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Distinct regulation of activity-dependent transcription of immediate early genes in cultured rat cortical neurons

Mamoru Fukuchi [a, b, *], Tomofumi Sanabe [b], Toshifumi Watanabe [b], Takane Kubota [b], Akiko Tabuchi [b], Masaaki Tsuda [b]

[a] Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki-shi, Gunma 370-0033, Japan
[b] Department of Biological Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama-shi, Toyama 930-0194, Japan

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ABSTRACT

The activity-regulated expression of immediate early genes (IEGs) contributes to long-lasting neuronal functions underlying long-term memory. However, their response properties following neuronal activity are unique and remain poorly understood. To address this knowledge gap, here we further investigated the response properties of two representative IEGs, *c-fos* and brain-derived neurotrophic factor (*Bdnf*). Treatment of cultured cortical cells with KCl produces a depolarization process that results in the increase of intracellular calcium concentration in a KCl concentration-dependent manner. Consistent with this increase, *c-fos* expression was induced in a KCl concentration-dependent manner. In contrast, however, *Bdnf* expression was optimally activated by both 25 and 50 mM concentration of KCl. Similar results were observed when the cells were treated with okadaic acid, which inhibits protein phosphatases and elicits the hyper-phosphorylation of signaling molecules. Thus, *Bdnf* expression is strictly regulated by a neuronal activity threshold in an all or nothing manner, whereas *c-fos* expression is activated in a neuronal activity-dependent manner. Our findings also suggest that these differential responses might be due to the presence or absence of a TATA box.

1. Introduction

Plasticity is a unique property of the nervous systems that allows it to flexibly change its functions in response to environmental stimuli. Long-term potentiation (LTP) and long-term depression (LTD) are well-known as molecular phenotypes of neuronal plasticity that underlie functions of learning and memory [1]. Long-lasting changes in neuronal functions are thought to constitute molecular mechanisms of long-term memory. Nowadays, it is widely accepted that neuronal activity-regulated *de novo* transcription participates in controlling the long-lasting changes of neuronal functions; that is, neuronal activity controls a group of genes called immediate early genes (IEGs) that are involved in the regulation of neuronal transcription and synaptic functions and subsequently modulate neuronal and synaptic properties. This communication between the synapse and the nucleus plays a crucial role in long-term memory [2–7].

Strong synaptic activation evoked by glutamatergic excitatory neurotransmission has been shown to regulate IEGs [8]. Major IEGs, the expression of which are generally controlled in a neuronal activity-dependent manner, constitute a set of transcription factors. For example, the expression of *c-fos* is well-known to be regulated by neuronal activity. Because of their fine response properties, *c-fos* promoter is available for labeling a set of activated cell populations [9]. In addition, because activity-regulated IEGs, such as activity-regulated cytoskeleton-associated protein (Arc), Homer1a/Vesl1S, and brain-derived neurotrophic factor (BDNF), also encode molecules involved in controlling synaptic structure and synaptic properties, the activity-regulated IEG expression actively participates in regulating synaptic functions.

A series of transcription factors involved in neuronal activity-dependent IEG expression have already been identified. For example, cAMP-response element (CRE)-binding protein (CREB) is a well-known master regulator of activity-dependent IEG

* Corresponding author. Laboratory of Molecular Neuroscience, Faculty of Pharmacy, Takasaki University of Health and Welfare, 60 Nakaorui-machi, Takasaki-shi, Gunma 370-0033, Japan.
E-mail address: fukuchi@takasaki-u.ac.jp (M. Fukuchi).

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expression [10] and contributes to long-term memory [11–13]. The serum response factor (SRF), which was originally identified in nuclear extracts prepared from Hela cells and participates in the activation of *c-fos* transcription in response to serum stimulation [14], is involved in the activity-dependent transcription of a set of IEGs [15,16].

Although the transcription factors regulating the activity-dependent IEG expression have been previously investigated, the response properties of IEG promoters following neuronal activity are unique and remain poorly understood. In the present study, we examined the response properties of *c-fos* and *Bdnf* promoters following neuronal activity using primary cultures of rat cortical cells.

2. Materials and methods

2.1. Cell culture

Primary cultures of cortical neurons were prepared from the cerebral cortices of 17-day-old Sprague-Dawley rat embryos (Japan SLC, Hamamatsu, Japan) as described previously [17–19]. Maternal rats and embryos were deeply anesthetized with sodium pentobarbital to obtain rat embryonic brains. Maternal rats were euthanatized with carbon dioxide and rat embryos were euthanatized by decapitation under deep anesthesia. All animal procedures were approved by the Animal Experiment Committee of the University of Toyama and were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Toyama.

2.2. Reporter assay

DNA transfection was performed at 3 days in culture as described previously [17–19]. A reporter plasmid, *Bdnf*-pI-Luc, *Bdnf*-pIV-Luc, *c-fos* promoter-Luc, CRE-Luc, SRE-Luc, UBE-Luc, or CaRE1,2,3-Luc, was co-transfected with the internal control vector pRL-EF1α using the calcium/phosphate/DNA co-precipitation method. Two days after the co-transfection, the cells were treated with either KCl or okadaic acid. Cell lysates were prepared 6–12 h after the treatment, and the activities of firefly and *Renilla* luciferases were measured by Dual-luciferase reporter assay system (Promega).

2.3. RT-PCR

Total cellular RNA was extracted with the acid guanidine phenol-chloroform method. The isolation of RNA from cultured cells or rat tissues is described in detail elsewhere [17–19]. One microgram of RNA was used for reverse transcription with SuperScript II (Invitrogen). Then, quantitative PCR was performed using the Stratagene Mx3000p Real-Time PCR system (Stratagene, La Jolla, CA) with the SYBR Green QPCR master mix (Stratagene), in

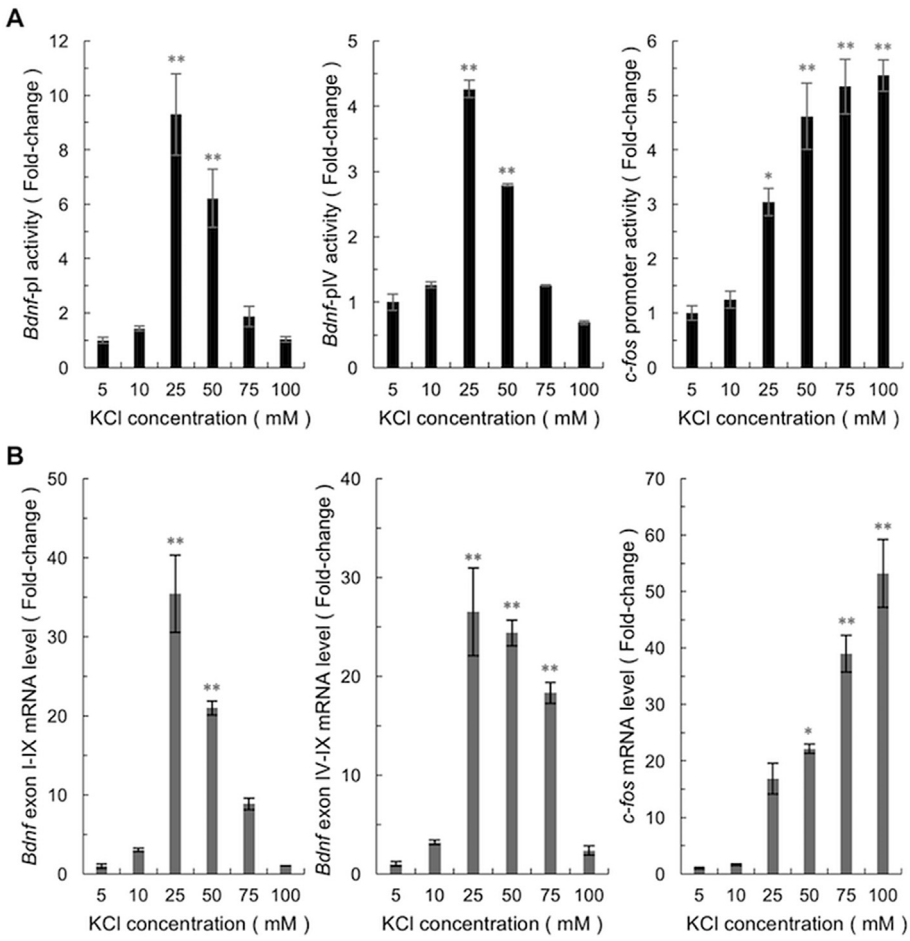

Fig. 1. Changes in *Bdnf* and *c-fos* expression after the treatment of cortical cells with different concentrations of KCl.
(A) The activity of *Bdnf*-pI, pIV, or *c-fos* promoter following KCl treatment was measured with a dual-luciferase reporter assay. (B) The expression level of *Bdnf* exon I-IX, IV-IX, or *c-fos* following KCl treatment. The expression level of each mRNA was investigated by quantitative RT-PCR analysis. The data are represented as the mean ± S.E.M. (n = 3). * $p < 0.05$ and ** $p < 0.01$ versus 5 mM KCl.

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