Published in final edited form as:

Eur J Pharmacol. 2008 October 10; 594(1-3): 109–116. doi:10.1016/j.ejphar.2008.07.023.

Antidepressant-like behavioral effects of IGF-I produced by enhanced serotonin transmission

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Abstract

Previous research has suggested that mobilization of neurotrophic factors, such as insulin-like growth factor I (IGF-I), can be involved in the effects of antidepressant treatments. The current experiments showed that IGF-I leads to antidepressant-like effects in the modified rat forced swim test when tested 3 days, but not 1 day, after i.c.v. administration. These effects were sustained longer than the antidepressants paroxetine and desipramine. In addition, blockade of the IGF-I receptor with the IGF-I antagonist JB1 30 min before IGF-I administration prevented the antidepressant-like effects of IGF-I. However, when JB1 was administered 3 days after IGF-I administration and 30 min prior to testing, the antidepressant-like effects of IGF-I were still present suggesting that IGF-I produces a long-term activation of neural systems involved in the antidepressant response. Because the pattern of antidepressant-like effects of IGF-I resembled those of selective serotonin reuptake inhibitors, the role of serotonin in the behavioral effects of IGF-I was studied. Depletion of serotonin, by the tryptophan hydroxylase inhibitor para-chlorophenylalanine, blocked the antidepressant-like effects of IGF-I. Administration of IGF-I increased basal serotonin levels in the ventral hippocampus and altered the effects of acute citalopram. IGF-I administration did not change hippocampal cell proliferation at the 3-day timepoint when behavioral effects were seen. In addition, IGF-I did not alter the expression of mRNA levels of tryptophan hydroxylase or SERT in the brain stem, or [³H] citalopram binding in the hippocampus or cortex. Thus, IGF-I administration initiates a long-lasting cascade of neurochemical effects involving increased serotonin levels that results in antidepressant-like behavioral effects.

Index words

depression; neurotrophins; animal models; neurogenesis; forced swim test; rat

1. Introduction

Major depressive disorder is currently one of the most prevalent and costly mood disorders (Crown et al., 2002). Neurotrophic factors play a key role in brain development, cell

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maintenance and survival (Dechant and Neumann, 2002), and their ability to facilitate neural signaling and plasticity in response to stress has led them to be proposed as mediators of the onset and treatment of depression (Duman, 2004). Evidence for the role of neurotrophic factors in depression comes from studies showing that brain derived neurotrophic factor (BDNF) expression and neurogenesis is decreased by chronic stress and increased following the chronic administration of a wide and varied range of antidepressant treatments (Duman and Monteggia, 2006). Further support derives from findings showing antidepressant-like behavioral effects for BDNF in animal tests predictive of antidepressant activity, like the rat forced swim test (FST) or learned helplessness (Hoshaw et al., 2005; Shirayama et al., 2002; Siuciak et al., 1997), and that mutant mice with reduced functioning *trkB* receptors are insensitive to the effects antidepressants in the mouse FST (Saarelainen et al., 2003). Furthermore, chronic administration of BDNF to adult rats increases neurogenesis in the hippocampus (Zigova et al., 1998). Since neurotrophic gene expression and neurogenesis is decreased by exposure to stressors and increased by chronic administration of antidepressants (Warner-Schmidt and Duman, 2006), compounds regulating the role of neurotrophins in the regulation of brain plasticity and adaptation to stress could be attractive candidates for the treatment of mood disorders.

Very little is known about the role of other neurotrophins in the treatment for depression. Insulin growth factor-I (IGF-I) is a 15 kD peptide that is secreted primarily by the liver under the control of growth hormone. The highest concentrations of IGF-I in the brain are present during early development where it stimulates neurons growth but IGF-I remains in the brain throughout the lifespan (Kar et al., 1993). IGF-I is regulated by a family of at least six binding proteins, which increase the half-life of the peptide, and regulate the affinity IGF-I for its receptor (Clemmons, 1992). The IGF-I receptor is a tyrosine kinase receptor, which autophosphorylates after ligand binding (Le Roith et al., 1995). Activation of the receptor leads to a downstream cascade of intracellular signals, which includes activation of mitogen activated protein (MAP) kinase, phosphatidylinositol (3,4,5)-trisphosphate (PIP3) kinase and activation of serine/threonine-specific protein kinase (AKT) signaling pathways (Clemmons, 1992). The major medicinal use of IGF-I is to counteract dwarfism associated with growth hormone deficiency (Laron, 2008), but important roles for IGF-I have been proposed in a number of diseases, including Alzheimer's disease, cancer, diabetes, cardiovascular disease and amyotrophic lateral sclerosis (Ezzat et al., 2008; Ryan and Goss, 2008; Torres-Aleman, 2007; Wilczak and de Keyser, 2005).

IGF-I promotes neurogenesis in the hippocampus (Zigova et al., 1998; Aberg et al., 2000) and exercise-induced increases in neurogenesis (Trejo et al., 2001), effects similar to those produced by chronic antidepressant treatment (Malberg et al., 2000). Recently our laboratory showed that central administration of IGF-I produced antidepressant-like effects that endured for at least 6 days in a well established model of antidepressant-like activity, the modified rat forced swim test (FST) (Hoshaw et al., 2005). In this study, the behavioral effects of IGF-I were similar to those of BDNF, indicating that neurotrophins may be an effective target for the development of novel antidepressants with sustained activity. Drugs that inhibit binding proteins for IGF-I, thereby increasing the effects of free IGF-I, can also produce antidepressant-like or anxiolytic effects in mice (Malberg et al., 2007).

The current set of experiments extends our findings on the enduring antidepressant-like effects of IGF-I by comparing its effects with two conventional antidepressant drugs. The antagonist JB-1 was used to determine whether the effects of the neurotrophin are mediated by IGF-I receptors and when the receptor blockade was effective. In addition, the interaction between IGF-I and the serotonergic system was examined in order to ascertain whether the behavioral effects of IGF-I functioned, in part, by modifying the effects of this neurotransmitter.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (Charles River) weighing 250–275 g were used for all of the experiments. The rats were housed in pairs in a temperature and humidity controlled facility maintained on a 12:12 light/dark cycle. Rats were given free access to food and water throughout the experiment. All Procedures were conducted with the approval of the University of Pennsylvania Institutional Animal Care and Use Committee and within the guidelines of The National Research Council's *Guide for the Care and Use of Laboratory Animals* (1996).

2.2. Modified rat forced swim test

For experiments examining the antidepressant-like effects of IGF-I (Experiments 2, 3, and 4), the modified version of the rat FST was run as previously described (Detke et al., 1995; Hoshaw et al., 2005). Briefly, on the first day the rats were placed in a container of water from which they could not escape (30 cm deep, 22–25°C) for 15 min. On the following day, the rats received an i.c.v. infusion of either artificial cerebrospinal fluid (aCSF) vehicle or IGF-I (1.0 µg). Finally, either 1 day (Experiment 3) or 3 days (Experiments 2 and 4) later, the rats were exposed to the FST again for 5 min. The final session was taped from an overhead view, and scored for the frequency of swimming, climbing and immobility, as previously described for conventional antidepressants (Detke et al., 1995).

2.3. Blockade of the behavioral effects of IGF-I with the IGF-I competitive antagonist JB1

The effects of pretreatment with the peptide IGF-I receptor antagonist JB1 (H-Cys-Tyr-Ala-Ala-Pro-Leu-Lys-Pro-Ala-Lys-Ser-Cys-OH; Bachem Biosciences; Willow Grove, PA) were examined on the antidepressant-like behavioral effects of IGF-I in the FST. The first study examined the effects of pretreatment with JB1 (5, 10 and 20 µg, i.c.v.) 30 min prior to administration of IGF-I (1.0 µg, i.c.v.). The effects of these doses on the FST were compared to two other groups, one receiving the aCSF vehicle and another receiving the highest dose of JB1 (20 µg) alone without IGF-I. The second study examined the effect of JB1 when given 3 days after the IGF-I infusion but just prior to the swim test. JB1 (20 µg) or the aCSF vehicle was administered 30 min before the FST (3 days after administration of either IGF-I or vehicle), and the behavioral effects in the FST were tested. The design of these studies was modeled after previous experiments in this lab showing that i.c.v. infusion of IGF-I (1.0 µg) leads to antidepressant-like effects in the FST 3 days after administration (Hoshaw et al., 2005).

2.4. Behavioral effects of IGF-I 1 day after administration

The previous studies on the behavioral effects of IGF-I found that the antidepressant-like effects were present 3 and 6, but not 12 days after i.c.v. administration (Hoshaw et al., 2005). Experiment 2 further examined this time course by testing the antidepressant-like effects 1 day after IGF-I administration.

2.5. Desipramine and paroxetine in the FST

In order to compare the effects of IGF-I with established antidepressants, the effects of the tricyclic antidepressant desipramine (20 mg/kg; i.p.) or the selective serotonin reuptake inhibitor paroxetine (20 mg/kg; i.p.) were tested in the standard rat FST (Detke et al., 1995). For this version of the FST, rats were exposed to two swim sessions. The first session was the pretest given for 15 min and the second session lasted for 5 min. Between the first and second sessions, rats were given three injections of either paroxetine (20 mg/kg), desipramine (20 mg/kg) or saline given 24.5, 5 and 1 h before the second swim session. The second FST session was recorded and scored for the presence of immobility, swimming and climbing. In addition

to testing the rats 1 h after the last injection, we also tested the same rats 3 days later, to see if the effects of systemically administered antidepressants were retained at this time.

2.6. Serotonin depletion and the antidepressant-like effects of IGF-I

Our previous study showed that IGF-I administration led to an increase in swimming as opposed to climbing in the FST (Hoshaw *et al.*, 2005), an effect indicative of the activation of serotonergic as opposed to noradrenergic mechanisms (Detke *et al.*, 1995). We next examined the effects of serotonin depletion on the antidepressant-like effects of IGF-I. In order to deplete serotonin, para-chlorophenylalanine (PCPA; 300 mg/kg) or saline was administered 24 h before the first 15-min FST session. Previous studies have shown that this dose of PCPA produced a decrease in central serotonin levels by 80–90% which lasted for at least 6 days (Koe and Weissman, 1966). Finally, 24 h later either IGF-I (1 µg) or aCSF was administered, and the effects on the FST were measured 3 days later.

2.7. The effects of IGF-I on serotonin levels in the ventral hippocampus

In order to measure the effects of IGF-I administration on central serotonin levels, a microdialysis probe (Kreiss and Lucki, 1997) was implanted into the ventral hippocampus of each rat (A/P: −5.6; M/L ±4.7; D/V: −4.4; (Paxinos and Watson, 1986) 2 days after i.c.v. administration of IGF-I (1 µg) or aCSF. Experiments were conducted 24 h after implantation. The microdialysis probes were continuously perfused with aCSF at a rate of 0.8 µl/min and samples of extracellular fluid were collected every 20 min for 2 h in order to measure a baseline level of serotonin. Next, citalopram (2.5 mg/kg, i.p.) was injected, and dialysate samples were collected every 20 min for an additional 3 h. Samples were frozen and stored at −80°C until analyzed. The samples were analyzed via high pressure liquid chromatography with electrochemical detection, as previously described (Page *et al.*, 2002). The mobile phase consisted of 12.4 mM citric acid, 39.85 mM NaPO₄, 10.0 mM NaCl, 0.25 mM EDTA, .737 mM 1-decanesulfonic acid, 0.2% triethylamine, and 19% methanol adjusted to pH of 4.1. Levels of serotonin measured at baseline and after citalopram administration were compared between the groups treated with IGF-I or vehicle. The overall effect of citalopram was measured as the net cumulative increase of serotonin for the entire session.

2.8. The effects of IGF-I administration on mRNA levels of TPH1, TPH2 and SERT

Additional examination of the effects of IGF-I on targets in the serotonin pathway was performed using RT-PCR to detect mRNA levels of tryptophan hydroxylase 1 and 2, as well as for the serotonin transporter (SERT). Rats were infused with either IGF-I (1.0 µg) or aCSF, and the brains were rapidly removed and frozen in isopentane (−80°C). For measurement of mRNA levels, frozen brain stem tissues were directly homogenized in Trizol reagent (Life Technologies, Bethesda, NY) at a ratio of 1 ml Trizol/100 mg tissue. Total RNA was extracted from Trizol homogenates following the manufacturer's instructions and treated with DNase to remove any contaminating genomic DNA. Around 5 micrograms of total RNA was then reverse transcribed using Superscript II (Life Technologies) enzyme according to manufacturer's instructions. After reverse transcription, samples were diluted 1:5 with deionized water and 2 µl of these dilutions were used in each SYBR Green PCR assay. The Real Time PCR reactions were carried out according to manufacturer instructions in an ABI Prism 7000 Sequence Detection System (PE, Applied Biosystems, Foster City, CA). The mRNA levels of the genes of interest were quantitated relative to an internal housekeeping gene (hypoxanthine-guanine phosphoribosyltransferase, HPRT) using the comparative Ct method according to manufacturer's instructions (Applied Biosystems). PCR primer sequences were designed to produce amplicons of 90–110 bp using Primer Express software (Applied Biosystems) and the consensus mRNA sequences from the GenBank database found on the NCBI website. Primer sequences (all written 5' to 3') were as follows: TPH1 forward- GTT CAG AAA CTG GCA

ACG TGC, TPH1 reverse- ACT GAT GGA AGA AAG CAG GCC; TPH2 forward- CAC AGA GTC CTC ATG TAC GGC A; TPH2 reverse- CCA TGG CCA CAT CCA CAA A; SERT forward- TTT ACA TGG AGC TCG CAC TGG; SERT reverse- ATG ATG CAG ATG GCG TAA CCA; HPRT forward- CAA GCT TGC TGG TGA AAA GGA C. Relative mRNA levels for TPH1, TPH2 and SERT were compared between IGF-I and vehicle groups via Student's t-test.

2.9. The effects of IGF-I on binding of [³H]citalopram

Individual samples (60–100 mg) were excised from the hippocampal and frontal cortical regions of frozen rat brains and homogenized in 0.32 M sucrose/50 mM Tris buffer (40 w/v) using a Polytron (Kinematica, Switzerland) (setting 6; 30 sec; 4°C). The homogenates were centrifuged (Beckman GS-6R 800 × g; 10 min; 4°C) and the supernatants decanted and diluted (3 v/v) in Tris assay buffer (50 mM Tris; 120 mM NaCl; 5 mM KCl; pH 7.4). Each sample was centrifuged (Beckman J2 Mi/JA –17 rotor 40,000 × g; 10 min; 4°C) and the pellets were resuspended in Tris assay buffer (300 vols) followed by centrifugation (Beckman J2 Mi/JA –17 rotor 40,000 × g; 10 min; 4°C); the latter operation was repeated once. The final membrane pellets were stored at –80°C. The protein content was determined by the Bradford method using a BioRad kit.

When assayed, cortical (35 µg protein/assay) and hippocampal (20 µg protein/assay) membranes were incubated for 90 min at RT in Tris assay buffer in the presence of increasing concentrations of [³H]citalopram (0.5–15 nM). Nonspecific binding was assessed in the presence of 1 µM fluoxetine. Final assay volume was 200 µl. Free ligand was eliminated with three filtration washes using ice-cold Tris buffer (50 mM Tris; 120 mM NaCl; pH 7.4) on 0.05% polyethylenimine pre-treated GF/B filters. Filters were counted in a scintillation counter (2200CA; PerkinElmer, Boston, MA) in the presence of 5 ml of a toluene-based scintillator. Each assay point represents the mean of duplicate determinations from three separate experiments. Equilibrium saturation binding data were analyzed with the GraphPad Prism program.

2.10. Effects of acute IGF-I administration on cell proliferation in the dentate gyrus of the hippocampus

The effects of acute administration of IGF-I were examined on cell proliferation in the hippocampus 3 days later. Separate groups of rats were injected with either IGF-I (1.0 µg, i.c.v.) or aCSF. In order to examine cell proliferation 3 days later, they were administered the thymidine analog bromodioxuridine (BrdU) (100 mg/kg, i.p.) 2 h before the subjects were given an overdose of pentobarbital (100 mg/kg, i.p.). The rats were transcardially perfused with 0.1 M PBS, followed by 4% paraformaldehyde. The brains were removed rapidly, and stored overnight in paraformaldehyde at 4° C, before being placed in a 30% sucrose solution at 4° C until sectioning. The entire hippocampus was sectioned (35 µm), mounted on slides (Fisher Scientific), and allowed to dry. Finally, immunohistochemistry for BrdU positive cells was performed, as described in (Malberg et al., 2000; Hoshaw et al., 2006).

2.11. Drugs

For all of the experiments, IGF-I and JB1 (Bachem Biosciences; King of Prussia, PA) were administered i.c.v. by microliter infusion pumps (Instech Laboratories; Plymouth Meeting, PA). IGF-I (1 µg) and JB1 (5, 10 or 20 µg) were dissolved in 2 µl of aCSF and administered over a period of 2 min, at a rate of 1 µl/min. All subjects in the vehicle groups received 2 µl of aCSF infused over 2 min. After the end of the infusion, the injectors remained in the cannula for an additional 2 min before the stylets were placed back in the cannula. The aCSF vehicle consisted of 147 mM NaCl, 1.7 mM CaCl₂, 0.90 mM MgCl₂, and 3.99 mM KCl. Paroxetine hydrochloride (20 mg/kg; SmithKline-Glaxo), desipramine hydrochloride (20 mg/kg; Sigma-

Aldrich; St. Louis MO), PCPA methyl ester hydrochloride (300 mg/kg; Sigma Aldrich; St. Louis MO) were dissolved in saline and injected i.p. at a concentration of 20 mg/ml for paroxetine and desipramine, or 300 mg/ml for PCPA. Doses of drugs were calculated according to molecular weight of the base.

For intraventricular injections, each rat was implanted with a guide cannula (Plastics One; Roanoke, VA) aimed at the lateral ventricle via stereotaxic surgery one week after arrival, as previously described (Hoshaw *et al.*, 2005). Briefly, rats were anesthetized with atropine sulfate (10 mg/kg) followed by pentobarbital hydrochloride (50 mg/kg). A small burr hole was drilled through the skull over the coordinates for the lateral ventricle (+1.0 mm A/P; ± 1.3 mm L/V; 4.5 mm D/V; (Paxinos and Watson, 1986). The cannulae were held in place by screws and dental cement, and stylets were placed in each cannula to prevent backup. All of the rats were given at least one week to recover from surgery before testing.

2.12. Statistical analysis

Depending on the treatment conditions, analysis of variance (ANOVA) using either one- or two-factors were used to examine the differences in the frequency of immobility, swimming and climbing behavior. Fisher's PLSD test was used when a post hoc test was required. The number of BrdU positive cells in the subgranular region of the dentate gyrus of the IGF-I and vehicle treated subjects was counted by an observer blind to the experimental procedure, and compared via a two-tailed Student's t-test.

3. Results

3.1. Blockade of the antidepressant-like effects of IGF-I with JB1 in the FST

IGF-I produced antidepressant-like effects when rats were tested in the FST 3 days after injection by decreasing immobility ($P < .01$) and increasing swimming ($P < .01$). Pretreatment with the IGF-I receptor antagonist JB1 30 min before IGF-I produced a dose-dependent blockade of the effects of IGF-I in the FST (Fig. 1A). The effects of IGF-I were completely blocked by pretreatment with the highest dose of JB1 (20 μ g), and the middle dose of JB1 (10 μ g) blocked the decrease in immobility but did not block the IGF-I-induced increase in swimming ($P < .02$). Finally, the highest dose of JB1 (20 μ g) administered by itself produced no effect on immobility, swimming or climbing.

When the highest dose of JB1 (20 μ g) was administered 30 min before the FST, the IGF-I receptor antagonist was unable to block the antidepressant-like effects of IGF-I in the FST (Fig. 1B). Although IGF-I decreased immobility ($P < .02$) and increased swimming ($P < .01$) significantly, pretreatment with 20 μ g of JB1 immediately before the FST was unable to block these effects. Therefore, JB1 administration blocked the antidepressant-like effects of IGF-I when administered before the neurotrophin 3 days prior to the FST, but JB1 was unable to block these effects when the antagonist was administered just before the FST.

3.2. Antidepressant-like effects of IGF-I 1 day after administration

IGF-I administered 24 h before testing did not produce antidepressant-like effects in the FST (Fig. 2). Comparison between the vehicle and IGF-I group revealed that there were no significant effects for immobility [$t_{(12)} = 1.01$, $P < .05$], swimming [$t_{(12)} = 0.95$, $P < .05$], or climbing [$t_{(12)} = 0.32$, $P < .05$]. Therefore, the antidepressant-like effects of IGF-I administered i.c.v. required more than a single day to emerge, although they were present 3 and 6 days after administration.

3.3. Antidepressant-like effects in the FST of paroxetine and desipramine

Both paroxetine and desipramine produced effects in the modified rat FST when tested immediately after 3 injections given within 24 h (Fig. 3a), the usual treatment paradigm for examining antidepressant activity in the rat FST. The SSRI paroxetine decreased immobility and increased swimming, while desipramine decreased immobility and increased climbing. These data agree with previous data showing different patterns of response in swimming and climbing behaviors by antidepressants that selectively inhibit the reuptake of serotonin or norepinephrine, respectively (Detke et al., 1995). However, when the same rats were tested 3 days later (Fig. 3b), there were no significant residual effects for immobility, swimming or climbing ($P > .05$ for all comparisons). Therefore, the effects of systemic antidepressant treatment do not appear to last as long as a single central administration of IGF-I (Hoshaw et al., 2005).

3.4. Serotonin depletion and the antidepressant-like effects of IGF-I

Depletion of serotonin produced by administration of PCPA completely blocked the behavioral effects of IGF-I in the FST (Fig. 4). Two-way ANOVA revealed that for drug condition (IGF-I vs. vehicle) there were significant effects for immobility [$F_{(1, 36)} = 9.96$, $P < .004$] and swimming [$F_{(1, 36)} = 10.83$, $P < .03$], and for pretreatment condition, there was a significant effect in immobility [$F_{(1, 36)} = 4.20$, $P < .05$]. Importantly, there was a significant interaction between drug and pretreatment conditions for both immobility [$F_{(1, 36)} = 4.25$, $P < .05$] and swimming [$F_{(1, 36)} = 4.94$, $P < .04$]. Post hoc tests revealed that IGF-I decreased immobility ($P < .02$) and increased swimming ($P < .03$), but these effects were not present after pretreatment with PCPA. Overall, there were no significant effects for climbing in any of the groups.

3.5. IGF-I administration and hippocampal serotonin levels

IGF-I (1 μ g) administration failed to alter basal serotonin levels 1 day after injection [$t(8) = 0.21$, $P > .05$] (Fig. 5A). Although cumulative output of 5-HT in response to citalopram administration increased from 7.23 ± 2.00 pg (N = 4) to 13.15 ± 1.88 pg after IGF-I (N = 6), the difference between groups failed to reach statistical significance [$t(8) = 2.09$, $P = .07$].

In contrast, when the rats were sampled 3 days after i.c.v. IGF-I administration (Fig. 5B), administration of IGF-I significantly increased basal extracellular serotonin levels nearly 4-fold compared to the vehicle group, [$t(9) = 3.13$, $P < .02$]. Administration of citalopram increased extracellular serotonin levels in both groups. However, rats treated with IGF-I showed a smaller cumulative output (3.04 pg ± 2.12 ; N = 5) than the aCSF vehicle (8.34 pg ± 1.90 ; N = 6), although the difference between groups failed to reach statistical significance [$t(9) = 1.87$, $P = .09$]. This may have been due to the larger baseline values in the IGF-I group. Overall, the increase in basal serotonin levels seen 3 days, but not 1 day, after IGF-I administration corresponds with the time course for the behavioral effects measured in the FST.

3.6. TPH1, TPH2 and SERT mRNA

Administration of IGF-I did not alter gene expression for tryptophan hydroxylase or SERT in the brain stem 3 days after administration (Table 1). There were no significant differences between vehicle and IGF-I groups for relative mRNA expression of tryptophan hydroxylase I, tryptophan hydroxylase II or the SERT ($P > .05$ for all comparisons). Therefore, the increase in serotonin levels was not due to an increase in either serotonin synthesis or expression of the SERT.

3.7. [³H]Citalopram Binding

Administration of IGF-I did not change [³H]citalopram binding *in vitro* (Table 2). [³H]citalopram binding was saturable and occurred to a single class of high-affinity binding sites. As shown in Table 2, no significant differences in [³H]citalopram binding were observed between vehicle and IGF-I-treated rats in membranes prepared from the hippocampus [$t_{(8)} = 0.21$, $P > .05$] or the cortex [$t_{(8)} = 0.21$, $P > .05$]. This indicated that the increase in serotonin levels was not due to a change in serotonin transporters.

3.8. Acute IGF-I administration and cell proliferation

Unlike chronic IGF-I administration (Aberg et al., 2000), a single i.c.v. treatment with IGF-I did not increase cell proliferation in the subgranular zone of the dentate gyrus 3 days after administration [$t_{(12)} = 0.63$, $P > .05$] (Fig. 6). Therefore, the antidepressant-like effects of IGF-I in the FST were not associated with an increase in cell proliferation in the hippocampus.

4. Discussion

The current set of data extend our previous findings on the enduring antidepressant-like effects of centrally administered IGF-I (Hoshaw et al., 2005). The antidepressant-like effects of IGF-I in the modified rat FST were present 3 and 6 days after central administration, although the current studies indicated that these effects required at least 24 h to emerge. The enduring effects of IGF-I were confirmed in studies comparing the duration of effect with conventional antidepressants, showing that the antidepressant-like effects of paroxetine and desipramine were confined to the period of drug treatment and were not present 3 days after the last injection. This enduring antidepressant-like effect of IGF-I parallels that of other neurotrophic factors, such as BDNF and neurotrophin-3 (Shirayama et al., 2002; Hoshaw et al., 2005). Those behavioral effects were first measured 3 days after administration and lasted up to 10 days in the learned helplessness paradigm. Recently, ketamine has been shown to produce rapid and enduring antidepressant effects in treatment-resistant patients that endured for at least one week (Zarate et al., 2006), and there is great interest in understanding the neural mechanisms that could underlie such unique psychotropic effects. Such enduring effects on clinical depression may be related to the ability of ketamine to increase BDNF levels in rat brain, and perhaps brain levels of other neurotrophins, after a single administration (Garcia et al., 2007). The preclinical research on animal models indicates that a rapid increase of central neurotrophin levels could sustain enduring antidepressant-like effects of ketamine, and would be expected to last longer than traditional antidepressants.

Administration of the IGF-I antagonist JB1 (Camarero et al., 2003) before IGF-I leads to a dose-dependent blockade of the behavioral effects of IGF-I in the FST, showing that the antidepressant-like effects of IGF-I are mediated specifically by IGF-I receptors. Similar effects were produced by JB1 pretreatment on the antidepressant and anxiolytic effects of IGF-I in the mouse (Malberg et al., 2007). Moreover, administration of JB-1 just prior to the FST (3 days after IGF-I administration) did not block the antidepressant-like effects of IGF-I. Therefore, the enduring behavioral effects of IGF-I are likely not mediated by the direct persistent activation of the IGF-I receptor but by a downstream cascade of cellular events that ultimately leads to sustained behavioral changes, as those measured in the FST. The IGF-I receptor is a tyrosine kinase that undergoes autophosphorylation after binding of IGF-I, and activates such secondary messengers as PI3K and MAPK (Aberg et al., 2006). Continued activation of one of these pathways by IGF-I could participate in the cascade of signaling events that would sustain the expression of behavioral effects. This would explain why JB1 was unable to block the maintenance of antidepressant-like effects after IGF-I was given but could block the emergence of the response when pretreated prior to initial treatment.

In the FST, intraventricular administration of IGF-I leads to a dose-dependent decrease in immobility, with a specific increase in swimming as opposed to climbing behavior (Hoshaw *et al.*, 2005; present study). Previous studies suggest that the selective increase in swimming behavior may be indicative of increased serotonergic transmission, since SSRIs increase swimming, but not climbing behavior (Detke *et al.*, 1995; Cryan *et al.*, 2005). In support of these data, we found that depletion of serotonin with the tryptophan hydroxylase inhibitor PCPA blocked the ability of IGF-I to decrease immobility and increase swimming behavior in the FST. PCPA was administered 2 days before IGF-I, which corresponds to 5 days before the final FST. As an irreversible inhibitor of tryptophan hydroxylase, a previous study with from our laboratory has shown that PCPA resulted in greater than 90% depletion of forebrain levels of serotonin measured 3 days after treatment (Page *et al.*, 1999), and that previous studies showed that an 80% or greater level of depletion of serotonin persisted at least 6 days after treatment with only minor effects on norepinephrine and dopamine levels (Koe and Weissman, 1966). These results are in agreement with previous findings that PCPA pretreatment blocked increased swimming produced by the SSRI fluoxetine, but not increased climbing by the norepinephrine reuptake inhibitor desipramine, in the modified rat FST (Page *et al.*, 1999). The blockade of the behavioral effects of IGF-I after serotonin depletion indicates that the antidepressant-like effects of IGF-I are mediated by serotonergic activity. Thus, IGF-1 joins neuropeptide Y and nitric oxide synthase inhibitors as novel compounds that produce antidepressant-like behavioral effects in the FST, but require intact serotonin neurotransmission to produce their effects (Harkin *et al.*, 2003; Redrobe *et al.*, 2005).

In agreement with the role of serotonin in the antidepressant-like effects of IGF-I, we also found that IGF-I increased basal levels of serotonin in the ventral hippocampus 3 days after administration. Therefore, it appears that IGF-I may produce antidepressant-like effects by increasing serotonin levels, at least in the hippocampus. The increased hippocampal serotonin levels corresponded to the time course for the behavioral effects of IGF-I, since serotonin levels were not increased 1 day after IGF-I administration, a time point when there were no antidepressant-like effects in the FST. This accord between the behavioral and serotonin promoting effects of IGF-I gives further evidence for the direct involvement of serotonin in the antidepressant-like effects of IGF-I. Interestingly, although IGF-I does not increase basal serotonin levels 1 day after administration, there is a trend for an additive effect between IGF-I and citalopram on serotonin levels in the ventral hippocampus. Although this effect did not reach significance, it may indicate that IGF-I does influence serotonergic activity even before the behavioral effects are fully present.

Although there is a clear role for serotonin in the antidepressant-like effects of IGF-I, at this time the mechanism for producing both of these effects is unknown. Because administration of IGF-I did not increase expression of mRNA expression for *tph1* or *tph2*, the increase in basal serotonin did not appear to be mediated by an increase in serotonin synthesis. Since there was also no change in expression of mRNA for the SERT 3 days after IGF-I administration, nor any difference in [³H]citalopram binding *in vitro*, the increase in serotonin was likely not mediated by changes in the SERT. Current studies in this lab are examining whether IGF-I receptors are present on serotonin cell bodies in the raphe, possibly leading to a direct activation of serotonin release. Studies on the expression of IGF-I receptors have indicated that these receptors are present in high density in the hippocampus, olfactory bulb and cerebellum (Aguado *et al.*, 1993; Bondy and Lee, 1993). The localization of IGF-I receptors, either on serotonin cell bodies in the raphé nuclei or co-localized in the hippocampus with projections from the raphé, could explain the mechanism of IGF-I-induced increases in serotonin levels in the ventral hippocampus.

Recently, much attention has been placed on the potential role of neurotrophic factors in the stimulation of neurogenesis and the clinical action of antidepressants (Altar, 1999; Sanger,

2001; Duman, 2004). Evidence for the role of neurotrophic factors in depression stems from their role in neuroplasticity and neuroprotection (Castren, 2004). In the case of IGF-I, chronic i.c.v. administration has been shown to increase neurogenesis in the hippocampus (Aberg et al., 2000) and disruption of IGF-I signaling produced a deficit in exercise-induced increases in neurogenesis in the hippocampus (Trejo *et al.*, 2001). The hippocampus is an area critical in the onset of depression (Campbell and Macqueen, 2004) and increases in hippocampal neurogenesis have been proposed to mediate some of the behavioral effects of chronic antidepressant treatments (Airan et al., 2007; Duman, 2004; Santarelli et al., 2003, but see Vollmayr et al., 2007). Since IGF-1 mediates the effects of exercise on neurogenesis (Trejo et al., 2001), and because IGF-1 increases extracellular levels of serotonin, it is appropriate to speculate that serotonergic mechanisms could play a key role in mediating the effects of chronic exercise on neurogenesis and depressive behavior through IGF-1 mechanisms. In the present study, the acute change in FST behavior was not associated with increased hippocampal cell proliferation. However, neurogenesis could participate in the effects of chronic IGF-1 administration. Aside from its effects on neurogenesis, IGF-I administration also increases cerebral blood flow in the hippocampus (Lopez-Lopez et al., 2004), and has pronounced anti-apoptotic effects, which could counteract the deleterious neuronal consequences of stress or injury (Guan et al., 2003). The combination of neural supportive and signaling actions in the hippocampus by this neurotrophin could participate in a potential palliative role in neuropsychiatric disease. Given the enduring effects of IGF-I administration, it appears that targeting this pathway for the development of novel antidepressants could lead to more effective medications for this disease (Schechter *et al.*, 2005). Future studies will focus on the effects of antidepressant treatment on IGF-I levels, as well as examination of the specific brain regions that mediate the antidepressant-like effects of IGF-I.

Acknowledgments

This research was supported by grant United States Public Health Service grants MH 14654 and MH 72832 as a National Cooperative Drug Discovery Group between the University of Pennsylvania and Wyeth Neuroscience.

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Fig. 1.

Blockade of antidepressant-like effects by the antagonist JB1 given before but not after IGF-I. **A**, Rats were pretreated i.c.v. with either vehicle (aCSF) or varied doses of JB1 (5 (n = 5), 10 (n = 5), or 20 μ g (n = 6); i.c.v.) 30 min before administration of either the vehicle or IGF-I (1 μ g; i.c.v.). The FST session (5 min) was run 3 days later. Administration of vehicle-IGF-I (n = 12) decreased immobility and increased swimming behavior compared to the vehicle-vehicle group (n = 11). This effect was blocked by pretreatment with JB1 30 min before IGF-I, with the highest dose of JB1 blocking both the decrease in immobility and increase in swimming. ANOVAs revealed significant overall effects for immobility [$F_{(5,39)} = 7.33$, $P < .001$] and swimming [$F_{(5,39)} = 6.25$, $P < .001$] but not for climbing [$F_{(5,39)} = 1.25$, $P > .05$]. **M**

B, Administration of JB1 3 days after IGF-I administration and 30 min before the FST, was not able to block the antidepressant-like effects of IGF-I. Rats were pretreated with either vehicle (aCSF) or IGF-I (1 μ g) 24 h after the pretest session (15-min), and then administered either vehicle (aCSF) or JB1 (20 μ g) 30 min before the FST session (5 min). Administration of IGF-I (n = 6) led to a significant decrease in immobility and a significant increase in swimming compared to the vehicle-vehicle group (n = 7), but administration of JB1 30 min before the FST (n = 7) did not block those behavioral effects. There were no significant differences between the vehicle-JB1 group (n = 6) and the vehicle-vehicle group. Two-factor ANOVA revealed that there was a significant effect for drug treatment (IGF-I vs. aCSF) in

immobility [$F_{(1,22)} = 19.32$, $P < .001$] and swimming [$F_{(1,22)} = 33.89$, $P < .0001$], but no effect for pretreatment group (JB1 vs. aCSF) for either immobility [$F_{(5,39)} = 0.58$, $P > .05$] or swimming [$F_{(5,39)} = 0.23$, $P > .05$]. Values represent mean $\pm$ 1 S.E.M. * indicates $P < .05$ compared with the vehicle-vehicle group.

Fig. 2.

IGF-I did not fully produce antidepressant-like effects in the FST when tested 1 day after administration. IGF-I (1 μ g; $n = 7$) or vehicle (aCSF; $n = 7$) was administered 24 h before the FST pretest (15-min), and the 5-min FST session was run 1 day after injection. Values represent mean ± 1 S.E.M.

Fig. 3.

Effects of antidepressants in the modified FST, either the SSRI paroxetine or the selective norepinephrine reuptake inhibitor desipramine. **A**, Rats were tested in the FST 1 h after the last of 3 injections with either paroxetine (20 mg/kg), desipramine (20 mg/kg) or saline ($n=10$ each). One-factor ANOVA revealed significant effects for immobility [$F_{(2,21)} = 5.96$, $P < .001$], swimming [$F_{(2,21)} = 8.35$, $P < .003$], and climbing [$F_{(2,21)} = 6.45$, $P < .007$]. Both drugs showed antidepressant-like effects. The SSRI paroxetine decreased immobility and increased swimming, while desipramine decreased immobility and increased climbing. **B**, Neither antidepressant was active in the FST when the rats were tested 3 days after the last injection. Values represent mean $\pm$ 1 S.E.M. * indicates $P < .05$ compared with saline.

Fig. 4.

Depletion of serotonin blocked the antidepressant-like effects of IGF-I in the FST. Serotonin synthesis was inhibited by administering the tryptophan hydroxylase inhibitor PCPA (300 mg/kg; i.p.) 24 h before the first 15-min session. Vehicle or IGF-I (1 μ g) was then administered 24 h later, and the rats were tested in the second session of the FST 3 days later, or 96 h after PCPA pretreatment. IGF-I (n = 9) produced a significant decrease in immobility and increase in swimming compared to the saline-vehicle group (n = 9). Pretreatment with PCPA before vehicle (n = 11) had no effect on any of the three behaviors compared to the saline-vehicle group. However, PCPA pretreatment blocked the antidepressant-like effects of IGF-I (n = 11). Therefore, serotonin transmission appears to be necessary for the antidepressant-like effects of IGF-I. Values represent mean $\pm$ 1 S.E.M. * indicates $P < .05$ compared with the saline-vehicle group.

Fig. 5.

IGF-I administration increased basal serotonin levels in the ventral hippocampus 3 days, but not 1 day, after administration as measured using *in vivo* microdialysis. Values represent mean (pg) $\pm$ 1 S.E.M. * indicates $P < .05$ compared with aCSF control. **A**, Hippocampal serotonin levels measured 1 day after IGF-I (1 μ g; $n = 6$) administration. Four 20-min samples were collected as a baseline measure before the systemic injection of citalopram (2.5 mg/kg; i.p.). IGF-I did not affect basal serotonin levels compared with vehicle (aCSF; $n = 4$). There was a trend towards an increase in the percentage change in serotonin levels after citalopram administration ($P = .07$). **B**, Hippocampal serotonin levels measured 3 days after administration of either vehicle (aCSF; $n = 6$) or IGF-I (1 μ g; $n = 5$) administration. The IGF-I-pretreated rats

had significantly higher basal serotonin levels ($P < .02$). Although the cumulative output after citalopram injection was smaller in rats treated with IGF-1, the difference was not significant ($P = .09$). The time course for the ability of IGF-I to increase serotonin levels in the ventral hippocampus coincides with the time course for the appearance of behavioral effects in the FST.

Fig. 6.

Cell proliferation in the dentate gyrus of the hippocampus was not affected 3 days after IGF-I administration. The number of cells labeled with BrdU in the subgranular zone of the dentate gyrus were counted and compared to vehicle controls. The thymidine analog BrdU (100 mg/kg, i.p.) was injected 3 days after a single administration of either vehicle or IGF-I (1 μ g; n = 7 each). Rats were perfused 2 h later and immunohistochemistry for BrdU performed to visualize labeled cells on the sectioned tissue. Values represent mean $\pm$ 1 S.E.M.

Table 1

Relative mRNA levels of TPH1, TPH2 or SERT in the brain stem after administration of IGF-I or vehicle, as measured by RT-PCR

Gene	Treatment	Relative mRNA level
TPH 1	Vehicle	100.0 $\pm$ 15.0
	IGF-I	99.0 $\pm$ 12.0
TPH 2	Vehicle	10.2 $\pm$ 2.8
	IGF-I	11.2 $\pm$ 2.3
SERT	Vehicle	30.3 $\pm$ 4.6
	IGF-I	29.0 $\pm$ 2.9

Relative expression levels of mRNA for genes for tryptophan hydroxylase (*tph1* and *tph2*) or the serotonin transporter (SERT) were measured in the brain stem 3 days after administration of either vehicle (aCSF; n = 9) or IGF-I (1 μ g; n = 8). The mRNA levels were measured by RT-PCR and quantified relative to an internal housekeeping gene (hypoxanthine-guanine phosphoribosyltransferase, HPRT).

Table 2

[³H] citalopram binding levels after administration of IGF-I or vehicle.

Measurement	Brain Region	Vehicle	IGF-I
Kd (nM)	Hippocampus	1.06 ± 0.14	1.17 ± 0.05
	Frontal Cortex	1.56 ± 0.09	1.55 ± 0.10
Bmax (pmol/mg protein)	Hippocampus	0.83 ± 0.06	0.68 ± 0.17
	Frontal Cortex	1.57 ± 0.01	1.42 ± 0.28

[³H]citalopram binding was not affected 3 days after IGF-I administration. Rats were administered either vehicle (aCSF; n = 9) or IGF-I (1 µg; n = 8) and then decapitated 3 days later. The hippocampus and frontal cortex were removed, and analyzed for [³H]citalopram binding *in vitro*.