRT-PCR with primers flanking the NRSEs of the BDNF and NaV1.2 genes or a sequence with no NRSE. Data are plotted as the quantity of DNA (in picograms) specifically precipitated. (**e**) ChIP was done on JTC-19 cells as in **d** with antibodies to acetylated, dimethylated or unmodified H3-K9 and with primers flanking the NRSE of BDNF. (**f**) Hippocampi from rats administered 2DG or saline for 2 weeks were subjected to ChIP with antibodies to CtBP1 and CtBP2 or sham antibody and primers flanking the NRSE of BDNF or the actin promoter. Data are plotted as in **d**.

(lanes 4–6 versus lane 1). We used a second reporter assay to assess the degree to which augmentation of repression by 2DG was due to the ability of 2DG to inhibit glycolysis rather than another, uncharacterized property of the molecule. With limiting quantities of Gal4-NRSF, 2DG resulted in increased repression when cells were given glucose or glucose and pyruvate as a carbon source (**Fig. 3c**, compare lanes 1 and 2 or 3 and 4). By contrast, 2DG failed to augment repression by NRSF in cells grown in pyruvate alone and thus not undergoing glycolysis[24] (**Fig. 3c**, lanes 5 and 6). These results show that inhibition of glycolysis is the mechanism by which 2DG increases NRSF-mediated repression.

Chromosomal NRSF target genes were also repressed by 2DG, as assessed by QRT-PCR, whereas actin was unaffected (**Supplementary Fig. 4** online). These results show that NRSF-mediated repression is under metabolic control and are consistent with the hypothesis that NRSF mediates the repressive effects of 2DG on hippocampal expression of BDNF and TrkB *in vivo*.

Because CtBP confers metabolic regulation on the transcription factors ZEB[19] and Hdm2 (ref. 25), we tested whether CtBP might also confer metabolic control on NRSF. Co-transfection of CtBP increased NRSF-dependent repression (**Fig. 4a**, compare lanes 1 and 3) and this repression was sensitive to hypoxia (compare lanes 3 and 4). CtBP exists as two isoforms in vertebrates (CtBP1 and CtBP2). Because expression of either CtBP1 or CtBP2 gave identical results (data not shown), all subsequent experiments were done with CtBP2. Coexpression of CtBP2 bearing a point mutation of a critical glycine residue required for NADH binding (G189A)[25] augmented NRSF repression (**Fig. 4a**, lanes 1 and 5), but hypoxia did not abrogate repression (compare lanes 5 and 6). This result suggests that NADH is the metabolite detected by the NRSF complex as a readout, or proxy, for metabolic state.

To test whether CtBP was required for NRSF repression of chromosomal genes, the expression of NRSF target genes was assayed in mouse embryonic fibroblasts (MEFs) heterozygous or homozygous for deletions of both CtBP1 and CtBP2. The NRSF target gene *Chrm4* was expressed in $CtBP1^{-/+}CtBP2^{-/+}$ cells but not in wild-type cells (**Fig. 4b**). Its expression was further increased in $CtBP1^{-/-}CtBP2^{-/-}$ cells, consistent with a dose-responsive co-repressor function for CtBP (**Fig. 4b**, compare lanes 1, 3 and 5). The same de-repression was observed for the NRSF target gene *Mbp* (**Supplementary Fig. 5** online). Notably, CHRM4 expression was repressed on glycolytic inhibition by 2DG in $CtBP1^{-/+}CtBP2^{-/+}$ cells (**Fig. 4b**, compare lanes 3 and 4), whereas expression was not altered by 2DG in $CtBP1^{-/-}CtBP2^{-/-}$ cells (lanes 5 and 6). In addition, short interfering RNA (siRNA) targeted to either NRSF or CtBP1 and CtBP2 in normal murine mammary gland cells (NMuMGs) relieved silencing of BDNF, showing that this NRSF target is also a CtBP target (**Fig. 4c**). These results show that CtBP is required both for repression of chromosomal NRSF targets and for their metabolic regulation, and implicate CtBP as a potential component of the regulatory mechanisms underlying the *in vivo* effects of 2DG.

If CtBP is a redox sensitive co-repressor for NRSF, then the two proteins would be predicted to interact. HEK cells transfected with plasmids expressing Myc-tagged NRSF and Flag-tagged CtBP2 were tested by co-immunoprecipitation. Myc-NRSF associated with CtBP2 (**Fig. 5a**), and glutathione *S*-transferase (GST) fused to the HZ4 fragment of NRSF[9] bound purified CtBP2 expressed in *Escherichia coli* under conditions in which GST alone did not (**Fig. 5b**), showing that NRSF and CtBP form complexes in living cells and interact directly. As NADH concentrations regulate CtBP2 binding to many transcription factors[19,25,26] and the non-NADH binding G189A CtBP2 mutant abolishes metabolic sensitivity of NRSF function (**Fig. 4a**), we tested CtBP2 binding to NRSF as a function of NADH concentration (**Fig. 5c**). Increasing concentrations of NADH disrupted binding of CtBP2 to NRSF in a dose-dependent manner. Half-maximal binding occurred at a physiologically relevant concentration of $\sim$ 70 nM (data not shown).

We used ChIP to assay direct binding of CtBP2 to NRSF target genes under conditions of glycolytic inhibition. Addition of pyruvate to the growth media (to reduce glycolysis and thus reduce cytoplasmic and/or nuclear NADH) increased CtBP2 occupancy at the NRSEs of NaV1.2 and BDNF (**Fig. 5d**). There was no change in CtBP2 occupancy at a 'random' site lacking an NRSE, indicating that CtBP2 recruitment is localized to the NRSE. To assess the impact of CtBP recruitment on chromatin status around the NRSE, we assessed acetylation and methylation at H3-K9. The reduction in glycolysis decreased H3-K9 acetylation and increased H3-K9 dimethylation around the NRSE of BDNF (**Fig. 5e**). These *in vitro* results recapitulate the chromatin alterations seen at the hippocampal BDNF locus *in vivo* on administration of 2DG.

These results predict that CtBP should be present at the NRSF-binding site of BDNF in rat hippocampus and that occupancy at this site should be augmented in the presence of 2DG. To test this prediction, we carried out ChIP assays using antibody to CtBP on chromatin isolated from 2DG-treated rats as above. CtBP was indeed present at the NRSE of BDNF in rat hippocampus and occupancy was increased in rats treated with 2DG (**Fig. 5f**). Together, these results show that glycolytic rate influences seizure threshold and regulates the expression of epilepsy-related genes and chromatin status *in vivo*. These effects are mediated through NADH-labile recruitment of the co-repressor CtBP to NRSF target genes.

DISCUSSION

2DG is an anticonvulsant, and downregulates BDNF and TrkB expression

We have shown that 2DG has anticonvulsant and antiepileptic properties as indicated by the increase in the AD threshold and decrease in the progression of kindling, respectively, suggesting that targeting pathways of energy metabolism can control seizures. 2DG differs from glucose in that a hydroxyl group at the C2 in glucose is replaced by a hydrogen atom. For over half a century[27], 2DG has been used as a specific inhibitor of glycolysis from bacteria to humans and has been shown to inhibit glycolysis in many tissues, including brain, by preventing glucose transport and the isomerization of glucose-6-phosphate to fructose-6-phosphate (see ref. 28 and references therein). We found that the effect of 2DG on transcriptional repression by NRSF requires ongoing glycolysis (**Fig. 3c**), and that 2DG treatment results in transcriptional changes in glycolytic genes that indicate reduced glycolysis *in vivo* (**Supplementary Fig. 1**). Although it remains possible that the effects of 2DG observed *in vivo* may result from an unidentified property of this molecule, our results are most easily explained by its glycolysis-inhibiting properties.

Conditional knockouts of BDNF and TrkB in mice have implicated neurotrophin signaling in the progression of kindling, and we have now shown that 2DG reduces expression of the NRSF target genes encoding BDNF and TrkB. 2DG decreases BDNF and TrkB expression in normal rats and prevents or reduces seizure-induced increases in BDNF and TrkB expression in rats during the early stages of kindling, such as after five afterdischarges. Because 2DG did not reduce expression in rats experiencing class V seizures, our results implicate downregulation of the BDNF and TrkB signaling pathway by 2DG in the therapeutic effects on kindling progression to class V seizures.

Mice heterozygous for BDNF show a twofold decrease in the rate of kindling, as measured by the number of afterdischarges to class V seizures[29]. 2DG treatment also results in an approximately twofold reduction in BDNF expression (which phenocopies expression in heterozygous mice) and a corresponding twofold reduction in the rate of kindling. The BDNF and TrkB signaling pathway has been implicated in various cellular alterations induced in neural circuits by experimental seizures and kindling[3,30], including mossy fiber sprouting, strengthening of mossy fiber–CA3 synapses[31–34], recurrent neuronal circuits that promote hyperexcitability[35–38], and synaptic (long-term) potentiation. Although the functional progression of kindled seizures is undoubtedly under complex regulatory control, our results provide additional support for the idea that BDNF and TrkB signaling is an important determinant of kindling progression.

The observation of anticonvulsant and antiepileptic effects suggest that 2DG may have considerable potential as a therapeutic agent for epilepsy. The therapeutic actions of the ketogenic diet[39] as a treatment for poorly controlled epilepsy may be partly due to metabolic regulation of NRSF function. A chief focus of epilepsy research and justification for elucidating the mechanisms behind epileptogenesis is the possibility of generating small molecules with which to treat epilepsy. Given that BDNF expression is increased in the hippocampus of individuals with temporal lobe epilepsy[40] and 2DG administration has been well tolerated in humans[41], we propose that 2DG may represent the founding member of a class of antiepileptic drugs that work by targeting energy metabolism to alter chromatin structure and gene expression.

CtBP is a NRSF co-repressor

We have shown that CtBP is a co-repressor of neuronal genes in both non-neuronal cells and neural tissue. Several observations lead us to define CtBP as a metabolism-sensing co-repressor of neuronal genes. First, CtBP recruitment to the NRSE of hippocampal BDNF *in vivo* was enhanced by glycolytic inhibition. Second, CtBP overexpression augmented the ability of NRSF to repress reporter gene expression in a metabolism-sensitive manner. Third, CtBP deletion in MEFs resulted in de-repression of endogenous, chromosomal neuronal genes. Last, CtBP and NRSF directly interacted in an NADH-labile manner. By recruiting CtBP as a co-repressor, the NRSF complex can respond to the metabolic state of the cell. Similar to the CtBP-bromodomain interaction[26], the CtBP-NRSF interaction was disrupted by increasing concentrations of NADH (by increasing glycolysis) and half-maximal binding occurred at 70 nM (data not shown). Given that the free NADH concentration is about 100 nM (ref. 19), the NRSF-CtBP complex is poised to respond to physiologically relevant dinucleotide concentrations.

The NAD^+-dependent class of histone deacetylases (SIRTs) have been shown to confer metabolic regulation on transcription and thus link caloric restriction with longevity, stress and cancer[18,42,43]. We have found that NRSF does not co-immunoprecipitate with SIRT1 and that NRSF-mediated repression is not sensitive to resveratrol. In addition, SIRT1 is not found at chromosomal NRSEs, as assessed by ChIP (data not shown). The sirtuin class of histone deacetylases are therefore not directly involved in metabolic regulation of NRSF-regulated genes.

Implications of NRSF/CtBP metabolic sensing for other disorders

TrkB and BDNF are upregulated in tumor cells to prevent anoikis (apoptosis on loss of cell-matrix contact), to promote cell survival and to aid metastasis[44,45]. As a consequence of CtBP-mediated metabolic control of NRSF repressor function, it is possible that the Warburg effect (an increase in glycolytic flux in transformed cells)[46] increases NADH and relieves NRSF repression of TrkB and BDNF. In addition, as NRSEs are present in genes implicated in drug resistance (such as *MDR3*), metastasis and migration (such as *MTA1*[7,47]), NADH-dependent loss of NRSF repression of neuronal genes may unify the observations of increased glycolysis in transformed cells, induction of neuronal markers, and acquisition of anoikis suppression and metastasis. These predictions suggest that inhibiting glycolysis with relatively nontoxic molecules such as 2DG or 3-bromopyruvate may re-establish silencing of metastasis-related genes and increase survival rates of individuals with cancer. The prediction that NRSF suppresses oncogenesis has been supported by isolation of NRSF as a tumor suppressor in a high-throughput screen for genes controlling cellular transformation[48].

In summary, the discovery that NRSF-mediated repression is dynamic and regulated by glycolysis will open avenues of therapeutic intervention for many diseases including neurological disorders and cancer.

METHODS

Cell line maintenance and transfections. HEK293, JTC-19 and MEFs cells were maintained and transfected as described[9].

ChIP. ChIP assays were done as described[14]. For CtBP ChIP assays using hippocampal tissue, equal quantities of CtBP1 and CtBP2 antibody were combined.

Immunoprecipitation and GST pull down. Immunoprecipitation and GST pull-down assays were done as described[9]. Details are available from the author on request.

Rat handling and kindling. Methods for *in vivo* recording and kindling have been described[49,50]. For details, see **Supplementary Fig. 6** online.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

A.R. conceived the idea, co-designed the study, contributed experimental data and co-wrote the paper. T.S. co-designed the study and co-wrote the paper. M.G.-C., B.S., R.Q., K.B., T.J.D., R.M.P., J.F.M., J.O. and C.S. contributed experimental data.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Neuroscience* website for details).

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