Available online at www.sciencedirect.com
SCIENCE DIRECT®
Neuroscience Letters 365 (2004) 132–136
Neuroscience Letters
www.elsevier.com/locate/neulet

Galantamine and memantine produce different degrees of neuroprotection in rat hippocampal slices subjected to oxygen–glucose deprivation

Mónica Sobrado [a,*], José M. Roda [d], Manuela G. López [a,c], Javier Egea [a], Antonio G. García [a,b,c]

[a] *Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo, 4, 28029 Madrid, Spain*
[b] *Servicio de Farmacología Clínica, Facultad de Medicina, Hospital Universitario de La Princesa, Madrid, Spain*
[c] *Instituto de Gerontología, Facultad de Medicina, Hospital Universitario de La Princesa, Madrid, Spain*
[d] *Unidad de Investigación Cerebrovascular, Cirugía Experimental, Hospital Universitario La Paz, Madrid, Spain*

Received 10 March 2004; received in revised form 26 April 2004; accepted 27 April 2004

Abstract

Recent clinical trials have shown that galantamine is efficacious in the treatment of mild to moderate Alzheimer's and vascular dementia, and memantine in severe stages of these diseases. Hence, the hypothesis that these two drugs might exert different degrees of neuroprotection has been tested. Rat hippocampal slices were subjected to oxygen and glucose deprivation (OGD) and to a re-oxygenation period. Neuronal damage was monitored using the lactate dehydrogenase (LDH) released into the Krebs-bicarbonate medium as an indicator. Galantamine, a mild acetylcholinesterase (AChE) blocker and nicotinic receptor modulator, given 30 min before and during OGD plus re-oxygenation (1, 2 and 3 h) significantly reduced LDH release by around 50%. Galantamine 5 μM reduced LDH release significantly during the re-oxygenation period while at 15 μM it afforded significant reduction of LDH release both during OGD and re-oxygenation. Memantine, a reversible blocker of NMDA receptors, at 10 μM only significantly reduced (40%) LDH release after 3 h re-oxygenation. The classical NMDA blocker MK-801 reduced LDH released around 40% at 1 μM at all re-oxygenation times studied. These data indicate that galantamine has a neuroprotective window against anoxia wider than memantine. Whether these differences can be clinically relevant remain to be studied in appropriate clinical trials.

Keywords: Oxygen–glucose deprivation; Galantamine; Memantine; Hippocampal slices

Recent clinical trials show therapeutic benefits in cognition, functional and behavioral symptoms exerted by galantamine and memantine in dementia of the Alzheimer's and vascular types. However, while galantamine has shown efficacy in patients at mild-to-moderate stages of these diseases [17,22], memantine shows efficacy in advanced stages [6]. Therefore, the question arises to whether these clinical differences are due to their different mechanisms of action and/or to different degrees of neuroprotection exerted by these two drugs.

Galantamine is a mild inhibitor of acetylcholinesterase (AChE) and an allosteric potentiator of neuronal nicotinic receptors [12], whereas memantine is a non-competitive, voltage-dependent, reversible blocker of NMDA receptors for glutamate [15]. Both compounds exhibit neuroprotective effects in vitro [3,15] as well as in vivo neuronal death models [4,15]. However, a study comparing simultaneously the neuroprotective effects of galantamine and memantine in the same model is lacking. Therefore, we decided to compare the neuroprotective effect of galantamine and memantine in an acute model of neuronal damage exerted by oxygen and glucose deprivation (OGD) in the rat hippocampal slice.

Abbreviations: OGD, oxygen and glucose deprivation; LDH, lactate dehydrogenase; AChE, acetylcholinesterase; i.p., intraperitoneal; mOD, mean optical density; APL, allosteric potentiator ligand; TRPM, Transition Receptor Potential Cation Channel

* Corresponding author. Tel.: +34-91-4975386; fax: +34-91-4975397.
E-mail address: monica.sobrado@uam.es (M. Sobrado).

0304-3940/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.04.067

All experiments were performed using the brain of adult male Sprague–Dawley rats (275–325 g) from a colony of our animal quarters; they were performed following the rules of the Ethical Committee for the Care and Use of Animals in Research, of our medical school. Rats were deeply anesthetized with sodium pentobarbital (60 mg/kg i.p.), decapitated, and each brain was rapidly removed from the skull and placed into ice-cold Krebs bicarbonate buffer, dissection buffer (pH 7.4), containing (in mM): NaCl 120, KCl 2, $CaCl_2$ 0.5, $NaHCO_3$ 26, $MgSO_4$ 10, KH_2PO_4 1.18, glucose 11 and sucrose 200 [13]. All chamber solutions were pre-bubbled with either 95% O_2/5% CO_2 or 95% N_2/5% CO_2 gas mixtures, for at least 45 min before slice immersion, to ensure O_2 saturation or O_2 removal as desired. The hippocampi were quickly dissected, glued down leaning vertically against agar blocks in a small chamber, submerged in cold, oxygenated dissection buffer and sectioned in transverse slices of 350 μm thick (dissection period) using a vibratome (Leica; Heidelberg, Germany). A basal and OGD group was included in all experiments. A maximum of three drug concentrations was tested in each experiment. Immediately after vibratome sectioning, the slices were transferred to vials of sucrose-free dissection buffer, bubbled with 95% O_2/5% CO_2 in a water bath at room temperature for 60 min to recover from slicing trauma before starting the experiments (equilibration period).

After an initial preincubation period of 30 min, the slices corresponding to the basal group were incubated 60 min in a normal Krebs solution, having the following composition (in mM): NaCl 120, KCl 2, $CaCl_2$ 2, $NaHCO_3$ 26, $MgSO_4$ 1.19, KH_2PO_4 1.18 and glucose 11; this solution was equilibrated with 95% O_2/5% CO_2. Oxygen and glucose deprivation was induced by incubating the slices for a 60 min period in a glucose-free Krebs solution, equilibrated with a 95% N_2/5% CO_2 gas mixture; glucose was replaced with 2-deoxyglucose. After this OGD period, the slices were returned back to an oxygenated normal Krebs solution containing glucose (re-oxygenation period). These experiments were performed at 36.5 °C. When used, memantine (1, 3, and 10 μM) and galantamine hydrobromide (0.3, 1, 5 and 15 μM) were added to the OGD slices during the 30 min of preincubation period and remained there during the OGD and re-oxygenation period (see protocol in Fig. 1A).

The viability of hippocampal slices was monitored by measuring the activity of the lactate dehydrogenase (LDH) released into the incubation media [9]. Samples of this solution were taken at the end of the preincubation period and at times 0, 60, 120 and 180 min of the re-oxygenation period (see protocol in Fig. 1). LDH activity was measured spectrophotometrically at 490–600 nm, using a microplate reader (Labsystems iEMS reader MF). The remaining LDH was obtained by incubating the slices with 1% Triton X-100 at the end of the experiment, for 30 min; then, the samples were centrifuged and an aliquot from the supernatant was taken to measure the intracellular LDH. LDH levels remaining in the basal and OGD-slices after 3 h of reperfusion were high and

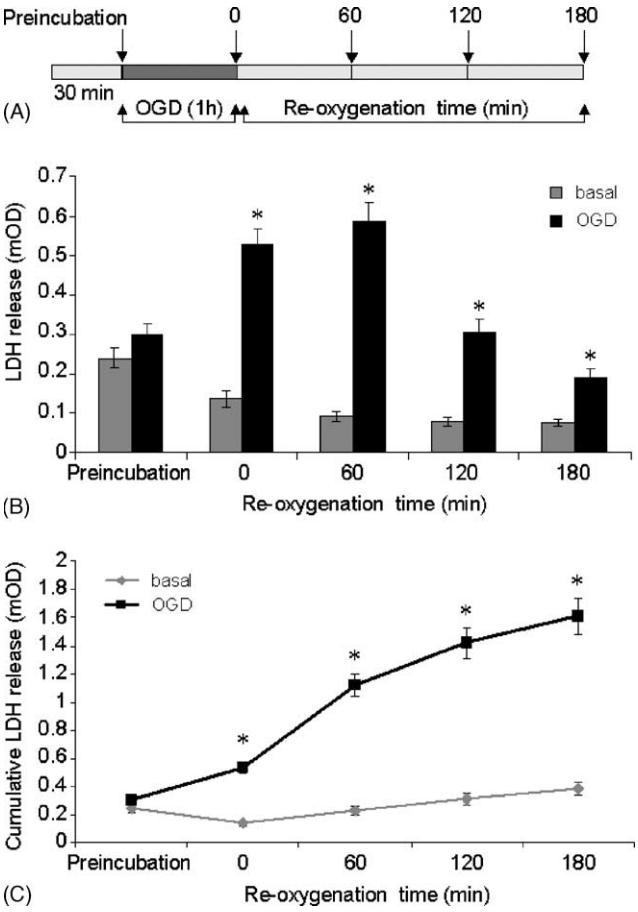

Fig. 1. Neuronal damage measured as LDH released into the medium, caused by oxygen and glucose deprivation (OGD) followed by re-oxygenation plus glucose reintroduction in rat hippocampal slices. Panel A shows the protocol (see methods). Panel B shows the basal LDH release and the release of LDH evoked by OGD plus re-oxygenation, in each experimental period (abscissa). Panel C shows the cumulative values of LDH released into the media (basal and OGD), calculated by adding the individual values of each collection period of incubation period from OGD to 3 h re-oxygenation, in each experiment. Data of LDH activity in (B) and (C) are expressed as mOD and they are means ± S.E.M. of 29 experiments. $^*P < 0.05$, compared with basal.

not significantly different from each other (4.008 ± 0.038 versus 4.002 ± 0.058 mean optical density (mOD) in basal and OGD-slices, respectively, $n = 8$, $P < 0.05$). Thus, only the LDH released into the medium was measured (see results).

MK-801 (1 μM), memantine (1, 3, and 10 μM) and galantamine hydrobromide (0.3, 1, 5 and 15 μM) were added to the OGD slices during the 30 min of preincubation period and remained during the OGD and re-oxygenation period. MK-801 was obtained from Tocris (Biogen Científica, Spain), memantine from Sigma (Aldrich, Spain) and galantamine hydrobromide from Janssen (Beerse, Belgium). They were dissolved in saline just before each experiment.

LDH efflux was expressed as the LDH activity present in the incubation solution measured as mOD. Data are represented as means ± S.E.M. Differences between treatments,

as a function of drug concentration and re-oxygenation time, were estimated by applying the analysis of variance (ANOVA) and Fisher's test. Differences were considered to be statistically different when $P = 0.05$.

In our present study, LDH release in basal conditions accounted for less than 25% of the maximum LDH released after OGD followed by 3 h reperfusion. Thus, we considered that there was a wide window to explore neuronal damage evoked by OGD/re-oxygenation, and the effects of galantamine and memantine on such damage.

These slices were subjected to the protocol of sequential incubations shown in Fig. 1A. Those incubations scarcely affected the total tissue LDH activity measured at the end of the experiment; this suggests that the overall tissue suffered little damage during the 4 h and 30 min period of the experiment. Hence, the total LDH activity remaining in the tissues at the end of the experiment was not measured in subsequent experiments. Only the LDH released into the different incubation media was taken as an indicator of neuronal tissue damage. It is likely that the LDH released into the media during OGD and re-oxygenation mostly came from the pyramidal neuron layer of the CA1 area of the hippocampus, which is known to be particularly sensitive to anoxia [19]. The basal LDH released into the incubation media from slices not subjected to OGD decreased with time (gray columns of Fig. 1B). LDH released rose as much as fourfold above basal after the 1 h OGD period (0 h of re-oxygenation in Fig. 1B). LDH released augmented further to sixfold above basal, during the first hour of re-oxygenation. Then, it gradually declined at 2 and 3 h re-oxygenation (black columns of Fig. 1B). The increments above basal LDH released into the media, elicited by OGD, were better seen if added in a cumulative manner from OGD to 3 h re-oxygenation, as Fig. 1C shows. Thus, the effects of drugs on LDH released were plotted in this cumulative manner in all subsequent experiments.

To study the effects of galantamine on the release of LDH from hippocampal slices subjected to OGD and re-oxygenation, the protocol performed was similar to that described in Fig. 1A. Every experiment included slices to measure the basal and the OGD plus re-oxygenation-induced LDH release; parallel slices contained various concentrations of galantamine, which were present during 30 min of the equilibration period, as well as throughout the OGD and re-oxygenation periods. Note that 0.3 and 1 μM galantamine slightly enhanced LDH release at 2 and 3 h re-oxygenation; however, this increase was not statistically significant. In contrast, 5 μM galantamine significantly reduced LDH release at 60, 120 and 180 min of re-oxygenation time; the reduction accounted for 45% after 1 h re-oxygenation, 51% after 2 h re-oxygenation, and 56% after 3 h re-oxygenation. Fifteen micromolar galantamine reduced LDH release by 56, 47, 50 and 54% at 0, 1, 2, 3 h re-oxygenation times, respectively (Fig. 2).

Experiments similar to those of galantamine were performed with various concentrations of memantine. Fig. 3A

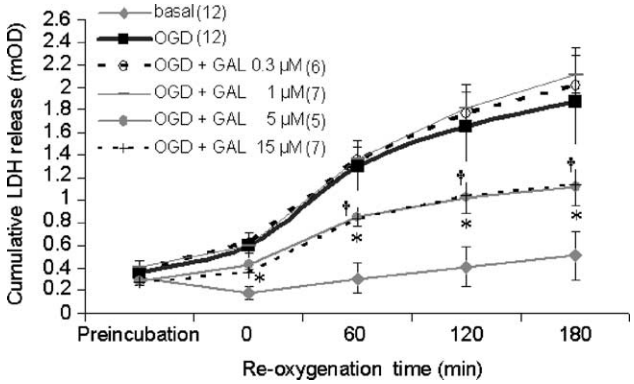

Fig. 2. Effects of galantamine (GAL) on the release of LDH from hippocampal slices subjected to OGD and re-oxygenation. Slices were run in parallel to measure the LDH released in basal or OGD conditions, in the absence or the presence of the indicated concentration of galantamine. Different concentrations of the drugs were tested in different experiments. LDH release is cumulatively expressed in the abscissa (see Fig. 1). Data are means ± S.E.M. of the number of experiments shown in parentheses. $^{*,\dagger}P < 0.05$, compared with OGD.

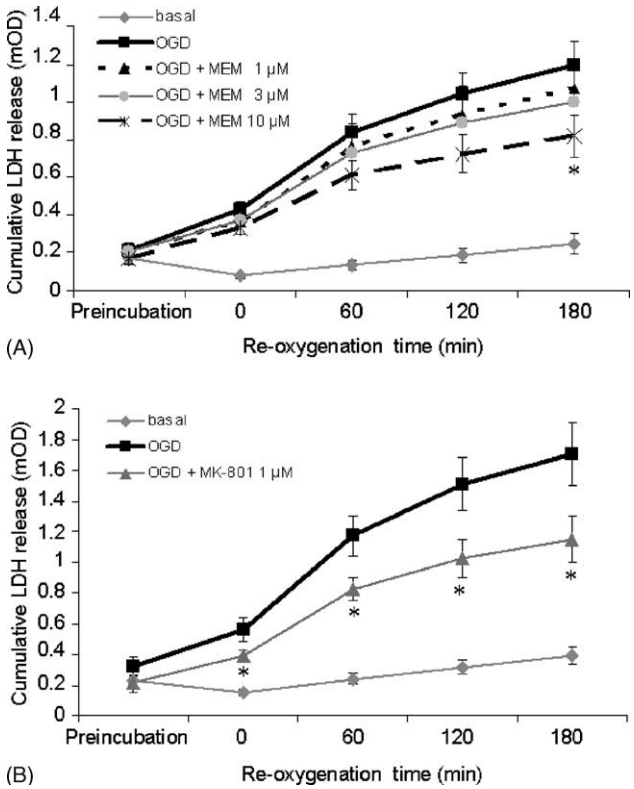

Fig. 3. Effects of memantine (MEM) and MK-801 on the release of LDH from hippocampal slices subjected to OGD. Slices were run in parallel to measure the LDH released in basal or OGD conditions, in the absence or the presence of the indicated concentrations of memantine (panel A) or MK-801 (panel B). Different concentrations of the drugs were tested in different experiments. Data correspond to means ± S.E.M. of eight experiments for the dose–response curve of memantine (A) and nine experiments for MK-801 (B). $^{*}P < 0.05$, compared with OGD.

shows that 1 and 3 μM of memantine reduced the release of LDH evoked by OGD plus re-oxygenation by 13–20%, but this reduction was not statistically significant. At 10 μM, memantine reduced LDH release by 28–37% at 0–2 h re-oxygenation and by 40% at 3 h re-oxygenation. We used the NMDA receptor blocker MK-801 for comparative purposes. At 1 μM, MK-801 significantly reduced LDH release at all times of re-oxygenation, reaching by 42% after OGD and 3 h re-oxygenation (Fig. 3). No further reduction was seen at higher concentrations of 10 and 30 μM (not shown).

This is the first study showing that an inhibitor of AChE, i.e. galantamine, has neuroprotective effects against neuronal damage caused by anoxia, in hippocampal slices. Furthermore, this is also the first study which compares in the same preparation (i.e. rat hippocampal slices subjected to OGD) the neuroprotective effects of galantamine and memantine, two drugs recently introduced in the clinic to treat patients with Alzheimer's and vascular type of dementia [11,18]. We have found in this study that galantamine is more efficacious than memantine as a neuroprotective agent which, considering their mechanism of action, was an unexpected result.

LDH release was originally used to measure neuronal cell death occurring via necrosis [9], although subsequent studies suggested that LDH release was also a good marker of apoptotic neuronal death [10]. More recent studies validated LDH release as an adequate index of necrotic tissue damage in brain slice preparations [5,13]. Although we tried other markers of neuronal death (i.e. MTT, propidium iodide), we found that LDH gave the highest and more reproducible window of injury.

It is well established that the hypoxia following a transient cerebral ischemic insult causes excessive accumulation of glutamate in the synapse, with the activation of the high Ca^{2+}-permeable NMDA receptors. This leads to Ca^{2+} overloading and neuronal cell death [21]. Thus, it is expected that non-competitive irreversible (i.e. MK-801) and reversible NMDA receptor blockers (i.e. memantine) should mitigate the neuronal damage due to ischemic–anoxic conflicts. This has been shown to be the case in a number of in vitro [20] and in vivo models [8,15]. In the present study, we have corroborated the neuroprotective effects of MK-801 and memantine in rat hippocampal slices subjected to anoxia stress. However, we found a surprising finding. This neuroprotective effect was mild and did not follow a clear concentration-dependence. Thus, 1 μM MK-801 afforded 42% of neuroprotection, while 10 μM did not offer further protection. The memantine case was even more striking, since 1–3 μM showed no protection and 10 μM exhibited only some protection after 3 h re-oxygenation; no protection was seen at 0–2 h re-oxygenation. This contrasts with the protective effects of memantine observed in other models of neuronal damage in this concentration range. For instance, in neuronal cultures exposed to hypoxic conditions, memantine exhibited clear neuroprotection at 1 μM [20]. It is possible that in our acute model of "cerebral ischemia," other mechanisms intervene in addition to the excessive activation of NMDA receptors by endogenously released glutamate. This is substantiated by the striking and powerful neuroprotective effects afforded by galantamine.

Galantamine has a dual mechanism of action that might explain its therapeutic benefits on cognition, functional activities and behavior in patients with dementia of the Alzheimer's and vascular types [17,22]. On the one hand, galantamine behaves as a mild inhibitor of AChE; on the other, it acts as an allosteric potentiator ligand (APL) at presynaptic nicotinic receptors [12]. We have recently shown that submicromolar concentrations of galantamine affords protection against cell damage in cultures of human neuroblastoma cells and bovine chromaffin cells, exposed to apoptotic agents such as thapsigargin or β-amyloid. Such protection was associated to α7 nicotinic receptors and the induction of the expression of the anti-apoptotic protein Bcl-2, and required 24–48 h preincubation periods with galantamine [3]. This could be explained by the APL effect of galantamine, which only appears at concentrations below or around 1 μM. In our present study, however, the neuroprotective effects of galantamine were visible rather at higher (10- to 20-fold) concentrations and developed in the time lapse of 4–5 h. Neuroprotection was similar at 5 and 15 μM, concentrations at which the APL effect of galantamine turns into a nicotinic receptor blockade [12]. Thus, its pronounced neuroprotective effects at 5–15 μM must be attributed to other galantamine effects, i.e. the inhibition of AChE. In fact, galantamine is a weak inhibitor of this enzyme, exhibiting an IC_{50} of about 3 μM [23], well in the range where the drug exerts its drastic neuroprotective action. It is also plausible that the inhibition of small-conductance Ca^{2+}-activated K^+ channels, recently discovered in our laboratory [2], might also contribute to its neuroprotective action. A new challenging hypothesis has been raised to explain why many compounds that show neuroprotective efficacy in animal models of focal cerebral ischemia, do not afford protection in clinical trials done in stroke patients. The hypothesis implies that after prolonged ischemic episodes (30 min or more) a "Transition Receptor Potential Cation Channel" (TRPM) opens following Ca^{2+} entry, cell Ca^{2+} overloading and neuronal cell death. This pore is a Ca^{2+} entry pathway that is not blocked by NMDA or AMPA receptor blockers [1]. Whether in our experimental conditions (i.e. 60 min OGD) this pore is contributing to neuronal damage is uncertain. But one possibility is that galantamine might be blocking such "TRPM."

It is interesting that galantamine and memantine show therapeutic benefit in both patients with dementia of Alzheimer's or vascular types, suggesting a common pathogenic mechanism. This may be linked to neuronal Ca^{2+} overloading and excess Ca^{2+} accumulation into mitochondria, leading to free radical overproduction and cell death. A Ca^{2+} dyshomeostasis causing a loss of Ca^{2+} from the endoplasmic reticulum, leading to apoptosis, has been implicated as an important metabolic cross-road in the pathogenesis of Alzheimer's disease [16]. In the case of

vascular dementia, the neuronal Ca^{2+} overload may come from excessive glutamate acting on NMDA receptors, as stated above. Both, in Alzheimer's disease [7] and during brain ischemic conditions [14] glutamatergic as well as cholinergic neurotransmission are affected. On other hand, free radicals seem to be involved in neurodegenerative process; in this context, it is interesting that a recent study reports the antioxidant properties of galantamine [24].

In conclusion, whatever the mechanism(s) involved, it seems clear that in rat hippocampal slices subjected to OGD and re-oxygenation, galantamine has a neuroprotection window wider than memantine. Our findings may orient the design of new comparative clinical trials between galantamine and memantine, in patients with Alzheimer's and/or vascular dementia, at different stages of the disease and with different dose ranges. They also suggest a possible neuroprotective role of galantamine in acute cerebral ischemia. We are presently testing this hypothesis in animal models of focal and global cerebral ischemia. If these experiments show that galantamine also exhibits neuroprotective properties in these in vivo models, a clinical trial in stroke patients may be indicated.

Acknowledgements

This work was supported in part by grants from La Caixa, Programa III PRICIT grupos estratégicos de la Comunidad de Madrid/UAM, DGICYT (no. PM99-005) and MCYT (no. BFI2003-02722) to A.G. García, as well as grants from Comunidad de Madrid (no. 08.5/0042/2001) and MCYCT (SAF 2003-04596) to Manuela G. López, and Janssen Pharmaceutica (USA). Mónica Sobrado Sanz is a fellow of Foundation Teófilo Hernando.

References

[1] M. Aarts, K. Iihara, W.L. Wei, Z.G. Xiong, M. Arundine, W. Cerwinski, J.F. MacDonald, M. Tymianski, A key role for TRPM7 channels in anoxic neuronal death, Cell 115 (2003) 863–877.

[2] E. Alés, E. Arias, M.G. Lopez, Apamin-like effects of galantamine in chromaffin cells, in: Proceedings of the 12th International Symposium on Chromaffin Cell Biology, La Palma, Canary Islands, Spain, 21–25 September 2003.

[3] E. Arias, E. Alés, N.H. Gabilán, M.F. Cano-Abad, M. Villarroya, A.G. García, M.G. López, Galantamine prevents apoptosis induced by β-amyloid and thapsigargin: involvement of nicotinic acetylcholine receptors, Neuropharmacology 46 (2004) 103–114.

[4] S. Capsoni, S. Giannotta, A. Cattaneo, Nerve growth factor and galantamine ameliorate early signs of neurodegeneration in anti-nerve growth factor mice, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 12432–12437.

[5] J. De Alba, A. Cárdenas, M.A. Moro, J. Leza, C.P. Lorenzo, I. Lizasoain, Use of brain slices in the study of pathogenic role of inducible nitric oxide synthase in cerebral ischemia-reperfusion, Gen. Pharmacol. 32 (1999) 577–581.

[6] D.J. Findlay, P.J. Connelly, Memantine (Ebixa) in the later stages of dementia, Hosp. Med. 64 (2003) 654–657.

[7] P.T. Francis, N.R. Sims, A.W. Procter, D.M. Bowen, Cortical pyramidal neurone loss may cause glutamatergic hypoactivity and cognitive impairment in Alzheimer's disease: investigative and therapeutic perspectives, J. Neurochem. 60 (1993) 1589–1604.

[8] A. Gorgulu, T. Kins, S. Cobanoglu, F. Unal, N.I. Izgi, B. Yanik, M. Kucuk, Reduction of edema and infarction by Memantine and MK-801 after focal cerebral ischaemia and reperfusion in rat, Acta Neurochir. (Wien) 142 (2000) 1287–1292.

[9] J.Y. Koh, D.W. Choi, Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay, J. Neurosci. Methods 20 (1987) 83–90.

[10] Y. Koh, C.W. Cotman, Programmed cell death: its possible contribution to neurotoxicity mediated by calcium channel antagonists, Brain Res. 587 (1992) 233–240.

[11] A.F. Kurz, T. Erkinjuntti, G.W. Small, S. Lilienfeld, C.R. Damaraju, Long-term safety and cognitive effects of galantamine in the treatment of probable vascular dementia or Alzheimer's disease with cerebrovascular disease, Eur. J. Neurol. 10 (2003) 633–640.

[12] A. Maelicke, A. Schrattenholz, M. Samochocki, M. Radina, E.X. Albuquerque, Allosterically potentiating ligands of nicotinic receptors as a treatment strategy for Alzheimer's disease, Behav. Brain Res. 113 (2000) 199–206.

[13] M.A. Moro, J. De Alba, A. Cárdenas, J. De Cristobal, J.C. Leza, I. Lizasoain, M.J. Díaz-Guerra, L. Bosca, P. Lorenzo, Mechanisms of the neuroprotective effect of aspirin after oxygen and glucose deprivation in rat forebrain, Neuropharmacology 39 (2000) 1309–1318.

[14] J.W. Ni, K. Matsumoto, H.B. Li, Y. Murakami, H. Watanabe, Neuronal damage and decrease of central acetylcholine level following permanent occlusion of bilateral common carotid arteries in rat, Brain Res. 673 (1995) 290–296.

[15] C.G. Parsons, W. Danysz, G. Quack, Memantine is a clinically well tolerated *N*-methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data, Neuropharmacology 38 (1999) 735–767.

[16] W. Paschen, Dependence of vital cell function on endoplasmic reticulum calcium levels: implications for the mechanisms underlying neuronal cell injury in different pathological states, Cell Calcium 29 (2001) 1–11.

[17] M.A. Raskind, E.R. Peskind, T. Wessel, W. Yuan, Galantamine in AD: a 6-month randomized, placebo-controlled trial with a 6-month extension. The Galantamine USA-1 Study Group, Neurology 54 (2000) 2261–2268.

[18] B. Reisberg, R. Doody, A. Stoffler, F. Schmitt, S. Ferris, H.J. Mobius, Memantine Study Group. Memantine in moderate-to-severe Alzheimer's disease, N. Engl. J. Med. 348 (2003) 1333–1341.

[19] A. Rytter, T. Cronberg, F. Asztely, S. Nemali, T. Wieloch, Mouse hippocampal organotypic tissue cultures exposed to in vitro ischemia show selective and delayed CA1 damage that is aggravated by glucose, J. Cereb. Blood Flow Metab. 23 (2003) 23–33.

[20] M. Seif-el-Nasr, B. Peruche, C. Rossberg, H.D. Mennel, J. Krieglstein, Neuroprotective effect of memantine demonstrated in vivo and in vitro, Eur. J. Pharmacol. 185 (1990) 19–24.

[21] B.K. Siesjo, Q. Zhao, K. Pahlmark, P. Siesjo, K. Katsura, J. Folbergrova, Glutamate, calcium, and free radicals as mediators of ischemic brain damage, Ann. Thorac. Surg. 59 (1995) 1316–1320.

[22] P.N. Tariot, P.R. Solomon, J.C. Morris, P. Kershaw, S. Lilienfeld, C. Ding, A 5-month, randomized, placebo-controlled trial of galantamine in AD. The galantamine USA-10 Study Group, Neurology 54 (2000) 2269–2276.

[23] T. Thomsen, B. Kaden, J.P. Fischer, U. Bickel, H. Barz, G. Gusztony, J. Cervos-Navarro, H. Kewitz, Inhibition of acetylcholinesterase activity in human brain tissue and erythrocytes by galanthamine, physostigmine and tacrine, Eur. J. Clin. Chem. Clin. Biochem. 29 (1991) 487–492.

[24] M. Traykova, T. Traykov, V. Hadjimitova, K. Krikorian, N. Bojadgieva, Antioxidant properties of galantamine hydrobromide, Z. Naturforsch. 58 (2003) 361–365.