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DEPOLARIZATION INDUCES ACETYLATION OF HISTONE H2B IN THE HIPPOCAMPUS

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Abstract—Phosphorylation is critically involved in synaptic plasticity and memory. Recent studies have shown that another posttranslational modification, acetylation, particularly of histone H3, also plays important roles in long-term potentiation and memory. However, activity-dependent modification of different histones of the nucleosome is not clearly understood. Here we show that depolarization enhances acetylation of histone H2B in the CA1 region of the hippocampus. Depolarization-induced H2B acetylation is dependent on calcium/calmodulin-dependent kinase and extracellular signal-regulated kinase activity. In addition, inhibition of DNA methyltransferase activity also abolishes depolarization-induced increase in H2B acetylation. These results show that acetylation of histone H2B is regulated in an activity-dependent manner by the molecular events important for synaptic plasticity and memory.

Key words: histone acetylation, CaM kinase, ERK, DNA methyltransferase.

Activity-dependent molecular changes in the neuronal cells play crucial roles in synaptic plasticity and memory. Long-term potentiation (LTP) is widely studied as a putative cellular mechanism of memory formation (Bliss and Collingridge, 1993; Lynch, 2004). The observations that long-lasting synaptic plasticity and long-term memory require transcription led to intense investigation of the mechanisms that may be involved in activity-dependent regulation of gene expression. Initial studies were mostly focused on the regulation of transcription factors. It is clear that in addition to transcription factors, the status of chromatin also plays important roles in gene regulation (Li et al., 2007). The nucleosome which consists of DNA wrapped around histones is the repeating unit of chromatin. Chromatin is not a static structure but undergoes modifications on both DNA as well as the associated histones. Histones are modified by several posttranslational modifications including acetylation (Strahl and Allis, 2000; Turner, 2002). Histone acetylation, a dynamic process regulated by the relative activities of histone acetyltransferase and histone deacetylase (HDAC), is typically associated with active transcription (Kouzarides, 2007).

Chromatin modification has been extensively studied with respect to development, drug abuse and cancer. Recent studies, however, point to the important role of chromatin modification, especially histone H3 acetylation and phosphorylation, in synaptic plasticity and memory (Levenson and Sweatt, 2005; Reul and Chandramohan, 2007; Barrett and Wood, 2008; Borrelli et al., 2008; Gräff and Mansuy, 2008; Sweatt, 2009). For example, Guan et al. (2002) have shown that 5HT which induces facilitation of sensory-motor neuron synapses in *Aplysia*, enhances acetylation of histones H3 and H4 at the promoter of C/EBP, an immediate early gene that critically regulates long-term facilitation (LTF) in *Aplysia* (Alberini et al., 1994). Furthermore, inhibition of HDACs with trichostatin A (TSA) facilitates the induction of LTF (Guan et al., 2002). In the mammalian system, histone acetylation and phosphorylation are regulated by memory training (Levenson et al., 2004; Chwang et al., 2006). Histone modifications are also regulated by pharmacological activation of neurotransmitter receptors, and activation of the protein kinase A (PKA) or the protein kinase C (PKC) pathway that have been implicated in LTP and memory (Crosio et al., 2003; Levenson et al., 2004; Chwang et al., 2006). In addition, HDAC inhibitors facilitate LTP and memory consolidation (Alarcón et al., 2004; Levenson et al., 2004; Yeh et al., 2004; Vecsey et al., 2007; Fontán-Lozano et al., 2008; Stefanko et al., 2009), and enhance extinction (Lattal et al., 2007; Bredy et al., 2007). Furthermore, inhibition of HDACs has been shown to ameliorate memory deficits due to neurodegeneration (Fischer et al., 2007).

Although the regulation of histone H3 and H4 acetylation has been examined in detail, activity-dependent regulation of other histones of the nucleosome is not clearly understood. In this study, we report depolarization-induced acetylation of histone H2B. We further examined the signaling molecules important for H2B acetylation.

EXPERIMENTAL PROCEDURES

Hippocampal slice preparation and treatment

Male Sprague-Dawley rats (6–8 weeks old), housed under light/dark (12 h/12 h) cycle with access to rodent chow and water *ad libitum*, were used for all the experiments. Animals were obtained from the animal facility of our Institute. Animals were sacrificed according to the procedure approved by the Institutional Animal Ethics Committee. Rats were anesthetized using halothane (Sigma) and sacrificed. Steps were taken to minimize the number of animals used and their suffering. Hippocampal slice preparation and recovery was performed essentially according to the procedure

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Abbreviations: aCSF, artificial cerebrospinal fluid; CaMK, calcium/calmodulin-dependent kinase; CS, cutting saline; DNMT, DNA methyltransferase; ERK, extracellular signal regulated kinase; HDAC, histone deacetylase; LTP, long-term potentiation; MSK, mitogen- and stress-activated kinase; PKA, protein kinase A; PKC, protein kinase C; PP1, protein phosphatase 1.

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described by Levenson et al. (2004). The brain was rapidly removed and immersed in oxygenated ice-cold cutting saline (CS: 110 mM sucrose, 60 mM NaCl, 3 mM KCl, 1.25 mM NaH_2PO_4, 28 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 7 mM $MgCl_2$, 5 mM glucose, 0.6 mM ascorbate) and hippocampi were isolated. Transverse hippocampal slices (350 μm) were prepared using Vibratome (The Vibratome Company) in ice-cold CS, and then equilibrated in 1:1 mixture of CS and artificial cerebrospinal fluid (aCSF: 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM glucose) for 45 min at room temperature. Slices were then transferred to aCSF and incubated at room temperature for 45 min. A final incubation was carried out in aCSF at 32 °C for 1 h. All the solutions were oxygenated by continuous bubbling with 95% O_2/5% CO_2. Slices from both the hippocampi of one animal were pooled, randomized, and divided into different treatment groups as required. Three-to-four slices were used for each group. Slices were treated with 90 mM KCl for 3 min (Wu et al., 2001) in aCSF with a corresponding reduction in the concentration of NaCl. Thus, composition of KCl-aCSF was: 37.5 mM NaCl, 90 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 25 mM glucose. Where pharmacological inhibitors were used, slices were pre-treated with the inhibitors during the last recovery period for 15–30 min before treatment with 90 mM KCl for 3 min. The inhibitors were present during the KCl treatment also. Calcium/calmodulin-dependent kinases (CaMK) inhibitor, KN93 (Calbiochem) was dissolved at 2 mM concentration in DMSO and used at 2 μM concentration. The stock solution of the MEK inhibitor, U0126 (Calbiochem) was prepared at 20 mM in DMSO, and was used at 20 μM concentration. The DNA methyltransferase (DNMT) inhibitor, 5-aza-2-deoxycytidine (Sigma) was dissolved in DMSO at 30 mM, and was used at a final concentration of 30 μM. All the inhibitors were diluted in aCSF to achieve the final concentration.

Immunofluorescence

After KCl treatment, slices were rinsed in chilled CS, fixed in 4% paraformaldehyde overnight at 4 °C, and equilibrated in 30% sucrose at 4 °C. Slices were further sectioned into 20 μm sections with cryotome and mounted on glass slides. The sections were washed with PBST [0.3% Triton X-100 in PBS (137 mM NaCl, 2.7 mM KCl, 10.14 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4)] and incubated in the blocking buffer [3% bovine serum albumin (BSA) in PBST] for 1 h. The sections were then incubated overnight at 4 °C with acetyl-H2B antibody (in PBST) that was raised against acetylated Lys-5, 12, 15 and 20 of histone H2B (Upstate). After washing, the sections were incubated with FITC-conjugated secondary antibody (Vector Laboratories) for 1 h at room temperature. The sections were cover-slipped with the hard set mounting media containing 4′,6′-Diamidino-2-phenylindole (DAPI) (Vector Laboratories). The images were acquired on a Zeiss Axioplan II microscope (Carl Zeiss Company).

Isolation of area CA1 and sample preparation

After different treatments, slices were rinsed in ice-cold CS and frozen immediately on dry ice. The CA1 region was dissected out under a dissecting microscope (Olympus) and lysed in the lysis buffer [50 mM Tris–Cl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM sodium butyrate, 2% sodium dodecyl sulfate, and a protease inhibitor cocktail (one tablet of Roche Diagnostics protease inhibitor mixture/25 ml)]. The samples were boiled for 2–5 min, centrifuged at 10,000 rpm in a microfuge for 10 min to remove any insoluble material, and stored frozen until use. Protein estimation was performed using the BCA reagent (Sigma).

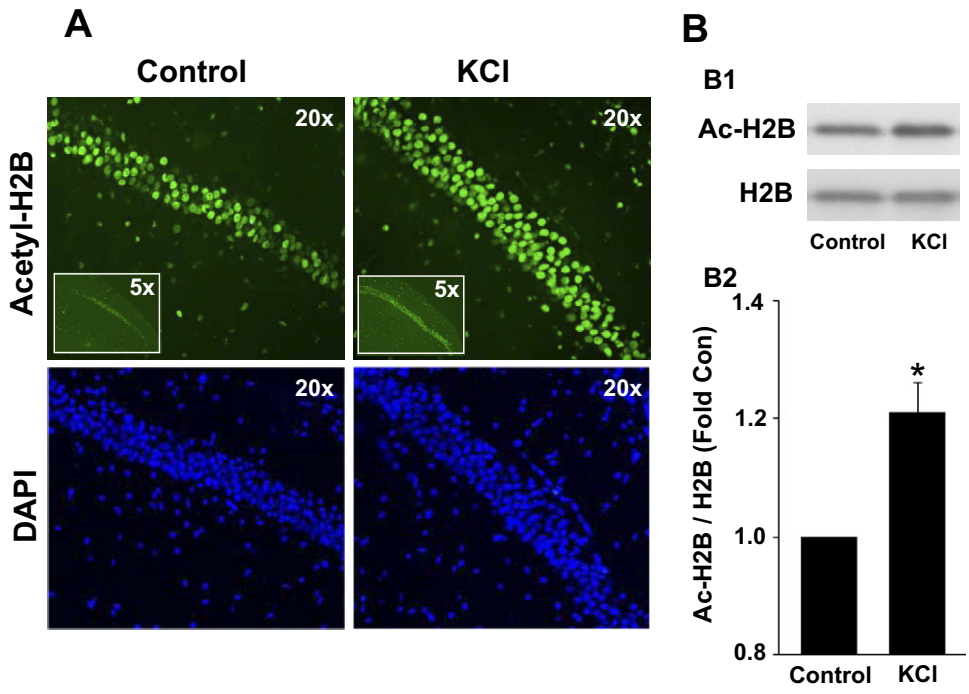

Fig. 1. KCl depolarization induces histone H2B acetylation. (A) Control or KCl treated acute hippocampal slices were re-sectioned (20 μm) and processed for immunofluorescence with acetyl-H2B antibody. KCl treatment enhanced H2B acetylation. CA1 regions of the acetyl-H2B antibody stained and DAPI stained hippocampal slices of control and KCl-treated groups are shown. (B) Histone H2B acetylation was examined in the CA1 region of control or KCl treated slices by Western blotting using acetyl-H2B and total H2B antibodies. KCl treatment significantly increased H2B acetylation. Representative immunoblots (B1) and summary data (B2, $n=8$) are shown. Asterisk denotes significant difference ($P<0.05$). For the color figure, the reader is referred to the Web version of this article.