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Research Report

Proteomic analysis of brain protein expression levels in NF-κβ p50 $^{-/-}$ homozygous knockout mice

Joshua B. Owen[a], *Wycliffe O. Opii*[a], *Charles Ramassamy*[b,c], *William M. Pierce*[d], *D. Allan Butterfield*[a,*]

[a]*Department of Chemistry, Center of Membrane Sciences and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40506-0055, USA*
[b]*INRS-Institut Armand-Frappier, Québec, Canada*
[c]*INAF, University Laval, Québec, Canada*
[d]*Department of Pharmacology, University of Louisville, Louisville, KY, USA*

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ABSTRACT

The role of nuclear factor kappa B (NF-κB) in oxidative stress, and most recently in pro- and anti-apoptotic-related mechanistic pathways, has well been established. Because of the dual nature of NF-κB, the wide range of genes it regulates and the plethora of stimuli that activate it, various studies addressing the functional role of NF-κB proteins have resulted in a number of differing findings. The present study examined the effect of a stimulus-free environment on the frontal cortex of mice brain with the p50 subunit of NF-κB knocked out p50 (–/–). Homozygous p50 mice knockout (KO) and wild type (WT) were used, and at 7–9 weeks they were sacrificed and various brain regions dissected. We analyzed the levels of oxidation in the frontal cortex of both the p50 (–/–) and WT mice. There was a significant reduction in the levels of protein-bound 4-hydroxynonenal (HNE) [a lipid peroxidation product], 3-nitrotyrosine (3NT), and protein carbonyls in the p50 (–/–) mice when compared to the WT. A proteomic profile analysis identified ATP synthase gamma chain, ubiquinol-cyt-C reductase, heat shock protein 10 (Hsp10), fructose bisphosphate aldolase C, and NADH–ubiquinone oxidoreductase as proteins whose expressions were significantly increased in the p50 (–/–) mice compared to the WT. With the reduction in the levels of oxidative stress and the increase in expression of key proteins in the p50 (–/–) brain, this study suggests that the p50 subunit can potentially be targeted for the development of therapeutic interventions in disorders in which oxidative stress plays a key role.

1. Introduction

Nuclear factor kappa B (NF-κB) is a key transcription factor that was earlier known to regulate immune and inflammatory processes and viral replication (Baeuerle and Henkel, 1994; Baldwin, 1996). The NF-κB family of transcription factors play key roles in the regulation of cell growth, activation, differentiation, and survival. In addition, NF-κB plays an important role in synaptic plasticity and long-term memory formation (Albensi and Mattson, 2000; Mattson et al., 2000; Santoro et al., 2003). The activation of NF-κB is responsible for transcription of various genes like: TNFα, interleukins, e.g., IL1, IL2, and IL6;

* *Corresponding author*. Fax: +1 859 257 5876.
E-mail address: dabcns@uky.edu (D.A. Butterfield).

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chemokines, adhesion molecules; enzymes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and proliferation-related proteins such as cyclin D1 among many others (Karin et al., 2002; Ferrucci et al., 2004; Chung et al., 2005; Donninger et al., 2004). In mammals, this family of NF-κB consists of several proteins, which include NF-κB1 (p50), NF-κB2 (p52), RelA (p65), c-Rel (Rel), and RelB (Hayden and Ghosh, 2004). A hallmark of this family of proteins is that they contain a highly conserved domain of ~300 amino acids, termed the *Rel* homology domain, which contains sequences important for dimerization, DNA binding, and nuclear localization (Grilli et al., 1993; Hayden and Ghosh, 2004). The classical and well-studied NF-κB heterodimer, which is also a potent activator of gene expression, is composed of p50 and p65 subunits. Unlike p65, p50 and p52 subunits lack transactivation domains and are produced either by the proteolytic processing of the precursor molecules p105 and p100 or cotranslationally from incompletely synthesized molecules by the proteasome (Ghosh et al., 1998; Sun and Andersson, 2002; Moorthy et al., 2006).

NF-κB is expressed in many cell types in the nervous system and is constitutively active in subsets of cells in the cortex and hippocampus of the rodent brain at comparably low levels (Kaltschmidt et al., 1994; O'Neill and Kaltschmidt, 1997). In resting cells, the classical NF-κB heterodimer p65/p50 is complexed with the inhibitor protein IκB, of which there are 5 known isoforms; IκBα, IκBβ, IκBγ, IκBε, and Bcl-3. NF-κB is sequestered as an inactive complex in the cytoplasm by IκB, until activation or stimulation of NF-κB in the cells leads to the phosphorylation and immediate proteasomal-mediated degradation of IκB. This leads to the nuclear translocation of NF-κB which then binds to the regulatory region of DNA and leads to transcription of various responsive genes (May and Ghosh, 1998; Santoro et al., 2003; O'Donnell et al., 2005). NF-κB can be activated in both transcriptional activating and repressing forms (Beg and Baltimore, 1996; May and Ghosh, 1998). This dual role of NF-κB has indeed presented considerable complexities and difficulties in studies that try to examine the mechanisms of action of this transcription factor. Hence, the precise role of NF-κB and its related mechanistic pathways have not been completely established.

Other than the classical heterodimer composed of the p50 and p65 subunits, little is known about gene regulation by other hetero- and homodimeric forms of NF-κB (Gadjeva et al., 2004). NF-κB knockouts have provided the best avenue for the investigation of the various roles of these subunits in the activation of NF-κB. It is known that p50-deficient mice develop normally, though they display functional defects in immune response (Beg et al., 1995). Neurons from p50 knockout mice survive well in culture and are no more sensitive or resistant to neuronal death than wild type neurons (Aleyasin et al., 2004). On the other hand, p65 knockouts have been shown to have defects during development and to die prematurely of liver apoptosis (Beg et al., 1995; Hayden and Ghosh, 2004). As a result, p50 knockout mice are preferred in studies that try to elucidate various mechanisms of NF-κB-related activity.

Considering the above, the current study involved further analysis of the p50 subunit of NF-κB. Since most studies on p50 knockout mice have so far been carried out using a wide range of stimuli (Weih et al., 1997; Iimuro et al., 1998; Pennypacker et al., 2001; Mabley et al., 2002; Gadjeva et al., 2004; Kassed and Herkenham, 2004), the present study provided a unique opportunity for investigating the role of p50 subunit in a stimulus-free experimental setting. We hypothesized that the homozygous knockout of the p50 (–/–) subunit could potentially be protective in a stimulus-free environment through an oxidative stress mechanism. To test our hypothesis, we measured the levels of oxidative stress biomarkers in the frontal cortex of WT and p50 (–/–) mice and observed a significant reduction in the levels of lipid peroxidation, as measured by the lipid peroxidation product HNE, and the levels of protein oxidation, as measured by the levels of 3-nitrotyrosine (3NT) and protein carbonyls, in the brains of p50 (–/–) mice compared to the WT. We further carried out a differential expression proteomic analysis on brain proteins of the p50 (–/–) mice, and we identified five proteins to be significantly increased in expression in the p50 (–/–) mice when compared to the WT. We determined that the lack of the p50 subunit makes the brain of mice less susceptible to oxidative stress and also activates the expression of key proteins involved in energy metabolism and antioxidant activity. Hence, this study has provided insights into the role of the p50 subunit of NF-κB and additional evidence on the mechanism of NF-κB activation.

2. Results

2.1. *Levels of oxidative stress*

The levels of protein carbonyls, 3NT and 4-hydroxnonenal (HNE) as indicators of oxidative stress were measured in the frontal cortex of p50 (–/–) mice and compared to WT. Fig. 1 shows total protein oxidation measured by the accumulation of protein carbonyls and 3NT and also the levels of lipid peroxidation as measured by protein-bound HNE. There was a significant decrease in the levels of all oxidative stress parameters measured in brains of the p50 (–/–) mice compared to that of WT.

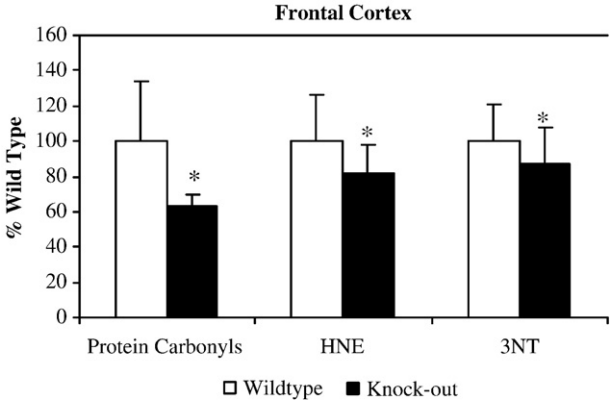

Fig. 1 – Protein carbonyl, 3NT and HNE levels in the frontal cortex of p50 (–/–) compared to wild type. There was a significant reduction in the levels of protein carbonyls, 3NT and HNE in the p50 (–/–) mice compared to the wild type. Data are represented as % wild type (WT); error bars indicate the SEM for each group measured (*$p < 0.05$ $n = 5$).