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Role of tetrahydrobiopterin in dopaminergic cell death: Relevance to Parkinson's disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting 1% of the population above the age of 65 and is characterized by a selective loss of dopaminergic neurons in the substantia nigra pars compacta. Although the underlying cause of dopaminergic cell death or the mechanism by which these cells degenerate is still not clearly understood, oxidative stress, mitochondrial dysfunction, and protein misfolding are thought to play important roles in the dopaminergic degeneration in PD.

Tetrahydrobiopterin (BH4) is synthesized exclusively in the monoaminergic, including dopaminergic, cells and serves as an endogenous and obligatory cofactor for syntheses of the potential oxidative stressors dopamine and nitric oxide. In addition to its contribution toward the syntheses of these two potentially toxic molecules, BH4 itself can directly generate oxidative stress. BH4 undergoes oxidation during the hydroxylation reaction as well as nonenzymatic autooxidation to produce hydrogen peroxide and superoxide radical. We have previously suggested BH4 as an endogenous molecule responsible for the dopaminergic neurodegeneration. BH4 exerts selective toxicity to dopamine-producing cells via generation of oxidative stress, mitochondrial dysfunction, and apoptosis. BH4 also induces morphological, biochemical, and behavioral characteristics associated with PD in vivo.

BH4 as well as enzyme activity and gene expression of GTP cyclohydrolase I, the rate-

limiting enzyme in BH4 synthesis pathway, are readily upregulated by cellular changes such as calcium influx and by various stimuli including stress situations. This points to the possibility that cellular availability of BH4 might be increased in aberrant conditions, leading to increased extracellular BH4 subsequent degeneration. The fact that BH4 is specifically and endogenously synthesized in dopaminergic cells, is readily upregulated, and generates oxidative stress-related cell death provides physical relevance of this molecule as an attractive candidate with which to explain the mechanism of pathogenesis of PD.

Results and Discussion

1. BH4 causes preferential death of dopaminergic cells in vitro and in vivo

BH4 given extracellularly is cytotoxic to dopamine-producing cells including CATH.a, but not to nondopaminergic cells. BH4-induced dopaminergic cell death is also observed in vivo and is related to motor deficit. In addition, both in vitro and in vivo, the cell death involves apoptosis, resembling that which occurs in PD.

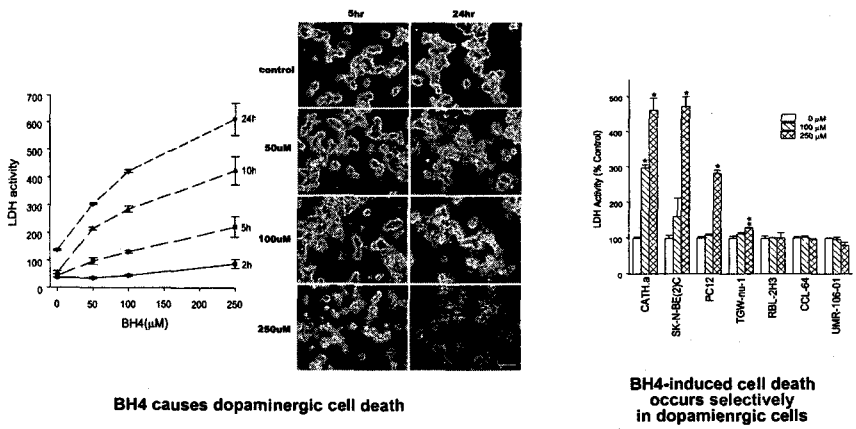


Fig. 1. BH4 causes dopaminergic cell death in vitro.

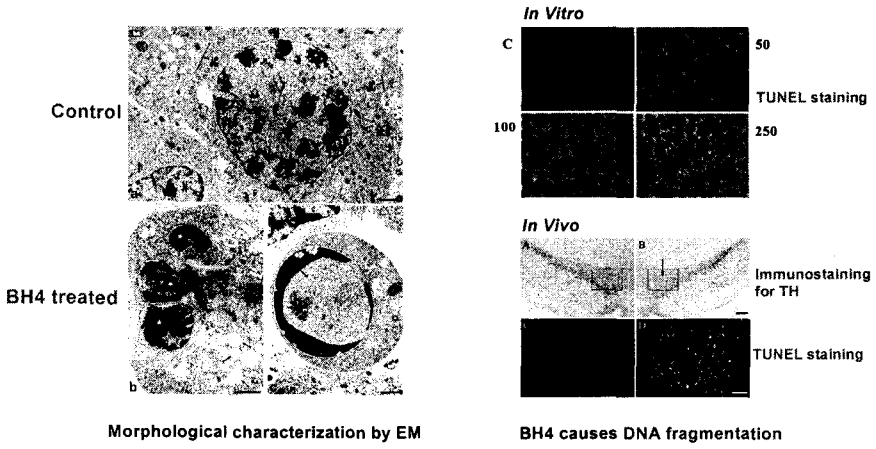


Fig. 2. Morphological analysis of the BH4-induced cell death and induction of DNA fragmentation by BH4 in vitro and in vivo.

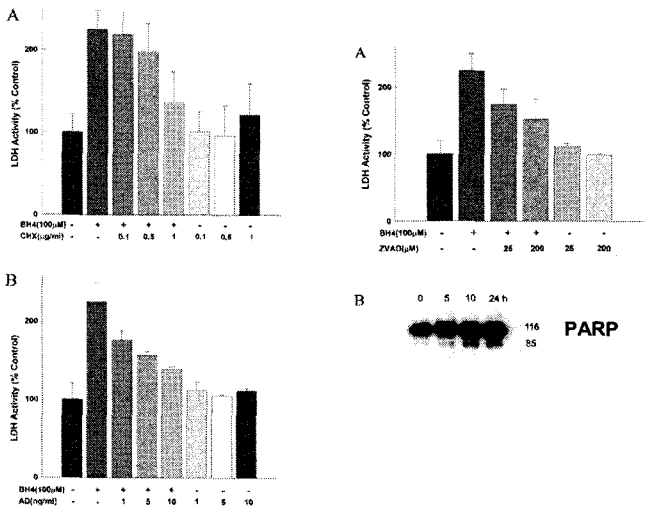


Fig. 3. Effects of macromolecule synthesis inhibitors and caspase inhibitor on BH4-induced cell death.

2. The presence of both dopamine and BH4 is important in rendering dopaminergic cells vulnerable and the toxicity involves oxidative stress

BH4-induced dopaminergic cell death is primarily mediated by dopamine, evidenced by findings that 1) BH4 toxicity is increased in proportion to cellular dopamine content; 2) non-dopaminergic cells become susceptible to BH4 upon exposure to dopamine; and 3) depletion of dopamine attenuates BH4 toxicity in dopamine-producing cells.

BH4 toxicity is caused by generation of reactive oxygen species, because various antioxidant enzymes, such as catalase, superoxide dismutase, and peroxidase, and thiol antioxidants protected cells from the BH4-induced demise. In addition, BH4 causes lipid peroxidation and increased formation of protein-bound quinones, suggesting involvement of oxidative stress.

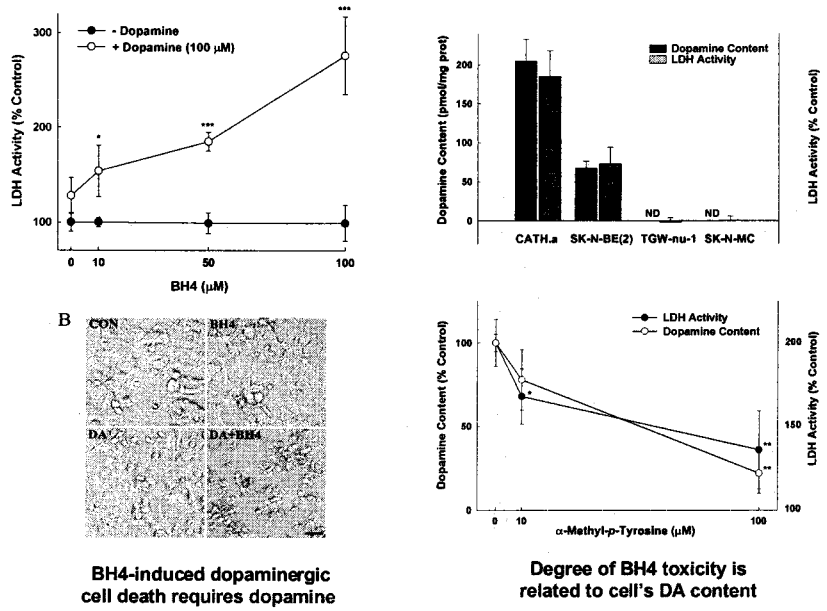
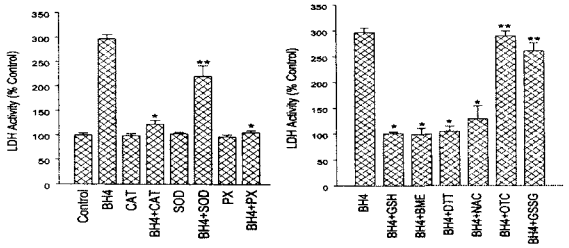
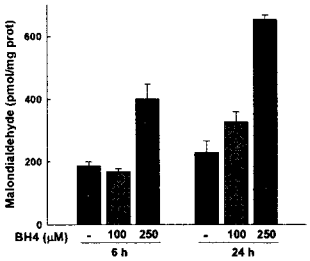


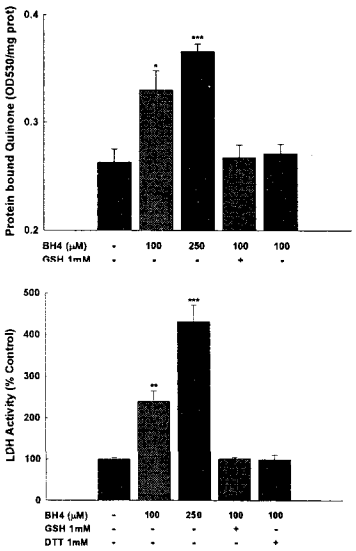
Fig. 4. Dopamine-dependent cytotoxcity of BH4.



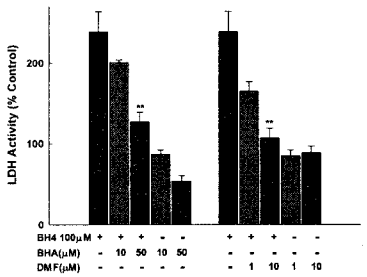
Antioxidants attenuate
BH4-induced cell death



BH4 causes lipid peroxidation



BH4 causes formation of
Protein-bound quinone



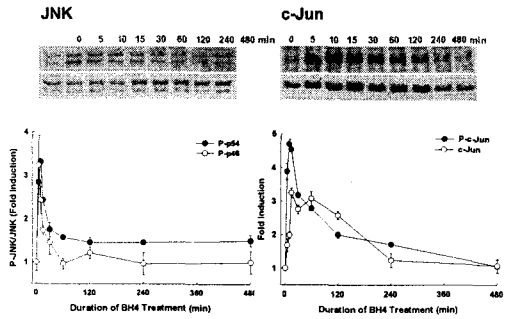
	Quinone Reductase Activity (umole/min/mg protein)
Control	67.005 ± 2.012
BHA 50uM	84.996 ± 3.005
BHA 50uM+Dicoumarol 10uM	59.069 ± 5.849
Dicoumarol 10uM	63.707 ± 6.210

Quinone reductase attenuates
BH4-induced cell death

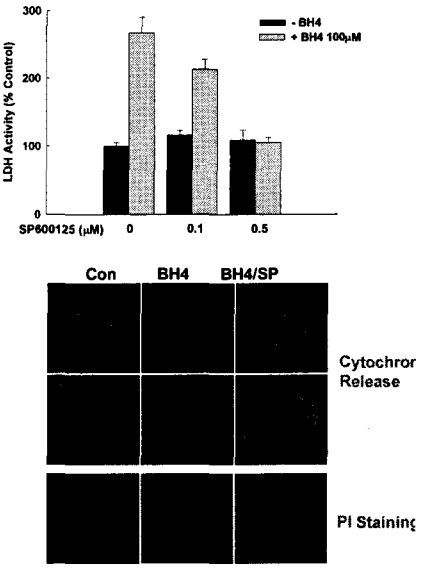
Fig. 5. BH4 toxicity is produced by generation of oxygen radicals.

3. Activation of the c-Jun N-terminal kinase (JNK) pathway is important in mediating BH4-induced dopaminergic cell death

JNK, but not extracellular signal-regulated kinase (ERK) or p38 mitogen-activated protein kinase (MAPK), is phosphorylated significantly by BH4 exposure. BH4 also leads to c-Jun phosphorylation and an increase in c-Jun protein level. The JNK inhibitor SP600125 protects cells against BH4 toxicity and inhibits cytochrome c release and apoptotic nuclear condensation induced by BH4.



BH4 causes phosphorylation of JNK & c-Jun

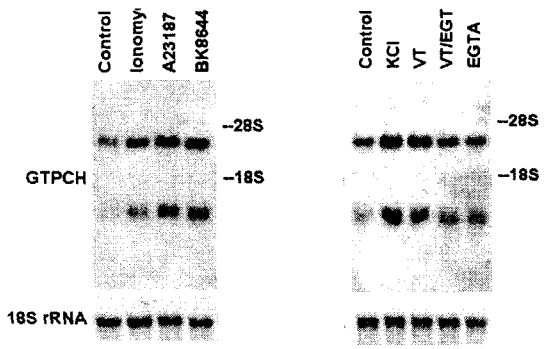


Inhibition of JNK protects cells against BH4-induced cell death

Fig. 6. BH4 causes phosphorylation of JNK and c-Jun, and inhibition of JNK protects cells against BH4-induced cell death.

4. Calcium influx up-regulates GTPCH gene expression and BH4 levels

Ionomycin, A23187 and BayK8644 dramatically up-regulates GTPCH mRNA level. Depolarization also increases GTPCH expression, which is abolished by calcium chelating. A231287 elevates GTPCH protein level, enzyme activity, and BH4 level.



GTPCH gene expression is upregulated by calcium influx

	Control	A23187	% Control
GTPCH activity	464.3 ± 11.6	655.1 ± 13.1	141.1
Intracellular BH4	472.0 ± 7.6	656.1 ± 11.1	139.0
Released BH4	12602 ± 767	17769 ± 971	142.0

Increases in BH4 synthesis & release by calcium influx

Fig. 7. Up-regulation of GTP cyclohydrolase I and BH4 by calcium influx

Our observation showed that BH4 leads to selective dopaminergic neurodegeneration both in vitro and in vivo in a manner of apoptosis, and the toxicity is mediated by generation of oxidative stress and mitochondrial dysfunction (data not shown). Together with the finding that BH4 is synthesized specifically and endogenously in monoaminergic, including dopaminergic, neurons in the brain and that it can be upregulated readily in aberrant conditions, these results suggests that when BH4 is present in excess, it may participate endogenously in dopaminergic neurodegeneration in PD.

References

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