Endocrine, liver-derived IGF-I is of importance for spatial learning and memory in old mice

J Svensson, M Diez¹, J Engel², C Wass², Å Tivesten, J-O Jansson, O Isaksson, T Archer³, T Hökfelt¹ and C Ohlsson

Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, Gröna Stråket 8, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden
¹Department of Neuroscience, Karolinska Institute, Stockholm, Sweden
²Department of Pharmacology, Göteborg University, Göteborg, Sweden
³Department of Psychology, Göteborg University, Göteborg, Sweden

(Requests for offprints should be addressed to J Svensson; Email: johan.svensson@medic.gu.se)

Abstract

IGF-I is a neuroprotective hormone, and neurodegenerative disorders, including Alzheimer’s disease, have been associated with decreased serum IGF-I concentration. In this study, IGF-I production was inactivated in the liver of adult mice (LI-IGF-I⁻/⁻), resulting in an approximately 80–85% reduction of circulating IGF-I concentrations. In young (6-month-old) mice there was no difference between the LI-IGF-I⁻/⁻ and the control mice in spatial learning and memory as measured using the Morris water maze test. In old (aged 15 and 18 months) LI-IGF-I⁻/⁻ mice, however, the acquisition of the spatial task was slower than in the controls. Furthermore, impaired spatial working as well as reference memory was observed in the old LI-IGF⁻/⁻ mice. Histochemical analyses revealed an increase in dynorphin and enkephalin immunoreactivities but decreased mRNA levels in the hippocampus of old LI-IGF-I⁻/⁻ mice. These mice also displayed astrocytosis and increased metabotropic glutamate receptor 7a-immunoreactivity. These neurochemical disturbances suggest synaptic dysfunction and early neurodegeneration in old LI-IGF-I⁻/⁻ mice. The decline in serum IGF-I with increasing age may therefore be important for the age-related decline in memory function.


Introduction

Alzheimer’s disease (AD) is the most common form of dementia and involves the parts of the brain that control thought, memory and language. It usually begins with a remarkable pure impairment of cognitive function, resulting in a loss of ability to encode new memories (Selkoe 2002). Early signs of AD include subtle alteration of hippocampal synaptic efficacy prior to neuronal degeneration and it is believed that the synaptic dysfunction might be caused by diffusible oligomeric assemblies of the amyloid-β (Aβ) peptide (Selkoe 2002).

Insulin-like growth factor-I (IGF-I) is a neuroprotective hormone. IGF-I protects neurones against the toxic effects of Aβ (Niikura et al. 2001), and circulating IGF-I increases the clearance of Aβ from the brain (Carro et al. 2002). Furthermore, circulating IGF-I can reach the brain (Reinhardt & Bondy 1994), and may mediate the beneficial effects of exercise on the function of the central nervous system (Carro et al. 2000, 2001, Trejo et al. 2001).


The major part of serum IGF-I is liver derived (Sjögren et al. 1999, Yakar et al. 1999). The serum levels of IGF-I are mainly regulated by growth hormone (GH) (Bengtsson et al. 1993), but are also affected by other factors, such as food intake, exercise and age (Landin-Wilhelmsen et al. 1994, Kaklamani et al. 1999, Ehrnhörn et al. 2003). It has been hypothesised that the age-dependent decrease in serum IGF-I levels is involved in the parallel age-dependent decrease in cognitive function (Dik et al. 2003, Trejo et al. 2004) as well as in the age-dependent increase in the incidence of AD (Amaducci & Tescio 1994, Busiguina et al. 2000). Patients with childhood-onset GH deficiency and low serum IGF-I concentration may have a cognitive disturbance (van Dam et al. 2005).

In experimental animals, intracerebroventricular administration of IGF ameliorates age-related behavioural deficits (Markowska et al. 1998), and systemic IGF-I...
administration can improve spatial memory (Sonntag et al. 2000, 2005). However, the specific, physiological role of liver-derived endocrine IGF-I for cognitive function in ageing is unknown.

The development of a mouse model with liver-specific, inducible inactivation of the IGF-I gene, using the Cre–LoxP conditional knockout system (LI-IGF-I−/− mice) (Sjögren et al. 1999, 2001, 2002, Wallenius et al. 2001, Tivesten et al. 2002), has made it possible to investigate the role of liver-derived IGF-I in adult mice. These mice do not have decreased IGF-I expression in the brain (Sjögren et al. 1999, Wallenius et al. 2001). Here we have assessed the role of adult expression of liver-derived IGF-I for spatial learning and memory, and have investigated to what extent this factor influences the expression patterns of a number of neuropeptides and other molecules associated with hippocampal function.

Materials and Methods

**Animals and serum IGF-I analysis**

The LI-IGF-I−/− mice were generated as previously described (Sjögren et al. 1999). Mice homozygous for LoxP (Liu et al. 1998) and heterozygous for Mx-Cre (Kuhn et al. 1995) were given polyinosinic-polycytidylic acid (PiPc; 6–25 µg/g body weight; Sigma-Aldrich Corp) in three i.p. injections at 6 weeks of age (mice aged 6 or 15 months at the water maze trials) or 10 weeks of age (mice aged 18 months at the water maze trials), to induce expression of the Cre protein in the liver (Kuhn et al. 1995). PiPc-treated littermates, homozygous for LoxP but lacking Mx-Cre, were used as controls. It is more practical to inactivate liver-derived IGF-I at 6 weeks of age when the mice are smaller and require a lower dose of PiPc. However, pre- and postnatal IGF-I expression is important for the development of the brain, and to definitely confirm that there was no interference with brain development, the LI-IGF-I−/− mice aged 18 months at the water maze trials were inactivated at 10 weeks of age.

Seven days after the first PiPc injection, blood was collected, and serum was assayed for IGF-I by a double-antibody IGF-binding protein-blocked RIA using a commercial kit (Mediagnost, Tubingen, Germany). In addition, in the 18-month-old mice, serum IGF-I (Mediagnost) was measured after the completion of the water maze tests. The animals had free access to fresh water and food pellets (B&K Universal AB, Sollentuna, Sweden). The study was approved by the ethical committees at the University of Göteborg and the Karolinska Institute, Stockholm, Sweden.

**Water maze**

Spatial learning and memory were tested in the water swim maze introduced by Morris (1981) and Morris et al. (1982). The apparatus and procedures for the determination of spatial learning and memory used in this and other studies (Sundström et al. 1988, Fredriksson et al. 2000, 2004, Archer et al. 2002) were similar, but not identical, to that originally described by Morris (1981) and Morris et al. (1982). All experiments were performed in a lighted room containing a number of two- and three-dimensional visual cues. Pool water was maintained at 24±1°C. The escape platform was submerged 1 cm below the surface of the water and hidden from sight by the use of dried milk powder. All extra maze cues were kept in a constant position through the test periods. The water maze experiments were performed, as described below, either with fixed or randomly changed release points (where the mice were let into the water).

**Water maze with fixed release point (working memory)** Mice were trained for 4 days (days 1–4) with five trials per day in a circular tank (95 cm in diameter; 30 cm water depth; escape platform 11×11 cm). The maximal swim time for each trial was 65 s followed by a 30-s rest on the platform. The mice were released from the same site during all trials on all days. One day after the last trial (day 5), additional trials were performed, with an unchanged release point but with a changed position of the escape platform. Except for the change in the position of the escape platform, the procedures during day 5 were similar to those during days 1–4. In our hands, the results during day 5, with changed position of the escape platform, have been a valid test for spatial working memory function (Sundström et al. 1988, Fredriksson et al. 2000, 2004, Archer et al. 2002).

**Water maze with random shifts in release point (reference memory)** Mice were trained for 5 days (days 1–5), with four trials per day, in a circular water tank (140 cm wide; 30 cm deep; 10 cm wide escape platform). The maximal swim time for each trial was 90 s followed by a 20-s rest on the platform. The four starting points were randomised for each day of training. Reference memory test (probe trial) was performed in the absence of the platform 1 day after the last trial (day 6). During the reference memory test, each animal was released from the position opposite to the target quadrant and allowed to swim for 60 s.

**Video equipment**

The latency to reach the platform, swim speed, distance and, for the reference memory tests, time spent in the different quadrants, were registered with a digital camera system connected to a computer (2020 Plus Tracking System; HVS Image, Bristol, UK).

**Immunohistochemistry**

For immunohistochemistry, brains from 22-month-old female LI-IGF-I−/− and control mice were used.
The mice that were killed were randomly selected from the mice aged 18 months at the water maze tests. Coronal sections (14 µm thick) of formalin–picric acid perfusion-fixed brains were cut at the level of the hippocampus, and the sections were processed for the tyramide signal amplification immunohistochemical method (Adams 1992) as described earlier (Diez et al. 2000). The sections were incubated with the following primary antisera/antibodies: (1) rabbit anti-enkephalin antiserum (1:4000) (Schultzb erg et al. 1979); (2) rabbit anti-dynorphin antiserum (1:4000) (Weber et al. 1982a,b); (3) rabbit anti-glial fibrillary acidic protein (anti-GFAP) (1:8000) (Sigma-Aldrich Corp); (4) rabbit anti-zinc transporter 3 (anti-ZnT3) (1:400) (Palmiter et al. 1996); (5) sheep anti-neuronal nitric oxide synthase (anti-nNOS) (1:20 000) (Herbison et al. 1996); (6) rabbit anti-metabotropic glutamate receptor 7a (anti-mGlutR7a) (1:4000) (Shigemoto et al. 1996); and (7) serotonin (1:8000) (Verhofstad et al. 1983).

**Results**

**Inactivation of liver-derived, endocrine IGF-I in adult mice**

To avoid interference with brain development, LI-IGF-I-/− mice were inactivated after sexual matura-
tion at 6–10 weeks of age. This complete and selective IGF-I inactivation in the liver of LI-IGF-I-/− mice was associated with a 78–85% reduction in circulating IGF-I (1 week after the inactivation serum IGF-I level was 61 ± 3 ng/ml in the LI-IGF-I-/− mice and 279 ± 14 ng/ml in the control mice aged 6 months at the water maze trials, P<0·001; 54 ± 3 ng/ml in the LI-IGF-I-/− mice and 267 ± 19 ng/ml in the control mice aged 15 months at the water maze trials, P<0·001; and 68 ± 3 ng/ml in the LI-IGF-I-/− mice and 439 ± 22 ng/ml in the control mice aged 18 months at the water maze trials, P<0·001). After the water maze trials were completed, serum IGF-I level was 33 ± 3 ng/ml in the 18-month-old LI-IGF-I-/− mice and 383 ± 48 ng/ml in the 18-month-old control mice, P<0·001. In the 6-month-old and the 15-month-old mice, serum IGF-I was only determined 1 week after the inactivation of liver-derived IGF-I. We did not consider it necessary to repeat the serum IGF-I measurements in these mice since we have found in several previous studies that the reduction in serum IGF-I level is sustained (Sjögren et al. 1999, 2001, 2002, Wallenius et al. 2001, Tivesten et al. 2002). In addition, we have unpublished measurements in other LI-IGF-I-/− mice aged 24 months, and the reduction in serum IGF-I level is still maintained in these old mice (data not shown).

**Behavioural analyses**

**Spatial working memory is decreased in old but not in young LI-IGF-I-/− mice** Spatial learning and working memory was first studied in 6-month-old LI-IGF-I-/− (n=20) and control (n=14) mice and in 18-month-old LI-IGF-I-/− (n=18) and control (n=20) mice using the Morris water maze swim test with a fixed release point. During the first 4 days, the escape platform had a fixed location in the water. The 6-month-old LI-IGF-I-/− and control mice had similar spatial memory learning during days 1–4 (two-way ANOVA for repeated measurements with group and time (days 1–4) as the independent variables, P=not significant; Fig. 1A). The acquisition curves in control mice (6-month-old and 18-month-old controls) demonstrated that spatial memory learning was slower in the old controls as compared with the young controls (Fig. 1A).

In the 18-month-old mice, a two-way ANOVA showed that the escape latency time differed between the two groups of old mice during days 1–4 (P<0·05; Fig. 1A). Post hoc analyses using the Student–Newman–Keul’s test showed that the 18-month-old LI-IGF-I-/− mice...
had longer escape latency time than the age-matched control mice at day 2 \((P<0.05)\), and also tended to have a longer latency time at day 3 \((P=0.08)\). At day 1 and day 4, there was no significant difference between the old LI-IGF-I\(^{-/-}\) mice and the controls in escape latency (Fig. 1A).

At day 5, with an unchanged release point, but after the location of the platform had been altered, the young (6-month-old) LI-IGF-I\(^{-/-}\) mice had similar latency to find the escape platform as the age-matched controls (Fig. 1B). In the 18-month-old mice, however, the latency to find the escape platform returned to baseline (=no training) values in the LI-IGF-I\(^{-/-}\) mice, whereas in the control mice the latency time was similar to that during day 4 of the experiment (Fig. 1B).

Analyses of swim speed showed similar swim speeds in the 6-month-old and 18-month-old LI-IGF-I\(^{-/-}\) mice as compared with the control mice of similar age during all the experiments using the water maze (Fig. 2).

**Decreased spatial reference memory in old LI-IGF-I\(^{-/-}\) mice** We next investigated the effect of circulating IGF-I on spatial learning and reference memory using the Morris water maze swim test with randomly changed release points. Fifteen-month-old LI-IGF-I\(^{-/-}\) \((n=9)\) and control \((n=9)\) mice were tested. A two-way ANOVA for repeated measurements, with group and time (days 1–5) as the independent variables, showed that the escape latency time differed between the two groups during the first 5 days \((P<0.05)\; \text{Fig. } 3\text{A}). Post hoc analyses using the Student–Newman–Keul’s test showed that the LI-IGF-I\(^{-/-}\) mice had longer escape latency time than the control mice at day 5 \((P<0.01)\). The probe tests for reference memory showed a lower \% of time spent in the trained quadrant in the LI-IGF-I\(^{-/-}\) mice \((P<0.05); \text{Fig. } 3\text{B}). Analyses of swim speed showed similar swim speed in the 15-month-old LI-IGF-I\(^{-/-}\) and control mice of a similar age during all the experiments using the water maze (data not shown).

**Histochemical analyses**

In order to characterise possible mechanisms underlying the impaired spatial learning and memory in old LI-IGF-I\(^{-/-}\) mice, the hippocampal expression of two neuropeptides, enkephalin and dynorphin, known to be...
affected in different AD mouse models, was analysed in 22-month-old animals. These analyses also included GFAP, ZnT3, serotonin, nNOS and mGlutR7a.

Increased number of glial fibrillary acidic protein-immunoreactive (IR) cells GFAP is a marker of astrocyte accumulation and gliosis. In control mice, weak GFAP-IR cells were scattered in all layers of the hippocampal formation (Fig. 4A). In LI-IGF-I−/− mice, GFAP-IR cells were increased in number and intensity compared with controls, and they were most abundant in strata oriens, radiatum, lacunosum moleculare and in the polymorph layer of dentate gyrus (Fig. 4B). No difference was seen for GFAP-IR cells in the cortex of the two groups (data not shown).

Enkephalin and dynorphin: increased peptide and decreased transcript levels Enkephalin-like immunoreactivity (LIR) was observed in a fairly dense network of fibres in the polymorph layer of the dentate gyrus and in the mossy fibres (Fig. 4C). A few weakly fluorescent fibres were also observed in strata oriens, molecular and lacunosum moleculare. In the LI-IGF-I−/− mice, varying degrees of increases of enkephalin-LIR were observed in the polymorph layer among individuals (Fig. 4D). In contrast, enkephalin mRNA levels were significantly decreased in the granule cell layer of the dentate gyrus of LI-IGF-I−/− mice as compared with controls (Table 1). However, no difference in transcript levels was seen in the CA1 region of the pyramidal cell layer (Table 1).

Dynorphin-LIR was mainly observed in fibres in the polymorph layer and in mossy fibres in the dentate gyrus (Fig. 4E). There was also a staining of processes in the visual/auditory cortex (Fig. 4G). In the LI-IGF-I−/− mice, dynorphin-LIR was increased in the polymorph layer of the dentate gyrus compared with controls (Fig. 4F). Dynorphin-positive processes and some dynorphin-positive neurones were observed in layer IV–V of the visual/auditory cortex of LI-IGF-I−/− mice (Fig. 4H). However, dynorphin mRNA levels were decreased in the granule cell layer (Table 1).

Unchanged ZnT3, serotonin and nNOS immuno-reactivities The expression of ZnT3 transporter was used to visualise changes (possibly sprouting) of the mossy fibre...
network in the dentate gyrus (Cole et al. 1999, Saito et al. 2000). In control mice, there was a dense network of ZnT3-IR fibres in the polymorph layer and in the mossy fibres of the dentate gyrus (Fig. 4I). In LI-IGF-I/p1/p1 mice, these fibres appeared similar to those in the controls (Fig. 4J), suggesting that sprouting of mossy fibres did not occur. Immunoreactivity for two markers for messenger molecules, serotonin and nNOS, was similar in the hippocampal region in LI-IGF-I/p1/p1 and control mice (data not shown).

Possible increase in mGlutR7a-IR

In control mice, there was a dense network of mGlutR7a-IR fibres in the polymorph layer and in the mossy fibres of dentate gyrus (Fig. 4K). In LI-IGF-I−/− mice, there was a tendency to increased mGlutR7a expression in the fibres of the polymorph layer (Fig. 4L), even though this increase varied among individuals.

Discussion

The present study has demonstrated that liver-derived, endocrine IGF-I is of importance for cognitive function, as old LI-IGF-I−/− mice display impaired spatial learning and memory. This is perhaps related to the observed disturbances in the expression of hippocampal neuropeptides, consistent with synaptic dysfunction and early neurodegeneration. The increased astrocytosis also supports this hypothesis.

Lifelong overexpression of IGF-I increases whereas inactivation of IGF-I or its receptor decreases brain size and the number of neurones (Carson et al. 1993, Dentremont et al. 1999, O’Kusky et al. 2000, Holzenberger et al. 2001, Vicario-Abejon et al. 2003). In humans, defects of IGF-I or IGF-I receptors due to gene mutations is associated with some mental retardation (Woods et al. 1996, Abuzzahab et al. 2003). Previous studies have shown that IGF-I is of importance for pre- and postnatal development of the brain, but they could not evaluate the role of IGF-I in adult animals because of the affected IGF-I activity during development. The LI-IGF-I−/− mice do not have decreased IGF-I expression in the brain (Sjögren et al. 1999, Wallenius et al. 2001) whereas the inactivation of liver-derived IGF-I at 6–10 weeks of age resulted in a marked reduction in serum IGF-I which was still maintained at 18 months of age. We can therefore exclude the possibility that the observed alterations were due to developmental changes in the brain.

The spatial acquisition process was slower in the old but not in the young LI-IGF-I−/− mice as compared with controls. Furthermore, in the 18-month-old LI-IGF-I−/− mice spatial working memory was impaired as the escape latency time in the LI-IGF-I−/− mice was longer than in the controls at day 5, after the position of the
Figure 4 Immunofluorescence micrographs of the dentate gyrus (A–F and I–L) and visual/auditory cortex (G and H) of 22-month-old Li-IGF-I−/− (B, D, F, H, J and L) and control mice (A, C, E, G, I and K) after incubation with antisera to GFAP (A and B), enkephalin (enkephalin-LR; C and D), dynorphin (dynorphin-LR; E–H), ZnT3 (I and J) or glutamate receptor 7a (GlutR7a-IR; K and L) antiserum. Further details of the analyses are given in Results. PoDG, polymorph layer of dentate gyrus; GrDG, granular layer of dentate gyrus; Mol, molecular layer of the dentate gyrus; wt, wild type; KO, knockout.
escape platform had been moved between days 4 and 5. The acquisition curve during days 1–4 (fixed release point) was not optimal in the 18-month-old control mice since, at day 4, the mean escape latency time was still over 30 s in the controls. The valid acquisition curve in the 6-month-old controls showed, however, that the sub-optimal acquisition curve in the old control mice was due to the high age of the animals. Furthermore, the additional water maze tests in 15-month-old mice, which included randomised release points, demonstrated that spatial reference memory was also decreased in old LI-IGF-I<sup>−/−</sup> mice.

As discussed above, the 15- and 18-month-old but not the 6-month-old LI-IGF-I<sup>−/−</sup> mice had reduced spatial learning and memory. This suggests that deficiency of serum IGF-I results in decreased cognitive function in old but not in young mice. The further analyses showed similar swim speeds for old LI-IGF-I<sup>−/−</sup> and control mice, suggesting that the between-group differences in latency time in the old mice were not due to confounding factors such as musculo-skeletal alterations.

The hippocampal formation is of importance for performance in the water maze test, especially when the release point is randomly changed (Morris et al. 1982, O’Hooge & De Deyn 2001). We observed increased inhibitory e-aminobutyric acid (GABA) interneurones (Segal 1977, Lee et al. 1980), thus causing disinhibition. Increase in enkephalin peptide together with decreased transcript levels in the granule cell–mossy fibre system could therefore lead to attenuated facilitatory mechanisms in the LI-IGF-I<sup>−/−</sup> mice. Taken together, the accumulation of dynorphin and enkephalin in mossy nerve endings combined with decreased mRNA levels suggest synaptic dysfunction in the hippocampus of the LI-IGF-I<sup>−/−</sup> mice. Increased dynorphin- and enkephalin-LIR has previously been observed in the mossy fibres of two AD mouse models (Diez et al. 2000, 2003), suggesting that intracellular accumulation of these peptides might be a common phenomenon in neurodegeneration.

The number and intensity of GFAP-IR cells were increased in 22-month-old LI-IGF-I<sup>−/−</sup> mice. This finding is in line with the results of a previous study (Carro et al. 2002), and suggests astrogliosis and neurodegeneration (Griffin et al. 1989, Unger 1998). Furthermore, mGlutR7a-IR was increased in the fibres of the polymorph layer of hippocampus in the LI-IGF-I<sup>−/−</sup> mice, indicating an imbalance in glutamatergic neurotransmission which is a feature described in AD and possibly involved in neurodegeneration (Selkoe 2002, Bleich et al. 2003).

Several lines of evidence indicate that synaptic dysfunction in AD is caused by diffusible oligomeric assemblies of the Aβ protein (Selkoe 2002). Circulating IGF-I, at the level of the blood–brain barrier, regulates brain Aβ levels by enhancing transport of Aβ carrier proteins such as albumin and transthyretin into the brain (Carro et al. 2002). However, it is also clear that IGF-I crosses the blood–brain barrier (Reinhardt & Bondy 1994), that the IGF-I receptor is expressed in the brain (Bondy et al. 1992, Kar et al. 1997, D’Ercole & O’Kusky 2002) and that IGF-I has the direct capacity to modulate synaptic efficacy in several in vitro studies (Nilsson et al. 1988, Araujo et al. 1989, Kar et al. 1997). There are therefore several possibilities as to how deficiency of liver-derived, endocrine IGF-I impairs spatial memory learning and synaptic function in old mice. At the current stage of knowledge, it is difficult to establish which mechanism is of most importance.

### Table 1

<table>
<thead>
<tr>
<th>Oligonucleotide probe</th>
<th>Area</th>
<th>Control (n=4)</th>
<th>LI-IGF-I&lt;sup&gt;−/−&lt;/sup&gt; (n=4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENK</td>
<td>DG</td>
<td>91.4 ± 1.3</td>
<td>86.8 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Py CA1</td>
<td>89.7 ± 4.4</td>
<td>89.7 ± 2.3</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>DYN</td>
<td>DG</td>
<td>85.7 ± 2.7</td>
<td>80.0 ± 3.3</td>
<td>0.02</td>
</tr>
</tbody>
</table>

All values are presented as the means ± s.d. DG, dentate gyrus; Py CA1, CA1 pyramidal cell layer.
In conclusion, old mice with liver-specific inactivation of IGF-I in young adult life display impaired spatial learning and memory associated with altered expression of hippocampal neuropeptides and some other markers consistent with synaptic dysfunction and with early signs of neurodegeneration. This observation provides further evidence that the decline in serum IGF-I with increasing age may be important for the age-related decline in memory function. The exact mechanism by which liver-derived endocrine IGF-I affects spatial learning and memory and synaptic function remains to be established however. Further studies are needed to evaluate the extent to which liver-derived endocrine IGF-I is involved in the pathogenesis of AD.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council, the Swedish Foundation for Strategic Research, the Lundberg Foundation, the Torsten and Ragnar Söderberg’s Foundation, the Emil and Vera Cornell Foundation, Petrus and Augusta Hedlund’s Foundation, the Novo Nordisk Foundation, the Marianne and Marcus Wallenberg Foundation, and an Unrestricted Bristol-Myers Squibb Neuroscience Grant. The authors are grateful to Professor Sven Ove Ögren and Eugenia Kuteeva for valuable advice concerning experiments with the Morris swim maze. They are also grateful to Maud Petersson, Anna-Lena Jirestedt and Anette Hansevi for excellent technical assistance. They would also like to thank SWEGENE Center for Bio-Imaging (CBI), University of Göteborg, for technical support. For their generous supply of antibodies the authors Drs L Terenius, Karolinska Institute, Stockholm (enkephalin), E Weber, CoCensys, Irvine, CA, USA (dynorphin), R Palmitier, University of Washington, Seattle, WA, USA (ZnT3), P Emson, University of Cambridge, Babraham, UK (NOS) and R. Shigemoto, National Institute for Physiological Sciences, Graduate University for Advanced Studies, Okazaki, Japan (mGlutR7a).

Conflicts of interest

O I is a co-founder of Tercica Inc, a company that produces IGF-I for use in children of short stature. No other author has any conflict of interest that would prejudice the impartiality of this scientific work.

References


Carro E, Trejo J, Busiguina S & Torres-Aleman I 2001 Circulating insulin-like growth factor-I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. Journal of Neuroscience 21 5678–5684.


Morris R 1981 Spatial localization does not require the presence of local cues. Learning and Motivation 12 239–260.


Received in final form 23 February 2006
Accepted 9 March 2006
Made available online as an Accepted Preprint 13 March 2006