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Short communication

Cabergoline prevents necrotic neuronal death in an in vitro model of oxidative stress

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Abstract

To study if cabergoline, a long-lasting specific dopamine D2 receptor agonist, has neuroprotective effects against oxidative stress, we exposed (3 h) SH-SY5Y human neuroblastoma cells to *tert*-butylhydroperoxide (*t*-BOOH; 500 μM). *t*-BOOH caused a 42 ± 4% neuronal death, which was prevented by cabergoline (2 h before) in a concentration-dependent manner (EC_{50}: 1.24 μM). This effect was not antagonised by haloperidol (concentration up to 10 μM), and was associated with an increased availability of intracellular GSH contents (+30 ± 11%) and a decrease in the membrane lipid peroxidation (−23 ± 9%). Our data suggest that cabergoline has neuroprotective effects useful for Parkinson's disease therapy.

Keywords: Dopamine receptor agonist; Neurodegeneration; Parkinson's disease; Anti-oxidant; Neuroprotection

1. Introduction

Despite the progress in the understanding of the pathogenesis of Parkinson's disease (Mouradian, 2002), there has been little progress in the pharmacotherapy of this disease and levodopa, although potentially neurotoxic, continues to be the most effective symptomatic drug. Recently, dopamine D2 receptor agonists (i.e., bromocriptine, pergolide, ropinirole, pramipexole and cabergoline) have been introduced into clinical practice for improving symptoms and preventing the development of levodopa-induced neurotoxic effects (Jankovic, 2001).

Most oral dopamine receptor agonists currently in use have relatively short durations of action and appear not to provide the continuous dopaminergic stimulation necessary for clinical efficacy of the therapy (Mouradian, 2002). Several drugs that could achieve this goal have been studied, and among them cabergoline may provide reasonably steady dopaminergic stimulation.

Cabergoline is an ergoline derivative with potent, selective, long-lasting agonist action at dopamine D2 receptors (Fariello, 1998). Administration of cabergoline reverses catalepsy in reserpine-treated rats (Miyagi et al., 1996), and improves the symptoms in animal models of Parkinson's disease (Nomoto et al., 1998). In healthy humans or hyperprolactinemic patients, oral cabergoline has a potent prolactin-lowering effect, lasting 4–7 days (Di Sarno et al., 2001). Cabergoline has a long plasma half-life (63–68 h in healthy volunteers), longer than that of pergolide, bromocriptine, and lisuride, and it is effective in Parkinson's disease therapy, either as monotherapy or combined with levodopa (Baas and Schueler, 2001).

Neuroprotection against oxidative stress is an important factor in the treatment of Parkinson's disease, since free radical-induced damage is one of the major possible mechanisms of nigrostriatal dopaminergic neurodegeneration (Lipton and Rosenberg, 1994). Some of the new dopamine receptor agonists have been shown to possess also neuroprotective properties (Gassen and Youdim, 1999), and we tested if cabergoline can prevent necrotic neuronal death in an in vitro model of oxidative stress.

For this purpose, SH-SY5Y human neuroblastoma cells, differentiated into neuron-like type by treatment with retinoic acid, were exposed to *tert*-butylhydroperoxide (*t*-BOOH), a membrane-permeant oxidant (Amoroso et al., 1999). Our data demonstrate that cabergoline protects SH-SY5Y cells from *t*-BOOH-induced oxidative stress by a

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non-receptor-mediated mechanism, probably linked to both an increased availability of intracellular thiol compounds, and a decrease of membrane lipid peroxidation.

2. Materials and methods

2.1. Cell culture

SH-SY5Y cells were cultured in 1:1 Ham's F-12:Dulbecco's modified Eagle medium (DMEM), supplemented with 10% foetal calf serum (GIBCO BRL, USA) and antibiotics, at 37 °C in 5% CO_2.

For differentiation, retinoic acid (10 μM) was added to the cell culture (60–70% cellular confluence/dish) medium (changed every day) for 1 week.

2.2. Drug treatment

For oxidative stress experiments, the cells were exposed (3 h at 37 °C) to 500 μM *t*-BOOH. Drugs were added to experimental buffer (in mM: NaCl 138, KCl 2.7, $CaCl_2$ 1.2, $MgCl_2$ 1.2, phosphate buffered saline (PBS) 10, glucose 10; pH 7.4): cabergoline (Pharmacia Italia S.p.a., Italy), haloperidol, bromocriptine and apomorphine 2 h before *t*-BOOH, vitamin E (α-tocopherol) 1 h before. Drugs were from Sigma Chemical Co. (USA); all other reagents were from Merck (Germany).

2.3. Cell viability

Cell survival was evaluated by the 3-(4,5-dimethylthiazo1-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983) and by fluorescein diacetate-propidium iodide staining (Jones and Senft, 1985) at the end of the oxidative insult.

2.4. Evaluation of intracellular GSH

Intracellular GSH content was measured on protein-free extracts according to Griffith (1980). GSH contents in each sample were quantified from a standard curve obtained with known amounts of GSH.

2.5. Evaluation of lipid peroxidation

Lipid peroxidation was evaluated by measuring the thiobarbituric acid-reacting substances in cells, according to the method described by Ohkawa et al. (1979).

2.6. Statistical analysis

Results are expressed as means ± S.E.M. of *n* experiments. Statistical significance was evaluated by Student's *t*-test for paired values. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Neuroprotective effects of cabergoline against oxidative stress

t-BOOH induces a rapid, progressive and dose-dependent increase of free radical production in the differentiated SH-SY5Y cells (Amoroso et al., 1999). As established in previous experiments, 3-h exposure to 500 μM *t*-BOOH in experimental buffer at 37 °C significantly ($P < 0.01$) induced the death of 42 ± 4% of neurons (MTT assay) (data not shown). During *t*-BOOH exposure, cell morphology was continuously observed by phase-contrast microscopy and progressive necrosis of the cell body and of neurites was evident.

Cell pretreatment (2 h before *t*-BOOH) with increasing concentrations (0.1–100 μM) of cabergoline was able to significantly ($P < 0.01$) prevent neuronal death. The maximum neuroprotection (56 ± 8% of cell survival, in comparison to cabergoline-untreated cells) was achieved at 10 μM cabergoline; the EC_{50} calculated was 1.24 μM. This effect was not abolished by cell pre-incubation (2 h) with haloperidol (concentration up to 10 μM), a specific dopamine D2 receptor antagonist, suggesting a non-receptor-mediated mechanism. In this case, the EC_{50} calculated was 1.20 μM.

t-BOOH-induced neuronal death was also prevented by cell pre-treatment (2 h before *t*-BOOH) with bromocriptine, another dopaminomimetic ergot derivative, although with a lower potency and efficacy than by cabergoline. The maximum effect calculated for this molecule was 35 ± 8% of cell survival at 10 μM, in comparison with a drug-untreated sample. The EC_{50} was 2.03 μM. Apomorphine (0.1–30 μM) did not have protective effects and was toxic at concentrations up to 30 μM in our experimental model.

When cells were pre-incubated (1 h before *t*-BOOH) with increasing concentrations (0.1–100 μM) of vitamin E, a lipid-soluble radical scavenger (Van Acker et al., 1993), we measured a high degree of neuroprotection (76 ± 10% cell survival, in comparison with drug-untreated cells). The EC_{50} calculated was 5.77 μM (Fig. 1A).

The cabergoline-induced neuroprotection was also confirmed by intravital fluorescein diacetate-propidium iodide staining. With this method the shape and the number of green staining vital cells, and of red bright staining dead cells, were examined by fluorescence microscopy. At the end of 3-h incubation with *t*-BOOH (500 μM), the number of dead neurons was 35 ± 7%, in vehicle-treated, 14 ± 3% in cabergoline (10 μM)-treated, and 12 ± 5% in vitamin E (50 μM)-treated cells, if compared with control cells not exposed to *t*-BOOH (Fig. 1B).

3.2. Effects of cabergoline on the GSH and thiobarbituric acid reacting substances levels

To study the mechanisms underlying cabergoline-induced neuroprotection against oxidative stress in SH-

SY5Y cells, we measured GSH and thiobarbituric acid reacting substances levels. *t*-BOOH (500 μM; 3 h) induced a significant ($P<0.01$) decrease ($-54\pm11\%$) in the intracellular GSH levels, and a significant ($P<0.01$) increase ($+326\pm35\%$) in the cellular thiobarbituric acid reacting substances contents if compared to those in vehicle-treated cells. Pre-treatment of cells with cabergoline (10 μM; 2 h) or vitamin E (50 μM; 1 h) significantly prevented *t*-BOOH-induced effects on both GSH and thiobarbituric acid reacting substances contents (Table 1).

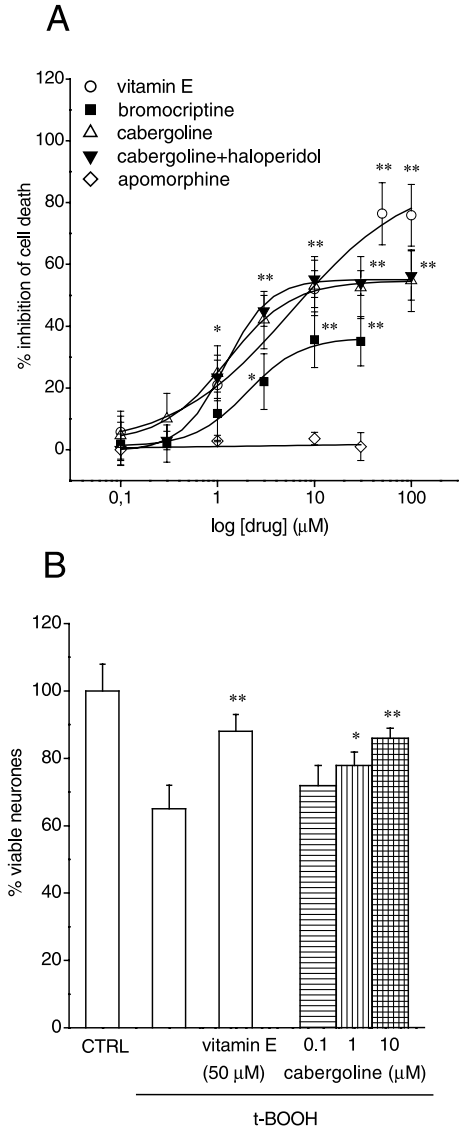

Fig. 1. Neuroprotective effects of dopamine receptor agonists on *t*-BOOH-induced oxidative stress. (A) Cabergoline (0.1–100 μM), haloperidol (10 μM), bromocriptine (0.1–30 μM) and apomorphine (0.1–30 μM) were added 2 h before *t*-BOOH (500 μM, 3 h), vitamin E (0.1–100 μM) 1 h before. The values represent the percentage inhibition of cell death (MTT assay, performed at the end of oxidative insult) over drug-untreated cells. The EC_{50s} calculated were: 1.24 μM for cabergoline, 1.20 μM for cabergoline + haloperidol, 2.03 μM for bromocriptine, and 5.77 μM for vitamin E. (B) Cabergoline (0.1–10 μM) or vitamin E (50 μM) was added 2 or 1 h before *t*-BOOH, respectively. Viable neurones were counted (four separate field-dish) by fluorescein diacetate-propidium iodide staining and are expressed as percentage of control cells not exposed to *t*-BOOH cells. The data represent the means ± S.E.M. of at least six experiments run in triplicate. *$P<0.05$; **$P<0.01$ vs. *t*-BOOH-treated cells.

Table 1
Effects of cabergoline and vitamin E on *t*-BOOH-induced oxidative stress

	% GSH decrease	% Lipid peroxidation increase
Vehicle	0	0
t-BOOH (500 μM)	-54 ± 11	326 ± 35
t-BOOH + cabergoline (10 μM)	-24 ± 2[a]	252 ± 23[b]
t-BOOH + vitamin E (50 μM)	0[a]	237 ± 5[b]

Cells were exposed to *t*-BOOH (500 μM) or vehicle for 3 h at 37 °C. Cabergoline (2 h before *t*-BOOH) or vitamin E (1 h before *t*-BOOH) were kept in the buffer until the end of the experiment. Intracellular GSH content and thiobarbituric acid reacting substances in vehicle-treated cells were 17.07 ± 2.35 nmol GSH/mg of proteins and 1.23 ± 0.02 nmol/mg of proteins, respectively.
The values represent the means ± S.E.M. of at least six experiments run in triplicate.
[a] $P<0.01$ vs. *t*-BOOH-treated samples.
[b] $P<0.05$ vs. *t*-BOOH-treated samples.

4. Discussion

Oxidative stress has been implicated in the progressive neuronal damage characteristic of Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (Lipton and Rosenberg, 1994). We selected as in vitro model of oxidative stress the exposure of cells to the membrane-permeant oxidant *t*-BOOH. In this model cell injury can only be attributed to the overproduction of free radicals, and therefore it represents a simple tool for studying new antioxidant compounds (i.e., cabergoline). SH-SY5Y cells were employed for our experiments, because these cells are sensitive to oxidative stress and express dopamine D2 receptors (Amoroso et al., 1999).

In our experimental model, cabergoline was the most potent dopamine receptor agonist able to prevent oxidative-induced neuronal death. The affinity of cabergoline for dopamine D2 receptors is higher than of both bromocriptine and apomorphine (Miyagi et al., 1996), and we might speculate that selective D2 receptor stimulation is necessary to induce intracellular event responsible for neuroprotection. However, the lack of antagonism by haloperidol, the prototypic D2 receptor antagonist, rules out the hypothesis that cabergoline prevents neuronal death by a dopamine receptor-mediated mechanism. In addition, apomorphine (0.1–30 μM), a non-selective dopamine receptor agonist, was ineffective in our model. Other authors reported different results in the same cell line by using H_2O_2 as oxidative agent (Uberti et al., 2002). It is possible that the type of oxidative pulse is important for the neuroprotective effect of apomorphine, or that the cells respond differently to apomorphine, when incubated into the buffer solution, as we did, or in the culture medium, as did the other authors.

From our results we may conclude that in vivo efficacy (Baas and Schueler, 2001) of cabergoline is due not only to its dopaminomimetic actions, but also to its ability to reduce both the oxidant damages (lipid peroxidation), and the intracellular GSH depletion. Data from the literature have also demonstrated that cabergoline is able to reduce basal lipid peroxide levels in vivo (Finotti et al., 2000), directly scavenges free radicals and activates GSH system in vitro and in vivo (Yoshioka et al., 2002). Therefore, overall the data suggest that cabergoline acts as radical scavenger and increases cellular responses against environmental stress conditions.

In conclusion, cabergoline is effective as anti-oxidant drug and prevents necrotic cell death. In our opinion, it may represent a favourable drug not only for Parkinson's disease therapy, but also for that of other neuropathological states related to accumulation of free radicals.

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