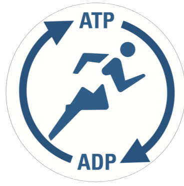

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Chronic exercise potentiates anorectic effects of leptin in hypothalamic Pomc neurons

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Objective Chronic imbalance of energy homeostasis leads to obesity and metabolic mellitus, which has developed as a major public health and economic burdens around the world. Disruption of "beige" fat mediated thermogenesis and hypothalamic neurons manipulated energy intake exaggerate this process. Multiple factors including hormonal regulation, fuel availability, and behavior contribute to energy utilization. The central nervous system (CNS) is critical for regulating energy balance and coordinating whole body metabolism. In the CNS, proopiomelanocortin (Pomc) neurons receive and integrate information about energy availability via responding to circulating hormones including insulin, leptin. Previous studies revealed that leptin receptors (LepRs) in arcuate Pomc neurons are required and sufficient for the proper regulation of energy balance and glucose homeostasis, including systemic insulin sensitivity and hepatic glucose production.
Exercise is an effective lifestyle intervention to combat obesity and metabolic diseases, which exerts many health benefits, including weight maintenance, appetite control, improved insulin sensitivity, improved mental health, and secondary prevention of chronic diseases such as obesity, type II diabetes mellitus, cancer, and hypertension. Moreover, the combined efficacy of exercise and dietary regimens on type two diabetes can surpass that of pharmacological interventions alone. Previous efforts aimed at identifying molecular mechanisms underlying the adaptive responses to exercise have mainly focused on the effects of exercise training in an organ or cell autonomous manner. However, the impact of exercise on performance, food intake after exercise, and more broadly, the healthy metabolic outcomes of exercise is not well-established. Despite the increased understanding of the importance of CNS underlying metabolic homeostasis, the specific neuronal groups and pathways that contribute to the metabolic responses during and following exercise remain largely unclear. In the current study, we aimed to investigate the role of exercise in mediating hypothalamic Pomc neuron activity, anorectic effects of leptin and glucose tolerance as well as insulin sensitivity.

Methods Animals

All mice were housed under standard laboratory conditions (12 h on/off; lights on at 7:00 a.m.) and temperature-controlled environment with food and water available ad libitum. All experiments were performed in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Slice preparation and whole-cell recordings

Male mice were deeply anesthetized with i.p. injection of 7% chloral hydrate and transcardially perfused with a modified ice-cold artificial CSF (ACSF) (described below). The mice were then decapitated, and the entire brain was removed and immediately submerged in ice-cold, carbogen-saturated (95% O_2 and 5% CO_2) ACSF (126 mM NaCl, 2.8 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 5 mM glucose). Coronal sections (250 μm) were cut with a Leica VT1000S Vibratome and then incubated in oxygenated ACSF at room temperature for at least 1 h before recording. The slices were bathed in oxygenated ACSF (32 °C–34 °C) at a flow rate of ~2 ml/min. All electrophysiology recordings were performed at room temperature.
The pipette solution for whole-cell recording was modified to include an intracellular dye (Alexa Fluor350 hydrazide dye) for whole-cell recording: 120 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 5

mM EGTA, 1 mM CaCl2, 1 mM MgCl2, and 2 mM MgATP, 0.03 mM Alexa Fluor 350 hydrazide dye (pH 7.3). Epifluorescence was briefly used to target fluorescent cells, at which time the light source was switched to infrared differential interference contrast imaging to obtain the whole-cell recording (Zeiss Axioskop FS2 Plus equipped with a fixed stage and a QuantEM:512SC electron-multiplying charge-coupled device camera). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices), low-pass filtered at 2–5 kHz, and analyzed offline on a PC with pCLAMP programs (Molecular Devices). Membrane potential and firing rate were measured by whole-cell current clamp recordings from Pomc neurons in brain slices. Recording electrodes had resistances of 2.5–5 MΩ when filled with the K-gluconate internal solution. Input resistance was assessed by measuring voltage deflection at the end of the response to a hyperpolarizing rectangular current pulse steps (500 ms of −10 to −50 pA).

Leptin (100 nM) was added to the ACSF for specific experiments. Solutions containing drug were typically perfused for 5 min. A drug effect was required to be associated temporally with peptide application, and the response had to be stable within a few minutes. A neuron was considered depolarized or hyperpolarized if a change in membrane potential was at least 2 mV in amplitude. Neurons were voltage-clamped at −75 mV (for excitatory postsynaptic currents) and −15 mV (for inhibitory postsynaptic currents). Frequency and peak amplitude were measured by using the Mini Analysis program (Synaptosoft, Inc.)

Exercise protocols

Motorized treadmills (Exer-6; Columbus Instruments, Columbus, OH) were used for exercise experiments. All mice were familiarized to the treadmills for 7 days prior to the exercise bout [Day 1: 5 min rest on the treadmill followed by running for 5 min at the speed of 8 m/min and then for 5 min at the speed of 10 m/min; Day 2-3: 5 min rest on the treadmill followed by running for 5 min at the speed of 10 m/min and then for 5 min at the speed of 12 m/min; Day 4-7: 5 min rest on the treadmill followed by running for 60 min at the speed of 12 m/min]. On Day 8, mice were subjected to a high intensity interval exercise (HIIE) bout to assess exercise-induced changes in plasma leptin, blood glucose, and food intake. Briefly, food was removed from all the mice at the start of the light cycle (7 AM) for a duration of 6 h, so as to eliminate any differences in food intake on the measured parameters. Mice were rested on the treadmill for 5 min prior to performing the 1 h of exercise consisting of 3 × 20 min intervals (5 min at the speed of 12 m/min, followed by 10 min at the speed of 17 m/min, and then 5 min at the speed of 22 m/min), without rest between intervals.

Tolerance test and food intake

For GTT, mice fasted for 16 h received an intraperitoneal injection of glucose (1 g/kg). For ITT, mice fasted for 6 h received an intraperitoneal injection of human insulin (0.75 IU/kg). Blood glucose concentrations were measured from tail blood at the indicated times using a One-Touch Ultra® glucometer (LifeScan Inc., Milpitas, CA). Food intake was measured hourly for 6 hours and then a single measurement at 24 hours.

Results To assess the predominant role of exercise on the neuronal activation of hypothalamic Pomc neuron, electrophysiology studies was conducted on transgenic mice after treadmill habitation for 7 days. And we found that exercise significantly reduced food intake and enhanced glucose tolerance as well as insulin sensitivity. Notably, chronic exercise dramatically potentiates leptin-induced depolarization of Pomc neurons and exerts leptin induced anorectic effects in vivo and in vitro. Furthermore, gene assay demonstrated an upregulation of sirtin1 after exercise, suggest-----ing a link between the exercise and key proteins involved in epigenetics, providing potential targets for the treatment of metabolic disease.

Conclusions Our results demonstrated chronic exercise potentiates anorectic effects of leptin in hypothalamic Pomc neurons. Moreover, these data provide evidence for sirtin1 as a substrate of exercise to regulate food intake and glucose tolerance as well as leptin sensitivity via activating Pomc neurons.