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Short communication

Exercise increases brain-derived neurotrophic factor level in serum of horses

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ABSTRACT

Serum-BDNF levels are known to reflect functions of the nervous system. It has been shown in humans that exercise increases serum-BDNF levels. However, how exercise training affects serum-BDNF in horses is unknown. Knowing how BDNF is altered in response to exercise training in horses will provide insight into the nervous system's response to exercise and may provide a novel indicator to improve the training program for sport horses. Here we investigated the effect of exercise on the level of serum-BDNF in horses by comparing BDNF levels in serum from sedentary horses to those from active horses that are trained and participated in polo matches. The level of total serum-BDNF was significantly higher in active horses compared with that of sedentary horses. Individual forms of BDNF (pro, truncated and mature BDNF) were also significantly higher in active horses, especially the truncated-BDNF. The findings suggest that exercise increases basal levels of horse serum-BDNF, indicating the positive response of the horse nervous system to physical activities.

1. Introduction

Several studies have reported the effects of exercise on physiological adaptation of horse's cardiovascular and skeletal muscle as well as articular cartilage (Evans and Rose, 1988; Firth, 2006; Rivero et al., 2007). However, the response of the horse nervous system to exercise has never been investigated. The nervous system is known to determine human and animal performances by controlling movement, sensation, balance and learning skills (Visser et al., 2003; Klintsova et al., 2004). Therefore, monitoring responses of this system to the training might benefit the physical performances of the horses.

Studies in rodent models found a positive relationship between motor learning and levels of neurotrophic factors in the brain, especially brain-derived neurotrophic factor (BDNF) (Sartorius et al., 2009; Fritsch et al., 2010). Up-regulation of BDNF in the brain is related to improved learning and cognitive performance (Bekinschtein et al., 2008). BDNF is a secreted protein, and is a member of the neurotrophin family of growth factors. It is highly expressed in the central nervous system (Connor et al., 1997), where it plays several roles in neuronal survival and plasticity (Lu, 2003; Waterhouse and Xu, 2009). Several studies have reported that low levels of serum-BDNF are correlated with motor impairment and poor cognitive performance in patients with neurological disorders, such as Parkinson's disease (Scalzo et al., 2010), Alzheimer's disease (Laske et al., 2006) and Schizophrenia (Grillo et al., 2007). In contrast, exercise has been shown to increase BDNF levels in the brain and serum of both rodent models and humans (Griesbach et al., 2009; Rasmussen et al., 2009; Griffin et al., 2011; Marlatt et al., 2012; Goda et al., 2013). However, the effect of exercise on the alteration of BDNF level in horses has never been investigated. Because BDNF can cross the blood brain barrier (Poduslo and Curran, 1996; Pan et al., 1998) and be detected in the serum, serum-BDNF levels may correlate with BDNF levels in the brain, and could therefore be used as an indicator for the nervous system responses to exercise. In this study we investigated the effect of exercise on the level of serum-BDNF in horses. The BDNF levels in serum of sedentary horses and active horses that are trained and participated in polo matches were compared. The changes in serum-BDNF levels in response to different intensities of exercise were also investigated.

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Table 1
Program and protocol for active horses conducted 6 days per week.

Program	Protocol
1. Low intensity (1 month)	Walk 1 h twice a day
2. Low to moderate intensity (1 month)	1st week: walk 25 min, Trot 5 min, walk 25 min, twice a day 2nd week: walk 25 min, Trot 10 min, walk 25 min, twice a day 3rd week: walk 20 min, Trot 15 min, walk 20 min, twice a day 4th week: walk 20 min, Trot 20 min, walk 20 min, twice a day
3. Moderate to high intensity (5 months)	Walk 20 min, Trot 20 min and walk 20 min, twice a day and participate in official polo match 4 days a week
4. Resting (5 months)	Graze in the paddock for 2 hours per day

2. Materials and methods

2.1. Animals, training program and blood sample collection

The animal protocol was approved by the committee on Animal Care and Use, Faculty of Science, Mahidol University (SCMU-ACU). All animal experiments were performed in accordance with the guidelines of National Laboratory Animal Center, Mahidol University. Five sedentary and nine active horses participated in this study, with the agreement from the owner (Thai polo and Equestrian Club and Pattaya, Chonburi, Thailand). These horses are Argentine polo ponies, which are crossbred horses between Thoroughbred horses and Criollo horses. All horses were housed separately within horse stables and were fed food pellets twice a day. They were sometimes left grazing in the paddock with hay and water ad libitum.

The training program for active horses was composed of four consecutive periods of different exercise intensities, which consisted of, low intensity, low to moderate intensity, high intensity and a resting period, respectively (Table 1). The length of the program was one year. Exercise training was performed six days per week, but matches were only four days per week. Within twenty four hours after the end of each training period, ten to fifteen milliliters of blood sample were collected from the jugular vein of each horse. They were left to clot at room temperature for 15 min and were centrifuged at $2000 \times g$ for 30 min. Serum was gently retrieved and stored at -80°.

2.2. Measurement of BDNF in serum

Total protein concentration in serum was measured by a BCA protein assay. Then, serum with equal amount of total protein was mixed with 5X Laemmli buffer and RIPA buffer, heated at 95 °C, and separated on 12% acrylamide gels. Electrophoresed samples were transferred to Polyvinylidene Fluoride (PVDF) membranes. The membranes were blocked for non-specific binding with 5% nonfat dry milk in 1 × TBST for 1 h at room temperature and incubated with anti-BDNF antibody overnight at 4 °C. The membranes were washed and incubated with secondary antibody in 5% nonfat dry milk in TBS for 1 h at room temperature. This antibody is light chain specific and was verified to have no cross-reactivity to horse IgG. Last, the signal from each sample was detected with HRP substrate and exposed onto hyperfilm. The intensity of each sample was analyzed with Image J software. Ponceau S was used as a loading control and for normalization.

2.3. Statistical analysis

All statistical tests were performed with GraphPad Prism 5.0. To quantify statistical differences in serum-BDNF levels between sedentary and active horses, results for each form of BDNF were analyzed with the Student *T*-test or one-way ANOVA. Statistical significance of the differences was set at a *p*-value of < 0.05.

3. Results

3.1. Levels of BDNF is higher in serum of active horses

To determine the effects of exercise on serum-BDNF in horses,

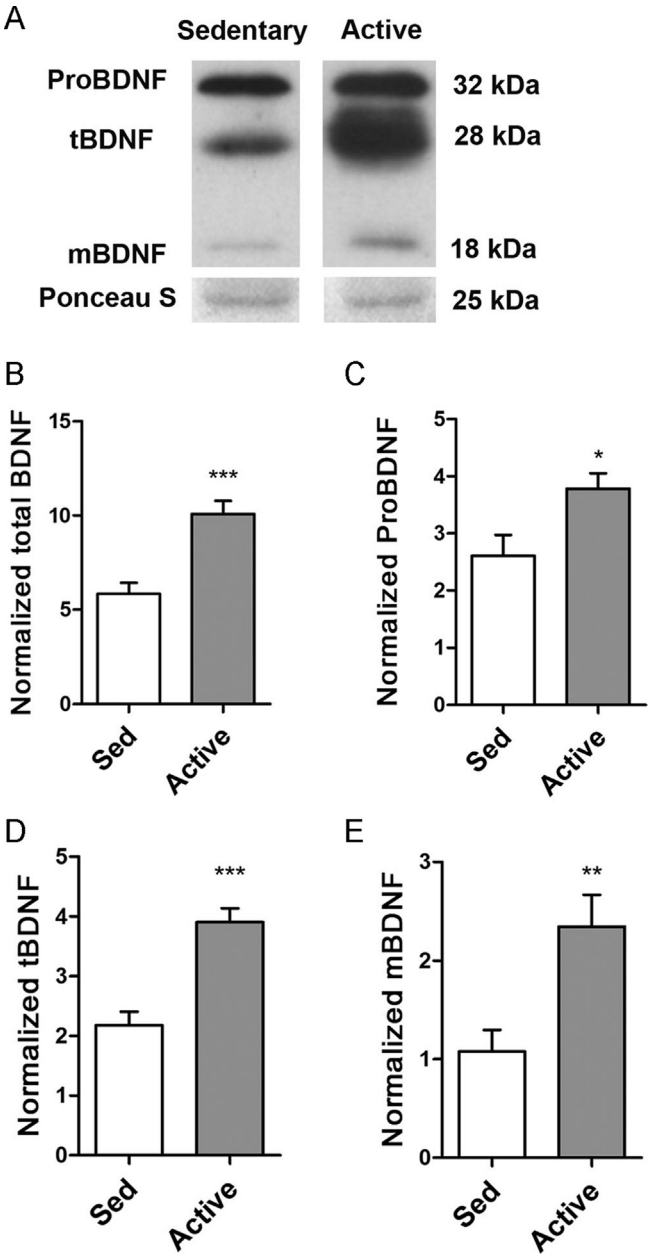

Fig. 1. Effect of exercise on horse serum-BDNF. Western blot of proBDNF, truncated BDNF (tBDNF) and mature BDNF (mBDNF) in serum of sedentary and active horses (A). Quantification showing that total and all forms of BDNF are significantly higher in the serum of active horses as compared to serum of sedentary horses (B–D) ($n=5$ sedentary and 9 active horses). Data are normalized with Ponceau S, and are expressed as mean ± SEM. ***, $P < 0.001$, **, $P < 0.01$, *, $P < 0.05$ (Student T-test).