The nootropic and neuroprotective proline-containing dipeptide noopept restores spatial memory and increases immunoreactivity to amyloid in an Alzheimer's disease model

Rita U. Ostrovskaya, Marina A. Gruden, Natalya A. Bobkova, Robert D. E. Sewell, Tatyana A. Gudasheva, Alexander N. Samokhin, Sergey B. Seredinin, Wim Noppe, Vladimir V. Sherstnev and Ludmilla A. Morozova-Roche

*J Psychopharmacol* 2007 21: 611
DOI: 10.1177/0269881106071335

The online version of this article can be found at: http://jop.sagepub.com/content/21/6/611
The nootropic and neuroprotective proline-containing dipeptide noopept restores spatial memory and increases immunoreactivity to amyloid in an Alzheimer’s disease model

Rita U. Ostrovskaya V. V. Zakusov State Institute of Pharmacology, Moscow, Russia.
Marina A. Gruden P. K. Anokhin State Institute of Normal Physiology, Moscow, Russia.
Natalya A. Bobkova Institute of Cell Biophysics, Moscow Region, Puschino, Russia.
Robert D. E. Sewell Welsh School of Pharmacy, Cardiff University, Cardiff, UK.
Tatyana A. Gudasheva V. V. Zakusov State Institute of Pharmacology, Moscow, Russia.
Alexander N. Samokhin Institute of Cell Biophysics, Moscow Region, Puschino, Russia.
Sergey B. Seredinin V. V. Zakusov State Institute of Pharmacology, Moscow, Russia.
Wim Noppe Interdisciplinary Research Center, Campus Kortrijk, KU Leuven, Kortrijk, Belgium.
Vladimir V. Sherstnev P. K. Anokhin State Institute of Normal Physiology, Moscow, Russia.
Ludmilla A. Morozova-Roche Department of Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden.

Abstract

The effects of the novel proline-containing nootropic and neuroprotective dipeptide, noopept (GVS-111, N-phenylacetyl-L-prolylglycine ethyl ester) were investigated in NMRI mice following olfactory bulbectomy. We have shown previously that these animals developed Alzheimer’s disease (AD)-like behaviour, morphology and biochemistry including impairment of spatial memory, regional neuronal degeneration and elevated Aβ peptide brain levels. In the current investigation, spatial memory was assessed using the Morris water maze and serum antibodies to in vitro morphologically characterized amyloid structures of both Aβ(25–35) Peptide and equine lysozyme, as well as to neurotrophic glial factor S100b, were analyzed by enzyme-linked immunosorbent assay (ELISA). Noopept (administered at a dose of 0.01 mg/kg for a period of 21 days and during a further 5 days training) restored spatial memory and increased serum antibody levels to oligomers of Aβ(25–35) peptide but not to equine lysozyme amyloid or S100b protein in bulbectomized animals. The positive immunotropic effect of noopept to Aβ(25–35) peptide prefibrillar aggregates was more marked in sham-operated compared to the bulbectomized subjects which were characterized by an overall suppression of immunoreactivity. Enhancement of the immune response to Aβ(25–35) peptide prefibrils caused by noopept may attenuate the neurotoxic consequences of amyloid fibrillation and also be associated with an improvement in spatial memory in bulbectomized mice. These actions of noopept, combined with it’s previously reported neuroprotective and cholinomimetic properties, suggests that this dipeptide may well be useful for improving cognitive deficits induced by neurodegenerative diseases.

Keywords

noopept (GVS-111), memory improvement, olfactory bulbectomy, Alzheimer’s disease, antibodies, Aβ(25–35) Peptide, amyloid
Introduction

AD is a prevalent neurodegenerative illness affecting millions of people worldwide (Maubach, 2003; Ross and Poirier, 2004). Currently, there is no effective therapy to prevent or halt the progression of the disease, although symptomatic treatments are available. Neurotransmitter-based therapy using acetyl cholinesterase inhibitors (Cacabelos et al., 2000) and NMDA receptor antagonists (Desai and Grossberg, 2005) are in current use. Anti-inflammatory and anti-oxidative approaches, as well as compounds devised to block Aβ peptide aggregation are being tested in the clinic. Furthermore, β- and γ-secretase inhibitors designed to reduce generation of Aβ peptide are under investigation in addition to the development of a vaccine against β-amyloid (Nitsch, 2004). The efficacy of these treatments is relatively modest, while the incidence of withdrawals due to adverse events is moderately high (Evans et al., 2004).

It is evident that AD has a complex multifactorial pathology, so it would be advantageous to explore novel therapeutic strategies (Vesey et al., 2002) focused on diverse mechanistic targets (Sewell et al., 2005). Nootropic drugs have received particular attention in this respect because they possess useful effects in the treatment of memory loss and age-related memory decline. Piracetam (Nootropil®) is the most well known first generation nootropic (Giurgea, 1972; Winblad, 2005), however, a significant proportion of AD patients appear to be unresponsive to it (Gualtieri et al., 2002), prompting the search for agents which surpass piracetam in AD neurodegeneration (Peskind et al., 2001) and we have demonstrated that antibodies to this biomarker are elevated in shorter duration AD sufferers. The aim of this study was to evaluate the behavioural efficacy of noopept on spatial memory and autoimmune responses to in vitro morphologically characterized amyloid structures of both Aβ(25-35) peptide and equine lysozyme as well as to protein S100b in bulbectomized animals.

Methods and materials

Drugs and proteins

Noopept (N-phenylacetyl-L-prolylglycine ethyl ester, MW = 319) was designed and synthesized at the State V. V. Zakusov Institute of Pharmacology, RAMS (Seredenin et al., 1995). Aβ(25-35) peptide was produced in the Pushchino Branch of academicians M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry RAS. Equine lysozyme was purified as described previously (Noppe et al., 1996). S100b was purchased from Sigma, USA. In order to produce amyloid structures, Aβ(25-35) peptide (20 mg/ml) was incubated in 50 mM PBS, pH 8.0 for 16 h at 8 °C (Gruden et al., 2004). Equine lysozyme (20 mg/ml) was incubated in 20 mM glycine buffer, pH 2.0 at 57 °C for 1 week and subsequently transferred to 2 mM sodium bicarbonate buffer, pH 8.0 (Malisauskas et al., 2003).

Animals

NMRI male mice weighing 25 ± 0.6 g were supplied by Charles Rivers Laboratories, USA. This strain has previously been shown to exhibit good performance in the water maze (Klapdor and van de Straay, 1996) and also displays inherent immunosuppression (Freisleben et al., 1994). Animals were allowed food and water ad libitum and housed in groups of eight in standard laboratory cages under 12 h: 12 h light–dark conditions (light from 8.00 AM) at 21–23 °C. All animal experiments were performed in accordance with the guidance of the National Institutes of Health for Care and Use of Laboratory Animals, NIH Publications No. 8023, revised 1978.

Olfactory bulbectomy procedure

Two groups of mice aged 10 weeks (n = 32) were employed in the experiments; one group underwent OB operation and the other...
group was sham operated (SO). Mice were anaesthetized with isoflurane and 0.5% Novocaine used for local anaesthesia of the scalp. A single burr hole of 2 mm diameter (2 mm anterior to bregma, 0 mm laterally from the midline) was drilled over the olfactory bulbs. Both olfactory bulbs were carefully aspirated through a rounded needle attached to a water pump. The extent of the lesion was assessed both visually and histologically at the end of the experimental study. SO mice were treated similarly, except that the olfactory bulbs were not removed.

**Drug treatment**

Four weeks following OB or SO procedures, mice were randomly divided into four groups of eight animals. Two groups denoted as SO + NP and OB + NP were treated with noopept (0.01 mg/kg i.p.) daily for 21 days. In the remaining two groups denoted as SO + saline and OB + saline, the animals were administrated with 0.9% saline (i.p.) for the same period. Either noopept (0.01 mg/kg) or saline was injected 1 h before training and memory testing (see Fig. 1). The 0.01 mg/kg dose of noopept was chosen from earlier studies using mice in cognitive tests (Seredenin et al., 1995) and the 21-day treatment regime was based on the fact that clinical effects of nootropics achieve significance within 2–3 weeks of administration (Waegemans et al., 2002).

**Morris water maze paradigm**

Experiments were performed in a test room with extra-maze cues to facilitate spatial learning. A circular swimming tank (80 cm diameter and 40 cm wall height with a hidden platform of 6 cm-diameter) – (State Institute of Biological Instrumentation, RAS, Russia) was filled to depth of 30 cm with water at 23 °C and rendered opaque by adding powdered milk. The dimensions of the tank were chosen according to a previous study by Klapdor and van de Staay (1996) who described good task acquisition with NMRI mice using these parameters. The tank was mentally rendered opaque by adding powdered milk. The dimensions of the tank were chosen according to a previous study by Klapdor and van de Staay (1996) who described good task acquisition with NMRI mice using these parameters. The tank was mentally divided into four quadrants: platform target quadrant (3), opposite quadrant (1), adjacent clockwise quadrant (4) and adjacent counterclockwise quadrant (2). The hidden platform was located in the middle of the third quadrant during training. It was submerged to a depth of 0.5 cm so as to be invisible to a swimming animal during the whole period of training. A video monitoring system (TSI, Germany) was used for recording the main behavioural parameters in the water-maze paradigm.

Fifty-four days after operation, learning ability and spatial memory were tested (Morris, 1984) as shown in Fig. 1. Initially, 34 OB and SO animals were assessed in the water maze to identify any inherent quadrant preference and those who showed some preference (2 mice) were eliminated from subsequent testing. Latency to reach the visible platform was then determined (three trials, 60 s each trial). Mice were then exposed to a total of 20 training trials over 5 days (i.e. four trials per day; 60 s each trial). During each trial, the latency to locate the hidden platform was evaluated up to a maximum of 60 s. Each animal was placed into the water facing the wall of the tank in one of three randomly selected quadrants other than that containing the hidden platform. If the animals failed to locate the platform within 60 s, they were placed on the platform for 10 s. Spatial memory was tested on the following day after completion of training with a single trial (60 s) in the absence of the hidden platform starting from a random position. During the test period, two parameters were recorded: quadrant occupancy time (s) spent in each quadrant and the number of entries in each quadrant expressed as a percentage of the total entries.

**Atomic force microscopy (AFM)**

The morphology of amyloid species of Aβ_{25–35} peptide and equine lysozyme was examined using a PicoPlus (Molecular Imaging, USA) atomic force microscope using the imaging and sample preparation procedures as described previously (Malisvaskas et al., 2003; Malisvaskas et al., 2005). The dimensions of protein species were measured in cross-sections of AFM height images by using PicoPlus software (Molecular Imaging, USA).

**ELISA assay**

The level of serum Abs to amyloid species of both Aβ_{25–35} peptide and equine lysozyme as well as to S100b protein in NMRI mice was quantified using solid phase ELISA as described previously (Gruden et al., 2004). One day after assessment of spatial memory, blood samples from all groups were obtained by interorbital venipuncture under general anaesthesia. Sera for ELISA were produced by centrifugation of blood in a microcentrifuge (Eppendorf, USA), 3500 rpm at 4 °C for 15 min. The titre of Abs to selected antigens in each serum sample was derived from the reciprocal of the greatest dilution at which the enzyme-substrate reaction gave an optical density value twice that of the mean optical density of control sera from naïve animals. The values of Abs titres at which we observed immunoreactivity to the antigens were expressed in relative dilution ratio units. Optical density was measured by using a microtitre plate reader (Flow Laboratory, USA). The mean serum level of Abs to amyloid species of both Aβ_{25–35} peptide, equine lysozyme and S100b protein in NMRI naïve mice were determined as 8.2 ± 2.0, 8.0 ± 2.0 and 10.2 ± 2.4, respectively.
Statistical analysis

Statistical analysis of the spatial memory training and testing was carried out with ANOVA using the program Statistics 6 and ANOVA statistical package (CSS). The p values were reported for repeated measures. The preference for platform target quadrant in comparison with other indifferent quadrants was assessed by post hoc analysis using a multiple-range LSD test. The statistical significance of the difference of immunological parameters in the ELISA experiments was evaluated using two-tailed Student’s t-test. All data were expressed as means ± sem.

Results

Behavioural experiments

In order to verify that there were no motor or visual impairments, latencies for all animals to locate a visible platform (in three trials) were screened in the Morris water maze before initiating the main spatial training schedule. Subsequent repeated measures ANOVA analysis between SO + saline, SO + NP, OB + saline and the OB + NP groups did not disclose any significant group effect (F = 1.64, p = 0.22).

During the succeeding 5-day course of water maze training, repeated measures ANOVA between all OB and SO groups indicated no significant group effect: F(3,124) = 6173, p = 0.605 but there was a significant outcome for the day of training: F(4,496) = 47.118, p = 0.000001. Hence, we observed a daily reduction in mean latency to locate the hidden platform in all OB, SO, saline and noopept treated groups. Post hoc examination revealed no statistical difference in learning abilities between any of the animal groups on each individual day of training as well as over the 5-day training course. This suggested that the speed of learning was comparable between all groups. In addition, noopept did not induce visual or motor deficits, neither did it modify water maze performance in OB or SO mice during training.

Spatial memory was tested on the day following completion of training. Two-way ANOVA was then performed in order to expose any possible link between group and quadrant as the main factors in the spatial memory test. This analysis of quadrant occupancy time revealed significant effects of quadrant (F(3,112) = 34.2; p < 0.0001), but not of group (F(3,112) = 0.014; p > 0.05) and there was a significant interaction between group and quadrant (F(9,112) = 6.8; p < 0.0001). A likewise analysis of number of entries also disclosed significant effects of quadrant, (F(3,112) = 36.1; p < 0.0001), no main effect of group (F(3,112) = 2.3; p > 0.05) and a significant interaction between group and quadrant (F(9,112) = 9.4; p < 0.0001). Consequently, a substantial link between quadrant and group in the water maze test was established using both quantified parameters of spatial memory (i.e. occupancy time and number of entries) in all groups of animals.

Group analyses of variance were performed to evaluate the differences in quadrant occupancy time and number of entries in each quadrant for each group, the results of which are presented in Table 1.

The data demonstrate that during spatial memory testing, when the platform was removed from the tank, a significant effect of quadrant as the main factor was verified both for quadrant occupancy time and number of quadrant entries in SO + saline, SO + NP and OB + NP groups. In contrast, the OB + saline animals did not exhibit preference for any of the four quadrants. Thus, bulbectomy impaired spatial memory in NMRI mice. Moreover, significant differences in quadrant preference were revealed in the OB + NP mice to both parameters (occupancy time, F(3,28) = 7.48; p ≤ 0.0008) and (number of entries, F(3,28) = 8.63; p ≤ 0.00325). High values of quadrant designation factor (for both occupancy time and number of entries) in the SO + NP group presumably reflects an increased homogeneity of the data under the influence of the dipeptide.

The results of post hoc analysis using LSD criteria are demonstrated in Fig. 2 (a and b). All experimental groups, except the OB + saline mice, searched the target platform quadrant (3) significantly more than other quadrants. This was true for both occupancy time and number of entries. In contrast, bullectomized animals did not display any preference for the target quadrant, thus manifesting a spatial memory deficit. Noopept (0.01 mg/kg administered for 21 days) on the other hand did not modify either parameter of spatial memory (occupancy time and number of entries) in the SO group, but clearly restored spatial memory impaired by bullectomy.

Table 1 Factor analysis of quadrant designation parameters in the water maze in OB and SO NMRI mice

<table>
<thead>
<tr>
<th>Animal treatment groups</th>
<th>Quadrant designation factor, occupancy time</th>
<th>Quadrant designation factor, number of entries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F (3,28)</td>
<td>p</td>
</tr>
<tr>
<td>SO + saline</td>
<td>9.73</td>
<td>0.0004*</td>
</tr>
<tr>
<td>SO + NP</td>
<td>22.671</td>
<td>0.00001*</td>
</tr>
<tr>
<td>OB + saline</td>
<td>0.811</td>
<td>0.503</td>
</tr>
<tr>
<td>OB + NP</td>
<td>7.48</td>
<td>0.0008*</td>
</tr>
</tbody>
</table>

* Denotes the significance of quadrant designation factor in water maze, p < 0.001.
The parameters of occupancy time and number of entries correspond to the time spent and entries into all quadrants by all animals of each group.
Atomic force microscopy imaging

The AFM analysis of amyloid species of Aβ<sub>(25-35)</sub> peptide and equine lysozyme were carried out before subjecting them to ELISA. This was particularly important since amyloid is characterized by an inherent diversity of species dependent on environmental conditions (Wang et al., 2001; Malisauskas et al., 2003). After incubation for 16 h in 50 mM PBS, pH 8.0 at 8 °C, Aβ<sub>(25-35)</sub> peptide formed round-shaped prefibrillar structures shown in Fig. 3a. They adhered to the mica surface as spherical-caps and their height estimated in cross-sections was equal to ca. 2 nm (Fig. 3c). The same images were acquired both in air and liquid environment indicating that the morphology and dimensions of the prefibrillar structures of Aβ<sub>(25-35)</sub> peptide were not affected by the mode and conditions of measurements.

Equine lysozyme was assembled into amyloid structures under the pH 2.0 and 57 °C conditions, transferred into ELISA 2 mM sodium bicarbonate buffer, pH 8.0 and subsequently assessed by AFM (Fig. 3b). The sample was a heterogeneous mixture of round-shaped oligomers of ca. 2 nm height measured in the cross-sections, linear and circular assemblies of oligomers and protofilaments of ca. 2 nm heights (Fig. 3d and e) with variable length from short stretches of ca. 20–40 nm to polymers of ca. 400 nm. The latter, nonetheless, did not proceed to thicker and longer mature fibrils. It is interesting to note, that in some linear and circular polymeric structures, the constituting ca. 2 nm height oligomers were still clearly identifiable (Fig. 3d and e).

Immunochemical experiments

The autoimmune response to amyloid species of Aβ<sub>(25-35)</sub> peptide and equine lysozyme as well as to S100b was measured by ELISA. The results for all groups of animals are presented in Table 2 as antibody titres. Both SO and OB animals, injected with saline, possessed low serum antibody titres to all antigens tested in our experiments and there was no statistically significant difference between any of these antibody levels. However, sub chronic administration of noopept to OB mice caused an increase in the Abs titre to Aβ<sub>(25-35)</sub> peptide prefibrillar amyloid by a two-fold factor but not to equine

** and *** denote the significance of quadrant designation factor in the water maze p < 0.01 and p < 0.001, respectively.
The nootropic and neuroprotective proline-containing dipeptide noopept restores spatial memory

Table 2  

| Animal treatment groups | Titres of antibodies to selected antigens  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± sem relative dilution ratio units)</td>
</tr>
<tr>
<td></td>
<td>A(_{\beta}(25-35)) peptide amyloid</td>
</tr>
<tr>
<td>SO + saline</td>
<td>10.0 ± 2.0</td>
</tr>
<tr>
<td>SO + NP</td>
<td>52.4 ± 10.2*</td>
</tr>
<tr>
<td>OB + saline</td>
<td>12.2 ± 2.4</td>
</tr>
<tr>
<td>0 + NP</td>
<td>26.8 ± 2.2*</td>
</tr>
</tbody>
</table>

*p < 0.05 – the level of antibodies in these groups of mice was compared to the antibody titres to A\(_{\beta}(25-35)\) peptide amyloid in saline injected animals.

lysozyme amyloid species nor to S100b protein. An even more pronounced effect was observed following noopept administration to SO mice, whereby the antibody titre to A\(_{\beta}(25-35)\) peptide amyloid was statistically elevated five-fold compared to saline treated SO animals (Table 2). Comparing the data obtained for each individual animal, we did not detect any direct correlation between the degree of memory deficit and the level of antibodies to the oligomers of A\(_{\beta}(25-35)\) peptide.

Discussion

Restorative effect of noopept on spatial memory impairment in OB mice

The present data revealed that OB mice developed spatial memory impairment in the water maze model. Thus OB animals failed to show rescue platform quadrant preference during the learning procedure and this concurs with previously reported data (Hozumi, 2003; Bobkova, 2004). The main finding of the current study was that reestablishment of preference to a platform target quadrant occurred in OB mice treated with noopept, a dipeptidal analogue of vasopressin and piracetam (Seredenin et al., 1995). Values of occupancy time in quadrant 3, which formerly contained the rescue platform, and the number of quadrant 3 entries in noopept treated OB animals were comparable to those of control SO mice injected with saline. It should be emphasized that bulbectomy itself did not change motor or visual functions in animals testifying to the specific action of bulbectomy on memory-related performance in the water maze paradigm. It should also be stressed that noopept did not affect the dynamics of learning in SO animals which supports the accepted view that nootropics are capable of restoring specific memory deficits without efficacy in the absence of mnemonic disturbances (Giurgea, 1972).

Currently, there is a need for predictive AD animal models in the screening and development of new drugs to treat this disorder. Apart from a number of newly developed genetic models (van Dooren et al., 2005), paradigms based on chemical, aspirative and electrolytic lesions of the brain remain in the focus of research endeavour (McDonald et al., 1998). There are several reports that OB lesions induce multiple morphological, biochemical and behavioural features of AD such as deposition of A\(_{\beta}\) peptide amyloid in the brain resulting in apoptotic neuronal cell death (Leung et al., 2003), a deficit of BDNF, choline uptake into various brain structures (Sohrabji et al., 2000), decreased stem cell production (Pagano et al., 2000) and also multiple suppression of the immune system (Novoselova et al., 2004). It is interesting to note, that clinical features of human AD also include the loss of olfactory discrimination and spatial disorientation (Kovacs et al., 2001). Moreover, it has been established that cholinergic dysfunction is conducive to impaired cognition associated with neuropathological hallmarks of AD (fibrous aggregates consisting of...
the Aβ peptide which develops into the amyloid plaques and paired helical filaments composed of hyperphosphorylated tau protein forming the neurofibrillary tangles (Friedhoff et al., 1999).

Furthermore, accumulation of senile plaques containing Aβ peptide and the formation of neurofibrillary tangles are commonly found in the bulbus olfactorius of AD patients (Esiri et al., 1984). Cholinergic deficit is regarded as one of the main sources of OB-induced memory impairment (Hozumi et al., 2003). This notion accords with the findings of Yamamoto (1997) who demonstrated the capability of acetylcholinesterase inhibitors to abolish memory disturbances caused by OB. Additional studies on noopept showed that it not only failed to inhibit cholinesterase activity in vivo, but that it also increased neuronal membrane sensitivity to acetylcholine applied microiontophoretically (10⁻⁵–10⁻¹¹ M) to isolated neurons of Helix lucorum (Ostrovskaya et al., 2002). Specific mechanisms underlying this effect (such as a direct cholinomimetic action and/or enhancement of acetylcholine release or synthesis) have yet to be clarified. However, the structural similarity of noopept to known cholinergic receptor allosteric modulators and channel activators such as ABT-418 and ABT-089 (Decker et al., 1997; Potter et al., 1999; Reuter et al., 2004) reinforce the possibility of a cholinomimetic mechanism in this dipeptide’s mnemotropic profile. Another conceivable mechanism underlying noopept memory enhancing properties focuses on its metabolism. Thus, a pharmacokinetic study on noopept has demonstrated that the parent molecule gives rise to the formation of a stable metabolite (cyco-propylglycine), which is an endogenous mnemotropic cyclic dipeptide (Gudasheva et al., 1996). These properties of noopept may well contribute towards restoration of spatial memory impaired by bullectomy. Noopet's ability to attenuate the OB-induced deficit of spatial memory in the water maze is consistent with its positive effect on another model of contextual-dependant behaviour, conditioned freezing (Vysotskii et al., 1999).

**Immunological effect of noopept in OB mice**

In ELISA experiments we have demonstrated that administration of noopept in bullectomized mice increased antibody production to the specific marker of AD, prefibrillar amyloid of Aβ(25-35) peptide by a two-fold factor compared to controls. The effect of noopept in SO animals was however even more pronounced, the level of these antibodies being raised five-fold. NMRI mice are characterized by low immune reactivity (Freisleben et al., 1994), while OB induces additional immunosuppression (Novoselova et al., 2004). This is consistent with a significant inhibition of immune reactions reported in advanced AD patients with severe cognitive impairment (Nitsch, 2004). It is noteworthy that using ELISA, we have analysed the pre fibrillar amyloid species of Aβ(25-35) peptide, the morphology of which was evaluated by AFM. The morphology of the Aβ(25-35) peptide oligomers produced in our studies closely resemble that of Aβ(1-40) and Aβ(1-42) peptides (Klein et al., 2002; Lashuel et al., 2003; Stine et al., 2003). It has also been demonstrated that the oligomers of both Aβ(25-35) and full-length Aβ peptides exert similar neurotoxic effects (Pike et al., 1995; Kirkradzke et al., 2002; Wu et al., 2004). Aβ peptide prefibrillar aggregates have not only been found in soluble brain extracts from AD patients but also in transgenic mouse models displaying signs of neurotoxicity and memory impairment (Lesne et al., 2006). They appear to disrupt synaptic signalling which induces an early-stage memory loss, leading subsequently to a broader scale effect including synaptic degeneration and nerve cell death (Wang et al., 2001; Kirkitadze et al., 2002). Immune-targeting of Aβ prefibrillar amyloid oligomers by vaccination has proved to be effective in neutralizing this type of neurotoxicity of amyloid oligomers in rabbits (Lambert et al., 2001; Klein, 2002) and also in attenuating AD-like pathology in transgenic mice (Schenk et al., 1999; Selkoe and Schenk, 2003; Butterini et al., 2005). Thus, clearance of primary pathogenic Aβ peptide amyloid species would be a particularly important step in AD treatment.

Comparison of the behavioural and immunological data obtained for each individual animal in this study did not disclose any significant direct correlation between the degree of memory deficit and the level of antibodies to oligomers of Aβ(25-35)peptide. The data obtained probably testify that noopept possesses dual activity both as a cognitive enhancer and an immune stimulator, but the mechanisms underlying the mnemotropic and immunotropic effects may be different and independent of each other.

In our experiments, the immune response to lysozyme amyloid, which has been selected as an AD non-specific marker of protein aggregation (Gruden et al., 2004), was not elevated either in OB or SO mice treated with noopept. Similar to Aβ(25-35) peptide, the amyloid sample of equine lysozyme contained only prefibrillar species including oligomers and protofilaments (Malisauskas et al., 2003). Amongst them, the transient oligomers of equine lysozyme shared the cytotoxic activity of Aβ peptides (Malisauskas et al., 2005). Thus, the immune reaction in both the AD and SO brain injury models was specific to Aβ(25-35) peptide prefibrillar species implicated in AD pathology but not to other amyloid aggregates.

We did not detect any significant changes in antibody level to S100b after noopept treatment either in OB or SO mice. However, S100b is associated with some features of AD where it is thought to play a role in neuritic pathology (Zimmer et al., 2005). Indeed, the brain level of S100b is augmented during AD development possibly via raised secretion or release from damaged astrocytes, even though this neurotrophic glial factor is rapidly utilized by neurons (Petzold et al., 2003; Rothermund et al., 2003). We suggest that the lack of immunoreactivity towards S100b in any of our operated animals may have arisen from an overall suppression of the immune system (Novoselova et al., 2004).

This study provides evidence that noopept restores spatial memory following bullectomy and that this action is accompanied by a positive immunomimetic effect with respect to Aβ peptide prefibrillar amyloid, known to play a causative role in the death of cholinergic neurons in AD development (Cacabelos et al., 2000). Current results on neurotropic and immunotropic activity of noopept together with its cholinimimetic activity (Ostrovskaya et al., 2002) and neuroprotective role against oxidative stress and Aβ peptide (Pealsman et al., 2003) suggest that the dipeptide may have different underlying molecular mechanisms of memory
improvement. The findings of this study endorse the idea that small antiapoptotic molecules of this nature hold promise for the causal treatment of neurodegenerative diseases (Waldmeier, 2003) and this includes AD-associated cognitive impairment.

Acknowledgements

We thank Elena Elistratova for technical assistance in conducting ELISA experiments. L.M.-R. was supported by the Swedish Medical Research Council. The research was supported by the Russian Foundation of Basis research (grants 03-04-49049 and 06-04-48325), the Russian Humanitarian Scientific Fund (grant 04-06-00165a), the Swedish Medical Research Council, the Royal Swedish Academy of Science and the Wellcome Trust (BRC grant 059823).

References


