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Research report

Peripheral insulin-like growth factor-I produces antidepressant-like behavior and contributes to the effect of exercise

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ABSTRACT

Growth factors in the brain are important to depression and it's treatment and we assessed the ability of peripherally administered insulin-like growth factor-I (IGF-I) to influence behavior related to depression. We found that mice that received chronic IGF-I treatment showed antidepressant-like behavior in forced-swim and novelty-induced hypophagia (NIH) tests and increased sucrose consumption after chronic mild unpredictable stress exposure. Additionally, peripheral anti-IGF-I administration blocked exercise-induced antidepressant effects in the forced-swim test (FST). These results support the functional relevance of neurotrophic mechanisms to depression and extend this idea to include neurotrophic factors in the periphery.

1. Introduction

Neurotrophic factors were originally identified for their roles in growth and development of the nervous system, but are now also considered important mediators of neuronal growth, survival and function in the adult [4,27]. Roles for neurotrophic factors have been demonstrated in learning and memory, synaptic facilitation and other plasticity-related processes [5,32,33]. Many diseases of the nervous system including depression are hypothesized to involve dysregulation of growth factor processes, and the therapeutic application of treatments that regulate neurotrophic actions is an active area of investigation [40,54].

Long-term antidepressant treatment increases the expression of neurotrophic factors in hippocampus [19]. This has been suggested to be functionally relevant due to the converse, down-regulation of neurotrophic factors by stress, which can be a precipitating factor for depression [20]. A loss of neurotrophic support could contribute to atrophy and loss of neurons observed in pre-clinical models, as well as volumetric and cellular abnormalities reported in post-mortem studies of depressed patients [7,41,47].

Insulin-like growth factor-I (IGF-I) is among the growth factors reported to be up-regulated in rat brain by antidepressant treatment [29]. IGF-I is a trophic factor that mediates actions of growth hormone and regulates cell growth and metabolism in the periphery [50]. IGF-I is expressed in many tissues, including the brain, where it is important to nerve growth and differentiation and neurotransmitter synthesis and release [1,14]. Actions of IGF-I are mediated primarily through the IGF-I type I receptor and are modulated by IGF-I binding proteins [12,50]. The adult brain contains high levels of IGF-I receptors, but unlike the case in developing brain, expression levels of IGF-I are low suggesting that the adult brain may utilize IGF-I from sources outside of the brain [6].

Circulating IGF-I, derived primarily from liver, can gain entry into the brain via transport across the blood–brain and blood–cerebrospinal fluid (CSF) barriers [42,43]. Physical exercise stimulates the expression and release of liver IGF-I and results in elevated brain uptake of IGF-I in rodents [8,56]. Moreover, peripheral IGF-I is necessary for exercise-induced hippocampal neurogenesis and for functional recovery after brain injury in rodent models [9,52]. These findings implicate peripheral IGF-I in the beneficial consequences of exercise on brain function and suggest that peripheral IGF-I might also be important to other central effects of exercise.

Chronic exercise can result in improvements in depression in humans and in antidepressant-like responses in rodent behavioral

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models [2,22,23,26]. In the experiments reported here, we aimed to assess the role of *peripheral* IGF-I in mediating antidepressant-like behavior under resting physiological conditions. We also investigated the extent to which IGF-I might contribute to antidepressant-like behavior in exercising mice.

2. Methods

2.1. Animals

Male C57Bl/6 mice were obtained at 10 weeks of age from Jackson Laboratories (Bar Harbor, ME) and were acclimated to the facility for 1 week before use in experiments. Mice were singly housed in standard mouse cages (Nalgene) with *ad libitum* access to food and water. Mice were maintained on a 12-h light-dark cycle with lights on at 7 am. Animal maintenance and use procedures were in accordance with *the NIH Guide for Care and Use of Laboratory Animals* and were approved by the Yale University Animal Care and Use Committee.

For exercise experiments, the cages of exercising mice were equipped with running wheels (34.5 cm diameter) attached to mechanical counters. The counters were connected to a CLOCKLAB data collection system (Actimetrics, Evanston, IL) and wheel-running activity was recorded continuously throughout the experiment. Chronic exercising mice were given wheel access for 4 weeks. Behavioral testing was performed 24 h following the last wheel access.

2.2. Treatments

Human recombinant IGF-I (GroPep Limited, Alelaide, Australia) was administered to mice via continuous subcutaneous osmotic minipump infusion. For chronic IGF-I treatments, Alzet model 1002 minipumps (Durect Corp., Cupertino, CA) were filled to deliver 50 μg/kg of IGF-I per day over a 14-day period. Control mice were implanted with osmotic minnipumps containing vehicle. Anti-IGF-I polyclonal antibody was a kind gift of Dr. Ignacio Torres-Aleman. This antibody effectively recognized IGF-I by Western blot analysis and has been used in published reports [9,31,52]. For the exercise experiments, anti-IGF-I was administered to mice throughout a 28-day period of running wheel access. Anti-IGF-I (20% in saline) was delivered by subcutaneous osmotic minipump infusion (Alzet 2004) at a rate of 6 μl/day. Control mice received similar minipump infusion of 20% normal rabbit serum in saline. Desipramine hydrochloride (Sigma, St. Louis, MO) was administered as a single acute dose of 15 mg/kg, i.p., given 30 min prior to testing and fluoxetine hydrochloride (gift of Eli Lily, Indianapolis IN) was administered in the drinking water at a dose of 20 mg/kg/day. Separate groups of mice were used for the antidepressant-responsive behavioral paradigms (forced-swim test (FST) and novelty-induced hypophagia (NIH)), the stress-exposure experiment and for the exercise experiment.

2.3. Behavioral procedures

2.3.1. Forced-swim test

The forced-swim test was performed according to standard published procedures with minor modifications [13,21]. Mice were placed in a glass cylinder (12 cm diameter) filled to a depth of 10 cm with water (23 °C). A 6 min test session was conducted and videotaped. Time spend immobile was defined as the absence of active/escape directed movements and was scored by a blind observer for the last 4 min of the test period.

2.3.2. Novelty-induced hypophagia

The novelty-induced hypophagia paradigm was conducted as described by Dulawa et al. [18]. After 3 days of habituation to a palatable solution (Carnation sweetened condensed milk 1:3 in water), mice were individually presented with the solution in the home cage and latencies to approach and drink were recorded as a control. On the subsequent day, mice were presented with the milk solution in a novel cage with bright lighting and the latency to approach and drink was recorded.

2.3.3. Chronic mild stress exposure

The chronic mild unpredictable stress (CUS) paradigm is a modification of published procedures [3,36]. The stress regimen consisted of exposure to the following physical and/or psychological stressors: confinement (1 h), cold exposure (4 °C, 45 min), new cagemates (3 h), exposure to rat odor (3 h), wet bedding (3 h), swim stress (10 min), periods of darkness during the light cycle (3 h), cage rotation (1 h), crowding (1 or 3 h), stroboscopic lighting (2 h), cage tilt (12 h), food deprivation (12 h), light on (overnight). Two different stressors were applied during each 24 h period, in a pseudo-random, and changing sequence. Non-stressed control mice were housed in a separate room.

2.3.4. Sucrose consumption

The consumption of a sucrose solution was conducted as previously published for mice [25]. Mice were habituated to sucrose over a 48 h period by replacing water bottles with bottles containing sucrose solution (1%). Sucrose consumption was then measured by presenting sucrose (1%) in the home cage for a 1 h test period that followed overnight water deprivation. Mice were tested in single-bottle tests in their home cages while their cagemates were temporarily removed. All mice were tested between 7:00 and 11:00 am. Sucrose consumption was quantified by weighing bottles before and after the test periods.

2.3.5. Locomotor activity

Locomotor activity was measured by video tracking (Ethovision Pro, Noldus Inc.) in standard cages. Data were analyzed with Ethovision behavioral analysis software.

2.4. Measurement of growth factors

Animals were decapitated at the end of the experiment and brains were rapidly removed and hemisected. Prefrontal cortex was dissected from one hemisphere and frozen on dry ice. The remaining hemisphere was frozen on dry ice and used for in situ hybridization analysis.

2.4.1. IGF-I protein

IGF-I in brain tissue was quantified by ELISA (R&D Systems, Minneapolis, MN) according to manufacturers instructions. Tissue homogenates were treated with an acidic dissociation solution in order to remove any IGF-I binding proteins and then aliquots of pre-treated samples and standards were added to a microplate precoated with a monoclonal antibody specific for IGF-I which binds IGF-I contained in the sample. Following washing, an enzyme-linked polyclonal antibody specific for IGF-I was added to the wells. After an additional wash, substrate solution that uses tetramethylbenzidine as a chromagen was added which develops color in proportion to the amount of IGF-I bound in the initial step. Color development was stopped by addition of sulfuric acid solution and optical density for individual samples was determined using a microplate reader set to 450 nm. Samples were run in duplicate.

2.4.2. In situ hybridization

In situ hybridization for IGF-I or for brain derived neurotrophic factor (BDNF) mRNA was carried out as described previously [37,38]. Cryostat cut coronal sections

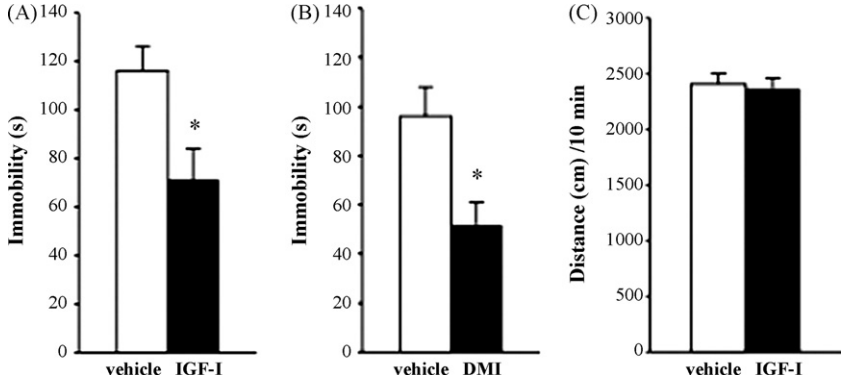

Fig. 1. Peripheral IGF-I administration results in an antidepressant-like behavioral response in the forced-swim test (FST). IGF-I (50 ug/kg/day) was administered subcutaneously via osmotic minipump for 14 days. (A) IGF-I-treated mice showed decreased immobility in the FST compared with mice that received similar administration of vehicle. Student's *t*-test, $^{*}p = 0.01$, $n = 9$–10. (B) Antidepressant response to desipramine in the FST is shown for comparison. Desipramine (DMI) (20 mg/kg, i.p.) was administered in a single acute dose 30 min prior to test. Student's *t*-test, $^{*}p = 0.01$, $n = 8$/group. (C) Baseline locomotor activity of IGF-I-treated mice was not different from the activity of control mice. Horizontal distance traveled was measured by video tracking in standard mouse cages. Bars represent the mean ± SEM for each experiment.