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Review

Histone modifications in status epilepticus induced by kainate

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Summary. Animal models of epilepsy have allowed the determination of the basic molecular and cellular mechanisms of epileptogenesis. Generalized limbic seizures and subsequent status epilepticus can be induced by either pilocarpine, the muscarinic acetylcholine receptor agonist or kainate, the glutamate receptor agonist. There has been increasing interest that chromatin remodeling might play a critical role in gene regulation even in non-dividing cells such as neurons. One form of chromatin remodeling is histone amino-terminal modification that can generate synergistic or antagonistic affinities for the interactions of transcriptional factors, in turn causing changes in gene activity. Two widely studied histone modification processes are histone acetylation and phosphorylation. While histone hyperacetylation indicates an increase in gene activity, its hypoacetylation marks gene repression. Both states are controlled by a dynamic interplay of histone acetyltransferase (HAT) and histone deacetylase (HDAC). We have found the upregulation of acetylation and phosphorylation of histones, coupled with status epilepticus after kainate administration. *c-fos* and *c-jun* mRNA have been sequentially induced in response to kainate, in different hippocampal subpopulations starting from the dentate gyrus and spreading to the cornus ammonis regions well correlated with the spatio-temporal distribution of histone H4 hyperacetylation. Both histone modifications are associated with the *c-fos* gene promoter after kainate stimulation, while only histone acetylation with the *c-jun* gene. Pretreatment with curcumin, which has a HAT inhibitory activity specific for CBP/p300, attenuates histone modifications, IEGs expression and also the severity of status epilepticus after kainate treatment. Histone modifications may have a crucial role in the development of epilepsy induced by kainate.

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Introduction

Seizures are the most extreme form of synchronous brain activity. When a person experiences repeated seizures, the condition is known as epilepsy. About 1% of the US population has epilepsy. The behavioral features of a seizure depend on the neurons involved. Most forms of generalized seizures show that consciousness is lost, while all muscle groups may be driven by tonic (ongoing) activity or by clonic (rhythmic) patterns or by both in sequence. Animal models of epilepsy have allowed the determination of the basic molecular and cellular mechanisms of epileptogenesis. Generalized limbic seizures and subsequent status epilepticus can be induced by either pilocarpine, the muscarinic acetylcholine receptor agonist or kainate, the glutamate receptor agonist (Turski et al., 1984; Ben-Ari, 1985). Systemic or intracerebral injection of kainate causes epileptiform seizures in the CA3 region of the hippocampus and these seizures propagate to other limbic structures. Several observations suggest that the epileptogenic effects of kainate in CA3 are largely caused by the activation of high-affinity kainate receptors that are expressed in the mossy fiber synaptic region (Monaphan and Cotman, 1982; Represa et al., 1987). The CA3 region is considered to be the pacemaker for the generation of synchronized activities.

Prolonged seizures induced by pilocarpine or kainate trigger numerous changes in gene expression that are thought to be the development of epilepsy (Elliott et al., 2003). The mechanisms underlying seizure-induced changes in gene expression are unknown but could involve regulation of transcription or mRNA stability. We and others have recently described that either drug-induced or electrostimulated seizures influence histone modifications in vivo (Huang et al., 2002; Crosio et al.,

2003; Tsankova et al., 2004; Sng et al., 2006). In this article, we will focus on recent findings suggesting the involvement of histone modifications induced by kainate not only in gene expression but also in the development of epilepsy.

Histone modifications in the regulation of gene expression

Chromatin remodeling, including histone modifications, might play a crucial role in gene regulation even in non-dividing cells such as neurons. Histones associate extensively with DNA in the nuclei of eukaryotic cells. DNA is packaged with an octamer of highly conserved histones, two H2A-H2B dimer and H3-H4 tetramer in every 1.75 turns of 146 bp, an assembly known as a nucleosome. Nucleosomes are connected to other nucleosomes with 10-90 bp of linker DNA and stabilized by binding of a fifth histone H1. There is further folding of the chromatin fiber into higher-order structure so as to package eukaryotic genomes within the nuclei.

Each core histone, H2A, H2B, H3 and H4, is composed of a structured domain and an unstructured amino-terminal tail of varying lengths from 16 amino acid residues for H2A, 32 for H2B, 44 for H3 and 26 for H4. The most compact form of chromatin is called heterochromatin or off state inaccessible to regulatory signals. To achieve a euchromatin or on state, the chromatin has to unpack to expose cis regulatory sequences such as enhancer-binding sites. The core histone amino-termini tails are susceptible to a wide range of covalent modifications including acetylation, phosphorylation, methylation and ADP-ribosylation (Jenuwein and Allis, 2001; Berger, 2002) (Fig. 1). Ubiquitination can occur at the carboxy-terminal tails of H2A and H2B. When subjected to covalent modifications by a variety of cellular factors, they are proposed to act as signals from DNA to cellular machinery for various processes including transcription and chromatin condensation.

Two widely studied histone modifications are histone acetylation and phosphorylation. Histone acetylation is a reversible modification of lysine residues within the amino-terminal tail domains of core histones. Histone acetyltransferase (HAT) transfers an acetyl-group from acetyl-coenzyme A to the ε-amino group of the lysine residue, while histone deacetylase (HDAC) acts in the reverse to remove the acetyl group (Mizzen et al., 1998; Davie and Spencer, 1999). Acetylation results in charge neutralization of the basic histone tails to weaken histone-DNA interactions for accessibility to gene locus for nuclear factors. Just like the effects of histone acetylation on the chromosomal structure, the potential effects of histone phosphorylation could be that the addition of a negatively charged phosphate group may disrupt electrostatic interactions between basic histone tails and DNA backbone. Phosphorylation of histones H1 and H3 also occur at the M-phase in which chromosomal condensation is observed. Nuclear MSK and RSK, downstream signaling molecules of ERK, yeast SNF1 kinase and drosophila Jil-1 kinase are identified as kinases that phosphorylate histone H3 (Cheung et al., 2000; Davie and Spencer, 2001). Conversely, protein phosphatase dephosphorylate from these residue sites.

Immediate early genes (IEGs) belong to a class of genes that are rapidly induced, usually in a transient fashion, in response to intracellular signaling cascades. Among IEGs, *c-fos* and *c-jun* genes induction by several different stimulations have been associated with histone modifications. *c-fos* induction in EGF-treated 10T1/2 cells is associated with histone H3 phosphorylation (Strelkov and Davie, 2002), while both of *c-fos* and *c-jun* inductions in anisomycin-treated 10T1/2 cells are associated with both histone H3 phosphorylation and H4 acetylation in vitro (Barratt et al., 1994; Clayton et al., 2000).

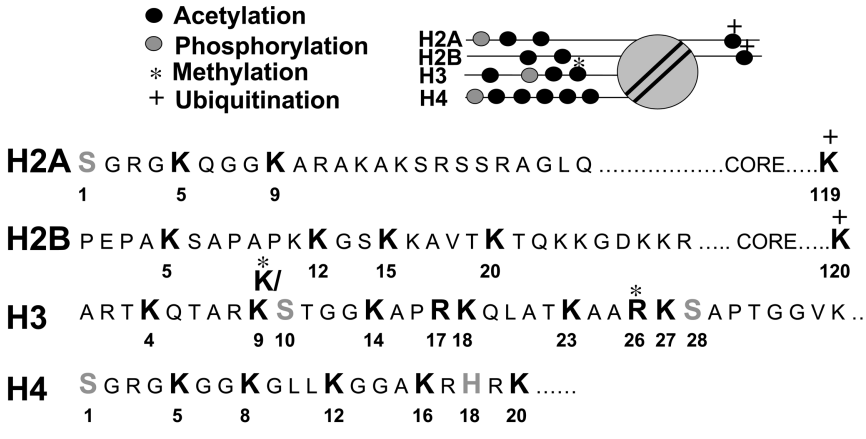

Fig. 1. Histone modifications. Histone tails are susceptible to a wide range of cocalent modifications including acetylation, phosphorylation, metylation and ubiquitination. The amino acid residues of histone tails represented as letters S for serine, K for lysine and R for arginine, are possible residues to modify. Histone H3 tail lysine 9 residue can be modified by both acetylation and metylation though not at the same time. Metylation can occur at lysine 79 in the core of histone H3. Ubiquitination occurs at histones H2A and H2B carboxyl tails of lysine residues 119 and 120, respectively.

Histone modifications and IEG expression in kainate-induced status epilepticus

We and others have recently described that either drug-induced or electrostimulated seizures influence histone modifications in vivo. Huang et al. have showed the reduction of histone H4 acetylation at the GluR2 gene promoter but increased at the BDNF gene promoter after induction of status epilepticus by pilocarpine in rat hippocampal CA3 neurons (Huang et al., 2002). Tsankova et al. have demonstrated that electroconvulsive seizures, produced by repetitive electronic stimulation, induce increased H4 acetylation at *c-fos* and BDNF genes promoters while decreasing at the CREB gene promoter in correlation with mRNA levels of these genes (Tsankova et al., 2004). Crosio et al. have reported a rapid, transient induction of histone H3 phosphorylation in the dentate gyrus (DG) of the hippocampus detectable 15 min after pilocarpine or kainate stimulation (Crosio et al. 2003). Induction of histone H3 phosphorylation and *c-fos* expression occurred concomitantly in the same hippocampal neurons in response to the drugs and a two-fold increase in the levels of phosphorylated H3 was detected on *c-fos* gene by chromatin immunoprecipitation assay (ChIP assay). We have observed not only transient increase in histone H3 phosphorylation but also histone H4 acetylation after kainate administration (Sng et al., 2006). After kainate injection, histone H3 phosphorylation was increased significantly by 9.6-fold at 0.5 h, its levels decreased to 2.9-fold at 3 h and returned to control values by 6 h. Histone H4 acetylation was also increased by 7.7-fold at 0.5 h, 13.2-fold at 3 h and sustained by 7.1-fold at 6 h. Seizure responses of mice after kainate injection can be evaluated by seizure score as follows: 1, arrest of motion; 2, myoclonic jerks of the head and neck, with brief twitching movements; 3, unilateral clonic activity; 4, bilateral forelimb tonic and clonic activity; 5, generalized tonic-clonic activity with loss of postural tone; 6, Death from continuous convulsions (Schauwecker and Steward, 1997; Yang et al., 1997). Within 15 min of injection, mice assumed a catatonic posture accompanied by staring behavior proceeding with myoclonic twitching and often frequently accompanied by rearing and falling (score at 0.5 h, 1.88±0.88). After 1 h of kainate administration, mice exhibited continuous tonic-clonic seizures, developing into full seizures in the next 2 h (score at 3 h, 5.10±0.8). Out of thirty mice analyzed during 0.5–3 h, six mice died from continuous convulsions. After 4-6 h, the mice were observed in a hunched position and remained immobile (score at 6 h, 2.56±0.6). We have observed that the seizure responses concomitantly occurred with histone modifications.

Kainate injection induces the expression of *c-fos* and *c-jun* mRNA. According to our observation, *c-fos* mRNA was induced from 0.5 h by 1.8-fold and continued to peak at 3 h by 13.1-fold in the hippocampus over control at 0 h. Expression kinetics of *c-jun* mRNA was congruent with that of *c-fos* with its significant increase by 6.0-fold at 3 h in the hippocampus after kainate stimulation. We have observed that *c-fos* mRNA induction was initiated in the DG at 0.5 h, increased by 19.2-fold relative to 0 h, sustained by 16.3-fold at 3 h and back to almost the basal level (1.6-fold) at 6 h by in situ hybridization analysis. In the CA regions, *c-fos* mRNA was induced significantly at 3 h by 11.6-fold in CA 1-2 and 10.4-fold in CA 3 regions, and continued to maintain its expression by 10-fold in all CA regions up to 6 h. *c-jun* mRNA expression was detected at 0 h (4.7-fold at 0 h in the DG and 2-fold in the CA regions), the fold increase of *c-jun* was only 2.8-fold (compared to 0 h) at 0.5 h in the DG, increasing by 3.6-fold at 3 h and decreasing its intensity to 1.8-fold by 6 h. The mRNA expression of *c-jun* was increased significantly by 4.4-fold in the CA 1-2 regions and 5.3-fold in the CA 3 region at 3 h. It continued to be maintained at 4.5-fold in the CA 1-2 regions and 4.8-fold in the CA 3 region at 6 h. The sequential inductions of both *c-fos* and *c-jun* genes within the hippocampal subregions are identical: The DG is the first structure to respond to kainate followed by the CA regions in the order of CA 3, CA 2 leading to CA 1. This phenomenon can be explained by the neural signals transmission to the hippocampus through a perforant pathway, which connects to an excitatory tri-synaptic chain propagating the onset of seizure to other limbic structures (Lothman et al., 1981; Le Gal La Salle, 1988; Liu et al., 1996).

Immunohistochemical analysis has shown a marked 4.6-fold increase in intensity of histone H4 acetylation immunostaining relative to 0 h in the DG and the CA regions at 0.5 h that continued to intensify at 3 h (DG, 4.3-fold; CA 1-2, 4.9-fold; CA 3, 3.4-fold) and returned back to almost the basal level by 6 h. In contrast, histone H3 phosphorylation transiently appeared at 0.5 h in the DG (3.4-fold). The spatial and temporal upregulation pattern of histone H4 acetylation and H3 phosphorylation is well correlated with the expressions of the *c-fos* and *c-jun* genes after kainate stimulation. Histone H4 acetylation at the *c-fos* gene in the hippocampus was rapidly increased to 5-fold over control values within 0.5 h after kainate treatment by ChIP assay. In addition, there was an early transient 6.6-fold increase of histone H3 phosphorylation at 0.5 h. At 3 h, H4 acetylation was 6.6-fold over control values and returned to almost its basal level by 6 h (1.6-fold over control values). Thus, kainate stimulation alters both phosphorylation and acetylation states of histones that are associated with the *c-fos* gene activation in hippocampal neurons. In contrast, there was no significant change in histone acetylation (1.2-1.3-fold over control values) at the *c-jun* gene over time after kainate injection. There was no upregulation of histone phosphorylation detected at the *c-jun* gene. Kainate stimulation does not induce significant changes in preexisting H4 acetylation and H3 phosphorylation at the *c-jun* gene.

Effects of trichostatin A (TSA), a HDAC inhibitor, and curcumin, a HAT inhibitor on genes expression and seizure behavior in kainate-induced status epilepticus

Histone acetylation is dynamically regulated by HAT and HDAC (Fig. 2). HAT transfers an acetyl-group to the ε-amino group of the lysine residue, while HDAC acts in the reverse to remove the acetyl group. TSA is a potent HDAC inhibitor that is frequently used to test the role of histone hyperacetylation in the transcriptional regulations (Yoshida et al, 1990). We have demonstrated that intraperitoneal administration of TSA in mice increased the basal level of acetylated histone H4 in hippocampus by 2-fold after 2 h (Sng et al., 2005), suggesting that the basal level of acetylated histone H4 is suppressed by HDAC. Pretreatment with TSA increased the sensitivities of *c-fos* and *c-jun* genes expression to kainate stimulation. The expressions of *c-fos* and *c-jun* mRNA by TSA pretreatment were induced by 1.55 and 1.76-fold, and 1.23 and 1.28-fold compared with vehicle pretreatment 3 and 6 h after kainate stimulation, respectively. There are almost no differences of seizure scores between TSA and vehicle pretreatment, but we have detected an increased seizure score significantly but slightly by TSA treatment at 6 h after kainate stimulation compared to vehicle pretreatment.

Curcumin is extracted from *Curcuma longa* rhizome, which is used as a yellow pigment in tumeric and curry. Curcumin has been found to inhibit different enzymes including HIV-1 integrase, JNK and p38 MAP kinase (Chen and Tan, 1998; Cheng et al., 2003). It has been found that curcumin inhibited specifically p300/CBP HAT activity but not other HAT enzymes, and effectively inhibits the acetylation of histones (Balasubramanyam et al., 2004). We have shown that curcumin pretreatment inhibited the kainate-induced histone H4 hyperacetylation (Sng et al., 2006). Its levels remained at 0.68-1.07-fold at all the time points over control at 0 h, while vehicle pretreatment showed kainate-induced hyperacetylation at the corresponding time points, 7.6-fold at 0.5 h, 13.7-fold at 3 h and 7.9-fold at 6 h. We have also found that curcumin pretreatment decreased the level of histone H3 phosphorylation, retaining between 0.97-1.2-fold change over control at 0 h after kainate stimulation. While vehicle-pretreated mice showed a higher induction of *c-fos* and *c-jun* mRNA levels compared to no treatment, we have found that curcumin-pretreated mice showed decreased *c-fos* mRNA expression at 0 h (0.52-fold over control at 0 h), 3.37-fold (in parallel to Vehicle, 6.2-fold) at 0.5 h and little or no expression at 6 h. On the other hand, only a significant decrease of *c-jun* mRNA at 3 h (5.0-fold compared to Vehicle, 9.1-fold) and 6 h (0.6-fold compared to Vehicle, 1.3-fold) were detected. Histone H4 acetylation seems to be necessary but not sufficient for the activation of *c-jun* transcription in kainate-induced status epilepticus because of only minor reduction of *c-jun* mRNA induction by curcumin pretreatment and a 1.2-fold increase in *c-jun* expression by TSA pretreatment compared with vehicle pretreatment after kainate administration. We have obtained unexpected results on the seizure severity by curcumin pretreatment. At 0.5 h, low dose curcumin (1.58± 0.53) at 3 mg/kg and high dose curcumin (1.15±0.34) at 30 mg/kg showed decreased seizure scores compared to vehicle pretreatment (2.05±0.37). Curcumin-pretreatment also reduced seizure severity at 3 h with low dose curcumin mice scoring 3.13±0.44 and high dose curcumin mice scoring 1.04±0.15 as compared to vehicle pretreatment with an average score of 5.04±1.29. At 6 h, vehicle and low dose curcumin pretreated-mice displayed almost similar scoring of an average of 3.80+0.63 and 3.67±1.13, respectively, but high dose curcumin-pretreated mice showed a return back to normal (1.04±0.15). Thus, curcumin pretreatment not only prevents the induction of kainate-induced histone modifications and the levels of *c-fos* and *c-jun* mRNA expression but also decreases the severity of kainate-induced epilepsy (Fig. 3).

Signal transduction molecules in kainate-induced status epilepticus

It has been demonstrated that status epilepticus induced either by kainate or pilovarpine rapidly induces a transient activation of extracellular regulated kinase (ERK) and p38 MAP kinase signal transduction pathways in the hippocampus (Berkley et al., 2002; Crosio et al., 2003; Jiang et al., 2005). Berkeley et al. have reported a robust increase in ERK activation

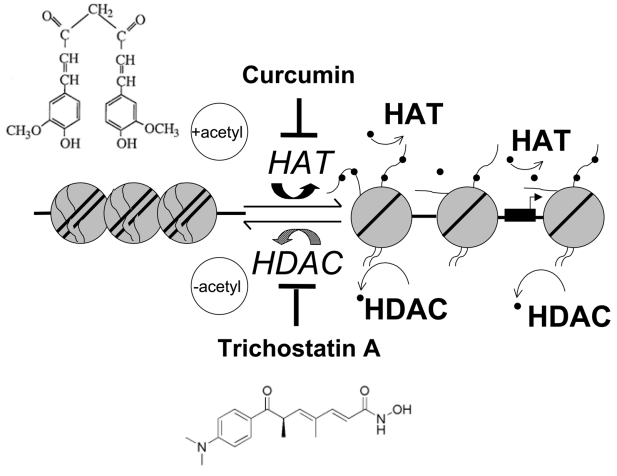

Fig. 2. Histone acetyltransferase and histone deacetylase. Histone acetyltransferase (HAT) interacts with lysine residues to catalyze the addition of acetyl group to lysine, and histone deacetylase (HDAC) acts in the reverse manner. Trichostatin A is a potent HDAC inhibitor, and curcumin inhibits HAT activity specific for CBP/p300. The chemical structures of trichostatin A and curcumin are also shown in the figure.

(phospho-ERK immunoreactivity) after 15 min in mice injected with pilocarpine in the hippocampus. SL327 is an inhibitor of MEK, the kinase directly upstream of ERK and responsible for its activation, which penetrates the blood-brain barrier. Although pretreatment with SL327 prevents pilocarpine-induced ERK activation in the hippocampus, they have observed that SL327 pretreatment resulted in a more severe seizure phenotype with higher mortality, suggesting that ERK might play a protective role during seizure (Berkeley et al., 2002). Crosio et al. have also found a rapid induction of ERK phosphorylation detected after 15 min and peaked at 1 h after kainate stimulation which occurs concomitantly with histone H3 phosphorylation in the DG (Crosio et al., 2003). Jiang et al. have studied the effects of the ERK1/2 phosphorylation inhibitor, PD98059, and the p38 MAP kinase inhibitor, SB203508, on kainate-induced seizure (Jiang et al., 2005). They observed intensive phosphorylated ERK1/2 and ATF2 immunoreactivities in the hippocampus 30 min after kainate stimulation. ATF2 is a substrate for activated p38 MAP kinase. Although pretreatment with ERK1/2 phosphorylation inhibitor PD98059 selectively blocked phosphorylated ERK1/2 immunolabeling without obvious effects on ATF2 phosphorylation and pretreatment with the p38 MAP kinase inhibitor SB203508 selectively blocked phosphorylated ATF2 without obvious effects on ERK1/2 phosphorylation, they could not detect significant inhibitory effects of both inhibitors on acute behavioral seizures induced by kainate.

The role of histone H3 phosphorylation in IEGs expression and epilepsy development is unclear. There was no correlation between H3 phosphorylation and IEGs expression in the CA regions of the hippocampus when IEGs expression and seizure severity are at their peaks. We have also shown the close correlation of H3 phosphorylation and IEGs expression in the DG of hippocampus and association of H3 phosphorylation on the *c-fos* gene at an early time point after kainate administration, suggesting the role of H3 phosphorylation in *c-fos* expression in the DG. The MAP kinase/ERK cascade is implicated in histone H3 phosphorylation. Although no report has identified histone H3 kinases in kainate-induced status epilepticus, two H3 kinase candidates have been postulated: MSK1/2, which is phosphorylated and activated by ERK or p38 MAP kinase (Thomson et al., 2001). Curcumin has been reported to inhibit different enzymatic activities including HIV-1 integrase, JNK activity and p38 MAP kinase (Chen and Tan, 1998; Cheng et al., 2003), suggesting that curcumin is not a specific inhibitor for HAT. The attenuation of kainate-induced H3 phosphorylation by curcumin may be caused by direct inhibition of p38 MAP kinase. Clayton et al. and Cheung et al. have demonstrated the synergistic coupling of H3 phosphorylation and H3 acetylation (Clayton et al., 2000; Cheung et al., 2000) but Thomson et al. have shown independent regulation of histone H3 phosphorylation and H4 acetylation in the activation of *c-fos* and *c-jun* genes (Thomson et al., 2001). Histone H3 phosphorylation may be necessary for induction of H4 acetylation, initial induction of IEGs in the DG or propagation of H4 acetylation to the CA regions of the hippocampus.

As for histone acetylation, different HAT families have been characterized into CBP and p300, the GNAT superfamily, the MYST family, TAF II p250 and TFIIIC family (Sterner and Berger, 2000; Roth et al., 2001). Among them, CBP is thought to be a good candidate for the involvement in kainate-induced IEGs expression. CBP is induced by calcium influx via NMDA receptors or voltage-sensitive calcium channels (Hardingham et al., 1999; Impey et al., 2002). Korzus et al. have reported that transgenic mice expressed mutant CBP, in which the HAT activity was eliminated, and exhibited reduced physiological level of *c-fos* gene expression in vivo (Korzus et al., 2004). We have also demonstrated that CBP was induced by kainate and its expression pattern was closely correlated with histone H4 hyperacetylation in the hippocampus. Curcumin has a HAT inhibitory activity, specific for CBP/p300. Curcumin pretreatment also inhibited the induction of CBP by kainate stimulation. Several studies have suggested that CaM kinase IV signaling activates CBP-dependent transcription. Impey et al. have suggested that CaM kinase IV-mediated phosphorylation of Ser 301 is required for the transcriptional activation of CBP by

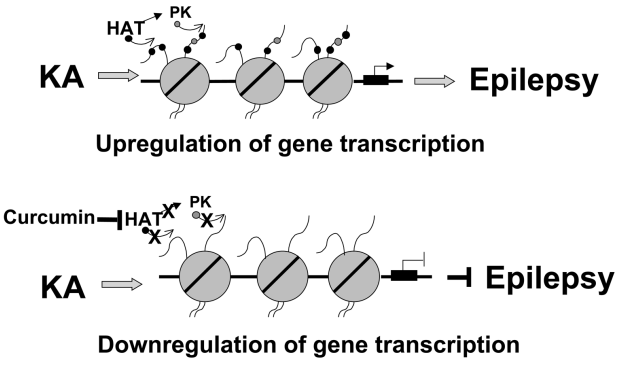

Fig. 3. A model depicting the involvement of histone modifications in kainate induced status epilepticus. After kainate stimulation, various cellular signaling pathways converge and direct at the chromatin, which are then translated as histone modifications on histone tails. Histone acetylation is regulated by histone acetyltransferase (HAT) such as CBP, which catalyzes the acetylation states of amino-acid residues on histone tails. Histone H3 phosphorylation is regulated by protein kinases (PK). Histone modifications disrupt higher folding of the chromatin and this allows transcriptional machinery access to the promoter regions of genes. The administration of curcumin inhibits the HAT activity that in turn, inhibits the acetylation of histones. This also affects the recruitment of protein kinase and prevents histone H3 phosphorylation directly or indirectly. Suppression of histone modifications by curcumin prevents genes transcription necessary for epileptogenesis and thus leads to the reduction of seizure severity.

NMDA signaling in primary rat hippocampal neurons (Impey et al., 2002). CBP is believed to regulate transcription through two mechanisms: by recruiting components of the core transcriptional machinery and by acetylating histones and other factors involved in gene activation and repression. They did not detect an increase in HAT activity after phosphorylation of CBP by CaM kinase IV in vitro; however, CBP phosphorylation could increase HAT activity indirectly by facilitating binding of another factor.

Concluding remarks

Our findings suggest the involvement of histone modifications induced by kainate not only in IEGs expression but also in the development of epilepsy. Induction of status epilepticus or prolonged seizures either by pilocarpine or kainate can trigger a myriad of gene upregulation, which could be regulated by histone modifications, and are thought to contribute to the development of epilepsy (Elliott et al., 2003). *c-fos* is a good candidate gene for involvement in the initiation and development of epilepsy because kainate-induced expression of *c-fos* concomitantly occurred with behavioral seizure and curcumin pretreatment attenuates both of kainate-induced *c-fos* expression and development of seizure. Several papers have reported contrary results; *c-fos* deficient mice exhibit more severe kainate-induced seizure (Jin et al., 2002; Zhang et al., 2002) but others showed anticonvulsant properties by anti-sense *c-fos* oligodeoxynucleotides treatment in kainate-induced seizures (Panegyres and Hughes, 1997). There could also be many other genes, other than *c-fos* or *c-jun*, which are involved in epileptogenesis and could have been induced by kainate stimulation and upregulated by the observed histone modifications. Our finding provides the possibility that curcumin may find therapeutic applicability for epilepsy and CBP as a new molecular target to develop potential therapeutic agents for epilepsy.

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