Neuroprotective signalling mechanisms in the mammalian brain
Dolga, Amalia Mihalea

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CHAPTER 6

Lovastatin-mediated \textit{in vivo} neuroprotection against excitotoxicity is PKB/Akt dependent

Amalia M. Dolga\textsuperscript{1}, Iviçka Granic\textsuperscript{1}, Ingrid M. Nijholt\textsuperscript{1}, Csaba Nyakas\textsuperscript{1}, Eddy A. van der Zee\textsuperscript{1}, Paul G. M. Luiten\textsuperscript{1,2} and Ulrich L. M. Eisel\textsuperscript{1}

\textsuperscript{1}Department of Molecular Neurobiology and \textsuperscript{2}Biological Psychiatry, Graduate School of Behavioral and Cognitive Neurosciences, University of Groningen, Kerklaan 30, 9751 NN, Haren, The Netherlands

Submitted
Abstract

Besides a beneficial cardiovascular effect, it was recently suggested that statins can also have neuroprotective effects. In a previous study we provided evidence that lovastatin treatment abates excitotoxic cell death in primary cortical neurons. Here, we investigated the neuroprotective effect of lovastatin in an in vivo mouse model. We found that administration of lovastatin (20 mg/kg) significantly protects cholinergic neurons and their cortical projections against N-methyl-D-aspartate (NMDA, 60 nmol) induced cell death in the magnocellular nucleus basalis (MNB). Furthermore, lovastatin-mediated neuroprotection was shown to be dependent on protein kinase B (PKB)/Akt signaling since inhibition of PKB/Akt with LY294002 blocked the lovastatin-induced neuroprotective effect. The loss of cholinergic neurons after the MNB lesion resulted in memory impairment as tested in a passive avoidance paradigm. This was reverted by pre- lesion lovastatin treatment. It is concluded that treatment with lovastatin may provide resistance against neuronal injury in excitotoxic conditions associated with neurodegenerative diseases.
6.1 Introduction

Statins or 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase inhibitors are commonly used as pharmaceutical compounds to lower cholesterol levels. Besides the beneficial cardiovascular effects statins exert pleiotropic actions in the central nervous system and were suggested to have neuroprotective properties. For example, in a model for brain injury statins (simvastatin and atorvastatin) were shown to increase neurogenesis in the hippocampal dentate gyrus and to reduce neuronal loss in the cornu ammonis 3 (Lu et al., 2007). Simvastatin enhanced neuronal survival in axotomized retinal ganglion cells after optic nerve injury via the overexpression of heat shock protein 27 (Kretz et al., 2006).

In a previous study we showed that lovastatin mediates neuroprotective effects against glutamate-induced excitotoxicity in cultured primary cortical neurons (Dolga et al., 2008). The neuroprotective effect of lovastatin against glutamate-induced excitotoxicity was, at least partly, mediated by the activation of tumor necrosis factor-receptor 2 (TNF-R2) signaling pathways, which include protein kinase B (PKB)/Akt phosphorylation and NF-κB activation (Dolga et al., 2008; Marchetti et al., 2004).

A well-established model to study excitotoxic brain damage in vivo was originally developed in rats (Dunnett et al., 1987; Luiten et al., 1995, 1987; Stuiver et al., 1996) and consists of the injection of a neurotoxic dose of the glutamate analog NMDA into the magnocellular nucleus basalis (MNB). Subsequently the neuronal damage is quantified. The basal forebrain cholinergic neurons and their projections to the cerebral cortex are susceptible to experimental conditions generally associated with cerebral ischemia and Alzheimer’s disease (AD), including exposure to glutamate, NMDA or amyloid beta peptides (Luiten et al., 1987; Harkany et al., 1999). Therefore, NMDA-induced lesion of the MNB is an effective in vivo method to study neurodegenerative mechanisms associated with NMDA receptor overstimulation (Luiten et al., 1987) and to assess the efficacy of potential therapeutic treatments that may interfere with neurodegenerative processes.

Interestingly, damage to cholinergic MNB neurons and to their cortical target areas is associated with a decline in memory function during aging and in AD and is in fact the basis of the acetylcholinesterase inhibition therapy in AD (Bartus et al., 1982). In the rat model NMDA-induced excitotoxicity in the MNB also led to cognitive deficits (Van der Zee et al., 1994). Hitherto the effects of statins on behavior remain rather unexplored. A few studies, however, showed that statins improve the spatial learning and memory deficits after hypoxic-ischemic or traumatic brain injuries (simvastatin and atorvastatin) in rats (Lu et al., 2007; Balduini et al., 2003) and increase learning and attention (lovastatin) in a mouse model of neurofibromatosis type I (Li et al., 2005). Simvastatin treatment reversed learning and memory deficits in a mouse model for AD (Tg2576 mice). However, simvastatin enhanced also learning and memory performance in the non-transgenic mice, independent of the levels of amyloid beta protein in the brain (Li et al., 2006).

In the current study, we aimed to substantiate our in vitro findings by testing the
protective potential of lovastatin against excitotoxic brain damage in vivo. Since our previous in vitro studies provided evidence for a role of PKB/Akt in the neuroprotective effect of lovastatin, we also investigated whether PKB/Akt activation is essential in the protective mechanism of lovastatin against NMDA-induced excitotoxicity in vivo. To extend the effects of lovastatin in our mouse model we tested memory performance of untreated and lovastatin-treated mice with and without NMDA-induced lesion.

6.2 Materials and methods

Animals and treatments. Experiments were carried out on male C57BL/6J mice (12 weeks, 25-30 g, Harlan, Horst, The Netherlands). The animals were individually housed and received a standard laboratory diet and tap water ad libitum with a 12/12 h dark-light cycle. All procedures were approved by the Ethical Committee for the use of experimental animals of the University of Groningen, The Netherlands (DEC 4681B).

Nucleus basalis lesion. Animals were anesthetized with avertin (tri-bromo-ethanol) and their heads positioned in a Kopf stereotactic frame adapted to mouse brain coordinates (Kopf Instruments model 900, Tujunga, CA, USA). Unilateral lesions were achieved by a slow (0.1 µl/min) injection of 0.4 µl and a total of 60 nmol NMDA (Sigma, St. Louis, MO, USA solved in phosphate buffered saline, PBS pH=7.4) into the MNB (0.6 mm posterior to bregma, 1.8 mm lateral to the sagittal suture, 4.6 mm and 4.4 mm ventral to the dura mater (Franklin and Paxinos, 1997) in two steps of 0.2 µl each with an interval of 5 min. Injections were performed with a Hamilton microsyringe (Hamilton, Bonaduz, Switzerland) in an infusion pump (TSE, Bad Homburg, Germany). Control lesions (0.4 µl PBS) were made in the contralateral MNB of each animal in a manner identical to the NMDA infusion procedure. Thereafter, mice were returned to their home cages and allowed to recover.

Neuroprotective action of lovastatin (experiment I). In the first experiment we investigated the neuroprotective action of lovastatin against neurotoxic damage induced by a unilateral injection of 60 nmol NMDA in the MBN. In pilot experiments 60 nmol NMDA was shown to induce approximately 50% reduction in cholinergic neurons and projection fibers which was similar to the results found in rat studies in our laboratory (Luiten et al., 1987).

Experiment I comprised the following four groups: (1) mice received 20 mg/kg monosodium salt lovastatin (Calbiochem, San Diego, CA, USA) i.p. for 5 consecutive days. Afterwards 60 nmol NMDA dissolved in PBS was injected in one hemisphere and PBS in the contralateral hemisphere. The PBS injected site served as an internal control. The lovastatin dose is based on the results of earlier pharmacokinetic studies (Cheng et al., 2002), (2) mice were injected with saline i.p. for 5 consecutive days
followed by an unilateral 60 nmol NMDA lesion and PBS infusion contralateral ($n = 7$), (3) mice received lovastatin and NMDA injections as in group 1) but the PKB/Akt inhibitor LY294002 (100 µM, Calbiochem, San Diego, CA, USA) was infused into the MNB together with NMDA. (4) mice were treated as in group 2) but received LY294002 together with NMDA into the MNB. All animals were sacrificed 7 days after the surgical procedure (Fig. 6.1(a)).

**Behavioral assessment (experiment II).** To study the behavioral consequences of lovastatin treatment and MNB lesions, four groups of animals were formed: (1) mice injected with saline i.p. for 5 consecutive days; (2) mice injected with 20 mg/kg lovastatin i.p. for 5 consecutive days; (3) mice receiving saline for 5 days i.p. followed by bilateral 60 nmol NMDA lesions on the next day; (4) mice receiving lovastatin for 5 days i.p. prior to bilateral 60 nmol NMDA lesions. 8 – 10 days after the last i.p injection, mice were tested in spontaneous alternation and passive avoidance paradigms (Fig. 6.1(b)).

**Behavioral testing.**

*Spontaneous alternation task.* Short-term spatial memory performance (working memory) was assessed by recording spontaneous alternation behavior in a Y-maze paradigm. The Y maze consisted of three tubular and transparent plexiglass arms forming the Y. All three arms were 5 cm in diameter, 27.5 cm long, and at a 120° angle from each other. The experimental room contained visual cues, which served as distal spatial cues.

The mouse (naïve to the maze) was placed into the centre of the Y maze and allowed to explore the maze freely during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when all four paws of the animal had entered the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The alternation percentage was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries minus two). Since this behavior is not reinforced it is considered “spontaneous”. The exploratory activity was assessed by counting the total number of arm entries.

*Passive avoidance paradigm.* For this test we used a two-compartment (one-way) step-through device. During the training trial, mice were placed in an illuminated compartment, which was separated from the dark compartment by a door. Mice were allowed to explore the light compartment for one minute before the door to the dark compartment was opened. The latency to step into the dark compartment was recorded (pre-shock latency). Upon entry into the dark compartment the door was closed and a mild foot shock (0.3 mA for 2 sec) was delivered through the grid floor. 30 sec after shock application the mice were returned to their home cages. Memory retention was checked 24 h later. The mice were placed in the illuminated compartment for 20 sec and after opening of the door the latency to step into the dark compartment was recorded (post-shock latency) up to a maximum of 8 min.
Tissue processing and ChAT histochemistry. Fixation of the brains was carried out under deep sodium pentobarbital anaesthesia by transcardial perfusion with 200 ml fixative composed of 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4), which was preceded by a short prerinse (50 ml) with ice-cold physiological salt solution. Brains were post-fixed for 24 h in the same fixative, cryoprotected by 3 days immersion in 30% sucrose in 0.1 M PB at 4 °C. Thereafter, 20 µm coronal sections were cut on a Leica cryostat microtome and collected in 0.01 M PBS containing 0.1% sodium azide (Sigma, St. Louis, MO, USA). Free floating sections were preincubated overnight at room temperature in 5% normal rabbit serum (NRS, Zymed, San Francisco, CA, USA) with 0.2% Triton-X. The following day, sections were incubated with goat anti choline-acetyltransferase (ChAT) primary antibody (Millipore, Billerica, MA, USA) diluted 1:500 in PBS for 2 – 4 days at 4 °C. Then, sections were exposed to biotinylated rabbit anti-goat IgG (Vector, Brunschwig Chemie, Amsterdam, The Netherlands) diluted 1:500 in PBS followed by an incubation step for 2 h with Vectastain Elite ABC kit, according to the manufacturer’s protocol (Vector, Brunschwig Chemie, Amsterdam, The Netherlands). For visualization, Sigma Fast 3,3’-diaminobenzidine tablets (Sigma, St. Louis, MO, USA) were used as chromogen with ammonium nickel sulphate (BDH Chemicals Ltd., UK) enhancement. The specificity of immunostaining was confirmed in stainings where incubation with the primary antibody was omitted (data not shown).

Quantitative image analysis. ChAT fiber density was measured in layer V of the posterior somatosensory cortex according to a standardized protocol using an Image Analysis System (Leica Quantimet, Cambridge, UK) with a Leica DFC 350FK camera. The images were analysed with Leica Qwin Image Analyse software. Surface area density of cortical ChAT positive fibers was measured in parietal cortical sections (0.6 mm posterior to bregma (Paxinos and Watson, 1998)), the cortical area that receives a strong cholinergic projection from the lesioned MNB region (Gaykema et al., 1990). After background subtraction and grey-scale threshold determination, the surface area of the skeletonized ChAT immunoreactive (ir) fibers ([the area covered by ChAT-ir fibers]/[total sampling area]) was computed in each parietal region using a 470 nm band pass filter. The relative value of ChAT fibers was calculated as the percentage difference between the surface area density of the lesioned hemisphere and the contralateral sham-operated control hemisphere.

6.3 Results

6.3.1 Lovastatin treatment protects against NMDA-induced lesions in MNB.

The sensitivity of cholinergic neurons to excitotoxicity can be quantified by the loss of the cholinergic cortical innervation that originates from the MNB (Gaykema et al., 1989) visualized with the cholinergic marker choline-acetyltransferase (ChAT,
6.3. Results

Figure 6.1: Schematic outline of the experimental setup. Mice were injected for 5 consecutive days with saline or lovastatin (20 mg/kg of body weight) for 5 consecutive days prior to unilateral NMDA lesions (60 nmol) into the magnocellular nucleus basalis. (a) Eight days after the last i.p injection mice were transcardial perfused (for histochemical analysis) and their brains were fixated with paraformaldehyde. (b) Memory performance was assessed using spontaneous alternation and one-way step-through passive avoidance paradigms. These tests were performed eight days after the drug delivery.

EC3.2.1.6) (Harkany et al., 2000). Histochemical detection of ChAT-ir projection fibers revealed a dense fiber network in the layer V of the somatosensory cortex with a smooth appearance and a layer specific homogenous staining pattern. The cholinergic fiber density decreased after NMDA infusion proportional to the loss of the number of neurons in MNB (Harkany et al., 2000). Characteristic morphological patterns of neuronal degeneration such as the appearance of shrunken or rounded somatic profiles without emanating dendrites, and fragmented cholinergic fibers ascertained the excitotoxic effect of NMDA-induced lesions (Figs. 6.2(a) and (b)). Quantification of ChAT positive projection fibers revealed a significant loss of cholinergic innervation in the NMDA-lesion hemisphere compared to the sham-injected hemisphere of 35.33 ± 5.77%. Pre-lesion administration of lovastatin significantly attenuated the NMDA-induced fiber reduction in the parietal cortex (11.25 ± 4.2%, p < 0.05 Univariate ANOVA), which means a neuroprotective effect of lovastatin to 60 nmol NMDA toxicity of 70.90% in adult mice (Fig. 6.2(c))
6.3.2 Lovastatin-mediated neuroprotection is dependent on PKB/Akt activation.

Our previous in vitro data already revealed an essential role of PKB/Akt pathways in lovastatin-induced protection in primary cortical neurons exposed to glutamate excitotoxicity (Dolga et al., 2008). To further elucidate the lovastatin-mediated neuroprotective pathways in vivo we included in our experiments LY294002 as a specific inhibitor of PKB/Akt signalling. The concentration used (100 µM) was based on previous in vivo studies, where 100 µM LY294002 delivered in rat brain by a single injection of 0.5 µl abolished PKB/Akt phosphorylation (Cheng et al., 2002). Lovastatin or saline was administered for 5 consecutive days prior to a combined NMDA and LY294002 infusion into MNB.

Quantitative analysis of the cholinergic fiber loss indicated no effect of the LY294002 treatment alone in the NMDA lesion model (35.33 ± 5.77% for NMDA plus LY294002 vs. 38.67 ± 3.33% for NMDA alone). In the lovastatin treated animals the ChAT-positive fiber loss in the NMDA plus LY294002 group (31.6 ± 5.41%) showed significantly more fiber loss than the NMDA lesioned cases which were not treated with LY294002 (11.25 ± 4.2%; p < 0.05). The lovastatin treated NMDA+LY294002 values were not statistically different from the saline-pretreated NMDA nor the saline pre-treated NMDA+LY294002 animals. It can thus be concluded that the neuroprotective lovastatin effect is almost completely blocked by PKB/Akt inhibition (Fig. 6.2(c)).

6.3.3 NMDA-induced lesions do not alter spatial short-term memory in a spontaneous alternation task.

Four experimental groups of mice (saline i.p.; lovastatin i.p.; saline i.p. and MNB lesion; lovastatin i.p. and MNB lesion) were tested for spontaneous alternation behavior and locomotor activity. The spontaneous alternation test in a Y maze is a task based on the natural tendency of rodents to alternate in their choices of arm visits (Dudchenko, 2004). Spontaneous alternation behavior is generally regarded as a measure of spatial working memory (Dudchenko, 2004; Senechal et al., 2008).

Alternation rates of the mice with MNB lesion showed no significant difference in a two-way ANOVA when compared with the non-lesioned animals. Moreover, lovastatin treatment in NMDA-induced lesioned mice did not significantly increase spontaneous alternation rates compared to the saline-pretreated animals (see Fig. 6.3(a)). Furthermore, we did not find a difference in the number of arm entries between any of the groups, which suggested that the exploratory activity was neither affected by lovastatin treatment nor by MNB lesions (Fig. 6.3(b)).

6.3.4 Lovastatin decreased lesion-induced memory deficits.

Neuronal projections from MNB directly modulate neocortical information processing. The passive avoidance task is a learning task which depends, in part, on proper neocortical information processing. In rats NMDA-induced lesions in MNB were indeed reported to result in memory impairments in a passive avoidance paradigm
6.3. Results

Figure 6.2: Lovastatin protects cholinergic fibers against NMDA-induced lesions. (a) ChAT-ir neuronal perikarya and proximal fiber branches in the MBN following NMDA or PBS infusion. Depicted representative region of intact cholinergic neurons (1, 2) after sham lesions in MBN whereas in (3, 4) it is shown the loss of proximal fiber branches emanating from ChAT-ir perikarya. Noteworthy is the loss of cholinergic neurons as a consequence of NMDA toxicity. Arrows show ChAT-ir stained cholinergic processes. (b) Distribution of ChAT-positive projection fibers in the posterior somatosensory cortex following NMDA or PBS infusion in the mouse MBN. (1) Depicted representative region of sham-operated control. Noteworthy is the loss of cholinergic projections as a consequence of (2) NMDA infusion. (3) Pre-lesion lovastatin administration (20 mg/kg) significantly attenuated the NMDA-induced damage whereas (4, 5) inhibition of PKB/Akt with LY294002 failed to rescue cholinergic projections in (5) lovastatin-treated mice. Horizontal bars in (1) represent layer V of the somatosensory cortex where the quantitative measurements were performed. (c) Quantitative measurements of cholinergic fiber densities in layer V of the somatosensory cortex. Whereas NMDA infusion into MBN induced a massive loss of ChAT fiber loss, lovastatin treatment significantly antagonized this NMDA detrimental effect. However, inhibition of PKB/Akt pathway with LY294002 did not rescue cholinergic fiber projections in saline- or lovastatin-treated mice. **p < 0.01, *p < 0.05 NMDA vs. all other groups examined (Univariate analysis of variance), n = 5–7 per group. Data represent means ±S.E.M.
Figure 6.3: NMDA-induced lesions do not cause changes in working memory. (a). In the spontaneous alternation task there is no difference in alternation rate between the four groups. Note, however that the pool of lovastatin-treated groups had a significantly higher alternation rate when compared to the pooled two saline-treated groups (p = 0.045, two-way ANOVA). (b). There was no significant difference in the number of arm entries. *p < 0.05, n = 6 – 7 per group. Data are expressed as means ± S.E.M.

(Harkany et al., 1999; Van der Zee et al., 1994). Therefore, we used this behavioral paradigm to investigate whether preserved cortical cholinergic function in lovastatin-treated mice is able to revert the cognitive deficits induced by NMDA lesions into MNB.

Altered retention mechanisms and acquisition were investigated at 8 days postsurgery or 8 days after the last intra-peritoneal injection in the passive avoidance paradigm. Pre-shock latencies did not exhibit significant differences among the experimental groups. NMDA infusion into the MNB resulted in a significant impairment of passive avoidance learning. This was indicated by shorter post-shock latencies in the retention trial 24 h after delivery of the foot-shock in the saline-treated NMDA lesion group (4.5 ± 0.75 min) in comparison to the saline-treated group (8 min). However, lovastatin pretreatment in NMDA lesioned mice significantly increased the postshock latency time compared to NMDA lesioned animals (6.5 ± 0.8 min vs. 4.5 ± 0.75 min; p < 0.05 Univariate analysis of variance) indicative of an improved memory performance in this test condition. Lovastatin treatment alone had no effect on memory performance.

6.4 Discussion

In the present study we show that administration of lovastatin protects cholinergic neurons and their cortical projections against excitotoxic damage in vivo. Decline of
ChAT positive neurons in the basal forebrain nucleus and their projection fibers in the parietal cortex as a result of excitotoxic stimuli is a well-established technique to test bioactive substances in vivo (Dunnett et al., 1987; Luiten et al., 1987). For the first time this method was applied in mice and we could demonstrate a similar effect of the NMDA lesion as compared with the well-established rat model (Luiten et al., 1995; Stuiver et al., 1996; Harkany et al., 2000). The advantage of this in vivo model is the precisely quantifiable neuronal lesion effect on cholinergic fiber loss. Moreover, because of the strictly unilateral projections of the MNB, the contralateral sham hemisphere can serve as a control within each individual case. Five days of lovastatin treatment induced a strong neuroprotection against NMDA excitotoxicity.

In a recent in vitro study, we have shown that lovastatin mediates the specific up-regulation of TNF-R2 in cultured neurons but not that of TNF-R1 (Dolga et al., 2008). Interestingly, this TNF-R2 up-regulation in combination with TNF signaling led to a strong neuroprotection. In several studies we have shown that neuroprotection initiated by activation of TNF-R2 is mediated by PKB/Akt (Marchetti et al., 2004; Fontaine et al., 2002). In vitro this PKB/Akt pathway was also shown to underlie lovastatin-mediated protection against glutamate-induced excitotoxicity (Dolga et al., 2008). To investigate if this is also the case in the in vivo situation, we used the inhibitor of PI3 kinase, LY294002, to block the activation of PKB/Akt. In the presently used experimental setup we show that this LY294002 treatment resulted in a strong reduction of lovastatin-mediated neuroprotection in vivo. The neuropro-
Lovastatin mediates \textit{in vivo} neuroprotection via PKB/Akt signaling

tective effect of PKB/Akt activation was also reported before in \textit{in vivo} models for brain injury. In a middle cerebral artery occlusion model (Pignataro et al., 2008; Prinz et al., 2008) PKB/Akt activation was found to be essential for neuroprotective signaling since neuroprotection after postconditioning was inhibited in the presence of PKB/Akt inhibitor LY294002. Our findings corroborate recently reported microarray and RT-PCR studies that identified several altered genes in statin-treated mice. Lovastatin treatment increased the expression levels of 26 genes, particularly genes related to apoptosis (c-myc, Bcl2) (Johnson-Anuna et al., 2005) and PKB/Akt phosphorylation (Li et al., 2006). Bcl2 proteins exhibit neuroprotective functions against various excitotoxic insults, such as glutamate or amyloid beta peptides. Bcl2 gene expression is of particular interest in this respect since this major anti-apoptotic gene is under direct PKB/Akt regulation (Pugazhenthi et al., 2000).

In order to assess whether the cholinergic neurons rescued by lovastatin treatment are able to counteract the behavioral deficits observed in NMDA-induced lesion mice we subjected the animals to a spontaneous alternation task and a passive avoidance memory test. To test the selectivity of the MNB lesion to cortical functions we used the spontaneous alternation paradigm for working memory, since this test is commonly associated with hippocampus and pre-frontal-related memory. We found no significant difference in alternation rates between NMDA-lesioned animals compared with non-lesioned control mice. This suggests that brain regions as the hippocampus are not affected by NMDA-induced lesions of MNB, which is in line with the known projection patterns of the MNB and medial septum complex (Luiten et al., 1987; Gaykema et al., 1989) and was confirmed by our anatomical data. Here, pre-lesion administration of lovastatin significantly attenuated the memory retention deficit induced by the NMDA lesion of the MNB. The passive avoidance paradigm is one of the test conditions of choice for the behavioral assessment of the integrity of the neocortical system. Thus, the neuroprotective effect of lovastatin on NMDA-induced damage to the MNB and its neocortical innervation are in line with the improved memory retention in lovastatin treated mice. In summary, our behavioral data indicate impaired behavioral performance associated with damage to the MNB that specifically affects neocortical denervation and its memory functions while leaving particular hippocampal innervation and its learning functions unaffected. Whereas lovastatin treatment reverted the memory deficits produced by NMDA lesions as observed in the passive avoidance paradigm, lovastatin treatment itself had a modest albeit significant effect on improving hippocampal memory and prefrontal cortex function irrespective of damage to cholinergic neocortical innervation.

It is unlikely that behavioral alteration of lovastatin-treated animals can be attributed to direct drug effect since statins are rapidly cleared from the brain within 6 hours after drug administration (Johnson-Anuma et al., 2005). We performed all behavioral testing 7 days after the last lovastatin administration. However, the exact underlying mechanisms of how statins can affect the brain remain to be elucidated (Wolozin, 2004). Statins such as lovastatin are able to cross the blood brain bar-
rier and reach the cerebral cortex within one hour after drug administration but are significantly reduced 6 hours later after a single drug application. It is not clear whether the effects of statins as found in the present study can be directly associated with cholesterol dependent processes in brain tissue. Brain cholesterol analyses in several studies showed conflicting results. Long-term treatment of simvastatin for 3 months did not change the total cholesterol levels in the brain although the plasma total cholesterol levels were strongly reduced (Li et al., 2006). Other studies report reduced brain cholesterol levels in young C57BL/6J mice treated for 3 weeks with simvastatin and pravastatin, but such a decline of cholesterol concentrations was not observed in the brains of mice treated with lovastatin (Johnson-Anuna et al., 2005).

In conclusion our data provide evidence for a neuroprotective role of lovastatin against NMDA-induced lesions of the MNB. Importantly, this beneficial effect was shown to be dependent on activation of the PKB/Akt pathway. Furthermore, lovastatin administration was able to attenuate impaired memory functions that can be associated with cholinergic cortical denervation that result from the damaged nucleus basalis-cortical projection pathway.

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