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Interference with activity-dependent transcriptional activation of BDNF gene depending upon the expanded polyglutamines in neurons

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Abstract

Expanded polyglutamines (polyQ) have been demonstrated to impair the CREB-dependent transcription in established cell lines. Since activity-dependent transcription in neurons, which plays an important role in forming neuronal plasticity, is largely controlled by CREB, it is important to study whether polyQ interferes with the activity-dependent transcriptional activation of genes in neurons. In cultured rat cortical neurons, over-expression of truncated dentatorubral-pallidoluysian atrophy proteins containing expanded polyQ, which form aggregation bodies in nucleus, reduced the calcium (Ca^{2+}) signal-mediated transcriptional activation of brain-derived neurotrophic factor, c-*fos*, and pituitary adenylate cyclase-activating polypeptide gene promoters in a dose-dependent manner. The interference with the transcriptional activation was dependent upon the presence of polyQ, the strength of which was increased as the length of polyQ stretches was expanded. Thus, polyQ interferes with the activity-dependent transcription in a polyQ-length-dependent manner, which may correspond to the severity of polyglutamine diseases.

Keywords: BDNF; PACAP; Calcium; Polyglutamine; Activity-dependent transcription; DRPLA

Huntington's disease (HD) and dentatorubral-pallidoluysian atrophy (DRPLA) are representative neurodegenerative disorders caused by polyglutamine (polyQ) expansions in the huntingtin and atrophin-1 proteins, respectively, in the causal genes of which the CAG trinucleotide repeats are expanded [1]. The clinical severity of these diseases is correlated with the length of expanded CAG repeats; that is, the longer the length of CAG repeats is, the earlier the age of onset and the severer the symptoms are [1]. Although precise mechanisms of polyglutamine pathogenesis remain unclear, evidence has been accumulating that the causal proteins containing expanded polyQ form neuronal intranuclear inclusions and aberrantly interact with nuclear proteins harboring polyglutamine stretches, resulting in transcriptional dysregulation [1–3]. Mainly using reporter plasmid constructs, it has already been demonstrated that the expression of expanded polyQ in cultured cells interferes with the CREB-, TATA box-binding protein (TBP)-associated factor (TAFII130)-, CREB-binding protein (CBP)-, and Sp1-dependent transcription [4–6].

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and plays a key role in neuronal survival, differentiation, and synaptic plasticity [7], whose mRNA expression is controlled in an activity-dependent manner [8,9]. Since Shimohata et al. [5]

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reported, using a cAMP-responsive element (CRE)-reporter plasmid construct, that expanded polyQ interfered with the CRE-binding protein (CREB)-dependent transcription through an interaction with TAFII130 in COS-7 cells, it can be speculated that polyQ impairs the activity-dependent transcription of a set of genes including BDNF and c-*fos* ones, whose activation is commonly controlled by CREB [10,11], resulting in a disruption of neuronal function.

In contrast to the interference observed with cultured cells, however, there has been a conflicting report with the interference observed with the Huntington's disease transgenic mice, in which, rather than repressing CREB-mediated transcription, mutant huntingtin facilitated transcription via CRE-dependent mechanism in vivo [12]. To confirm the interference with the CREB-dependent transcription by polyQ, it is needed to study whether or not the interference with transcription by expanded polyQ is also detected with the transcription of natural gene promoters in primary neurons, whose activation is highly attributable to CRE, and, furthermore, directly depends upon the polyQ portion. Using the cultured rat cortical neuronal cells, therefore, we investigated the effect of polyQ on the activity-dependent transcription of BDNF gene promoters I and III and that of c-*fos* and PACAP gene promoters, especially focusing on the dependency of the interference upon the dose and the length of polyQ.

Materials and methods

Plasmid construction. Plasmid DNAs containing rat BDNF gene promoters I and III, pGL3-BDNFpI and pGL3-BDNFpIII, respectively, were prepared by inserting the amplified DNA fragments covering the regions of BDNF promoters I (−528 to +138) and III (−629 to +281) into the *Hin*dIII site of the pGL3-basic firefly luciferase reporter vector (Promega) [9]. The pGL3-pc-fos and pGL3-pPACAP containing the human c-*fos* promoter (−404 to +41) and rat PACAP gene promoter (−787 to +376), respectively, were also constructed by inserting the corresponding DNA fragments into pGL3-basic vector after DNA amplification by PCR [13]. For the overexpression experiments of polyQ proteins, pEGFP-polyQ-S(19), pEGFP-polyQ-L(57), and pcDNA3.1-polyQ-L(57) were generated by inserting the *Xho*I/*Bam*HI DNA fragment of pT-DRPLAQ57-NLS-GFP, pT-DRPLAQ19-NLS-GFP, and pT-DRPLAQ0-NLS-GFP [5] into pEGFP-polyQ-(0) or pcDNA3.1(−) vector plasmid (Invitrogen). These plasmids all accommodate the nuclear location signal (NLS) fused to the enhanced green fluorescence (EGFP) gene (Fig. 1A). As an empty vector for the DNA transfection, pEGFP-1 (Clontech) and pcDNA3.1(−) vectors were used.

Cell culture. Primary cultures of rat cortical neurons were prepared from the cerebral cortexes of 17- to 18-day-old rat (Sprague–Dawley) embryos, as described previously [13]. Briefly, the cells were seeded at about 5×10^6 cells in a 60-mm (diameter) culture dishes (Iwaki) after dissociating the cells from small pieces of cerebral cortex with enzymatic treatments. The cells were grown for 48 h in Dulbecco's Modified Eagle's Medium (DMEM, Nissui) containing 10% fetal calf serum and then the medium was replaced with serum-free DMEM containing glucose (4.5 mg/ml), tranferrin (5 mg/ml), insulin (5 mg/ml), sodium selenite (5 ng/ml), bovine serum albumin (1 mg/ml) and kanamycin sulfate (100 mg/ml) (TIS medium). Before DNA transfection, the medium was replaced with fresh TIS medium.

DNA transfection and reporter gene assays. The procedures for DNA transfection using calcium-phosphate-mediated DNA precipitation method were previously described in detail [13]. Briefly, DNA transfection was carried out 3 days after culturing, with the precipitates which were prepared by mixing the plasmid DNAs (totally 8 μg; reporter vector/expression vector = 1:7, pGL3-firefly luciferase vector/EF1α-β-gal = 10:1) with $CaCl_2$. After culturing for another 40 h in a TIS medium, the transfected cells were stimulated with 25 mM KCl or vehicle for 12 h and the cell lysates were prepared for measuring the luciferase and β-galactosidase activities. In the case of c-*fos* promoter analysis, the cells were stimulated with 25 mM KCl for 6 h. The efficiency of DNA transfection by calcium-phosphate-mediated DNA precipitation method was approximately 5%, which was calculated by dividing the numbers of green fluorescent protein (GFP)-positive cells by those of cultured cells counted (data not shown). More than 90% of the GFP-positive cells were stained with the antibodies specific to microtubule-associated protein 2 (MAP2) protein (data not shown),

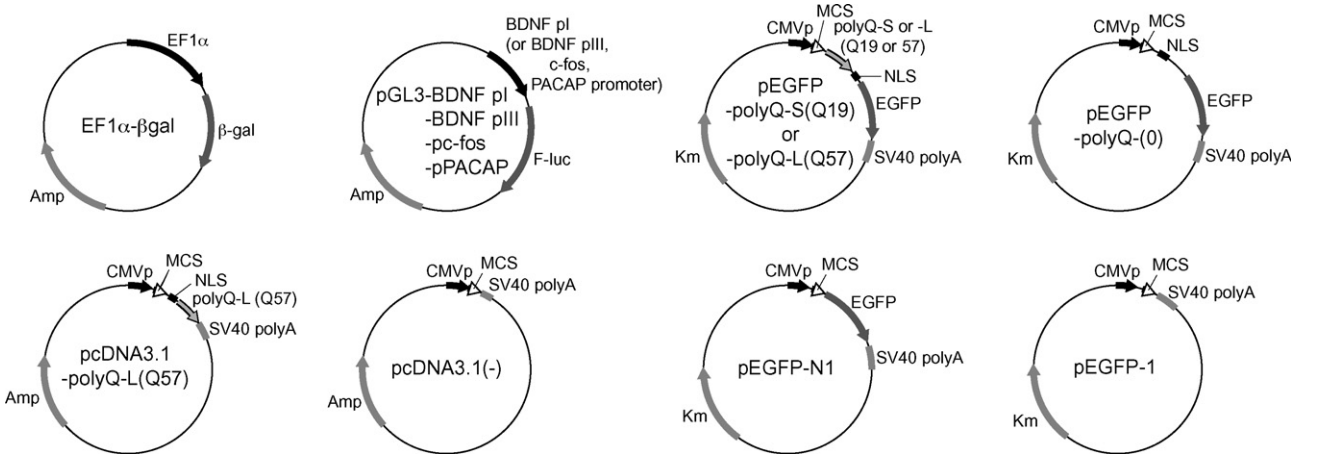

Fig. 1. Plasmid DNA constructs. Schematic representation of reporter and expression plasmid constructs. Construction of plasmids was described under Materials and methods. The reporter plasmids, pGL3-BDNFpI, pGL3-pc-*fos*, and pGL3-pPACAP, were constructed by replacing the BDNF promoter III (BDNF-pIII) of pGL3-BDNFpIII with the promoters of BDNF promoter I, c-*fos*, and PACAP gene promoter, respectively. MCS, multi-cloning site. NLS, nuclear location signal. CMV-p, cytomegalovirus promoter.