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Effects of insulin-like growth factor 1 on synaptic excitability in cultured rat hippocampal neurons

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Abstract

Insulin-like growth factor 1 (IGF-1) has important functions in the brain, including metabolic, neurotrophic, neuromodulatory and neuroendocrine actions, and it also prevents β amyloid-induced death of hippocampal neurons. However, its functions in the synaptic excitability remain uncertain. Here we investigated the effects of IGF-1 on synaptic excitability in cultured rat hippocampal neurons using whole-cell patch clamp recordings. Incubation the hippocampal neurons with different concentrations of IGF-1 for 24 h or 30 min significantly increased the frequency of spontaneous excitatory postsynaptic currents (sEPSCs), but had no effect on the frequency of miniature EPSCs (mEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs). The mean amplitudes, rise, and decay kinetics of sEPSCs, mEPSCs, and sIPSCs were not significantly affected by IGF-1, indicating that IGF-1 increased the probability of neurotransmitter release but did not modulate postsynaptic receptors. The effects of IGF-1 were mediated by mitogen-activated protein kinase (MAPK). IGF-1 activated the ERK1/2 signaling pathway in cultured hippocampal neurons, and the inhibitor PD98059 blocked the enhancement of sEPSCs induced by IGF-1. These results demonstrated the regulatory function of IGF-1 on synaptic excitability in hippocampal neurons and its underlying signaling mechanism.

Keywords: Insulin-like growth factor 1; Excitatory postsynaptic currents; Inhibitory postsynaptic currents; Patch clamp; MAPK

Introduction

Insulin-like growth factor 1 (IGF-1) is a pleiotropic factor with structural and functional homologies to IGF-2 and insulin. IGF-1 and insulin play an important role in the regulation of physiological functions such as glucose and energy metabolism and growth (Gasparini and Xu, 2003). In addition, insulin and IGF-1 have important functions in the brain, including metabolic, neurotrophic, neuromodulatory and neuroendocrine actions (Torres-Aleman, 2000). Insulin, IGF-1, and their receptors are all present in rodent and human brain (Craft et al., 1998; Frolich et al., 1998; Schulingkamp et al., 2000). It is now known that insulin and IGF-1 are actively transported across the blood–brain barrier and possibly even produced locally in the brain (Schulingkamp et al., 2000). IGF-1 can promote the survival, proliferation and maturation of cultured neurons (DiCicco-Bloom and Black, 1988), reduce neuronal loss in adult rat brain following hypoxic–ischemic injury (Guan et al., 1993), induce the differentiation of oligodendrocytes (McMorris et al., 1993), stimulate DNA synthesis (Lenoir and Honegger, 1983) and neurite outgrowth (Ruiz et al., 1992), direct the sprouting of spared afferents into a deafferented hippocampus (Guthrie et al., 1995), and modulate hippocampal acetylcholine release (Seto et al., 2002).

Recently, IGF-1 has gained increasing attention for the pathogenesis of age-related neurodegenerative diseases, such as Alzheimer's disease (AD) (Gasparini et al., 2002). AD

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patients show changes in insulin and IGF-1 levels and their response to insulin is defective. IGF-1 has been found to protect hippocampal neurons against the toxicity of β amyloid protein (Aβ) (Dore et al., 1997a), and has a direct effect on the metabolism and clearance of Aβ. Therefore, IGF-1 is considered as a potential therapeutic agent for AD.

Although several reports describe different actions of IGF-1 on synaptic plasticity (Kakizawa et al., 2003; Nunez et al., 2003; Ramsey et al., 2004, 2005), modulation of neuronal excitability in response to IGF-1 is not well understood. In this study, we have investigated the roles of IGF-1 in the regulation of excitatory and inhibitory synaptic transmission using whole-cell patch-clamp recordings in cultured rat hippocampal neurons. Furthermore, we determine the intracellular signaling mechanisms mediating these effects.

Materials and methods

Reagents

Media, fetal bovine serum (FBS), B27 supplements, 0.25% trypsin–EDTA, and poly-D-lysine for cell culture were from Invitrogen (Carlsbad, CA). Equine serum (ES) and L-glutamine were from Hyclone (Logan, Utah). IGF-1 was purchased from PeproTech Inc. (Rocky Hill, NJ) and dissolved in sterile ddH_2O. Anti-ERK1/2, anti-active-JNK, and anti-active-p38 antibodies were purchased from Promega (Madison, WI). Anti-phospho-ERK antibody was purchased from Santa Cruz (California, USA). Anti-SAPK/JNK and anti-p38 antibodies were purchased from Cell Singaling Technology (Beverly, MA). PD98059 and other reagents were purchased from Sigma (St. Louis, MO).

Hippocampal neurons culture and treatments

Hippocampal neuron cultures were prepared as described previously (Evans et al., 1998). Briefly, pregnant Wistar rats were anesthetized (i.p.) with 30 mg/kg of pentobarbital and the E18–19 embryos delivered by cesarean section. The hippocampi were dissected and incubated with 0.25% trypsin–EDTA for 15 min at 37 °C and mechanically dissociated. The resulting single cell suspension was diluted at a density of 1×10^5 cells/ml in high glucose DMEM containing 10% FBS, 5% ES and 2 mM L-glutamine, then plated in 35 mm-cell plates coated with poly-D-lysine (20 μg/ml). Cells were incubated at 37 °C in a humidified incubator with 5% CO_2. After approximately 20 h, the medium was replaced by serum-free Neurobasal medium containing B27 supplement and 0.5 mM L-glutamine to inhibit the growth of glia cells. In the cultures, the percentage of glia cells was below 5%. Every 3–4 days half of the media was replaced and the cultures were used for experiments 10–14 days after plating.

To determine the effects of IGF-1, we added different concentrations of IGF-1 (1, 10 and 100 ng/ml) to cultured hippocampal neurons, and continued to incubate for 24 h or 30 min. Then the media with IGF-1 was discarded, and culture dishes were rinsed twice and perfused with extracellular solution containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 10 Glucose, 10 Hepes, pH 7.4. The changes in their physiological properties were measured using whole-cell patch clamp recordings. Control experiments were done to determine the specificity of the IGF-1 effects. In the inhibitor experiment, cultures were pretreated with inhibitor PD98059 (10 μM) for 1 h prior to addition of 10 ng/ml IGF-1 for 30 min. For Western blot analysis, the hippocampal neurons were incubated with different concentrations of IGF-1 (1, 10 and 100 ng/ml) for 24 h or with 10 ng/ml IGF-1 for various times.

Whole-cell patch clamp recording

The whole-cell voltage-clamp technique was used to record current. The patch electrodes of thick-walled boro-silicate glass (VWR Scientific, West Chester, PA) were pulled on a PP-83 micropipette puller (Narishige, Japan). The patch-pipette solution contained (in mM): 140 KCl, 10 Hepes, 10 EGTA, 2 $MgCl_2$, 2 Na_2ATP, 1 $CaCl_2$, pH 7.3. The typical resistance of glass electrodes was 3–5 MΩ when filled with intracellular pipette solution. Data were collected with an Axopatch 200B amplifier (Axon Instruments, Forster City, CA) and acquired and analyzed using pCLAMP 9 (Axon Instruments). Fast and slow capacitances were neutralized and series resistance was always compensated (about 70%).

Spontaneous inhibitory and excitatory postsynaptic currents (sIPSCs and sEPSCs) were isolated by the application of antagonists of the excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors, 20 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 50 μM aminophosphonobutyrate (APV), respectively, or the antagonist of the inhibitory γ-aminobutyric acid ($GABA_A$) receptor, 50 μM bicuculline. Miniature EPSCs (mEPSCs) were recorded using 50 μM bicuculline and 1 μM TTX added to the extracellular solution. sEPSCs, mEPSCs, and sIPSCs were recorded without synaptic stimulation at a holding potential of −70 mV from cultured hippocampal neurons for at least 5 min. Cells were chosen for recording based on their morphology and density of surrounding cells. The criterion was that they were similar to hippocampal pyramidal neurons. Relatively isolated or spherically shaped cells were avoided.

Western blotting analysis

MAPK activation was determined by Western blot. Cells were lysed in buffer containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1% SDS, 50 nM okadaic acid, 2 mM dithiothreitol (DTT), 10% glycerol and Complete Mini protease inhibitors (1 tablet/10 ml buffer; Roche, Mannheim, Germany). Insoluble materials were removed by centrifugation. The protein concentrations of the lysates were determined by the colorimetric bicinchoninic acid analysis (Pierce Chemical Company, Rockford, IL, USA). Equal amount of protein were separated in a 10% SDS-PAGE (20 μg/lane) for approximately 1.5–2 h at 120 V, and then transferred onto nitrocellucose membranes at constant voltage (100 V) for 1.5 h. The membranes were blocked with 5% non-fat dry milk and 50 mM NaF in TTBS (20 mM Tris–HCl, pH

7.4, 150 mM NaCl, 0.05% Tween 20) and then probed with different primary antibodies at 4 °C overnight. After washing, the membrane was incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature, followed by enhanced chemiluminescence detection (Roche).

Statistical analysis

Data were expressed as the means±S.E.M. One-way ANOVA and post hoc tests, as indicated in Results, were used to compare difference (SPSS, Chicago, IL, USA). Statistical significance was taken at the level of $p<0.05$. In all cases, n referred to the number of neurons studied.

Results

IGF-1 potentiated the frequency of sEPSCs of cultured hippocampal neurons, but did not influence the amplitude

Twenty-four hours of IGF-1 treatment significantly increased the frequency of sEPSCs in cultured hippocampal neurons, but did not influence the mean amplitude (Fig. 1). Quantitative analysis of all neurons recorded indicated that sEPSCs burst frequency increased by 49.7–63.3%. The average burst frequency of the control group ($n=12$) was 0.04±0.005 Hz. After 24 h treatment of 1, 10, and 100 ng/ml of IGF-1, the frequency increased to 0.07±0.007 Hz ($n=10$), 0.07±0.006 Hz ($n=10$), and 0.06±0.005 Hz ($n=11$), respectively ($p<0.05$ compared with control group, Tukey HSD test). We also counted the number of sEPSCs per burst, and there was no significant difference between the control and IGF-1-treated groups. For the control group, the number per burst was 8.11± 0.68. After incubation with 1, 10, and 100 ng/ml IGF-1 for 24 h, the number per burst was 8.95±0.72, 8.14± 0.94, and 7.53± 1.02, respectively (Table 1).

Further, the kinetic properties of individual sEPSCs, including rise time constant and decay time constant, were analyzed using Clampfit 9.0 (Axon Instruments). The rise and decay kinetics of sEPSCs was similar for the control and IGF-1-treated hippocampal neurons, and there was no significant difference between two groups. The details were reported in Table 1.

IGF-1 had no effects on mEPSCs and sIPSCs

The average values of the frequency and mean amplitude of mEPSCs in the control group ($n=9$) were 0.019±0.004 Hz and −26.18±1.97 pA, respectively. After treatment with 1, 10, and 100 ng/ml of IGF-1 for 24 h, the frequency of mEPSCs was 0.018±0.003 Hz ($n=7$), 0.022±0.006 Hz ($n=7$), and 0.017± 0.005 Hz ($n=8$), and the mean amplitude of mEPSCs was −24.50±1.99 pA, −28.63±4.77 pA, and −30.76±2.78 pA, respectively. There was no significant difference between the IGF-1-treated and control groups.

The average values of the frequency and mean amplitude of sIPSCs in the control neurons ($n=10$) were 3.37±0.33 Hz and −1020.45±126.4 pA, respectively. Application of IGF-1 did not significantly modify the frequency and mean amplitude of

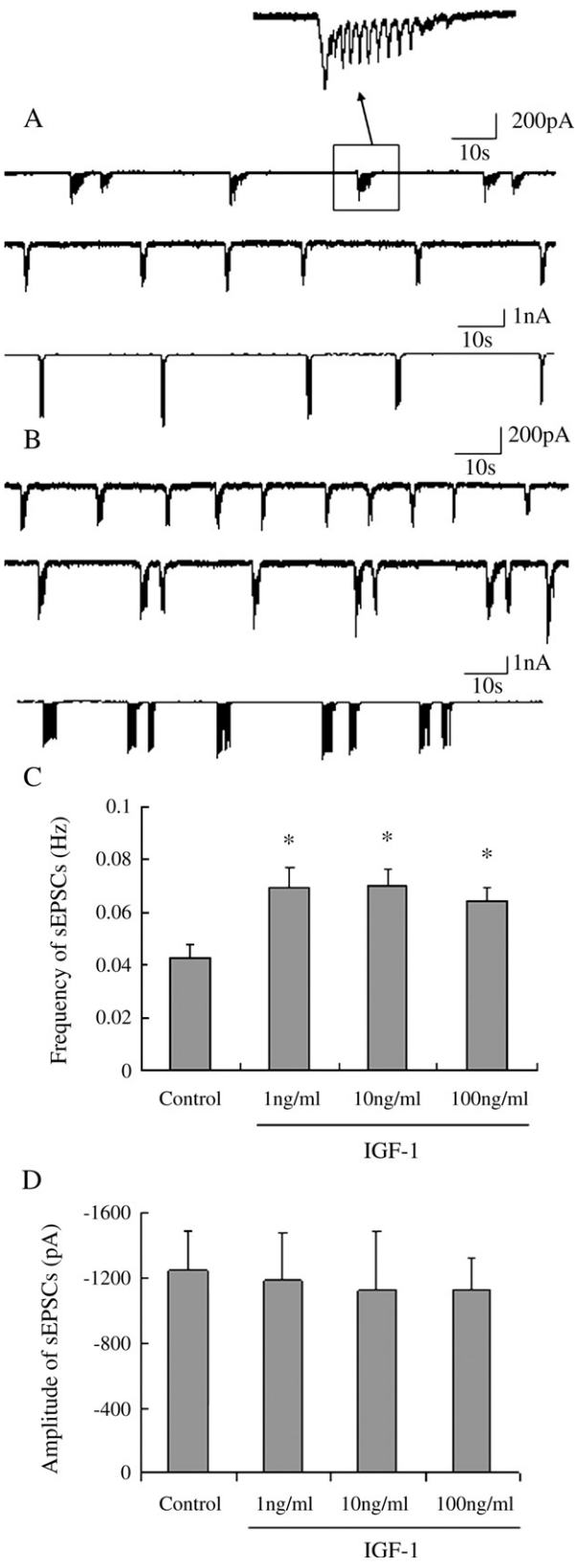

Fig. 1. IGF-1 potentiates the frequency of sEPSCs, but has no effect on the mean amplitude. Primary hippocampal neurons (10–14 days old) were treated with different concentrations of IGF-1 for 24 h. (A–B) Representative traces from three different control cells ($n=12$, A) and 10 ng/ml IGF-1-treated cells ($n=10$, B). (C–D) Bar graph of the frequency (C) and mean amplitude (D) of sEPSCs. ∗, $p<0.05$ compared with the control group.

Table 1
Summary of quantitative data

	Control		IGF-1 10 ng/ml	
	Mean±S.E.M.	*n*	Mean±S.E.M.	*n*
sEPSCs				
Frequency (Hz)	0.04±0.005	12	0.07±0.006*	10
Mean peak amplitude (pA)	−1245.01±240.3	12	−1122.03±360.0	10
Numbers/burst	8.11±0.68	12	8.14±0.94	10
Rise time constant (ms)	14.95±1.58	12	12.27±2.43	10
Decay time constant (ms)	23.71±2.79	12	23.34±3.92	10
sIPSCs				
Frequency (Hz)	3.37±0.33	10	3.14±0.69	9
Mean peak amplitude (pA)	−1020.45±126.4	10	−1138.94±361.4	9
Time to peak (ms)	5.01±0.28	10	4.81±0.27	9
Rise time constant (ms)	4.55±0.75	10	4.72±0.83	9
Decay time constant (ms)	40.00±3.32	10	42.01±3.40	9

* $p<0.05$ compared with control group.

sIPSCs of cultured hippocampal neurons (Fig. 2). In addition, the kinetic properties of sIPSCs were analyzed, including time to peak, rise time constant, and decay time constant. The rise and decay kinetics of sIPSCs was similar for the control and IGF-1-treated hippocampal neurons, and there was no significant difference between two groups. The details were reported in Table 1.

IGF-1 activated ERK1/2 signaling pathway in cultured hippocampal neurons

To study the synaptic cell signaling pathways influenced by IGF-1, we measured activations of mitogen-activated protein kinase (MAPK) pathways in cultured hippocampal neurons. Based on the sequence similarity, mechanisms of upstream regulation and, to a lesser extent, substrate specificity, MAPK pathways could be divided into several subgroups including extracellular signal-regulated kinase (ERK) 1 and 2, c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SAPK) and p38 (Chang and Karin, 2001).

Cells lysates from cultured hippocampal neurons treated with different concentrations of IGF-1 (Fig. 3) for specific intervals (Fig. 4) were analyzed by Western blot using anti-phospho-ERK, -active-JNK and -active-p38 antibodies to detect phosphorylated ERK1/2, JNK, and p38, respectively (upper panel). Duplicate blots were probed with antibodies recognizing total ERK1/2, SAPK/JNK, and p38 to verify equal protein loading in the samples (lower panel). Treatment with different concentrations of IGF-1 for 24 h strongly activated ERK1/2, whereas JNK and p38 were not been activated. Quantity analysis of the optical density of p-ERK1/2 bands showed that there was no significant difference among different concentrations of IGF-1 treatments. This result suggested that ERK1/2 but not JNK and p38 kinase activation may be involved in the effect of IGF-1 on the frequency of sEPSCs. To evaluate the progress of activation, ERK1/2 phosphorylation was measured at various times after 10 ng/ml IGF-1 treatment. As shown in Fig. 4, 10 ng/ml of IGF-1 led to increased phosphorylation of ERK1/2 within 30 min and this phosphorylation remained elevated for 24 h.

IGF-1 acutely increased the frequency of sEPSCs, and PD98059 blocked this effect

Since the effect of IGF-1 on sEPSCs involved the activation of ERK1/2, and IGF-1 induced ERK1/2 phosphorylation as

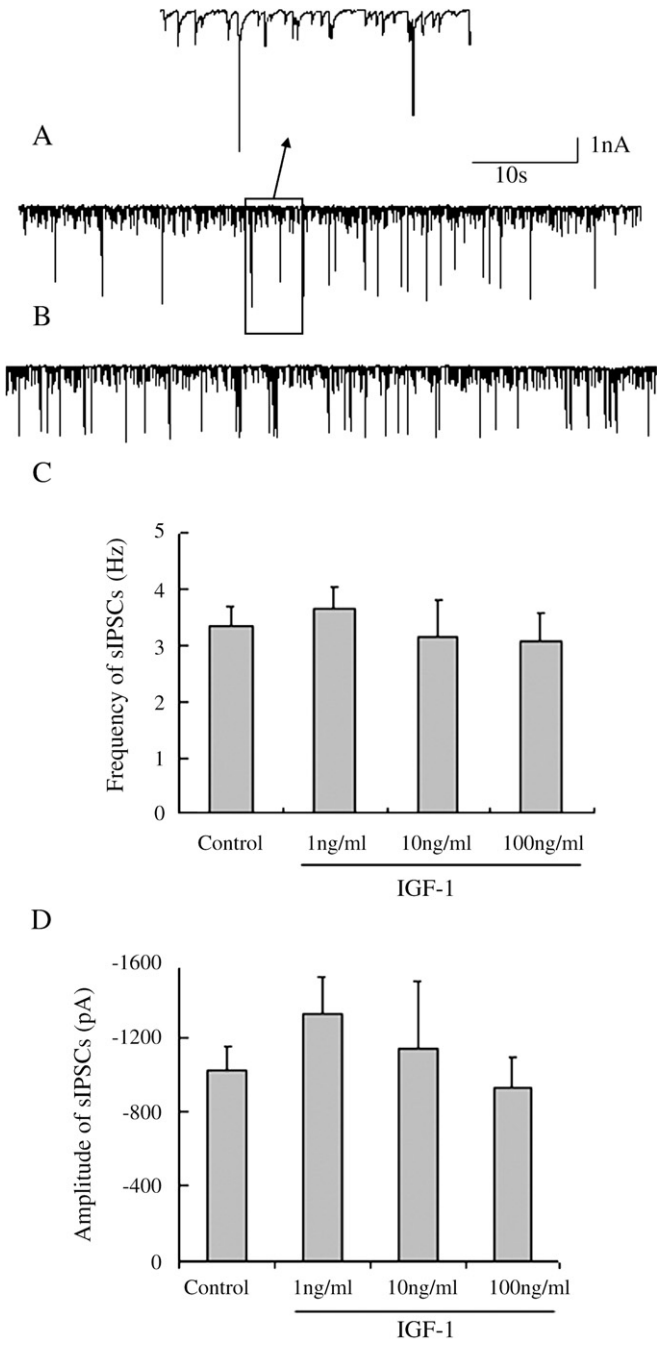

Fig. 2. IGF-1 has no effects on the frequency and mean amplitude of sIPSCS. Primary hippocampal neurons (10–14 days old) were treated with different concentrations of IGF-1 for 24 h. (A–B) Representative traces from the control cells ($n=10$, A) and 100 ng/ml IGF-1-treated cells ($n=9$, B). (C–D) Bar graph of the frequency (C) and mean amplitude (D) of sIPSCs. There is no significant difference between the groups.

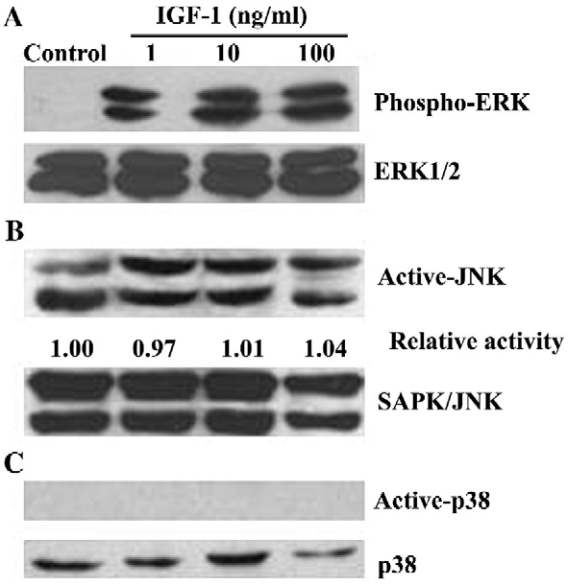

Fig. 3. Western blot analysis of MAPK activation in cultured hippocampal neurons treated with 1, 10, and 100 ng/ml of IGF-1 for 24 h. (A) ERK1/2 phosphorylation. (B) No JNK phosphorylation. (C) No p38 phosphorylation. The blots are representative of three separate experiments. Upper panels in A–C are Western blots using phosphorylation specific antibodies; the lower panels are Western blots with antibodies recognizing total ERK1/2, JNK/SAPK, and p38.

early as 30 min, we examined whether acute application of IGF-1 could potentiate sEPSCs. Treatment of the hippocampal neurons with 10 ng/ml IGF-1 for 30 min significantly increased the frequency of sEPSCs (Fig. 5), but had no effect on the mean amplitude. The frequency of control group ($n=8$) was 0.05±0.007 Hz. After treatment with 10 ng/ml IGF-1 for 30 min ($n=8$), the frequency significantly increased to 0.10±0.003 Hz ($p<0.01$ compared with control group, Tukey HSD test). Pretreatment with 10 μM of PD98059 for 1 h prior to IGF-1 application ($n=10$) could block the action of IGF-1 (Fig. 5), and the frequency was 0.07±0.004 Hz ($p<0.01$ compared with IGF-1 group, $p=0.162$ compared with control group, Tukey HSD test).

We also counted the number of sEPSCs per burst. There was no significant difference between the control and IGF-1-treated group. For the control group, the number per burst was 6.70±0.96. After incubation with 10 ng/ml IGF-1 for 30 min, the number per burst was 7.13±0.74. In addition, the acute application of IGF-1 did not change the rise and decay kinetic of sEPSCs. The details were reported in Table 2.

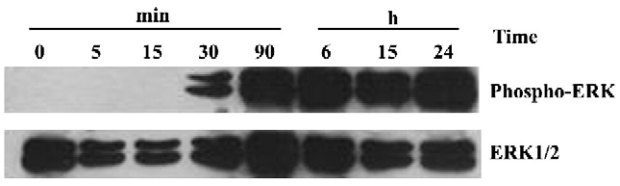

Fig. 4. Western blot analysis of ERK1/2 activation in cultured hippocampal neurons treated with 10 ng/ml of IGF-1 for the indicated times. The blots are representative of three separate experiments. Upper panel is blots using phosphorylation-specific ERK antibody; the lower panel is blots with antibody recognizing total ERK1/2 kinase.

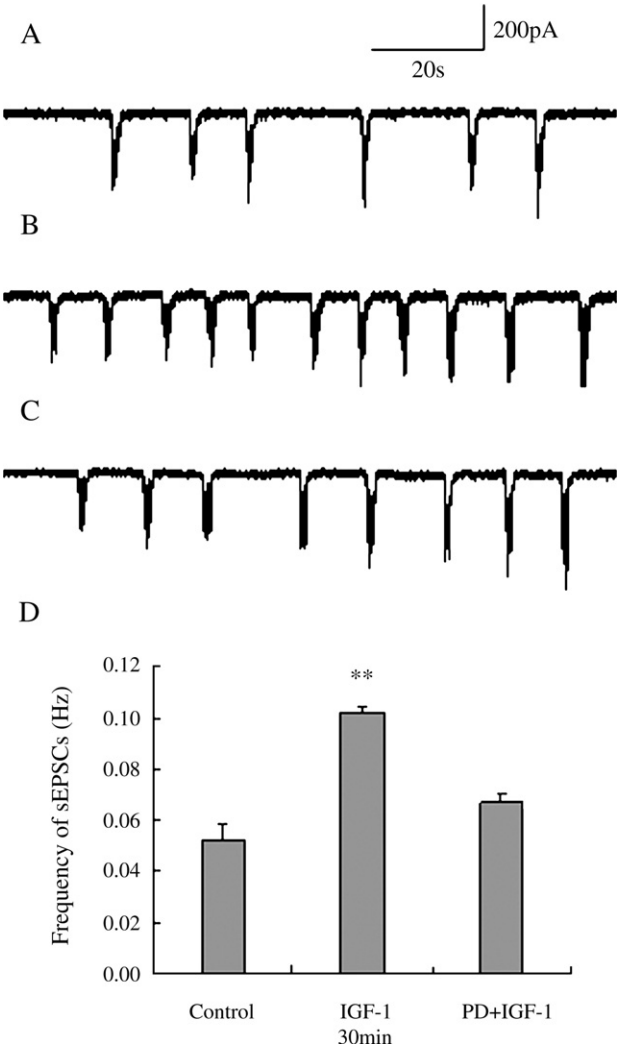

Fig. 5. IGF-1 acutely increases the frequency of sEPSCs, and PD98059 blocks this effect. Primary hippocampal neurons (10–14 days old) were treated with 10 ng/ml IGF-1 for 30 min or pretreated with 10 μM PD98059 for 1 h prior to addition of IGF-1. (A–C) Representative traces from the control cells ($n=8$, A), IGF-1 treated cells ($n=8$, C), and the cells pretreated with PD98059 for 1 h prior to IGF-1 incubation ($n=10$, D). (D) Bar graph of the frequency of sEPSCs. **, $p<0.01$ compared with the control group.

Discussion

This study represented the characterization of the effects of IGF-1 on hippocampal excitatory and inhibitory synaptic transmission. First, acute and chronic application of IGF-1 significantly increased the frequency of sEPSCs in cultured hippocampal neurons, but had no effect on the mean amplitude, and rise or decay kinetics. Second, IGF-1 did not affect mEPSCs and sIPSCs of hippocampal neurons. Finally, experiment of inhibitor PD98059 demonstrated that the effect of IGF-1 on excitatory transmission may be mediated by MAPK pathways. These findings revealed the regulation of

Table 2
Summary of quantitative data of sEPSCs

sEPSCs	Control		IGF-1 30 min		PD+IGF-1	
	Mean±S.E.M.	*n*	Mean±S.E.M.	*n*	Mean±S.E.M.	*n*
Frequency (Hz)	0.05±0.007	8	0.10±0.003 *	8	0.07±0.004	10
Mean peak amplitude (pA)	−684.99±161.64	8	−515.43±119.99	8	−616.48±206.11	10
Numbers/burst	6.70±0.96	8	7.13±0.74	8	6.93±0.62	10
Rise time constant (ms)	12.48±1.85	8	13.96±1.77	8	13.83±1.28	10
Decay time constant (ms)	22.19±3.98	8	25.34±2.30	8	22.90±2.19	10

* $p<0.01$ compared with control group.

IGF-1 on the synaptic function in normal hippocampal neurons and the signaling mechanism involved.

Neurotrophic factors have recently been shown to act as rapid and potent modulators of neuronal activity in the adult brain (Thoenen, 2000). IGF-1 can modulate many ion channels (Blair and Marshall, 1997; Kanzaki et al., 1999; Kelsch et al., 2001) and neurotransmitter receptor activity (Gonzalez de la Vega et al., 2001; Savchenko et al., 2001) in different types of cell. Additional studies have focused on IGF-1-mediated changes in hippocampus, a brain region that has an important role in learning and memory and is severely affected in AD (Crews et al., 1992; Dore et al., 1997b). In the present study, we determined a role for IGF-1 in modulation of synaptic excitability in cultured rat hippocampal neurons.

Our results indicated that IGF-1 also modulated the excitability of hippocampal neurons. Acute and chronic application of IGF-1 both significantly enhanced the excitability of cultured rat hippocampal neurons. In the current study, IGF-1 increased the frequency of sEPSCs but did not affect the frequency of mEPSCs, indicating that the effects of IGF-1 on excitatory synaptic transmission may be action potential-related (i.e., TTX-sensitive). To further clarify the action site of IGF-1-induced excitability in hippocampal neurons, we determined the amplitude and rise and decay kinetic properties of sEPSCs and mEPSCs which primarily depended on the numbers and activity of postsynaptic receptors. But no differences were found. This may indicate that IGF-1 substantially enhanced release of glutamate at synapses from a subset of presynaptic excitatory neurons, or increased the number of excitatory neurons in functional synaptic contact between hippocampal neurons, but did not interact with postsynaptic NMDA or non-NMDA receptors.

In addition, we also elucidated the role of IGF-1 in inhibitory synaptic transmission. In the normal hippocampal neurons, the inhibitory synaptic properties were unaffected by IGF-1. But in another study we did (Xing et al., 2005), IGF-1 could block the effect of Aβ-induced reduction of the frequency of sIPSCs in cultured hippocampal neurons. It was likely that the multiple regulatory actions of IGF-1 on sIPSCs were probably due to different physiologic and pathological situations.

The IGF-1-induced enhancement of hippocampal excitatory synapses that we observed is consistent with some other investigations. IGF-1 may act over hours or days to promote neuronal differentiation and survival, or exert critical effects on synaptic transmission and plasticity within minutes. IGF-1 produces an increase in the excitability of different types of neurons. Acute in vitro application of IGF-1 potentiates kainate receptor-mediated currents via a phosphoinositide 3-kinase (PI3K)-dependent mechanism in cerebellar granule neurons (Gonzalez de la Vega et al., 2001). IGF-1 increases the excitability of dorsal column nuclei (DCN) cells in vivo and in vitro by a presynaptic process dependent on MAPK activation (Carro et al., 2000; Nunez et al., 2003). IGF-1 elicits long-term depression in cultured Purkinje neurons by down-regulating AMPA receptor signaling (Wang and Linden, 2000). The acute enhancement effect of des-IGF-1 on excitatory synaptic transmission in the CA1 region is due to a postsynaptic mechanism involving AMPA but not NMDA receptors (Ramsey et al., 2005). These studies demonstrate the diverse actions of IGF-1 on excitatory neurotransmission with respect to different brain regions. However, the different experimental settings may result in some different results between the published study (Ramsey et al., 2005) and our present study, including the preparation used (hippocampal slice vs. cultured hippocampal neurons), patch clamp recording (evoked EPSC vs. spontaneous EPSC), agents (des-IGF-1 vs. IGF-1), etc.

IGF-1 receptor possesses tyrosine kinase activity, and can regulate many downstream effectors including ras, MAPK, PI3K, and Akt, as well as the adaptor proteins shc and Grb2 (Friedman and Greene, 1999; Fukudome et al., 2003). Although the PI3K/Akt pathway is a well-documented cascade initiated by activation of the IGF-1 receptor (De Meyts et al., 1995; Zheng and Quirion, 2004), MAPK activation by IGF-1 on target cells is also a very common step in IGF-1 signaling (LeRoith, 2000). In our study, application of IGF-1 strongly activated ERK1/2 at a concentration as low as 1 ng/ml. This phosphorylation of ERK1/2 appeared within 30 min, and remained elevated for 24 h. Under our experimental conditions, IGF-1 did not activate the JNK and p38 pathway. Furthermore, we examined whether inhibition of MAPK activity prevented the IGF-1 modulation of sEPSCs. Indeed, pretreatment with PD98059, MAPK/ERK kinase (MEK) inhibitor, blocked the enhanced excitability of hippocampal neurons evoked by IGF-1, and the frequency of sEPSCs decreased to the normal level. Thus, MAPK appeared to be a likely candidate pathway because inhibition of this pathway blocked the modulation of IGF-1 of synaptic excitability in the hippocampal neurons.

Glutamatergic neurotransmission, an important process in learning and memory, is severely disrupted in patients with AD. IGF-1 increases hippocampal NMDAR2A and R2B subunit expression (Sonntag et al., 2000), ameliorates the age-related cognitive deficits and changes of short-term plasticity in hippocampus (Ramsey et al., 2004). Additionally, IGF-1 has been reported to protect hippocampal neurons against Aβ-induced toxicity. Together with the anti-apoptotic and the anti-inflammatory effects of IGF-1 on nerve tissue, enhancement of neuronal excitability evoked by IGF-1 may contribute to the known neuroprotective effects of IGF-1 (Torres-Aleman, 2000). It is conceivable that the potentiation of neuronal excitability and an increase in glutamate release induced by IGF-1 may lead to an enhancement in learning and memory. These results may also imply that IGF-1 may be a promising target for treatment to AD.

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