Insulin-like growth factor I protects and rescues hippocampal neurons against β-amyloid- and human amylin-induced toxicity

(Sylvain Dore, Satyabrata Kar, and Remi Quirion)

Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, PQ Canada, H4H 1R3

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ABSTRACT Insulin-like growth factors (IGF-I and IGF-II) are well known trophic factors and their specific receptors are uniquely distributed throughout the brain, being especially concentrated in the hippocampal formation. IGFs possess neurotrophic activities in the hippocampus, an area severely affected in Alzheimer disease. These data, together with the evidence that β-amyloid (Aβ)-derived peptides likely play an important role in the neurodegenerative process observed in Alzheimer disease, lead us to investigate if IGFs could be neuroprotective to hippocampal neurons against toxicity induced by amyloidogenic derivatives. Exposure of rat primary hippocampal neurons to different concentrations of Aβ25–35, Aβ1–40, Aβ1–42, and human amylin produced marked toxicity, while similar concentrations of two control Aβ peptides—reverse (Aβ40–1) and scrambled sequence (Aβ25–35)—and rat amylin failed to exhibit any significant effect on neuronal survival. IGF-I (10–100 nM) significantly protected hippocampal neurons against neurotoxicity induced by Aβ derivatives and human amylin. The homolog IGF-II was also effective although less potent than IGF-I suggesting the involvement of a typical IGF-I receptor in the observed neuroprotective effect. Most interestingly, IGF-I (10–100 nM) was even able to rescue neurons pre-exposed (up to 4 days) to amyloidogenic peptides. Other neurotrophic factors are reported to lack such rescuing abilities. These results suggest that IGF-I may have unique properties as a potent neuroprotective and neurorescuing agent against amyloid-related neurotoxicity.

β-Amyloid (Aβ1–40, Aβ1–42) is believed to play a role in the neurodegenerative process occurring in Alzheimer disease (AD) (1, 2). This protein is found deposited in extracellular neuritic plaques, one of the hallmark of the AD brain along with the presence of neurofibrillary tangles and cell losses in various regions, including the basal forebrain (3). Aβ is derived proteolytically from a larger transmembrane protein, the amyloid protein precursor, which is expressed widely throughout the brain (4, 5). The direct, toxic properties of Aβ-related fragments (Aβ1–40, Aβ1–42, Aβ25–35) in cultured rat and human neurons and in vivo are well established (6–8), although the mechanism of action involved remains to be established.

Insulin-like growth factors (IGF-I and IGF-II) play an important role in the normal development and maintenance of the cellular integrity of the organism, including the central nervous system (9). Both trophic factors are selectively localized in the brain and their specific receptors are uniquely distributed in various neuroanatomical regions, being especially concentrated in the hippocampal formation (10). The IGF-I receptor is composed of two α chains where the ligand binds and two β chains possessing a tyrosine kinase domain (11). In contrast, the IGF-II receptor is made of a single transmembrane segment containing a binding site for IGF-II and another for mannose-6-phosphate residues. Both receptors bind specifically to their cognate ligands but can also recognize the other with lower affinity. We have recently shown that cultured hippocampal neurons are highly enriched with IGF-I and IGF-II receptors each being differentially internalized (12), and serving distinct functions (13). Earlier studies have shown that IGFs possess neurotrophic activities in the hippocampus (14–16), an area severely affected in AD. Interestingly, it was also observed that the levels of IGF-I binding sites are significantly increased in cortical areas of AD brains (17). It is unclear if these increases in IGF-I receptors represent a protective and/or compensatory mechanism against neuronal losses.

Considering the broad actions of IGFs on the maintenance of normal cellular functions and the presence of high levels of IGF receptors in the hippocampus, we investigated the potential neuroprotective effects of IGFs against Aβ-induced toxicity in rat hippocampal neurons. Human amylin, which also has amyloidogenic properties (18), was also studied for comparison. Our results show that IGF-I is able not only to protect neurons against Aβ-induced toxicity but even to rescue them up to a few days following exposure to Aβ derivatives and human amylin. Preliminary results were presented in abstract form (58).

MATERIALS AND METHODS

Materials. Different fragments of Aβ peptides including Aβ25–35, lot no. ZM501; Aβ1–28, lot no. ZK792; Aβ1–40, lot no. ZM365; Aβ40–1, lot no. ZL511; Aβ1–42, lot no. ZN052 (Figs. 1–3) and Aβ1–42, lot no. ZM823 (Figs. 4–6) were purchased from Bachem. The scrambled sequence of Aβ25–35 was generously provided by P. Gaudreau (Notre-Dame Hospital Research Center, Montreal). Rat and human amylin were bought from Peninsula Laboratories. Recombinant human (rh)IGF-I was obtained from Genentech while hIGF-II was bought from Lilly Research Laboratories (Indianapolis). Materials used for cell culture were obtained from Gibco/BRL. Unless stated otherwise, all other chemicals were purchased from Sigma.

Hippocampal Neuron Cultures and Experimental Treatments. Hippocampal neuronal cells, as described earlier (12), were prepared from fetuses (embryonic day 19) obtained from time-pregnant Sprague–Dawley rats (Charles River Breeding Laboratories). Animal care was according to protocols and guidelines approved by the McGill University Animal Care Committee and the Canadian Council for Animal Care.

Following dissection, hippocampal cells were plated at high density (7.5 × 10⁵ cells per well) in 16-mm tissue culture dishes coated with poly-d-lysine (10 μg/ml) under serum-free con-
Neurobiology: Doré et al.

Neuroprotective Effects of IGF-I and IGF-II Against Aβ-Related Peptides. Rat primary hippocampal neurons were treated for 6 days with different concentrations of Aβ1–28, Aβ1–40, the control reversed sequence Aβ40–1, Aβ1–45 (Upper) and Aβ25–35, and its control scrambled sequence (Aβ25–35 scrambled) (Lower). *P < 0.01 as control treatments.

Neurorescuing Effects of IGF-I Against Aβ-Induced Toxicity. As shown in Fig. 2, increasing concentrations of IGF-I and IGF-II are able to protect hippocampal neurons against Aβ25–35-induced toxicity (30 μM Aβ25–35; 44% of control values). IGF-I is found to be more potent than IGF-II (Fig. 2). When treated alone with either IGF-I or IGF-II, the survival of neurons is not found to be significantly affected (Fig. 2, top lines).

Neurorescuing Properties of IGF-I Against Aβ-Induced Toxicity. Fig. 3 shows that IGF-I at 100 nM is able to significantly protect neurons against toxicity induced by 30 μM Aβ25–35 even if added up to 3–5 days post-Aβ treatments. Neurons incubated with Aβ alone were highly affected with MTT values down to 34% of control. When the cells were incubated with IGF-I immediately or up to 2 days after Aβ exposure, IGF-I rescued neurons with MTT values being significantly increased between 68–72% of controls. At subsequent days (3–5), the rescuing effect was still significant but less evident indicating that neurons are too affected to fully benefit from IGF-I rescuing properties. At the same concentration, IGF-II had a slight protective effect when incubated simultaneously with Aβ but failed to demonstrate any rescuing abilities (Fig. 3).

Morphological Features of Neurons Exposed to Aβ1–42 and/or IGF-I. Fig. 4 summarizes the morphological features of exposure to IGF-I, Aβ, and their combination (simultaneously or post-Aβ treatment). Aβ1–42, at a concentration of 5 μM, induced degeneration with shrinkage of cell soma, neuronal clustering, and debris. IGF-I added simultaneously at 10, 30, and 100 nM was clearly neuroprotective (Fig. 4, second row). Moreover, IGF-I (30 nM) was able to rescue neurons against Aβ1–42 toxicity even when added 1, 2, or 4 days later (Fig. 4, bottom row).
Neuroprotective and Neurorescuing Effects of IGF-I Against Amylin-Induced Toxicity. Human amylin was highly toxic to cultured hippocampal neurons as shown by decreased concentration-dependent MTT values (Fig. 5). In contrast, rat amylin was unable to induce toxicity, in accordance with an earlier report (18). Fig. 6 Upper shows that increasing concentrations of IGF-I were able to protect neurons against toxicity induced by 30 μM human amylin. Neurons incubated with human amylin alone (35 μM) were highly affected with MTT values down to 26% of control (Fig. 6 Lower). IGF-I (100 nM) was able to significantly rescue neurons for up to 4 days after treatment with human amylin, with important rescuing abilities observed after 2 days. At subsequent days, the rescuing ability of IGF-I was still significant but less evident (Fig. 6).

DISCUSSION

The major finding of the present study relates to the neuroprotective and neurorescuing properties of IGF-I against Aβ-induced toxicity. While various neurotrophins and neurotrophic factors (24) have been shown to block the toxic effect of Aβ derivatives in vitro and in vivo, the rescuing action of IGF-I is, to our knowledge, unique. Indeed, we observed that the incubation of IGF-I was able to rescue primary rat hippocampal neurons pre-exposed to Aβ for up to 4–5 days. This is particularly interesting in the clinical context and suggests that the mechanism of action of Aβ-induced toxicity involved a rather long process allowing for various pharmacological interventions such as the use of IGF-I, its mimetics, or molecules activating the IGF-I receptor signaling pathway.

It is now rather well established that various Aβ derivatives, especially in their aggregated forms, are toxic to many cell types, including neurons both in vitro and in vivo (8, 25, 26). We confirmed and extended these findings in the present study. The main amyloidogenic components of the neuritic plaques are the Aβ1–42 and Aβ1–40 fragments (1, 27). Aβ1–40, Aβ1–42 fragments and the Aβ25–35 peptide (which contains the active toxic domain) are highly toxic to rat primary hippocampal neurons as exemplified by the reduction in MTT values and the altered morphological features of the culture. In contrast, the nonamyloidogenic fragment Aβ1–28 and the controls (including the random sequence of the Aβ25–35 and the reverse sequence peptide, Aβ40–1) did not affect neuronal survival. These results confirm that the neurotoxic properties of Aβ derivatives are highly sequence-dependent.
dependent and possibly related to their amphiphilic nature. This hypothesis is supported further by the fact human, but not rat, amylin was found to be neurotoxic in various models (18,28), including ours. Human amylin, which is present in the brain (28), is a highly amphiphilic peptide that has a high tendency to aggregate in contrast to rat amylin that is nontoxic (29).

The initial event leading to Aβ-induced toxicity is unknown at present but may involve plasma membrane receptors for advanced glycation end products (RAGE; ref. 30), class A scavenger receptor-related proteins (31), certain O-proteins (32), heparan sulfate (33, 34), and r2-macroglobulin extracellular domains (35). Whether IGF-I can directly interact with these various receptor sites to block Aβ-induced toxicity remains to be established. Moreover, we cannot rule out the possibility that IGF-I could interfere with the aggregation of Aβ or human amylin. Preliminary experiments, however, failed to provide evidence for differential fibril formation in the presence (Aβ25-35; 30 μM plus IGF-I, 100 nM) or absence (Aβ alone) of IGF-I (unpublished results).

We observed that both IGF-I and IGF-II were able to protect rat hippocampal neurons against Aβ-induced neurotoxicity. However, IGF-I was clearly more potent than IGF-II. Additionally, IGF-II, at the concentration tested (100 nM), was unable to rescue neurons previously exposed to Aβ25-35. Taken together, these results suggest the involvement of the IGF-I receptor subtype known to be activated by both IGF-I and IGF-II, the latter with markedly lower potency.

Other trophic factors have been reported to protect neurons against various types of insults. For example, nerve growth factor (NGF) (36), basic fibroblast growth factor (bFGF) (37), and transforming growth factors (TNF) α and β (38) have been shown to protect cultured hippocampal neurons against Aβ-induced toxicity. However, none of these trophic factors were effective post-Aβ-treatment hence lacking neurorescuing properties. Interestingly, TGFβ1 and TGFβ2, but not TGFβ3, were apparently able to have a slight protective effect in neurons pre-exposed to Aβ25-35 for 24 hr while such effect was not observed with TNF, NGF, aFGF, or bFGF (39). The rescuing ability of TGFβ1 and TGFβ2 was not as pronounced as the one observed with IGF-I and was not as effective against longer exposures to Aβ25-35. Moreover, preliminary results with TGFβ (100 nM) and NGF (10 to 1,000 nM) failed to reveal any rescuing abilities of these two factors against toxicity induced by Aβ25-35 in our model (unpublished results).
together, it appears that the neuroprotective/neurorescuing abilities of IGF-I against Aβ- and human amylin-induced toxicities observed in the present study are rather unique.

The mechanism of action involves in neuroprotective and especially neurorescuing properties of IGF-I against Aβ-induced toxicity remains to be clarified. The activation of the IGF-I tyrosine kinase receptor induces protein phosphorylation followed by a cascade of intracellular events that include the activation of insulin receptor substrates 1 and 2, and phosphoinositol 3-kinase, phosphotyrosine phosphatases, S6 kinase, Ras-mitogen-activating protein kinase and transcription factors leading to alterations in Ca²⁺ storage and mobilization, and mitochondrial respiration (40, 41). It has been proposed that Aβ-induced toxicity is due to free radicals production/oxidative stress (2, 42) and/or increased free intracellular Ca²⁺ levels (43) leading to necrosis and cell death (21). The toxic properties of Aβ-derivatives could also relate to their abilities to stimulate apoptotic genes/cellular events (7, 44, 45). Accordingly, IGF-I could interfere at different stages of the necrotic or apoptotic pathway to block and even rescue neurons against Aβ-induced cell death. Interestingly, IGF-I has already been shown to block programmed cell death in various models. For example, IGF-I and the IGF-I receptor prevent topoisomerase I inhibitor etoposide-induced apoptosis in 3T3 cells (46), inhibit interleukin-3-dependent cell death in various cell lines (47), inhibit apoptosis induced by either TNFα, radiation and dysregulated c-myc expression (48) and protect differentiated PC12 cells against cell death following NGF withdrawal (49). IGF-I has also been shown to prevent apoptosis associated with K⁺-deprivation in cerebellar granule neurons, other factors tested including aFGF, bFGF, platelet-derived growth factor, and neurotrophin 3 being inactive (49). Similar protective effects were observed in a hybrid dopaminergic cell line against oxidation and hypoglycemia-induced cell death, IGF-I being more potent than bFGF, epidermal growth factor, and NGF (50). More recently, it was demonstrated that IGF-I (and supraphysiologic concentrations of insulin) activates a phosphoinositol 3-kinase and the serine-threonine protein kinase Akt to promote the survival of rat primary cerebellar neurons after induction of apoptosis by serum deprivation (51). It would be of interest to investigate if this pathway is also involved in the neuroprotective/neurorescuing properties of IGF-I observed in the present study. In vivo, IGF-I has been shown to reduce neuronal cell losses observed following hypoxic-ischemic insults (15, 52) and is beneficial in the treatment of amyotrophic lateral sclerosis patients (53). Hence, IGF-I by acting on necrotic and/or apoptotic cellular events could protect and more importantly rescue neurons against Aβ- and human amylin-induced toxicity.

It is well established that insulin-like family members are critically involved in maintenance of body homeostasis. Aging is associated with changes in basic functions among which glucose metabolism is central to the nervous system. In aged rats and
humans, poor cognitive performances have been correlated with impaired glucose regulation (54). In sporadic AD cases, significant reductions in glucose utilization have been reported (55) and neuroglucopenia reduced the formation rate of ATP and acetate from 54% to 47% of control values, respectively (55). Moreover, IGF-I receptor binding levels are apparently increased in affected areas of the AD brain (17), may be as an attempt to counteract energy metabolism deficits and cell losses. Interestingly, IGF-I is also known to be neuroprotective against toxicity induced by glucose deprivation (14, 50, 56) and it has been shown that exposure to subthreshold doses of Aβ derivatives render neurons more susceptible to glucose deprivation (57). The unique capacity of IGF-I to protect and rescue neurons against Aβ-induced toxicity, in parallel to its well-known involvement in various metabolic pathways, suggest that the development of IGF-I-related mimetics could be a promising strategy toward the treatment of various neurodegenerative diseases including AD.

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