[Frontiers in Bioscience 9, 100-103, January 1, 2004]

CREB AND NEURODEGENERATION

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1. ABSTRACT

Programmed cell death (PCD) is major concept in neurobiology and transcription factors are pivotal in switching on the nerve cell death program. More recently, the transcriptional control of programmed cell life (PCL) is beginning to be understood. This work began in studies of the activation of the CREB transcription factor in stroke models where it was shown that CREB is phosphorylated (and presumably activated) in neurons that survive this insult. In this review I will describe this data and also discuss the up-stream and down-stream pathways in this CREB neuroprotective transcriptional cassette. Finally, I will discuss studies showing that this CREB survival pathway may be inactivated by neurotoxins and genes involved in neurodegenerative disorders.

2. INTRODUCTION

Selective vulnerability of neuronal populations to various insults suggests that different signal transduction pathways are operating in different neuronal systems to either promote death and/or promote nerve cell survival. Many years ago we, and others, showed that the transcription factor c-Jun was induced for prolonged periods in CA1 neurons that undergo apoptosis after hypoxia-ischemia and status epilepticus (1). Many reports have now confirmed that c-Jun is important in switching on the neuronal apoptosis cascade. To understand the up-stream elements that might switch on Jun expression in dying neurons we undertook studies of CREB activation. CREB is activated by phosphorylation on serine-133 and using a phospho-specific CREB antibody we were unable to show CREB phosphorylation in dying neurons indicating that CREB was not up-stream of c-Jun in the death pathway (2). However, CREB was activated but only in neurons (dentate granule cells) that survived these insults. We suggested on the basis of these results that CREB switches on PCL in neurons. To directly test the role of CREB in neuronal survival we transfected Neuro2A and PC12 cells with a CREB expressing plasmid and then exposed cells to the toxin okadaic acid. We found that CREB but not LacZ expressing cells resisted okadaic acid induced apoptosis (3). This combined work, as well as that by others, has been previously reviewed in Walton and Dragunow (2000) and readers are directed to that manuscript for further details.

More recently, it has become clear that CREB is also involved in neuronal differentiation and survival of newly formed neurons. A number of years ago we discovered that putative neuronal stem cells in the sub-granular zone of the dentate granule cells expressed high basal levels of phosphorylated (active) CREB (4,5). More recently, Pons *et al* (6) showed that neuronal differentiation of cerebellar granule cells was controlled by vibronectin-induced CREB phosphorylation. In PC12 cells a gain-of-function CREB mutant (that is constitutively active) facilitates nerve growth factor - induced neuronal differentiation (7). Furthermore, Nakagawa *et al* (8) recently reported that the phosphodiesterase inhibitor rolipram induced proliferation and neuronal differentiation of granule cells in hippocampus via activation of CREB. Bender *et al* (9) have shown a correlation between CREB phosphorylation and granule cell differentiation. Also, chronic lithium, which promotes neurogenesis faciltates CREB DNA binding activity (10). Son *et al* (11) found that CREB was phosphorylated during differentiation of HiB5 cells, an immortalized hippocampal cell line, following treatment with a combination of forskolin and KCL. Sung *et al* (12) found that bFGF-induced neuronal differentiation of immortalized hippocampal progenitor cells (H19-7) was accompanied by prolonged CREB phosphorylation and CRE-mediated gene transcription. Furthermore, the dual specificity kinase Dyrk1A promotes neuronal differentiation of H19-7 cells by activating CREB (13). Thus CREB plays a pivotal role in neuronal differentiation/survival. I recently observed that P19 embryonic carcinoma cells, and NT-2 cells when induced to differentiate into post-mitotic neurons with retinoic acid show strong CREB phosphorylation (Figures 1 & 2). This CREB activation is most likely related to both the neural differentiation of these cells and to their survival.

CREB may also mediate the survival promoting effects of neurotrophins and other molecules (reviewed in reference 4). We have observed using TrkB-expressing PC12 cells that brain-derived neurotrophic factor (BDNF) promotes neuronal differentiation and neuroprotection, and also drives CRE-mediated luciferase gene expression (see Figure 3).

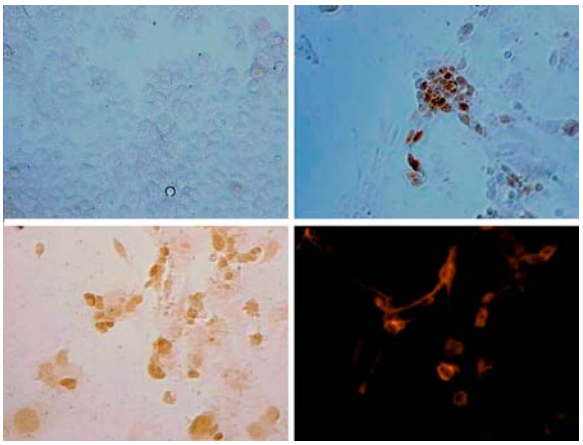

Figure 1. Photomicrographs showing lack of CREB phosphorylation in undifferentiated P19 cells (top left) and strong CREB phosphorylation in retinoic acid differentiated P19 cells (top right). Bottom photomicrographs show differentiated P19 cells immunostained with pCREB (left, DAB) and with MAP-2 (cy3). Note that MAP-2 positive post-mitotic neurons express high levels of pCREB.

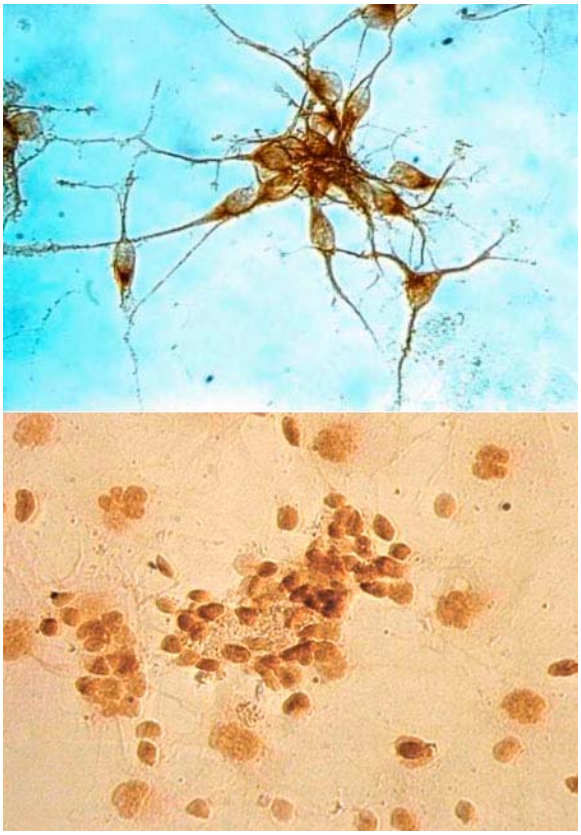

Figure 2. Photomicrographs showing differentiated hNT neurons immunostained with MAP-2 (top) and pCREB (bottom).

The recent report by Mantamadiotis *et al* (14) showing that neurodegeneration in brain occurs after the disruption of CREB function confirms many previous studies and demonstrates also *in vivo* that CREB is critical for nerve cell survival. Their work shows that both CREB and CREM can promote nerve cell survival globally in developing brain but more selectively in adult brain. Others have recently shown that activation of CREB also mediates the pro-survival effects of the neurotransmitter glutamate, and ischemic tolerance produced by preconditioning ischemia (15). Ischemic tolerance in the brain occurs when a brief period of ischemia protects the brain from a subsequent normally damaging period of ischemia. Researchers have known for many years that this ischemic tolerance in the brain is mediated by the activation of the NMDA-type glutamate receptor (16). On the other hand activation of NMDA receptors has also been shown to be mediate death of neurons after ischemia. A recent report suggests that opposing actions on CREB may mediate both the neuroprotective and neurotoxic effects of glutamate-mediated NMDA receptor activation on neurons (17). In an elegant set of studies, Hardingham *et al* (17) showed that activation of synaptic versus extra-synaptic NMDA receptors has opposite effects on CREB activity that mirror their effects on nerve cell survival. Activation of synaptic NMDA receptors leads to CREB activation and cell survival (this presumably mediates ischemic preconditioning), whereas extra-synaptic NMDA receptor activation by glutamate leads to a CREB block and cell death (17).

Neuronal death caused by oxidative stress (18) and during amyloid precursor protein-mediated neuronal death (19) may also be mediated by impaired CREB-mediated neuroprotection. This toxin-mediated CREB shut-off may explain why neurons that die after ischemia do not show any CREB phosphorylation although why some neurons resist this shut-off is unclear, but may be due to differences in NMDA receptor sub-unit composition. Furthermore, recent studies show that there is cross-talk between caspase 3 and CREB as caspase 3 can cleave CREB (20), perhaps silencing this pathway during caspase 3-mediated apoptosis. Additionally, prolonged ERK phosphorylation, which is associated with nerve cell death (21) negatively controls CREB activity (22).

These combined studies demonstrate that CREB plays an important role in promoting neuronal survival and that interference with this cell life pathway may be involved in a number of neurodegenerative disorders including protein misfolding disorders (23) and disorders that involve glutamatergic neurotransmission and oxidative stress. Future studies aimed at augmenting CREB-mediated neuronal survival pathways may provide pharmaceuticals with broad neuroprotective efficacy, and indeed a novel phenylpyrimidine derivative may protect against cerebral ischemic injury by causing persistent neuronal CREB phosphorylation (24). Drugs that promote CREB-mediated neuroprotection may also enhance cognition, given the important role of CREB in memory formation (25,26). Interestingly, we have recently shown that activation of muscarinic receptors on human neuroblastoma cells leads to CREB phosphorylation (26), and perhaps this is related to both the memory enhancing effects of muscarinic agonists (26) and to their neuroprotective effects (27).

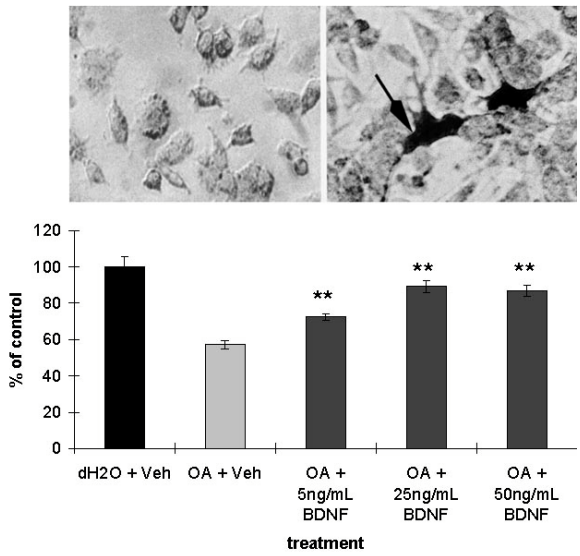

Figure 3. Photomicrograph showing that BDNF (50 ng/ml, RBI) drives CRE-luciferase gene expression in TrkB-expressing PC12 cells (top) and graph showing that BDNF reverses okadaic acid-induced cell death measured with MTT (bottom).

Thus, these drugs may be a novel class of neuroprotective cognitive enhancers, particularly useful for treating neurodegenerative disorders that involve cognitive imapirment (eg: Alzheimer's disease). Given that CREB mediates both memory formation and neuroprotection, perhaps "learning to protect your neurons" (ie: cognitive activity promoting nerve cell survival) is mediated by the CREB pathway?

3. ACKNOWLEDGEMENTS

I would like to thank Eric Shooter for providing the PC12 TrkB-expressing cell line, and the following students, collaborators and research technicians for their help with many studies described in this review:- Simon O'Carroll, Claire Henderson, Marshall Walton. This work was supported by a Programme Grant from the New Zealand Health Research Council.

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Key Words: Apoptosis, Programmed Cell Life, Neurogenesis, Review

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