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Antidepressant effects of exercise are produced via suppression of hypocretin/orexin and melanin-concentrating hormone in the basolateral amygdala

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ABSTRACT

Physical exercise is considered beneficial in the treatment of depression, but the underlying mechanism is not clearly understood. In the present study, we investigated the mechanism regulating antidepressant effects of exercise by focusing on the role of the amygdala using a well-defined animal model of depression. C57BL/6 mice treated with repeated restraint showed depression-like behaviors, which was counteracted by post-stress treatment with physical exercise. The two neuropeptides hypocretin/orexin (Hcrt/Orx) and melanin-concentrating hormone (MCH) were transcriptionally upregulated in the BLA after repeated stress, and their enhanced expression was downregulated by treatment with exercise, mirroring stress-induced depression-like behaviors and their reversal by exercise. Stereotaxic injection of either Hcrt/Orx peptide or MCH peptide within the BLA commonly increased phospho-CaMKIIα level and produced depression-like behaviors, mimicking the neural states in the BLA of mice subjected to repeated stress. In contrast, siRNA-mediated suppression of Hcrt/Orx or MCH in the BLA blocked stress-induced depression-like behaviors. Furthermore, siRNA-mediated inhibition of CaMKIIα in the BLA also counteracted stress-induced depression-like behaviors. Local injection of Hcrt/Orx peptide or MCH peptide within the BLA in exercise-treated animals blocked antidepressant-like effects of exercise. Together these results suggest that exercise produces antidepressant effects via suppression of Hcrt/Orx and MCH neural systems in the BLA.

Introduction

Depression is a mental illness characterized by a wide range of neuropathological symptoms. Because of the diverse causes and multiple symptoms of depression, various therapeutic strategies need to be developed (Nestler and Hyman, 2010). Clinical and animal studies have suggested that physical exercise helps relieve depression. Recent meta-analyses show that physical exercise is beneficial for those who show mild to moderate depression symptoms (Josefsson et al., 2014; Krogh et al., 2011). However, the molecular mechanism mediating exercise effects is not fully understood.

Hypocretins 1 and 2 (Hcrt-1 and -2), also called orexins A and B (Orx-A and -B), and melanin-concentrating hormone (MCH) have been identified as hypothalamic neuropeptides mediating hypophyseal functions (Guyon et al., 2009; Tsujino and Sakurai, 2013). Hcrt/Orx and MCH are expressed mainly in the lateral hypothalamus that projects to many brain regions including the cerebral cortex, hippocampus and amygdala (Hervieu, 2003; Peyron et al., 1998). The hypothalamic Hcrt/Orx plays a key role in arousal/narcolepsy, the control of energy metabolism, cardiovascular responses, food intake, and drug reward responses (Harris et al., 2005; Tsujino and Sakurai, 2009). The hypothalamic MCH increases feeding (Qu et al., 1996; Rossi et al., 1997) and regulates motivation (Duncan et al., 2005). Being independent from these energy metabolism and food-intake-related functions, several lines of evidence based on human and animal studies suggest that the Hcrt/Orx and MCH systems play also a role in depression. Patients with major depression have decreased levels of Hcrt/Orx-1 in the cerebrospinal fluid (Brundin et al., 2007), and in the blood (Rotter et al., 2011). In line with these studies, it has been proposed that activation of the Hcrt/Orx system

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in the brain will be beneficial for relief of depression (Borgland et al., 2010).

However, wildtype mice treated with Hcrt/Orx antagonist display a significant reduction in immobility time in the FST and TST. Hcrt/Orx receptor-1 knockout (HcrtR1 KO) mice also display a similar reduction in immobility time in the FST and TST (Scott et al., 2011; Nollet and Leman, 2013). Injection of the Hcrt/Orx receptor antagonist almorexant in mice showing depressive-like behaviors after treatment with chronic stress produces antidepressant-like effects (Nollet et al., 2012). In contrast, HcrtR2 KO mice show depressive behaviors (Scott et al., 2011). Concerning the MCH system, female, but not male, mice lacking the MCH receptor-1 (MCHR1) show antidepressant-like behaviors (Roy et al., 2007). MCHR1 antagonists produce antidepressant-like effects (Borowsky et al., 2002; Chaki et al., 2005). Thus available evidence supports the roles of Hcrt/Orx and MCH in depression-related behaviors, but detailed mechanisms of how the two distinct neuropeptide systems regulate depression states are too complicated and are inconsistent in some cases. These complex results might be attributed in part to the possibility that pharmacological or genetic inhibitions produce systemic effects, while the Hcrt/Orx and MCH neural systems in specific brain regions may have some distinct function. Mice with high immobility in the FST show an increased expression of Hcrt/Orx and Orx1 receptors in the amygdala, while a decreased expression of Hcrt/Orx in the hippocampus (Arendt et al., 2013). However, the functional significance of the Hcrt/Orx and MCH in the amygdala in depression-related behaviors has not been explored.

Clinical studies and animal models have suggested that the amygdala is an important brain region for regulating depression (Drevets, 1999; Krishnan and Nestler, 2011). Functional neuroimaging studies show that patients with depression displayed sustained amygdala reactivity on emotional tasks (Ressler and Mayberg, 2007; Siegle et al., 2007). Studies for stimulus-dependent c-fos induction in animal models show that the amygdala is one of the brain regions that are sensitively activated by stress (Kim and Han, 2009) and exercise (Greenwood et al., 2012). Among several subregions of the amygdala, the basolateral amygdala (BLA) has a functional connectivity to the prefrontal cortex, the nucleus accumbens and the hippocampus (McDonald, 1991), which all are core parts of the corticolimbic system that is important for mood controls. Thus, the amygdala posits to integrate various sensory information produced by stress and exercise, and regulates emotional states. However, few studies have investigated whether antidepressant effects of exercise are produced through functional changes in the amygdala and how the amygdala regulates stress-induced depressive behaviors.

In the present study, using complementary molecular, pharmacological, and genetic tools, we demonstrated that chronic stress produces depression-like behaviors via up-regulation of the Hcrt/Orx and MCH neural systems in the BLA, whereas exercise produces antidepressant effects via downregulation of the BLA Hcrt/Orx and MCH systems.

Materials and methods

Animals and restraints

Seven-week-old C57BL/6 mice were obtained from Daehan BioLink (Eumsung, Chungbuk, Republic of Korea). Hcrt/Orx knockout (KO) mice (Chemelli et al., 1999) and MCH KO mice (Shimada et al., 1998) used were backcrossed to C57BL/6J for more than, respectively, 12 and 23 generations. The genotyping of Hcrt/Orx KO mice and MCH KO mice was performed using PCR with the primer set: 5′-CGCCTTCTTG ACGAGTTC-3′ 5′-GACGACGGCCTCAGACTT-3′ and 5′-TCACCCCCTTGG GATAGC-3′ for Hcrt/Orx KO mice; 5′-TGTGAACAGGTTTTGCTGTTG-3′ 5′-CACCCCGTAGAGCCTTTGTA-3′ and 5′-GCCAGAGGCCACTTGTGTAG-3′ for MCH KO mice. Male and female mice were used as indicated. Mice were housed in pairs in a standard clear plastic cage in a temperature- and humidity-controlled room. All animals were handled in accordance with the animal care guidelines of Ewha Womans University (IACUC 2013-01-007).

Mice were restrained for 2 h daily for 14 days as described previously (Park et al., 2014; Seo et al., 2012). In brief, mice were individually placed in a well-ventilated, 50-ml polypropylene conical tube and were completely restrained within this tube for 2 h daily starting from 10 am. After each session of restraint, they were returned to their home cages with free access to food and water. This procedure was repeated for the indicated days.

Forced exercise using a running wheel

Forced wheel running exercise was carried out as described recently (Kim et al., in press). In brief, the running wheel consisted of a rotating drum with two circular ventilated plastic walls (with 20 cm of inner diameter) with a track (7 cm width) that was made of evenly-spaced aluminum bars (each with 2 mm-width, spaced 0.65 cm apart). When placed on a wheel rotating, mice were running in most of the time at a low speed, but they were also coasting by holding on inside a rotating wheel even at a low speed. The higher the rotating speed, the more the coasting time. After pre-training for 20 min daily for 5 days, mice spent >95% of the 60-min exercise period running at the speed of 6 m/min and 84% of the 60-min exercise period running at 9 m/min, but they did far less than 50% of the 60-min exercise period running at 12 m/min, so that the total distance of running for the 60-min period at 9 m/min was higher than that at 6 or 12 m/min (Fig. 1A). At 9 m/min, the total distance of running on each trial was stably maintained (~449 m/day) for 21 days of repeated exercise (Fig. 1B). Based on these tests, all mice that were put on the exercise regime were pre-trained to run on the rotating wheel at a constant speed of 9 m/min for 20 min daily for 5 days. This procedure was given to animals prior to the stress treatment period and was regarded as a part of habituation. The pre-training alone did not suppress stress-induced depressive behaviors. Mice were individually placed on the wheel rotating at 9 m/min for 60 min per day starting at 10:00 am in the light phase, and this treatment was chosen as a daily standard exercise procedure and was repeated for indicated days.

All exercise performances for 21 days of the exercise regime were videotaped, and the amounts of time each animal run or coasted were analyzed. To control the amount of exercise given to each mouse, when normal mice ran on the running wheel less than 80% of the daily exercise time for more than any two consecutive days during 21 days of exercise, they were excluded from the final data set. Usually 0–2 animals were excluded for each group due to this reason.

We noted that when mice were placed in the unrotating wheel for 60 min, they showed stress responses, resulting in an increase of corticosterone release in the blood. Therefore, after initial grouping, sedentary control mice were placed in home cages without disturbance until behavioral tests or tissue preparation.

Drug administration

Imipramine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Hcrt/Oxt and MCH peptides were purchased from Tocris Bioscience (Bristol, U.K.). Imipramine, Hcrt/Oxt and MCH peptides were diluted in 0.9% saline. Imipramine was intraperitoneally injected at 20 mg/Kg/day (i.p.). in a volume of 120 μl each for indicated periods. For peptide injection, mice were anesthetized with 1.2% isoflurane. Hcrt/Orx1 (0.53 ng, each side) or MCH (0.12 ng, each side) in a volume of 0.5 μl was injected into each BLA (stereotaxic coordinate: AP, −1.4; ML, ±3.0; DV, −4.8 mm) at the speed of 0.2 μl/min using a 30 G needle. After 50 min of injection, the U-field test was performed, followed by the FST. After behavioral analysis, injection sites were histologically examined and mice with mislocalized injections were excluded from the final data.