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Changes in DNA binding pattern of transcription factor YY1 in neuronal degeneration

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Abstract

Molecular events under the neuronal degeneration are widely studied but still not defined. Here we compared the effects of both excitotoxic and apoptotic insults on the DNA binding profile of multifunctional transcription factor YY1 protein in cultured cerebellar granule neurons. We report that L-glutamate-induced excitotoxic insult but not ionophore A23187 treatment caused the disappearance of the larger DNA binding complex of YY1 and a simultaneous appearance of the smaller YY1 complex in cerebellar granule neurons. MK-801 (NMDA receptor antagonist) as well as benzamide (PARP inhibitor), MDL 28170 (calpain inhibitor) and roscovitine (cyclin-dependent kinase inhibitor) inhibited the glutamate response to the YY1 complexes. Herbimycin, PD169316, wortmannin, JAK3 inhibitor, KN-93, H-7 and LY294002 were not effective. Apoptosis induced by okadaic acid but not that induced by etoposide or trichostatin A caused a similar excitotoxic reorganization in YY1 complexes. We suggest that despite the different cell death mechanisms, glutamate and okadaic acid activate signalling cascades that affect the formation of YY1 complexes and probably YY1-mediated gene regulation.

Keywords: Aging; Apoptosis; Calcium signalling; Excitotoxicity; Neurodegeneration

Transcription factor YY1 is a ubiquitously expressed multifunctional zinc finger protein which can either activate or repress gene transcription [18]. The role of YY1 as a transcriptional activator or repressor is suggested to be regulated by the proteins which associate to YY1, such as HDACs 1–3, Rb, PARP and CREB. YY1 factor regulates the expression of several important neuronal genes, such as p53, BACE1 and neuron specific Fe65 [15,18].

Glutamate is the most abundant excitatory neurotransmitter in the brain but in certain conditions it becomes toxic and causes neuronal cell death, either an apoptotic or necrotic one. Glutamate receptor-mediated intracellular calcium overload has a key role in neuronal degeneration after acute insults, such as stroke, epilepsy and trauma. Calcium-related signalling cascades which regulate both cell death and survival responses, are still poorly defined [6,11]. Apoptosis participates in brain development, but it has also an important role in neurodegenerative diseases [7,16].

We aimed to compare the influence of excitotoxicity and apoptosis on the DNA binding profiles of transcription factor YY1 protein in the cultured cerebellar granule neurons. Insight into the regulation cascades of these cell death processes is necessary to be able to find novel targets to treat and prevent neuronal degeneration.

Primary cerebellar granule cells were isolated and cultured as described earlier [4]. Cultured cerebellar granule cells were exposed to L-glutamate in Locke's salt solution after 10 days in culture with a final concentration of 5, 25 or 50 μM for 15 min at RT. This glutamate shock was stopped by washing the cells with Locke's salt solution containing 100 mM Mg^{2+}, the old medium was returned to cell plates, and the samples were collected after 1–2 h. Ionophore A23187 (calcimycin) was added to the cerebellar granule cells in Locke's salt solu-

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tion and incubated for 1 h. All drugs were added in Locke's solution 15 min before L-glutamate exposure and were present during treatment. Drug concentrations were tested to be non-toxic in short-term cerebellar granule cell culture conditions.

Apoptosis in cultured cerebellar granule cells was induced after 7 days in culture by okadaic acid, etoposide, or trichostatin A (TSA). Exposure times were 12 and 24 h. The activity of caspase-3 enzyme from the total cell lysate or from the cytosolic extracts was assayed as we have described earlier [19].

Soluble nuclear proteins were isolated for electrophoretic mobility shift assays (EMSA) as described earlier [12]. EMSA assays were performed as we have described earlier [12,13]. Rabbit polyclonal anti-YY1 antibody used for the supershift of YY1 complex was from Santa Cruz (C-20X). Consensus and mutated double-stranded oligonucleotides were from Santa Cruz and labelled according to their protocol.

Our preliminary study [13] showed that the exposure of cultured rat primary cerebellar and hippocampal neurons to L-glutamate insult affects the DNA-binding of YY1 complex. Here we have extended these experiments to study the regulation of complex formation in respect to excitotoxic and apoptotic insults in cultured primary cerebellar granule neurons. Figs. 1 and 2 show that the soluble nuclear proteins of cultured cerebellar granular neurons form one major YY1 complex and a faint smaller complex. L-Glutamate treatment induced a dramatic appearance of a smaller (fast-moving) YY1 complex. Simultaneously, the formation of the larger YY1 binding complex was prominently reduced. Fig. 1B shows that the response is concentration dependent since it appeared at the 5 μM concentration level and increased at the higher 25 μM concentration. Both YY1 complexes were supershifted with specific antibodies showing that YY1 protein was included in the both complexes (Fig. 1C). Antibody against NF-kB p52 protein did not affect the YY1 complex (Fig. 1C). We also verified that the cytoplasmic proteins do not form any specific or unspecific YY1 complexes which could "contaminate" nuclear protein fraction after L-glutamate treatment (data not shown).

Next, we studied whether the L-glutamate-induced response in the formation of YY1 complexes could be directly related to calcium overload in primary cerebellar granule cells. Interestingly, Fig. 1A shows that the ionophore A23187 (calcimycin) treatment of the cerebellar granule cells did not cause any changes in the DNA binding complexes of YY1 protein at the concentrations between 5 and 25 μM although it caused neurotoxic effects in the morphology of cerebellar granule cells. It is known that spatially distinct calcium signals in neurons can induce differential control of transcription [1]. For instance, L-glutamate and ionophores differentially regulate MAP kinase pathway [14].

We also studied whether the reduction of the toxicity of L-glutamate treatment by NMDA receptor antagonist, MK-801 [9], or poly(ADP-ribose)polymerase (PARP) inhibitor, benzamide [2], affect the L-glutamate response in YY1 binding complexes. Fig. 1A shows that MK-801 and benzamide

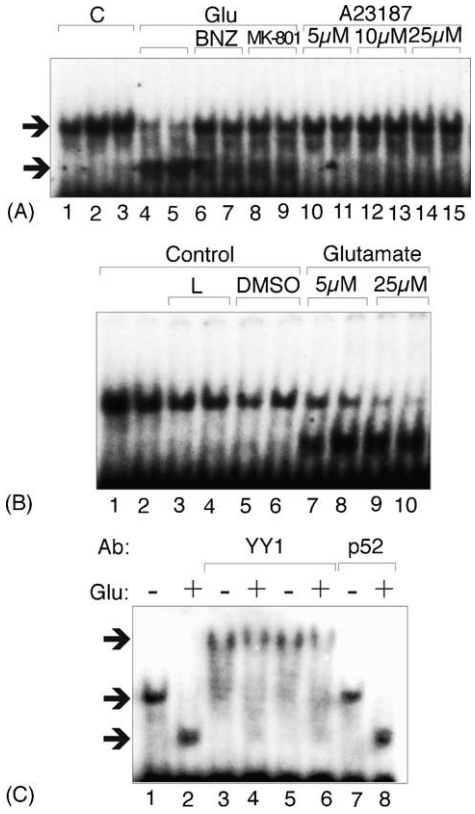

Fig. 1. Effects of L-glutamate and ionophore A23187 on the formation of YY1 complex in cerebellar granule neurons. (A) Lanes 1 and 2 show the control samples, lane 3 represents DMSO control in Locke's salt solution, lanes 4 and 5 contain L-glutamate-treated cells (50 μM for 15 min, RT). Lanes 6 and 7 represents cells preincubated with 5 mM benzamide (BNZ). Lanes 8 and 9 contain samples from cells preincubated with MK-801 (10 μM). Lanes 10–15 show cells incubated with ionophore A23187 in Locke's salt solution with different concentration of drug. (B) L-Glutamate response appeared at the concentration of 5 μM and increased at the higher 25 μM concentration. (C) A supershift study with control and excitotoxic samples. Lane 1 is a control and lane 2 is L-glutamate-treated sample from cerebellar granule cells. Lanes 3 and 5 contain control samples with antibody against YY1 (C-20X) and lanes 4 and 6 have glutamate-treated samples with the same antibody. Lanes 7 and 8 contain a control and glutamate sample, respectively, with antibody against NF-κB p52 (K-27).

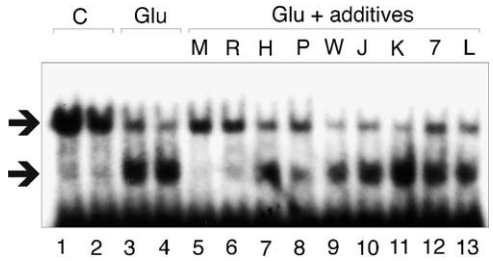

Fig. 2. Regulation of L-glutamate-induced excitotoxic profile of YY1 complexes by different signalling inhibitors. Cerebellar granule neurons were exposed to 50 μM L-glutamate for 15 min and samples were collected 2 h after treatment. Drug concentrations: MDL 28170 (100 μM) (lane 5), roscovitine (20 μM) (lane 6), herbimycin (100 nM) (lane 7), PD169316 (10 μM) (lane 8), wortmannin (1 μM) (lane 9), JAK3 inhibitor (10 μM) (lane 10), KN-93 (10 μM) (lane 11), H-7 (100 μM) (lane 12) and LY294002 (50 μM) (lane 13).