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Monosialoganglioside Increases Catalase Activity in Cerebral Cortex of Rats

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Monosialoganglioside (GM1) is a neuroprotective agent that has been reported to scavenge free radicals generated during reperfusion and to protect receptors and enzymes from oxidative damage. However, only a few studies have attempted to investigate the effects of GM1 on enzymatic antioxidant defenses of the brain. In the present study, we evaluate the effects of the systemic administration of GM1 on the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and on spontaneous chemiluminescence and total radical-trapping potential (TRAP) in cerebral cortex of rats *ex vivo*. The effects of GM1 on CAT activity and spontaneous chemiluminescence *in vitro* were also determined.

Animals received two injections of GM1 (50 mg/kg, i.p.) or saline (0.85% NaCl, i.p.) spaced 24 h apart. Thirty minutes after the second injection the animals were sacrificed and enzyme activities and spontaneous chemiluminescence and TRAP were measured in cell-free homogenates. GM1 administration reduced spontaneous chemiluminescence and increased catalase activity *ex vivo*, but had no effect on TRAP, SOD or GSH-Px activities. GM1, at high concentrations, reduced CAT activity *in vitro*. We suggest that the antioxidant activity of GM1 ganglioside in the cerebral cortex may be due to an increased catalase activity.

Keywords: GM1 ganglioside; Total radical-trapping antioxidant potential; Antioxidant enzymes; Spontaneous chemiluminescence

INTRODUCTION

Gangliosides comprehend a group of glycosphingolipids characterized by the presence of one or more sialic acid residues in the oligosaccharide chain, which are synthesized in the Golgi apparatus and then transported and incorporated into the plasma membrane.[1] Gangliosides have been found virtually in every vertebrate tissue, particularly in the brain, where they represent about 10% of the lipid content.[2] It has been reported that gangliosides play a modulatory role in different events associated with adaptive functions[3–6] neuronal plasticity[7,8] memory formation[9–12] and neuroprotection against various neurotoxic agents or conditions, such as excitotoxic amino acid exposure, anoxia/ischemia, Parkinson's and Alzheimer's diseases,[13–17] accompanied by an apparent absence of side effects. Accordingly, GM1 inhibits lipid peroxidation and intracellular calcium overloading,[15,17,18] and it has been argued that GM1 may directly scavenge free radicals generated during reperfusion and protect receptors and enzymes from oxidative damage.[2,19] In addition, we have recently demonstrated that GM1 attenuates methylmalonate-induced convulsions and thiobarbituric acid-reactive substances (TBARS) generation and increases ascorbic acid levels in the striatum of the rats *ex vivo*, further supporting a possible antioxidant role for gangliosides in the central nervous system (CNS).[17]

Considering that superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) constitute the major intracellular antioxidant protection system of the CNS, and that little is known about

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the effects of GM1 on these antioxidant enzymes, we decided to investigate whether GM1 alters SOD, CAT and GSH-Px activities *ex vivo*. Since GM1 administration altered CAT activity and spontaneous chemiluminescence *ex vivo*, the effects of GM1 on catalase activity and spontaneous chemiluminescence *in vitro* were also determined.

MATERIALS AND METHODS

Reagents and Equipment

All chemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA, except for the RANSOD kit, which was purchased from RANDOX, and GM1 ganglioside, which was kindly donated by TRB Pharma. Chemiluminescence and total radical-trapping potential (TRAP) were assayed using a beta liquid scintillation spectrometer (Tricarb 2100TR and Wallac model 1409, respectively) and enzyme activities were measured with a double-beam spectrophotometer with temperature control.

Animals

Adult male Wistar rats (105–120 days, 350–380 g) bred in the animal house of the UFRGS, were used. Rats had free access to water and to a standard lab chow (Germani, Porto Alegre, RS, Brazil). Temperature was maintained at 24 ± 1°C, with a 12/12 h light/dark cycle. The "Principles of laboratory animal care" (NIH publication no 85-23, revised 1985) were followed throughout the experiments.

Treatment and Tissue Preparation *Ex Vivo*

Animals received two injections of GM1 (50 mg/kg, i.p.) or saline (NaCl 0.9%, i.p.) spaced 24 h apart. Thirty minutes after the second GM1 or saline injection the animals were killed by decapitation and the brain was immediately removed. This experimental protocol of GM1 administration has been shown to be effective in decreasing methylmalonate-induced TBARS production in rat striatum.[17] The cerebral cortex was dissected, weighed and homogenized in the incubation medium specified for each technique and centrifuged at 1000*g* for 10 min at 4°C. The supernatant was immediately used for the measurements.

Spontaneous Chemiluminescence

Samples were assayed for chemiluminescence in a dark room by the method of Gonzalez-Flecha *et al.*[20] Incubation flasks contained 3.5 ml of medium consisting of 20 mM sodium phosphate, pH 7.4, and 140 mM KCl. The background chemiluminescence was measured, and 0.5 ml of cell-free homogenate immediately added. Chemiluminescence was measured for 30 min at room temperature. The background chemiluminescence was subtracted from the total value and the results are presented as cps/mg of protein. *In vitro* experiments were carried out as described above, except that cell-free cerebral cortex homogenates were previously incubated for 1 h at 37°C in the absence or presence of GM1 (10^{-2} to 10^{4} nM).

Total Radical-trapping Antioxidant Potential

TRAP represents the total antioxidant capacity of the tissue and was determined by measuring the luminol chemiluminescence induced by 2,2′-azo-bis (2-amidinopropane) (ABAP) at room temperature by the method of Lissi *et al.*[21] Tissue was homogenized 1:10 (w/v) in 0.1 M glycine buffer (pH 8.6), and ABAP was added to the vial and the background chemiluminescence measured. Luminol was subsequently added to the vial and the chemiluminescence was measured again. The chemiluminescence value obtained in the presence of luminol was considered the initial value. Afterwards, Trolox or cerebral homogenates were added to the medium and chemiluminescence was measured until it reached the initial value. The induction time was considered as the time elapsed between adding Trolox or cerebral homogenates and the total recovery of initial chemiluminescence levels. The induction time is directly proportional to the antioxidant capacity of the tissue and was compared to the induction time of Trolox. The results are reported as nmol of Trolox/mg of protein. *In vitro* experiments were carried out as described above, except that cell-free cortex homogenates were previously incubated for 1 h at 37°C in the absence or presence of GM1 (10^{-2} to 10^{4} nM).

Catalase Assay

CAT activity was assayed by the method of Aebi,[22] which is based on the disappearance of H_2O_2 at 240 nm. Brain tissue was homogenized 1:10 (w/v) in 10 mM potassium phosphate buffer, pH 7.6. One unit of enzyme activity was defined as 1 μmol of hydrogen peroxide consumed per minute, and the specific activity reported as units/mg protein.

Superoxide Dismutase Assay

The assay of SOD activity was carried out with the RANSOD kit (Randox, USA), which is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride and superoxide radical. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample.

A 50% inhibition was defined as one unit of SOD and specific activity reported as units/mg of protein.

Glutathione Peroxidase Assay

GSH-Px activity was measured according to Wendel,[23] except for the concentration of NADPH, which was adjusted to 0.1 mM after previous tests performed in our laboratory. NADPH disappearance was monitored at 340 nm and one GSH-Px unit was defined as 1 μmol of NADPH consumed per min and specific activity reported as units/mg of protein.

Protein Determination

Protein was measured by the method of Lowry *et al.*,[24] using bovine serum albumin as standard.

Statistical Analysis

Data were analyzed by the Student's *t*-test or by one-way analysis of variance. *Post hoc* analysis was carried out by the Student-Newman-Keuls test, when appropriate.

RESULTS

Ex Vivo Experiments

Table I shows the effect of systemic GM1 administration on the spontaneous chemiluminescence and on the CAT activity of cerebral cortex of rats *ex vivo*. Statistical analysis revealed that GM1 administration reduced spontaneous chemiluminescence [$t(7) = 2.64$; $p < 0.05$] and increased CAT activity [$t(14) = 2.72$; $p < 0.01$] in cerebral cortex homogenates, as compared with saline.

Table II shows that the systemic GM1 administration had no effect on TRAP [$t(9) = 0.38$; $p > 0.05$], SOD [$t(12) = 1.83$; $p > 0.05$] and GSH-Px [$t(10) = 1.29$; $p > 0.05$] activities in cortex homogenates, as compared with saline.

In Vitro Experiments

Figure 1 shows the effect of GM1 on CAT activity *in vitro*. Statistical analysis revealed that high concentrations of GM1 (10^3 to 10^4 nM) in the incubation medium inhibited CAT activity *in vitro* [$F(7, 21) = 6.8$; $p < 0.001$], as compared with saline. In contrast, GM1 had no effect on spontaneous chemiluminescence [$t(3) = 2.65$; $p > 0.05$] of cortex homogenates *in vitro* (data not shown).

TABLE I Effect of GM1 ganglioside (50 mg/kg, i.p.) on the spontaneous chemiluminescence (Chem) and catalase (CAT) activity in brain homogenates from rats *ex vivo*

	Chem (cps/mg protein)	CAT (unit/mg protein)
Saline	170 ± 10.1	3.8 ± 1.5
GM1	135 ± 5.2*	5.2 ± 1.5*

Data are mean SEM and $n = 9$–10 per group. Saline = NaCl 0.9%; GM1 = monosialoganglioside. *$p < 0.05$ compared with saline group (Student *t*-test).

TABLE II Absence of effect of GM1 ganglioside (50 mg/kg, i.p.) on total radical-trapping antioxidant potential (TRAP), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in brain homogenates from rats *ex vivo*

	TRAP (nmol/mg protein)	SOD	GHS-Px
Saline	12.5 ± 1.52	32.1 ± 1.12	32.5 ± 1.8
GM1	11.6 ± 1.53	34.4 ± 2.2	36.6 ± 2.2

Data are mean SEM and $n = 6$–7 per group. Saline = NaCl 0.9%; GM1 = monosialoganglioside.

DISCUSSION

In the present study, we show that GM1 administration increased CAT activity and reduced spontaneous chemiluminescence of cerebral cortex homogenates *ex vivo*. Conversely, GM1 ganglioside had no effect on SOD and GSH-Px activities and on total TRAP. Interestingly, GM1 ganglioside (10^3 to 10^4 nM) addition to the incubation medium reduced CAT activity *in vitro* and had no affect spontaneous chemiluminescence *in vitro*, suggesting that GM1-induced CAT activation *ex vivo* is not due to a direct effect of the ganglioside on the enzyme.

The brain is particularly susceptible to oxidation by reactive species because of its dependency on aerobic metabolism, large content of polyunsaturated lipid in the mitochondrial and plasma membranes of brain cells and its low antioxidant defenses.[25] Mitochondrial dysfunction and consequent ATP depletion are a major cause of oxidative stress and calcium homeostasis alterations[26] in the CNS, ultimately producing

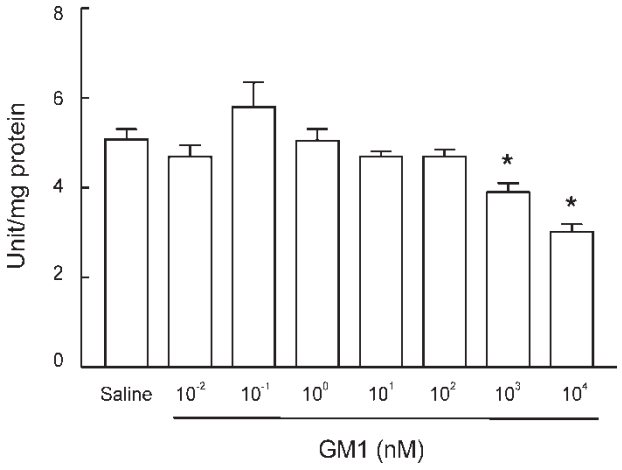

FIGURE 1 Effect of GM1 on catalase activity *in vitro*. Data are mean + SEM and $n = 6$ per group. *$p < 0.05$ compared with saline (Student-Newman-Keuls Test).

loss of cellular integrity and cell death. Indeed, various neurodegenerative disorders, including Parkinson, Huntington and Alzheimer's diseases have been associated with mitochondrial dysfunction, activation of excitotoxic mechanisms and free radical generation.[26,27] The integrity and existence of an organism depend on proper cellular homeostatic regulation. Under normal physiological conditions, cellular homeostasis is incessantly challenged by stressors and, among them, are the products generated from oxygen metabolism. The cell detoxifies these free radicals via its own antioxidant defense system,[26] which includes the antioxidant enzymes SOD, CAT and GSH-Px.[28] In fact, most of the evidence for a protective role for antioxidant defenses in limiting injury in the CNS depends on the activation antioxidant enzymes like GSH-Px,[26] SOD[29,30] or CAT[31] or both.[32–36] Various factors may regulate the expression of antioxidants enzymes, such as copper deficiency,[37] oxidative stress[26] and neuronal growth factor (NGF) administration.[31,38,39] In the CNS, NGF stimulates cellular resistance to oxidative stress, protects PC12 cells from the toxic effects of reactive oxygen species and increases CAT and GSH-Px mRNA levels in PC12 cells in a time- and dose-dependent manner.[39,40] In addition to NGF, gangliosides, are also known to exert neuritogenic and neurotrophic effects both *in vitro* and *in vivo*[41] and further increase the already elevated levels of catalase after ischemia in the cerebral cortex of rats.[42] Exogenously administered gangliosides, particularly GM1, have been shown to stimulate neurite outgrowth in cell culture.[43–46] Moreover, GM1 has been shown to potentiate the NGF-induced recovery of neurochemical markers following cortical devascularisation[47] or others injuries,[48] suggesting that it can be used as a useful tool to promote functional recovery of the CNS. It has been suggested that GM1 promotes neuronal survival by activating NGF receptor tyrosine kinase activity,[49] what indicates that GM1 and NGF effects are closely related. Therefore, it may be speculated that the presently reported increase in CAT activity by GM1 involves an increased transcription of the enzyme, which is possibly mediated by neurotrophic factors. Indeed, the presently reported lack of activating effect of GM1 ganglioside on CAT activity *in vitro* supports the view that GM1 ganglioside activating effect on CAT depends on the cellular integrity of the system. This result matches previous studies from our group, since we have previously reported that the inhibitory effect of GM1 on methylmalonate-induced striatal TBARS generation depends on the cellular integrity of the system.[17] Therefore, it is tempting to propose that the presently reported increase of CAT activity by GM1 underlies its antioxidant effects in the CNS, although a GM1-induced increase in the ascorbate content of the CNS has been also reported.[17]

Although some of the antioxidant actions of GM1 (including the presently described increase in CAT activity) seem to depend on the cellular integrity of the system, their magnitude seem to afford important functional effects. For example, systemic administration of GM1, at lower doses than or the same doses as that used in the present study, was effective in inhibiting methylmalonate-induced convulsions[17] as well as reserpine-induced orofacial movement[6] in rats. Importantly, the physiopathogenesis of these behavioral phenomena have been extensively related to increased oxidative stress.[50–55]

At last, it should be pointed out that CAT, due to its low activity in the CNS, has been considered a secondary enzyme in controlling free radical-induced damage,[25] a fact that could diminish the relative importance of the presently reported GM1-induced increase of CAT activity as the mechanism underlying GM1-induced antioxidant action. On the other hand, it has been reported that local enzyme activities determine regional susceptibility to neurotoxic agents.[56] Accordingly, highly immunoreactive cells for CAT correspond to neurons known to be resistant to ischemia-reperfusion injury, whereas weakly stained cells correspond to neurons that are more susceptible to ischemic damage. This finding indicates that CAT may be critical for a protective effect against oxidative stress under pathological conditions, such as ischemia-reperfusion injury.[57] Further evidence for a critical role for CAT as a protective agent against oxidative stress in the CNS come from the studies that have demonstrated that inhibition of CAT activity increases Alzheimer's β-amyloid peptide neurotoxicity[58] and that CAT activity is decreased, but SOD activity is unchanged, in brains of patients with dementia of Alzheimer type.[59] Therefore, one might propose that CAT, due to its decreased activity in the brain, is a point of vulnerability in the cerebral antioxidant system, which has had its importance overlooked in the literature. We think that this is a matter of significant discussion, and that the relative importance of CAT activity as a determinant of the CNS susceptibility to reactive oxygen species is still to be established.

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References

[1] Leeden, R.W. (1983) "Gangliosides", In: Lajtha, A., ed, *Handbook of Neurochemistry* (Plenum Press, New York), pp 41–90.

[2] Maulik, N., Das, D.K., Gogineni, M., Cordis, G.A., Avrova, N. and Denisova, N. (1993) "Reduction of myocardial ischemic reperfusion injury by sialylated glycosphingolipids, gangliosides", *J. Cardiovasc. Pharmacol.* **22**, 74–81.

[3] Bellot, R.G., Camarini, R., Vital, M.A.B.F., Palermo-Neto, J., Leyton, V. and Frussa-Filho, R. (1996) "Monosialoganglioside attenuates the excitatory and behavioural sensitization effects of ethanol", *Eur. J. Pharmacol.* **313**, 175–179.

[4] Bellot, R.G., Vital, M.A.B.F., Palermo-Neto, J. and Frussa-Filho, R. (1997) "Repeated monosialoganglioside administration attenuates behavioral sensitization to amphetamine", *Brain Res.* **747**, 169–172.

[5] Vital, M.A.B.F., Frussa-Filho, R. and Palermo-Neto, J. (1995) "Effects of monosialoganglioside on dopaminergic supersensitivity", *Life Sci.* **56**, 2299–2307.

[6] Vital, M.A.B.F., Frussa-Filho, R. and Palermo-Neto, J. (1997) "Effects of monosialoganglioside on a new model of tardive dyskinesia", *Prog. Neuropsychopharmacol. Biol. Psychiatry* **21**, 1169–1179.

[7] Palermo-Neto, J., Frussa-Filho, R. and Vital, M.A.B.F. (1999) "Kinetic analysis of GM1 effects on haloperidol-induced dopaminergic supersensitivity", *Gen. Pharmacol.* **32**, 265–270.

[8] Vital, M.A.B.F., Flório, J.C., Frussa-Filho, R., De Lucia, R., Tufik, S. and Palermo-Neto, J. (1998) "Effects of haloperidol and GM1 ganglioside treatment on striatal D_2 receptor binding and dopamine turnover", *Life Sci.* **62**, 1161–1169.

[9] Silva, R.H., Felicio, L.F., Nasello, G., Vital, M.A.B.F. and Frussa-Filho, R. (1996) "Effects of ganglioside GM1 on memory in senescent rats", *Neurobiol. Aging* **144**, 582–586.

[10] Silva, R.H., Bellot, R.G., Vital, M.A.B.F. and Frussa-Filho, R. (1997) "Effects of long-term ganglioside GM1 administration on a new discriminative avoidance test in normal adult mice", *Psychopharmacology* **129**, 322–328.

[11] Silva, R.H., Felício, L.F. and Frussa-Filho, R. (1999) "Ganglioside, GM1 attenuates scopolamine-induced amnesia in rats and mice", *Psychopharmacology* **141**, 111–117.

[12] Silva, R.H., Bergamo, M. and Frussa-Filho, R. (2000) "Effects of neonatal ganglioside GM1 administration on memory in adult and old rats", *Pharmacol. Toxicol.* **87**, 120–125.

[13] Zoli, M., Ruggeri, M., Zini, I., Grimaldi, R., Pich, E.M., Toffano, G., Fuxe, K. and Agnati, L.F. (1990) "Aspects of neural plasticity in the central nervous system-VI. Studies of the effects of gangliosides on brain metabolic lesions", *Neurochem. Int.* **16**, 469–478.

[14] Carolei, A., Fieschi, C., Bruno, R. and Toffano, G. (1991) "Monosialoganglioside GM1 in cerebral ischemia", *Cerebrovasc. Brain Metab. Rev.* **3**, 134–157.

[15] Phillis, J.W. and O'Regan, M.H. (1995) "GM1 Ganglioside inhibits ischemic release of amino acid neurotransmitters from rat cortex", *Neuroreport* **6**, 2010–2012.

[16] Tajrine, G., Garofalo, L., Cuello, A.C. and Ribeiro-da-Silva, A. (1997) "Responses of cortical noradrenergic and somatostinergic fibers and terminals to adjacent strokes and subsequent treatment with NGF and/or the ganglioside GM1", *J. Neurosci. Res.* **50**, 627–642.

[17] Fighera, M.R., Bonini, J.S., Oliveira, T.G., Rocha, J.B.T., Dutra-Filho, C.S., Rubin, M.A. and Mello, C.F. (2003) "GM1 ganglioside attenuates convulsions and thiobarbituric acid reactive substances production induced by the intrastriatal injection of methylmalonic acid", *Int. J. Biochem. Cell Biol.* **35**, 465–473.

[18] Tanaka, K., Dora, E., Greenberg, J., Toffano, G. and Reivich, M. (1986) "Effect of the Ganglioside on cerebral metabolism, recovery kinetics of EcoG and histology during the recovery period following focal ischemia in cats", *Stroke* **17**, 1170–1178.

[19] Tyurin, V.A., Kuznetsova, L.A., Tyurina, Y.Y., Erin, A.N., Avrova, N.F. and Kagan, V.E. (1991) "Role of Gangliosides in protection of beta-adrenoceptors against damage by lipid peroxidation in synaptosomal membranes", *Bull. Exp. Biol. Med. USSR* **111**, 779–783.

[20] Gonzalez-Flecha, B., Llesuy, S. and Boveris, A. (1991) "Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle", *Free Radic. Biol. Med.* **10**, 93–100.

[21] Lissi, E., Pascual, C. and Castilho, M.D. Del (1992) "Luminol luminescence induced by 2,2′-azo-bis (2-amidinopropane) thermolysis", *Free Radic. Res. Commun.* **17**, 299–311.

[22] Aebi, H. (1984) "Catalase *in vitro*", *Methods Enzymol.* **105**, 121–126.

[23] Wendel, A. (1981) "Glutathione peroxidase", *Methods Enzymol.* **77**, 325–332.

[24] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) "Protein measurement with Folin phenol reagent", *J. Biol. Chem.* **193**, 265–267.

[25] Reiter, R.J. (1995) "Oxidative processes and antioxidative defense mechanisms in the aging brain", *FASEB J.* **9**, 526–553.

[26] Cassarino, D.S. and Bennett, J.P. (1999) "An evaluation of the role of mitochondrial in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration", *Brain Res. Rev.* **29**, 1–25.

[27] Beal, M.F. (2000) "Energetics in the pathogenesis of neurodegenerative diseases", *Trends Neurosci.* **23**, 298–304.

[28] Onodera, K., Omoi, Nao-Omi, Fukui, K., Hayasaka, T., Shinkai, T., Suzuki, S., Abe, K. and Urano, S. (2003) "Oxidative damage of rat cerebral cotex and hippocampus, and changes in antioxidative defense systems caused by hyperoxia", *Free Radic. Res.* **37**, 367–372.

[29] Troy, C.M. and Chelanski, M.L. (1994) "Downregulation of Cu–Zn superoxide dismutase cause apoptotic death in PC12 neuronal cells", *Proc. Natl Acad. Sci. USA* **91**, 6384–6387.

[30] Rothstein, J.D., Bristol, L.A., Hosler, B.A., Brown, R.H. and Kunel, R.W. (1994) "Chronic inhibition of superoxide dismutase produces apoptotic death of spinal neurons", *Proc. Natl Acad. Sci. USA* **91**, 4155–4199.

[31] Sampath, D., Holets, V. and Perz-Polo, J.R. (1995) "Effect of a spinal cord photolesion injury on catalase", *Int. J. Dev. Neurosci.* **13**, 645–654.

[32] Chan, P.H. (1992) "Antioxidant-dependent amelioration of brain injury: role of Cu,Zn-superoxide dismutase", *J. Neurotrauma* **9**(Suppl. 2), S417–S423.

[33] Farbiszewski, R., Chwiecko, M. and Ustymowicz, J. (1994) "The 21-aminosteroid U-74389G protects the antioxidant enzymes in the ischemia/reperfusion-induced rat brain damage", *Eur. J. Pharmacol.* **270**, 263–265.

[34] Liu, D., Yang, R., Yan, X. and McAdoo, D.J. (1994) "Hydroxyl radicals generated *in vivo* kill neurons in the rat spinal cord: electrophysiological, histological, and neurochemical results", *J. Neurochem.* **62**, 37–44.

[35] Truelove, D., Shuaib, A., Ijaz, S., Richardson, S. and Kalra, J. (1994) "Superoxide dismutase, catalase, and U78517F attenuate neuronal damage in gerbils with repeated brief ischemic insults", *Neurochem. Res.* **19**, 665–671.

[36] Truelove, D., Shuaib, A., Ijaz, S., Ishaqzay, R. and Kalra, J. (1994) "Neuronal protection with superoxide dismutase in repetitive forebrain ischemia in gerbils", *Free Radic. Biol. Med.* **17**, 445–450.

[37] Lai, C.C., Huang, W.H., Askari, A., Klevay, L.M. and Chiu, T.H. (1995) "Expression of glutathione peroxidase and catalase in copper-deficient rat liver and heart", *J. Nutr. Biochem.* **6**, 256–262.

[38] Satoh, T., Yamagata, T., Ishikawa, E., Yamada, M., Uchiyama, Y. and Hatanaka, H. (1999) "Regulation of reactive oxygen species by nerve growth factor but not Bcl-2 as a novel mechanism of protection of PC12 cells front superoxide anion-induced death", *J. Biochem.* **125**, 952–959.

[39] Yamagata, T., Satoh, T., Ishikawa, Y., Nakatani, A., Yamada, M., Ikeuchi, T. and Hatanaka, H. (1999) "Brain-derived neurotrophic factor prevents superoxide anion-induced death of PC12 cells stably expressing TrkB receptor via modulation of reactive oxygen species", *Neurosci. Res.* **35**, 9–17.

[40] Sampath, D., Jackson, G.R., Werrbach-Perez, K. and Perez-Polo, J.R. (1994) "Effects of nerve growth factor on glutathione peroxidase and catalase in PC 12 cells", *J. Neurochem.* **62**, 2476–2479.

[41] Hadjiconstantinon, M. and Neff, N.H. (1998) "GM1 ganglioside: *in vivo* and *in vitro* trophic actions on central neurotransmitters systems", *J. Neurochem.* **70**, 1335–1345.

[42] Mahadik, S.P., Makar, T.K., Murthy, J.N., Ortiz, A., Wakade, C.G. and Karpiak, S.E. (1993) "Temporal changes in superoxide dismutase, glutathione peroxidase, and catalase levels in primary and peri-ischemic tissue. Monosialoganglioside (GM1) treatment effects", *Mol. Chem. Neuropathol.* **18**, 1–14.
[43] Doherty, P., Ashton, S.V., Skaper, S.D., Leon, A. and Walsh, F.S. (1992) "Ganglioside modulation of neural cell adhesion molecule and N-cadherin-dependent neurite outgrowth", *J. Cell Biol.* **117**, 1093–1099.
[44] Ferrari, G., Fabris, M. and Gorio, A. (1983) "Gangliosides enhance neurite outgrowth in PC12 cells", *Brain Res.* **284**, 215–221.
[45] Skaper, S.D., Katoh-Semba, R. and Varon, S. (1985) "GM1 ganglioside accelerates neurite outgrowth from primary peripheral and central neurons under selected culture conditions", *Brain Res.* **355**, 19–26.
[46] Wu, G. and Ledeen, R.W. (1991) "Stimulation of neurite outgrowth in neuroblastoma cells by neuraminidase: putative role of GM1 ganglioside in differentiation", *J. Neurochem.* **56**, 95–104.
[47] Cuello, A.C., Garofalo, L., Kenigsberg, R.L. and Maysinger, D. (1989) "Gangliosides potentiate *in vivo* and *in vitro* effects of nerve growth factor on central cholinergic neurons", *Proc. Natl Acad. Sci. USA.* **86**, 2056–2060.
[48] Vantini, G., Fusco, M., Bigon, E. and Leon, A. (1988) "GM1 ganglioside potentiates the effect of nerve growth factor in preventing vinblastine-induced sympathectomy in newborn rats", *Brain Res.* **448**, 252–258.
[49] Ferrari, G. and Greene, L.A. (1996) "Prevention of neuronal apoptotic death by neurotrophic agents and ganglioside GM1: insights and speculations regarding a common mechanisms", *Perspect. Dev. Neurobiol.* **3**, 93–100.
[50] Fighera, M.R., Queiroz, C.M., Stracke, M.P., Nin Brauer, M.C., González-Rodríguez, L.L., Frussa- Filho, R., Wajner, M. and de Mello, C.F. (1999) "Ascorbic acid and α-tocopherol attenuate methylmalonic acid-induced convulsion", *Neuroreport* **10**, 2039–2043.
[51] Bergamo, M., Abílio, V.C., Queiroz, C.M.T., Barbosa-Júnior, H.N., Abdanur, L.R.A. and Frussa-Filho, R. (1997) "Effects of age on a new animal model of tardive dyskinesia", *Neurobiol. Aging* **18**, 623–629.
[52] Calvente, P.R.V., Araújo, C.C.S., Bergamo, M., Abílio, V.C., D'Almeida, V., Ribeiro, R.D.A. and Frussa-Filho, R. (2002) "The mitochondrial toxin 3-nitropropionic acid aggravates reserpine-induced oral dyskinesia in rats", *Prog. Neuropsychopharmacol. Biol. Psychiatry* **26**, 1–5.
[53] Abílio, V.C., Vera, J.A.R., Ferreira, L.S.M., Duarte, C.R.M., Carvalho, R.C., Grassl, C., Martins, C.R., Torres-Leite, D., Bignotto, M., Tufik, S., Ribeiro, R.D. and Frussa-Filho, R. (2002) "Effects of melatonin on orofacial movements in rats", *Psychopharmacology* **161**, 340–347.
[54] Bürger, M.E., Alves, A., Callegari, L., Athayde, F.R., Nogueira, C.W., Zeni, G. and Rocha, J.B.T. (2003) "Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum", *Prog. Neuropsychopharmacol. Biol. Psychiatry* **27**, 135–140.
[55] Marisco, P.C., Ribeiro, M.C.P., Bonini, J.S., Lima, T.T.F., Mann, K.C., Brenner, G.M., Dutra-Filho, C.S. and Mello, C.F. (2003) "Ammonia potentiates methylmalonic acid-induced convulsions and TBARS production", *Exp. Neurol.* **182**, 455–460.
[56] Davolio, C. and Greenamyre, J.T. (1995) "Selective vulnerability of the CA 1 region of hippocampus to the indirect excitotoxic effects of malonic acid", *Neurosci. Lett.* **192**, 29–32.
[57] Moreno, S., Mugnaini, E. and Ceru, M.P. (1995) "Immunocytochemical localization of catalase in the central nervous system of the rat", *J. Histochem. Cytochem.* **43**, 1253–1267.
[58] Milton, N.G.N. (2001) "Inhibition of catalase activity with 3-amino-triazole enhances the cytotoxicity of the Alzheimer's amyloid-β peptide", *Neurotoxicology* **22**, 767–774.
[59] Gsell, W., Conrad, R., Hickethier, M., Sofic, E., Frolich, L., Wichart, I., Jellinger, K., Moll, G., Ransmayr, G. and Beckmann, H. (1995) "Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type", *J. Neurochem.* **64**, 1216–1223.