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# A novel mechanism for pergolide-induced neuroprotection: inhibition of NF-κB nuclear translocation

D. Uberti[a], T. Carsana[a], S. Francisconi[a], G. Ferrari Toninelli[a], P.L. Canonico[b], M. Memo[a,*]

[a]*Department of Biomedical Sciences and Biotechnologies, School of Medicine, University of Brescia, Viale Europa 11, 25123 Brescia, Italy*
[b]*DiSCAFF Department, University of Piemonte Orientale, Novara, Italy (P.L.C.)*

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**Abstract**

We previously demonstrated that the dopaminergic agonist pergolide, independently from its DA agonist activity, can exert neuroprotective effects against cell death induced in SH-SY5Y neural cells by $H_2O_2$ treatment. Since oxidative stress in SH-SY5Y neural cells is known to activate the NF-κB pathway we tested the hypothesis that pergolide may interfere with NF-κB activity. Based on Western blot analysis and immunocytochemistry, pergolide was found to prevent $H_2O_2$-induced apoptosis by inhibiting NF-κB nuclear translocation and activation of p53 signalling pathway. Similarly, the cell-permeable SN50 peptide, which is known to block NF-κB nuclear translocation, prevented both $H_2O_2$-induced p53 expression and apoptosis. The mechanism of action of pergolide responsible for neuroprotection differed from that of antioxidants. In fact, Vitamin E, contrary to pergolide and SN50, rescued neuronal cells from $H_2O_2$-induced apoptosis acting upstream NF-κB activation, as demonstrated by the prevention of $H_2O_2$-induced IκB degradation. These data suggest a novel site of action of pergolide that may account for additional pharmacological properties of this drug.


*Keywords:* Parkinson's disease; Oxidative stress; Dopamine agonists; Human neuroblastoma cell line; Tumour suppressor protein p53

## 1. Introduction

Pergolide mesylate, a synthetic ergoline derivative endowed with dopamine (DA) agonist activity [1,2], is one of the drugs successfully used in clinical therapy of Parkinson's disease (PD), a chronic and progressive neurological disease characterized by selective degeneration of DA neurons in the substantia nigra. Several double-blind, controlled studies have demonstrated the efficacy of pergolide as adjunctive therapy in the treatment of PD [3], and recent studies have indicated pergolide monotherapy as an efficacious and well-tolerated first-line treatment in patients with early-stage PD [4]. Although, it is generally believed that treatment of PD with drugs acting as agonists at the DA receptor level, such as pergolide, is symptomatic rather than curative, increasing evidence indicates that these drugs may elicit neuroprotective properties, in experimental models [5–11] and in patients [12]. Thus, it is very important to address this claim because, at present, no effective treatments are available for slowing the progression of the disease.

Among factors involved in idiopathic PD, oxidative stress, as the result of inefficacious antioxidant defense mechanism as well as an excessive production of reactive oxygen species (ROS), is considered an important cause/factor in the pathogenesis of the disease (see [13,14] as reviews). Experimental studies demonstrated that enhanced dopamine turnover, occurring to compensate for dopamine depletion in PD, is associated with increased formation of oxidized glutathione, and is prevented by inhibitors of DA metabolism [15]. Postmortem studies show increased lipid hydroperoxide and reactive carbonyl levels in the substantia nigra of PD compared with normal subjects [16,17]. Further insight on the oxidative stress hypothesis in PD has been achieved experimentally using the neurotoxin MPTP, which produces irreversible clinical, biochemical and neuropathological effects similar to those found in PD. MPTP, through the formation of $MPP^+$, causes an impairment in the function of the mitochondrial respiratory chain with generation of ROS in nigral DA-containing neurons [18].

*Abbreviations:* DA, dopamine; NF-κB, nuclear factor kappaB; PD, Parkinson's disease; ROS, reactive oxygen species

* Corresponding author. Tel.: +39-030-3717516; fax: +39-030-3717407.
*E-mail address:* memo@med.unibs.it (M. Memo).

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Different studies ascribed to pergolide antioxidant effects [11,19], and this property could explain its protective activity. In this line, we previously demonstrated that pergolide, independently of its DA agonist activity, can exert neuroprotective effects against cell death induced by ROS [20]. However, the mechanism by which this drug exerts antioxidant and neuroprotective effects has not yet been completely clarified. We hypothesized that pergolide may interfere with the NF-κB transcriptional activity. NF-κB pathway is known to be activated by enhanced ROS generation in different cell phenotypes, including the neural cell lines used in the present study. Nuclear translocation of NF-κB was also found to be increased in DA neurons of PD patients [21]. Here we show that pergolide prevented $H_2O_2$-induced apoptosis and inhibited NF-κB nuclear translocation without affecting IκB degradation. A similar pattern of results was obtained using the cell-permeable SN50 peptide, which is known to block NF-κB nuclear translocation, but not with the antioxidant Vitamin E. These data suggest novel sites of action of pergolide that may account for additional pharmacological properties of this drug.

## 2. Experimental procedures

### 2.1. Cell culture

Neuroblastoma cell line SH-SY5Y was routinely cultured in 1:1 Ham's F12:Dulbecco modified Eagle's medium supplemented with 10% (v/v) foetal calf serum, 2 mM glutamine, 50 μg/ml penicillin, and 100 μg/ml streptomycin and was kept at 37 °C in humidified 5% $CO_2$/95% air. For differentiation, cultures were seeded at approximately $5 \times 10^4$ cells/cm$^2$ and retinoic acid was added to a final concentration of 10 μM. Medium was changed every day and cultures were allowed to differentiate for 2 weeks.

### 2.2. Drug treatment

In the oxidative stress paradigm, cells were exposed to 1 mM $H_2O_2$ in PBS or PBS for 15 min. Pergolide mesylate (1 μM, kindly provided by Eli Lilly, Sesto Fiorentino, Italy) was added to the culture media 2 h before the addition of the cytotoxic agent. The cell permeable inhibitory peptide SN50 (100 μg/ml) and the inactive analog SN50M (100 μg/ml) (both from Biomol Research Laboratories, Plymouth Meeting, PA, USA) were added to the culture media 30 min before $H_2O_2$. Vitamin E (Sigma Aldrich, Saint Louis, Missouri, USA) 1 μg/ml was added to the culture simultaneously to $H_2O_2$. Briefly, culture cells were washed with PBS and exposed to $H_2O_2$ for 15 min. After this period, cells were returned to their original conditioned media for an additional time according to the experiments.

### 2.3. Comet assay

Cells growing in dish were washed with PBS and detached with scraper; about $4 \times 10^3$ cells/cm$^2$ were transferred to an eppendorf tube and spun down at $150 \times g$, 3 min at 4 °C. Cells were then suspended in 80 μl of 0.5% low melting agarose in PBS kept at 37 °C, and transferred onto slides, precoated with 1% agarose. Slides were then incubated in cold lysis buffer (2.5 M NaCl, 100 mM $Na_2EDTA$, 10 mM Tris, pH 10, and 1% Triton X-100 added immediately before use) for 1 h to remove cellular proteins. This treatment leaves residual nuclei embedded in the agarose gel. Slides were then washed briefly in PBS, prior to being placed in a horizontal electrophoresis tank filled with cold electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) and left at 4 °C for 40 min to unwind the DNA. Electrophoresis was conducted for 30 min at 25 V and 300 mA in an ice-cooled tank. After electrophoresis the slides were drained and washed three times in neutralization buffer at 4 °C (0.4 M Tris–HCl, pH 7.5). After neutralization comet DNA was visualized with 50 μl of 20 μg/ml ethidium-bromide in PBS. The slides under coverslip were visualized in a fluorescence microscope. One hundred cells of five fields per sample, visualized with a 40× objective, were counted. Data are expressed as the percentage of cells with a tail (comet).

### 2.4. Chromosomal condensation

Chromosomal condensation and DNA fragmentation were determined using the chromatin dye Hoechst 33258. After treatments, cells were stained with 1 μM Hoechst 33258 in PBS for 5 min, washed and then fixed with 4% paraformaldehyde in PBS for 30 min at 4 °C. Cells were then analysed under a fluorescence microscope. One hundred cells of five field per sample, visualized with 40× objective, were counted. Data are expressed as the percentage of apoptotic nuclei.

### 2.5. Immunocytochemistry

Cultures were fixed for 5 min in cold methanol. Cells were raised in PBS and then incubated overnight with the polyclonal anti-p50 antibody (C19, Santa Cruz Biotechnology, Santa Cruz, CA, USA), used at 1:200 dilution and a monoclonal anti-p53 antibody (Ab 5, Oncogene, Cambridge, MA, USA), used at 1:300 dilution. Fluorescein-conjugated secondary antibodies were used for detection. Slides were mounted with moviol and examined using a fluorescence microscope.

### 2.6. Western blot analysis

Cells were harvested in 100 μl of lysis buffer containing 50 mM Tris, pH 7.6, 150 mM NaCl, 5 mM EDTA, 1 mM phenyl-methyl-sulfonyl-fluoride, 0.5 μg/μl leupeptin,

5 μg/μl aprotinin, 1 μg/ml pepstatin. The samples were sonicated and centrifuged at 15,000 × *g* for 30 min at 4 °C. The resulting supernatants were collected and protein content determined by a conventional method (BCA protein assay Kit, Pierce, Rockford, IL, USA). Nuclear and cytosolic protein fractions were separated according to Uberti et al. [22]. Fifteen micrograms of total protein or nuclear or cytoplasmic extracts were electrophoresed on 12% SDS–PAGE, and transferred to nitrocellulose paper (Schleicher and Schuell, Dassel, Germany). Filters were incubated at RT overnight with anti-p50 (same used for immunohistochemistry), anti-p53 (same used for immunohistochemistry), anti-p65 (C-20 Santa Cruz Biotechnology, Santa Cruz, CA, USA) anti-IκBα (C-21 Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p21 (F5, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Bax (B9, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-GADD45 (C4, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-MSH2 (Ab2 Oncogene, San Diego, CA, USA) anti-actin (Sigma, Saint Louis, MO, USA), or anti-tubulin (Ab3, Neo Markers, Fremont, CA, USA) antibody in 3% non-fat dried milk (Sigma, Saint Louis, MO, USA). The secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a chemiluminescence blotting substrate kit (Boehringer, Mannheim, Germany) were used for immunodetection. Evaluation of immunoreactivity was performed on immunoblots by densitometric analysis using a LKB 2222-020 Ultra Scan XL laser densitometer at a wavelength of 633 nm.

### 2.7. Statistical evaluation

Results in cell viability and densitometric analysis of the immunoblots are given as mean ± standard error mean values. Statistical significance of differences was determined by mean values of the ANOVA.

## 3. Results

Human SH-SY5Y neuroblastoma cells were differentiated by treatment with retinoic acid for 2 weeks to acquire morphological, neurochemical and electrophysiological properties characteristic of a neural cell line. Exposure of these cells to increasing concentrations of $H_2O_2$, ranging from 0.5 to 2 mM, for different periods of time, ranging from 1 to 20 min, resulted in a concentration- and time-dependent apoptosis which was easily detectable 24 h after the insult (data not shown). Based on the results obtained in the above mentioned experiments, cells were routinely exposed to 1 mM $H_2O_2$ for 15 min to obtain submaximal cytotoxicity. As shown in Fig. 1, cells in apoptosis were detected by Hoechst 33258 staining (left panel, B) and comet assay (right panel, F).

This experimental model of neurotoxicity was challenged with pergolide. Based on previous experiments, the drug was added to the culture media 2 h before the $H_2O_2$ pulse at 1 μM concentration. Fig. 1 shows representative micrographs illustrating the antiapoptotic effects of 1 μM pergolide as evaluated by the lack of pyknotic and shrinking nuclei (panel C) and comets (panel G).

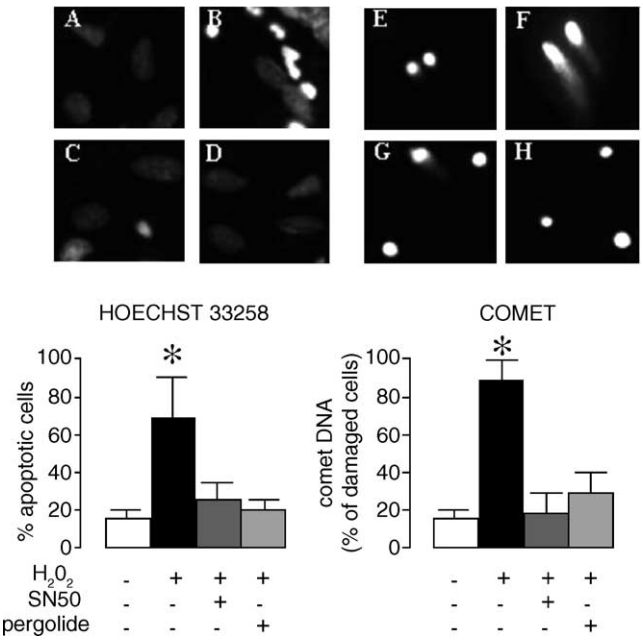


Fig. 1. Pergolide and SN50 protect SH-SY5Y neural cells from $H_2O_2$-induced apoptosis. Apoptotic cell death was determined morphologically by Hoechst 33258 staining (left, upper panel) and by measuring DNA degradation with comet assay (right, upper panel). Cells were treated with PBS (A, E) or 1 mM $H_2O_2$ (B–D, F–H) for 15 min in the absence (B, F) or presence of pergolide (C, G) or SN50 peptide (D, H). Pergolide or SN50 were added to the culture media 2 h or 30 min before $H_2O_2$, respectively. Lower panels represent the quantitative analysis of apoptotic cells (left) or comet DNA (right). Data represent means ± S.E.M. of at least three different experiments and are from three separate cell preparations. $^*P < 0.01$ vs. the corresponding control values.

Cells were also challenged with the cell-permeable peptide SN50. This peptide is known to inhibit subcellular traffic of NF-κB complexes from the cytoplasm to the nucleus [23], and this effect has been well described in several cell phenotypes [24–30]. SN50 peptide was added to the culture medium at the final concentration of 100 μg/ml, 30 min before the $H_2O_2$ pulse. Cells were then evaluated for Hoechst 33258 staining and comet assay. As shown in representative pictures reported in Fig. 1, panels D and H, SN50 was able to prevent $H_2O_2$-induced apoptosis. Quantitative analysis of three different experiments by counting the number of cells with condensed nuclei (Fig. 1, left lower panel) or the presence of comet tails (Fig. 1, right lower panel) revealed that both pergolide and SN50, in a statistically significant and reproducible manner, protected against $H_2O_2$.

Fig. 2 shows representative immunofluorescence micrographs carried out with an antibody against the NF-κB p50 subunit in cells pretreated with SN50 (100 μg/ml) or the inactive analog SN50M (100 μg/ml), and then challenged

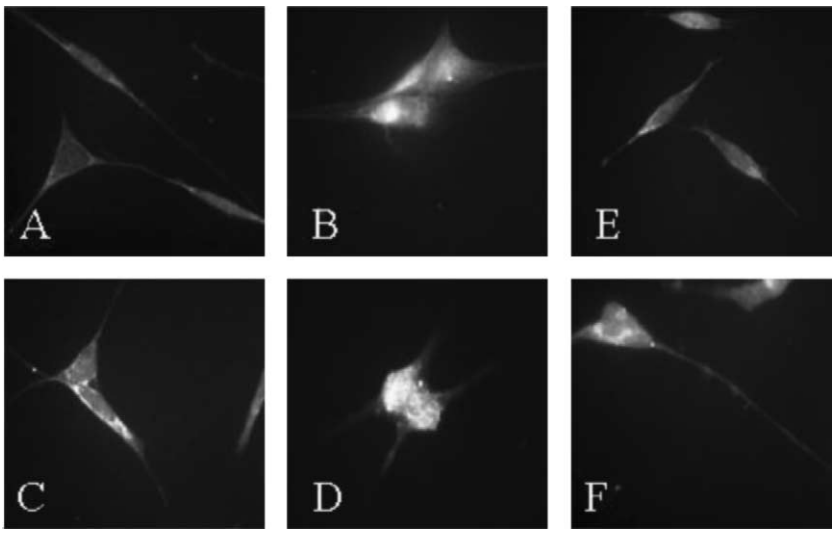


Fig. 2. Pergolide and SN50 inhibit p50 NF-κB nuclear translocation in SH-SY5Y neural cells exposed to $H_2O_2$. Cells were exposed to PBS (A), pergolide alone (E) or 1 mM $H_2O_2$ for 15 min (B–D, F) in the presence of 100 μg/ml SN50 (C), 100 μg/ml SN50M (D), or 1 μM pergolide (F). Pergolide was added to the culture media 2 h before $H_2O_2$. SN50 or SN50M were added to the culture media 30 min before $H_2O_2$. Immunofluorescence was performed 60 min after $H_2O_2$ treatment. Pictures are representative images. Similar results were obtained from three different experiments using cells from three separate preparations.

with 1 mM $H_2O_2$ for 15 min. Exposure of the cells to $H_2O_2$ induced a marked enhancement of p50 immunoreactivity which was already significant 60 min after the oxidative treatment. Immunofluorescence analysis clearly showed the nuclear location of p50 immunoreactivity in $H_2O_2$-treated cells (Fig. 2, panel B). As expected, pretreatment of the cells with SN50 blocked $H_2O_2$-induced p50 nuclear translocation (Fig. 2, panel C). On the contrary, cells pretreated with the inactive analog SN50M prior to the $H_2O_2$ pulse displayed p50 nuclear immunostaining similar to that found in $H_2O_2$ alone-treated cells (panel D). Pretreatment of the cells with 1 μM pergolide 2 h prior to the $H_2O_2$ pulse prevented p50 nuclear translocation (Fig. 2, panel F).

The effects of pergolide on $H_2O_2$-induced NF-κB translocation was also studied by Western blot analysis. Nuclear proteins from cells treated with $H_2O_2$ alone or pretreated with either SN50 peptide or pergolide were extracted 60 min after the oxidative injury. Fig. 3A shows representative immunoblots carried out with an anti-p50 or an anti-p65 antibody (upper panel), and the densitometric analysis of three different experiments (lower panel). Exposure of

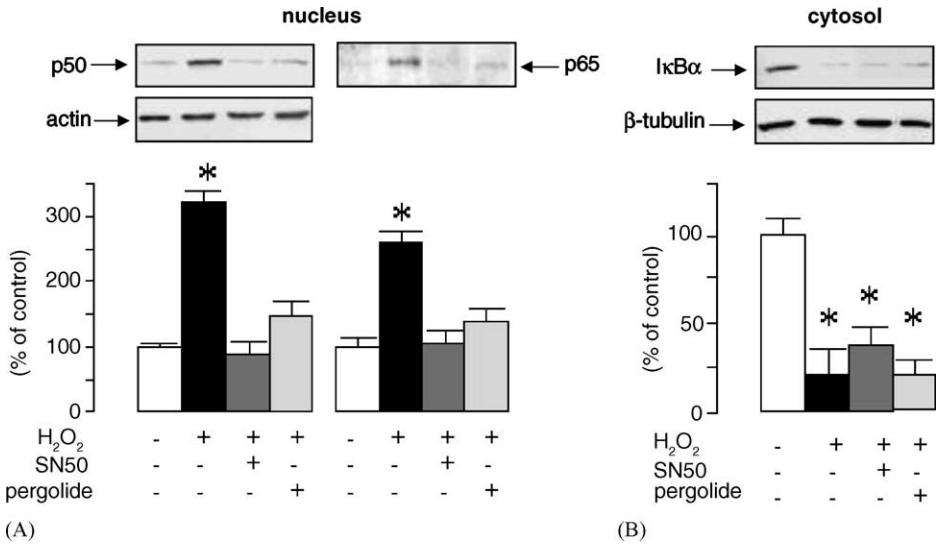


Fig. 3. Induction of nuclear p50 and p65 NF-κB subunits and degradation of IκBα by $H_2O_2$ in SH-SY5Y neural cells. Cells were exposed to 1 mM $H_2O_2$ for 15 min in the absence or presence of 1 μM pergolide or 100 μg/ml SN50 peptide as described in Fig. 2. Cytosolic or nuclear protein extracts were prepared from cells 60 min after $H_2O_2$ treatment, electrophoresed, transferred to nitrocellulose paper and immunoblotted with anti-p50 (panel A), anti-p65 (panel A), or anti-IκBα (panel B) antibodies. Anti-actin and anti-β-tubulin antibodies were used to normalize the sample in the nuclear or cytosolic fraction, respectively. Values are expressed as densitometric arbitrary units with 100 corresponding to control and represent means ± S.E.M. of at least three different experiments and are from three separate cell preparations. $^{*}P < 0.01$ vs. the corresponding control values.

SH-SY5Y neural cells to $H_2O_2$ increased both p50 and p65 protein levels of nuclear fraction. Pretreatment with pergolide or SN50 peptide prevented $H_2O_2$-induced p50 and p65 nuclear translocation.

We also studied the expression of IκBα, the inhibitory protein that by binding to NF-κB dimers makes them inactive. 60 min after $H_2O_2$ pulse, IκBα protein levels almost completely disappeared in the cytoplasmic fraction, in comparison with controls (Fig. 3B). As expected, $H_2O_2$-induced IκBα degradation was not affected in the cells pretreated with SN50 peptide. In fact, under these experimental conditions cytosolic levels of IκBα decreased 60 min after $H_2O_2$ pulse in a manner similar to that found in the absence of the peptide. Interestingly, a decrease in IκBα protein levels were also observed in the cytoplasmic fraction derived from cells pretreated with pergolide and then challenged with $H_2O_2$ (Fig. 3B). These data suggest that, similarly to SN50, pergolide does not affect IκBα and the subsequent release of NFκB subunits from the NFκB/IκB complex induced by $H_2O_2$. On the contrary, pretreatment of the cells with the antioxidant Vitamin E, besides its neuroprotective action (Fig. 4, panel B), completely prevented $H_2O_2$-induced IκBα degradation (Fig. 4, panel A).

One of the target genes of NF-κB is the tumour suppressor p53 [31–33]. Although still controversial [34], a link between NF-κB transcriptional activity and the p53 pathway in neural death has been demonstrated [35–37]. We thus evaluated the contribution of p53 in $H_2O_2$-induced apoptosis by immunofluorescence and Western blot analysis. As shown in Fig. 5, a significant increase of the protein levels was observed in many nuclei at 60 min following $H_2O_2$ exposure (panel B). Pre-treatment of the cells with 1 μM pergolide or 100 μg/ml SN50 prior

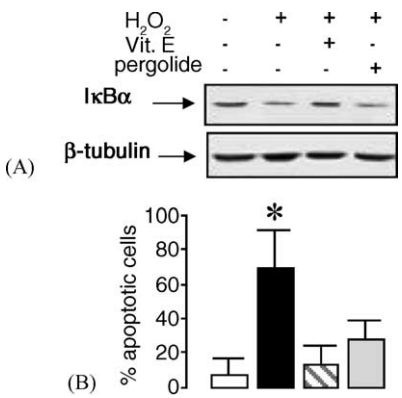


Fig. 4. Vitamin E but not pergolide prevented IkB-α degradation in SH-SY5Y neural cells exposed to $H_2O_2$. Cells were exposed to 1 mM $H_2O_2$ for 15 min in the absence or presence of 1 μM pergolide or 1 μg/ml Vitamin E. (A) Cytosolic protein extracts were prepared from cells 60 min after $H_2O_2$ treatment, electrophoresed, transferred to nitrocellulose paper and immunoblotted with anti-IκBα antibody. Anti β-tubulin antibody was used to normalize the sample. (B) Quantitative analysis of apoptotic cells, determined by Hoechst 33258 staining. Data represent means ± S.E.M. of at least three different experiments and are from three separate cell preparations. $^*P < 0.01$ vs. the corresponding control values.

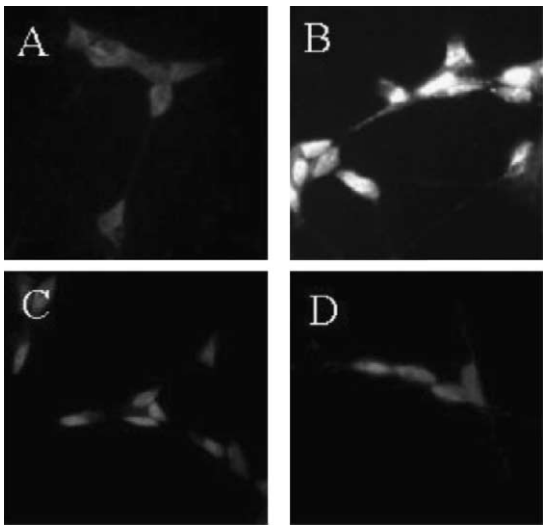


Fig. 5. Pergolide and SN50 inhibit p53 expression in SH-SY5Y neuronal cells exposed to $H_2O_2$. Cells were exposed to PBS (A), or 1 mM $H_2O_2$ for 15 min (B–D) in the absence (B) or presence of 1 μM pergolide (C) or 100 μg/ml SN50 (D). Pergolide, and SN50 were added to the culture media 2 h or 30 min before $H_2O_2$, respectively. Immunofluorescence was performed 60 min after $H_2O_2$ treatment. Pictures are representative images. Similar results were obtained from three different experiments using cells from three separate preparations.

$H_2O_2$ exposure resulted in a complete prevention of the $H_2O_2$-induced enhancement of p53 immunoreactivity (panels C and D).

Protein extracts from cells pretreated with SN50 (100 μg/ml) or pergolide (1 μM), and then challenged with 1 mM $H_2O_2$ pulse, were electrophoresed and immunoblotted with antibodies against p53 and its target gene products such as p21, GADD45, MSH2, and Bax. Representative results and densitometric analysis of the data are in Fig. 6. Exposure of SH-SY5Y neural cells to $H_2O_2$ increased p53 levels by about 3–4-fold. Similarly, the oxidative insult increased the expression of all the p53-target genes studied, i.e. p21 (plus 150% over basal), GADD45 (plus 350% over basal), MSH2 (plus 150% over basal) and Bax (plus 200% over basal) (Fig. 6). The induction of p53 and p53-target genes induced by $H_2O_2$ was completely prevented by pretreatment of the cells with either 100 μg/ml SN50 or 1 μM pergolide (Fig. 6).

## 4. Discussion

This study investigated the molecular mechanisms by which pergolide exerts its neuroprotective effects using a human neural cell line and an oxidative insult as the experimental paradigm. We found that pergolide, contrary to the antioxidant Vitamin E and similarly to the peptide SN50, prevented NF-κB pathway activation by inhibiting nuclear translocation.

NF-κB proteins are ubiquitous transcription factors that are activated in response to oxidative stress [38–43].

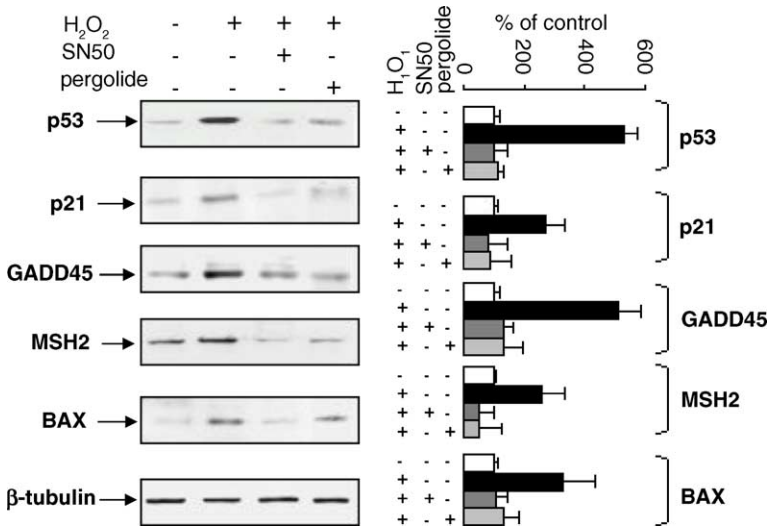


Fig. 6. Pergolide and SN50 inhibit p53 transcriptional activity in SH-SY5Y neural cells exposed to $H_2O_2$. Cells were exposed to 1 mM $H_2O_2$ for 15 min in the absence or presence of 1 μM pergolide or 100 μg/ml SN50 peptide as described in Section 2. Protein extracts were prepared from cells 60 min after $H_2O_2$ treatment, electrophoresed, transferred to nitrocellulose paper and immunoblotted with anti-p53, anti-p21, anti-GADD45, anti-MSH2, or anti-Bax antibodies as described in Section 2. Anti β-tubulin antibody was used to normalized the samples. Values are expressed as densitometric arbitrary units with 100 corresponding to control and represent means ± S.E.M. of at least five different experiments and are from three separate cell preparations.

In particular, the NF-κB family consists of at least five different subunits known as p50, p65 (RelA), C-Rel, p52 and RelB constitutively maintained in a latent form in the cytoplasm by binding to an inhibitory protein Iκβ [44]. Multiple mammalian forms of IκB also exist, namely IκBα, β, γ (p105), δ (p100), ϵ and Bcl-3 [45]. NF-κB is known to function as key regulator of either cell death or survival in both neural and peripheral cells [26,28–30,32–34,37,46]. NF-κB activation involves the phosphorylation and subsequent degradation of the inhibitory protein. Thus, the released NF-κB dimer can translocate into the nucleus and activate target genes. Based on Western blot analysis and immunocytochemistry, we found that upon stimulation by $H_2O_2$ cytosolic IκBα is rapidly degraded and both p50 and p65 NF-κB subunits translocate to the nucleus. The NF-κB C-Rel subunit was not involved in $H_2O_2$-induced transcriptional activation in SH-SY5Y neuronal cells (data not shown). Treatment of the cells with the SN50 peptide was ineffective in preventing $H_2O_2$-induced IκBα degradation but inhibited p50/p65 nuclear translocation. Inhibition of NF-κB nuclear translocation by SN50 was specific since the inactive mutant isoform of the peptide (SN50M) was devoid of effects. Pergolide behaved similarly to SN50. Exposure of cells to pergolide before the $H_2O_2$ pulse resulted in IκBα degradation and inhibition of p50 and p65 nuclear translocation. These data suggest that IκBα degradation, which possibly occurs as a consequence of its phosphorylation in serine S32 and S36 [47], is not affected by pergolide. Vitamin E behaved differently from pergolide or SN50. $H_2O_2$-induced IκBα degradation was completely prevented in cells pretreated with Vitamin E. Thus, the site of action of pergolide appears to be different from that of other NF-κB inhibiting agents, such as antioxidants, that act by scavenging newly generated ROS [48], and aspirin, that is known to interfere with IκB phosphorylation [49]. In line with our data, Galea et al. [50], demonstrated that NF-κB transcriptional activity induced by IL-1β in brain endothelium was inhibited by estrogens without interfering with IκB degradation.

Among the NF-κB-target genes is the tumour suppressor p53 [31]. p53 is a cell-cycle related protein which senses DNA damage and activates DNA repair and/or apoptosis [51–56]. NF-κB and p53 have been previously proposed to be relevant contributors to neuronal apoptosis [35,37] although controversial reports exist on their reciprocal interaction, depending on cell type and experimental conditions [32,34,57]. Data obtained from Western blot analysis and immunocytochemistry indicate that SN50, as well as pergolide, prevents the increased protein expression of p53 and various p53 target genes, including the cyclin kinase inhibitor p21, the cell cycle regulator GADD45, the DNA mismatch repair factor MSH2 and the proapoptotic Bax. These effects may be responsible, at least in part, for the abortion of the $H_2O_2$-induced apoptotic program. In this line, Quin et al. [37] have demonstrated that SN50 significantly inhibits quinolinic acid-induced elevation of p53 mRNA and protein levels and prevents apoptosis in striatal medium spiny neurons.

In summary, we found that pergolide protects human SH-SY5Y neuronal cells from $H_2O_2$-induced apoptosis by inhibiting NF-κB nuclear translocation. This effect may represent an additional pharmacological property of this drug contributing, together with its DA agonist and antioxidant activities, to its clinical efficacy in PD.

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