Increasing the Levels of Insulin-Like Growth Factor-I by an IGF Binding Protein Inhibitor Produces Anxiolytic and Antidepressant-Like Effects

Jessica E Malberg*,[1], Brian Platt[1], Stacey J Sukoff Rizzo[1], Robert H Ring[1], Irwin Lucki[2], Lee E Schechter[1] and Sharon Rosenzweig-Lipson[1]

[1]Wyeth Research, Discovery Neuroscience, Princeton, NJ, USA; [2]Department of Psychiatry, University of Pennsylvania, Philadelphia, PA, USA

The present studies were conducted to determine if increasing central levels of the neurotrophic factor insulin-like growth factor-I (IGF-I) either directly or indirectly produces anxiolytic and antidepressant-like effects in the mouse. Central levels of IGF-I can be increased directly, by administering IGF-I, or indirectly by blocking the insulin-like growth factor binding proteins (IGFBPs). The IGFBP family has the unique ability to regulate IGF-I levels by sequestering IGF-I into an inactive complex. Therefore, an IGFBP inhibitor increases the level of IGF-I available to bind to its receptor. Intracerebroventricular (icv) administration of the nonspecific IGFBP inhibitor NBI-31772 (10–30 μg) increases the number of punished crossings in the four-plate test and NBI-31772 (0.3–10 μg) increases time spent in the open quadrant of the elevated zero maze (EZM), indicative of anxiolytic-like effects. NBI-31772 (3–30 μg) also decreases immobility time in the tail suspension test, indicative of antidepressant-like effects. Similarly, icv administration of IGF-I (0.1 μg) produces anxiolytic-like effects in the four-plate test and IGF-I (0.3–1 μg) produces anxiolytic-like effects in the EZM. IGF-I (10 μg) also produces antidepressant-like effects in the tail suspension test. Coadministration of the IGF-I receptor antagonist JB1 with NBI-31772 or IGF-I blocks the anxiolytic-like and antidepressant-like effects of these compounds. These results suggest that NBI-31772 produces behavioral effects by increasing levels of IGF-I that in turn activate the IGF-I receptor. The present studies demonstrate that an IGFBP inhibitor mimics the behavioral effects of IGF-I and that IGFBP inhibition may represent a novel mechanism by which to increase IGF-I to treat depression and anxiety.
Neuropsychopharmacology (2007) **32,** 2360–2368; doi:10.1038/sj.npp.1301358; published online 7 March 2007

INTRODUCTION

Despite the high prevalence of depressive and anxiety disorders in the population (Berton and Nestler, 2006; Kessler *et al*, 2005; Malberg and Schechter, 2005), our understanding of the mechanisms by which antidepressant and anxiolytic drugs produce their effects are limited. The current generation of antidepressant drugs primarily modulate neurotransmitter systems by indirectly elevating levels of monoamines (Schechter *et al*, 2005). More recent clinical and preclinical research in depression (Duman, 2004; Nestler *et al*, 2002) has begun to focus on the role of growth factors and neurotrophic factors in depression and antidepressant action. Clinically effective antidepressant drugs increase adult hippocampal neurogenesis in rodents, and a current hypothesis is that neurotrophins or drugs that modulate plasticity-related proteins or growth factors may provide the next generation of antidepressant or anxiolytic drugs (Malberg and Schechter, 2005).

*Correspondence: Dr JE Malberg, Psychogenics, Inc., 765 Old Saw Mill River Road, Tarrytown, NY 10591, USA, Tel: 914 406 8022, Fax: 914 593 0645, E-mail: Jessica.Malberg@psychogenics.com

Insulin-like growth factor-1 (IGF-I) has a number of growth-promoting effects in the central nervous system (CNS), which qualify this molecule as a neurotrophin (Kim *et al*, 1998, 2004; Kurihara *et al*, 2000). IGF-I was first named 'somatomedin C' owing to its ability to mediate the effect of somatotropin (growth hormone) (Le Roith *et al*, 2001). In the late 1970s somatomedin C was renamed IGF-I because of its sequence similarity with insulin. Both central (icv) and systemic administration of IGF-I increases hippocampal cell proliferation and neurogenesis in the adult rat (Aberg *et al*, 2000; Anderson *et al*, 2002). These same effects are seen after administration of clinically effective antidepressant drugs. Moreover, central administration of IGF-I produces antidepressant-like effects in the rat forced swim test (Hoshaw *et al*, 2005).

An IGFBP inhibitor can be used to increase IGF-I levels owing to the unique properties of the IGFBP family (Wetterau *et al*, 1999). The IGFBP family exerts a precise regulation of IGF-I levels throughout the CNS and peripheral nervous systems (for a review, see Wetterau *et al*, 1999). The majority of circulating IGF-I is not available in a 'free pool' for IGF-I receptor (IGF-IR) binding and activation. Instead, the majority of IGF-I exists in an inactive IGF-I:IGFBP complex. The insulin-like growth

factor binding proteins (IGFBPs) act as functional antagonists by sequestering IGF-I into the inactive IGF-I:IGFBP complex. An IGFBP inhibitor has the net effect of releasing free IGF-I from the inactive IGF-I:IGFBP complex and increasing the amount of free IGF-I available for IGF-IR binding.

NBI-31772 is a nonspecific IGFBP inhibitor, which has been shown to mimic the effects of IGF-I in both in vitro and in vivo assays (Liu et al, 2001; Mackay et al, 2003). This nonpeptide ligand binds with high affinity to all six members of the IGFBP family (Liu et al, 2001) and has only low affinity for the IGF-IR and IGF-IIR (Chen et al, 2001; Loddick et al, 1998). NBI-31772 has been shown to displace IGF-I from the inactive IGF-I:IGFBP complex at low nanomolar concentrations, resulting in active IGF-I. In an IGF-I-mediated cell proliferation assay, the IGFBPs produced an inhibition of cell proliferation, which was reversed by NBI-31772. In vivo, NBI-31772 produced neuroprotective effects in a similar manner to exogenously administered IGF-I (Mackay et al, 2003).

In the present studies, NBI-31772 was administered centrally (icv) to determine whether inhibition of the IGFBPs can be used to produce antidepressant or anxiolytic-like effects similar to IGF-I. Additional studies were conducted to determine whether the IGF-IR antagonist, JB1, could block the effects of IGF-I and NBI-31772 to confirm that IGF-I and NBI-31772 produce their behavioral effects via activation of IGF-IR. The present studies demonstrate that increasing central levels of IGF-I by administration of an IGFBP inhibitor produces anxiolytic-like and antidepressant-like behavioral effects in the mouse in vivo, similar to IGF-I administration. Moreover, these effects are blocked by the IGF-IR antagonist JB1, indicating that these effects are mediated via increased IGF-I acting at the IGF-IR. Taken together, these results suggest that increasing IGF-I levels via an IGFBP inhibitor may represent a novel treatment for depression and anxiety.

MATERIALS AND METHODS

Animals

Male mice weighing 18–24 g were housed in groups of four in hanging wire cages, allowed access to food and water ad libitum, and maintained on a 12-h light–dark cycle, with lights on at 0600. All behavioral testing was performed during the light cycle. Separate strains of mice were used in different behavioral tests based on the best combination of baseline behavior and response to reference compounds. Swiss–Webster mice were used for the four-plate and tail suspension tests and Balb/C mice were used for the elevated zero maze (EZM). All studies were previously approved by the Institutional Animal Care and Use Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Test Compounds

IGF-I (recombinant human IGF-I; Bachem), JB1 (Bachem), and NBI-31772 (Calbiochem) were all dissolved in a water vehicle.

ICV Injections

Mice were lightly anesthetized with halothane. Test compounds were administered into either the left or right ventricle by visual location, and all compounds were administered 20 min before the test session. For experiments where two icv injections were given, an animal received an injection in both the left and right ventricles, with the second injection given immediately after the first injection. A 26-gauge Hamilton syringe with 3 mm needle was used for injections and the injection site was visualized by locating the middle of the invisible line that runs diagonally from the left eye to the right ear. Test compounds were injected in a 2 μl total volume. This injection method has been previously validated in our laboratory (Ring et al, 2006).

Four-Plate Test

The four-plate apparatus consists of a Plexiglas chamber ($18 \times 25 \times 16\,cm^3$) with a floor consisting of four rectangular metal plates ($8 \times 11\,cm^2$), which are separated from one another by a 4 mm gap. All four plates are wired to a shock generator (Med Associates). In each experiment, the mouse is placed into the chamber and given an 18-s habituation period followed by a 1-min test. During the test, the animal's innate tendency to explore the novel environment is suppressed by the delivery of a mild foot shock (0.8 mA, 3 s) every time the animal moves from one plate to another (referred to as a 'punished crossing'). During each shock, there is a 3-s time out where no further shocks are administered by the investigator and crossings are not recorded during this time period. Experimental sessions are 1 min in length and the number of punished crossings is recorded by a computer attached to the shocker device. Clinically effective classes of anxiolytic compounds such as benzodiazepines, selective serotonin reuptake inhibitors, or partial 5-HT$_{1A}$ agonists produce increases in punished crossings, which is indicative of anxiolytic-like activity (Aron et al, 1971; Bourin et al, 1992; Hascoet et al, 2000).

EZM

The EZM is a modification of the elevated plus maze and consists of a circular platform (outer diameter approximately 60 cm, width 5 cm) that is elevated 55 cm above the floor, and made of black Perspex (Shepherd et al, 1994). The EZM features two open and two enclosed (closed) quadrants. The closed quadrants have walls extending 20 cm above the surface of the maze, whereas the open quadrants have a lip, constructed of clear Perspex, extending 3 mm above the surface of the maze. In this paradigm, a mouse is placed onto the EZM for a 5-min test period and has free access to explore all quadrants of the maze. This location and all subsequent movement is recorded using Ethovision video tracking software (Noldus IT, Netherlands). The percentage of time the animal spends in the open or closed quadrants is recorded and analyzed. In this paradigm, as a result of the aversive properties of the open quadrants, animals spend a greater proportion of time in the closed quadrants. Compounds that increase the percentage time an animal spends in the open quadrants

of the EZM, compared to the closed quadrants, are considered to exhibit anxiolytic-like activity (Kash et al, 1999; Pellow et al, 1985).

Tail Suspension Test

The procedure followed in this study is a variant of the one originally described by Steru et al (1985). In this test, mice are suspended upside down by taping their tails with adhesive laboratory tape (VWR International) to a flat metal bar connected to a force transducer within a sound-attenuating chamber (Med Associates). The bar is positioned such that a mouse is not able to grasp the top or the sides of the chamber. The total time spent immobile during a 6-min test session is automatically recorded. Compounds that decrease the total immobility time compared to control animals are predicted to have antidepressant-like effects. In each study, eight mice were tested simultaneously in separate chambers.

Locomotor Activity Test

The locomotor activity chambers are Plexiglas chambers (16 in × 16 in × 20 in). During each test, mice are individually placed into the chamber and spontaneous locomotor activity is measured for a 6-min period using an automated infrared beam system (Versamax; Accuscan, Columbus,

OH). The computer and software converts the infrared beam breaks into total activity counts for analysis.
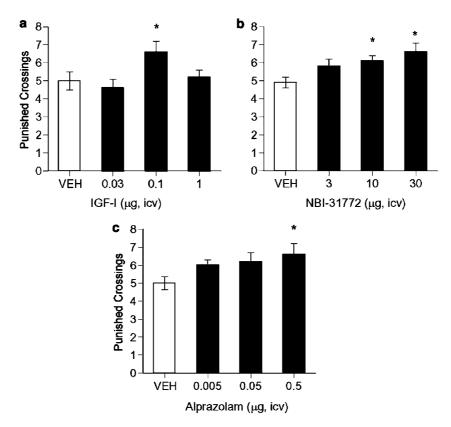
Statistical Analysis

One-way analysis of variance (ANOVA) was performed on behavioral data to determine effects of test compound treatments, followed by least significant difference tests for a post hoc analysis. All data in figures are the mean ± SEM.

RESULTS

Anxiolytic-Like Effects of IGF-I and the IGFBP Inhibitor NBI-31772 in the Mouse Four-Plate Test

In the mouse four-plate test, central administration of IGF-I (0.03–1 μg) produced a significant overall effect on punished crossings (Figure 1a; $F(3,36) = 3.153$, $p < 0.05$). Post hoc analysis revealed a significant increase in punished crossings after the 0.1 μg dose of IGF-I (32% increase from vehicle; $p < 0.03$). This increase in punished crossings indicates putative anxiolytic-like activity of IGF-I.
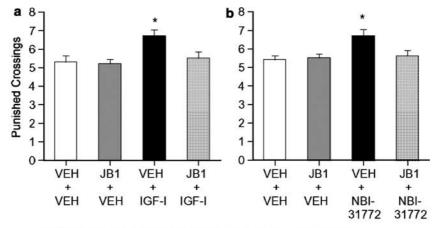
NBI-31772 (3–30 μg, icv) also produced a dose-dependent increase in punished crossings in this model (Figure 1b; $F(5,74) = 13.215$, $p < 0.001$). Post hoc analysis revealed a significant increase in punished crossings at 10 and 30 μg (22 and 32% for 10 and 30 μg, respectively; $p < 0.05$). At

*p<0.05 compared to vehicle (VEH) treated group, n=10 per group

Figure 1 The effects of IGF-I or NBI-31772 or alprazolam on behavior in the mouse four-plate test. Compounds were administered 20 min before testing and the number of punished crosses during the 1-min test was recorded. (a) IGF-I (0.1 μg) produced a significant increase in punished crossings, which is indicative of an anxiolytic-like effect. (b) NBI-31772 produced a dose–dependent increase in punished crossings, which reached significance at the 10 and 30 μg doses. (c) The reference anxiolytic compound alprazolam produced a dose–dependent increase in punished crossings, which reached significance at 0.5 μg.

*p<0.05 compared to vehicle (VEH) treated group, n=10 per group

Figure 2 The effects of the combination of IGF-I or NBI-31772 with the IGF-IR antagonist JB1 on behavior in the mouse four-plate test. Compounds were administered 20 min before testing and the number of punished crosses during the 1-min test was recorded. (a) IGF-I (0.1 µg) produced an anxiolytic-like increase in punished crossings, which was completely blocked by coadministration of JB1 (30 µg). (b) NBI-31772 (30 µg) produced an anxiolytic-like increase in punished crossings, which was completely blocked by coadministration of JB1 (10 µg).

higher doses of NBI-31772 (56 and 100 µg), an increase in punished crossings was observed, although seizures were observed in a percentage of animals in both groups (20 and 40% of animals in the 56 and 100 µg group, respectively) and these doses were not used for analysis. The increase in punished crossings at 10 and 30 µg indicates putative anxiolytic-like activity of NBI-31772.

In comparison, central administration of the reference anxiolytic compound alprazolam (0.005–0.5 µg, icv) produced a dose–dependent increase in punished crossings (Figure 1c). Although the overall ANOVA did not reach significance ($p = 0.10$), planned *post hoc* comparison tests indicated that the 0.5 µg dose produced a significant increase in punished crossings compared to vehicle (32%, $p < 0.05$).

Blockade of the Anxiolytic-Like Effects of IGF-I or NBI-31772 by the IGF-I Antagonist JB1

Combination studies with the IGF-1 antagonist JB1 and IGF-1 were conducted in the mouse four-plate test to determine whether the anxiolytic-like effects of IGF-I are mediated by the IGF-IR. Experiments performed in our laboratory had determined that centrally administered JB1 (3–30 µg) had no effect on punished crossings (data not shown; $p > 0.05$ compared to vehicle group at all doses tested). IGF-I (0.1 µg) produced the expected anxiolytic-like effect of increased punished crossings (Figure 2a; $F(3,36) = 4.785$, $p < 0.01$; 25% increase compared to vehicle group; $p < 0.004$) and JB1 (30 µg) had no effect on punished crossings. When IGF-I was administered in combination with JB1, the anxiolytic-like effects of IGF-I were completely blocked, suggesting that the anxiolytic-like effects of IGF-I are mediated through the IGF-IR.

Similar studies were conducted to determine if the IGF-IR mediates the anxiolytic-like effects of NBI-31772 in a similar manner to those seen with IGF-I. Under these conditions, NBI-31772 (30 µg) increased punished crossings (Figure 2b; $F(3,36) = 8.634$, $p < 0.005$; 24% increase compared to vehicle group; $p < 0.003$), but when administered in combination with JB1 (10 µg), the anxiolytic-like effects of NBI-31772 were

completely blocked. Taken together, these data suggest that NBI-31772 produces its anxiolytic-like effects by increasing free active IGF-I to bind to and activate the IGF-IR.

Anxiolytic-Like Effects of IGF-1 and NBI-31772 in the Mouse EZM

In the mouse EZM, central administration of IGF-I (0.1–1 µg) produced a significant overall effect on the percent time spent in the open arms of the maze (Figure 3a, $F(3,36) = 4.789$, $p < 0.01$). *Post hoc* analysis revealed that the 0.3 and 1 µg doses significantly increased the percent time spent in the open arms compared to the vehicle group (91 and 159% respectively, $p < 0.05$). This increase in percent time in the open arms of the EZM is indicative of an anxiolytic-like effect of IGF-1.
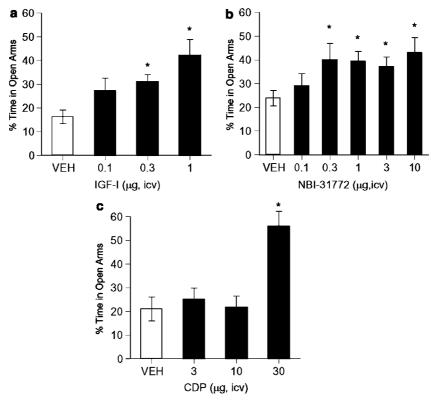
Centrally administered NBI-31772 (0.1–10 µg, icv) also produced a dose–dependent increase in the percent time spent in the open arms of the maze (Figure 3b; $F(5,81) = 3.613$, $p < 0.05$). *Post hoc* analysis revealed that the 0.3–10 µg doses produced significant increases in the percent time in the open arms compared to the vehicle group (67, 65, 55, and 79% increase in the 0.3, 1, 3, and 10 µg groups, respectively, $p < 0.05$). These effects are indicative of anxiolytic-like activity of NBI-31772 in the EZM.

In comparison, central administration of the reference anxiolytic compound chlordiazepoxide (CDP; 3–30 µg) increased the percent time spent in the open arms of the EZM (Figure 3c; $F(3,35) = 10.221$, $p < 0.001$), with the 30 µg dose producing a significant increase in the percent time spent in the open arms compared to the vehicle group (165% increase, $p < 0.0001$).

Antidepressant-Like Effects of IGF-I and NBI-31772 in the Mouse Tail Suspension Test

In the mouse tail suspension test, IGF-I (1–10 µg) produced a dose-dependent decrease in immobility (Figure 4a; $F(3,43) = 3.71$, $p < 0.05$). *Post hoc* analysis revealed that the 10 µg dose produced a significant decrease in immobility

Figure 3 The effects of IGF-I or NBI-31772 or CDP on behavior in the mouse EZM. Compounds were administered 20 min before testing and the percent time spent in the open arms of the EZM during the 5-min test was recorded. (a) IGF-I produced a dose–dependent increase in the percent time spent in the open arms of the EZM, which reached significance at 0.3 and 1 μg. These data are indicative of an anxiolytic-like effect. (b) NBI-31772 produced a dose–dependent increase in the percent time spent in the open arms, which reached significance at 0.3–10 μg. (c) The reference anxiolytic compound CDP produced an increase in the percent time spent in the open arms of the EZM, which reached significance at 30 μg.

(33% decrease from vehicle; $p < 0.003$). This decrease in immobility indicates a putative antidepressant-like effect and is in agreement with previous data demonstrating antidepressant-like activity of IGF-I in the rat (Hoshaw *et al*, 2005).

Similarly, NBI-31772 (0.3–30 μg) produced a dose-dependent decrease in immobility (Figure 4b; $F_{(5,70)} = 5.222$, $p < 0.005$). *Post hoc* analysis revealed that the three highest doses (3–30 μg) produced a significant decrease in immobility (32, 35, and 38% decrease in the 3, 10, and 30 μg groups, respectively; $p < 0.03$). This decrease in immobility indicates antidepressant-like effects similar to that seen with IGF-I.

In comparison, the reference antidepressant compound fluoxetine (10–30 μg) produced a dose–dependent decrease in immobility (Figure 4c; $F_{(3,44)} = 11.306$; $p < 0.001$), which reached significance at 30 μg (54% decrease compared to vehicle, $p < 0.001$).

Blockade of the Antidepressant-Like Effects of IGF-I or NBI-31772 by the IGF-I Antagonist JB1

Studies were conducted in the mouse tail suspension test to determine if the antidepressant-like effects of IGF-I are mediated by the IGF-IR. IGF-I (10 μg) produced the expected antidepressant-like effects of decreased immobility time (Figure 5a; $F_{(3.36)} = 5.129$, $p < 0.005$; 22% decrease compared to vehicle group, $p < 0.02$). Although JB1 (30 μg) alone had no effect on immobility time in this model, JB1 blocked the antidepressant-like effects of IGF-I. This

suggests that the antidepressant-like effects of IGF-I are mediated by the IGF-IR.
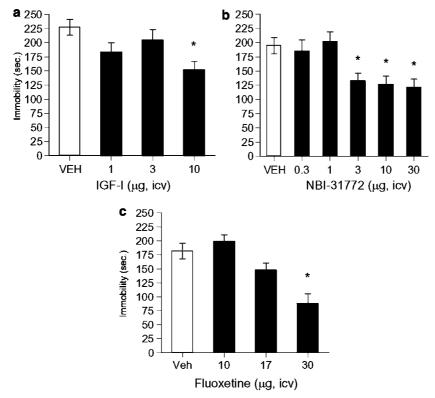
To determine if the IGF-IR also mediates the antidepressant-like effect of NBI-31772 in the tail suspension test, a similar study was conducted as with IGF-I. NBI-31772 (10 μg) produced a significant decrease in immobility time as expected (Figure 5b; $F_{(3,36)} = 3.228$, $p < 0.05$; 47% decrease compared to vehicle group, $p < 0.02$). Coadministration of JB1 (30 μg) blocked the antidepressant-like effects of NBI-31772 ($p < 0.05$ compared to vehicle group). These data indicate that NBI-31772 produces its antidepressant-like effects by increasing IGF-I to activate the IGF-IR, in a similar mechanism by which it produces anxiolytic-like effects.

IGF-I and NBI-31772 have no Effect on Spontaneous Locomotor Activity

IGF-I (0.03–10 μg, icv) had no effect on spontaneous locomotor activity, as measured by activity counts, compared to vehicle-treated controls ($p > 0.05$; Figure 6a). Similarly, NBI-31772 (3–30 μg, icv) had no effect on locomotor activity at any dose tested ($p > 0.05$ compared to vehicle; Figure 6b).
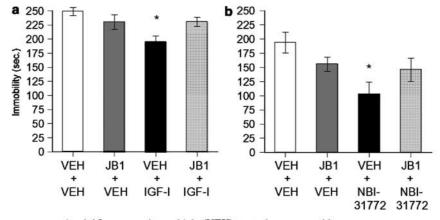
DISCUSSION

The present studies demonstrate that increasing the level of IGF-I in the CNS via an IGFBP inhibitor produces both

Figure 4 The effects of IGF-I or NBI-31772 or fluoxetine on behavior in the mouse tail suspension test. IGF-I or NBI-31772 was administered 20 min before testing and the total time (seconds) spent immobile was calculated. (a) IGF-I produced a dose–dependent decrease in immobility, which reached significance at 10 μg. These data are indicative of an antidepressant-like effect. (b) NBI-31772 produced a dose–dependent decrease in immobility, which reached significance at 3–30 μg. (c) The reference antidepressant compound fluoxetine produced a dose-dependent decrease in immobility, which reached significance at 56 μg.

Figure 5 The effects of the combination of IGF-I or NBI-31772 with the IGF-IR antagonist JB1 on behavior in the mouse tail suspension test. Compounds were administered 20 min before testing and the total time (seconds) spent immobile was calculated. (a) IGF-I (10 μg) produced an antidepressant-like decrease in immobility, which was blocked by co-administration of JB1 (30 μg). (b) NBI-31772 (10 μg) produced an antidepressant-like decrease in immobility, which was blocked by coadministration of JB1 (30 μg).

anxiolytic and antidepressant-like effects in the mouse, similar to those observed after IGF-I administration. Further, these effects are blocked by administration of an IGF-IR antagonist suggesting that these effects may be mediated by activation of the IGF-IR.

The IGFBP inhibitor, NBI-31772, binds with high affinity to all six IGFBPs, and is inactive at the IGF-I and IGF-II receptors (Chen et al, 2001; Loddick et al, 1998; Mackay et al, 2003). By blocking the ability of IGF-I to bind to the IGFBPs, NBI-31772 breaks apart or inhibits the IGF-I:IGFBP complex, releasing IGF-I into the CSF. The released IGF-I can, in turn, activate both IGF-IR and IGF-IIR. Thus, it is possible that the anxiolytic-like effect of NBI-31772 is not owing to direct activation of the IGF-IR or IGF-IIR but as a result of increasing the levels of IGF-I, which then activates the IGF-IR or IGF-II (Figure 7).
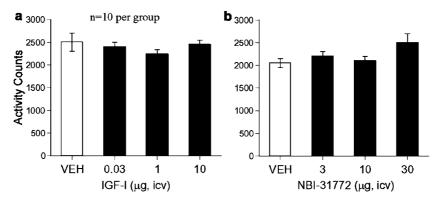
Figure 6 The effects of IGF-I or NBI-31772 on spontaneous locomotor activity. IGF-I or NBI-31772 was administered 20 before testing and the total activity count (number of beam breaks) was calculated. (a) IGF-I had no effect on locomotor activity at any dose tested. (b) NBI-31772 had no effect on locomotor activity at any dose tested.

Figure 7 Schematic of anxiolytic-like actions of IGF-I and the IGF binding protein inhibitor NBI-31772. (a) IGF-IR activation is responsible for the anxiolytic-like effect of IGF-I, based on the finding that the IGF-IR antagonist JB1 blocks the anxiolytic-like effect of IGF-I in the mouse four-plate test. (b) For NBI-31772, we hypothesize that the compound inhibits or breaks apart the inactive IGF-I:IGFBP complex, releasing active IGF-I. The released IGF-I then activates the IGF-IR, producing an anxiolytic-like effect that can be blocked by the IGF-IR antagonist JB1, in a similar manner to IGF-I.

IGF-I signals through the IGF-IR via a multiprotein signaling complex (Ye and D'Ercole, 2006). This receptor signaling pathway activates both the Ras/mitogen-activated protein kinase pathway and the PI3 kinase–AKT pathways (Brunet et al, 2001; Ye and D'Ercole, 2006). These pathways share a high degree of overlap with the signaling pathways of 5-HT and BDNF, which are also implicated in antidepressant action (Mattson et al, 2004). It has also been shown that chronic icv infusion of IGF-I increases hippocampal 5-HT levels (Malberg et al, 2005). Therefore, IGF-IR activation may involve other growth factors as well neurotransmitter release.

IGF-I also binds to the IGF-IIR, also known as the IGF-II/mannose-6-phospate receptor, albeit at a much reduced potency (>100-fold difference) than for the IGF-I (Loddick et al, 1998). In contrast to the IGF-IR, the IGF-IIR primarily acts on intracellular trafficking of enzymes and IGF-II clearance (Hawkes and Kar, 2003; Yee, 2006), although recently Hawkes et al (2006) demonstrated that the IGF-IIR activates a G-protein sensitive, protein kinase C dependent pathway.

JB1 is an IGF-1R antagonist, which is highly selective for the IGF-IR relative to the IGF-IIR (Elmlinger et al, 1998). JB1 completely blocked both the anxiolytic and antidepressant-like effects of IGF-1, consistent with IGF-1R mediation of these behavioral effects. JB1 also completely blocked the effects of the IGFBP inhibitor NBI-31772, suggesting that the behavioral effects of NBI-31772 may be mediated via increased levels of IGF-1 that act at the IGF-1R. Although a role for the IGF-IIR cannot be excluded, the results with JB1 are suggestive that the IGF-IR plays a critical role in these effects (Figure 7). Taken together, this demonstrates that

NBI-31772 mimics the behavioral effects of IGF-I and that the IGF-IR may mediate these effects.

NBI-31772 has been shown to have functional effects of mimicking IGF-I administration both *in vivo* and *in vitro*. *In vivo*, NBI-31772 produces neuroprotective effects in a similar manner to exogenously administered IGF-I (Mackay et al, 2003). *In vitro*, NBI-31772 increases cell proliferation in a similar manner to IGF-I (Liu et al, 2001). In addition, NBI-31772 can antagonize the inhibitory effect of IGFBP3 on anabolic responses in human chondrocytes (De Ceuninck et al, 2004). This indicates that the IGFBPs act as inhibitory functional regulators of IGF-I and that NBI-31772 can substitute for exogenous administration of IGF-I.

Given that NBI-31772 is a nonspecific inhibitor of all six IGFBPs, it is currently unknown which IGFBPs may mediate the observed anxiolytic-like and antidepressant-like responses. There are six members of the IGFBP family, which are found throughout the peripheral and central nervous systems (Shimasaki and Ling, 1991). Although also found in the periphery, IGFBP2 and IGFBP4 are highly localized in the CSF, hippocampus, amygdala, cortex, thalamus, and basal ganglia, and are expressed in adult hippocampal progenitor cell cultures (Aberg et al, 2003; Brar and Chernausek, 1993; Logan et al, 1994). Further research is clearly needed to delineate the roles of each of the IGFBPs.

The demonstration that an IGFBP inhibitor can be used to functionally mimic IGF-I may allow for specificity in elevating IGF-I in brain regions of interest without elevating IGF-I peripherally. IGF-I induces cell proliferation in multiple cell types and organ systems that may result in undesirable side effects (Yee, 2006). Therefore, inhibition of CNS-specific IGFBPs may produce increases in brain IGF-I without affecting peripheral IGF-I *in vivo*.

In the present studies, the anxiolytic-like and antidepressant-like behavioral effects of icv IGF-I occur within 20 min of infusion. This is in contrast to our previous results in the rat forced swim test (Hoshaw et al, 2005), where IGF-1 was infused 3 days before testing in order to produce an antidepressant-like effect. The delayed response to IGF-1 in the rat forced swim test suggests that a long-term cascade is involved in that effect, whereas the current findings are consistent with a more rapid effect of IGF-1.

A recent study by Nagaraja et al (2005) has investigated the time course of IGF-I clearance in the rat after a single icv

infusion of radiolabeled IGF-I. These investigators demonstrated that within minutes after the infusion, IGF-I spreads from the lateral ventricle into the 3rd and 4th ventricles. At the 20-min timepoint, which corresponds to the timepoint that the animals in the present study were tested, the majority of IGF-I was cleared out of the CSF, with 1.5 mm penetration into the caudate–putamen and periaqueductal grey of the rat brain. Although IGF-1R mRNA and protein expression has been demonstrated in multiple regions of the CNS, it is currently unknown where the IGF-I binds to the IGF-IR to exert the observed behavioral effects. However, in the mouse used in this study, IGF-1 may be hypothesized to reach additional structures such as the hippocampus and the amygdala, which are areas implicated in depression and antidepressant action (Nestler *et al*, 2002) and express IGF-1R mRNA (Araujo *et al*, 1989). Further research will determine the cellular localization of the relevant IGF-1Rs and the specific IGF-1-related pathways that mediate the acute antidepressant and anxiolytic-like effects of IGF-1.

It has been shown that IGF-I produces increases in cell proliferation and neurogenesis *in vivo* (Aberg *et al*, 2000). However, the short time course (20 min) needed to produce the behavioral effects of IGF-1 contrasts with the longer time course necessary for IGF-1 to increase cell proliferation. This indicates that the behavioral and neurogenic effects of IGF-1 may be differentially regulated. This difference in time course between the acute behavioral effects and the chronic administration necessary for cell proliferation has also been seen with clinically effective antidepressant such as fluoxetine (Malberg *et al*, 2000). An understanding of the relationship between IGF-1-induced changes in behavior and IGF-1-induced changes in neurogenesis is necessary and currently under investigation by a number of laboratories.

Clinically, reduced serum IGF-I levels are seen in patients with growth hormone (GH) deficiency and depressive symptoms have been reported in these populations (Wallymahmed *et al*, 1996), although this has not been seen in all studies (Zenker *et al*, 2002). In these patients, GH therapy is the standard treatment, which normalizes IGF-I levels. Interestingly, Pavel *et al* (2003) have demonstrated that adult patients who received GH treatment had an improvement in mood. In contrast, cessation of GH treatment, which produced a decrease in IGF-I levels, also produced an increase in depression and other negative symptoms and complaints (McMillan *et al*, 2003; Stouthart *et al*, 2003). This effect can be counteracted in young adults by restarting GH treatment and once again normalizing IGF-I levels (Stouthart *et al*, 2003). In addition, children with GH deficiency (compared to children with normal GH but short stature) exhibit both depression and anxiety, and these conditions are reduced by GH treatment (Stabler, 2001). Taken together, it can be seen that patient populations with IGF-I deficiency exhibit anxious or depressed moods, which can be treated by GH therapy that increases IGF-I levels.

In summary, we report that increasing IGF-I levels via an IGFBP inhibitor produces anxiolytic-like and antidepressant-like effects, which are likely mediated by IGF-IR activation. Importantly, an IGFBP inhibitor mimics the effect of exogenous administration of IGF-I in these tests. Taken together, these data point to inhibition of IGFBPs as a novel mechanism for anxiolytic and antidepressant therapy.

REFERENCES

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS (2000). Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J Neurosci* **20**: 2896–2903.

Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P *et al* (2003). IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol Cell Neurosci* **24**: 23–40.

Anderson MF, Aberg MA, Nilsson M, Eriksson PS (2002). Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. *Brain Res Dev Brain Res* **134**: 115–122.

Araujo DM, Lapchak PA, Collier B, Chabot JG, Quirion R (1989). Insulin-like growth factor (somatomedin-C) receptors in the rat brain: distribution and interaction with the hippocampal cholinergic system. *Brain Res* **484**: 130–138.

Aron C, Simon P, Larousse C, Boissier JR (1971). Evaluation of a rapid technique for detecting minor tranquilizers. *Neuropharmacology* **10**: 459–469.

Berton O, Nestler EJ (2006). New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci* **7**: 137–151.

Bourin M, Hascoet M, Mansouri B, Colombel MC, Bradwejn J (1992). Comparison of behavioral effects after single and repeated administrations of four benzodiazepines in three mice behavioral models. *J Psychiatry Neurosci* **17**: 72–77.

Brar AK, Chernausek SD (1993). Localization of insulin-like growth factor binding protein-4 expression in the developing and adult rat brain: analysis by *in situ* hybridization. *J Neurosci Res* **35**: 103–114.

Brunet A, Datta SR, Greenberg ME (2001). Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. *Curr Opin Neurobiol* **11**: 297–305.

Chen C, Zhu YF, Liu XJ, Lu ZX, Xie Q, Ling N (2001). Discovery of a series of nonpeptide small molecules that inhibit the binding of insulin-like growth factor (IGF) to IGF-binding proteins. *J Med Chem* **44**: 4001–4010.

De Ceuninck F, Caliez A, Dassencourt L, Anract P, Renard P (2004). Pharmacological disruption of insulin-like growth factor 1 binding to IGF-binding proteins restores anabolic responses in human osteoarthritic chondrocytes. *Arthritis Res Ther* **6**: R393–R403.

Duman RS (2004). Role of neurotrophic factors in the etiology and treatment of mood disorders. *Neuromolecular Med* **5**: 11–25.

Elmlinger MW, Sanatani MS, Bell M, Dannecker GE, Ranke MB (1998). Elevated insulin-like growth factor (IGF) binding protein (IGFBP)-2 and IGFBP-4 expression of leukemic T-cells is affected by autocrine/paracrine IGF-II action but not by IGF type I receptor expression. *Eur J Endocrinol* **138**: 337–343.

Hascoet M, Bourin M, Colombel MC, Fiocco AJ, Baker GB (2000). Anxiolytic-like effects of antidepressants after acute administration in a four-plate test in mice. *Pharmacol Biochem Behav* **65**: 339–344.

Hawkes C, Kar S (2003). Insulin-like growth factor-II/mannose-6-phosphate receptor: widespread distribution in neurons of the central nervous system including those expressing cholinergic phenotype. *J Comp Neurol* **458**: 113–127.

Hawkes C, Jhamandas JH, Harris KH, Fu W, MacDonald RG, Kar S (2006). Single transmembrane domain insulin-like growth factor-II/mannose-6-phosphate receptor regulates central cholinergic function by activating a G-protein-sensitive, protein kinase C-dependent pathway. *J Neurosci* **26**: 585–596.

Hoshaw BA, Malberg JE, Lucki I (2005). Central administration of IGF-I and BDNF leads to long-lasting antidepressant-like effects. *Brain Res* **1037**: 204–208.

Kash SF, Tecott LH, Hodge C, Baekkeskov S (1999). Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci USA* **96**: 1698–1703.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* **62**: 593–602.

Kim B, Leventhal PS, White MF, Feldman EL (1998). Differential regulation of insulin receptor substrate-2 and mitogen-activated protein kinase tyrosine phosphorylation by phosphatidylinositol 3-kinase inhibitors in SH-SY5Y human neuroblastoma cells. *Endocrinology* **139**: 4881–4889.

Kim B, van Golen CM, Feldman EL (2004). Insulin-like growth factor-I signaling in human neuroblastoma cells. *Oncogene* **23**: 130–141.

Kurihara S, Hakuno F, Takahashi S (2000). Insulin-like growth factor-I-dependent signal transduction pathways leading to the induction of cell growth and differentiation of human neuroblastoma cell line SH-SY5Y: the roles of MAP kinase pathway and PI 3-kinase pathway. *Endocr J* **47**: 739–751.

Le Roith D, Bondy C, Yakar S, Liu JL, Butler A (2001). The somatomedin hypothesis: 2001. *Endocr Rev* **22**: 53–74.

Liu XJ, Xie Q, Zhu YF, Chen C, Ling N (2001). Identification of a nonpeptide ligand that releases bioactive insulin-like growth factor-I from its binding protein complex. *J Biol Chem* **276**: 32419–32422.

Loddick SA, Liu XJ, Lu ZX, Liu C, Behan DP, Chalmers DC et al (1998). Displacement of insulin-like growth factors from their binding proteins as a potential treatment for stroke. *Proc Natl Acad Sci USA* **95**: 1894–1898.

Logan A, Gonzalez AM, Hill DJ, Berry M, Gregson NA, Baird A (1994). Coordinated pattern of expression and localization of insulin-like growth factor-II (IGF-II) and IGF-binding protein-2 in the adult rat brain. *Endocrinology* **135**: 2255–2264.

Mackay KB, Loddick SA, Naeve GS, Vana AM, Verge GM, Foster AC (2003). Neuroprotective effects of insulin-like growth factor-binding protein ligand inhibitors in vitro and *in vivo*. *J Cereb Blood Flow Metab* **23**: 1160–1167.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* **20**: 9104–9110.

Malberg JE, Schechter LE (2005). Increasing hippocampal neurogenesis: a novel mechanism for antidepressant drugs. *Curr Pharm Des* **11**: 145–155.

Malberg JE, Luo B, Lin Q, Lucki I, Schechter LE, Rosenzweig-Lipson S et al (2005). Increasing insulin-like growth factor-1 (IGF-1) produces anxiolytic-like and antidepressant-like effects in rodents. Paper Presented at the Society for Neuroscience Abstracts, Washington, DC.

Mattson MP, Maudsley S, Martin B (2004). A neural signaling triumvirate that influences ageing and age-related disease: insulin/IGF-1, BDNF and serotonin. *Ageing Res Rev* **3**: 445–464.

McMillan CV, Bradley C, Gibney J, Healy ML, Russell-Jones DL, Sonksen PH (2003). Psychological effects of withdrawal of growth hormone therapy from adults with growth hormone deficiency. *Clin Endocrinol (Oxf)* **59**: 467–475.

Nagaraja TN, Patel P, Gorski M, Gorevic PD, Patlak CS, Fenstermacher JD (2005). In normal rat, intraventricularly administered insulin-like growth factor-1 is rapidly cleared from CSF with limited distribution into brain. *Cerebrospinal Fluid Res* **2**: 5.

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM (2002). Neurobiology of depression. *Neuron* **34**: 13–25.

Pavel ME, Lohmann T, Hahn EG, Hoffmann M (2003). Impact of growth hormone on central nervous activity, vigilance, and tiredness after short-term therapy in growth hormone-deficient adults. *Horm Metab Res* **35**: 114–119.

Pellow S, Chopin P, File SE, Briley M (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* **14**: 149–167.

Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, Luo B et al (2006). Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. *Psychopharmacology (Berl)* **185**: 218–225.

Schechter LE, Ring RH, Beyer CE, Hughes ZA, Khawaja X, Malberg JE et al (2005). Innovative approaches for the development of antidepressant drugs: current and future strategies. *NeuroRx* **2**: 590–611.

Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT (1994). Behavioural and pharmacological characterisation of the elevated 'zero-maze' as an animal model of anxiety. *Psychopharmacology (Berl)* **116**: 56–64.

Shimasaki S, Ling N (1991). Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). *Prog in Growth Factor Res* **3**: 243–266.

Stabler B (2001). Impact of growth hormone (GH) therapy on quality of life along the lifespan of GH-treated patients. *Horm Res* **56**(Suppl 1): 55–58.

Steru L, Chermat R, Thierry B, Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**: 367–370.

Stouthart PJ, Deijen JB, Roffel M, Delemarre-van de Waal HA (2003). Quality of life of growth hormone (GH) deficient young adults during discontinuation and restart of GH therapy. *Psychoneuroendocrinology* **28**: 612–626.

Wallymahmed ME, Baker GA, Humphris G, Dewey M, MacFarlane IA (1996). The development, reliability and validity of a disease specific quality of life model for adults with growth hormone deficiency. *Clin Endocrinol (Oxf)* **44**: 403–411.

Wetterau LA, Moore MG, Lee KW, Shim ML, Cohen P (1999). Novel aspects of the insulin-like growth factor binding proteins. *Mol Genet Metab* **68**: 161–181.

Ye P, D'Ercole AJ (2006). Insulin-like growth factor actions during development of neural stem cells and progenitors in the central nervous system. *J Neurosci Res* **83**: 1–6.

Yee D (2006). Targeting insulin-like growth factor pathways. *Br J Cancer* **94**: 465–468.

Zenker S, Haverkamp F, Klingmuller D (2002). Growth hormone deficiency in pituitary disease: relationship to depression, apathy and somatic complaints. *Eur J Endocrinol* **147**: 165–171.