AGE (2013) 35:1575–1587
DOI 10.1007/s11357-012-9460-4

Growth hormone and insulin-like growth factor-I alter hippocampal excitatory synaptic transmission in young and old rats

Doris P. Molina · Olusegun J. Ariwodola · Jeff L. Weiner · Judy K. Brunso-Bechtold · Michelle M. Adams

Received: 13 March 2012 / Accepted: 11 July 2012 / Published online: 1 August 2012

Abstract In rats, as in humans, normal aging is characterized by a decline in hippocampal-dependent learning and memory, as well as in glutamatergic function. Both growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels have been reported to decrease with age, and treatment with either GH or IGF-I can ameliorate age-related cognitive decline. Interestingly, acute GH and IGF-I treatments enhance glutamatergic synaptic transmission in the rat hippocampus of juvenile animals. However, whether this enhancement also occurs in old rats, when cognitive impairment is ameliorated by GH and IGF-I (*des*-IGF-I), remains to be determined. To address this issue, we used an in vitro CA1 hippocampal slice preparation and extracellular recording techniques to study the effects of acute application of GH and IGF-I on compound field excitatory postsynaptic potentials (fEPSPs), as well as AMPA- and NMDA-dependent fEPSPs, in young adult (10 months) and old (28 months) rats. The results indicated that both GH and IGF-I increased compound-, AMPA-, and NMDA-dependent fEPSPs to a similar extent in slices from both age groups and that this augmentation was likely mediated via a postsynaptic mechanism. Initial characterization of the signaling cascades underlying these effects revealed that the GH-induced enhancement was not mediated by the JAK2 signaling element in either young adult or old rats but that the IGF-I-induced enhancement involved a PI3K-mediated mechanism in old, but not young adults. The present findings are consistent with a role for a GH- or IGF-I-induced enhancement of glutamatergic transmission in mitigating age-related cognitive impairment in old rats.

Keywords Plasticity · Postsynaptic · Phosphorylation · Glutamate receptors · Cell signaling

D. P. Molina · J. K. Brunso-Bechtold · M. M. Adams
Departments of Neurobiology and Anatomy,
Wake Forest University School of Medicine,
Medical Center Boulevard,
Winston-Salem, NC 27157-1010, USA

O. J. Ariwodola · J. L. Weiner
Department of Physiology and Pharmacology,
Wake Forest University School of Medicine,
Medical Center Boulevard,
Winston-Salem, NC 27157-1010, USA

J. K. Brunso-Bechtold · M. M. Adams
Roena Kulynych Center for Memory and Cognition
Research, Wake Forest University School of Medicine,
Medical Center Boulevard,
Winston-Salem, NC 27157-1010, USA

M. M. Adams (✉)
Department of Psychology, Bilkent University,
06800 Bilkent,
Ankara, Turkey
e-mail: michelle@bilkent.edu.tr

Introduction

It is well-established that both growth hormone (GH) and insulin-like growth factor-I (IGF-I) have

important roles in mammalian growth and development. These polypeptide hormones play major roles in the regulation of cellular processes in tissues throughout the body (Gahete et al. 2009) including, but not limited to, protein synthesis (Lo and Ney 1996), regulation of bone metabolism (Ohlsson et al. 1998), immune and cardiovascular function (Cittadini et al. 1999; Conti et al. 2008; Hattori 2009). However, in addition to the peripheral effects of GH and IGF-I, these factors also impact the central nervous system, in particular, the hippocampus of adult animals (Lobie et al. 2000).

Cognitive function, including hippocampal-dependent learning and memory, declines with advancing age (Frick et al. 1995; Rosenzweig et al. 1997; Ramsey et al. 2004). Parallel to this age-related decline in hippocampal-dependent cognition, the levels of GH and IGF-I also decrease across lifespan (Sonntag et al. 1980; Richman et al. 1981; Carter et al. 2002; Sonntag et al. 2005; Ramsey et al. 2004). Importantly, GH and IGF-I supplementation in rodents have been reported to ameliorate hippocampal-dependent cognitive deficits associated with normal aging (Markowska et al. 1998; Thornton et al. 2000; Ramsey et al. 2004), and there has been debate on whether there is a direct role of GH in mediating these effects or whether GH acts through its anabolic mediator, IGF-I (Molina et al. 2011). One potential mechanism for the improvement of age-related cognitive impairment by GH and IGF-I is an enhancement of excitatory synaptic transmission. Excitatory synaptic transmission in the hippocampus is mediated primarily by glutamatergic receptors, principally the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA) types of the glutamate receptor. Subunits that comprise the AMPA and NMDA receptors in the hippocampus have been reported to decline with age (Foster 2002; Shi et al. 2007; Adams et al. 2008; Newton et al. 2008) and AMPA and NMDA receptor-mediated synaptic transmission is reduced as well (Barnes 1990; Barnes et al. 1992, 1997, 2000a, b; Bauman et al. 1992; Deupree et al. 1993; Papatheodoropoulos and Kostopoulos 1996; Jovenceau et al. 1998). Moreover, administration of either GH or IGF-I has been reported to increase mRNA and protein levels of those subunits (Sonntag et al. 2000; Le Grevès et al. 2005). Notably, GH and IGF-I can also enhance glutamatergic synaptic transmission in rat hippocampal slices of juvenile animals (Ramsey et al. 2005; Mahmoud and Grover 2006), an effect that may contribute to improved cognitive performance (Porrino et al. 2005; Goff et al. 2008; Hamlyn et al. 2009; Hampson et al. 2009).

The cognitive benefits of GH and IGF-I supplementation have been demonstrated in aged animals; however, the enhancement of hippocampal synaptic transmission by GH and IGF-I has been shown only in juvenile rats, and little is known about the effects of GH or IGF-I on glutamatergic synaptic transmission in adulthood or old age. The question then arises as to whether GH and IGF-I can augment synaptic transmission in old animals when chronic supplementation with these hormones ameliorates impaired hippocampal-dependent cognitive function. Thus, in the present study, we used extracellular recording methods to evaluate the effect of acute GH and IGF-I application on hippocampal glutamatergic synaptic transmission in brain slices from young adult and old rats. The results demonstrate that both GH and IGF-I enhance AMPA and NMDA function to a similar extent in young adult and old rats, supporting the notion that acute effects of either hormone may contribute to the reported GH and IGF-I mediated amelioration of age-related cognitive impairment. Our findings also provide initial evidence of possible age-dependent differences in the signaling cascades that mediate the effects of these hormones on rat glutamatergic synapses.

Methods

Experimental subjects and slice preparation

Juvenile (1–2 months), young (10 months) and old (28 months) male Fisher 344 × Brown Norway rats (F344 × BN) rats from the National Institute of Aging Colony at Harlan Sprague–Dawley were anesthetized with halothane and decapitated. The brain was removed and submerged in chilled, oxygenated artificial cerebrospinal fluid (aCSF) containing 124 mM NaCl, 3.3 mM KCl, 2.4 mM $MgCl_2$, 2.5 mM Ca Cl_2, 1.2 mM KH_2PO_4, 10-D-glucose, and 25.9 mM $NaHCO_3$ and saturated with 95 % O_2–5 % CO_2. The right and left hemispheres were separated and cut coronally into 400-μm thick slices using a vibratome (Leica

VT1000S; Vashaw Scientific, Atlanta, GA). The hippocampus in each slice was dissected from the surrounding tissue and was equilibrated in an incubation chamber with oxygenated aCSF at 20–25 °C for 90 min. During experiments, slices were placed in a recording chamber and perfused with oxygenated aCSF at a flow rate of 2 mL/min at 32 °C. All experiments were conducted in accordance with the Guidelines for the Care and Use of Experimental Animals and approved by the Institutional Animal Care and Use Committee.

Field excitatory postsynaptic potential recordings

Electrodes were prepared from filamented borosilicate glass capillary tubes (0.86 mm ID) using a horizontal micropipette puller (Sutter P-97; Sutter, Novato, CA) and filled with aCSF. Slices were transferred to a recording chamber, a twisted bipolar stimulating electrode (FHC, Bowdoinham, ME) was placed in the Schaffer collaterals of CA3, and Field excitatory postsynaptic potential (fEPSPs) were recorded from the stratum radiatum of CA1. Stimulation was adjusted to elicit ~30 % of the peak amplitude (pAmp), and responses were recorded every 20 s, prior to growth hormone (GH) or *des*-insulin-like growth factor-I (*des*-IGF-I) application. Compound (CMPD) responses were defined as fEPSPs elicited in the absence of either AMPA or NMDA inhibitors. For experiments evaluating AMPA- and NMDA-mediated synaptic transmission, appropriate inhibitors were added for 20 min (see "Drug preparation") before a baseline was recorded. To ensure that NMDA and AMPA were effectively isolated AMPA- and NMDA-dependent fEPSPs were blocked using AMPA and NMDA receptor antagonists, respectively. In addition, CMPD responses were completely abolished by bath application of AMPA and NMDA receptor antagonists supporting that the CMPD response consisted of AMPA and NMDA fESPSPs, consistent with previous studies (Foy et al. 1999; Frazier et al. 1998; Alkondon et al. 2003; McQuiston 2010; data not shown). Baseline, under all conditions was defined as 10 min of stable recordings where the pAmp remained within 10 % of the mean. In the case of AMPA- and NMDA-dependent recordings, the baseline was recorded after 20 min of inhibitor application. GH or *des*-IGF-I was applied for 30 min following baseline recordings. The enhancement of CMPD, AMPA- and NMDA-dependent fEPSPs after GH or *des*-IGF-I application in young adult and old hippocampal slices was determined by comparing the last 5 min of GH or IGF-I application to the last 5 min of baseline. The same procedure for determining the GH and IGF-I enhancement was followed for all conditions. Paired-pulse ratio studies were conducted by delivering two consecutive stimuli with an interpulse interval of 50 ms. The ratio between the second stimulus pulse (P2) and the first stimulus (P1) was calculated in the presence and absence of GH or *des*-IGF-I.

Drug preparation

The selective NMDA receptor blocker D-(-)2-amino-5-phosphonovaleric acid (APV, Sigma, St. Louis, MO; 20 μM) was used for the pharmacological isolation of AMPA-dependent fEPSPs. To isolate NMDA-dependent fEPSPs, 6,7-dinitroquinoxaline-2,3-dione (DNQX, Sigma, St. Louis, MO; 50 μM), a selective AMPA/KA receptor blocker, combined with bicuculline methylbromide (BIC, Sigma, St. Louis, MO; 30 μM) a gamma-aminobutyric acid type A ($GABA_A$) channel blocker, and glycine (Sigma, St. Louis, MO; 1 μM), an allosteric modulator of NMDA receptors were used. The drugs were prepared as stock solutions in dimethyl sulfoxide (DMSO; final DMSO concentration <0.05 %, Sigma, St. Louis, MO) for DNQX or in deionized water for APV and BIC.

Porcine GH (Ozbiopharm, Knoxfield, Australia) was prepared as a stock solution in deionized water. Human *des*(1-3)-IGF-I (Gropep, Thebarton, Australia) was prepared as a stock solution in 0.1 N glacial acetic acid (final concentration 0.1 acetic acid <0.005 %). Des-IGF-I is a truncated form of IGF-I that does not interact with the IGF-I binding proteins that normally sequester circulating IGF-I (Ballard et al. 1996). Furthermore, while *des*-IGF-I has been shown to more potent than IGF-I in vitro, both *des*-IGF-I and IGF-I have similar effects (Russo and Werther 1994; Guan et al. 1996). Drugs used to block signaling components involved in GH- and IGF-I-mediated effects were: tyrphostin AG 490 (Sigma, St. Louis, MO; 10 μM), a Janus kinase 2 (JAK2) inhibitor and wortmannin

(Sigma, St. Louis, MO; 1 μM), a phosphoinositide 3-kinase (PI3K) inhibitor. Both inhibitors were made up as stock solutions in DMSO (final DMSO concentration <0.05 %). During recordings, all drugs were applied with aCSF in known concentrations via calibrated syringe pumps (Razel, Stamford, CT).

Statistics

Post-treatment effects of GH and *des*-IGF-I on pAmp of fEPSPs were defined as a percent of pre-treatment baseline values. The mean of the last 5 min post-treatment was compared to the mean of the last 5 min pre-treatment baseline. Paired Student's *t* tests were used to compare post-treatment to pre-treatment values for compound, AMPA- and NMDA-dependent fEPSPs. Signaling cascade inhibitor effects on GH- and *des*-IGF-I-mediated changes in fEPSPs pAmp were analyzed by one-way ANOVA followed by Newman–Keuls test for pairwise comparison, when appropriate. All statistical analyses were performed using Sigmastat version 3.5 (SYSTAT Software, Point Richmond, CA). Statistical significance was defined as $p<0.05$. All error bars in the figures represent standard error. Values for dose response studies are represented as mean percent pAmp above baseline ± standard error. Values for percent potentiation for GH and IGF-I enhancement and percent potentiation for signaling studies are represented as mean percent potentiation ± standard error.

Results

Concentration–response curves

In the first set of experiments, concentration–response curves were generated to determine the optimal GH and *des*-IGF-I concentrations for the present studies of the effects of these hormones on excitatory synaptic transmission in the CA1 field of hippocampal slices from young adult and old rats (Fig. 1a–d). Statistical assessment of age versus drug effects for both GH and IGF-I demonstrated that there was an overall concentration effect for both GH ($p<0.005$) and *des*-IGF-I ($p<0.0001$); however, there was no significant age effect at any of the concentrations ($p=0.7737$). Concentrations of 88 ng/mL of GH and 80 ng/mL of *des*-IGF-I were selected because both were intermediate among the concentrations tested and both exerted significant enhancement above baseline in young adult and old rats (Fig. 1a–d).

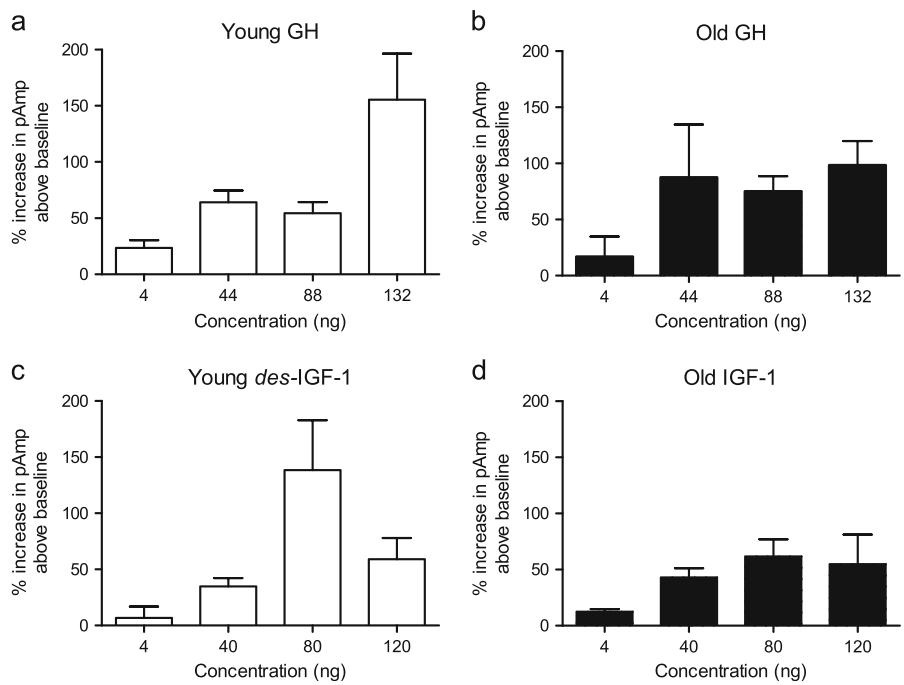

Fig. 1 Concentration–response curves. Concentration-dependence of GH (**a**, **b**) and *des*-IGF-I (**c**, **d**) potentiation of compound fEPSPs in young adult (**a**, **c**) and old (**b**, **d**) rats

GH and *des*-IGF-I enhance fEPSPs in young adult and old rats

Age-related differences in the effect of GH or *des*-IGF-I on glutamatergic synaptic transmission were assessed in hippocampal slices (Figs. 2 and 3). Baseline recordings were performed for each slice during which pAmp remained within 10 % of the mean value for 10 min. CMPD and AMPA-dependent fEPSPs were characterized by short latency negative-deflecting potentials (Karnup and Stelzer 1999), whereas NMDA-dependent fEPSPs were characterized by prolonged negative-deflecting potentials preceeded by an initial, rapid negative deflection. These NMDA-dependent fEPSPs were similar in appearance to those described previously (Eckles-Smith et al. 2000). Our results reveal that GH induced a statistically significant enhancement above baseline in CMPD (144±7 %, n=18), AMPA- (142±15 %, n=8), and NMDA-dependent (145±15 %, n=7) fEPSPs in slices from young adult rats (Fig. 2a, b). A similar, significant GH-induced enhancement of CMPD (161±7 %, n=14), AMPA- (138±15 %, n=8), and NMDA-dependent (178±17 %, n=7) fEPSPs was also observed in slices from old rats (Fig. 2a, b). *Des*-IGF-I also produced a statistically significant enhancement above baseline in young adult, CMPD (140±8 %, n=20), AMPA- (123±13 %, n=12), and NMDA- (133±8 %, n=12,) dependent synaptic transmission, as well as in old CMPD (159±11 %, n=17), AMPA (118±2 %, n=11) and NMDA (122±3 %, n=12) fEPSPs (Fig. 2c, d). Furthermore, time–course waveforms of average fEPSPs across time for GH (Fig. 3a, b) and *des*-IGF-I (Fig. 3c, d) under, AMPA- and NMDA-dependent conditions were similar for young and old slices. In addition, in Fig. 3, the arrow indicates the onset of the drug application and since these time courses are not different directly following the onset of the drug this likely indicates the enhancement is not due to a change in the speed of the release of the neurotransmitter. Thus, our results demonstrate that CMPD, AMPA-, and NMDA-dependent synaptic transmission was increased above baseline by either GH or *des*-IGF-I and that the enhancement was comparable in young adult and old rats (p values: GH CMPD, p=0.08; AMPA, p=0.83; NMDA, p=0.18; p

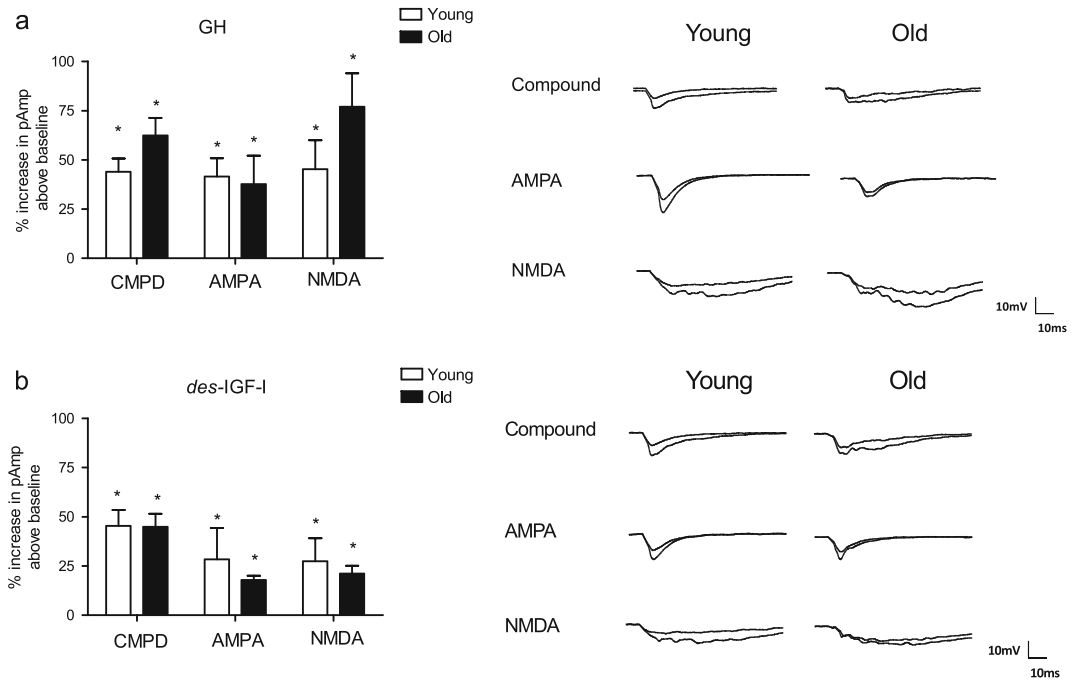

Fig. 2 GH and *des*-IGF-I enhancement of excitatory transmission in young and old rats. Effect of GH (**a**) and *des*-IGF-I (**b**) on compound (CMPD) fEPSPs and pharmacologically isolated AMPA- and NMDA-mediated fEPSPs in young adult and old hippocampal slices. Field EPSP enhancement above baseline (100 %) was significant in both young adult and old F344 × BN rats (all p values <0.05) and there were no significant effects of age under any of the recording conditions (all p values >0.05)

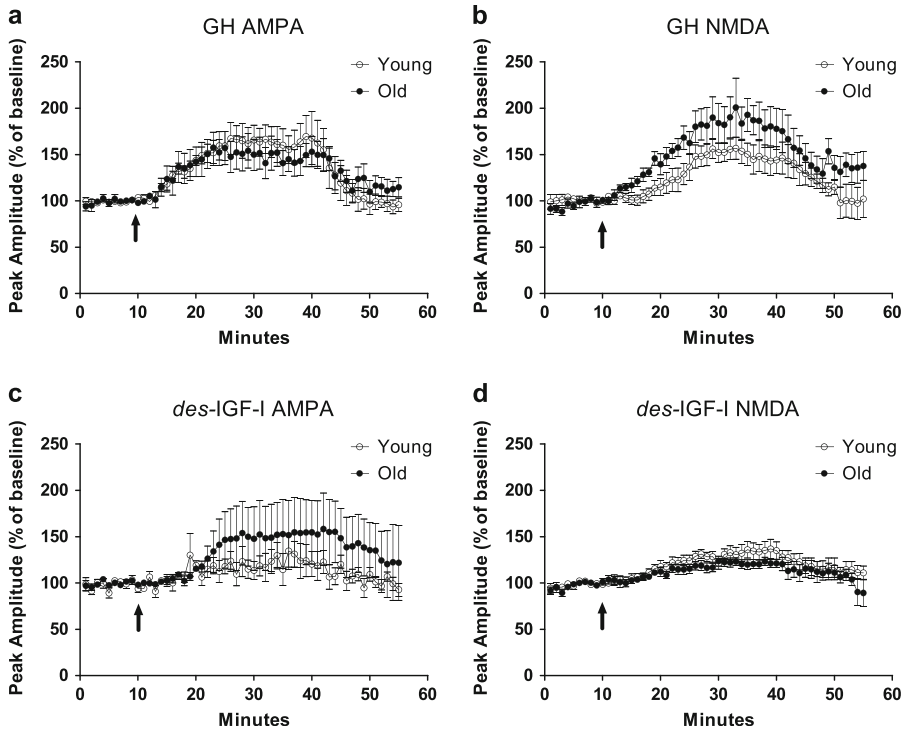

Fig. 3 GH and *des*-IGF-I effects on excitatory transmission in young and old rats. Time course for GH (**a**, **b**) and *des*-IGF-I (**c**, **d**) application on pharmacologically isolated AMPA- and NMDA-mediated fEPSPs in young adult and old hippocampal slices. Application onset of GH and IGF-I is depicted by the *arrow*. Comparison of time courses for young adult and old hippocampal slices demonstrated no significant age-related differences in the response to GH or *des*-IGF-I

values: *des*-IGF-I CMPD, $p=0.18$; AMPA, $p=0.72$; NMDA, $p=0.25$ (Fig. 2).

Paired-pulse facilitation is unaltered by GH and IGF-I

Since GH and *des*-IGF-1 significantly enhanced glutamatergic synaptic transmission in slices from young adult and old animals, we next sought to characterize the synaptic locus underlying these effects. Alterations in the paired-pulse ratio (PPR) of fEPSPs can be used as an indicator of changes in neurotransmitter release and thus may reveal changes that are of a presynaptic origin (Foster and McNaughton 1991; Schultz et al. 1994). Therefore, we measured the PPR of CMPD fEPSPs following direct application of these hormones (Fig. 4). Although bath application of GH significantly potentiated the fEPSPs, GH had no effect on the PPR in recordings from either young adult (pre-treatment GH=1.311; post-treatment GH 1.217; $n=4$; Fig. 4a) or old rats (pre-treatment GH=1.635; post-treatment GH=1.299, $n=7$; Fig. 4a). Similarly, *des*-IGF-I application also enhanced CMPD fEPSPs with no effect on PPR in either young adult (pre-treatment *des*-IGF-I=1.394; post-treatment *des*-IGF-I=1.435; $n=5$; Fig. 4b) or old hippocampal slices (pre-treatment *des*-IGF-I=1.524, $n=7$; post-treatment *des*-IGF-I=1.034, $n=7$; Fig. 4b). The observation that neither hormone altered the PPR is consistent with the notion that GH and *des*-IGF-I enhancement of synaptic transmission in young adult and old hippocampal slices is not mediated by an increase in glutamate release and instead likely involves postsynaptic mechanisms.

GH and IGF-I signaling cascade elements are altered with age

In light of the comparable enhancement of glutamatergic transmission observed in young adult and old hippocampal slices treated with GH or *des*-IGF-I, we also conducted initial studies to investigate whether there were any age-dependent changes in the dominant elements of the GH and *des*-IGF-I signaling cascades that could underlie these effects. Studies indicate that the non-intrinsic tyrosine kinase GH receptor dimerizes and associates with a non-receptor cytoplasmic tyrosine kinase JAK2 for its activation (Carter-Su et al. 1996). Furthermore, JAK2 phosphorylation has been proposed to be critical for GH signal transduction (Jin et al. 2008). To compare the role of this element of the GH signaling cascade in young adult and old rats, the JAK2 inhibitor tyrphostin AG 490 was added to slices from both groups 20 min prior to and throughout GH

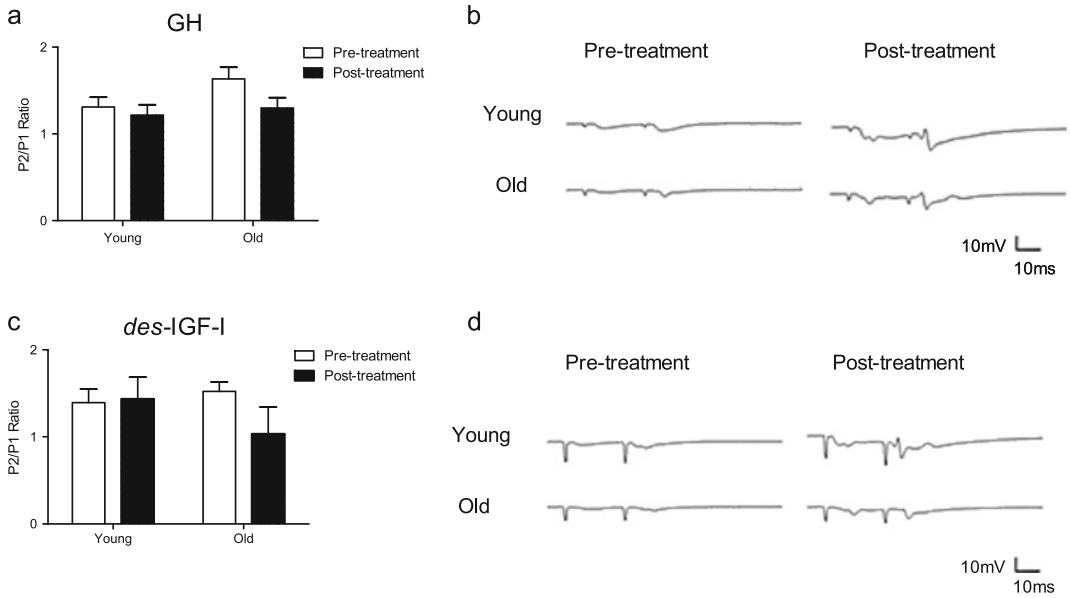

Fig. 4 GH and *des*-IGF-1 effects on presynaptic facilitation in young and old rats. Effect of GH (**a**) and *des*-IGF-I (**b**) on paired-pulse ratios of CMPD fEPSPs. A paired-pulse stimulus train was generated in the hippocampal slices at an interpulse interval of 50 ms. The ratio of the second (P2) pulse over the first pulse (P1) was determined under baseline conditions (pre-treatment) and after GH and *des*-IGF-I application (post-treatment). Neither GH nor IGF-I application altered paired-pulse ratios significantly in young adult or old rats

application. The results demonstrated that tyrphostin AG 490 did not block GH enhancement in either young adult (GH=144±6 %, n=19, GH + Tyrphostin AG 490=143±5 %; n=10; Fig. 5a) or old slices (GH=161±7 %, n=14, GH + Tyrphostin AG 490=167±7 %, n=4; Fig. 5a). These findings are in contrast to reports that GH enhancement is diminished by tyrphostin AG 490 in 2–3-month-old juvenile rats (Mahmoud and Grover 2006). Therefore, in order to ensure the effectiveness of our inhibitor, we assessed inhibition of the JAK2 signaling cascade by tyrphostin AG 490 in slices from rats in that age range. We found a significant inhibition of GH-induced enhancement similar to that described by Mahmoud and Grover (2006), (GH=188±18 %, n=7, GH + tyrphostin AG 490=136±9, n=9, p=0.0171; data not shown). These data indicate that tyrphostin AG 490 can inhibit JAK2 in juvenile but not in young or old rats. This suggests that the signaling cascade mediating GH enhancement in young adult and old rats is not dependent on JAK2, and based on current evidence from the literature, a second tyrosine kinase, a Src family kinase, that activates the Ras/MAP kinase pathway may be involved (Zhu et al. 2002; Barclay et al. 2010).

One of the major signaling cascades initiated by IGF-I receptor activation is the phosphatidylinositol 3-kinase (PI3K) signaling cascade. It has been proposed that the PI3K-dependent pathway contributes to the maintenance of cognitive function (Sun et al. 2005) and this pathway is involved in the IGF-I potentiation of hippocampal AMPA EPSCs in juvenile rats (Ramsey et al. 2005). To determine if there were age-dependent changes in this signaling cascade, we tested the effect of the PI3K inhibitor wortmannin on *des*-IGF-I potentiation of fEPSPs in young adult and old rats. The results demonstrated that wortmannin reduced *des*-IGF-I enhancement in slices prepared from old (IGF-I=151±8 %, n=16, IGF-I+wortmannin 124±7, n=5, p=0.0262; Fig. 5b) but not young adult rats (IGF-I=140±9 %, n=15, IGF-I=141±11 %, n=5; Fig. 5b). Thus, our data suggest that the IGF-I-dependent enhancement may be partially mediated by PI3K in old rats, but not in young. In young animals, the IGF-I enhancement may be mediated by a parallel pathway, the Ras/MAP kinase pathway, through the recruitment of GRB2/SOS and its interaction with a phosphorylated IRS-1 or SHC that

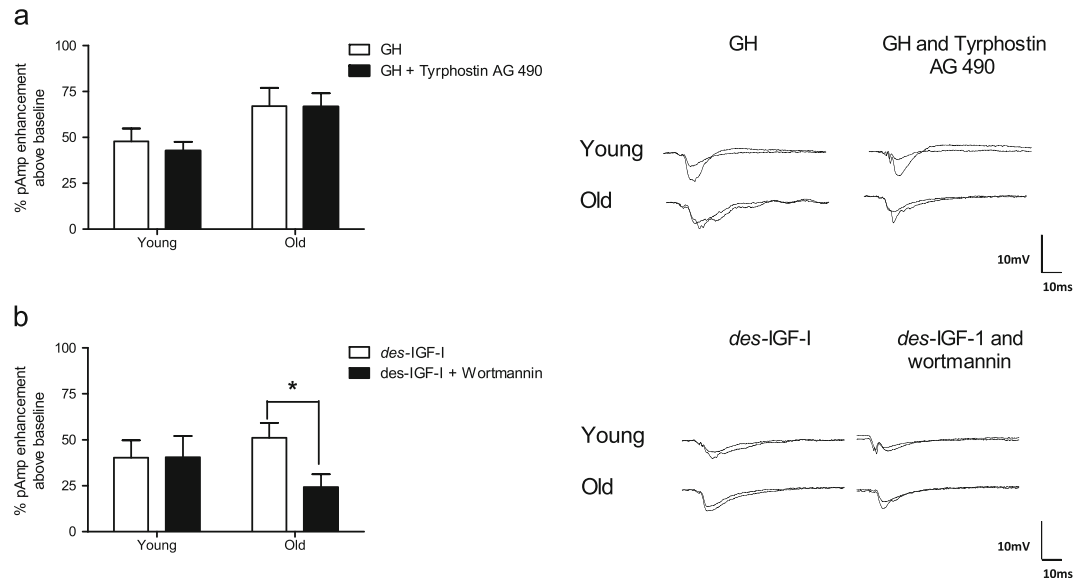

Fig. 5 Effect of age on the signaling cascades responsible for GH and *des*-IGF-1 potentiation of excitatory transmission. For blocking the GH-induced JAK-STAT pathway (**a**), tyrphostin AG 490 was used in slices from young adult and old rats. Tyrphostin AG 490 did not block the GH enhancement of fEPSPs. For blocking the *des*-IGF-1-induced PI3K pathway wortmannin was used (**b**). Wortmannin partially blocked *des-IGF-1* enhancement in slices from young adult but not old rats (*p values <0.05)

is activated by the phosphorylation of the IGF-I receptor (Neuman-Haefelin et al. 2008; Perrini et al. 2010).

Discussion

The results of this study provide the first evidence that both acute GH and *des*-IGF-I application significantly augment excitatory synaptic transmission in the hippocampus of young adult and old rats. Isolation of AMPA- and NMDA-dependent fEPSPs demonstrated that both hormones significantly increased AMPA- and NMDA-mediated synaptic transmission in young adult and old rats. PPR experiments suggested that the GH- and *des*-IGF-I-induced enhancement is not mediated by a presynaptic facilitation of glutamate release. Finally, our data provided initial evidence of significant age-dependent changes in the signaling cascades responsible for these effects. Although the JAK2 pathway is required for GH potentiation of fEPSPs in juvenile rats, blockade of this pathway had no effect on GH modulation of glutamatergic transmission in slices from young adult and old rats. Moreover, inhibition of PI3K revealed that *des*-IGF-I enhancement involves the PI3K signaling cascade in old rats, but not in young adults, suggesting a shift such that the PI3K pathway is utilized in juvenile and old rats, but not young animals.

The present results indicate that application of GH or *des*-IGF-I potentiates excitatory synaptic transmission in the hippocampus of young adult and old rats. A previous study reported that the GH augmentation involved both AMPA and NMDA receptors in hippocampal slices from juvenile rats (Mahmoud and Grover 2006). The present study extends those findings by demonstrating that (1) GH also increased AMPA- and NMDA-dependent synaptic transmission in young adult and old rats and (2) the level of enhancement was comparable in young adult and old rats. A similar change in excitatory glutamatergic transmission was also evident following application of *des*-IGF-I in slices from adult and old rats. Importantly, we found that *des*-IGF-I enhances both AMPA- and NMDA-dependent fEPSPs. This finding is in contrast to a previous report that *des*-IGF-I potentiated AMPA-dependent, but not NMDA-dependent synaptic transmission in juvenile rats (Ramsey et al. 2005). These data, taken together with the present results, suggest that there may be age-

related changes in the glutamate receptors responsible for the *des*-IGF-I augmentation of hippocampal excitatory synaptic transmission. Specifically, the *des*-IGF-I enhancement appears to only involve AMPA receptors early in life (1–2 months; Ramsey et al. 2005) but utilizes both AMPA and NMDA receptors by young adulthood (10 months of age, present findings). This developmental change could be due to the addition or maturation of NMDA receptor subunit complexes. NMDA receptors are comprised of heteromeric complexes consisting of an NR1 subunit and a complement of NR2 subunits (A-D) which exhibit distinct physiological and pharmacological properties (Meguro et al. 1992; Kutsuwada et al. 1992; Cull-Candy et al. 2001), and the relative proportions of these subunits have been documented to demonstrate functionally significant changes during development (Monyer et al. 1994; Portera-Cailliau et al. 1996; Bellone and Nicoll 2007).

The enhancement of hippocampal glutamatergic synaptic transmission by GH and *des*-IGF-I demonstrated in the present study provides a mechanism for the reported amelioration of age-related cognitive impairment by GH and IGF-I supplementation. Specifically, we show here that GH- and *des*-IGF-I each can directly modulate hippocampal synaptic transmission not only in young adults, but also at old age when cognitive performance can be improved by either GH or IGF-I administration (Markowska et al. 1998; Ramsey et al. 2004). Compromised AMPA- and NMDA-dependent synaptic transmission has been reported in rats with cognitive deficits (Porrino et al. 2005; Liu et al. 2008; Goff et al. 2008; Kessels and Malinow 2009; Lin and Tsien 2009; Hampson et al. 2009; Hamlyn et al. 2009), and increased activation or expression of glutamatergic receptors maintains cognitive function (Tang et al. 1999; Porrino et al. 2005; Cao et al. 2007; Hampson et al. 2009; Hamlyn et al. 2009). While GH and IGF-I levels were not measured in the animals in the present study, it has been well-documented in the literature that they decline with age (Sonntag et al. 1980; Richman et al. 1981; Carter et al. 2002; Sonntag et al. 2005; Ramsey et al. 2004), and these declines may contribute to age-related cognitive deficits. Future studies will be directed at correlating the amount of the enhancement in excitatory synaptic transmission with the hormone levels within individual subjects to determine whether animals with and without cognitive deficits are still responsive to GH and/or IGF-I. Finally, it has been recently shown by our group that in two well-characterized models of decreased circulating GH and IGF-I levels, the local levels of these hormones remain constant (Adams et al. 2009; Molina et al. 2011). Thus, it will be important in future studies to document whether the local levels of these hormones also change and whether these levels correlate with alterations in synaptic plasticity.

The results of our PPR experiments, taken together with the data indicating no change in the speed of release of the neurotransmitter, suggest that the effects of GH and *des*-IGF-I observed here may not involve increased glutamate release and may instead reflect changes in the glutamate receptors in the postsynaptic membrane. For example, increased trafficking of NMDA or AMPA subunits to the postsynaptic membrane could occur following GH or IGF-I administration. In the cerebellum, IGF-I mediates NMDA receptor trafficking by subunit phosphorylation (Chen and Roche 2007) and other growth factors also have been shown to enhance NMDA subunit phosphorylation (Suen et al. 1998; Lin et al. 1998, 1999). Moreover, the expression of both NR2A and NR2B subunit protein levels in old rats is enhanced by IGF-I (Sonntag et al. 2000) and GH increases the level of NMDA receptor expression in the hippocampus (Le Grevès et al. 2005). Finally, the literature suggests that AMPA subunit phosphorylation and insertion at the synapse increases the receptor fEPSP amplitude (Malinow and Malenka 2002) and increases in NMDA receptor levels occur simultaneously with an increase in the NMDA-mediated receptor fEPSP (Eckles-Smith et al. 2000). Thus, the effects of GH and/or IGF-I on subunit phosphorylation, as well as expression, may be essential in the enhanced synaptic transmission reported here.

Although the ability of GH and *des*-IGF-I to enhance synaptic transmission does not change appreciably between 10 and 28 months of age, the signaling pathway implicated in the IGF-I-mediated enhancement appears to be modified during that time. Specifically, blocking the PI3K, a signaling pathway involved in the maintenance of cognitive function (Sun et al. 2005), reduces the *des*-IGF-I-mediated enhancement in old, but not in young adult rats. In addition to IGF-I downstream signaling events being mediated through the phosphorylation of PI3K, activation of the Ras/MAP kinase

pathway through SHC adaptor protein phosphorylation has been observed to occur in parallel to PI3K (Neuman-Haefelin et al. 2008; Perrini et al. 2010). It may be that as animals age there is a switch from a dependence on an SHC-MAPK signaling pathway, which is more utilized in young adult rats (Kim et al. 1997; Sullivan et al. 2008) to a greater reliance on the PI3K pathway in old rats. This type of age-related switch has been reported to occur in the expression patterns of neurotrophins TrkA and p75NTR and is thought to involve IGF-I receptor activation (Costantini et al. 2006). In contrast, blocking the JAK2 pathway, reported to be a major signaling pathway for GH induced effects, was equally ineffective in reducing GH-mediated synaptic enhancement in young adult and old rats. While most of GH-dependent effects have been thought to occur through the phosphorylation of JAK2 that occurs after the GH receptor activation, several studies have demonstrated that some of GH's functions are independent of JAK2 phosphorylation (Zhu et al. 2002; Barclay et al. 2010). It is possible that in young adult and old rats, GH mediates its effects primarily by recruiting other tyrosine kinases such as c-Src, which activates the Ras/MAP kinase pathway, and that the effects mediated through the recruitment of c-Src are independent of JAK2 (Zhu et al. 2002). Furthermore, it has been demonstrated that some GH events GH can induce an increase in calcium entry independent of JAK2 (Billestrub et al. 1995). Thus, a GH-induced increase in intracellular Ca^{2+} might be responsible for the enhancement of synaptic transmission during JAK2 inhibition (Zhang et al. 2004). While JAK2 has a much greater response to GH receptor activation, JAK1 has also been observed to be phosphorylated by JAK1 activation (Smit et al. 1996), and therefore, the AMPA- and NMDA-dependent results that we observe could be mediated by JAK1. In addition, 17β-estradiol has been observed to exert rapid action on glutamatergic, currents in the hippocampus (Gu et al. 1999; Kim et al. 2006). It is possible that the GH-mediated enhancement that we observed on AMPA- and NMDA-dependent fEPSPs might be as a result of rapid action of GH on AMPA and NMDA receptors without requiring post-translational modification. This would occur by GH directly modulating AMPA and NMDA receptor-dependent functions.

IGF-I is known to be the anabolic mediator of the biological effects of GH. Interestingly, we previously showed that chronic GH treatment enhances excitatory synaptic transmission in the absence of an increase in IGF-I levels in aged rats and suggested that GH may be having a direct effect on the brain. Thus, the current data demonstrating that acute GH application enhances compound and pharmacologically isolated NMDA- and AMPA-receptor fEPSPs in young adult and old animals, together with evidence of the presence of GH receptor mRNA in the brain (Fraser et al. 1990; Walsh et al. 1990; Burton et al. 1992; Lobie et al. 1993, 2000; Zhai et al. 1994; Hull and Harvey 1998) and a demonstration that GH crosses the blood–brain barrier (Pan et al. 2005), support the conclusion that GH may directly influence glutamate receptor function. This also provides a potential mechanism for the GH-induced amelioration of cognitive function in aged rats.

Summary

Taken together, the results presented here demonstrate that either GH or *des*-IGF-I application to hippocampal slices enhanced synaptic transmission in both young adult and old rats. These findings reveal for the first time that both GH and IGF-I have direct effects on AMPA and NMDA-dependent synaptic transmission at a time in the lifespan that administration of either GH or IGF-I is able to ameliorate age-related cognitive decline. Understanding the mechanisms by which GH and IGF-I mediate synaptic enhancement is essential for understanding how the chronic administration of these factors ameliorates the age-related decline cognitive function. The involvement of AMPA and NMDA receptors in the GH and *des*-IGF-I-induced enhancement and the alteration to the IGF-I signaling cascade between young adulthood and old age provide opportunities for understanding the targets of the GH and/or IGF-I-induced amelioration of cognitive impairment in the elderly, and thus contribute to the development of alternative therapeutic strategies that could benefit this population.

Acknowledgments This work was supported by NIH grants: NIA PO1 AG11370 and KO1 AG027828. M.M. Adams is currently supported by an Installation Grant from the European Molecular Biology Organization.

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