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Review

Activation of NF-κB by Reactive Oxygen Intermediates in the Nervous System

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ABSTRACT

Nuclear factor kappa B (NF-κB) is a transcription factor crucially involved in glial and neuronal function. NF-κB is ubiquitously distributed within the nervous system, and its inducible activity can be discerned from constitutive activity. Prototypic inducible NF-κB in the nervous system is composed of the DNA-binding subunits p50 and p65 complexed with an inhibitory IκB-α molecule. A number of signals from the cell surface can lead to rapid activation of NK-κB, thus releasing the inhibition by IκB. This activates translocation of NF-κB to the nucleus, where it binds to κB motifs of target genes and activates transcription. Previous findings have identified reactive oxygen intermediates (ROI) as a common denominator of NF-κB activating signals. More specifically, hydrogen peroxide (H_2O_2) might be used as second messenger in the NF-κB system, despite its cytotoxicity. Analysis of pathways leading to NF-κB activation in the nervous system has identified a number of ROI-dependent pathways such as cytokine- and neurotrophin-mediated activation, glutamatergic signal transduction, and various diseases with crucial ROI involvement (*e.g.*, Alzheimer's disease, Parkinson's disease, experimental autoimmune encephalomyelitis, multiple sclerosis, amyotrophic lateral sclerosis, and injury). A number of NF-κB-specific target genes contribute to the production of ROI or are involved in detoxification of ROIs. In this review, possible mechanisms and regulatory pathways of ROI-mediated NF-κB activation are discussed. Antiox. Redox Signal. 1, 129–144, 1999.

INTRODUCTION

NUCLEAR FACTOR KAPPA B (NF-κB) is a transcription factor with inducible activity that is present in many neuronal cell types (for review, see O'Neill and Kaltschmidt, 1997). To date five mammalian NF-κB DNA-binding subunits are known: p50, p52, p65 (RelA), c-Rel, and RelB (Baeuerle and Baltimore, 1996; Baldwin, 1996). The p65 subunit contains extremely strong transactivation domains (Schmitz and Baeuerle, 1991). A knock-out of p65 with embryonic lethality underscores the imminent role of the transactivating p65 subunit. Inhibitory proteins are IκB-α, IκB-β, IκB-γ (p105), IκB-δ (p100), and IκB-ε (Whiteside and Israel, 1997). Within the nervous system, heteromeric NF-κB is most frequently composed of two DNA-binding subunits (*e.g.*, p50 or p65/RelA), which are either constitutively active or are complexed with the inhibitory subunit IκB-α (Kaltschmidt *et al.*, 1993b, 1994, 1995a,b, 1997; Rattner *et al.*, 1993; Guerrini *et al.*, 1995). Interaction of ankyrin obstructs the nuclear localization signals p65 and p50 (Jacobs and Harrison, 1998; Malek *et al.*, 1998), thus

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keeping the complex in the cytoplasm. Activation of NF-κB results in the degradation of IκB, which in turn exposes the nuclear localization signals, resulting in the nuclear import of NF-κB (Baeuerle and Henkel, 1994). In neurons, NF-κB can be activated by stimuli such as glutamate (Guerrini *et al.*, 1995; Kaltschmidt *et al.*, 1995a) or nerve growth factor (NGF) (Wood, 1995), which can involve activation of p75NTR (Carter *et al.*, 1996). In some subtypes of neurons, NF-κB is constitutively activated (Rattner *et al.*, 1993; Kaltschmidt *et al.*, 1994b; Schmidt-Ullrich *et al.*, 1996). The specific post-translational regulation qualifies NF-κB as an immediate signal transducer for transmitting short-term external signals to the nucleus. Thus, NF-κB is a transcription factor that was found in synaptic compartments (Kaltschmidt *et al.*, 1993b; Guerrini *et al.*, 1995; Meberg *et al.*, 1996; Suzuki *et al.*, 1997) and in *Aplysia* axoplasm (Povelones *et al.*, 1997). Localization and signals that activate NF-κB qualify this factor as an ideal candidate for transporting information from the synapse to the nucleus. We have suggested that NF-κB functions as a retrograde signal transducer that unifies signal perception at distant sites (dendrites, axons, synapses) with an effector function and thus is as a molecular switch for turning on gene expression (Kaltschmidt *et al.*, 1993a; O'Neill and Kaltschmidt, 1997; Kaltschmidt and Kaltschmidt, 1998).

SIGNAL TRANSDUCTION VIA TUMOR NECROSIS FACTOR ACTIVATES NF-κB

In terms of understanding NF-κB-relevant signal transduction pathways, most progress has been achieved with tumor necrosis factor (TNF). Therefore, we will try to summarize briefly current knowledge (Fig. 1). TNF receptors (p55 and p75) are expressed in the nervous system both in neurons and glia. Recent findings suggest that TNF modulates neuronal responses to excitotoxic and hypoxic insults in the nervous system (for review, see O'Neill and Kaltschmidt, 1997). In contrast to the protective effects of TNF, there are also reports that identify TNF-α as a potent mediator of microgliosis, astrogliosis, and cell death (Feuerstein *et al.*, 1997). Neuronal cell death and breakdown of the blood–brain barrier and demyelination were described in a transgenic model expressing TNF in astrocytes (Probert *et al.*, 1997). Hence, TNF plays an important role in the nervous system; most signal transduction pathways were characterized in the immune system (for review, see Ashkenazi and Dixit, 1998). As summarized in Fig. 1, binding of soluble TNF to the trimeric TNF-receptor triggers the recruitment of various adapter molecules. For clarity, the TNF-dependent caspase pathway will not be discussed in this context. NF-κB activation is mediated via the interaction of the adapter TRAF-2 with the kinase RIP. This, in turn, activates the kinase NIK, which is part of a high-molecular-weight complex. Recent results show that a scaffold protein is mediating the assembly of NIK and IκB-kinase (IKKα) and IKKβ. This protein IKAP (IKK-complex-associated protein) can function to link the kinases NIK, Ikkα, and Ikkβ physically (Zandi *et al.*, 1998). This physical interaction mediated by the scaffold IKAP might be essential for the

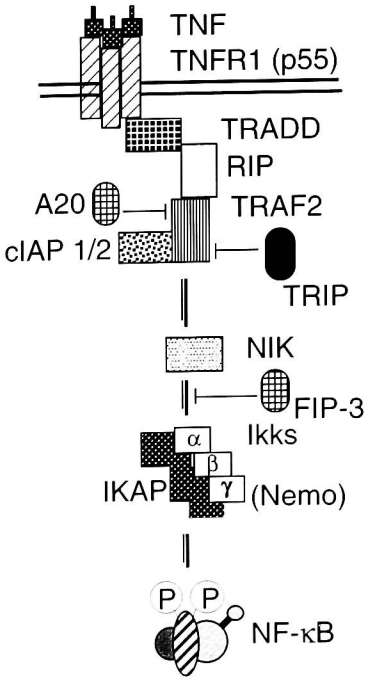

FIG. 1. TNF-mediated NF-κB activation. Binding of TNF-α activates IKKs through the signal cascade shown. This activation leads to phosphorylation of IκB-α **(striped elliptical circle)** and degradation resulting in the activation of NF-κB. For details see text. The caspase pathway was omitted for clarity.

function of cytokine-inducible IKK complexes, to impose tight control by NIK over IKKs. Finally, a homo- or heterodimeric complex of Ikkα or Ikkβ is activated via the action of NIK. The Ikk complex can phosphorylate IκB bound by NF-κB more efficiently than free IκB. Thus, this preference for bound IκB provides a mechanism to turn off activated NF-κB via the accumulation of unphosphorylated free IκB (Zandi *et al.*, 1998). Recently a third kinase, NEMO/IKKγ (Yamaoka *et al.*, 1998), was discovered. It must be expressed to transduce NF-κB activation by TNF, and interleukin-1 (IL-1). Therefore, NEMO/IKKγ might function to link the IκB kinases physically to upstream activators. Perhaps IKAP and NEMO/Ikkγ are part of the same holoenzyme complex. Several inhibitors of TNF-mediated signaling were recently identified, including the zinc finger protein A20 (Song *et al.*, 1996), TRIP (TRAF-interacting protein), which contains a RING finger motif (Lee and Choi, 1997), and FIP-3, which contains a zinc finger and multiple leucine zippers (Li *et al.*, 1999). In contrast to the other inhibitors, FIP-3 expression induces apoptosis on its own in HEK 293 cells, presumably due to inhibition of NF-κB (Li *et al.*, 1999). In contrast, the proteins cIAP and TRAF were recently identified as NF-κB target genes mediating the antiapoptotic action of activated NF-κB (Wang *et al.*, 1998).

CHECKPOINTS IN THE ACTIVATION OF NF-κB: THE ROLE OF REACTIVE OXYGEN INTERMEDIATES

Pioneering work from the lab of P.A. Baeuerle identified the reactive oxygen intermediate (ROI) hydrogen peroxide (H_2O_2) as a second messenger, integrating several signal transduction pathways (Schreck *et al.*, 1991; Schreck and Baeuerle, 1991; Baeuerle *et al.*, 1996). It has been shown that TNF can induce the production and release of ROIs in fibroblasts. Similarly phorbol myristate acetate (PMA), IL-1, lipopolysaccharide (LPS), viral infection, ultraviolet (UV)-light, γ-immunoglobulin M rays, anti-(IgM), and T-cell mitogens leads to the production of H_2O_2 and causes NF-κB activation (for review, see Schreck *et al.*, 1992a). Mitochondria were identified as a subcellular compartment essential for TNF-mediated ROI production (Schulze-Osthoff *et al.*, 1992, 1993). Depletion of mitochondria abrogated TNF-mediated ROI production, resulting in a repression of TNF-mediated cytotoxicity and inhibition of NF-κB-dependent IL-6 production. Furthermore, an inhibition of electron transport at complex III (cytochrome *c* reductase) by antimycin A resulted in a marked potentiation of TNF-mediated injury in the L929 fibroblast cell line. Antimycin A induced increased ROI generation, potentiating TNF-mediated NF-κB activation.

Another argument for the crucial role of ROIs in the activation process of NF-κB stems from blocking NF-κB activation with small-molecule antioxidants. In systems other than the nervous system, it was shown that thiol compounds such as *N*-acetyl-L-cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC), iron chelators, vitamin E, quinone derivates, and α-lipoic acid could repress stimulus-dependent NF-κB activation (see Schreck *et al.*, 1992a for overview). The activation of the latent complex of NF-κB was shown to rely on the production of ROIs. Exogenously provided H_2O_2, which can rapidly diffuse through membranes, was shown to activate the latent binding activity (Schreck *et al.*, 1991). Later, it was shown that PDTC blocks this activation process in living cells (Schreck *et al.*, 1992b). This is due to an antioxidative action of PDTC, as shown by Schmidt *et al.* (1996). Interestingly, PDTC also blocks DNA binding of NF-κB but not of AP-1 *in vitro* (Meyer *et al.*, 1993). Other antioxidants, such as NAC, which replenish the glutathione (GSH) pool, can also act to block NF-κB activation (Staal *et al.*, 1990; Mihm *et al.*, 1995). Similarly, the action of many known activators of NF-κB, *e.g.*, cytokines TNF, IL-1, LPS, or phorbolesters, rely on ROI. This activation can be blocked by overexpressed or exogenously added catalase, pointing to an important role of H_2O_2 in this process (Schmidt *et al.*, 1995). In the activation process of NF-κB, thus far the phosphorylation of IκB has been identified as a molecular target of ROIs that could be blocked with the antioxidant PDTC (Traenckner *et al.*, 1995).

Taken together these results speak in favor

of an oxidative environment necessary for the NF-κB activation in the cytoplasm (see Fig. 2). Several steps with different requirements for redox processes are important for NF-κB activation. As summarized in Fig. 2, the control of NF-κB activation can operate on three potential redox checkpoints and directly on the NF-κB complex, in addition to the multiple possibilities of controlling upstream signal transduction pathways. These checkpoints might represent the degradation of IκB (Fig. 2a), nuclear import (Fig. 2b), and DNA binding (Fig. 2c). The first checkpoint is entered via the IKK-mediated phosphorylation of IκB at Ser-32 and Ser-36. This phosphorylated IκB is a substrate of the IκB ubiquitin ligase E3, which recognizes IκB due to its interaction with a receptor protein (E3RSIκB) (Yaron *et al.*, 1998). Ubiquitin conjugation takes place on lysines 21 and 22 (for review, see Baldwin, 1996). The ubiquitinated IκB is thus ultimately targeted for proteolytic degradation, providing free NF-κB for nuclear import, and thus entering the second checkpoint (for review, see Baeuerle and Baltimore, 1996). Degradation of the ubiquitinated IκB (Fig. 2) takes place within the proteasome (Palombella *et al.*, 1994; Traenckner *et al.*, 1994).

Recent data about nuclear import as the second redox-checkpoint were gathered using an activity-specific monoclonal antibody. This antibody recognizes the activated form of NF-κB due to interaction with its nuclear localization signal (see below for detailed discussion). Using the neurotoxic amyloid beta peptide (Aβ), an inverted U-shape dose dependence of NF-κB activation could be shown (Kaltschmidt *et al.*, 1997) using this antibody. High amounts of Aβ still activated the degradation of IκB and thus allowed the antibody to bind, but all p65

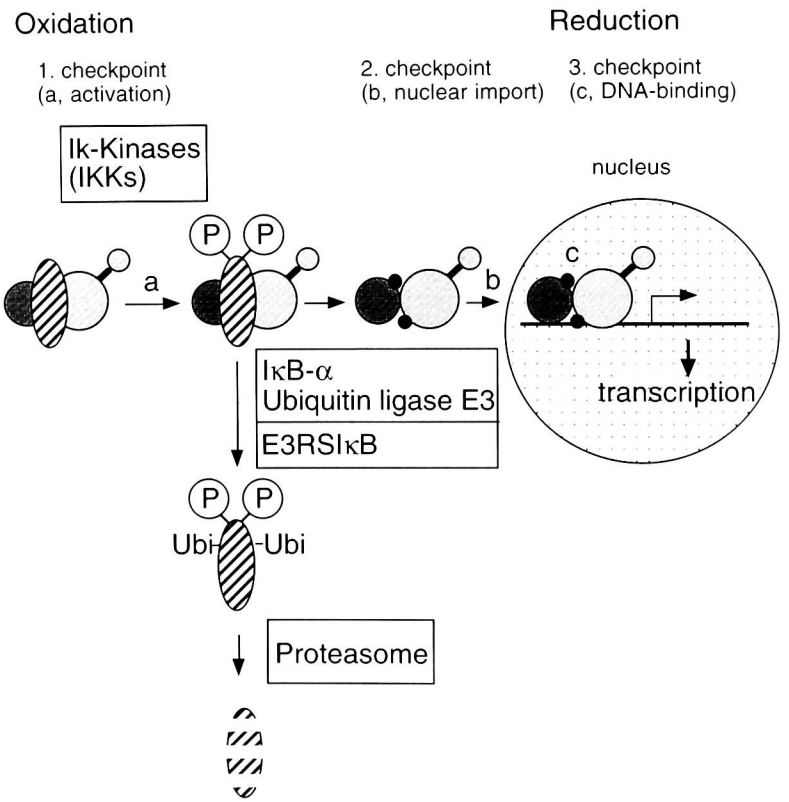

FIG. 2. Activation of NF-κB is controlled by three potential redox checkpoints. The first checkpoint is entered by phosphorylation of IκB **(striped)** via IKKs, followed by ubiquitinilation and degradation of the inhibitor **(striped)** within the proteasome. This results in free NF-κB **(dark grey circle,** p50; **grey circle,** p65; **grey dot,** transactivation domain) with exposed nuclear translocation signals **(black dots)**. This activated NF-κB molecule can enter the nucleus (second checkpoint). The third checkpoint controls the binding of NF-κB to its cognate DNA binding sequence in the nucleus, resulting in transcription of NF-κB target genes (for details see text).

immunoreactivity was now concentrated in perinuclear aggregates (Kaltschmidt *et al.*, 1997). This finding suggests that massive overoxidation interferes with nuclear import. It was reported that both activation and nuclear import are inhibited in T47D cells overexpressing GSH peroxidase (Kretz-Remy *et al.*, 1996). Within the nucleus, NF-κB is bound to its cognate target sequences, resulting in enhanced transcription of its target genes.

MOLECULAR EVIDENCE FOR REDOX-SENSITIVE DNA-BINDING

Redox regulation of the DNA–protein interaction can play an important role in controlling the actions of many mammalian transcription factors and thus provides a means to control gene expression. Two eminent transcription factors regulated by redox-changes were described: AP-1 (Abate *et al.*, 1990) and NF-κB (Staal *et al.*, 1990; Schreck *et al.*, 1991). The DNA-binding activity of AP-1 (*e.g.*, Fos-Jun heterodimers) is also regulated by a redox mechanism (Abate *et al.*, 1990). NF-κB is an example of transcription factor activated by ROIs (Schreck *et al.*, 1991), whereas AP-1 DNA binding and AP-1-dependent transcription can be activated by antioxidants, (*e.g.*, PDTC (Meyer *et al.*, 1993). NF-κB binding activity is stimulated by reducing agents (Zabel *et al.*, 1991; Schenk *et al.*, 1994) (*e.g.*, dithiothreitol, 2-mercaptoethanol, thioredoxin). DNA binding is abolished by sulfhydryl-modifying agents, *e.g.*, diamide, *N*-ethylmaleimide) (Toledano and Leonard, 1991). In contrast, in the activation process the requirements for DNA-binding demand a reducing environment (see Fig. 2c; DNA binding). Similarly, it was shown that the inhibition of DNA binding by increased amounts of oxidants could be rescued via reduction with β-mercaptoethanol in extracts derived from intact cells (Toledano and Leonard, 1991; Brennan and O'Neill, 1996), thus pointing to a role of reactive cysteines in DNA binding.

Using recombinant p50, R. Hay and co-workers have shown a crucial role for Cys-62 as a redox-sensitive site. Mutation of this amino acid rendered the protein insensitive to the inactivating action of thiol-group modifiers. Similarly, thioredoxin was able to stimulate DNA binding of wild type (wt) p50 *in vitro* and enhance the human immunodeficiency virus long terminal repeat (HIV-LTR)-driven reporter gene expression *in vivo* (Matthews *et al.*, 1992). Also, it was shown that Cys-59 of (mouse sequence) p50 and Cys-38 of p65 subunit (mouse sequence) are probably the two most critical for redox-controlled cysteine residues involved in DNA binding (see Fig. 3) (Toledano *et al.*, 1993). Acetylation of Cys-59 inhibits DNA binding of p50 but not its mutation to serine. All redox-sensitive cysteines are found in conserved sequence motifs present in all Rel proteins (Toledano *et al.*, 1993). Interestingly, p50 from human and mouse contain the same sequence motif as p65 from human and mouse (see Table 1). Recent progress in solving the molecular structures of p50 and p65 bound to DNA (Chen *et al.*, 1998a) explains the data obtained from mutational analysis (see Fig. 3). As shown schematically in Fig. 3, both cysteines are making key backbone contacts to DNA. Identical contacts in the homodimer structures (p50/DNA and p65/DNA complexes; Ghosh *et al.*, 1995; Chen *et al.*, 1998b) were also observed (G. Ghosh, personal communication).

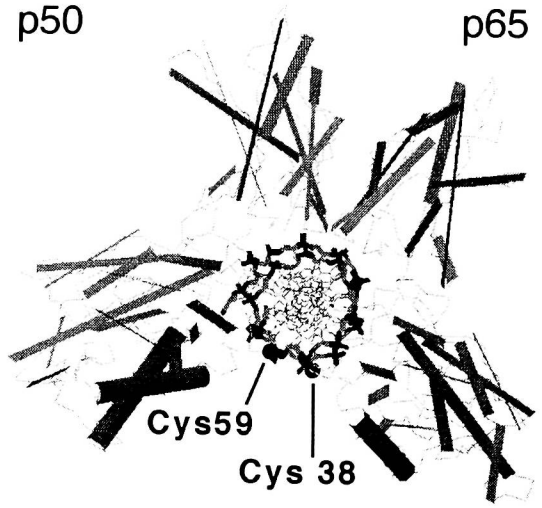

FIG. 3. Crystal structure of a typical NF-κB heterodimer consisting of the subunits p50 and p65 (Chen *et al.*, 1998); BHDB: 1VKX. The DNA helix can be seen in the center. Note the redox-sensitive Cys residues 59 from p50 and Cys-38 from the p65 subunit in close proximity to DNA backbone. Details are discussed in the text.

TABLE 1. CONSERVED REDOX-SENSITIVE CYSTEINE RESIDUES IN THE REL HOMOLOGY DOMAIN OF NF-κB SUBUNITS

	Cys position	*Sequence*	*Accession number*
Mouse p50	59	FRFRYV**C**EGP	BPDB: 1VKX
Human p50	57	FRFRYG**C**EGP	GB: X61498
Mouse p65	38	MRFRYK**C**EGR	GB: M61909
Human p65	38	MRFRYK**C**EGR	GB: M62399

PATHWAYS GENERATING OXIDATIVE STRESS WITHIN THE NERVOUS SYSTEM

Several pathophysiological conditions (inflammation or neurological diseases) can increase ROI production (Halliwell, 1992). The brain is selectively vulnerable to oxidative stress. Principal reasons (Halliwell and Gutteridge, 1989) for this might be: (1) mainly oxidative metabolism producing a lot of reactive oxygen species as byproducts of normal physiological processes; (2) frequent use of signaling pathways generating a lot of ROIs, *e.g.* involving glutamate or calcium (see Fig. 4; Coyle and Puttfarcken, 1993; (3) elaborate membrane systems prone to destruction via lipid peroxidation; (4) high content of pro-oxidant iron ions, at least in selective brain regions; (5) low antioxidant defense capacities in extracellular fluids.

The following ROIs are most frequently involved in neuronal damage: superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}OH$), and H_2O_2, all of which are generated as byproducts of normal biochemical processes using molecular oxygen (O_2). About 1–2% of electrons transported in the respiratory chain leak to generate superoxide anion in reaction. Therefore, mitochondria are considered as major sites of ROI production (see Fig. 4). Adenosine triphosphate (ATP) produced via the mitochondrial respiratory chain is crucial for the function of the deliberate structures of the nervous system. Consequently, mitochondria are found in many cellular compartments such as cell bodies as well as in neuronal processes (dendrites, axons, synaptic boutons), where they produce ROIs in addition to energy. Several enzymes in the nervous system, such as monoamine oxidase (MAO; Fig. 4), tyrosine hydroxylase, and L-amino oxidase, produce H_2O_2 as a byproduct. Endogenous substances like ascorbic acid or catecholamines could contribute to the production of H_2O_2 via autooxidation (Halliwell and Gutteridge, 1989). An important calcium-dependent signaling pathway involves the activation of phospholipase A2 (PLA_2), which releases arachidonic acid (AA) from membrane lipids. Arachidonic acid is the substrate for either cycloxygenases, which release prostaglandins together with radicals, or lipoxygenase to form leukotrienes.

ROI-DEPENDENT PATHWAYS ACTIVATING NF-κB IN THE NERVOUS SYSTEM

Within the nervous system, a considerable amount of knowledge has been gathered about ROI-dependent pathways leading to NF-κB activation (see Fig. 5 and O'Neill and Kaltschmidt, 1997). When starting our analysis of the NF-κB system in the nervous system, we made some predictions about a potential involvement of NF-κB in several ROI-dependent neurological diseases such as Alzheimer's disease (AD), Parkinson's disease, multiple scle-

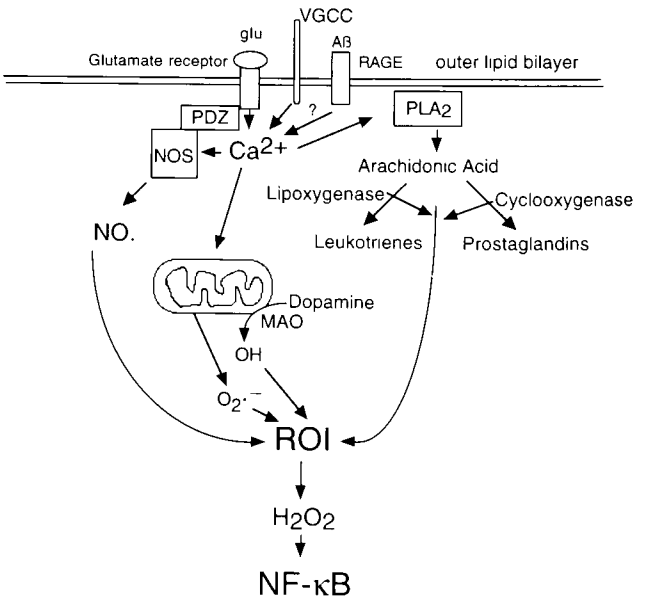

FIG. 4. Mechanisms of ROI generation within the nervous system. See text for details.

rosis, and amyotropic lateral sclerosis (ALS) (Kaltschmidt *et al.*, 1993a). Now we will discuss multiple studies in line with our initial predictions. Some studies were performed using single-cell analysis of NF-κB activation. This was achieved with a monoclonal antibody, specifically recognizing the IκB free form of p65 in fixed cells (Kaltschmidt *et al.*, 1995b).

Recent evidence from structural data (Huxford *et al.*, 1998; Jacobs and Harrison, 1998) explained the molecular basis of this recognition (see Baeuerle, 1998 for discussion). The nuclear localization signal of p65 is unstructured when p65 is free of IκB. Thus, a monoclonal antibody directed against a peptide comprising the nuclear localization signal (NLS) epitope can bind this IκB-free form. In contrast, when p65 is complexed with IκB, the NLS adopts an α-helical conformation. Hence it cannot be recognized by the activity-specific antibody.

Previous studies relying on DNA-binding assays could not easily discriminate between the activation process per se, which takes place in the cytoplasm, and the influence of ROIs on DNA binding. DNA binding is first induced via activation of NF-κB followed by nuclear entry, but on the other hand it was found that massive oxidation results in inhibition of DNA binding (see above, Fig. 2). Recently, using an antibody specific for the activated form of NF-

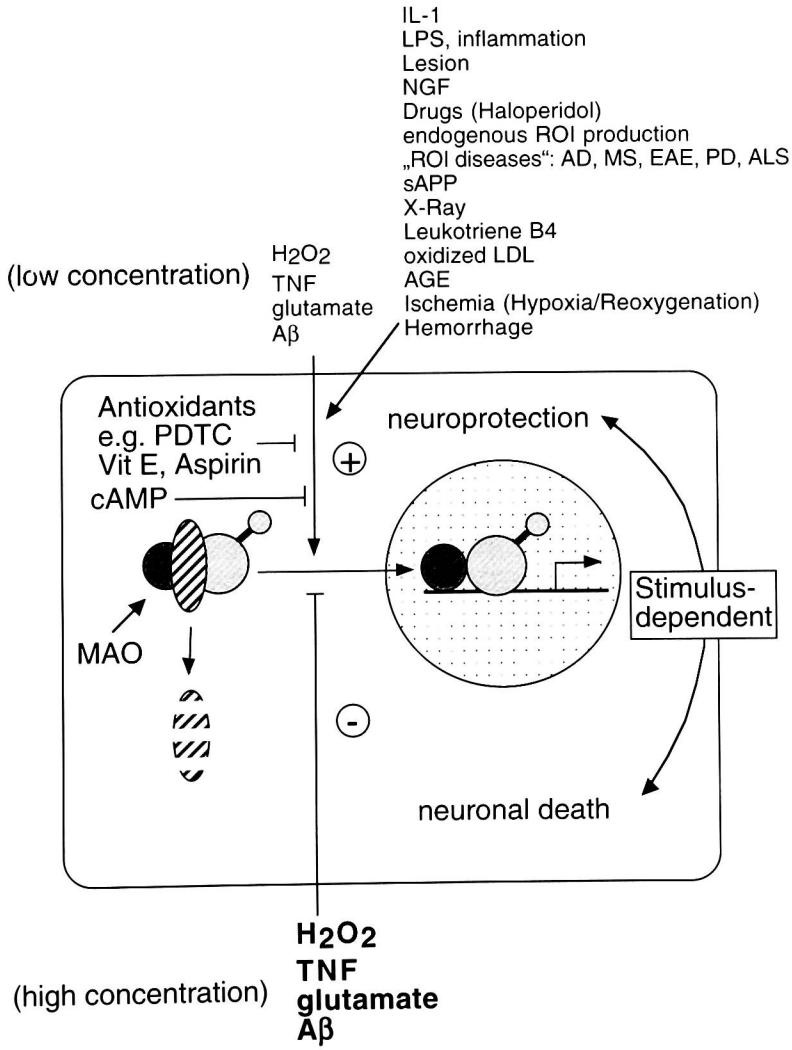

FIG. 5. Concentration-dependent NF-κB activation via ROI-dependent pathways. Note that the same stimulus can either activate or inhibit NF-κB, depending on the concentration. In addition NF-κB activation can lead to stimulus dependent neuronal death or neuronal survival.

κB p65, it was found that also the nuclear import of NF-κB could be inhibited by massive oxidation (Kaltschmidt *et al.*, 1997). The oxidative ROIs could be either applied extracellularly as H_2O_2, or the ROI production could be induced via Aβ (Fig. 5). This translated to a dose-dependent activation of NF-κB, where increasing amounts inhibited nuclear import and/or activation of NF-κB. For primary cerebellar granule cells, a concentration of 10 μM H_2O_2 or 0.1 μM Aβ resulted in an optimal activation of NF-κB, which was abolished with higher concentrations (Kaltschmidt *et al.*, 1997).

Along these lines, it was previously reported that cholinergic stimulation could activate NF-κB and that this activation could be blocked by H_2O_2 (Li *et al.*, 1996). Cholinergic system deficits are reported in AD and are also detected during normal aging. Thus, the constitutively activated form of NF-κB was analyzed during aging in rat hippocampus and basal forebrain. Surprisingly, an increase of NF-κB-specific binding activity was detected (Toliver-Kinsky *et al.*, 1997), perhaps suggesting an increase of NF-κB-activating ROI during aging. Similarly, ROI production by TNF could be abolished by PDTC pretreatment in primary cerebellar granule cells and TNF showed a similar dose-dependence (Kaltschmidt *et al.*, submitted). Interestingly, these dose-dependent effects translate into physiologically relevant responses (see Fig. 5): Preactivation of NF-κB with low doses of Aβ or TNF translated into neuroprotection against high amounts of Aβ, whereas preactivation with a non-NF-κB-activating dose did not induce neuroprotection (Kaltschmidt *et al.*, submitted). Similarly, it was found that NF-κB could be very efficiently activated with nanomolar doses of glutamate in cerebellar granule cells via the NMDA-receptor (Guerrini *et al.*, 1995), but higher amounts (500 μM) resulted in only a weak NF-κB activation in the same culture paradigm (Kaltschmidt *et al.*, 1995a). In contrast, the glutamate agonist kainate, specific for non-NMDA receptors, resulted in a concentration of 100 μM in a very efficient activation of NF-κB (Kaltschmidt *et al.*, 1995a). NF-κB activation induced by ceramide was protective against glutamate-mediated excitotoxicity (Goodman and Mattson, 1996). In contrast, in a quinolinic acid-induced model of striatal excitotoxicity, inhibition of NF-κB via striatal administration of an NLS peptide decoy reduced apoptotic cell death (Qin *et al.*, 1998). In this respect, a recent study showed NF-κB activation via kainate in the hippocampal fields CA1/CA3 and in the pyriform/entorhinal cortex sensitive to neurotoxicity (Perez *et al.*, 1996), albeit in astrocytes.

Behl and co-workers have shown that several antioxidants including α-tocopherol (vitamin E), act as neuroprotective agents (Behl *et al.*, 1992). Similarly, the anti-inflammatory agent aspirin, which can either act as an antioxidant or as a specific inhibitor of IKKβ (Yin *et al.*, 1998), was shown to be neuroprotective in an excitotoxicity paradigm presumably due to inhibition of NF-κB (Grilli *et al.*, 1996).

It could be shown that Aβ toxicity is mediated by the production of H_2O_2, which could activate NF-κB (Behl *et al.*, 1994). This H_2O_2 neurotoxic action of Aβ could be inhibited by antioxidants. PC-12 cell clones resistant to Aβ could be isolated (Behl *et al.*, 1994). These PC-12 cell clones were shown to withstand the apoptotic effects of ROI generation (hydroxyl radical or H_2O_2) via Aβ, amylin, 6-hydroxydopamine, and H_2O_2, but show no resistance to other forms of apoptotic cell death or to necrotic cell death (Mazziotti and Perlmutter, 1998). Interestingly, the molecular mechanisms of Aβ resistance rely on the constitutive activation of NF-κB (Lezoualc'h *et al.*, 1998). Resistance of H_2O_2 was abolished via overexpression of a transdominant negative IκB or by the synthetic glucocorticoid dexamethasone, leading to increased expression of endogenous IκB. These data point to a crucial role of NF-κB in protecting neurons from the neurotoxic action of Aβ.

In this line, analysis of AD patients brains has shown that neurons located in concentric rings around early plaque stages contain constitutively activated NF-κB, in contrast to neurons in plaque-free regions (Kaltschmidt *et al.*, 1997). Constitutively activated NF-κB was also shown to mediate neuronal survival of cortical cultures when challenged with Aβ (Bales *et al.*, 1998). Early-onset AD could be genetically linked to mutations of the transmembrane proteins called presenilins. These mutations lead to increased proteolytic production of the neu-

rotoxic peptide Aβ due to a protein interaction with the Aβ precursor protein (APP) (Weidemann *et al.*, 1997). In addition to advanced glycation end products (AGE), Aβ can also bind to the receptor of advanced glycation end products and activate NF-κB (for review, see O'Neill and Kaltschmidt, 1997). Recently, it could be shown that RAGE activation resulted in the increased expression of macrophage colony-stimulating factor by an oxidant-sensitive NF-κB-dependent pathway (Du Yan *et al.*, 1997). Oxidative stress induced by overexpression of the mutated presenilin-1 could be counteracted by treatment with the soluble form of APP. This counteraction could be blocked in PC-12 cells with decoy oligonucleotides comprising κB motifs (Guo *et al.*, 1998).

Similarly it was shown that TNF-mediated neuroprotection involves an NF-κB-dependent increase in Ca^{2+} buffering (Barger *et al.*, 1995). Reduction of protein nitration was correlated with an NF-κB-dependent increase in manganese superoxide dismutase (MnSOD) expression (Mattson *et al.*, 1997). Despite the large amount of evidence for a role of NF-κB in neuroprotection, there is also the notion that NF-κB activation is linked to neurodegeneration. In astrocytes, NF-κB can be also activated by Aβ, which in turn activates the expression of inducible nitric oxide synthase (iNOS) (Akama *et al.*, 1998). The iNOS-induced NO production might contribute to the oxidative stress known to play a crucial role in AD. On the other hand, the autoregulatory loop present in astrocytic iNOS expression might also switch off increased iNOS production (see Fig. 6). Similarly LPS and cytokine-mediated NF-κB-dependent iNOS regulation in astrocytes is blocked by cAMP, in contrast to macrophages where NF-κB can be activated by cAMP (Pahan *et al.*, 1997).

Parkinson's disease is manifested by the loss of dopaminergic neurons located in the substantia nigra in front of the brainstem. Substantia nigra neurons project to the striatum, a region involved in movement control, therefore explaining the patient's deficits such as shaking of the hands and the head. A clue for the involvement of ROIs in this disease became evident from the Parkinson-like disease of some young drug addicts. The reason for this phenotype was found to be a contaminating neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrridine (MPTP), which is metabolized in glial cells via MAO, resulting in ROI production (for review, see Halliwell and Gutteridge, 1989).

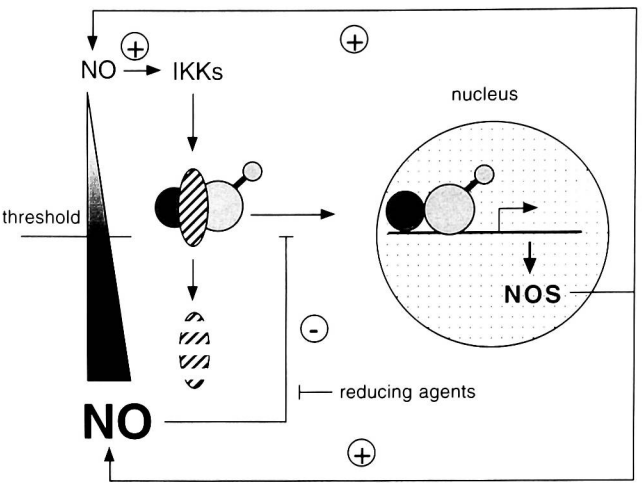

FIG. 6. Molecular feedback-loop regulating NO via modulation of NF-κB activity. Low amounts of NO can activate NF-κB whereas high amounts inhibit NF-κB and therefore also the expression of iNOS resulting in a net reduction of cellular NO concentration.

Related to this, it was shown that haloperidol, a dopamine receptor antagonist, is cytotoxic to mouse clonal hippocampal HT22 cells and causes cell death by oxidative stress (Post *et al.*, 1998). Consequently, NF-κB was activated by haloperidol; blocking of NF-κB was partially cytoprotective. Further on, dopaminergic cell death was shown to be ROI-dependent apoptosis (Hunot *et al.*, 1997). NF-κB was found to be constitutively activated in dopaminergic neurons of Parkinson patients. In primary cultures, ROI-dependent apoptosis induced via C2-ceramide correlated with NF-κB activation. On the other hand, ceramide-induced NF-κB activation was protective in hippocampal neurons against excitotoxic and oxidative insults (Goodman and Mattson, 1996).

Other diseases with increased ROI amounts include experimental autoimmune encephalomyelitis (EAE), which is an animal model of multiple sclerosis. Increased ROIs are also found in multiple sclerosis (MS) itself. In these diseases, with their mainly inflammatory character, microglia and macrophages might be crucially important for the increased ROI production and cytotoxicity (for review, see Banati

et al., 1993). In this line, constitutively activated NF-κB could be detected in EAE, which correlated with the maximal disease effects (Kaltschmidt *et al.*, 1994a). Similarly activated NF-κB was found in macrophages present in actively demyelinating plaques of MS patients' brains (Gveric *et al.*, 1998).

Interestingly, NF-κB is activated in astrocytes and not in motor neurons in ALS (Migheli *et al.*, 1997), suggesting that ROI-dependent cell death of motor neurons is the consequence of missing NF-κB-directed neuroprotection. Ionizing radiation also increases ROI production and leads to NF-κB activation in PC12 cells, which in turn might activate IL-6 production (Abeyama *et al.*, 1995). This radiation-induced IL-6 can act as differentiating agent. Previously, we have shown that NGF activates NF-κB via $p75^{NTR}$ (Carter *et al.*, 1996). NGF has multiple functions, *e.g.*, it can act to induce the differentiation of the pheochromocytoma cell line PC12 or induce cell death (Frade and Barde, 1998). Deprivation of NGF results in increased ROI production.

Inhibition of NF-κB in PC12 cells induces apoptosis that was resistant to NFG (Taglialatela *et al.*, 1997). Similarly inhibition of NF-κB in the central nervous system via proteasome inhibitors results in increased apoptosis (Taglialatela *et al.*, 1998), speaking for an essential role of constitutively activated NF-κB (Kaltschmidt *et al.*, 1994b) for neuronal survival *in vivo*.

Inflammatory responses are a major component of secondary injury, and ROI-mediated NF-κB activation might be crucially involved. Using the activity-dependent monoclonal antibody specific for p65, it was found that activated NF-κB is present within the nuclei of macrophages/microglia, endothelial cells, and neurons of traumatic spinal cord injury (Bethea *et al.*, 1998). In addition, upregulation of the NF-κB target gene iNOS was detected in this model of traumatic injury. Increased NF-κB binding activity was detected in a cortical trauma paradigm (Yang *et al.*, 1995). Another report presents data on a downregulation of p65 immunoreactivity after crush-lesion of the rat sciatic nerve (Doyle and Hunt, 1997). Similarly, crush-lesion of *Aplysia* pedal neurons results in a downregulation of axoplasmic NF-κB binding activity (Povelones *et al.*, 1997). We have suggested that this downregulation might be a consequence of ROI-mediated processes (Kaltschmidt and Kaltschmidt, 1998), but so far there is no direct experimental proof. Additional potential important activators of NF-κB are listed in Fig. 5 (see Baeuerle and Baichwal, 1997; O'Neill and Kaltschmidt, 1997).

Regarding relevant target gene expression, recently an NF-κB-dependent enhancer was identified on an intronic enhancer regulating the TNF- and IL-1-induced expression of MnSOD (Jones *et al.*, 1997). Moreover, it was also shown that MnSOD overexpression in liver resulted in a repression of NF-κB binding activity (Zwacka *et al.*, 1998), suggesting an autoregulatory loop. Other NF-κB target genes that contribute to ROI production (see Fig. 4) include the NO-producing enzyme iNOS, cyclooxygenase 2, 12-lipooxygenase, PLA, the iron-binding protein ferritin H-chain, and NAD(P)H:quinone oxidoreductase (for a recent review, see Baeuerle and Baichwal, 1997).

An important NF-κB target gene with a direct link to apoptosis is CD95L (Fas ligand), which can be induced via ROI-dependent NF-κB activation H_2O_2 or hypoxia/reoxygenation) in microglial cells (Vogt *et al.*, 1998). An important *in vivo* model for hypoxia is forebrain ischemia, and ROI-dependent NF-κB activation could be detected using this model (Salminen *et al.*, 1995; Clemens *et al.*, 1997). This ROI-induced NF-κB activity could be repressed by the disease-ameliorating antioxidants LY231617 (Clemens *et al.*, 1998) and NAC (Carroll *et al.*, 1998) or α-lipoic acid (Packer, 1998).

AUTOREGULATORY LOOPS IN NO PRODUCTION

Another radical with second-messenger function is NO, which was first described as endothelial relaxing factor (for review, see Moncada, 1997). This radical plays an important role in neurotransmission. It serves as a retrograde messenger connecting pre- with post-synaptic events (see Arancio *et al.*, 1996). Dawson and co-workers have shown that NO can be generated by NMDA receptor-mediated calcium ion influx activating NOS (for review,

see Dawson *et al.*, 1992), thus connecting glutamatergic signal transduction with NO production. Previously a cross-coupling of NO signaling and the regulation of NF-κB activation became evident. Two NO-mediated effects were reported—a co-stimulatory effect and an inhibitory effect (see Fig. 6).

A co-stimulatory action of NO leads to a strong enhancement of other stimuli, *e.g.*, PMA. Schmitz and colleagues (Umansky *et al.*, 1998) have shown that low doses of NO that could not stimulate NF-κB on its own strongly augment the NF-κB activation by PMA as assayed by DNA-binding and transactivation. Because the inducible NO is under NF-κB control (Chao *et al.*, 1997), this provides an important feed-forward loop. On the other hand, increased amounts of NO resulted in strong inhibition of TNF-mediated NF-κB activation. These findings strengthen the emerging picture of a dose-dependent, ROI-mediated NF-κB activation, which is operative in neurons (see above). NO donors inhibited the DNA binding activity of recombinant NF-κB p50 and p65 homodimers and p50-p65 heterodimers (Matthews *et al.*, 1996). This involves a covalent modification of the conserved redox-sensitive Cys-62 residue of p50. Biophysical analysis of a synthetic NF-κB p50 peptide suggested that NO could modify Cys-62 by *S*-nitrosylation. Similarly, it was recently reported that NO produced by the neuronal form of NOS, which is constitutively expressed in astrocytes, results in an inhibition of astrocytic NF-κB activity (Togashi *et al.*, 1997). Moreover, inhibition by NO synthase with the competitive NOS inhibitor L-NMMA results in a slow but robust activation of NF-κB. This effect was completely reversible by L-Arg, the substrate of NOS.

In astrocyte cultures, PDTC does not inhibit LPS- or TNF-mediated NF-κB activation. Treatment with PDTC resulted in an activation of astrocytic NF-κB (Togashi *et al.*, 1997), presumably due to an action of NO scavenger resulting in the formation of mononitrosyl iron complexes. Park *et al.* show that the effect of NO does not interfere with the activation and translocation of NF-κB in astroglial cells (Park *et al.*, 1997). But NO inhibits binding of NF-κB to DNA, presumably due to the modification of redox-sensitive cysteines. Inhibited NF-κB binding could be restored by the reducing agent dithiothreitol. In this respect, it is interesting to note that the LPS- and interferon-γ-mediated activation of NF-κB, which translates in the expression of iNOS in astroglial cells, can be inhibited by benzoquinones (Niwa *et al.*, 1997). Thus, a high amount of NO might tonically inhibit the expression of proinflammatory genes such as TNF, IL-6, or iNOS (see O'Neill and Kaltschmidt, 1997) in the nervous system.

CONCLUSION

A large body of evidence suggesting a crucial role of ROI-mediated activation of NF-κB in the nervous system has accumulated. Although data are available for disease models such as AD, Parkinson's disease, EAE, MS, and inflammation, *in vitro* studies have shown a wealth of specific activators known to rely on oxidative stress both for neurons and glia. Conflicting results about the role of NF-κB in neuroprotection of neurodegeneration might reflect a stimulus-specific complexity or could be due to different experimental paradigms. Taken together, the exquisite sensitivity of NF-κB to redox changes qualifies this transcription factor as a key regulator mediating either normal physiological signals or triggering a pathological gene expression program.

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ABBREVIATIONS

AA, arachidonic acid; A20, zinc finger protein involved in TNF signalling; cAMP, cyclic

adenosine monophosphate; APP, amyloid beta precursor protein; ATP, adenosine triphosphate; AP-1, activator protein 1; Ab, amyloid beta peptide; AD, Alzheimer's disease; AGE, advanced glycation endproducts; ALS, amyotropic lateral sclerosis; cIAP, cellular inhibitor of apoptosis; CA $^1/_2$, cornu amunis fields of the hippocampus; CD95L, CD95 (Fas) ligand; Cys, cysteine; COX, cyclooxygenase; EAE, experimental autoimmune encephalomyelitis; E3RSIkB, phospho-IkBa-E3 receptor subunit; FIP, 14.7K interacting protein; glu, glutamate; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; IgM, immunoglobulin M; IKAP, IKK-complex-associated protein; IκB, inhibitory protein for NF-κB; IKK, IκB kinase; IL-1, interleukin 1; IL-6, interleukin 6; iNOS, inducible nitric oxide synthase; L-Arg, L-arginine; LDL, low density lipoprotein; LPS, lipopolysaccharide; MAO, monoamine oxidase; MnSOD, manganese-containing superoxide dismutase; MS, multiple sclerosis; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyrridine; NAC, N-acetyl-L-cysteine; NEMO, NF-κB Essential MOdulator (IKKg); NF-κB, nuclear factor kappa B; NGF, nerve growth factor; NIK, NF-κB inducing kinase; NLS, nuclear localization signal; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; O_2, molecular oxygen; $^{\bullet}O_2^-$, superoxide anion, $^{\bullet}OH$, hydroxyl radical; p100, precursor of p52 (IκB-d); p105, precursor of p50 (IκB-g); p50, 50 kDa DNA-binding subunit of NF-κB; p52, 52 kDa DNA-binding subunit of NF-κB; p65, RelA; p75 NTR, 75 kDa neurotrophin receptor; PD, Parkinson's disease; PDTC, pyrrolidinedithiocarbamate; PDZ, protein family (PSD-95, DIg, ZO-1); PLA2, phospholipase A2; PMA, phorbol myristate acetate; RAGE, receptor of advanced glycation endproducts; Rel, protein family with homology to the avian reticuloendotheliosis virus strain T (REV-T) viral oncogene Rel; RIP, receptor interacting protein; ROI, reactive oxygen intermediates; Ser, serine; sAPP, soluble amyloid precursor protein; TNF, tumor necrosis factor; TNFR1, TNF receptor 1 (p55); TRADD, TNFR-associated death domain protein; TRAF, TNF receptor-associated factor; TRIP, TRAF-interacting protein; Ubi, ubiquitin; VGCC, voltage gated calcium channels; VitE, vitamin E; X-Ray, gamma radiation.

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