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Exercise Induces BDNF and Synapsin I to Specific Hippocampal Subfields

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To assess the relationship between brain-derived neurotrophic factor (BDNF) and synapsin I in the hippocampus during exercise, we employed a novel microsphere injection method to block the action of BDNF through its tyrosine kinase (Trk) receptor and subsequently measure the mRNA levels of synapsin I, using real-time TaqMan RT-PCR for RNA quantification. After establishing a causal link between BDNF and exercise-induced synapsin I mRNA levels, we studied the exercise-induced distribution of BDNF and synapsin I in the rodent hippocampus. Quantitative immunohistochemical analysis revealed increases of BDNF and synapsin I in CA3 stratum lucidum and dentate gyrus, and synapsin I alone in CA1 stratum radiatum and stratum laconosum moleculare. These results indicate that exercise induces plasticity of select hippocampal transsynaptic circuitry, possibly comprising a spatial restriction on synapsin I regulation by BDNF.

Key words: exercise; hippocampus; circuitry; BDNF; synapsin I; immunohistochemistry

Exercise has been shown to increase hippocampal brain-derived neurotrophic factor (BDNF) levels (Gómez-Pinilla et al., 2001a; Molteni et al., 2002). BDNF is a powerful modulator of synaptic plasticity, able to modify neuronal excitability and synaptic transmission (Causing et al., 1997; Lu and Figurov, 1997; Kafitz et al., 1999). The capacity of exercise to benefit neuronal function, i.e., promote learning and memory (Fordyce and Wehner, 1993; Kramer et al., 1999), induce neurogenesis (van Praag et al., 1999), and support functional recovery from brain injury (Grealy et al., 1999), is likely due to BDNF-induced synaptic plasticity. Notably, the hippocampus, an area crucial for learning and memory, expresses an abundance of BDNF and its receptor (Rocamora et al., 1996) and is responsive to manipulations in BDNF levels. For instance, BDNF gene inhibition or deletion (Figurovet al., 1996, Kang et al., 1997) produces a deficit in hippocampal long-term potentiation (LTP), an electrophysiologic correlate of learning and memory (Nguyen and Kandel, 1996). Reinstitution of hippocampal BDNF by exogenous application (Patterson et al., 1992) or overexpression (Korte et al., 1995) is able to correct this deficit in hippocampal synaptic function. Importantly, exercise is able to modulate BDNF levels as BDNF is synthesized (Patterson et al., 1992; Rocamora et al., 1996) and secreted in an activity-dependent manner (Goodman et al., 1996) and thus in response to exercise (Neeper et al., 1995, 1996).

The ability of BDNF to influence neural function may be intrinsic to its ability to modulate synaptic transmission by regulating synapsin I through its tyrosine kinase B (TrkB) receptor (Jovanovic et al., 2000). Synapsin I is a vesicle-associated phosphoprotein that modulates transmitter release (Jovanovic et al., 2000), the formation and maintenance of the presynaptic structure (Takei et al., 1995), and axonal elongation (Akagi et al., 1996). The finding that BDNF is localized in close proximity to synapsin I (Haubensak et al., 1998) suggests that the hippocampal subfield localization of BDNF and synapsin I may be an important determinant as to which hippocampal regions are subject to regulation by BDNF during exercise.

Pathologically or electrically induced paradigms of activity, such as seizures and LTP, respectively, have noted changes in BDNF and synapsin I expression in specific hippocampal subfields. Specifically, seizure activity has been shown to increase BDNF mRNA in CA3 and dentate gyrus (DG) (Kim et al., 2001). In addition, seizure activity and LTP both have been documented to increase synapsin I in CA3 and DG (Suemaru et al., 2000; Sato et al., 2002, respectively). Accordingly, we wanted to determine whether BDNF mediates exercise-induced increases in hippocampal mRNA levels and whether this relationship manifests itself as an overlapping protein distribution in hippocampal subregions in response to exercise. Although exercise has been shown to increase overall hippocampal BDNF and synapsin I levels (Gómez-Pinilla et al., 2001a; Molteni et al., 2002), determining regional

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changes may reveal important information concerning plasticity changes in hippocampal neural networks in response to exercise. Our study shows that overlapping increases in BDNF and synapsin I, in distinct hippocampal subregions, are induced by physiologic levels of neuronal activity but show a spatial restriction on BDNF regulation of synapsin I.

MATERIAL AND METHODS

Exercise Paradigm

As a well-adopted paradigm of experience-based change in synaptic plasticity (Gómez-Pinilla et al., 2001a; Molteni et al., 2002), we chose voluntary exercise because it simulated aspects of voluntary human behavior in that animals chose how much to run. Adult male Sprague-Dawley (Charles River) rats (approximately 3 months of age) were assigned randomly to four groups designed to study the relationship between BDNF and synapsin I by using the Trk inhibitor K252a ($n = 5$ animals per group). The four groups comprised a K252a/exercise group, a cytochrome C (cytC) control/exercise group, a K252a/sedentary group, and a cytC control/sedentary group. The K252a/exercise and cytC/exercise rats were given 3 days of exercise. A separate group of adult male Sprague-Dawley (Charles River) rats (approximately 3 months of age) was used to identify the immunohistochemical localization of BDNF and synapsin I in the hippocampus. These were divided randomly into a control or a 7-day exercise group ($n = 5$ animals per group). All rats were housed individually in standard polyethylene cages in a 12/12-hr light/dark cycle at 22–24°C, with food and water ad lib. The exercise rats were given access to a wheel (diameter = 31.8 cm, width = 10 cm), which freely rotated against a resistance of 100 grams attached to a receiver that monitored revolutions every 3 min (VitalViewer data acquisition system software; Mini-Mitter, Sunriver, OR). All exercised rats were given 3 or 7 nights of wheel running, whereas control rats were housed in a cage with no access to a wheel for the same time period as their exercised counterparts. On the morning after the last running period, all rats were sacrificed and brain tissue was collected. For biochemical assays, animals were killed by decapitation and their hippocampi were rapidly dissected out, placed immediately on dry ice, and stored at −70°C. For immunohistochemistry, animals were anesthetized deeply (Nembutal, 100 mg/kg intraperitoneally [i.p.]) and killed by intracardial perfusion on the morning after the last running period. All procedures were approved by UCLA Animal Research Committees and followed the guidelines of the American Physiological Society of Animal Care.

Blocking Protocol

Rats were divided randomly into 3-day exercise and sedentary control groups ($n = 5$ rats per group) and were pretreated with inhibitor or cytochrome C (cytC), a standard control (Lom and Cohen-Cory, 1999). All drugs were administered by injecting fluorescent latex microspheres directly into the right hippocampus, resulting in a consistent and effective blockade of synapsin I. Successful delivery of bioactive agents, such as neurotrophins and neurotransmitter agonists/antagonists, into highly localized brain regions by the microsphere injection method has been described previously (Quattrochi et al., 1989; Riddle et al., 1995, 1997; Lom and Cohen-Cory, 1999). The injection of neurotrophins using microspheres as a vehicle has provided neurotrophic activity comparable to that of free neurotrophin, and has been shown to retain activity for at least 4 days (Riddle et al., 1997).

We blocked the action of BDNF through its Trk receptor using K252a, a Trk receptor inhibitor. This inhibitor has been shown to effectively block or attenuate the action of BDNF; K252a has been shown to block BDNF-evoked excitatory potentials in hippocampal neurons (Kafitz et al., 1999). We used 46.8 ng/μl, a 1,000× magnification of the 100 nM dose used in vitro (Kafitz et al., 1999), because this amount was more consistent with the 100 ng/μl dose of neurotrophin used to coat microspheres for the in vivo application (Riddle et al., 1997; Lom and Cohen-Cory, 1999).

We prepared microspheres (Lumafluor, New York, NY) by methods described previously (Riddle et al., 1997; Lom and Cohen-Cory, 1999). Microspheres were coated with either K252a or cytC via passive absorbency by incubating overnight at 4°C with a 1:5 mix of microspheres to K252a (46.8 ng/μl sterile water; Kafitz et al., 1999), or cytC (100 ng/μl sterile water; Lom and Cohen-Cory, 1999). The next morning, the solution was centrifuged at 14,000 × *g* for 30 min and the microspheres were resuspended in sterile water at a 10% concentration.

Exercise and sedentary rats received K252a or the standard control cytC (Calbiochem Bioscience, La Jolla, CA; Lom and Cohen-Cory, 1999) once before the 3-day running period, administered early in the morning, such that an ample recovery time permitted all animals to begin running that same evening. All animals were anesthetized (i.p.), with a combination of 70 mg/kg of ketamine hydrochloride and 10 mg/kg of xylazine, once before receiving the drug injection. We used a stereotaxic apparatus to secure the animal and measure the sight for microsphere injection. Microspheres were injected into the right hippocampus (3.8 mm from Bregma, 1 mm from the midline, and 3.7 mm vertically) with either 2 μl of K252a, or cytC. Histological examination was employed to verify the location of microsphere injection, as described previously (Quattrochi et al., 1989; Riddle et al., 1995), and to insure that there was no obvious tissue damage from the injections.

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) as per the manufacturer's protocol, and quantification was carried out by absorption at 260 nm. Brain tissue was examined for synapsin I mRNA by real-time quantitative RT-PCR using a PE ABI PRISM 7700 sequence detection instrument (Applied Biosystems, Foster City, CA), 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 glyceraldyehyde-3-phosphate dehydrogenase (GAPDH) gene, which has been used previously as a successful endogenous control for the assay (Greisbach et al., 2002; Molteni et al., 2002). Total RNA (100 ng) was converted into cDNA using TaqMan EZ RT-PCR Core reagents (Perkin-Elmer, Branch-

burg, NJ). The sequences of probes, forward and reverse primers (designed by Integrated DNA Technologies, Coralville, IA), for synapsin I were: 5′-CATGGCACGTAATGGAGACTAC-CGCA-3′; forward, 5′-CCGCCAGCATGCCTTC-3′;and reverse, 5′-TGCAGCCCAATG ACCAAA-3′, respectively.

The endogenous control probe, specific for the GAPDH gene, 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-sec period at 94°C and a 1-min period at 62°C.

Statistical Analyses

We used GAPDH 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 semilogarithmic scale. A threshold cycle (C_T) was designated as the amplification cycle at which the first significant increase in fluorescence occurred. The C_T value of each sample was compared to that of the internal standard. 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 for synapsin I were corrected by subtracting values for GAPDH as described previously (Greisbach et al., 2002; Molteni et al., 2002). These corrected values were used to make between-group comparisons. Mean values for mRNA levels were computed for the 3-day cytC control and exercise groups that received surgical intervention and were compared using analysis of variance (ANOVA). A Fisher's test was used for between-group comparisons. To ensure that there was no significant difference in running ability due to drug treatment, the mean values of the running distances in kilometers were computed for the 3-day exercise groups and compared by *t*-test. Results were expressed as the mean percent of control values for graphic clarity and represent the mean ± standard error of the mean (SEM).

Immunohistochemistry

Sections from the 7-day exercise and control groups (n = 5 animals per group) were processed for BDNF and synapsin I immunohistochemistry as described previously (Gómez-Pinilla et al., 2001b). Brain tissue was sliced in the sagittal plane (30 μm) in a cryostat and collected free-floating in cold phosphate-buffered saline (PBS). Tissue sections were treated initially with 1% hydrogen peroxide in PBS to deactivate endogenous peroxidase activity. Sections were then incubated at 25°C overnight in a solution containing the primary antibody, a 1:1,000 dilution of rabbit polyclonal anti-BDNF antisera (Chemicon, Temecula, CA) and a 1:1,000 dilution of the goat polyclonal anti-synapsin I a/b antisera (Santa Cruz Biotechnology, Santa Cruz, CA), in PBS, 2% bovine serum albumin (BSA), and 0.1% Triton X-100, pH 7.3. Sections were rinsed thoroughly in PBS and incubated for 2 hr with biotinylated anti-rabbit IgG (1:1,000). After being washed with PBS, sections were incubated for 1 hr with avidin-biotin-horseradish peroxidase (HRP) complex (Vector, Burlingame, CA). Finally, sections were rinsed for 15 min in PBS, reacted with 3,3′-diaminobenzidine (DAB; 05 mg/ml) in 0.1 Tris, pH 7.6, 0.01% H_2O_2, and 0.001 NiCl for a more intense reaction product, and rinsed in PBS. We quantified changes in BDNF and synapsin I in hippocampal subfields CA1 (stratum pyramidale, stratum radiatum, and stratum lacunosum moleculare), CA3 (stratum lucidum and stratum pyramidale of CA3) and the dentate gyrus hilus. The selection of these subfields was based on previous findings showing that BDNF and synapsin I are induced in response to seizure or electrical activity in CA3 and DG (Suemaru et al., 2000; Kim et al., 2001; Sato et al., 2002). Specifically, synapsin I has also been shown to increase in CA1 (Suemaru et al., 2000) in response to pathologically induced activity. Interestingly, learning-impaired rats show a decrease in BDNF localized to CA3 and DG (Wu et al., 2003). We examined the DG hilus and CA3, because it comprises a transsynaptic circuitry, which relays information from the granule cells via the mossy fiber projections to CA3 stratum lucidum. Additionally, we examined the CA1, as it is part of the circuitry receiving input from the DG via the Schaffer collaterals. Quantitative immunohistochemical analysis was carried out as described previously (Suemaru et al., 2000). The relative optical density (ROD) of the hippocampal staining for BDNF and synapsin I was measured by a computer-based analysis as an average of the gray value between white (0) and black (255). Sections were imaged (10× objective) using a digital camera (Olympus Camedia C-2020 ZOOM) attached to a microscope (Olympus BX51) and images were captured and digitized onto a computer. The means of the gray areas of the hippocampus subfields were analyzed using image software (NIH Image). Average ROD levels from the corpus callosum were subtracted out from the areas of interest, as the corpus callosum is considered representative of background levels (Neeper et al., 1996). Measurements were made in five adjacent coronal hippocampal sections (100-μm apart) for each animal (n = 5), approximately 3.8 mm posterior to Bregma. The mean ROD values were computed for control (sedentary) and exercise groups and statistically analyzed using ANOVA and Fisher's test. Statistical differences were considered significant when $P < 0.05$. Results were expressed as the mean percent of control ROD values for graphic clarity and represent the mean ± SEM.

RESULTS

Effect of Inhibiting BDNF Action on Synapsin I mRNA Levels

BDNF has been shown to regulate synapsin I through its TrkB receptor (Jovanovic et al., 2000). To determine the involvement of BDNF action through the Trk receptor in the pathways through which exercise regulates synapsin I, we therefore used K252a (46.8 ng/μl), which has been shown to block the action of BDNF through the TrkB receptor in the hippocampus in vitro (Kafitz et al., 1999).

To insure that K252a did not adversely affect the ability of animals to run, we recorded the distances that the animals ran in the 3-day exercise groups. We noted that

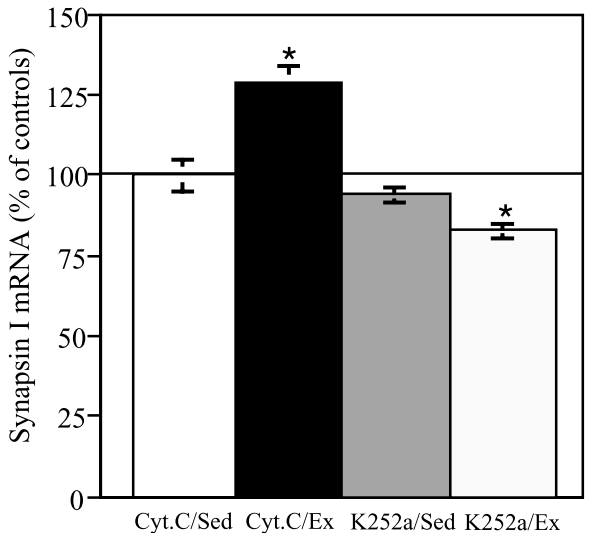

Fig. 1. Effects of the inhibitor K252a on synapsin I mRNA levels in the hippocampus after 3 days of voluntary exercise (K252a/Ex). Results are displayed as percentage of sedentary controls (Sed). Exercise increased synapsin I mRNA levels significantly above those of sedentary controls (cytC/Ex; 129 ± 5%, $P < 0.05$). Inhibiting the action of BDNF with K252a completely abolished exercise-induced increase in synapsin I mRNA levels to levels below those of sedentary controls (K252a/Ex; 83 ± 2%, $P < 0.05$). The application of the inhibitor K252a had no significant effect on sedentary animals (K252a/Sed; 94 ± 2%). CytC/Sed controls are represented by the 100% horizontal line; the statistical significance of mRNA levels for K252a/Sed animals is represented as compared to this line. Each value represents the mean ± SEM. $\star P < 0.05$; ANOVA, Fisher's test.

K252a did not alter the amount that animals chose to run, as the running distances for the K252a/exercise group (1.51 ± 0.27 km) did not differ significantly from the cytC/exercise group (1.63 ± 0.18 km). Exercise significantly ($P < 0.05$) increased synapsin I mRNA levels in the hippocampus (129 ± 5%) after only 3 days of wheel running. Furthermore, blocking the action of BDNF through its Trk receptor with K252a consistently and completely abolished the exercise-induced increases in synapsin I mRNA levels (83 ± 2%; $P < 0.05$) to below the levels in sentary controls. Conversely, K252a application did not significantly alter synapsin I mRNA levels in sedentary animals (94 ± 2%) from levels in sedentary/cytC controls (Fig. 1).

BDNF and Synapsin I Distribution in Hippocampal Subfields After 7 Days of Exercise

Immunohistochemical analysis in sedentary controls showed light BDNF labeling in CA1, which was more prominent along the mossy fiber system that runs between CA3 and dentate gyrus (DG) (Fig. 2A). Synapsin I immunostaining was localized to the stratum pyramidale layers, was light in CA1 dendritic layers, sr and slm, and light along the mossy fibers, CA3 to DG (Fig. 2C).

BDNF and synapsin I levels in rats exercised for 7 days were increased compared to that in sedentary control animals. Increases in BDNF and synapsin I in response to exercise affected select hippocampal subfield layers, such that quantitative immunohistochemical analysis revealed confluent increases ($P < 0.01$) of BDNF and synapsin I in CA3 stratum lucidum (sl) and DG hilus. With exercise, BDNF immunoreactivity increased (183 ± 11%, $P < 0.01$) in the DG hilus, and CA3 sl (164 ± 4%, $P < 0.01$) above sedentary controls (Fig. 3A). Histological evaluation of synapsin I immunoreactivity after 7 days of exercise showed that exercise increased ($P < 0.01$) synapsin I in CA3 sl (228 ± 12%) above controls. With exercise, synapsin I immunoreactivity showed striking and significant increases ($P < 0.01$) in CA1 dendritic layers, CA1 sr (180 ± 12%), CA1 slm (168 ± 6%), and DG hilus (213 ± 12%). In contrast, the pyramidale layer of CA1 (sp) showed a significant decrease ($P < 0.05$, 80 ± 7%, Fig. 3B).

DISCUSSION

Previous work in this area has documented that pathologically or electrically induced forms of activity, such as seizures and LTP, respectively, induce changes in BDNF and synapsin I expression in specific hippocampal subfields. We have shown here that physiologic activity in the form of voluntary exercise is able to induce a specific hippocampal subfield distribution of BDNF and one of its downstream effectors on synaptic plasticity, synapsin I. Exercise has been noted to benefit neural function, in both humans (Kramer et al., 1999) and animals (Samorajski et al., 1985; Fordyce and Farrar, 1991; Fordyce and Wehner, 1993), to maintain and reduce the cognitive decline associated with aging (Laurin et al., 2001), to promote functional recovery after central nervous system (CNS) damage (Mattson, 2000), and even induce neurogenesis in the adult hippocampus (van Praag et al., 1999). This ability of exercise to benefit neural function is likely subject to the action of BDNF and its downstream effect on molecules of synaptic plasticity. Our current study revealed that in the hippocampus during exercise, BDNF regulates the mRNA levels of synapsin I, a phosphoprotein important for vesicular release. Consequently, our finding that BDNF and synapsin I hold a particular molecular anatomic localization may underscore the relationship BDNF and synapsin I hold in modulating hippocampal synaptic plasticity in certain subfields. Our findings show also that there may be a spatial restriction on the regulation of synapsin I by BDNF. Importantly, the specific subfield increases in BDNF and synapsin I reflect a potential for plasticity changes in specific hippocampal transsynaptic neural networks.

We found that exercise increased synapsin I mRNA levels and that blocking the action of BDNF through its Trk receptor with K252a reduced the exercise-induced synapsin I mRNA levels. This ability of BDNF to regulate synapsin I mRNA levels in the hippocampus seems an activity-dependent phenomena, induced by exercise, because we found that blocking BDNF action in sedentary animals did not significantly decrease synapsin I mRNA levels (Fig. 1). In fact, we found that blocking BDNF

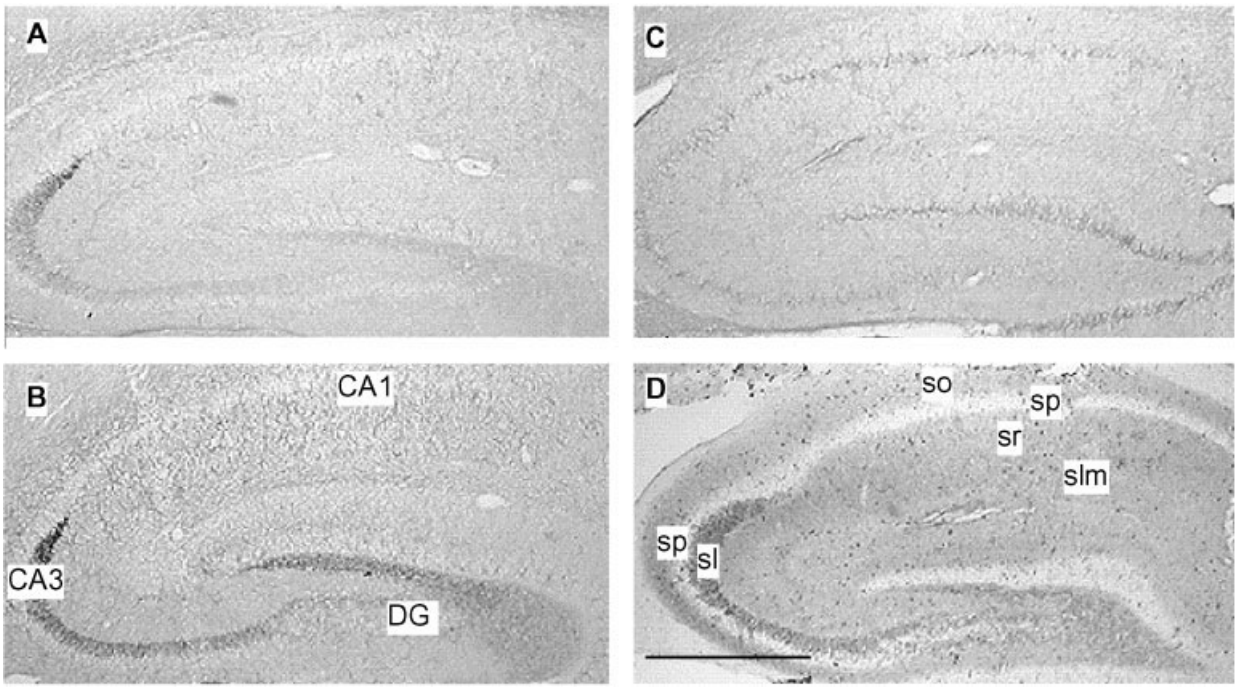

Fig. 2. Immunohistochemical staining in sagittal sections of the hippocampus showing increases in BDNF and synapsin I after 7 days of exercise. The photomicrograph shows BDNF immunoreactivity in sedentary controls (**A**) and exercised rats (**B**) and synapsin I immunoreactivity in controls (**C**) and exercised rats (**D**). CA1, CA3, DG, stratum oriens (so), stratum pyramidal (sp), stratum radiatum (sr), stratum lacunosum moleculare (slm), and stratum lucidum (sl) have been labeled. Scale bar = 1,000 μm (for A–D).

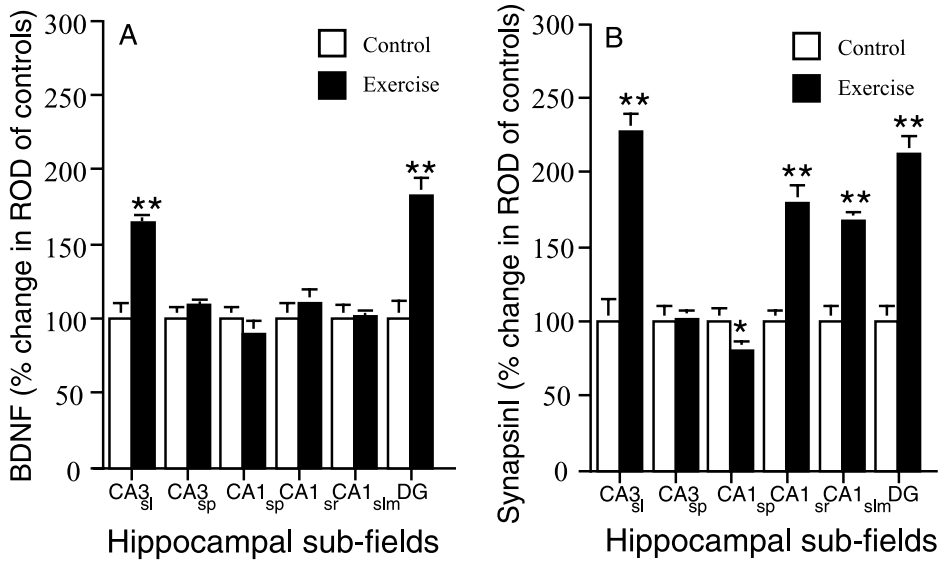

Fig. 3. Quantitative immunohistochemical analysis was carried out in subregions of the hippocampus: CA1 (stratum pyramidale, stratum radiatum, and stratum lacunosum moleculare); CA3 (stratum lucidum and stratum pyramidale of CA3); and the DG hilus. **A:** Quantitative analysis of hippocampal BDNF after 7 days of exercise. Significant increases in BDNF immunoreactivity occurred in stratum lucidum (sl) of CA3 and the DG hilus. **B:** Quantitative analysis of hippocampal synapsin I after 7 days of exercise showed significant increases in stratum lucidum of CA3, stratum pyramidale and stratum lacunosum moleculare of CA1, and the DG hilus. Each value represents the mean ± SEM. $\star P < 0.05$, $\star\star P < 0.01$; ANOVA, Fisher's test.

action during exercise decreased synapsin I mRNA levels below that of sedentary control (Fig. 1). This decrease to below sedentary controls could be an effect of the activity-dependent relationship between BDNF and synapsin I. If the activity-dependent regulation of synapsin I by BDNF was to gain ascendancy over other synapsin I regulatory mechanisms during exercise, then blocking BDNF action in exercising animals could possibly reduce synapsin I mRNA levels below sedentary control levels. BDNF this modulates synapsin I mRNA levels in the hippocampus during exercise. Subsequently, we found that the BDNF/synapsin I relationship manifests itself on the protein level in specific hippocampal subfields after 7 days of exercise. Specifically, exercise significantly increased both BDNF and synapsin I in CA3 sl and DG hilus (Fig. 3A and 3B, respectively). Our finding that BDNF modulates synapsin I mRNA levels during exercise is in accordance with previous research that has shown that BDNF is localized to synaptic junctions, in close proximity to synapsin I. This localization places BDNF in a position to act as an acute modulator of synaptic transmission (Haubensak et al., 1998). In fact, BDNF is able to enhance synaptic transmission by regulating synapsin I through its TrkB receptor (Jovanovic et al., 2000) and has been shown to potentiate excitatory synaptic transmission and excitability in CA3 and DG (Scharfman, 1997; Messaoudi et al., 1998). DG granule cell axons project via mossy fibers to CA3 cells (Amaral and Witter, 1989) with the stratum lucidum comprising the axon terminal region for these dentate granule cells. The finding that BDNF modulates synapsin I mRNA levels during exercise (Fig. 1) and the localization of BDNF and synapsin I to the DG hilus and the CA3 sl, (Fig. 3A and 3B, respectively) intimates an activation of a transsynaptic circuitry leading from the DG granule cells to enhance synaptic transmission to CA3 (Suominen-Troyer et al., 1986).

Studies using models of neuronal plasticity such as kindled seizures (Suemaru et al., 2000) and LTP (Sato et al., 2002), have reported that hippocampal activity increases synapsin I levels in CA3 sl and DG. Furthermore, both kindled seizures (Cavazos et al., 1991) and LTP (Adams et al., 1997) induce gross mossy fiber sprouting. Warranted by the ability of synapsin I to regulate neurite development (Melloni et al., 1994; Zurmohle et al., 1996), the formation and maintenance of the presynaptic structure (Sato et al., 2002), axonal elongation (Akagi et al., 1996) and new synaptic formation (Ferreira et al., 1998), it is presumed that increases in synapsin I lead to changes in synaptic circuitry (Greengard et al., 1993; Morimoto et al., 1998; Suemaru et al., 2000). Exercise-induced increases in synapsin I in CA3 and DG thus may be indicative of mossy fiber sprouting. Interestingly, neuronal activity such as seizures induces BDNF mRNA in both CA3 and DG (Kim et al., 2001) and increases mossy fiber sprouting via BDNF (Scharfman et al., 1999). Because we found that BDNF regulates synapsin I mRNA levels during exercise, these exercise-induced changes in synaptic reorganization may be orchestrated via synapsin I regulation by BDNF.

Exercise induces significant ($P < 0.01$) increases in synapsin I immunoreactivity in dendritic layers CA1 sr and CA1 slm congruous with increases seen in kindled seizures (Suemaru et al., 2000). Synapsin I increases in CA1 were not accompanied by increases in BDNF immunoreactivity, suggesting that synapsin I may be regulated by another factor in CA1. The *N*-methyl-D-aspartate (NMDA) receptor is a likely candidate. CA1 would be the most likely area for this regulation, because NMDA receptors (NMDA-Rs), although scarce in CA3 (Watanabe et al., 1998), are abundant in CA1 (Geddes et al., 1986). In contrast, BDNF immunoreactivity is light in CA1 and intense in both CA3 and DG (Connor et al., 1997). Interestingly, we found a shift in synapsin I immunoreactivity, a decrease in the CA1 pyramidale layer, seen previously to be prominent in the sedentary control animals, to an increase in CA1 dendritic layers sr and slm with exercise. As synapsin I is a presynaptic protein, its increase in CA1 dendritic layers may indicate synaptogenesis and neural reorganization, possibly induced by activity relayed from the DG through transsynaptic circuitry from the DG to CA1. In conclusion, physiologic levels of activity produced by exercise increase BDNF and synapsin I levels to specific hippocampal subfields, indicating activation of specific hippocampal circuitry, which may include a spatial restriction on BDNF-mediated regulation of synapsin I. This ability of BDNF to mediate exercise-induced hippocampal synaptic plasticity by regulating synapsin I in specific hippocampal subfields may underlie the improved neural function seen with exercise.

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