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IGF-1 exacerbates the neurotoxicity of the mitochondrial inhibitor 3NP in rats

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Abstract

Insulin-like Growth Factor 1 (IGF-1) has broad-range neuroprotective effects and is a therapeutic candidate for Huntington's disease (HD). IGF-1 protects striatal neurons from the toxicity of mutated huntingtin *in vitro* and improves neuronal survival *in vivo* in a phenotypic model of HD involving excitotoxic cell death. Because HD is a multifactorial disease, it is important to evaluate the neuroprotective role of IGF-1 in other pathological situations involved in HD progression. We have evaluated the neuroprotective effects of IGF-1 *in vivo*, using the 3-nitropropionic acid (3NP) rat model which replicates the mitochondrial dysfunction observed in HD. Continuous intracerebroventricular infusion of recombinant IGF-1 at a low dose (0.025 μg/h for 5 days) did not alleviate motor impairment and weight loss induced by 3NP treatment. In addition, histological evaluation and quantification of DNA fragmentation evidenced no improvement in neuronal survival. Of interest, we found that a higher concentration of IGF-1 (0.25 μg/h) resulted in an exacerbation of 3NP toxicity on striatal neurons. These results suggest that intracerebral delivery of IGF-1 may not provide a fully effective therapeutic strategy for HD or other disorders involving mitochondrial impairment.
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Huntington's disease (HD) is an inherited neurodegenerative disease characterized by the preferential death of striatal neurons. HD patients exhibit progressive motor, psychiatric and cognitive abnormalities, and usually die within 10–20 years. The mutation responsible for HD was identified more than 10 years ago as an expanded polyglutamine tract in the N-terminal part of a protein named huntingtin (Htt) [27]. In spite of this well-defined molecular alteration, HD appears as a highly complex and multifactorial disease [17]. Many factors have been implicated in the degeneration of striatal neurons in HD including excitotoxicity, oxidative stress, protein misfolding as well as alterations in Ca^{2+} homeostasis, transcription, intracellular signaling, axonal transport and synaptic transmission [17]. Impairment in energy metabolism and mitochondrial defects also seems to play a central role in HD pathogenesis [7].

Metabolic alterations in HD are evidenced by the fact that patients are cachexic and exhibit striatal hypometabolism as well as increased lactate levels before marked atrophy of the striatum [6]. Recent studies in patients and genetic models of HD have evidenced several mitochondrial anomalies involved in disease progression [1,4,25]. These include preferential defects in mitochondrial complex II (succinate dehydrogenase, SDH) [4,25], which is consistent with the observation that intoxication of rodents and primates with 3-nitropropionic acid (3NP), an irreversible inhibitor of SDH, reproduces several histopathological and clinical features of HD [7].

There is currently no effective treatment for HD despite intense efforts to develop alternative therapeutic strategies. The 3NP models do not reproduce the genetic mutation responsible for HD. However, unlike transgenic models of HD, they involve a marked degeneration of the striatum that makes them valuable tools to evaluate neuroprotective strategies [7]. So far, these models have allowed for the assessment of several neuroprotective approaches such as inhibition of glutamate signaling [24], supplementation with energetic substrates [22],

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inhibition of proteases [5], and gene transfer of neurotrophic factors [23].

Along these lines, insulin-like growth factor 1 (IGF-1) is an interesting neuroprotective candidate for HD. IGF-1 reduces the toxicity of mutated Htt towards striatal neurons by inducing its phosphorylation [16]. In addition, we have previously shown that an intracerebroventricular (i.c.v.) infusion of recombinant IGF-1 has neuroprotective effects against excitoxicity using the quinolinate rat model of HD [12]. Here, we aimed to further evaluate neuroprotective effects of IGF-1 in the 3NP rat model of HD.

Three-month-old male Lewis rats (weighing ~400 g, IFFA Credo, France) were used. For surgical procedure, animals were anesthetized with a mixture of ketamine (15 mg/kg) and xylazine (1.5 mg/kg). All experimental procedures were carried out in compliance with the recommendations of the EEC (86/609/EEC) and the guidelines of the French National Committee (87/848) for the care and use of laboratory animals. All chemicals were purchased from Sigma (Saint-Louis, MO) unless otherwise specified.

On day 0, rats were simultaneously implanted in the left lateral ventricle with a canula connected to a minipump filled with either IGF-1 at 0.025 μg/μl ($n = 10$), IGF-1 at 0.25 μg/μl ($n = 10$) or vehicle ($n = 10$) and with a subcutaneous pump filled with 3NP. Additional controls included sham-operated animals ($n = 9$).

On days 4 and 5, rats were evaluated using a clinical index as described previously (normal = 0, very symptomatic = 8) [23]. On day 5, animals were killed by decapitation, and the brain was rapidly removed from the skull. The left hemisphere was frozen in isopentane, and used for histochemistry to assess the activities of cytochrome oxidase (COX) and succinate dehydrogenase (SDH). The striatum and somatosensory cortex were dissected out from the right hemisphere in five animals per group for the purpose of biochemical analysis (quantification of IGF-1 and free oligonucleosomes levels).

Lyophilized recombinant human IGF-1 (R&D systems Inc., Minneapolis, MN) was dissolved to a final concentration of 0.25 and 0.025 μg/μl in a vehicle composed of 10 mM acetic acid with 0.1% bovine serum albumin in 0.1 M phosphate buffer saline (PBS). Continuous i.c.v. delivery of IGF-1 was made via an osmotic minipump (1 μl/h, 2001 model, Alzet, Palo Alto, CA) connected to a canula (stereotaxic coordinates: Anteroposterior, −0.7 mm; lateral, 1.5 mm left from bregma; ventral, 3.6 mm from dura with tooth bar set at −3.3 mm; "brain infusion kit", Alzet) which was secured to the skull bone with dental cement as previously described [5]. Previous experiments have shown that IGF-1 degradation in the minipump was minimal, at least during the first 2 days after implantation [12].

3NP (Fluka, Saint-Louis, MO) was prepared as previously described and systemically administered via subcutaneous osmotic pumps (2ML1 model, Alzet) delivering 56 mg/kg/day for 5 days [5]. Control rats (no 3NP treatment) were similarly anesthetized and subject to identical surgical procedures. One rat of the group receiving the highest dose of IGF-1 was found dead on day 5 and was excluded for the calculation of the behavioral score at day 4.

The lateral striatum and the somatosensory cortex were rapidly dissected out from acutely prepared coronal sections (2 mm thick) prepared with a steel rat-brain matrix. Tissue samples were homogenized using a 1 ml glass–Teflon-homogenizer (900 rpm, 20 strokes) in 300 μl of buffer (25 mM HEPES pH 7.6, 0.1% Triton X-100, 5 mM $MgCl_2$, 1.3 mM EDTA, 1 mM EGTA, with protease inhibitor cocktail "complete", Roche, Indianapolis, IN). Homogenates were pooled by experimental group and centrifuged at 10,000 × g for 30 min at 4 °C. The supernatant was collected and stored at −80 °C until analysis.

Striatal and cortical supernatants were used for the quantification of IGF-1 concentrations using the Quantikine Human IGF-1 colorimetric Sandwich ELISA kit (R&D) as described previously [12]. Quantification was made in duplicate for each group and each cerebral region.

Striatal supernatants were used for the quantification of free oligonucleosomes using the Cell Death detection ELISA plus kit (Roche) [5]. The measure was made in triplicate and was normalized to the protein content of each sample as measured by the microBCA kit (Pierce, Rockford, IL).

The left frozen hemispheres were cut serially into 40 μm-thick sections using a cryostat and stored at –20 °C before histochemistry. For each animal, 10–12 serial sections encompassing the striatum were examined (interspace 400 μm). SDH and COX histochemistry were performed as previously described, to quantify the regional V_{max} of these enzymes [5,14]. Non-specific staining was evaluated by incubating adjacent sections without substrate (succinate and cytochrome c, respectively). To precisely evaluate the loss of striatal COX activity due to striatal neurodegeneration, striatal COX activity was normalized to COX activity in the cortex, because cortical cells are generally unaffected by 3NP treatment.

Sections labeled for COX histochemistry were also used to evaluate the volume of striatal lesions. Lesioned areas were measured by delineating the external border of the lesion seen as a pale staining on digitized images. From these areas, the volume of the striatal lesion was determined using the Cavalieri method as previously described [12].

The blood was collected from decapitated animals and the plasma was recovered by a 3 min centrifugation at 3000 rpm. Samples were stored at −80 °C until analysis. Plasma glucose concentrations were measured using the glucose oxidase method (Beckman Coulter, Fullerton, CA, USA).

Results are expressed as mean values ± S.E.M. Statistical analysis included one-way analysis of variance (ANOVA) followed by a *post-hoc* Scheffé's test. Motor scores were compared using non-parametric tests: Kruskall–Wallis followed by a U Mann–Whitney test. The level of significance was set at $p < 0.05$.

On day 5 of 3NP treatment, IGF-1 concentration was quantified in striatal and cortical homogenates. 3NP treatment associated with an i.c.v. infusion of the vehicle did not alter IGF-1 basal concentrations ($p > 0.6$ between Control and vehicle groups). Chronic infusion of IGF-1 at a rate of 0.025 and 0.25 μg/h in the lateral ventricle increased IGF-1 striatal concentration by 30% and 144%, respectively (Table 1). A significant increase in IGF-1 concentration was also detected in the

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