Acute exercise suppresses hypothalamic PTP1B protein level and improves insulin and leptin signaling in obese rats

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It has been proposed that obesity is associated with hypothalamic inflammation and dysfunction in animal models (5, 9, 46) and in humans (37, 40). An imbalance between caloric intake and energy expenditure is generated as a consequence of low-grade inflammation, leading to the hypothalamic insulin and leptin resistance through distinct intracellular mechanisms (3, 5, 6, 9, 46). During the past decade, protein tyrosine phosphatase 1B (PTP1B) was investigated as a key phosphatase induced by hyperphagia, and obesity. In this scenario, hypothalamic protein tyrosine phosphatase 1B (PTP1B) has emerged as the key phosphatase induced by inflammation that is responsible for the central insulin and leptin resistance. Here, we demonstrated that acute exercise reduced inflammation and PTP1B protein level/activity in the hypothalamus of obese rodents. Exercise disrupted the interaction between PTP1B with proteins involved in the early steps of insulin (IRβ and IRS-1) and leptin (JAK2) signaling, increased the tyrosine phosphorylation of these molecules, and restored the anorexigenic effects of insulin and leptin in obese rats. Interestingly, the anti-inflammatory action and the reduction of PTP1B activity mediated by exercise occurred in an interleukin-6 (IL-6)-dependent manner because exercise failed to reduce inflammation and PTP1B protein level after the disruption of hypothalamic-specific IL-6 action in obese rats. Conversely, intracerebroventricular administration of recombinant IL-6 reproduced the effects of exercise, improving hypothalamic insulin and leptin action by reducing the inflammatory signaling and PTP1B activity in obese rats at rest. Taken together, our study reports that physical exercise restores insulin and leptin signaling, at least in part, by reducing hypothalamic PTP1B protein level through the central anti-inflammatory response.

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MATERIALS AND METHODS

Animals and Diets

Male 4-wk-old Wistar rats were obtained from the University of Campinas Breeding Center. The investigation was approved by the university’s ethics committee and followed the university guidelines for the use of animals in experimental studies, and our experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996). The animals were maintained in 12:12-h artificial light-dark cycles, with lights on at 0600, and housed in individual cages. Rats were randomly divided into the following groups: control, fed standard rodent chow (3.948 kcal/kg), or fed HFD (5.358 kcal/kg) ad libitum for 3 mo. The HFD composition has been described previously (23).

Male (10-wk-old) ob/ob and db/db mice and their respective control C57BL/6J background mice were obtained from The Jackson Laboratory and provided by the University of São Paulo. The mice were bred under specific pathogen-free conditions at the Central Breeding Center of the University of Campinas and were fed standard rodent chow (3.948 kcal/kg).

Antibodies and Chemicals

Anti-phospho-JAK2 antibody (rabbit polyclonal, AB3805) was from Upstate Biotechnology (Charlottesville, VA). Anti-JAK2 (rabbit polyclonal, SC-278), anti-phospho-IRβ (rabbit polyclonal, SC-25103), anti-IRβ (rabbit polyclonal, SC-711), anti-phospho-IRS-1 (rabbit polyclonal, SC-17199), anti-IRS-1 (rabbit polyclonal, SC-559), anti-IL-6 (rabbit polyclonal, SC-7920), anti-IL6Rα (rabbit polyclonal, sc-13947), anti-SOCS3 (rabbit polyclonal, sc-9023), and anti-PTP1B (goat polyclonal, SC1718) antibodies were from Santa Cruz Biotechnology. Anti-PTP1B (AB-1 mouse polyclonal) was purchased from Calbiochem (La Jolla, CA). Anti-β-tubulin (rabbit polyclonal, no. 2146), anti-phospho-IKKα/β (rabbit polyclonal, no. 2687), and anti-IκBα (rabbit polyclonal, no. 9242) were from Cell Signaling Technology (Beverly, MA). Leptin and recombinant IL-6 were from Calbiochem (San Diego, CA). Protein A-Sepharose 6 MB and nitrocellulose paper (Hybond ECL, 0.45 mm) were from Amersham Pharmacia Biotech United Kingdom (Buckinghamshire, UK). Routine reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise specified.

Intracerebroventricular Cannulation

After intraperitoneal injection of a mix of ketamin (10 mg) and diazepam (0.07 mg, 0.2 ml/100 g body wt), the rats were stereotaxically instrumented with a chronic 26-gauge stainless-steel indwelling guide cannula placed aseptically into the third ventricle at the midline coordinates of 0.5 mm posterior to the bregma and 8.5 mm below the surface of the skull of the rats, using the Stoelting stereotaxic apparatus as described previously (32). After a 5-day recovery period, cannula placement was confirmed by a positive drinking response after administration of angiotensin II (40 ng/2 μl), and animals that did not drink 5 ml of water within 15 min after angiotensin injection were not included in the experiments.

Exercise Protocols

Swimming. Animals were acclimated to swimming for 2 days (10 min/day). Water temperature was maintained at 32–34°C. Rats performed two 3-h exercise bouts, which were separated by one 45-min rest period. The rats swam in groups of three in plastic barrels of 45 cm in diameter that were filled to a depth of 50 cm. This protocol was conducted between 11 AM and 6 PM, as described previously (32). Details of the exercise protocol are shown in the experimental design in Fig. 1A. Mice performed four 30-min exercise bouts that were separated by 5-min rest periods. The mice swam in groups of four in plastic barrels of 40 cm in diameter that were filled to a depth of 20 cm. This protocol was conducted between 3 and 6 PM. Both exercise protocols were finished at 6 PM for evaluation of food intake and analysis of hypothalamic tissue, as described previously (31).

Intracerebroventricular Injections

Rats were deprived of food for 2 h with free access to water and received 2 μl of bolus injection into the third ventricle (see below).

Insulin and leptin injections. Rats received icv infusion of vehicle (saline), insulin (200 μU), or leptin (10−6 M) at 6 PM to evaluate the food consumption. Food intake was determined by measuring the difference between the weight of chow given and the weight of chow at the end of a 4- and 12-h period, as shown in Fig. 2G. For insulin and leptin signaling evaluation, the hypothalamic tissue was removed 15 min after insulin or leptin icv injection, as described previously (13, 31).

Recombinant IL-6. For Western blot analysis, icv recombinant IL-6 (50, 100, or 200 ng) was injected into the third ventricle, and the hypothalamus was excised 6 h later. To evaluate the effects of IL-6 on food intake in obese rats, icv injection of IL-6 (200 ng) was performed at 12 PM and 6 h later (6 PM), rats received icv injection of vehicle, insulin, or leptin, and then the food consumption was monitored for 4 and 12 h, as shown in Fig. 3L.

IL-6-neutralizing antibody. Rats were randomly selected for saline, rabbit preimmune serum (RPS), or rabbit antiserum against IL-6 (50 ng; anti-IL-6) icv injection. This dose was obtained in dose response experiments published previously (13, 31). Anti-IL-6 was injected into the third ventricle of the rats 30 min before the exercise protocol and after the first bout of swimming exercise, as shown in Fig. 5A.

Serum Analysis

Blood was collected from the cava vein 15 min after the exercise protocols. Plasma was separated by centrifugation (1,100 g) for 15 min at 4°C and stored at −80°C until the assay. RIA was employed to measure serum insulin. Leptin and IL-6 concentrations were determined using a commercially available enzyme-linked immunosorbent assay kit (Crystal Chem, Chicago, IL). Blood lactate was measured using Accutrend Plus equipment (Roche, Egham, UK). Sample blood was obtained from the tails every 60 min during the swimming exercise.

Corticosterone Analysis

Corticosterone levels were determined using urine samples obtained from rats, using a specific metabolic cage for 24 h after the exercise protocols. The corticosterone level was determined using an EIA kit from Cayman Chemical (Ann Arbor, MI).

Immunohistochemistry

Paraformaldehyde-fixed hypothalami were sectioned (5 μm). The sections were obtained from the hypothalami of five rats per group in the same localization (antero-posterior = −1.78 from bregma) and were subjected to regular single- or double-immunofluorescence staining using DAPI, anti-IL-6 receptor-α, and anti-PTP1B antibodies according to a previously described protocol (31). Analysis and photodocumentation of results were performed using a LSM 510 laser confocal microscope (Zeiss, Jena, Germany). The anatomic correlations were made according to the landmarks given in a stereotaxic atlas. The frequency of positive cells was determined in 100 randomly counted cells using ImageJlab Analysis software (version 2.4).

Dissection of the Hypothalamic Regions

Four hours after the exercise protocol, hypothalamic nuclei of obese rats were quickly dissected in a stainless-steel matrix with razor
blades and frozen in liquid nitrogen. Later on, each region of the hypothalamus was dissected from 1-mm-thick sagittal sections of fresh brain. Paraventricular nucleus (PVN), arcuate nucleus (ARC), and ventromedial hypothalamus (VMH) plus dorsomedial hypothalamus (DMH) were dissected from the first sections from the midline of the brain. Coordinates for each hypothalamic region are as follows: PVN: square area with anterior margin (posterior region of anterior commissure), dorsal margin (border with thalamus), ventral margin, and posterior margin (white matter separating PVN/anterior hypothalamus and VMH/DMH); VMH plus DMH: triangular area with anterior margin (border with mamillary body), and ventral margin (border with ARC); ARC: ventral part of the medial hypothalamus with anterior and dorsal margin and posterior margin (border with mammillary body).

Western Blotting Analysis and Immunoprecipitation

Four hours after the exercise protocol or 6 h after IL-6 injection, hypothalami were removed and homogenized in solubilization buffer containing 1% Triton X-100, 100 mM Tris, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM phenyl methylsulphonyl fluoride, and 0.1 mg aprotinin) suitable for preserving the phosphorylation states of enzymes. Western blotting was performed as described previously (33).

The β-subunits of the insulin receptor (IRβ), IRS-1, JAK2, and PTP1B were immunoprecipitated from rat hypothalami. Antibodies used for immunoblotting were anti-IR, anti-IRS-1, anti-JAK2, and anti-PTP1B. Blots were exposed to preflashed Kodak XAR film. Band intensities were quantified by optical densitometry (Scion Image software; Scion, Frederick, MD) of the developed autoradiographs.

PTP Activity Assay

The in vitro PTP1B activity assay was conducted based on a protocol described previously by Taghibiglou et al. (36). After 6 h of IL-6 icv injection, hypothalami were removed and homogenized in solubilization buffer containing 1% Triton X-100, 20 mM Tris (pH 7.6), 5 mM EDTA, 2 mM PMSF, 0.1 mg aprotinin/ml, 1 mM EGTA, and 130 mM NaCl. Lysates were centrifuged (15,000 rpm, 40 min, 4°C), and the supernatants were collected for immunoprecipitation with anti-PTP1B antibody. Immunoprecipitates were washed in PTP assay buffer [100 mM HEPES (pH 7.6), 2 mM EDTA, 1 mM dithiothreitol, 150 mM NaCl, and 0.5 mg/ml bovine serum albumin]. The pp56c-src COOH-terminal phospho-regulatory peptide (TSTEPQYQPGENLL) was added to a final concentration of 200 μM in a total reaction volume of 60 μl in a PTP assay buffer for immunoprecipitation. The activity of total extracts (125 μg) was measured in the same manner in a total reaction volume of 60 μl in a PTP assay buffer, adding the peptide to a final concentration 200 μM. The reaction was then allowed to proceed for 1 h at 30°C. At the end of the reaction, 40-μl aliquots were placed into 96-well plates, 100 μl of Biomol Green Reagent (Enzo Life Sciences) was added, and the absorbance was measured at 660 nm.

Fig. 1. Exercise (EXE) reduces hypothalamic protein tyrosine phosphatase 1B (PTP1B) protein levels in different models of obesity. A: schematic representation of experimental design. B: representative blots show hypothalamic PTP1B protein level in control and in diet-obesity induced rats (n = 6). C: PTP1B activity assay was performed by using hypothalamic samples from Wistar rats in different experimental conditions (n = 5). D: hypothalamic PTP1B protein level 4 h after acute exercise in ob/ob and db/db mice (n = 6). E: hypothalamic suppressor of cytokine signaling 3 (SOCS3) protein level in Wistar rats (n = 4). F: median of blood lactate during EXE protocol. Samples were obtained for 60 min each during swimming exercise protocol (n = 6). G: body weight. H: 24-h evaluation of urine corticosterone (ng/ml). Bars represent means ± SE. *P < 0.05 vs. the control group (chow) at rest; #P < 0.05 vs. obese group at rest. HFD, high-fat diet; IB, immunoblot.
**RESULTS**

**Acute Exercise Reduces PTP1B Protein Levels in the Hypothalamus of Obese Rodents**

First, we sought to determine the effects of exercise on hypothalamic PTP1B protein levels and activity in obese animals. The experiment design is demonstrated in the Fig. 1A...
(Fig. 1A). By using Western blotting analysis, high PTP1B protein levels were found in the hypothalamic samples of HFD-fed rats, as expected (Fig. 1B). Interestingly, 4 h after the acute protocol of exercise, we observed a significant reduction in hypothalamic PTP1B levels in obese rats (∼35%) compared with obese animals at rest (Fig. 1B); however, exercise did not change PTP1B protein levels in the hypothalamus of control rats (Fig. 1B). To confirm the suppressive effect of exercise on hypothalamic PTP1B in obese animals, a PTP1B activity assay was performed 4 h after the acute protocol of exercise, and in fact, exercise did not change PTP1B activity in the hypothalamus of lean rats but reduced PTP1B activity in the hypothalamus of obese rats by ∼30% (Fig. 1C). In addition, we employed an acute swimming exercise in ob/ob and db/db mice to evaluate the hypothalamic PTP1B protein levels 4 h after the acute exercise. As observed in obese rats, swimming exercise reduced PTP1B protein levels in the hypothalamus of both ob/ob and db/db mice (Fig. 1D). We also monitored the protein levels of suppressor of cytokine signaling 3 (SOCS3) in the hypothalamus of Wistar rats. We observed that SOCS3 protein levels were increased in the hypothalamus of obese animals, but exercise did not change the SOCS3 levels in the hypothalamus of either lean or obese groups (Fig. 1E).

Blood lactate analysis demonstrated that the swimming exercise protocol presented moderate intensity for both lean and obese rats (Fig. 1F), because previously, a maximal lactate steady state in rats was estimated at 5.5 mmol/l (15). In addition, acute swimming exercise did not change the total body weight in lean or obese rats compared with the respective control groups at rest (Fig. 1G). Corticosterone levels were increased slightly in obese animals compared with the lean group, and the urine obtained after the exercise protocol revealed that corticosterone levels did not change after the exercise protocol compared with obese rats at rest (Fig. 1H). These data suggest that moderate exercise was sufficient to reduce PTP1B protein levels in the hypothalamus of obese rats independent of body weight changes and stressful effects.

**Exercise Disrupts the Association Between PTP1B, IRβ, IRS-1, and JAK2 in the Hypothalamus of Obese Rats**

The effects of acute swimming exercise on PTP1B interactions with early proteins involved in insulin and leptin signaling were examined. Immunoprecipitation assays demonstrated a strong interaction between PTP1B, IRβ, IRS1, and JAK2 in the hypothalamus of obese animals compared with the control group (Fig. 2, A–C). However, in the hypothalamic samples obtained 4 h after swimming exercise, we observed that exercise disrupted the PTP1B association with IRβ, IRS1, and JAK2 in the hypothalamus of obese animals compared with the control group (Fig. 2, A–C). Exercise did not change the PTP1B interaction with IRβ, IRS1, and JAK2 in the hypothalamus of lean rats (data not shown).

Thereafter, we examined the insulin and leptin sensitivity in the hypothalamus of obese rats after acute exercise. Four hours after the exercise protocol, we performed an icv injection of insulin or leptin in obese animals. We note that swimming exercise increased insulin-induced IRβ and IRS-1 tyrosine phosphorylation (Fig. 2, D and E) and leptin-induced JAK2 tyrosine phosphorylation in the hypothalamus of obese rats compared with the obese group at rest (Fig. 2F). Consistent with these results, we injected insulin and leptin in the third ventricle of obese rats immediately after the exercise protocol to evaluate the anorexigenic actions of these hormones in obese animals, as demonstrated in the experimental design (Fig. 2G).

We observed that exercise restored insulin’s and leptin’s anorexigenic actions by reducing food intake in obese animals (Fig. 2, H and I, respectively). Blood samples obtained 15 min after the exercise protocol revealed that exercise reduced insulin but did not change the leptin serum levels in obese rats compared with obese animals under resting conditions (Fig. 2, J and K, respectively). These data demonstrated that acute exercise reduced PTP1B association with IRβ, IRS1, and JAK2 in the hypothalamus of obese animals and improved insulin and leptin signaling in the hypothalamic tissue.

**IL-6 Anti-inflammatory Activity Reduces PTP1B Protein Levels in the Hypothalamus**

Because potent anti-inflammatory effects of IL-6 were observed and IL-6 was produced during exercise, we hypothesized that IL-6 is involved in the reduction of hypothalamic PTP1B protein levels in exercised rats. We performed a Western blot to evaluate IL-6 and PTP1B protein levels in obese rats at three different time-points: at rest, immediately after acute exercise, and 4 h after acute exercise. Interestingly, hypothalamic PTP1B protein levels are inversely correlated with IL-6 levels in the obese rats at rest, immediately after acute exercise, and 4 h after acute exercise (Fig. 3A).

To determine whether IL-6 is directly involved in the reduction of PTP1B protein levels in the hypothalamus, we performed a dose response experiment in vivo by injecting recombinant IL-6 into the third hypothalamic ventricle of obese rats at rest. Six hours after IL-6 injections, we observed that recombinant IL-6 (100 and 200 ng) reduced the hypothalamic PTP1B protein levels in obese rats (Fig. 3B). Furthermore, 200 ng of recombinant IL-6 infusion reduced the PTP1B activity by 33% in the hypothalamus of obese rats compared with obese animals that received saline (2 μl) icv injection (Fig. 3C).

It has been proposed that NF-κB activity controls PTP1B protein levels under inflammatory conditions (28, 44). In accord with this notion, we observed that high levels of PTP1B activity in the hypothalamus of obese animals were accompanied by the activation of inflammatory signaling compared with the lean group (Fig. 3, D and E). Thereafter, we sought to determine the role of IL-6 on upstream proteins involved in NF-κB activation. We observed that icv recombinant IL-6 injection reduced IKKβ serine phosphorylation and increased IκBα protein levels in the hypothalamic tissue of obese rats at rest (Fig. 3, D and E).

Similarly to the exercise protocol, acute recombinant IL-6 injection disrupted interactions between PTP1B, IRβ, IRS-1, and JAK2 in the hypothalamus of obese animals compared with saline injection (Fig. 3, F–H). IL-6 icv injection increased insulin-induced IRβ and IRS-1 tyrosine phosphorylation and leptin-induced JAK2 tyrosine phosphorylation in obese rats (Fig. 3, I–K). In addition, we examined whether IL-6 improves the effects of insulin or leptin on food intake in obese rats by using the experimental design demonstrated in Fig. 3L. Intracerebroventricular IL-6 injection promoted a similar effect on food intake in obese rats compared with insulin or leptin alone (Fig. 3, M and N), but recombinant IL-6 was able to increase...
the anorexigenic actions of insulin (Fig. 3M) and leptin (Fig. 3N) by reducing the food consumption in obese rats at rest. Thereafter, the accuracy of the dissection of the hypothalamus was assessed by measuring the protein levels of PTP1B and IL-6Rα in specific hypothalamic nuclei of obese rats at rest. We observed abundant protein levels of both PTP1B and IL-6Rα in the ARC compared with DMH/VMH and PVN (Fig. 4A). In addition, we evaluated PTP1B expression in the ARC of rats at
rest or 4 h after acute exercise. Exercise was able to reduce PTP1B protein levels in the ARC of obese rats (Fig. 4B). Next, we performed immunohistochemical analyses to confirm the colocalization of IL-6Rα and PTP1B in the ARC of obese rats. Double-staining confocal microscopy showed that IL-6Rα is expressed in a majority of cells in the ARC. Interestingly, we observed that most cells expressing IL-6Rα in the ARC were shown to possess PTP1B (Fig. 4C), suggesting a possible interaction between these molecules.

**Exercise Requires IL-6 Hypothalamic Action to Reduce PTP1B Protein Levels**

We hypothesized that exercise requires IL-6 action to reduce PTP1B protein levels in the hypothalamus. To test this hypothesis, we developed an experimental strategy to block the central action of IL-6 in obese animals during physical exercise. Thus, we injected an anti-IL-6 antibody or RPIS into the third hypothalamic ventricle in obese animals 30 min before the exercise protocol and during the resting period between the two bouts of exercise (Fig. 5A). Interestingly, exercise failed to reduce PTP1B protein levels (Fig. 5B) and activity (Fig. 5C) in the hypothalamus of obese rats injected with anti-IL-6 antibody compared with exercised animals that received RPIS. We also observed that anti-IL-6 icv pretreatment blunted, at least in part, the anti-inflammatory effects of exercise in the hypothalamus in obese rats (Fig. 5, D and E). Moreover, high levels of PTP1B/IRβ, PTP1B/IRS-1, and PTP1B/JAK2 interactions were found in the hypothalamic samples obtained from exercised obese rats that received anti-IL-6 antibody compared with exercised animals injected only with RPIS (Fig. 5, F–H). Finally, exercise failed to improve insulin and leptin signaling in the hypothalamus of obese animals that received anti-IL-6 antibody pretreatment (Fig. 5, I and K). It is important to note

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**Fig. 4.** PTP1B and IL-6 receptor (IL-6R) colocalization in the hypothalamus of obese rats. **A:** representative blots show PTP1B and IL-6Rα protein levels in the arcuate (ARC), dorsomedial/vetromedial (DMH/VMH), and paraventricular (PVN) nuclei of obese rats at rest. **B:** PTP1B protein levels in the ARC of obese rats at rest and 4 h after swimming exercise protocol (bottom), with 200 magnification (scale bar, 20 μm). Blue arrows indicate PTP1B-positive cells, white arrows indicate IL-6Rα-positive cells, and yellow arrows indicate PTP1B/IL-6Rα colocalization. Positive cells were quantified in 100 randomly counted cells. Data were expressed by using means ± SE. §P < 0.05 vs. DMH/VMH and PVN; ⋆P < 0.05 vs. obese group; ●P < 0.05 vs. PTP1B of obese group at rest; ▲P < 0.05 vs. merge of obese group at rest (n = 5 animals/group).
that all of these events occurred even in the presence of high levels of circulating IL-6 in exercised obese animals (Fig. 5L).

**DISCUSSION**

In the present study, we investigated the effects of acute exercise on PTP1B protein levels/activity in the hypothalamus of obese rodents. Our data demonstrated that swimming exercise was sufficient to reduce the protein levels of hypothalamic PTP1B in different models of obesity. In parallel, we observed that exercise reduced PTP1B interactions with proteins involved in the insulin- and leptin-signaling cascade, improving the anorexigenic action of
these hormones. We also determined that exercise reduced hypothalamic PTP1B protein levels through the anti-inflammatory response mediated by IL-6. These data are important since inflammation inhibition in the central nervous system is a potential target therapy to combat obesity and that most anti-inflammatory therapies have limited direct effects on neuronal inflammation. Our study provides substantial evidence that acute exercise could help to reorganize the anorexigenic signals and, therefore, aid in counteracting the energy imbalance induced by obesity-reducing hypothalamic PTP1B protein levels through the central anti-inflammatory response.

PTP1B plays a critical role in the metabolic processes of mammals (4, 38). This phosphatase negatively regulates insulin signaling by directly dephosphorylating the IR and IRS-1 (11, 17, 35) and leptin signal transduction by dephosphorylating JAK2 tyrosine phosphorylation in vivo (43). The expression of this enzyme is upregulated in different insulin- and leptin-sensitive tissues in obesity, diabetes, dyslipidemia, and metabolic syndrome (16, 38). Interestingly, PTP1B knockout mice display high insulin (11) and leptin (43) sensitivity and are resistant to developing obesity under high-fat diet treatment. Thus, PTP1B was characterized as a core inhibitor of insulin and leptin signaling in different cell types, including in hypothalamic neurons. Consistent with this notion, strategies to reduce the aberrant PTP1B activation in the hypothalamus are of great interest to improve central insulin and leptin actions and prevent or treat obesity.

Several studies were performed to elucidate the role of neuronal PTP1B in the control of food intake and energy expenditure. In an elegant study, Bence et al. (2) used a genetic approach to determine that loss of PTP1B protein levels specifically in neurons causes a marked decrease in adiposity in mice. Rodents with POMC neuron-specific deletion of the gene encoding PTP1B presented reduced fat mass, improved leptin sensitivity, and increased energy expenditure (1). Similar results were found in mice lacking PTP1B in leptin receptor-expressing neurons (39). Moreover, it was demonstrated that transient reduction of PTP1B in discrete hypothalamic nuclei by infusion of an antisense oligonucleotide in obese rats increased insulin and leptin sensitivity and resulted in decreased food intake, body weight, and adiposity after high-fat diet feeding (27). In addition to these genetic strategies, our data demonstrated that an acute physiological stimulus such as swimming exercise was sufficient to reduce PTP1B protein levels in the hypothalamic tissue of obese and diabetic rodents and the PTP1B/IRβ, PTP1B/IRS-1, and PTP1B/JAK2 interactions in obese rats. It is important to note that moderate physical exercise reduced PTP1B protein levels and restored the anorexigenic effects of insulin and leptin without changing the total body weight and adiposity.

Inflammatory signaling has been proposed as the main mechanism responsible for inducing PTP1B overexpression in obese and diabetic mice. Zabolotny et al. (44) determined that TNFα administration increased PTP1B mRNA levels in adipose tissue, liver, skeletal muscle, and the hypothalamic arcuate nucleus and PTP1B protein levels in the livers of mice. Furthermore, it was demonstrated that TNFα induced the recruitment of NF-κB subunit p65 to the PTP1B promoter in vitro and in vivo. In accord with these data, we observed a consistent activation of IKKβ and IκBα degradation in the hypothalamus of rats after high-fat diet treatment, with substantial elevation of PTP1B protein levels. Surprisingly, acute exercise disrupts inflammatory signaling and reduces PTP1B protein levels/activity, restoring insulin and leptin anorexigenic effects in obese animals. Previously, we demonstrated that long-term acute exercise improved insulin sensitivity in the skeletal muscle of obese (8, 34) and aged animals (24) by reducing IKKβ signaling and PTP1B activity. These results suggest that physical exercise suppresses PTP1B protein levels through anti-inflammatory mechanisms in different tissues.

Physical exercise elicits the production and secretion of proteins from skeletal muscle during contraction. These molecules can induce metabolic changes in other tissues or organs, such as the liver, adipose tissue, pancreas, and hypothalamus (12, 25). The anti-inflammatory response mediated by exercise is carried out by IL-10, IL-1 receptor antagonist, and soluble TNF receptors and in particular by IL-6 (26). IL-6 is most often classified as a proinflammatory cytokine, although consistent data also demonstrate that IL-6 induces an anti-inflammatory response and may attenuate the inflammation of acute-phase responses (26). Recently, it has been reported that physical exercise suppressed hyperphagia in obese animals by reducing hypothalamic IKKβ/ NF-κB and endoplasmic reticulum stress activation through IL-6 and IL-10 anti-inflammatory activity (31). The IL-6/IL-10 anti-inflammatory axis induced by exercise was observed in different models of rodents and humans (7, 22, 29, 45). In the present study, we observed that exercise diminished hypothalamic PTP1B protein levels through the anti-inflammatory effects of IL-6 in obese rats. For example, exercise failed to reduce IKKβ phosphorylation and PTP1B protein levels after the disruption of hypothalamic-specific IL-6 action by anti-IL-6 antibody injection. Conversely, icv injection of recombinant IL-6 mimicked the exercise effects in obese rats at rest, reducing the inflammatory signaling and PTP1B protein levels. Although we and others have demonstrated a consistent anti-inflammatory action mediated by IL-6 (26, 31) in the present study, we cannot exclude the possibility that IL-6 acts directly as an anorexigenic factor in the hypothalamus.

The role of hypothalamic IL-6 in the control of energy balance remains uncertain. It has been proposed that centrally acting IL-6 exerts anti-obesity effects in rodents. An interesting study investigated the impact of a loss of IL-6 on body composition in mice lacking the gene encoding IL-6 (IL-6−/− mice); those authors found that mature-onset obesity observed in IL-6−/− mice was reversed partly by IL-6 replacement and that this effect occurred through the central action of IL-6 (41). Previously, we demonstrated that the IL-6 receptor (IL6R) is expressed largely in the arcuate nucleus of the hypothalamus of rats in both orexigenic (neuropeptide Y and agouti-related protein) and anorexigenic (POMC) neurons (30, 31). In the present study, immunohistochemical analyses demonstrated the colocalization of IL-6R and PTP1B in the hypothalamus of obese rats at rest. These analyses confirmed that exercise reduced PTP1B protein expression in the arcuate nucleus of obese rats, demonstrating that the IL-6 anti-inflammatory action occurred in the same cell types where PTP1B was expressed.

Although skeletal muscle contraction delivers IL-6 into the circulation during exercise, it is not possible to affirm the source of the protein levels of IL-6 observed in the hypothalamus of exercised animals. Alternatively, several studies have
demonstrated that exercise increases IL-6 production in the brain of rodents (14) and humans (21). In addition, we determined previously that the same exercise protocol used in the present study was sufficient to increase IL-6 mRNA and protein levels in the hypothalamus of obese rats (31). In addition, it was reported that icv, but not intraperitoneal IL-6 treatment, increased energy expenditure in obese mice, demonstrating that centrally acting IL-6 exerts anti-obesity effects in rodents (41). Consistent with these ideas, we observed that icv anti-IL-6 pretreatment blocked the effects of exercise even in the presence of high levels of serum IL-6. However, the mechanism by which exercise induces IL-6 production in the brain remains unclear and requires further investigation.

Collectively, our study demonstrates that prolonged acute exercise induced an anti-inflammatory response in the hypothalamus of obese rodents, reducing PTP1B protein levels/activity, reducing PTP1B interactions with IBβ, IRS-1, and JAK2, and improving insulin and leptin signaling and sensitivity in an IL-6-dependent manner, whereas exogenous icv infusion of IL-6 reproduced these effects in obese rats. Thus, the effects of acute exercise on PTP1B protein levels seems to depend on hypothalamic IL-6 action, and this phenomenon may help to reorganize the set point of nutritional balance during the obesity state.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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