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INSULIN-LIKE GROWTH FACTOR I INTERFACES WITH BRAIN-DERIVED NEUROTROPHIC FACTOR-MEDIATED SYNAPTIC PLASTICITY TO MODULATE ASPECTS OF EXERCISE-INDUCED COGNITIVE FUNCTION

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Abstract—The ability of exercise to benefit neuronal and cognitive plasticity is well recognized. This study reveals that the effects of exercise on brain neuronal and cognitive plasticity are in part modulated by a central source of insulin-like growth factor-I. Exercise selectively increased insulin-like growth factor-I expression without affecting insulin-like growth factor-II expression in the rat hippocampus. To determine the role that insulin-like growth factor-I holds in mediating exercise-induced neuronal and cognitive enhancement, a specific antibody against the insulin-like growth factor-I receptor was used to block the action of insulin-like growth factor-I in the hippocampus during a 5-day voluntary exercise period. A two-trial-per-day Morris water maze was performed for five consecutive days, succeeded by a probe trial 2 days later. Blocking hippocampal insulin-like growth factor-I receptors did not significantly attenuate the ability of exercise to enhance learning acquisition, but abolished the effect of exercise on augmenting recall. Blocking the insulin-like growth factor-I receptor significantly reversed the exercise-induced increase in the levels of brain-derived neurotrophic factor mRNA and protein and pro-brain-derived neurotrophic factor protein, suggesting that the effects of insulin-like growth factor-I may be partially accomplished by modulating the precursor to the mature brain-derived neurotrophic factor. A molecular analysis revealed that exercise significantly elevated proteins downstream to brain-derived neurotrophic factor activation important for synaptic function, i.e. synapsin I, and signal transduction cascades associated with memory processes, i.e. phosphorylated calcium/calmodulin protein kinase II and phosphorylated mitogen-activated protein kinase II. Blocking the insulin-like growth factor-I receptor abolished these exercise-induced increases. Our results illustrate a possible mechanism by which insulin-like growth factor-I interfaces with the brain-derived neurotrophic factor system to mediate exercise-induced synaptic and cognitive plasticity. © 2006 Published by Elsevier Ltd on behalf of IBRO.

Key words: pro-BDNF, synapsin I, MAPKII, CAMKII, memory.

Experimental evidence from both animals and humans supports the beneficial effects of exercise on cognitive function. Physical activity promotes learning and memory (Fordyce and Wehner, 1993; Kramer et al., 1999), facilitates functional recovery following brain injury (Grealy et al., 1999), and counteracts the mental decline associated with senescence (Laurin et al., 2001). The principle finding is that exercise increases brain-derived neurotrophic factor (BDNF) in the hippocampus, an area involved in learning and memory formation (Neeper et al., 1996; Gomez-Pinilla et al., 2002; Vaynman et al., 2003). However, given the integral effects of exercise on physiology, additional molecular mechanisms and signaling pathways through which exercise enhances cognitive function remain to be explored.

Insulin-like growth factor-I (IGF-I) is important for nerve growth and differentiation, neurotransmitter synthesis and release (Anlar et al., 1999), and has been shown to support cognitive function in cases of brain dysfunction associated with brain trauma (Saatman et al., 1997; Carro et al., 2001), diabetes (Lupien et al., 2003) and old age (Markowska et al., 1998; Sonntag et al., 2000). Given the prominent role of IGF-I in supporting cognitive function, IGF-I may play a significant part in the mechanisms subserving exercise-induced cognitive enhancement.

The role that IGF-I plays during exercise may be associated with the action of BDNF, a critical modulator of synaptic-plasticity in the adult brain (Lo, 1995) and a mediator of exercise-induced cognitive enhancement in the hippocampus (Vaynman et al., 2004). IGF-I entrains similar downstream pathways to BDNF action (Yamada et al., 1997; Roudabush et al., 2000), and like BDNF, its receptors are abundant in the hippocampus (Bohannon et al., 1988; Araujo et al., 1989; Bondy et al., 1992). Previous studies have indicated that the IGF-I signaling pathway may interact with those composing the BDNF system in the hippocampus. IGF-I has been shown to result in a qualitative increment in hippocampal BDNF immunostaining (Carro et al., 2001), mimic the effects of exercise on brain

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; C_T, threshold cycle; DG, dentate gyrus; Exc, exercise receiving the control cytochrome C solution; Exc/ab, exercise/insulin-like growth factor-I receptor neutralization; GAPDH, glyceraldyehyde-3-phosphate dehydrogenase; IGF-I, insulin-like growth factor-I; MWM, Morris water maze; p-CAMKII, phosphorylated calcium/calmodulin protein kinase II; PI3-K, phosphoinositol 3-kinase; PLC, phospholipase; p-MAPKII, phosphorylated mitogen-activated protein kinase II; RE-1, repressor element-1; Sed, sedentary receiving the control cytochrome C solution; Sed/ab, sedentary/insulin-like growth factor-1 receptor neutralization; S.E.M., standard error of the mean; slm, stratum lacunosum moleculare; TRKB, tyrosine kinase B; UNG, uracil glycosylase.

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c-Fos and BDNF mRNA expression (Iwamoto et al., 1996; Liste et al., 1997), and even partially reverse injury-induced decreases in BDNF (Kazanis et al., 2004). Given these findings, this study aimed to address the following questions. First, does exercise enhance IGF-I expression in the hippocampus? Second, does IGF-I play a role in mediating the exercise-induced enhancement in learning and memory? And third, does IGF-I interact with the BDNF system in the hippocampus during exercise? Our results showed that the IGF-I pathway may have a circumscribed role in mediating the effects of exercise on cognitive function as blocking the IGF-I receptor in the hippocampus during exercise abolished the exercise-enhancement in memory recall but failed to have a significant effect on learning acquisition.

EXPERIMENTAL PROCEDURES

Exercise paradigm

Adult male Sprague–Dawley rats (210±10 g; Charles River, Wilmington, MA, USA) were randomly assigned to four groups (n=8 animals per group), i.e. sedentary receiving the control cytochrome C solution (Sed); exercise receiving the control cytochrome C solution (Exc); sedentary/insulin-like growth factor-I receptor neutralization (Sed/ab); and exercise/insulin-like growth factor-I receptor neutralization (Exc/ab). These groups were exposed to five days of exercise after which their learning and memory were tested on the Morris water maze (MWM) task. To determine the effects of IGF-I receptor neutralization on exercise-induced hippocampal mRNA and protein expression, four separate groups of rats (n=6 animals per group; Sed, Exc, Sed/ab, and Exc/ab) were killed immediately after five days of exercise and used for molecular analysis. The exercise rats were housed individually and given access to a wheel (diameter=31.8 cm, width=10 cm) that freely rotated against a resistance of 100 g for five consecutive nights. The revolutions were monitored every hour by Vitalviewer Data Acquisition System Software (Minimitter Company, Inc., Sunriver, OR, USA). Sedentary rats were housed individually in standard polyethylene cages without access to a running wheel. During the experiments, all groups were exposed to a 12-h light/dark cycle at 22–24 °C, with food and water *ad libitum*.

Hippocampal IGF-I receptor neutralization

We used latex microbeads (Lumafluor Corporation, Naples, FL, USA) to deliver the specific IGF-I receptor binding antibody alphaIR3 (Oncogene Research Products, San Diego, CA, USA) into the hippocampus in order to neutralize local IGF-I receptors. AlphaIR3 has been reported to successfully block the IGF-I receptor (Scotlandi et al., 1996, 1998). AlphaIR3 is "a specific IGF-IR binding antibody" (Scotlandi et al., 1996) that blocks the binding site of the IGF-I receptor (Pavelic et al., 2005) and tyrosine phosphorylation of the IGF receptor (Shi et al., 2005). AlphaIR3 application has been shown to block IGF-I receptor-mediated cell proliferation (Pavelic et al., 2005) and IGF-I-induced cell migration in human neuroblastoma cells (Puglianiello et al., 2000) *in vitro*. Importantly, this antibody has been used to block IGF-I receptor function and inhibit IGF-I receptor mediated cell proliferation *in vivo* (Seely et al., 2002). A dose of 15 ng/μl was used in our experiment based on the work of Puglianiello et al. (2000). Exc and Sed control groups received cytochrome C (100 μg/ml; Sigma, St. Louis, MO, USA) as the control drug since it has been successfully used as a standard control for microbead injections (Vaynman et al., 2003, 2004; Lom and Cohen-Cory, 1999). Microbeads were incubated overnight at 4 °C in a 1:5 mixture of microbeads to solution (IGF-I receptor antibody or control solution). The following morning, microbeads were centrifuged at 14,000×g for 30 min and resuspended in cold sterile water for a final 10% microbead concentration. A stereotaxic apparatus was used to locate the injection site in the hippocampus (right hippocampus, 3.8 mm from Bregma, 2.6 mm from the midline and 3.7 mm vertically). Each rat was injected with 2 μl of antibody or control solution into the right side of hippocampus using a Hamilton syringe over a period of 15 min. The microbead injection site was verified by fluorescence microscopy (shown in Fig. 1). Animals were killed by decapitation and their hippocampi were rapidly dissected out, immediately placed on dry ice, and stored at −70 °C. All procedures were approved by UCLA animal research committees and followed the guidelines of the American Physiology Society of Animal Care. Anesthesia was not used during animal decapitation; the synthesis of trophic factors has been shown to be regulated by several neurotransmitter systems and metabolic mediators that are the targets of anesthetics (Zafra et al., 1991).

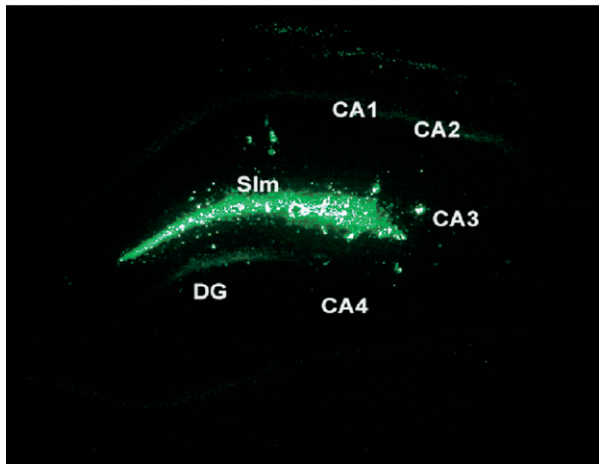

Fig. 1. Tissue section in the saggital plane showing the site of microbead injection in the right hippocampus, concentrated in the slm. For convenience, hippocampal areas CA1, CA2, CA3, and the DG have been labeled.

MWM experiment

To evaluate the effects of exercise and IGF-I receptor blocking on memory functions, all rats were tested on the MWM for spatial memory acquisition and retention (Morris et al., 1982; Sutherland et al., 1982). The swimming pool (150 cm diameter, 60 cm height) was divided into four quadrants. As previously described in Molteni et al. (2002), the quadrant housing the escape platform (12 cm diameter) was designated as the target zone, such that the escape platform was fixed in a permanent position 2 cm under the water surface during the course of the MWM procedures. The other three quadrants were designated as left, right and opposite to the target zone. The water was kept at a steady 22±2 °C and was made opaque with white non-toxic biodegradable dye to prevent the rats from seeing the platform. Spatial reference cues around the pool were maintained in their fixed positions through out the duration of the MWM experiments. We used a stringent two-trial-per-day, 5-day MWM training protocol, identified as a good discriminative test for the effect of exercise on learning and memory (Vaynman et al., 2004). Each rat was given two trials per day for five consecutive days. The animals were placed into the tank facing the wall from one of four equally spaced start locations and each release point was randomly altered every trial. Each trial lasted until the rat found the platform or for a maximum duration of 60 s. Animals who failed to find the platform within the allocated time were gently guided to the platform. At the end of each trial, animals were allowed to rest on the platform for 10 s. The escape

latency (platform search time) for each trial was recorded. A spatial probe test was performed 2 days after the last acquisition trial, during which time the platform was removed. As previously described (Molteni et al., 2002, 2004), rats were allowed to swim for 60 s during which the percentage of time spent by each animal in each quadrant was calculated and swim paths were semi-automatically recorded by a video tracking system (Smart: Spontaneous Motor Activity Recording and Tracking. #35E4F-FA9, Pan Laboratory s.l.).

Protein measurements

Hippocampal extracts were prepared in lysis buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP-40, 10% glycerol 1 mM phenylmethyl sulfonyl fluoride, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate). Homogenates were centrifuged to remove insoluble material (12,500$\times g$ for 20 min at 4 °C) and total protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA). The free mature BDNF protein was quantified using an enzyme-linked immunosorbent assay without acid treatment (ELISA; BDNF Emax Immuno Assay System kit, Promega Inc., Madison, WI, USA). Proteins for the BDNF precursor (pro-BDNF), synapsin I, phosphorylated calcium/calmodulin protein kinase II (p-CAMKII), phosphorylated mitogen-activated protein kinase II (p-MAPKII), and phosphoinositol 3-kinase (PI3-K) activated Akt were analyzed by Western blot and quantified by densitometric film scanning normalized for actin levels. Membranes were incubated with the following primary antibodies: anti-BDNF (N-20), 1:500 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA); anti-synapsin I, 1:2000 (Santa Cruz Biotechnology Inc.); anti-actin, 1:4000 (Santa Cruz Biotechnology Inc.); anti-p-MAPKII (Santa Cruz Biotechnology Inc.), 1:2000; anti-p-CAMKII, 1:5000 (Santa Cruz Biotechnology Inc.); anti-p-Akt, 1:1000 (Cell Signaling, Danvers, MA, USA) and followed by an anti-primary IgG horseradish peroxidase conjugate. Immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using NIH image software.

Isolation of total RNA and real-time quantitative RT-PCR

Total RNA was isolated using an RNA STAT-60 kit (TEL-TEST, Inc., Friendswood, TX, USA) as per manufacturer's protocol, and quantification was carried out by absorption at 260 nm. Hippocampal tissue was examined for the mRNA of IGF-I, IGF-II, BDNF, and synapsin I. The method used for mRNA quantification was real-time quantitative RT-PCR using a PE ABI PRISM 7700 sequence detection instrument (Applied Biosystems, Foster City, CA, USA), which directly detects the RT-PCR product without downstream processing by monitoring the increase in fluorescence of a dye-labeled DNA probe specific for the factor of interest. As the control, we employed a probe specific for the glyceraldyehyde-3-phosphate dehydrogenase (GAPDH) gene, which has been used previously as a successful endogenous control for the assay (Griesbach et al., 2002; Molteni et al., 2002). Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer, Branchburg, NJ, USA). The sequences of probes, forward and reverse primers, designed by Integrated DNA Technologies (Coralville, IA, USA) were as follows: IGF-I: (5′-CCGGACCAGAGACCCTTTGCGG-3′); forward: (5′-CTTTACCAGCTCGGCCACA-3′); reverse: (5′-TTGGTCCACACACGAACTGAAG-3′); IGF-II: (5′-CTTGTGCTGCATCGCTGCTTACCG-3′); forward: (5′-TGCTTCTCATCTCTTTGGCCTT-3′); reverse: (5′-TCCGCACAGAGTCTCGCTG-3′); BDNF: (5′-AGTCATTTGCGCACAACTTTAAAAGTCTGCATT-3′); forward: (5′-GGACATATCCATGACCAGAAAGAAA-3′); reverse: (5′-GCAACAAACCACAACATTATCGAG-3′); synapsin I: (5′-CATGGCACGTAATGGAGACTACCGCA-3′); forward: (5′-CCGCCAGCATGCCTTC-3′); reverse: (5′-TGCAGCCCAATGACCAAA-3′). The endogenous control probe, specific for the GAPDH gene, was served to standardize the amount of RNA sample and consisted of the oligonucleotide sequence 5-CCGACTCTTGCCCTTCGAAC-3. RT reaction steps consisted of an initial 2-min incubation step at 50 °C to activate uracil glycosylase (UNG) and were followed by 30 min of reverse transcription at 60 °C. A completion step for UNG deactivation was carried out for 5 min at 95 °C. The 40 cycles of two-step PCR reaction consisted of a 20-s period at 94 °C and a 1-min period at 62 °C.

Statistical analysis

GAPDH was used for RT-PCR as an internal standard as described previously (Molteni et al., 2002). Quantification of the TaqMan RT-PCR results was carried out by plotting fluorescent signal intensities against the number of PCR cycles on a semi logarithmic scale. A threshold cycle (C_T) was designated as the amplification cycle during which the first significant increase in fluorescence occurred. The C_T value of each sample was compared with that of the internal standard GAPDH. These processes were fully automated and carried out using the ABI sequence detector software version 1.6.3 (PE Biosystem). TaqMan EZ RT-PCR values were corrected by subtracting values for GAPDH as described previously (Griesbach et al., 2002; Molteni et al., 2002). Actin was used as internal standard for Western blot analysis. An analysis of variance (ANOVA) was conducted for data between multiple groups. Statistical differences were considered significant when $P<0.05$. We used the mean value of the sedentary control group to calculate the % for each animal in all the other groups. Values represent the mean±standard error of the mean (S.E.M.). A Fisher's test was used for between-group comparisons.

RESULTS

Microbead injection site

The exact injection site of microbeads in the hippocampus was investigated by fluorescent imaging. Microbead injection was concentrated in the stratum lacunosum moleculare (slm; Fig. 1). Hippocampal areas CA1, CA3 and the dentate gyrus (DG) have been labeled for reference purposes. This location is consistent with previous microbead infusion studies (Vaynman et al., 2003, 2004) as well as with previous drug infusion protocols that evaluated exogenous stimulators (Messaoudi et al., 1998) or inhibitors (Zhang et al., 2001) on hippocampal function.

Exercise increases IGF-I expression in the hippocampus under the direction of exercise

We found that exercise significantly ($P<0.05$) increased IGF-I mRNA levels in the hippocampus (138±13%; Fig. 2A) above Sed control levels. This effect was specific to IGF-I, as exercise did not increase basal levels of IGF-II mRNA in the hippocampus (98±8%; Fig. 2B). Blocking the IGF-I receptor during exercise significantly abrogated the exercise-induced increase in IGF-I expression below Sed controls (68±6%; Fig. 2A), but did not affect IGF-II expression in exercising animals (102±6%; Fig. 2A). Blocking the IGF-I receptor did not have any significant effect on IGF-I and IGF-II expression in sedentary animals (101±20% and 104±11%, respectively; Fig. 2A, B).

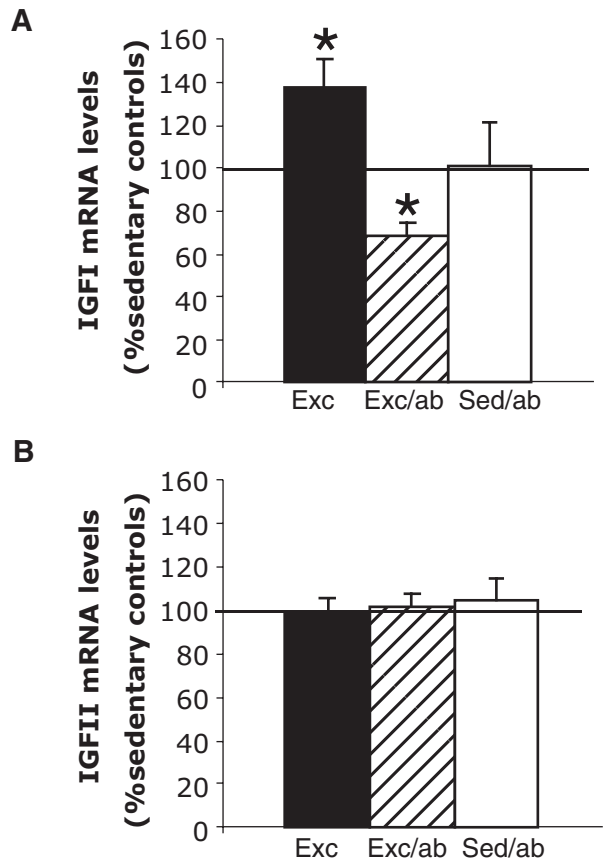

Fig. 2. Physical activity selectively increases the expression of IGF-I in the hippocampus. Five days of exercise selectively increased the mRNA levels of IGF-I above sedentary controls (A) without significantly altering the expression of IGF-II in the hippocampus (B). Blocking the IGF-I receptor abolished the exercise-induced increase in IGF-I mRNA expression below sedentary control levels, while having no significant effect on IGF-I expression in sedentary animals (A) or IGF-II in exercise and sedentary animals (B). Results are displayed as percentages of sedentary control levels (represented by the 100% line). Each value represents the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$).

IGF-I receptor blockade abolished the effects of exercise on memory retention but not on learning acquisition

To assess spatial learning acquisition, we used a challenging two-trial-per-day, 5-day MWM paradigm, which is sufficiently sensitive for discerning learning differences between exercise and sedentary animals (Vaynman et al., 2004). Results showed that the escape latencies were similar between all four groups at the beginning of MWM training (Fig. 3). Consistent with our previous findings for the effect of exercise on promoting learning acquisition (Vaynman et al., 2004), we found that exercise significantly ($P<0.05$) decreased the latency to locate the platform as compared with Sed controls. Specifically, this effect of exercise manifested as a decrease in latency to find the platform on days 3 (16.4±1.8 s, $P<0.01$), 4 (15.3±1.9 s, $P<0.01$), and 5 (8.7±1.1 s, $P<0.01$) of MWM training as compared with Sed control rats (32.5±3.4 s, 23.4±1.3 s, and 21.2±1.7 s, respectively; Fig. 3). Blocking the IGF-I receptor in exercising animals failed to significantly alter the exercise-induced effect on learning acquisition on days 3–5 of MWM training; there was no significant difference for the escape latencies of Exc/ab animals on days 3 (18.9±2.4 s), 4 (19.3±1.3 s), and 5 (12.7±1.4 s) from Exc animals (16.4±1.8 s, 15.3±1.9 s, and 8.7±1.1 s, respectively). Blocking the IGF-I receptor did not significantly alter the escape latencies in the sedentary condition; the Sed/ab group had similar escape latencies on days 3 (33.1±2.9 s), 4 (28.6±1.4 s), and 5 (19.6±2.2 s) to those of Sed controls (Fig. 3).

To evaluate memory retention, we performed a probe trial 2 days after the last MWM training day. Rats were allowed to swim for 60 s in the pool in which they received their training, but with the escape platform removed. The percentage of time spent in the probe quadrant, which previously housed the platform (quadrant P), was calculated for each animal. We found that the Exc group showed a clear preference for the P quadrant over Sed rats, as they spent a significantly ($P<0.02$) greater percentage of time in quadrant P (42.2±4.0%) than Sed controls (27.4±3.3%). Blocking the IGF-I receptor fully prevented the exercise-induced preference for the target quadrant, such that there was no difference between the amount of time in quadrant P spent by Exc/ab rats (29.9±4.6%) and Sed controls (Fig. 4A). Blocking the IGF-I receptor did not signifi-

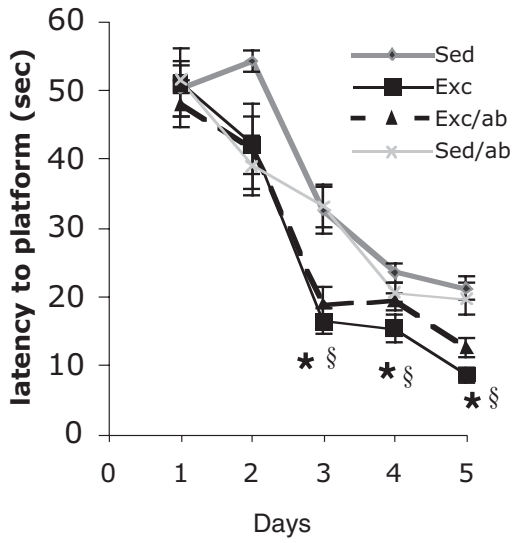

Fig. 3. Effect of blocking the IGF-I receptor in the hippocampus during exercise on learning acquisition as measured by the MWM task. Exercise improved learning ability, as exercise animals took significantly less time to learn the location of the platform than their sedentary control counterparts (Exc vs. Sed). Blocking the IGF-I receptor during exercise had no significant effect on learning ability, as exercise animals given the IGF-I receptor blocker had similar escape latencies to exercise control animals (Exc/ab vs. Exc). Blocking the IGF-I receptor had no significant effect in sedentary animals; sedentary animals given the IGF-I receptor blocker had similar escape latencies to sedentary controls (Sed/ab vs. Sed) and significantly longer escape latencies than exercise animals (Sed/ab vs. Exc). Data are expressed as the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$). * Represents comparison between the Exc and Sed Control groups. § Represents comparison between the Exc/ab and Sed control groups.

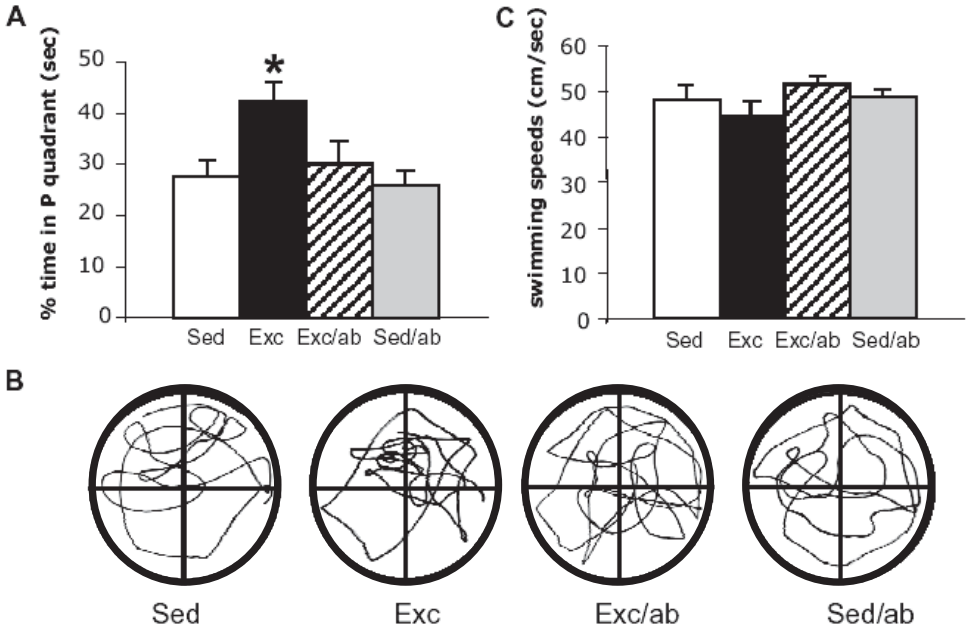

Fig. 4. Effect of blocking the IGF-I receptor in the hippocampus during exercise on memory retention, as measured by the MWM task. (A) Exercise enhanced memory retention on the probe trial of the MWM task; exercise animals spent significantly more time in the P quadrant than their sedentary control counterparts (Exc vs. Sed). Blocking the IGF-I receptor during exercise abolished the exercise-induced preference for the P quadrant; exercise animals given the IGF-I receptor blocker spent as much time in the P quadrant as their sedentary control counterparts (Exc/ab. vs. Sed). Blocking the IGF-I receptor did not significantly affect the preference of sedentary animals for the P quadrant as compared with sedentary controls (Sed/ab vs. Sed). (B) Representative sample of paths taken during the probe test, illustrating the marked preference by exercising animals for the P quadrant as compared with all other groups. Data are expressed as the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$).

cantly affect the preference of Sed/ab rats for quadrant P, as there was no significant difference in the percentage of time spent in quadrant P between Sed/ab (26±2.8%) and Sed control rats (Fig. 4A). Fig. 4B shows the representative path taken by each group, illustrating the effect of exercise on promoting a preference for quadrant P. To control for differences in MWM performance, we recorded each animal's swimming speed. We found no difference in swimming speeds between all four groups; Exc (44.3±3.2 cm/s), Sed (48.1±2.9 cm/s), Exc/ab (51.6±1.5 cm/s), and Sed/ab (48.9±1.6 cm/s; Fig. 4C). We recorded the running distances for each exercise group and found that the average distance (K_m) run per day did not significantly differ between the exercise groups, Exc (2.8±0.36) and Exc/ab (2.6±0.30).

IGF-I receptor blockade abolished the effects of exercise on hippocampal BDNF

We found that while exercise significantly ($P<0.05$) increased the mRNA levels of BDNF (159±24%) in the hippocampus above Sed controls (100±13%), blocking the IGF-I receptor fully abolished the exercise-induced increase in BDNF mRNA levels (159±24% to 88±8% $P<0.05$; Fig. 5A). Blocking the IGF-I receptor did not have an effect on the BDNF mRNA levels of sedentary animals (92±6%). Exercise also significantly ($P<0.01$) increased the BDNF protein levels in the hippocampus (133±5%) above Sed controls (100±3%; Fig. 5B). Blocking the IGF-I receptor significantly ($P<0.05$) reduced the exercise-induced increase in BDNF protein (115±7%) to those of Sed controls. Blocking the IGF-I receptor did not have any significant effect on BDNF protein levels in sedentary animals (102±1%; Fig. 5B).

BDNF is synthesized as a precursor and then processed to its mature form by prohormone convertases (Lee et al., 2001; Chen et al., 2004; Pang et al., 2004) such that the conversion of pro-BDNF to BDNF has been shown to participate in hippocampal synaptic plasticity (Pang et al., 2004). Therefore, we evaluated whether exercise increases BDNF levels by modulating pro-BDNF levels through the IGF-I receptor. We found that exercise significantly ($P<0.05$) increased the levels of pro-BDNF (125±10.6%) above Sed controls (100±7.3%; Fig. 5C). Blocking the IGF-I receptor in exercising animals significantly abrogated the effect of exercise on pro-BDNF levels (125±10.6% to 98.3±12.9%). Moreover, IGF-I receptor inhibition did not significantly alter pro-BDNF levels in sedentary animals (107.5±7.5%; Fig. 5C).

IGF-I receptor blockade abolished the effects of exercise on hippocampal synapsin I

Recent evidence suggests that IGF-I may play a role in regulating the synaptic vesicle protein synapsin I (Di Toro et al., 2005). To explore the possible role of IGF in promoting the exercise-induced increase in hippocampal synapsin I expression, we studied synapsin I changes under IGF-I receptor blockade. Our results showed that exercise significantly ($P<0.05$) increased the mRNA (137±9%) and protein levels (144±13%) of synapsin I above Sed controls (100±13% and

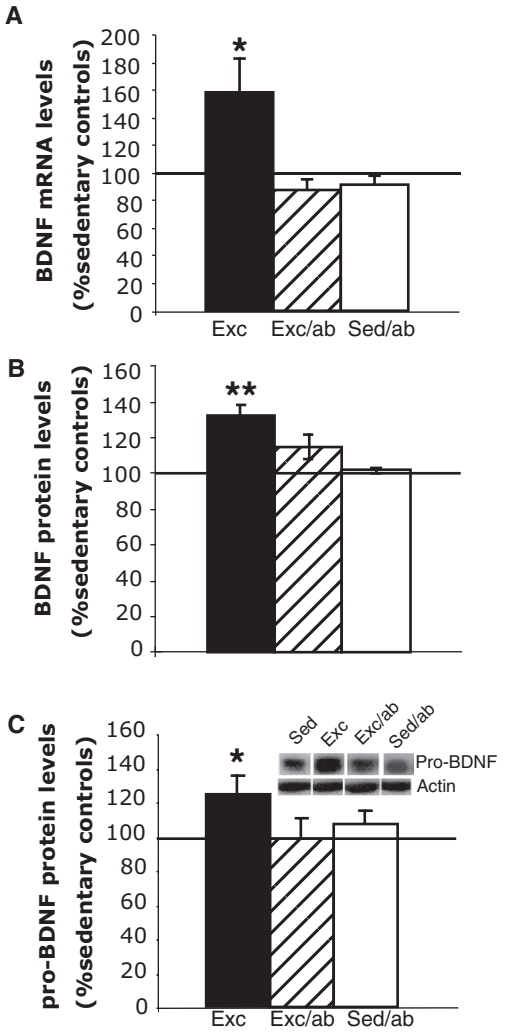

Fig. 5. Effect of blocking the IGF-I receptor in the hippocampus during exercise on the expression of BDNF mRNA, BDNF protein, and pro-BDNF protein levels. Exercise significantly increased the levels of (A) BDNF mRNA, (B) BDNF protein, and (C) pro-BDNF protein levels above sedentary control levels. Blocking the IGF-I receptor during exercise prevented the exercise-induced increase in BDNF mRNA levels (A), BDNF protein levels (B), and pro-BDNF levels. (A–C) Blocking the IGF-I receptor had no significant effect on the expression of BDNF mRNA, BDNF protein, and pro-BDNF protein levels in sedentary animals. Representative immunoblot for pro-BDNF I is shown in the upper hand right corner of (C). Results are displayed as percentages of sedentary control levels (represented by the 100% line). Each value represents the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$, ** $P<0.01$).

100±12%, respectively) and that blocking the IGF-I receptor completely abrogated this exercise-induced increase on the mRNA (91±9%) and protein (96±3%) level (Fig. 6A–B). Neutralizing the IGF-I receptor had no significant effect in sedentary animals on modulating synapsin I mRNA (102±19%) and protein (96±6%) levels (Fig. 6A–B).

IGF-I receptor blockade abolished the effects of exercise on hippocampal CAMKII and MAPKII

Previously we found that the CAMKII signal transduction cascade is activated during exercise to regulate the expression of molecules mediating synaptic plasticity in the hippocampus (Vaynman et al., 2003). CAMKII is highly expressed in postnatal forebrain structures, especially in the hippocampus (Erondu and Kennedy, 1985), and may be an essential element in modulating activity-dependent synaptic plasticity and learning and memory (Silva et al., 1992; Giese et al., 1998). As activation of the IGF-I receptor results in phospholipase activation (PLC), CAMKII may be a downstream target for kinase phosphorylation (Blanquet et al., 2003). Therefore, we wanted to determine if IGF-I plays a role in modulating the levels of p-CAMKII in the hippocampus. Our results show that exercise significantly ($P<0.05$) increased the active form of the CAMKII, p-CAMKII (164±14%) above Sed control levels (100±12%). Blocking the IGF-I receptor abrogated the exercise-induced increase in p-CAMKII levels, thereby reducing them to Sed control levels (164±14% to 106±9%). Blocking the IGF-I receptor seemed to have no significant effect on p-CAMKII levels in sedentary animals (98±8%; Fig. 7A).

Several studies have implicated MAPK as an essential component of signal transduction cascades underlying learning and memory (Selcher et al., 1999; Sharma et al., 2003). It has been reported that physical activity elicits sustained activation of MAPK in the rat hippocampus (Shen et al., 2001). Considering that canonical signaling

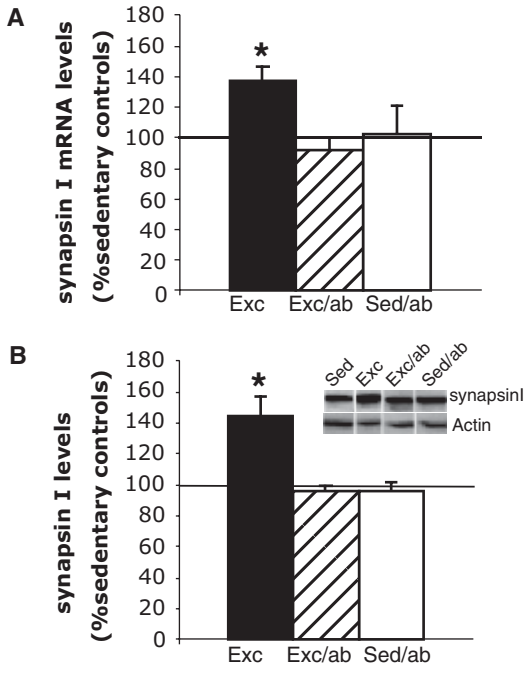

Fig. 6. Effect of blocking the IGF-I receptor in the hippocampus during exercise on synapsin I mRNA and protein levels. Exercise significantly enhanced synapsin I mRNA (A) and protein (B) levels in the hippocampus above sedentary controls. Blocking the IGF-I receptor had no significant effect on synapsin I mRNA and protein levels in exercise and sedentary animals (A, B). Representative immunoblot for synapsin I is shown in the upper hand right corner of (B). Results are displayed as percentages of sedentary control levels (represented by the 100% line). Each value represents the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$).

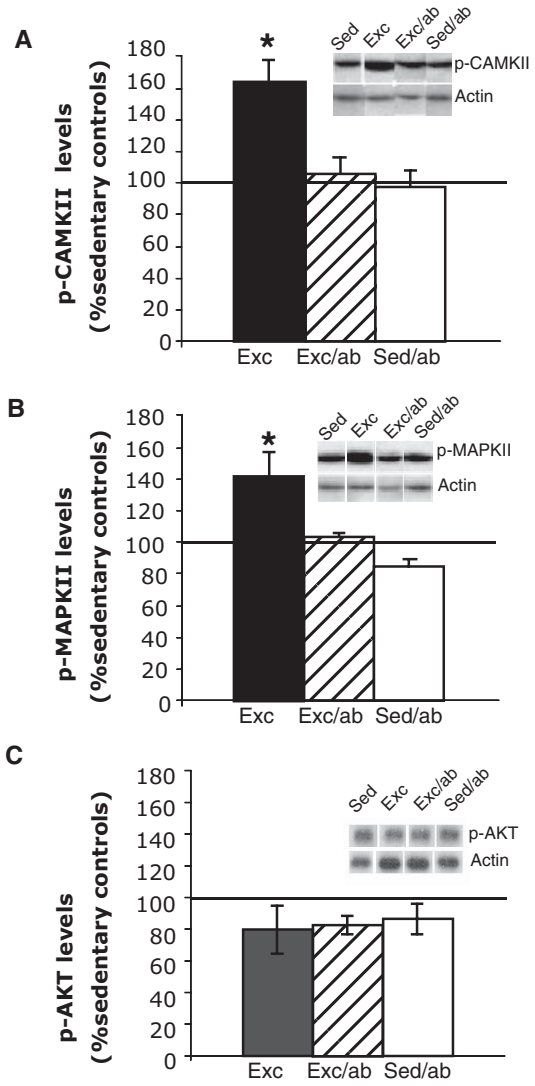

Fig. 7. Effect of blocking the IGF-I receptor in the hippocampus during exercise on p-CAMKII, p-MAPKII, and p-Akt levels. Exercise significantly increased (A) p-CAMKII and (B) p-MAPKII protein levels in the hippocampus above sedentary controls. Blocking the IGF-I receptor had no significant effect on the levels of p-CAMKII and p-MAPKII in exercise and sedentary animals (A, B). Neither exercise nor blocking the IGF-I receptor significantly affected the levels of p-Akt as compared with sedentary controls (C). Representative immunoblots for p-CAMKII, p-MAPKII, and p-Akt are shown in the upper hand right corner of (A), (B), and (C) respectively. Results are displayed as percentages of sedentary control levels (represented by the 100% line). Each value represents the mean±S.E.M. (ANOVA, Fischer's test, * $P<0.05$).

through the IGF-I receptor consists of MAPK pathway activation (Roudabush et al., 2000; Cardona-Gomez et al., 2001; Pugazhenthi et al., 1999), it was of primary interest to determine whether this pathway is sensitive to the activity-dependent regulation imposed by exercise. Exercise significantly ($P<0.05$) increased p-MAPKII levels (141±16%) above Sed control levels (100±8%). Blocking the IGF-I receptor in the hippocampus completely abolished this exercise-induced increase in p-MAPKII levels (141±16% to 103±3%). Blocking the IGF-I receptor in sedentary animals had no significant effect on p-MAPKII levels (85±4%; Fig. 7B).

To determine a level of specificity for the affects of IGF-I receptor activation on p-CAMKII and p-MAPKII during exercise, we measured p-Akt levels as a control. Akt, also known as Akt-PI3, is a major downstream factor activated by the IGF-I receptor through PI3-K and acts to mediate the effects of IGF-I on cell survival (Aikin et al., 2000). We found that neither exercise nor IGF-I receptor inhibition significantly affected the levels of p-Akt as compared with sedentary control animals (Fig. 7C).

DISCUSSION

This study provides novel evidence that exercise selectively increases IGF-I mRNA levels in the hippocampus under the direction of the IGF-I receptor. Our findings show that IGF-I signaling may serve a circumscribed role in mediating the effects of exercise on cognitive function, selectively operating on the exercise-induced enhancement in memory retention. Blocking the hippocampal IGF-I receptor during the exercise period was sufficient to prevent the exercise-induced enhancement in memory. We found that IGF-I receptor blockade abrogated the exercise-induced increase in the mRNA and proteins levels of BDNF and its precursor (pro-BDNF). The conversion of pro-BDNF to BDNF is postulated to play a role in hippocampal synaptic plasticity (Pang et al., 2004). Additionally, our results show that the IGF-I pathway modulates a consummate end-product of BDNF action, i.e. synapsin I, and signal transduction cascades downstream to BDNF activation, i.e. p-CAMKII and p-MAPKII.

Exercise increases IGF-I expression in the hippocampus

IGF-I is a trophic factor with various roles in neuronal plasticity, among which figure the regulation of nerve growth and differentiation, neurotransmitter synthesis and release (Anlar et al., 1999), the potentiation of CA1 hippocampal synapses (Ramsey et al., 2005), and learning and memory (Lupien et al., 2003). Therefore, the ability of exercise to enhance neuronal and cognitive plasticity may be in part mediated by IGF-I. It has been shown that blocking peripheral IGF-I entry into the brain prevents the effect of exercise on neurogenesis in the hippocampus (Trejo et al., 2001). We found that exercise affects the production of IGF-I in the CNS, specifically increasing IGF-I expression in the hippocampus (Fig. 2A). The effect of exercise in the hippocampus was found to be specific to IGF-I as exercise failed to increase IGF-II expression (Fig. 2B). The mechanism by which exercise increases IGF-I expression may be analogous to that reported for BDNF, one that is activity-dependent and linked to the electrical activity of hippocampal neurons (Zafra et al., 1990). In fact, recent evidence from retinal neurons shows that IGF-I expression is regulated by electrical activity (Morimoto et al., 2005). Comparable to the regulation of BDNF expression by its cognate tyrosine kinase B (TRKB) receptor during exercise (Vaynman et al., 2003), our results show

that the ability of exercise to augment IGF-I mRNA levels in the hippocampus is mediated through the IGF-I receptor (Fig. 2A). Accordingly, hippocampal sources of IGF-I could act in a paracrine fashion on IGF-I expression. Since our inhibitor to IGF-I is "a specific IGF-IR binding antibody" (Scotlandi et al., 1996) that blocks the binding site of the IGF-I receptor (Pavelic et al., 2005), we cannot discard the possibility that peripheral sources of IGF-I can influence exercise-induced plasticity in the hippocampus (Carro et al., 2001). However, our study, focused on IGF-I mRNA, indicates that exercise affects IGF-I synthesized in the brain.

The role of the IGF-I pathway in mediating exercise-induced enhancement in learning and memory

We found that exercise was able to enhance learning acquisition; exercise rats had shorter escape latencies to find the platform than their sedentary counterparts (Exc vs. Sed; Fig. 3). Blocking the IGF-I receptor during exercise did not significantly alter the latency to acquire the MWM task from that of exercise controls (Exc/ab vs. Exc; Fig. 3). Moreover, exercise enhanced the recall ability of animals above sedentary control levels; exercise animals spent significantly more time in quadrant P than their sedentary counterparts (Exc vs. Sed; Fig. 4A, C). Neutralizing the IGF-I receptor during the exercise period effectively abrogated this exercise-induced recall effect (Exc/ab vs. Exc; Fig. 4A) without affecting group swim speed (Fig. 4C). Importantly, blocking the IGF-I receptor was selective for exercise as it did not affect the acquisition or recall abilities of sedentary animals (Sed/ab vs. Sed; Fig. 3, Fig. 4A).

The IGF-I pathway modulates the levels of the BDNF precursor under the direction of exercise; implications for learning and memory formation

Previous studies have indicated that IGF-I signaling may interact with the BDNF signaling system in the hippocampus. Peripheral IGF-I infusion has been shown to qualitatively increase BDNF immunohistochemical staining in the hippocampus (Carro et al., 2001) and even partially reverse injury-induced decreases in BDNF (Kazanis et al., 2004). Previously, we showed that blocking the action of BDNF in the hippocampus during exercise prevents the exercise-induced increase in both learning acquisition and memory retention (Vaynman et al., 2004). Our current results indicate a partial role for IGF-I signaling in mediating exercise-induced plasticity; blocking the IGF-I receptor abrogated the exercise-induced enhancement in memory recall without disrupting the effect of exercise on learning acquisition (Figs. 3, 4A). The ability of IGF-I to selectively impact memory and not learning acquisition may be partially due to the capacity of the IGF-I pathway to modulate pro-BDNF; Fig. 5C). It is possible that pro-BDNF processing into the mature form by enzymatic reactions may occur after the learning acquisition phase, thereby enabling IGF-I-induced pro-BDNF levels to only become functional to support memory retention. In fact, it has been found that pro-BDNF contributes to more long-term hippocampal synaptic plasticity, such as late long-term potentiation, and that pro-BDNF cleavage is essential for this process to occur (Pang et al., 2004). However, conducting future experiments on the role of pro-BDNF by extracellular processing is necessary to provide insight for deducing a possible differential regulation by IGF-I on BDNF and cognitive function.

Systems entrained by the IGF-I pathway under the direction of exercise

IGF-I and BDNF both feed into a conserved signaling system that activates parallel and converging pathways (Fig. 8). Upon activation, both the BDNF TRKB receptor and the IGF-I receptor can recruit the insulin receptor substrate (IRS-1) adaptor protein, which possesses tyrosine kinase activity (Yamada et al., 1997) and provides a mechanism for either BDNF or IGF-I to compliment one another's effect on synaptic plasticity. Previous studies in our laboratory have documented the strong regulatory control of BDNF over synapsin I during exercise (Vaynman et al., 2003, 2004) and the importance of CAMKII and MAPKII activation in mediating exercise-induced hippocampal synaptic plasticity (Vaynman et al., 2003). Our current results show that the IGF-I pathway is also involved in modulating synapsin I mRNA and protein levels under the direction of exercise (Fig. 6A–B) and in activating the CAMKII and MAPKII signaling cascades (Fig. 7A–B), which are canonical downstream signals to BDNF activation. We found that exercise-induced IGF-I receptor activation induces p-CAMKII and P-MAPKII downstream pathways with a degree of selectively, as neither exercise nor the IGF-I receptor inhibitor significantly altered the levels of p-Akt, another major downstream pathway of IGF-I receptor activation (Fig. 7C). The effects of IGF-I are largely mediated by the IGF-I receptor, a member of the growth factor tyrosine kinase receptor family that signals through the Ras-MAPK cascade (Cardona-Gomez et al., 2001; Pugazhenthi et al., 1999). MAP-K promotes a wide range of plasticity changes: nuclear signaling (Adams et al., 2000), long-term potentiation (English and Sweatt, 1997), and learning and memory formation (Blum et al., 1999; Bozon et al., 2003). Likewise, CAMKII plays an important role in various neuronal adaptive processes in the brain (Hamdy et al., 2004) and has an established reputation for being a molecular memory switch (Lisman et al., 2002) based upon its critical role in learning and memory (Silva et al., 1992; Giese et al., 1998; Cho et al., 1998). Thus, both CAMKII and MAPKII may contribute to the ability of exercise to enhance cognitive function.

Our findings show that the IGF-I receptor pathway may facilitate the synapse by regulating synapsin I (Fig. 6A–B). This is consistent with the ability of IGF-I to modulate neuronal synapse size and vesicle translocation (Torres-Aleman, 1999) as synapsin I is involved in the formation and maintenance of the presynaptic structure (Takei et al., 1995) and in axonal elongation (Akagi et al., 1996). The synapsin I promoter contains a repressor element-1 (RE-1) motif, enabling synapsin I transcription to be repressed by the RE-1 silencing transcription factor (REST), which is regulated by IGF-I in a time dependent manner; a decrease in REST and concomitant increase in synapsin I

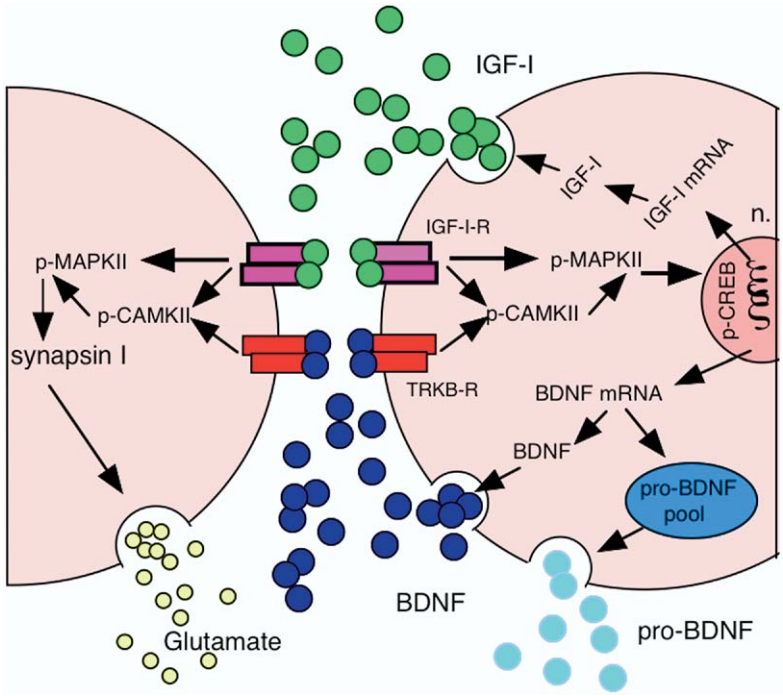

Fig. 8. Hypothetical mechanism through which IGF-I signaling may interface with BDNF-mediated synaptic plasticity in the hippocampus during exercise. Exercise can induce IGF-I production in the hippocampus. IGF-I and BDNF are shown to have similar downstream signaling mechanisms, incorporating both p-CAMKII and p-MAPKII signaling cascades. The regulation of IGF-I and BDNF mRNA expression, BDNF, and pro-BDNF protein is illustrated on the postsynaptic membrane for concise purposes, although this type of regulation likely occurs pre-synaptically as well; n, nucleus.

occur with neurite extension (Di Toro et al., 2005). Alternatively, IGF-I may act on CAMKII to modulate synapsin I levels as our results show that IGF-I mediates the activation of CAMKII (p-CAMKII, Fig. 6A), a pathway previously found to be critical for modulating the expression of synapsin I under exercise (Vaynman et al., 2003). The IGF-I receptor pathway may activate CAMKII by increasing cytosolic free calcium ions through the PLC-IP3 pathway (Blair et al., 1999). Consistent with Shen et al. (2001), we also found that exercise increases the active form of MAPKII in the hippocampus. Given the previous finding that inhibiting CAMKII, but not MAP-K, abolishes the exercise-induced increase in synapsin I expression (Vaynman et al., 2003), the IGF-I/CAMKII pathway may be more responsible for synapsin I mRNA regulation. As IGF-I modulates p-MAPKII (Fig. 7B), which regulates synapsin I phosphorylation (Jovanovic et al., 1996), the IGF-I/MAPK pathway may regulate the phosphorylation of synapsin I during exercise to facilitate neurotransmitter release.

CONCLUSIONS

Implications of IGF-I signaling pathway for synaptic plasticity

The finding that the IGF-I pathway has a common regulatory capacity over CAMKII, MAPKII, and synapsin I to that of BDNF, implies the convergence of multiple signaling pathways mediating synaptic plasticity and cognitive function. BDNF regulates various aspects of synaptic plasticity during both development and adulthood to include axonal and dendritic branching and remodeling (Lom and Cohen-Cory, 1999; McAllister et al., 1999), the efficacy of synaptic transmission (Boulanger and Poo, 1999; Kafitz et al., 1999), and learning and memory (Falkenberg et al., 1992; Kesslak et al., 1998; Mu et al., 1999). Similarly, the IGF-I pathway is important for nerve growth and differentiation, neurotransmitter synthesis and release (Anlar et al., 1999; Niblock et al., 2000), and has been shown to support cognitive function (Saatman et al., 1997; Carro et al., 2001; Markowska et al., 1998; Sonntag et al., 2000). It has been previously suggested that peripheral sources of IGF-I supplied to the brain can mediate the effect of exercise on neuronal plasticity (Carro et al., 2001). However, here we show new evidence that CNS-derived IGF-I may be responsible for mediating the effects of exercise on synaptic and cognitive plasticity. IGF-I signaling may serve one way by which mechanisms activated by exercise interact with brain specific systems, such as BDNF in the hippocampus, to impact cognitive function.

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