Growth Hormone Enhances Excitatory Synaptic Transmission in Area CA1 of Rat Hippocampus

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Mahmoud, Ghada S. and Lawrence M. Grover. Growth hormone enhances excitatory synaptic transmission in area CA1 of rat hippocampus. J Neurophysiol 95: 2962–2974, 2006. First published February 15, 2006; doi:10.1152/jn.00947.2005. The hippocampus produces growth hormone (GH) and contains GH receptors, suggesting a potential role for GH signaling in the regulation of hippocampal function. In agreement with this possibility, previous investigations have found altered hippocampal function and hippocampal-dependent learning and memory after chronic GH administration or deficiency. In this study we applied GH to in vitro rat hippocampal brain slices, to determine whether GH has short-term effects on hippocampal function in addition to previously documented chronic effects. We found that GH enhanced both AMPA- and NMDA-receptor-mediated excitatory postsynaptic potentials (EPSPs) in hippocampal area CA1, but did not alter GABA<sub>A</sub>-receptor–mediated inhibitory synaptic transmission. GH enhancement of excitatory synaptic transmission was gradual, requiring 60–70 min to reach maximum, and occurred without any change in paired-pulse facilitation, suggesting a possible postsynaptic site of action. In CA1 pyramidal neurons, GH enhancement of EPSPs was correlated with significant hyperpolarization and decreased input resistance. GH enhancement of EPSPs required Janus kinase 2 (JAK2), phosphatidylinositol-3-kinase (PI3 kinase), mitogen-activated protein (MAP) kinase kinase (MEK), and synthesis of new proteins. Although PI3 kinase and MEK were required for initiation of GH effects on excitatory synaptic transmission, they were not required for maintained enhancement of EPSPs. GH treatment and tetanus-induced long-term potentiation were mutually occluding, suggesting a common mechanism or mechanisms in both forms of synaptic enhancement. Our results demonstrate that GH has powerful short-term effects on hippocampal function, and extend the timescale for potential roles of GH in regulating hippocampal function and hippocampal-dependent behaviors.

INTRODUCTION

Growth hormone (GH, somatotropin) is essential for normal growth and development of the nervous system (reviewed in Harvey and Hull 2003; Noguchi 1996; Scheepens et al. 2000) and decreased GH levels are associated with a variety of cognitive impairments including memory disruption (reviewed in van Dam et al. 2000). Although the primary source of GH is the anterior pituitary, there are other sites for GH production, including the hippocampus (Gossard et al. 1987; Hojvat et al. 1982). In addition to producing GH, the hippocampus also contains GH receptors (Burton et al. 1992; Lobie et al. 1993; Mustafa et al. 1994a).

Beyond its role in development, GH also plays a role in adult memory processing. GH modulates long-term memory (Schneider-Rivas et al. 1995) and may attenuate effects of aging on memory (Ramsey et al. 2004; Thornton et al. 2000). The effects of GH on memory may be mediated by the hippocampus (Nyberg 2000). Interestingly, hippocampal GH expression is upregulated during learning (Donahue et al. 2002), suggesting a possible autocrine or paracrine function for GH in the hippocampus. Chronic GH treatment alters N-methyl-D-aspartate (NMDA) receptor gene expression in the hippocampus (Le Greves et al. 2002) and this could at least partially explain the effects of GH on memory function. Although previous studies have examined effects of long-term GH treatment and GH deficiency, acute effects of GH on hippocampal function have not been investigated.

The GH receptor (GHR) belongs to the cytokine receptor superfamily. GH stimulation causes receptor dimerization, activation of the Janus kinase 2 (JAK2) tyrosine kinase, and phosphorylation of signal transducer and activator of transcription 5 (STAT5; reviewed in Herrington and Carter-Su 2001; Moutoussamy et al. 1998). Other downstream signaling events include stimulation of mitogen-activated protein kinase (MAP kinase) and phosphatidylinositol-3-kinase (PI3 kinase) (Anderson 1992; Argetsinger et al. 1995; Jeay et al. 2001; Shoba et al. 2001; Sotiropoulos et al. 1994). GH is well known for its ability to rapidly stimulate protein synthesis (Dreskin and Kostyo 1980; Jeffersson et al. 1975; Kostyo and Nutting 1973; Mowbray et al. 1975). Our main objective in this study was to determine whether somatotropic signaling exerts short-term effects on hippocampal function and, if so, which signaling pathway or pathways are required.

We found that GH enhanced both a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)- and NMDA-receptor–mediated excitatory postsynaptic potentials (EPSPs), but did not alter γ-aminobutyric acid type A (GABA<sub>A</sub>)–receptor–mediated inhibitory synaptic transmission. The effects of GH on excitatory synaptic transmission required JAK2, MAP kinase, PI3 kinase, and protein synthesis. MAP kinase and PI3 kinase were required for initial enhancement of EPSPs, but were not required for maintained enhancement of EPSPs.

METHODS

Slice preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Marshall University. Hippocampal slices were prepared from 1.5- to 3-mo-old male Sprague–Dawley rats (Hilltop Laboratory Animals). Animals were sedated by inhalation of isoflurane and maintained under general anesthesia with 1% isoflurane in 99% oxygen for surgical preparation. The rats were allowed to recover for 1 h before being transferred to the recording chambers. The chamber was maintained at a temperature of 37°C and had a flow of 1% isoflurane in 99% oxygen during the experiment. Slices were held in a recording chamber with a perfusion rate of 2.5 ml/min. The recording solution was composed of (in mM): NaCl 124, KCl 5, MgCl<sub>2</sub> 2, CaCl<sub>2</sub> 1.5, NaH<sub>2</sub>PO<sub>4</sub> 1, NaHCO<sub>3</sub> 26, glucose 10, and HEPES 25. The slices were superfused with the recording solution at a constant temperature of 37°C and a pH of 7.35–7.45. The slices were exposed to GH in a concentration of 100 nM for 1 h.

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tion of a CO2/air mixture and decapitated. The skull was opened and the brain was removed and submerged in chilled, oxygenated (95% O2-5% CO2), low Ca2+/high Mg2+ artificial cerebrospinal fluid (ACSF) composed of: 124 mM NaCl, 26 mM NaHCO3, 3 mM KCl, 0.5 mM CaCl2, 5.0 mM MgSO4, and 10 mM glucose. While submerged in chilled low Ca2+/high Mg2+ ACSF the brain was trimmed to a block containing both hippocampi. The block was glued to the stage of a vibrating microtome (Campden Instruments), immersed in a bath of chilled, oxygenated, low Ca2+/high Mg2+ ACSF, and 400-μm coronal sections were cut. Sections containing the hippocampus in transverse profile were selected and transferred to a small petri dish, where they were further dissected to free the hippocampus from surrounding tissue. The CA3 region was removed from slices which were later treated with the GABAA receptor antagonist bicuculline. After dissection, hippocampal slices were transferred to a holding chamber where they were stored for later use.

Slices were maintained in the holding chamber at room temperature (20–22°C) at the ACSF/atmosphere (95% O2-5% CO2) interface. The holding chamber was filled with standard ACSF composed of 124 mM NaCl, 26 mM NaHCO3, 3.4 mM KCl, 1.2 mM NaHPO4, 2.0 mM CaCl2, 2.0 mM MgSO4, and 10 mM glucose. Slices were incubated in the holding chamber for a minimum of 1 h before use.

Slices were withdrawn from the holding chamber as needed and placed in a low-volume (about 200 μL) interface recording chamber, where they were continuously perfused at a rate of 1.5 ml/min with standard ACSF. The recording chamber was kept at a temperature of 25 ± 0.5°C. A minimum 30-min period was allowed for recovery after transferring slices from the holding chamber to the recording chamber.

Field potential recording

Extracellular potentials were recorded through low-impedance (3–4 MΩ) glass micropipettes filled with ACSF and placed into the stratum radiatum of area CA1. Signals were amplified (gain 1,000) and filtered (0.1–3,000 Hz) using a WPI DAM50 amplifier, then digitized (10 kHz; National Instruments) and stored on a personal computer.

Whole cell patch-clamp recording

Whole cell recordings were obtained from the somata of CA1 pyramidal neurons by the method of Blanton et al. (1989). Patch electrodes (3–4 MΩ) were filled with a solution of 140 mM cesium or potassium gluconate, 10 mM sodium HEPES (N-[2-hydroxyethyl]-piperazine-N’-[2-ethanesulfonic acid]), and 3 mM MgCl2. Positive pressure was applied to the back of the patch electrodes as they were lowered into the somatic layer of area CA1, and the electrode resistance was continuously monitored. When electrode resistance increased, positive pressure was released, and gentle negative pressure was applied to form a high-resistance seal (>1 GΩ, typically 2–5 GΩ) with the cell membrane. The membrane patch was then ruptured to obtain the whole cell recording configuration. Most recordings were done in current-clamp mode, although some recordings were made in whole cell voltage-clamp mode with membrane potential clamped to the initial level resting level throughout the recording.

Membrane potentials were measured with an Axoclamp 2B (Axon Instruments) operating in continuous current-clamp mode. Access resistance was measured and compensated using the Axoclamp bridge balance circuitry. Cell input resistance was monitored throughout experiments by passing small hyperpolarizing and depolarizing currents into the cell (±50–100 pA). Cells were discarded if access or input resistances showed large, abrupt, irreversible changes. Reported membrane potentials were compensated for a liquid junction potential of 10 mV. Series resistance compensation was not applied during whole cell voltage-clamp recordings.

Synaptic stimulation

Postsynaptic potentials were evoked by delivery of constant-voltage stimuli through a bipolar stimulating electrode placed into the stratum radiatum. Stimuli were delivered at a 15-s interval. In some field potential recordings, paired stimuli (50-ms interstimulus interval) were delivered to measure paired-pulse facilitation (PPF), which was quantified as the ratio of the second response divided by first response.

Postynaptic potentials evoked in standard ACSF were quantified by measuring the slope of the linear portion of the initial response. In some recordings we isolated synaptic responses mediated by NMDA receptors or GABA receptors. NMDA receptor-mediated EPSPs were isolated by perfusing slices with the AMPA receptor antagonist DQNX (30 μM) and the GABAA receptor antagonist bicuculline (bicuculline methiodide, 10 μM). GABA-receptor-mediated inhibitory postsynaptic potentials (IPSPs) were isolated by perfusing slices with the AMPA-receptor antagonist DQNX (6,7-dinitroquinoxaline-2,3-dione, 30 μM) and the NMDA-receptor antagonist (2-aminoo-5-phosphonopenta genoic acid, 50 μM). Isolated synaptic responses were quantified by measuring peak amplitude.

Occlusion test

We tested for mutual occlusion between effects of GH treatment and high-frequency (tetanic) stimulation, by applying two trains of 100-Hz, 1-s stimuli at an intertrain interval of 30 s. One group of slices was first pretreated with growth hormone (60–120 min), washed in standard ACSF for 30–60 min, and then stimulated with two trains of 100-Hz stimuli as described above. A second group of slices received repeated rounds of tetanic stimulation until potentiation reached a stable, maximal amplitude, followed 20–60 min later with application of GH. Results from these two groups of slices were compared with slices receiving tetanic stimulation alone or GH alone.

Reagents

Reagents used in this study were: recombinant human GH (Bachem); recombinant rat GH (Cell Sciences); bicuculline methiodide, d-AP5, DQNX (Tocris); 1,4-diamino-2,3-dicyano-1,4-bis(2-amino-phenylthio)-butadiene (U-0126), tyrphostin AG 490 (LC Labs); wortmanin, cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2,3-dione, 30 M); GABA-receptor–mediated inhibitory postsynaptic potentials (IPSPs) were isolated by perfusing slices with the AMPA-receptor antagonist DQNX (6,7-dinitroquinoxaline-2,3-dione, 30 μM) and the NMDA-receptor antagonist d-AP5 (d-2-amino-5-phosphonopentanoic acid, 50 μM). Isolated synaptic responses were quantified by measuring peak amplitude.

Data analysis

Changes in synaptic response caused by GH treatment or tetanization were expressed as percentage of baseline before treatment. Synaptic responses and membrane potentials were recorded and initially analyzed using the WinWCP program (Strathclyde Electrophysiology Software, John Dempster, University of Strathclyde). Further data analysis was performed with Origin (Microcal Software) and Excel (Microsoft). Statistical significance was assessed by paired or unpaired t-tests, as appropriate, with P < 0.05 (two-tailed) considered significant.

RESULTS

GH enhanced field EPSPs

We used field potential recordings to determine whether recombinant human GH (rhGH) application affects excitatory
synaptic transmission. Application of rhGH (22 ng/ml) produced a slow increase in EPSP slope (Fig. 1). EPSPs began to increase within a few minutes of rhGH application, but required ≥60 min to reach a stable level. EPSPs remained enhanced throughout continued rhGH application. In slices where a presynaptic fiber volley could be clearly resolved, the increase in EPSP slope occurred without any change in the fiber volley (Fig. 1).

To test for possible nonspecific effects of rhGH, we conducted two control experiments. In the first of these control experiments, we applied boiled rhGH to slices. As shown in Fig. 2, boiling largely destroyed the activity of the GH. Slices treated with boiled rhGH (n = 6) showed significantly reduced change in EPSP (135.0 ± 10.4% of the baseline value of −0.26 ± 0.04 V/s after 60 min of application) compared with slices treated with intact rhGH (254.7 ± 34.1% of the baseline value of −0.16 ± 0.01 V/s at 60 min, n = 12, P < 0.05). Boiled rhGH appeared to retain some residual activity. This residual activity may reflect incomplete denaturation and retained biological activity of GH after heating denaturation (Bewley 1982; Gomez-Orellana et al. 1998; Li 1962; Pfund et al. 1996). In a second control experiment we compared rhGH to recombinant rat GH (rrGH). Primate GHs, including rhGH, are effective ligands at both GH and prolactin receptors, whereas nonprimate GHs, which retain activity at GH receptors, are ineffective ligands at prolactin receptors (reviewed in

FIG. 1. Recombinant human GH caused enhancement of field excitatory poststimulus potential (EPSP) without affecting the presynaptic fiber volley. A glass micropipette filled with artificial cerebrospinal fluid (ACSF) was placed in midstratum radiatum to record field EPSPs evoked by stimulation of Schaffer collateral/commisural afferents in stratum radiatum (15-s interstimulus interval). EPSPs were evoked for a 15-min baseline period in standard ACSF followed by ACSF + 22 ng/ml growth hormone (GH) for 150 min. EPSP slopes were measured from the initial negative going phase of the waveform after the fiber volley (fiber volley is indicated by small upward pointing arrow). EPSP slopes were normalized by the mean of the baseline slope and plotted against time as percentage of baseline (bottom). Application of GH caused a progressive increase in EPSP slope, which began within a few minutes of the start of GH application but required about 60 min to reach a stable, maximal level. After reaching a stable level, EPSPs remained enhanced for the duration of the recording. Averaged EPSP waveforms from different points during the recording are shown at the top: (1) during the 15 min baseline period before GH application, and during GH application, (2) after 25–30 min, (3) 55–60 min, and (4) 115–120 min. GH induced increase in EPSPs occurred without any change in fiber volley (upward pointing arrow) indicating no change in presynaptic afferent excitability.

FIG. 2. Recombinant human growth hormone (rhGH, open squares) and recombinant rat growth hormone (rrGH, filled squares) caused equivalent increases in field EPSP slope. Heating rhGH in a boiling water bath for 60 min (boiled rhGH) significantly impaired the ability of rhGH to increase EPSPs. EPSP slopes were measured during a 15-min baseline period, and during a 60-min period of GH application (22 ng/ml). Mean EPSP slopes during the pre-GH baseline were averaged and plotted against time as mean ± 1 SE (bottom). Sample waveforms shown at the top are 5-min averages from the end of the baseline period (1) and the end of the 60-min GH application (2) from one rhGH treated slice (top left) and one rrGH treated slice (top middle) and one boiled rhGH treated slice (top right).
Goffin et al. 1996). To verify that the EPSP enhancement we observed in response to rhGH was caused by GH receptor stimulation, and not prolactin receptor stimulation, we compared the effects of rhGH with rrGH. The results are summarized in Fig. 2. Both GHs enhanced EPSPs, with similar time course and magnitude. EPSPs from slices treated with rrGH (n = 7) increased to 224.6 ± 25.3% of baseline (−0.26 ± 0.07 V/s) after 60 min of treatment. This value was not significantly different from that obtained with rhGH (P > 0.99). Because rhGH and rrGH produced comparable results, subsequent recordings were done using rhGH.

To determine the duration of the GH effect, we applied GH to slices for 4 h (Fig. 3). Because slice viability is a potential issue with such long duration recordings, we recorded from a second set of slices that were perfused with ACSF alone for 4 h. These long-duration recordings revealed that maximal enhancement was obtained after about 60–80 min of GH application. Although there was some decrease between 120 and 240 min of GH application, slices perfused with ACSF alone also showed decreased responses over this time period, indicating that the GH enhancement of EPSPs persists essentially unabated for ≥4 h. After 4 h of GH application, EPSPs averaged 217.2 ± 30.4% of the initial baseline (−0.12 ± 0.2 V/s, n = 8), a value significantly greater than that from slices perfused with ACSF alone for an equivalent period of time (71.4 ± 8.7% of the initial baseline level of −0.15 ± 0.03 V/s, n = 4, P < 0.002).

The GH-induced increase in EPSP slope could be attributable to enhanced postsynaptic response to glutamate, or increased presynaptic release of glutamate. Increased probability of transmitter release is accompanied by decreased PPF (Dunwiddie and Haas 1985; Hess et al. 1987; Otmakhov et al. 1993; Zucker 1989). To determine whether increased probability of glutamate release might underlie the GH enhancement, we examined PPF during GH application. Although 1 h of GH application caused a substantial and significant increase in EPSP slope to 218.1 ± 18.6% of the initial baseline (−0.21 ± 0.03 V/s, P < 0.0001), there was no significant change in PPF ratio (n = 15, Fig. 4A). Before GH application, the PPF ratio averaged 1.38 ± 0.09, and after 60 min of GH treatment, the PPF ratio was 1.30 ± 0.08 (P > 0.27). Our failure to observe a change in PPF during GH application is not the result of a lack of sensitivity in our methods because changes in PPF were easily observed during application of high-Ca²⁺ ACSF (Fig. 4B) and during the posttetanic facilitation that follows high-frequency tetanic stimulation (data not shown; see also Grover 1998). Because GH caused a significant increase in EPSPs without causing significant alteration in PPF, it seems unlikely that increased probability of glutamate release underlies the GH enhancement of excitatory synaptic transmission. Our PPF data do not, however, argue against other changes in presynaptic function that do not involve altered probability of release.

Because synaptic potentials recorded in standard ACSF are mediated almost entirely by AMPA receptors (Andreasen et al. 1989; Collingridge et al. 1983; Davies and Collingridge 1989; Koerner and Cotman 1982), the changes in field potentials that we observed during GH treatment most likely reflect alterations in AMPA-receptor–mediated synaptic transmission. To determine whether this is, in fact, the case, we conducted an additional set of recordings where we blocked NMDA receptors with the antagonist D-AP5 (50 μM) before applying GH to slices. As shown in Fig. 5, we observed a large increase in AMPA-receptor EPSPs during GH application. After 60 min of GH treatment, EPSPs averaged 389.6 ± 101.5% of the initial baseline (−0.19 ± 0.03 V/s, n = 5). This increase was significant (P < 0.05).

Whole cell recordings

We used whole cell recordings to examine the effects of GH on EPSPs, pharmacologically isolated NMDA-receptor–mediated EPSPs (NMDA-EPSPs), and pharmacologically isolated IPSPs. We also assessed possible effects of GH on resting membrane potential and input resistance (Rm).

![Fig. 3. GH enhancement of EPSPs persisted for ≥4 h. RhGH was applied to one group of slices (filled squares) for 4 h, after a 15-min baseline recording period. A second group of slices (open circles) was perfused with ACSF alone for the same period. Slices treated with rhGH showed a gradual increase in field EPSPs (bottom), as shown in Figs. 1 and 2. GH enhancement of EPSP peaked after 60–80 min of application, and then was followed by a very gradual decline in EPSP slope up to the end of the recording period. EPSP slopes were appreciably enhanced even at the end of 4 h of GH treatment. Slices perfused with ACSF alone showed a gradual decline in EPSP slope, beginning after nearly 90 min of recording. For clarity, error bars are plotted for only one out of every 20 points. Sample waveforms shown at the top are 5-min averages from the end of the baseline period (1) and after 60 min of rhGH application or equivalent period of ACSF alone (2).](http://jn.physiology.org/doi/10.1152/jn.00821.2005)
GH ENHANCED NMDA-EPSPs. EPSPs evoked in normal ACSF are almost entirely mediated by AMPA receptors (Andreasen et al. 1989; Collingridge et al. 1983; Davies and Collingridge 1989; Koerner and Cotman 1982), and the enhancement of these EPSPs by GH indicates an effect on AMPA-receptor–dependent synaptic transmission, a conclusion supported by our earlier field potential recordings (Fig. 5). To determine whether NMDA-receptor–dependent synaptic function is also affected by GH, we treated slices with the AMPA-receptor antagonist DNQX and the GABA<sub>B</sub> receptor antagonist bicuculline. Afferent stimulation under these conditions results in a PSP consisting of an NMDA-receptor–mediated EPSP (NMDA-EPSP) followed by a GABA<sub>B</sub> IPSP. In preliminary recordings (not shown), we found that the GABA<sub>B</sub> IPSPs ran down substantially during the first 30 min of whole cell recording. Because the NMDA-EPSP and GABA<sub>B</sub> IPSP partially overlap in time, rundown of the GABA<sub>B</sub> IPSP allows the NMDA-EPSP to increase. Therefore to avoid possible confounding effects of GABA<sub>B</sub> rundown and GH, we either 1) increased the duration of the baseline recording before application of GH to 30 min to allow GABA<sub>B</sub> IPSP rundown to complete, or 2) substituted Cs<sup>+</sup> for K<sup>+</sup> in our whole cell pipette solution to block the GABA<sub>B</sub> conductance (Ling and Benardo 1994; Otis et al. 1993). Under these conditions, NMDA-EPSPs were stable before GH application, and it was possible to determine whether GH altered the NMDA-EPSP. Because the NMDA-EPSP is voltage-dependent, in these recordings we injected DC holding current to maintain the original resting membrane potential (−65 to −75 mV) and compensate for any GH-induced hyperpolarization. As shown in Fig. 7A, GH application caused enhancement of isolated NMDA-EPSPs similar to its effect on EPSPs evoked in standard ACSF (compare Figs. 6 and 7). In the presence of GH, NMDA-EPSPs slowly increased over time, reaching a mean level of 171 ± 10% of baseline (initial amplitude 1.17 ± 0.21 mV, n = 3) after 30 min of application (P < 0.01, Fig. 9A). Similar results were obtained during whole cell voltage-clamp recordings (Fig. 7B). Isolated NMDA-receptor–mediated EPSCs increased from a mean of −64.3 ± 36.3 to −109.7 ± 47.1 pA after 30 min of GH treatment (n = 3).

GH DID NOT AFFECT IPSPS. In contrast to its effect on EPSPs, GH did not alter GABA<sub>A</sub>-receptor–mediated IPSPs (Figs. 8 and 9A). After 30 min of GH application, IPSPs averaged 104 ± 13% of the pre-GH baseline (−4.56 ± 0.69 mV, n = 6, P > 0.90). Because the Cl<sup>−</sup> equilibrium potential is near the resting membrane potential of CA1 pyramidal neurons, small changes in membrane potential, similar to those that occur during GH application (Figs. 3B and 6B) could have a considerable effect on GABA<sub>A</sub>-IPSP amplitude. Therefore in these recordings we used DC current injection to compensate for the GH-induced hyperpolarization (as in the preceding, NMDA-EPSP experiment).

GH HAS A NET EXCITATORY EFFECT. GH enhanced isolated AMPA- and NMDA-receptor–mediated EPSPs (Figs. 5, 7, and 9) without altering GABA<sub>A</sub>-receptor–mediated inhibition, suggesting a net excitatory effect on CA1 neurons. However, GH also hyperpolarized neurons by an average of about 3 mV, and this might counteract the net excitatory effect of enhanced excitatory synaptic transmission. Several observations argue against this possibility. First, in our whole cell current-clamp recordings, EPSPs increased on average by more than the hyperpolarization in resting potential. EPSPs increased from 3.7 ± 0.9 to 7.3 ± 2.2 mV (mean increase of 3.6 mV), whereas on average membrane potential hyperpolarized from −67.9 ± 0.69 mV, an increase of 3.6 mV, whereas
1.2 to \(-70.8 \pm 1.8\) mV (mean of 2.9 mV). Second, field potential recordings frequently showed the appearance of population spikes after GH application when they were not initially present (Figs. 1–3, 12, 14, and 15), indicating a net increase in synaptic excitation. Finally, a majority of cells (four of seven) showed action potential firing during synaptic stimulation after GH application, whereas synaptic stimulation before GH treatment was subthreshold. One example is shown in Fig. 10.

**Signaling pathway for GH enhancement of EPSPs**

Our whole cell and field potential recordings revealed pronounced effects of GH on both AMPA- and NMDA-receptor–mediated EPSPs, membrane potential, and input resistance, but no effect on GABA_A-receptor–mediated IPSPs. To begin characterizing the signaling pathway responsible for GH enhancement of EPSPs, we applied specific inhibitors of JAK2, PI3 kinase, or MAP kinase kinase (MEK) along with GH, and examined effects on field EPSPs. The JAK inhibitor tyrphostin AG 490 (10 nM), applied beginning 30 min before and during GH, caused a significant decrease in the normal EPSP enhancement seen during GH (Fig. 11); after 60 min of GH, EPSPs averaged 153 \(\pm\) 22% of baseline (\(-0.17 \pm 0.02\) V/s, \(n = 6\)), compared with 255 \(\pm\) 27% when GH was applied alone (\(P < 0.05, n = 12\)). As shown in Fig. 11, complete inhibition was seen when GH was applied with 20 \(\mu\)M of the MEK inhibitor U0126 (96 \(\pm\) 7% of baseline, A1 and B1).
of the PI3 kinase inhibitor wortmanin (105 ± 8% of baseline, −0.21 ± 0.02 V/s, n = 6, P < 0.01 vs. GH alone). GH signaling in area CA1 of the hippocampus therefore involves both PI3-kinase and MAP-kinase signaling pathways, as shown previously in other tissues (Anderson 1992; Argetsinger et al. 1995; Jeay et al. 2001; Shoba et al. 2001; Sotiropoulos et al. 1994).

Our previous experiments indicated that GH-induced enhancement of EPSPs requires both PI3- and MAP-kinase signaling pathways. It is possible that the maintained enhancement of EPSPs we observed during continuous GH application (Figs. 1–7 and 10) requires continuous activity in these signaling pathways. Alternatively, activation of these signaling pathways may be required initially, but after some period of time continued EPSP enhancement may become independent of PI3-kinase or MEK activity. To determine whether continuous activity in these pathways is required, we applied wortmanin and U0126 to slices beginning 30 min after the start of GH application. If continuous activity of PI3 kinase or MEK is required to maintain the GH enhancement of EPSPs, then application of the inhibitor should cause a reversal of the EPSP enhancement despite continued application of GH. On the other hand, if inhibitor application fails to reverse the EPSP enhancement, this would suggest a temporally limited signaling requirement.

In support of a temporally limited requirement for both PI3- and MAP-kinase signaling pathways, the addition of either wortmanin (50 nM) or U0126 (10 μM) during the final 30 min of a 60-min period of GH treatment failed to affect the EPSP enhancement (Fig. 12). EPSPs were increased to 244 ± 65% of baseline (initially 0.16 ± 0.02 V/s, n = 5) in slices treated with GH followed by wortmanin (P > 0.30 compared with GH alone) and were increased to

FIG. 7. Isolated N-methyl-D-aspartate (NMDA)–receptor-mediated EPSPs and excitatory postsynaptic currents (EPSCs) were increased by GH application. Synaptic responses were isolated by bath application of the AMPA receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 30 μM) and the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor antagonist bicuculline methiodide (10 μM); in addition, cesium was used instead of potassium in the whole cell pipette solution, to block GABA<sub>B</sub> inhibitory postsynaptic potentials (IPSPs). During the current-clamp recording shown on the left, membrane potential was maintained at a steady level (−70 mV) by DC current injection to prevent voltage-dependent changes in NMDA-EPSPs. At the end of the recording the NMDA receptor antagonist D-AP5 (50 μM) was applied to verify that the response was mediated by NMDA receptors. Averaged NMDA-EPSPs from the baseline period (1), the end of GH application (2), and after application of D-AP5 (3) are shown in the top inset. During the voltage-clamp recording shown on the right, membrane potential was clamped to −75 mV. Averaged NMDA-EPSCs from the baseline period (1) and the end of GH application (2) are shown at the top. Treatment with GH caused a gradual increase in the amplitude of isolated NMDA-receptor–mediated synaptic responses, similar to those seen in previous whole cell and field potential recordings (Figs. 1–6). Data shown in this figure are from 2 different CA1 pyramidal neurons.

FIG. 8. Isolated fast (GABA<sub>A</sub>) IPSPs were not affected by GH. IPSPs were isolated by application of DNQX (30 μM) and D-AP5 (50 μM). Peak amplitude of the fast IPSP is plotted against time at the bottom. GH was applied for 55 min, but there was no change in the amplitude of the GABA<sub>A</sub>-receptor–mediated fast IPSP. Membrane potential was maintained at −72 mV by DC current injection. Inset, top right: averaged IPSPs from the baseline period (1) and the end of the GH application (2). Peak amplitude of the IPSP was not affected by GH application. Decrease in amplitude of the late component of the IPSP (GABA<sub>B</sub>-receptor–mediated IPSP) was an artifact of the recording technique used because identical decreases were seen during prolonged recording without application of GH (see text for further discussion). Data shown in this figure are from a single CA1 pyramidal neuron.
212 ± 16% of baseline (initially −0.14 ± 0.01 V/s, n = 5) in slices treated with GH followed by U0126 (P > 0.50 compared with GH alone, n = 12).

GH is capable of rapidly stimulating protein synthesis (Dreskin and Kostyo 1980; Jeffereson et al. 1975; Kostyo and Nutting 1973; Mowbray et al. 1975). To determine whether synthesis of new proteins contributes to GH enhancement of EPSPs, we pretreated slices with the protein synthesis inhibitor cycloheximide (60 μM) for 30 min before GH treatment, and continued to apply cycloheximide with GH for 60 min. To control for possible nonspecific effects of cycloheximide on synaptic responses, we treated a second group of slices with cycloheximide alone. Results from these two groups of slices were compared with results from slices treated with GH alone for 60 min. The control group treated with cycloheximide alone showed no change in field EPSP (at 60-min averaging 94 ± 14% of the initial baseline of −0.18 ± 0.02 V/s, n = 5, P > 0.60; see Fig. 13). Slices treated with GH + cycloheximide failed to show the EPSP enhancement normally seen during GH application. At the end of the 60-min GH + cycloheximide application, EPSPs were unchanged from baseline (99 ± 22% of the initial baseline of −0.18 ± 0.04 V/s, n = 6, P > 0.90) and were indistinguishable from the cycloheximide-alone control slices. Slices treated with GH alone (no cycloheximide, n = 12) showed significant EPSP enhancement in comparison with both the GH + cycloheximide and cycloheximide-alone groups (P values all <0.001).

Our results suggest that GH enhancement of EPSPs requires both PI3- and MAP-kinase signaling pathways, as well as synthesis of new proteins. Long-term potentiation (LTP), another form of long-lasting synaptic enhancement,
well-documented and prominent in hippocampal area CA1, may share these signaling pathways (reviewed in Lynch 2004; Sweatt 2001). To test whether common signaling mechanisms are involved in GH enhancement of EPSPs and LTP, we conducted a final set of experiments. In these experiments we looked for mutual occlusion between GH and LTP induced by high-frequency (100-Hz) stimulation. In the first experiment we pretreated slices with GH, then washed GH from the tissue and attempted to induce LTP by 100-Hz tetanization; results were compared with slices that received 100-Hz tetanization without prior GH treatment. Sample results from two slices, one receiving 100-Hz tetanization without prior GH treatment and one receiving 100-Hz tetanization after GH treatment, are shown in Fig. 14, A and B. Slices receiving 100-Hz tetanization showed robust LTP, as expected, averaging 160 ± 17% of baseline (0.15 ± 0.02 V/s, n = 9; Fig. 14C). In contrast, slices pretreated with GH showed posttetanic potentiation (PTP) only, and no LTP (at 50 min posttetanus, EPSPs averaged 97 ± 4% of the initial baseline, 0.21 ± 0.04 V/s, n = 6; P < 0.01; Fig. 15C). The difference between these two groups, at the 50-min posttetanus time point, was significant.
(P < 0.01). In a second experiment, we reversed the order of treatment, first inducing maximal LTP by repeated delivery of 100-Hz stimulation, then applying GH; results were compared with slices receiving GH with no prior tetanization. Sample results from two slices are shown in Fig. 15, A and B to illustrate the protocols used. Prior induction of LTP, by repeated 100-Hz stimulation, completely abolished the normal response to GH; after 60 min of treatment (Fig. 15C); EPSPs averaged 109 ± 5% of the initial baseline (–0.30 ± 0.07 V/s, n = 5), a value significantly less (P < 0.0002) than that obtained in slices treated with GH without prior tetanization (255 ± 27% of baseline, n = 12).

**DISCUSSION**

The hippocampus contains GH receptors (Burton et al. 1992; Lобie et al. 1993; Mustafa et al. 1994a) and hippocampal function is affected by chronic GH administration or deficiency (Le Greves et al. 2002; Ramsey et al. 2004; van Dam et al. 2000). The effects of GH on memory may be mediated by the hippocampus (Nyberg 2000). Our results demonstrate that hippocampal function is affected by GH over a much shorter timescale—minutes to hours—than previously appreciated. GH administration caused a selective enhancement of excitatory synaptic transmission in area CA1 (Figs. 1–7, 9, and 10).
without affecting fast GABA_A-receptor-mediated synaptic inhibition (Figs. 8 and 9). GH enhanced both AMPA- and NMDA-receptor-mediated excitatory synaptic transmission (Figs. 5, 7, and 9) without altering paired-pulse facilitation, an index of presynaptic function that changes when the probability of transmitter release is altered (Fig. 4). This increase in excitatory synaptic function was correlated with significant changes in postsynaptic neuron membrane function: hyperpolarization and decreased input resistance (Figs. 6, 9, and 10). However, these changes in postsynaptic neuron membrane function do not explain the increase in EPSPs. Decreased input resistance might lead to smaller EPSPs, but would not cause enhancement of EPSPs, and EPSP enhancement, at least for NMDA-receptor-mediated EPSP/CS was observed when changes in membrane potential were prevented (Figs. 7 and 9). Although our findings indicate that GH does affect postsynaptic membrane function, decreasing input resistance and hyperpolarizing CA1 neurons, and we found no change in PPF during GH application, suggesting that the probability of glutamate release from presynaptic terminals is not altered, we cannot exclude the possibility that GH has presynaptic effects. For example, GH could increase the number of presynaptic release sites without changing PPF so long as the new release sites have the same overall probability of release as the original release sites.

GH enhancement of EPSPs requires JAK2, PI3 kinase, and MEK (Fig. 11) as well as synthesis of new proteins (Fig. 13). Although PI3-kinase, MEK, and protein synthesis are required for GH effects on excitatory synaptic transmission, the requirement is temporally limited because application of inhibitors beginning 30 min after the start of GH application failed to reverse the EPSP enhancement (Figs. 11 and 13). On the contrary, EPSPs continued to increase during inhibitor application (Fig. 13). That EPSPs continued to increase during maintained GH application for \( \leq 60 – 70\) min, either with or without inhibitors present (Figs. 1–5 and 13), may indicate the existence of additional downstream signaling targets whose activity outlasts initial signaling events. Alternatively, the continuing increase in EPSPs beyond the first 30 min of GH application may simply reflect the final consequences of signaling events that occur during the first 30 min. For example, the final delivery of new proteins that are synthesized within the first 30 min of GH stimulation could require \( >30\) min.

GH signaling has been studied extensively in several non-neuronal tissues (reviewed in Herrington and Carter-Su 2001; Kelly et al. 2001; Piwien-Pilipuk et al. 2002). GH binding to GH receptors induces receptor dimerization and activation of JAK2. In response to GH receptor-JAK2 activation, several distinct signaling pathways are stimulated, including the STAT (primarily STAT5, but also STAT1 and STAT3), PI3-kinase, and MAP-kinase pathways. GH signaling in hippocampus requires at least JAK2, PI3 kinase, and MEK, indicating substantial similarity with other tissues. We have not yet investigated the possible role of STATs in GH enhancement of excitatory synaptic transmission. In response to GH, STATs are phosphorylated, dimerize, and stimulate transcription of specific genes (Herrington and Carter-Su 2001; Kelly et al. 2001; Piwien-Pilipuk et al. 2002), leading eventually to synthesis of new proteins. Considering the time required for formation of new proteins in response to STAT activation and the transport of these new proteins from somatic to synaptic regions of the neuron, it seems unlikely that STATs could participate during the initial response to GH, which begins within a few minutes of the start of GH application (see Figs. 1–7 and 10). However, it is possible that the sustained response to GH, which persists for \( \geq 4\) h, might require activation of one or more of the STATs. The possible contributions of one or more of the STATs will need to be addressed in future work.
Future experiments will also need to determine which of the GH-stimulated pathways is required for GH effects on postsynaptic membrane function (membrane potential and input resistance).

Future experiments should also address the possible contribution of insulin-like growth factor I (IGF-I) to the acute GH effects we observed. IGF-I expression is induced by GH and IGF-I in turn is responsible for mediating many but by no means all of the effects of GH (reviewed in Jones and Clemmons 1995; LeRoith et al. 2001). Previous studies in the hippocampus suggest that IGF-I can act as a modulator of synaptic transmission (Huang et al. 2004; Ramsey et al. 2005). Although IGF-I might contribute to GH effects on hippocampal function, it seems unlikely that IGF-I acts as a mediator during the initial response to GH, for the same reason that the STATs seem unlikely to contribute during the early phase of the response. IGF-I might, however, contribute to the maintained effects of GH several hours after the initial application.

Primates, but not nonprimates, can bind to and activate prolactin receptors (Goffin et al. 1996; Kossiakoff et al. 1994). The hippocampus contains prolactin receptors (Fechner and Buntin 1989; Lai et al. 1992; Mustafa et al. 1994a,b) in addition to GH receptors, and they might contribute to effects of rhGH. We therefore examined rGH, which does not bind the prolactin receptor (Amit et al. 1987; Møldrop et al. 1990). We found identical effects for both rGH and rhGH, indicating that prolactin receptors are not required for GH effects in the hippocampus.

Our results indicate a number of similarities between GH enhancement of EPSPs and other forms of long-lasting synaptic enhancement in hippocampal area CA1, including high-frequency stimulation-induced (tetanus-induced) long-term potentiation (LTP) and brain-derived neurotrophic (BDNF)-induced potentiation. We found that GH enhancement of EPSPs required activation of both the PI3-kinase and MAP-kinase pathways, and also required synthesis of new proteins. Similarly, tetanus- and neurotrophin-induced LTP also require PI3-kinase and MAP-kinase signaling, and synthesis of new proteins (reviewed in Kelleher et al. 2004; Lynch 2004). It appears that GH, like BDNF, shares at least some of the signaling mechanisms involved in tetanus-induced LTP. In agreement with this conclusion, we found mutual occlusion between the effects of GH treatment and high-frequency stimulation-induced LTP (Figs. 14 and 15).

Previous studies have shown that GH is required for normal development of the brain (reviewed in Harvey and Hull 2003; Noguchi 1996; Scheepens et al. 2000), and memory deficiency including both short- and long-term memory is a well known syndrome in GH-deficient patients (Aleman et al. 2000; Deijen et al. 1996). Chronic (3-mo) GH supplementation improved attentional performance in adult patients suffering from hypopituitarism with GH deficiency (Oertel et al. 2004). An age-related decrease of GH is associated with defects in spatial memory in animals (van Dam et al. 2000) and may contribute to age-related decline in memory function during normal human aging (Creyghton et al. 2004; van Dam et al. 2000). The hippocampus contains receptors for GH (Burton et al. 1992; Lobbie et al. 1993; Mustafa et al. 1994a) and chronic, systemic treatment with GH alters the pattern of expression of NMDA-receptor subunits in the hippocampus (Le Greves et al. 2002), alters GABA \( \alpha_1 \) subunit expression, short-term synaptic plasticity (paired-pulse inhibition), and improves hippocampal-dependent spatial learning (Ramsey et al. 2004). The beneficial effects of GH on memory function may result from the alteration of glutamate and GABA-receptor expression during long-term treatment with GH. In addition, our results suggest more immediate effects of GH on hippocampal synaptic function, which might also contribute to the ability of GH to modulate hippocampal-dependent memory function. In addition to GH receptors, GH itself is produced in the hippocampus (Gossard et al. 1987; Hojvat et al. 1982). In a recent study, Donahue et al. (2002) showed upregulation of hippocampal GH mRNA after a hippocampal-dependent learning task (trace eye-blink conditioning). This finding together with our results suggests that endogenous hippocampal GH might act in an autocrine or paracrine manner to enhance or help maintain altered synaptic function during memory formation. GH may therefore influence hippocampal function in multiple ways, over both short and long timescales.

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