Exercise intensity influences the temporal profile of growth factors involved in neuronal plasticity following focal ischemia

Michelle Ploughman, Shirley Granter-Button, Garry Chernenko, Zachary Attwood, Budd A. Tucker, Karen M. Mearow, Dale Corbett*

Basic Medical Science, Faculty of Medicine, Memorial University, St. John’s NL, Canada A1B 3V6

ARTICLE INFO

Article history:
Accepted 27 February 2007
Available online 2 March 2007

Keywords:
BDNF
pCREB
Synapsin-I
Corticosterone
Telemetry
Stroke rehabilitation

ABSTRACT

Exercise increases brain-derived neurotrophic factor (BDNF), phosphorylated cAMP response-element binding protein (pCREB), insulin-like growth factor (IGF-I) and synapsin-I, each of which has been implicated in neuroplastic processes underlying recovery from ischemia. In this study we examined the temporal profile (0, 30, 60 and 120 min following exercise) of these proteins in the hippocampus and sensorimotor cortex following both motorized (60 min) and voluntary (12 h) running, 2 weeks after focal ischemia. Our goal was to identify the optimal training paradigms (intensity, duration and frequency) needed to integrate endurance exercise in stroke rehabilitation. Therefore we utilized telemetry to measure changes in heart rate with both exercise methods. Our findings show that although the more intense, motorized running exercise induced a rapid increase in BDNF, the elevation was more short-lived than with voluntary running. Motorized running was also associated with higher levels of synapsin-I in several brain regions but simultaneously, a more pronounced increase in the stress hormone, corticosterone. Furthermore, both forms of exercise resulted in decreased phosphorylation of CREB and downregulation of synapsin-I in hippocampus beginning 30 to 60 min after the exercise bout. This phenomenon was more robust after motorized running, the method that generated higher heart rate and serum corticosterone levels. This immediate stress response is likely specific to acute exercise and may diminish with repeated exercise exposure. The present data illustrate a complex interaction between different forms of exercise and proteins implicated in neuroplasticity. For clinical application, frequent lower intensity exercise episodes (as in voluntary running wheels), which may be safer to provide to patients with stroke, has a delayed but sustained effect on BDNF that may support brain remodeling after stroke.

© 2007 Elsevier B.V. All rights reserved.

1. Introduction

The majority of individuals who survive stroke have enduring motor deficits that affect productivity and quality of life (Duncan et al., 1994; Mayo et al., 2002) and presently, rehabilitative therapy provides the only chance for stroke survivors to improve outcome. Endurance exercise enhances neurogenesis and improves memory and learning (Anderson et al., 2000; Radak et al., 2001; Van Praag et al., 1999), in part by upregulating brain-derived neurotrophic factor (BDNF) which
in turn elevates cAMP response element binding protein (CREB) and synapsin-I (Vaynman et al., 2004), BDNF is one of a family of neurotrophins that supports neuronal survival, reduces the threshold for long term potentiation (LTP; Kiprianova et al., 1999; Zhou et al., 2000) and facilitates motor recovery after stroke (Schabitz et al., 2004). BDNF by phosphorylating its trkB receptor, activates synapsin-I, a neuron-specific protein that tethers synaptic vesicles to the actin cytoskeleton (Jovanovic et al., 2000). Synapsin-I is upregulated by exercise in normal animals and may contribute to improved performance in cognitive tasks (Vaynman et al., 2004). The transcriptional protein CREB is not only involved in learning and memory but also plays a regulatory role in dendritic spine and filopodium dynamics (Ji et al., 2005).

Exercise, by elevating these critical proteins, may facilitate plastic processes that underlie recovery from brain injury. Unfortunately, stroke patients engage in endurance exercise only a few minutes each day (MacKay-Lyons and Makrides, 2002) and the optimal exercise parameters required to enhance these proteins are not known.

We previously reported that, following focal ischemia, motorized running exercise for 30 or 60 min is more effective than voluntary overnight running in the upregulation of BDNF and synapsin-I in the hippocampus and sensorimotor cortex of the intact hemisphere, measured immediately following exercise (Ploughman et al., 2005). Since these brain regions in the contralateral hemisphere contribute to stroke recovery (Biernaskie and Corbett, 2001; Biernaskie et al., 2005), we were interested in optimizing mediators of synaptic plasticity in these areas. For example, the temporal expression of BDNF in the ischemic brain within the first 1–2 h of training has not been investigated. If levels are downregulated rapidly following exercise then rehabilitative training should be scheduled repeatedly so that therapeutic levels are optimized.

In our previous study, the stress hormone corticosterone was elevated immediately following exercise. Since this hormone has been shown to attenuate BDNF expression (Smith et al., 1995; Schaff et al., 1998; Kuipers et al., 2003), we speculated that serum corticosterone could have a delayed deleterious effect on synaptic plasticity mediators such as CREB, IGF-I and synapsin-I. IGF-I, like BDNF, is a neurotrophin that has been implicated in recovery, angiogenesis (Lopez-Lopez et al., 2004) and protection from brain injury (Carro et al., 2001). Since it is present in both the circulation and parenchyma, we examined both brain and serum levels of IGF-I.

Finally, no study has measured the physiological intensity (i.e. heart rate) of exercise required to increase growth factors and other proteins involved in neuronal plasticity. In order to utilize endurance exercise training to enhance these processes in clinical rehabilitation, the indicators of exercise intensity must be defined. The most common physiological measure of exercise intensity is heart rate, therefore we used telemetry probes to determine resting and exercise heart rate responses to different endurance training methods.

2. Results

Application of endothelin-1 resulted in greater than 50% damage to the cortex and striatum. There were no differences in ischemic scores between groups in the cortex (2.77±0.09) or striatum (2.2±0.17). Two animals with minimal injury, scoring 1 or 0 in the cortex and striatum, were excluded from analysis, and the remaining animals’ cortical infarcts scored between 2 and 4 (Fig. 1).

Animals in the voluntary running groups ran 1109.86 m±162.31 in 12 h while animals in the motorized wheels ran 726 m in 60 min. One animal that ran less than 500 m in 12 h in the voluntary wheel was omitted from the study. There were no differences in running distances in the voluntary running groups (F3,16=1.24; p=0.34).

2.1. Hippocampal and cortical BDNF following exercise

Motorized and voluntary endurance training induced different temporal profiles of BDNF expression directly following exercise in the ischemic hippocampus. There was a significant treatment effect on BDNF levels in the hippocampus of the ischemic hemisphere (Fig. 2; F8,62=2.66; p<0.01) but not in the intact side (F8,62=1.47; p=0.18). Following motorized running, elevation of hippocampal BDNF in the ischemic hemisphere was very brief, peaking directly after the exercise challenge (141.16±7.8%; p<0.01) but falling to sedentary levels over subsequent sample intervals. In contrast, following the 12 h voluntary run, BDNF elevation in the hippocampus of the ischemic side is sustained, remaining significantly elevated at 30 (136.68±9.62%) and 120 min (140.40±10.14%; p<0.05). Motorized exercise did not affect hippocampal BDNF in the intact hemisphere, however after completion of voluntary running, hippocampal BDNF in the intact hemisphere was elevated both immediately (120.35±7.1%) and at 60 min (132.14±10.8%; p<0.05).

In the cortex of the intact hemisphere, ANOVA showed no overall effect of group on BDNF (F8,41=1.66; p=0.14) except for a brief surge at 30 min (121.28±17.12%; p<0.05) following the motorized run (data not shown).
2.2. Temporal effects of exercise on pCREB

CREB, as a transcription factor, activates many genes that may contribute to plasticity processes (Molteni et al., 2002). Activated CREB was not altered by exercise in the ischemic hippocampus (Fig. 3) or the intact sensorimotor cortex (data not shown) however, in the intact hippocampus, following both motorized and voluntary running, there was a period of decreased CREB phosphorylation (Fig. 3; $F_{8,41}=2.84; p<0.01$). At 30 min ($89.4\pm 5.4\%$, $p<0.05$) and 60 min ($89.2\pm 5.5\%$, $p<0.01$) following motorized running, pCREB levels were significantly below sedentary values. The pattern was similar following the voluntary run. Thirty minutes ($89.3\pm 3.8\%$) after completing the voluntary run, pCREB values fell significantly below both sedentary and Vol/0 levels ($108.06\pm 4.38\%$, $p<0.05$). Except for a slight rebound at 60 min, pCREB values at 120 min ($90.9\pm 4.7\%$) remained significantly below those directly following the running episode ($p<0.05$).

2.3. The effects of exercise on the temporal expression of synapsin-I

Synapsin-I tethers synaptic vesicles to the actin cytoskeleton and is elevated by exercise (Vaynman et al., 2003, 2004). Both exercise methods induced time-dependent changes in hippocampal synapsin-I levels in the ischemic brain (Fig. 4A; ischemic side $F_{8,42}=4.37; p<0.001$; intact side $F_{8,42}=2.5; p<0.05$). There was a robust phasic response after motorized exercise in which synapsin-I was rapidly upregulated immediately following exercise in both hippocampi (ischemic side $130.0\pm 8.22\%$, intact side $130.82\pm 13.97\%$, $p<0.005$) but fell below sedentary levels briefly at 30 min ($70.59\pm 7.47\%$ ischemic side; $p<0.005$, $76.99\pm 9.99\%$ intact side; $p<0.05$) and rebounded again at 60 min. Other than decreasing synapsin-I in the ischemic hippocampus at 60 min ($88.48\pm 12.97\%$, $p<0.05$), voluntary exercise did not markedly affect hippocampal synapsin-I.

Although there was no overall group effect of exercise on synapsin-I in the intact sensorimotor cortex (Fig. 4B; $F_{8,43}=2.06; p<0.06$), synapsin-I was significantly elevated directly following motorized running ($124.4\pm 15.19\%$) compared to sedentary ($p<0.05$) and to 30 min ($90.38\pm 36.11\%$, $p<0.01$). Synapsin-I was also significantly higher directly following motorized exercise than at the same interval after voluntary exercise ($83.44\pm 6.89\%$, $p<0.005$).

2.4. Serum and brain levels of IGF-I after exercise

Since IGF-I supports angiogenesis after ischemia (Lopez-Lopez et al., 2004) and is likely extracted from the circulation with exercise (Carro et al., 2000), levels of IGF-I were measured in both the brain and blood following exercise intervention. There was no significant group effect on serum IGF-I levels ($F_{8,53}=1.52; p=0.17$), levels of IGF-I in the hippocampi (ischemic side $F_{8,42}=1.17; p=0.34$, intact side $F_{8,42}=1.97; p=0.07$) or the intact sensorimotor cortex ($F_{8,43}=0.69; p=0.69$; data not shown).

2.5. Time course of serum corticosterone after exercise

The stress hormone corticosterone has been implicated in downregulation of BDNF (Schaaf et al., 1998) and both voluntary and motorized endurance exercise elevated serum corticosterone immediately after exercise (Fig. 5; $F_{8,55}=14.04; p<0.0001$). Motorized running resulted in higher corticosterone levels than voluntary running ($477.98\pm 39.18\ ng/ml$ and $365.94\pm 19.04\ ng/ml$, respectively; $p<0.05$). In fact, corticosterone levels in the Motor/0 group were significantly higher than all other time

Fig. 3 – pCREB levels measured in the hippocampi from the ischemic and intact hemispheres 0, 30, 60 and 120 min following either 60 min motorized or 12 h voluntary running. Values are expressed as a percentage of BDNF levels in the hippocampus of the intact side in sedentary animals. Values are mean ± S.E.M. ($^*p<0.05$ versus Sedentary; **$p<0.01$ versus Motor/60, Motor/120 and Sedentary groups).
points for both exercise methods and the sedentary group (154.65 ng/ml±26.68; p<0.0001). However, 30 min after either exercise method, serum corticosterone returned to sedentary levels. There was no correlation between serum corticosterone and brain levels of BDNF in any of the groups (data not shown).

2.6. Heart rate responses to voluntary and motorized exercise

Since heart rate is the most common method to monitor exercise intensity, we were interested in the physiological response to voluntary and motorized running in order to determine intensity levels that could be translated to the clinical rehabilitation setting. To have a comparative baseline, it is important to determine both maximum and resting heart rate in normal and ischemic animals. Maximum heart rate (HR) was determined from a separate group of uninjured rats of similar weight and size as animals from the first experiment. There was no difference in values of two consecutive exercise challenges so the average maximum heart rate (HRmax) was based on the first trial (554.75±5.39 bpm). All HR parameters measured in ischemic animals, resting (355.16 bpm±14.02), motorized, voluntary and home cage HR (average and peak) were significantly different from each other (Fig. 6; F6,48=83.21 p<0.0001) except average motor HR was not different than peak home cage HR. Although both motorized and voluntary exercise peak heart rate values (535.53 bpm±5.4 and 505.06 bpm±9.11 respectively) were in the upper quartile of intensity, average heart rate for motorized exercise was in the 3rd quartile of intensity while voluntary was just in the 2nd quartile (468.47 bpm±5.18 and 402.08 bpm±6.74 respectively). Voluntary wheel running was a less intense form of endurance exercise, and in fact, peak home cage heart rate (444.26 bpm±12.73) was significantly higher than the average voluntary exercise heart rate (p<0.05). Activity measurements confirmed that rats performing voluntary

Fig. 4 – Synapsin-I levels measured in the hippocampi from the ischemic and intact hemispheres, A, or in the intact sensorimotor cortex, B, 0, 30, 60, and 120 min of either 60 min motorized or 12 h voluntary running. Panels above graph are representative Western blot images from each of the groups. Values are expressed as a percentage of sedentary levels in the hippocampus of the intact hemisphere or in the cortex as a percentage of sedentary levels and are mean±S.E.M. (*p<0.05 versus Motor/60; **p<0.05 versus Sedentary; †p<0.01 versus Vol/0 and Sedentary; ‡p<0.005 versus Motor/60 and Sedentary; §p<0.001 versus Motor/30 and Motor/120; §§p<0.005 versus Motor/30 and Motor/120).

Fig. 5 – Serum corticosterone levels measured 0, 30, 60 and 120 min after either 60 min motorized or 12 h voluntary run. Values are ng/ml and are mean±S.E.M. (*p<0.05 versus Vol/0; **p<0.0001 versus all Motor groups and Sedentary; †p<0.0001 versus all Voluntary groups and Sedentary).
exercise ran and stopped frequently whereas motorized exercise was continuous (data not shown).

3. Discussion

Since exercise enhances learning and memory likely via a BDNF-pCREB mediated mechanism (Vaynman et al., 2004) in the normal rat brain and BDNF infusion promotes recovery in a focal model of stroke (Schabitz et al., 2004), it is conceivable that exercise can be used to enhance the neuroplastic milieu to facilitate motor relearning after stroke. The optimal temporal arrangement of exercise and level of exercise (i.e., intensity, duration, frequency) within a rehabilitative therapy session must be determined in order to engage BDNF and other proteins involved in neuronal plasticity.

3.1. BDNF levels may be related to exercise intensity and duration

We have previously shown in a rat model of focal ischemia, that BDNF in the hippocampus of the intact hemisphere was elevated immediately after one episode of 30 min running on a motorized wheel or following one 12 h voluntary run (Ploughman et al., 2005). In this experiment, the temporal profile (0, 30, 60 and 120 min after exercise) of BDNF following voluntary and motorized running differed by hemisphere (ischemic versus intact) and the method of exercise. In the hippocampus of the ischemic side, BDNF was upregulated for up to 2 h following the voluntary run but only 30 min following 60 min in the motorized wheel (Fig. 2). In terms of exercise intensity, heart rate analysis showed that although voluntary exercise was performed for a longer duration, animals took frequent rests making it intermittent and less intense than motorized exercise (Fig. 6). The range of heart rate values, from resting to maximum, was comparable to previous reports in the rat (Bolter and Atkinson, 1988). This suggests that short-term elevation of BDNF occurs with short periods of intense exercise (motorized) but more prolonged upregulation results from lower intensity endurance exercise, such as voluntary running, interspersed with rest periods. This has important implications for the structure of rehabilitation for stroke. Running exercise, applied on its own, has had limited benefit in animal brain injury models (Hicks et al., 1998; Marin et al., 2003; Yang et al., 2003) however, exercise paired appropriately with language, cognitive or skilled motor learning tasks, by enhancing neurotrophins, may have synergistic beneficial effects on these outcomes.

Motor learning is associated with motor map reorganization (Kleim et al., 1998) and synaptogenesis (Kleim et al., 2002a), and the motor map is considered labile and responsive to BDNF (Kleim et al., 2003). Although our results show robust effects in the hippocampus rather than the cortex, the hippocampus is important in transferring contextual memory so it can be compared with incoming sensory information processed in the cortex (Zhang et al., 2005). The role of the hippocampus in motor learning is not known. Kleim and colleagues (Kleim et al., 2004) have shown that motor map reorganization occurs 10 days after a skilled task is learned suggesting that other structures (cerebellum, striatum and possibly hippocampus) may contribute to the early phases of learning. We show that acute episodes of endurance exercise elevate BDNF (and synapsin-I), which when paired with complex tasks, may act as a trigger to form and modify dendritic spines (Schrott et al., 2006; Ji et al., 2005) that may contribute to motor map reorganization. Future studies should examine how endurance exercise can enhance subsequent skilled learning tasks and corresponding motor topography in normal and ischemic brain.

Regions within the intact hemisphere may play a role in recovery after stroke (Biermasz et al., 2005) so we were interested in not only hippocampal response to exercise but also that of intact sensorimotor cortex. The sensorimotor cortex within the intact hemisphere was more resistant to exercise modulation than the hippocampi. Acute exercise using either method only briefly increased BDNF and synapsin-I and did not affect pCREB or IGF-I, suggesting that perhaps different training periods are required to alter these proteins in this region. We previously found that only a 30 min motorized exercise episode at a walking speed (compared to 60 min walk, 30 min run and 12 h voluntary run) increased BDNF and IGF-I in the intact sensorimotor cortex suggesting that the neurotrophin response in the cortex may be both time and intensity sensitive (Ploughman et al., 2005). However, Kleim et al. also found that prolonged running exercise, despite increasing angiogenesis, did not alter topographic maps within the primary motor cortex of rats (Kleim et al., 2002b). The effect of chronic exercise training on growth factors and other mediators of synaptic plasticity within the remaining intact cortex has not been identified and awaits further study.

3.2. Is acute exercise stressful?

Activation of CREB by neurotrophins is believed to regulate neuronal survival, differentiation, and dendritic spine dyna-
mics (Ji et al., 2005). Synaptic activity quickly and simultaneously influences both CREB phosphorylation and dephosphorylation (Bito et al., 1996) suggesting that CREB dephosphorylation may decrease gene expression and therefore recruitment of proteins involved in synaptic plasticity (Koch et al., 2003). Although others have shown a positive correlation between exercise, BDNF, and pCREB (Shen et al., 2001; Vaynman et al., 2003) in the hippocampus of normal rat brain, we have found that following a bout of either motorized or voluntary running after stroke, there is a period of decreased CREB phosphorylation in the hippocampus of the intact hemisphere (Fig. 3). This occurs even though BDNF and synapsin-I levels are near sedentary levels in the same brain region (Figs. 2 and 4A). Perhaps acute running exercise is initially stressful and causes short term CREB dephosphorylation. In support of this idea, forced swimming (15 min) produces a similar phasic response; pCREB elevation occurs within 30 min of exercise followed by a 1-2 h period of dephosphorylation in the hippocampal dentate gyrus and superficial layers of the cortex (Billang-Bleuel et al., 2002). These authors show that this response is specific (to exercise) and not produced by other stimuli such as cold or ether exposure. It is interesting that, in rat models of brain injury, running exercise provides little or no functional benefit (Hicks et al., 1998; Marin et al., 2003; Yang et al., 2003). In fact, running attenuates ischemia-induced neurogenesis and is associated with poorer functional outcome (Lee et al., 2003; Komitova et al., 2005). Furthermore, early, but not late, voluntary running decreases BDNF and pCREB and worsens functional outcome in a rat model of traumatic brain injury (Griesbach et al., 2004a, b). Our findings support the notion that the ischemic brain may be more sensitive to the stressful effects of acute exercise than normal brain. However, since pCREB was measured only after one episode of exercise, pCREB could be decreased in the short term, but undergo a form of adaptation with repeated bouts of exercise such that pCREB levels rebound. Consistent with this notion, others have shown that CREB is elevated after chronic exercise training in uninjured animals (Vaynman et al., 2004). Further, although others have shown that expression of hippocampal BDNF, pCREB and synapsin-I are correlated following 3 days of exercise (Vaynman et al., 2003), we have previously shown that directly following either motorized or voluntary exercise, these proteins are not correlated (Ploughman et al., 2005). This study is the first to examine the temporal profile of growth factors and related proteins directly after acute exercise and suggests that acute and chronic exercise may have different effects on pCREB.

Corticosterone has been implicated in the inhibition of LTP via a BDNF-related mechanism (Zhou et al., 2000) and our previous work has shown a rapid and robust increase in serum corticosterone with exercise (Ploughman et al., 2005). Although corticosterone is elevated after only one episode of either motorized or voluntary running, the effect is short-lived; falling to control levels after 30 min (Fig. 5). Others have shown that corticosterone is not altered by exercise (Dahlqvist et al., 2003; Shaw et al., 2003), however, unless serum levels are measured immediately following exercise, changes may be missed. Motorized exercise results in significantly higher corticosterone levels than the more intermittent voluntary running indicating that forced running in the motorized wheels, even at this moderate pace, is more stressful. The increased serum corticosterone after motorized exercise coincides with greater HR and longer suppression of CREB phosphorylation in the hippocampus, a potentially negative phenomenon during recovery from stroke. The forced swimming stress paradigm also shows a similar robust increase in serum corticosterone (over 500 ng/ml) that peaks at 60 min then falls to sedentary levels (Billang-Bleuel et al., 2002).

Bachmann et al. (2005) have further examined forced swimming in the rat and have found that blocking glucocorticoid receptors prevents the decline in hippocampal pCREB. Others have shown that restricted access to running (30-50%) reduces corticosterone and reverses the deleterious effects of long term running on neurogenesis and pCREB activation (Naylor et al., 2005). Stranahan and colleagues (Stranahan et al., 2006) have shown that singly housed rats exposed to voluntary running have decreased hippocampal neurogenesis compared to their group-housed counterparts related to higher serum corticosterone. Interestingly, voluntary exercise prevents the decline in BDNF associated with restraint stress even in the presence of high corticosterone (Adlard and Cotman, 2004). We also found that BDNF levels were maintained despite high serum corticosterone. These results support the notion that a short period of intense exercise may be stressful to the ischemic brain, at least in the short term. However since BDNF and synapsin-I are elevated after the same interventions, it is quite possible that overall, exercise in beneficial. Nevertheless, it may be important to use graduated exercise protocols after ischemia to mitigate any deleterious stress response.

3.3. Temporal responses of synapsin-I and IGF-I after exercise

Our previous study showed that synapsin-I levels are elevated in the intact hippocampus and sensorimotor cortex with a 60 min motorized run (Ploughman et al., 2005). In this study, synapsin-I levels in the hippocampi spike immediately following motorized exercise, then are rapidly and robustly down-regulated in a cyclic manner, rising and falling in discrete 30 min phases (Fig. 4A). This phasic response is pronounced after the more intense motorized exercise and in the hippocampus rather than in the intact sensorimotor cortex. Synapsin-I is elevated in both oxygen/glucose deprived hippocampal slices (Jung et al., 2004) and following gerbil forebrain ischemia (Martí et al., 1999). The mechanisms underlying ischemia-induced elevation may include both presynaptic activation in response to ischemia and synaptic remodeling (Jung et al., 2004). Voluntary exercise does increase the length and complexity of dendate granule cell dendrites as well as the density of dendritic spines (Eadie et al., 2005); structural changes that may account for the increase in synapsin-I.

Serum IGF-I has been shown to cross the blood–brain barrier and mediate the protective effects of exercise in both ischemic and degenerative injury models (Carro et al., 2001). IGF-I increases neurogenesis and dendritic growth in the developing brain (Cheng et al., 2003; Popken et al., 2004), important processes that also occur after ischemia. In our previous study examining different exercise parameters, we found that serum IGF-I depletion was correlated with
increasing concentrations of brain IGF-I suggesting that the protein may be entering the brain parenchyma from the circulation (Ploughman et al., 2005). In the present study, there was no significant overall effect on IGF-I, suggesting that one episode of exercise may be insufficient to affect brain and serum levels of IGF-I in a substantive way.

4. Conclusion

In summary, the expression of BDNF, pCREB, synapsin-I in the ischemic brain immediately after periods of voluntary and motorized endurance exercise are quite different. Intermittent, lower intensity (as reflected by heart rate) voluntary exercise induces a sustained increase in BDNF while an acute episode of more intense motorized exercise produces a brief BDNF elevation but results in more robust downregulation of pCREB and elevated serum corticosterone. Therefore, acute exercise may have both positive (elevation of BDNF) and negative (elevation of corticosterone) consequences post-stroke, however, chronic exercise may result in adaptation and attenuation of the stress response. This study is the first to examine the temporal profile of growth factors implicated in neuronal plasticity directly after acute exercise and helps to outline intensity, duration and frequency of exercise required to alter neurotrophins after stroke.

5. Experimental procedures

5.1. Experiment 1

5.1.1. Subjects

Sixty-six male Sprague–Dawley rats (Charles River Laboratories, Montreal, Quebec, Canada) weighing 390–440 g (approximately 4 months of age) at the time of surgery were used in this study. Animals were socially housed (2–3 rats per cage) on a 12 h reverse light/dark cycle in clear plexiglas cages with water and food ad libitum. All procedures were performed during the animals’ dark phase. All efforts were made to minimize suffering and reduce the number of animals used. Experimental protocols were in accordance with established guidelines determined by the Canadian Council on Animal Care with approval of the Memorial University Animal Care Committee.

5.1.2. Training

The training regimens were chosen to compare typical training methods in clinical rehabilitation (as in treadmill walking) and training in rat models of stroke (voluntary wheel). Final motorized wheel speed (11 m/min) was at a fast walking pace, a speed we had previously shown to upregulate proteins of interest (BDNF, synapsin-I; Ploughman et al., 2005). All animals (regardless of later group assignment) except sedentary controls received a graduated exercise training program on both motorized and voluntary wheels (36 cm diameter × 13 cm wide) to familiarize animals with the apparatus and the task. Motorized running progressed from 4 m/min for 5 min to 9 m/min for 20 min over 7 days. Similarly, voluntary running began at 5 min progressing to 60 min over 7 days. Total pre-training amounted to 45 min (500 m) on motorized wheels and 135 min (474±39 m) in voluntary wheels over 7 days. This was followed by a wash out period for 5 days to eliminate any potential protective effects of exercise against stroke. Sedentary animals received daily handling (n=12) equivalent to that of exercised animals. All animals then underwent endothelin-1 induced focal ischemia (Biernaskie and Corbett, 2001) followed by 4 days recovery. Post-surgery retraining on the exercise programs began 4 days post-stroke with the same progression as pre-surgery (for 7 days), followed by another 4 day wash out period. Exercising animals were then randomized to either motorized (11 m/min for 60 min) or voluntary running wheels (12 h) and further randomized to be sacrificed at 0 (n=10), 30 (n=6), 60 (n=6) and 120 (n=5) minutes following the designated exercise method. Exercise sessions were performed during the animals’ dark cycle. Revolutions in the voluntary wheels were recorded from mechanical counters.

5.1.3. Surgery

Anesthesia was induced using a mixture of 3% isoflurane in 30% oxygen and 70% nitrous oxide and animals were maintained with 1.5% isoflurane. Focal ischemia was induced by stereotaxic injection of the vasoconstrictive peptide endothelin-1 (Calbiochem, Temecula, CA, USA), 1200 pmol in 3 μl sterile water, adjacent to the middle cerebral artery (MCA) over 13 min at coordinates anteroposterior +0.9 mm, mediolateral −5.2 mm, dorsoventral −8.7 mm from bregma (Paxinos and Watson, 1997) as described previously (Biernaskie and Corbett, 2001; Biernaskie et al., 2001). The needle was kept in position for 5 min to minimize backflow. Rectal temperature was maintained between 36.5 and 37.5 °C during surgery using a self-regulating heating blanket (Harvard Apparatus, Holliston, MA, USA). After surgery, animals were placed on a heating blanket overnight. They were assessed 1 h post-stroke using a 3-item neurological assessment: retraction of contralateral paw, circling to contralateral side, and decreased resistance to perturbation on the contralateral side. Using the neurological assessment, animals were stratified by severity of impairment (total score 1; mild impairment, 2; moderate impairment, 3: severe impairment) then randomized to exercise groups.

5.1.4. Tissue processing

All animals were anesthetized with isoflurane and sacrificed by quick decapitation 2 weeks after stroke. Sacrifice occurred at 0, 30, 60 and 120 min following the running session during the dark cycle, ensuring animals from different groups were processed together to control for diurnal variation of BDNF and corticosterone (Schaaf et al., 2000). Sedentary control animals were sacrificed at the same time. The brain was quickly removed and hippocampi dissected. A wedge shaped section of cortex from the intact hemisphere, corresponding to the forelimb and hindlimb areas to the depth of the corpus callosum (approximate coordinates bregma −1 mm to +4 mm anteroposterior; Paxinos and Watson, 1997) was also excised in a subgroup of animals. Dissected tissue was weighed, frozen in liquid nitrogen and stored at −80 °C. Remaining brain was immediately placed in cold formalin for later infant assessment. Trunk blood was collected in the same subgroup.
of animals, centrifuged at 3000 rpm, the serum removed, and stored at −20 °C.

5.1.5. **Immun assay**

Tissue from hippocampus and cortex was homogenized as previously described (Ploughman et al., 2005). The activated form of CREB and BDNF were quantified in duplicate using enzyme-linked immunosorbent assay (ELISA; ActiveMotif, Carlsbad, CA and Chemicon, Temecula, CA, respectively) as per manufacturer’s protocols. The mean optical densities of unknown samples, in duplicate, were expressed as BDNF concentrations by plotting against known BDNF concentrations (7.8–500 pg/ml). Control animals were analyzed on every plate. Values were expressed as a percentage of protein in the intact hippocampus (or intact cortex) of sedentary animals.

5.1.6. **Radioim munoassay**

Competitive radioimmunoassay was used to quantify serum corticosterone and serum IGF-I (Cost-A-Count, DPC, Los Angeles, CA and DSL, Webster, TX, respectively) as per manufacturers’ protocols. Unknown samples were compared, in duplicate, to a known calibration curve and adjusted for non-specific binding. Corticosterone is expressed as ng/ml and IGF-I is presented as a percentage of sedentary control levels.

5.1.7. **Immunob lott ing**

Brain tissue was also analyzed by Western blot. The supernatants were removed, placed in fresh Eppendorf tubes, and protein concentrations were determined using the BCA protein assay (Pierce Chemicals, Rockford, IL). Equivalent amounts of protein (50 μg) were subjected to SDS-PAGE (8–10% acrylamide). Following transfer to nitrocellulose, the blots were stained with Ponceau Red to confirm transfer of proteins. Blots were subsequently probed with IGF-I antibody (Upstate Biotech Inc, Lake Placid, NY) and synapsin-I antibody (Chemicon, Temecula, CA). Blots were then visualized with ECL reagents (NEN, Boston, MA) and exposed to X-ray film (Cronex micon, Temecula, CA). Blots were subsequently digitized and densitometrically analyzed with Image J. Digital images of the blots were used to make composite figures with Adobe Photoshop graphics software (Adobe Corp, Mountain View, CA). Analysis was performed in triplicate.

5.1.8. **Infarct assessment**

Frozen 40 μm brain sections were cut on a cryostat and every eighth section was mounted on coated slides and stained with cresyl violet. Previous brain tissue dissection prevented the quantitative measurement of ischemic volume. A damage score, as described previously (Ploughman et al., 2005), was used to identify successful cases of stroke and to ensure that each treatment group had, on average, the same degree of injury. Briefly, a damage score from the section with maximal injury in each brain was assigned as follows: 0; no ischemic damage; 1: 1–25% damage; 2: 26–50% damage; 3: 51–75% damage and 4: >75% damage, compared to the intact cortex and striatum separately. This graded scoring system correlates well with infarct volume measurement done using quantitative stereological methods (D. Corbett, unpublished observations).

5.2. **Experiment 2**

5.2.1. **Subjects**

Fourteen male Sprague–Dawley rats weighing 300–350 g were used in this experiment. Nine rats were treated identically to animals in experiment 1. They received exercise training and endothelin-1 surgery but were not processed for immunohistochemistry. Two days following endothelin-1 surgery, animals were implanted with telemetry probes (Physiotel Implant TA10CA-F40, Data Sciences International, St. Paul, MN, USA) to determine heart rate responses during both methods of running and during resting while singly housed in plexiglas cages. A group of animals (n=5), not receiving endothelin-1 induced ischemia, weighing 300–320 g, were implanted with telemetry probes to determine normal maximum heart rate.

5.2.2. **Surgery**

Animals were anesthetized using isoflurane as in Experiment 1. Telemetry probes were inserted as described by Sgoifo (Sgoifo et al., 1996), using a procedure previously shown to provide an accurate measure of cardiac function measurements in awake and moving animals (Nadziejko et al., 2002). Briefly, the body of the transmitter was inserted subcutaneously in the abdominal region. The positive lead was sutured to the posterior aspect of the xiphoid process while the negative lead was placed parallel to the trachea into the anterior mediastinum. Animals were allowed to recover for 1 week before testing.

5.2.3. **Heart rate and activity data collection**

Heart rate and activity measurements were gathered by receivers (RA1010, Data Sciences International, St. Paul, MN, USA) for 6 s each minute and stored on a linked computer for later analysis. During the recovery period, we observed animals to ensure that the monitored heart rate reflected activity levels. Animals underwent two episodes (9:00 am and 1:00 pm) of motorized running (60 min at 11 m/min) and two 6 h voluntary running periods on alternating days as well as two samples of cage activity (3 h each at 9:00 am and 1:00 pm) during the dark cycle. Maximum heart rate was determined from the second group of normal animals using motorized wheels. These animals received identical training but only on the motorized wheels after which they underwent two exercise challenges at progressive speeds until they could no longer maintain the wheel pace (19 m/min). Heart rate range was set so that outlying heart rate data points below 100 beats per min (bpm) and above 1000 bpm were excluded. Average exercise heart rate was calculated in voluntary running wheels by excluding data points when the activity measure was zero. Resting heart rate was calculated from all heart rate measures when activity scores were zero, taken while animals were in their home cages.

5.2.4. **Statistics**

Statview software (SAS Institute, Cary, NC, USA) was used for data analyses. One way ANOVA was used for analysis and Fisher’s PLSD post-hoc comparisons were used where appropriate. Repeated measures ANOVA was used to analyze heart rate measures. Significance was set at p < 0.05 for all analyses and values are expressed as mean±S.E.M.
**Acknowledgments**

This research was supported by a CIHR grant to DC and by a Heart and Stroke Foundation/CIHR/Canadian Stroke Network/AstraZeneca Focus on Stroke Doctoral Research Award to MP. DC holds a Canada Research Chair in Stroke and Neuroplasticity. The authors thank Dr Bruce VanVleet for his helpful advice.

**References**


Lopez-Lopez, C., LeRoith, D., Torres-Aleman, I., 2004. Insulin-like...


