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ORIGINAL ARTICLE

Sodium tungstate regulates food intake and body weight through activation of the hypothalamic leptin pathway

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Aims: Sodium tungstate is an anti-obesity drug targeting peripheral tissues. *In vivo*, sodium tungstate reduces body weight gain and food intake through increasing energy expenditure and lipid oxidation, but it also modulates hypothalamic gene expression when orally administered, raising the possibility of a direct effect of sodium tungstate on the central nervous system.

Methods: Sodium tungstate was administered intraperitoneally (ip) to Wistar rats, and its levels were measured in cerebrospinal fluid through mass spectrometry. Body weight gain and food intake were monitored for 24 h after its administration in the third ventricle. Hypothalamic protein was obtained and subjected to western blot. *In vitro*, hypothalamic N29/4 cells were treated with 100 μM sodium tungstate or 1 nM leptin, and protein and neural gene expression were analysed.

Results: Sodium tungstate crossed the blood–brain barrier, reaching a concentration of 1.31 ± 0.07 mg/l in cerebrospinal fluid 30 min after ip injection. When centrally administered, sodium tungstate decreased body weight gain and food intake and increased the phosphorylation state of the main kinases and proteins involved in leptin signalling. *In vitro*, sodium tungstate increased the phosphorylation of janus kinase-2 (JAK2) and extracellular signal-regulated kinase-1/2 (ERK1/2), but the activation of each kinase did not depend on each other. It regulated *c-myc* gene expression through the JAK2/STAT system and *c-fos* and *AgRP* (agouti-related peptide) gene expression through the ERK1/2 pathway simultaneously and independently.

Conclusions: Sodium tungstate increased the activity of several kinases involved in the leptin signalling system in an independent way, making it a suitable and promising candidate as a leptin-mimetic compound in order to manage obesity.

Keywords: AgRP, appetite control, anti-obesity drug, drug mechanism, energy regulation, ERK1/2, hypothalamus, JAK2, leptin, neuropharmacology, NPY, obesity, obesity therapy, sodium tungstate

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Introduction

Obesity is broadly considered a global epidemic that aggravates other severe pathologies with the overall consequence of reduced quality of life and shortened lifespan [1]. This pathology develops when energy intake exceeds energy expenditure, favouring the accumulation of fat. Both processes, energy intake and expenditure, are strictly regulated by the central nervous system (CNS). The hypothalamus integrates several peripheral and central signals in order to maintain a correct energy balance [2], with leptin playing a key role in managing this balance.

Leptin is an adipocyte-secreted hormone [3] that reports the energetic status in the body to the brain and centrally regulates food intake and energy expenditure [4]. These processes are significantly altered when leptin signalling is absent due to the mutation of leptin or of leptin receptor genes, leading to severe obesity both in animals and humans [4,5].

Leptin receptor is widely expressed in hypothalamic neurons [6], found mainly in the arcuate nucleus, paraventricular nucleus and lateral area [2,7]. Thus, leptin is a negative and positive regulator of the orexigenic neuropeptides NPY (neuropeptide Y) and AgRP (agouti-related peptide) [8], and of the anorexigenic neuropeptides proopiomelanocortin and cocaine and amphetamine-regulated transcript [9,10], respectively.

Leptin binding to its receptor in the hypothalamus activates a well-established signalling cascade involving the janus kinase/signal transducer and activator of transcription (JAK/STAT) system [11]. JAK2 binds to and phosphorylates leptin receptor upon ligand binding. The phosphorylated leptin receptor provides a binding site for STAT3, allowing for its phosphorylation on Tyr705 (pSTAT3-Y^{705}) and activation through JAK2. Activated STAT3 then translocates to the nucleus and stimulates transcription [12]. In addition to phosphorylation of Tyr705, STAT3 can also be phosphorylated

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at Ser727 (pSTAT3-S^{727}), modulating its transcriptional activity, at least in certain systems [13]. This Ser-phosphorylation seems to be the consequence of the activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway after leptin binds to its receptor [14]. Recently, it has been shown that hypothalamic ERK1/2 is a mediator of leptin regulation of food intake and body weight, highlighting the role of these kinases in leptin signalling [15]. In addition, leptin activates STAT3-independent events, in part through ERK1/2. For example, the upregulation of *c-fos* gene expression, a very well-known marker of neural activity [16], depends on ERK1/2 *in vivo* and *in vitro* [16,17].

The aim of obesity management is primarily to reduce energy intake and increase energy expenditure, mainly through lifestyle modification accompanied by pharmacological treatments [18]. Sodium tungstate is a new and promising compound with anti-obesity and antidiabetic effects [19,20]. Sodium tungstate blocks adipocyte differentiation and increases mitochondrial oxygen consumption *in vitro* [21]. *In vivo*, sodium tungstate enhances thermogenesis in brown adipose tissue and lipid oxidation in white adipose tissue of diet-induced obese animals [19,22,23]. It also modulates hypothalamic gene expression [24], raising the possibility of a direct effect of sodium tungstate on the CNS, with no evidence of toxicity or other adverse side effects [25–28].

This study attempts to depict the possible central effects of sodium tungstate on obesity, on a neurophysiologic and molecular level.

Methods

Animals

Twelve-week-old male Wistar rats (Charles River Laboratories, Sta. Perpètua de la Mogoda, Spain) were housed under standard conditions of light (12 h light/dark cycles) and temperature (21 °C). Animals were fed *ad libitum* with standard chow diet (type A04 from Panlab, Barcelona, Spain). The studies received the approval of the University of Barcelona Ethics Committee, complying with current Spanish legislation.

Determination of Sodium Tungstate in Cerebrospinal Fluid

Wistar rats were intraperitoneally (ip) treated with sodium tungstate (10 mg/kg; SIGMA, St. Louis, MO, USA) or phosphate buffer solution (PBS). After 30 min, cerebrospinal fluid was obtained from *cisterna magna*, and sodium tungstate was determined by mass spectrometry in the Microbiology and Environment Laboratory, CNRS-UMR 5254 (Pau, France).

Surgery and Drug Administration

Under anaesthesia with pentobarbital, rats underwent stereotaxic surgery to implant a chronic stainless steel cannula (Plastics One, Roanoke, VA, USA). The third cerebral ventricle was targeted using the following coordinates from Bregma: −2.64 mm anterior–posterior; 0 mm lateral to mid-sagittal sinus and −10.2 mm below the skull surface. The cannula was fixed to the skull using dental cement. Rats were housed individually and were allowed 1 week for recovery before experiment. Injections in the third cerebral ventricle were performed in awake rats. Injections consisted of 4 µl of artificial cerebrospinal fluid (aCSF; NaCl 125 mM, KCl 2.5 mM, $CaCl_2 \cdot 2H_2O$ 1.26 mM, $MgCl_2 \cdot 6H_2O$ 1.18 mM dissolved in water) as a control, sodium tungstate (5 µg) or leptin (10 µg) (SIGMA), both dissolved in aCSF. Injections were performed over 1 min. Following the infusion, the guide cannula was kept in place for an additional 30 s to allow the drugs to diffuse away from the cannula tip.

Food Intake and Body Weight Gain Measurements

Food was removed 1 h before intracerebroventricular (icv) injection. Leptin or sodium tungstate were injected 4 h before the onset of darkness (16:00 hours). Just prior to the beginning of the dark period (20:00 hours), food was offered to rats. Twenty-four hours later, food intake and body weight were measured.

Tissue Collection

Intracerebroventricular injections of leptin or sodium tungstate were performed following the same protocol as before, in order to perform protein assays. Five and fifteen minutes after the injections, rats were killed by decapitation and the hypothalamus was quickly removed from each, frozen in liquid nitrogen and kept at −80 °C until use.

Cell Culture and Treatments

Embryonic mouse hypothalamic cell line N29/4 [29] was purchased from Cellutions Biosystems (Toronto, ON, Canada). Cells were grown in a 5% CO_2 environment at 37 °C and in dulbecco's modified eagle medium (DMEM) supplemented with 10% foetal bovine serum, 1 mM sodium pyruvate, 20 mM glucose and antibiotics. At confluence, cells were treated with 1 nM leptin or 100 µM sodium tungstate at the indicated time points. When kinase inhibitors were used (all of which were diluted in dimethyl sulfoxide, DMSO), cells were pretreated for 1 h with 20 µM mitogen-activated protein kinase (MEK) inhibitor U0126 (SIGMA), or for 2 h with 20 µM JAK2 inhibitor IV (Calbiochem, San Diego, CA, USA), and control cells with equal amounts of DMSO for the same period of time. Each experiment was performed at least in duplicate.

RNA Isolation and Quantitative RT-PCR Analysis

Total RNA from N29 cells was purified with Trizol solution and reverse-transcribed using First Strand cDNA synthesis, following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). Quantitative RT-PCR was performed (7900HT Fast Real-Time PCR System, Applied Biosystems, Foster City, CA, USA) using Power SYBR Green PCR Master Mix (Applied Biosystems). Cycle threshold (Ct) values were calculated using AbiPrism SDS 2.1 software (Applied Biosystems). Data were normalized to the amount of Cyclophilin A cDNA. Results were expressed as a ratio of the values obtained for untreated cells. Each experiment was performed

at least in triplicate. The primers used were as follows: *Cyclophilin A* forward 5′-GATGAGAACTTCATCCTAAAGCATACA-3′, reverse 5′-TCAGTCTTGGCAGTGCAGATAAA-3′; *c-fos* forward 5′-CGGGTTTCAACGCCGACTA-3′, reverse 5′-TTGGCACTAGAGACG GACAGA-3′; *c-myc* forward 5′-TCTCCATCCTATGTTGCGTC-3′, reverse 5′-TCCAAGTAACTCGGTCATCATCT-3′; *NPY* forward 5′-ATGCTAGGTAACAAGCGAATGG-3′, reverse 5′-TGTCGCAGAGCGGAG TAGTAT-3′; *AgRP* forward 5′-GTTCCCAGAGTTCCCAGGTCTA-3′; reverse 5′-TTCTGCTCGGTCTGCAGTTG-3′ and *STAT3* forward 5′-GCAGTTTGGAAATAACGGTGAAG-3′; reverse 5′- ACTCCGAGGTCAGA TCCATGTC-3′.

Protein Extraction and Western Blot

Protein isolation from the hypothalamus was performed in a protein lysis buffer containing HEPES 10 nM, KCl 10 nM, sucrose 240 mM, ethylenediaminetetraacetic acid (EDTA) 2 mM, dithiothreitol (DTT) 0.5 mM, phosphatase inhibitors (sodium orthovanadate 10 mM, phenylmethylsulfonyl fluoride (PMSF) 0.5 mM and sodium pyrophosphate 149 mM) and 1 × protease inhibitor cocktail (SIGMA). Tissues were homogenized and centrifuged, and supernatants were collected. Protein isolation from cell culture was performed as previously described [21]. Proteins were quantified and subjected to western blot separation in an 8% acrylamide gel and transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were probed with 1/1000 dilution of rabbit anti-Phospho-JAK2 antibody, anti-Phospho-p44/42 ERK1/2, anti-pSTAT3-Y^{705} (Cell Signalling, Beverly, MA, USA), anti-pSTAT3-S^{727} (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-β actin (SIGMA).

Statistical Analysis

Data are presented as mean ± standard error of the mean. A one-way analysis of variance followed by Tukey's multiple-comparison test was used to assess the statistical difference between groups.

Results

Sodium Tungstate Crosses the Blood–Brain Barrier

As a first step to answer whether sodium tungstate can directly affect the CNS, we assessed the sodium tungstate concentration in the cerebrospinal fluid of Wistar rats after 10 mg/kg ip administration. In these conditions, the concentration range of sodium tungstate in the cerebrospinal fluid was 1.31 ± 0.07 mg/l (3.98 µM) as soon as 30 min after ip injection. Thus, sodium tungstate, when peripherally administered, is able to cross the blood–brain barrier (BBB).

Central Administration of Sodium Tungstate Decreases Food Intake and Body Weight Gain

We assessed the direct sodium tungstate effects on the CNS by icv administration of a single 5-µg dose in the third cerebral ventricle. Since leptin acts on the CNS, decreasing food intake and body weight gain, we injected 10 µg leptin and aCSF as positive and negative controls, respectively. Twenty-four hours after injection, sodium tungstate decreased body weight gain (figure 1A) and food intake (figure 1B) to the same extent as leptin did, suggesting that sodium tungstate plays a central role.

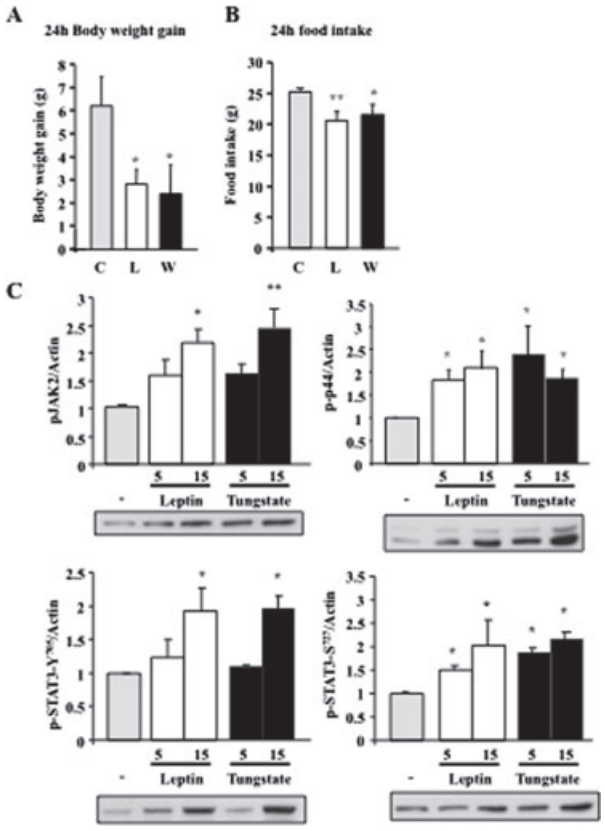

Figure 1. *In vivo* sodium tungstate effects. Five micro-grams of sodium tungstate or 10 µg of leptin was administered to rats via intracerebroventricular (icv) injection, and the body weight gain (A) and food intake (B) were measured 24 h after food presentation (n = 8–13 rats per group). (C) Leptin-signalling pathway was activated in the hypothalamus of sodium tungstate-treated animals as soon as 5 min after icv injection. Representative western blot quantification of hypothalamic proteins of treated rats (n = 8 animals per group), 5 and 15 min after icv injection, showing the changes in the phosphorylation state of janus kinase-2 (JAK2), extracellular signal-regulated kinase (ERK) and signal transducer and activator of transcription 3 (STAT3; either in tyroine 706 and serine 727). $^{*}p < 0.05$; $^{**}p < 0.01$ vs. control rats. C, control; L, leptin; W, sodium tungstate.

Sodium Tungstate Activates the Leptin-Signalling Pathway *In Vivo*

To verify whether sodium tungstate was able to activate the leptin pathway *in vivo*, we analysed the leptin cascade after an icv administration of leptin, sodium tungstate and aCSF to cannulated and awake rats, in the third ventricle. As shown in figure 1C, both leptin and sodium tungstate induced the phosphorylation of JAK2, and, consequently, of pSTAT3-Y^{705}, indicating that sodium tungstate activated the main leptin-signalling pathway, that is, the JAK/STAT system.

Leptin binding to its receptor can activate ERK1/2 [14,15]. Rats treated with either leptin or sodium tungstate activated the

hypothalamic phosphorylation of ERK1/2 and of pSTAT3-S^{727} (figure 1C), subsequently. Thus, sodium tungstate can act *in vivo* as a leptin-mimetic compound.

Characterization of the Hypothalamic Cell Line N29/4

The hypothalamic cell line N29/4 has been previously characterized and expresses different markers of neural cells [29]. When a confluence of these cells takes place, they acquire a neuronal morphology. A time course of expression showed that, at 70% confluence, they expressed hypothalamic gene markers (figure 2A), reaching maximum levels once they reached 100% of confluence. When cells were treated with leptin at different doses for 5 min, they were able to increase the phosphorylation state of STAT3, indicating that this cell type was sensitive to leptin (figure 2B). The lowest concentration of leptin was most effective in inducing pSTAT3-Y^{705}. This concentration was chosen for the rest of experiments.

Sodium Tungstate Activates the Leptin-Signalling Pathway *In Vitro*

To verify whether sodium tungstate was able to activate the signalling pathway related to leptin in N29/4 cells, we tested different concentrations. As leptin did, sodium tungstate increased the phosphorylation of pSTAT3-Y^{705}, with 100 µM being the most efficient dose (figure 2C). Thus, this dose was chosen for the following experiments.

To further explore the molecular mechanisms of sodium tungstate action, we treated the hypothalamic cell line N29/4 [29] with 100 µM sodium tungstate or 1 nM leptin at different time points. Also *in vitro*, sodium tungstate increased the phosphorylation state of all the leptin-signalling proteins, that is, pJAK2, pERK1/2, pSTAT3-Y^{705} and pSTAT3-S^{727} (figure 3A). No cooperation or synergism was observed when cells were treated with both leptin and sodium tungstate (data not shown). The maximum effect of sodium tungstate was observed as soon as 5 min after treatment, decreasing afterwards (figure 3A). Thus, all the following protein studies were performed at this time point.

Sodium Tungstate Regulates Neural Gene Expression

We assessed the mRNA expression of leptin target genes in treated N29/4 cells. Sodium tungstate raised the mRNA expression of *c-fos* and *c-myc* (a transcription factor directly regulated by STAT3) as soon as 30 min after treatment (figure 3B). Furthermore, as leptin treatment did, it decreased the mRNA expression of the *AgRP* gene. Leptin decreased *NPY* mRNA expression at 30 min, but sodium tungstate did not. These rapid changes in mRNA expression were normalized 1–2 h after leptin or sodium tungstate treatments. Longer periods of treatment (from 6 to 24 h) did not change the mRNA expression of any studied gene (data not shown).

Sodium Tungstate Acts Through Both JAK2 and ERK1/2 Signalling Pathways

In order to define the sodium tungstate molecular target, we used different inhibitors of the leptin pathway. When JAK2 was inhibited with JAK2 Inhibitor IV, pSTAT3-Y^{705} was completely blocked regardless of the treatment used, but pSTAT3-S^{727} was not affected by sodium tungstate and slightly decreased with leptin treatment (figure 4A). Furthermore, JAK2 Inhibitor IV blocked the leptin-dependent increase in *c-fos* mRNA expression, whereas sodium tungstate-treated cells still retained their ability to increase *c-fos* mRNA (figure 4B). These data suggested that part of the sodium tungstate effects were independent of the activation of the JAK2 cascade and that sodium tungstate could bypass the first steps of this pathway. Only *c-myc* mRNA expression was entirely reversed when blocking JAK2 activity, either in leptin- or sodium tungstate-treated cells, indicating that its expression was completely dependent on a full, active JAK2 system (figure 4B). Neither *AgRP* mRNA expression nor *NPY* mRNA expression was affected when JAK2 inhibitor IV was used (figure 4B).

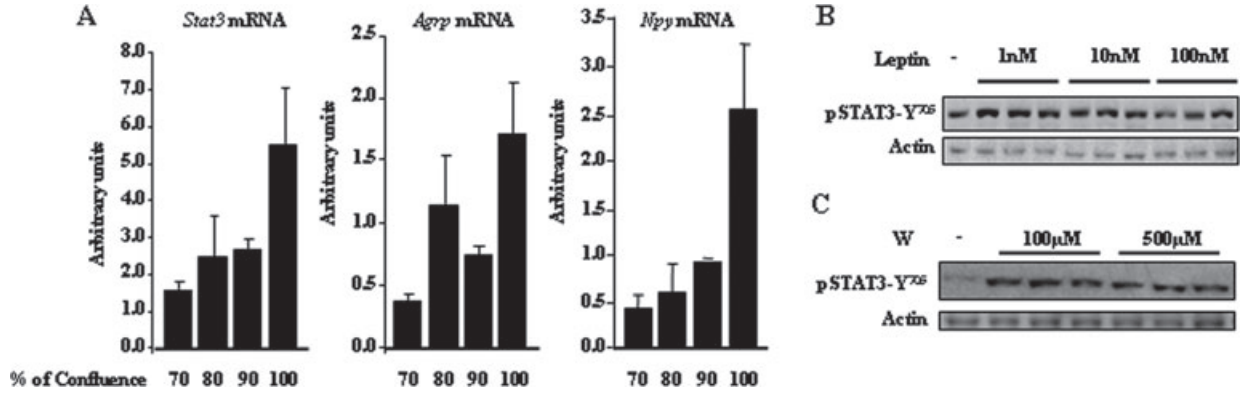

Figure 2. Hypothalamic N29/4 cells expressed genes regulated by leptin (A) such as signal transducer and activator of transcription 3 (*STAT3*), agouti-related peptide (*AgRP*) and neuropeptide Y (*NPY*) (three independent experiments). Cells were allowed to proliferate and at different percentage of confluence gene expression was assessed by real-time PCR. (B) N29/4 cells were sensitive to leptin treatment. Different doses of leptin were used and the phosphorylation state of STAT3 was assessed after 5 min of leptin treatment by western blot (representative western blot of two independent experiments). (C) N29/4 cells were also sensitive to sodium tungstate. Different doses of sodium tungstate induced an increase in pSTAT3-Y^{705} after 5 min of treatment (representative western blot of two independent experiments).

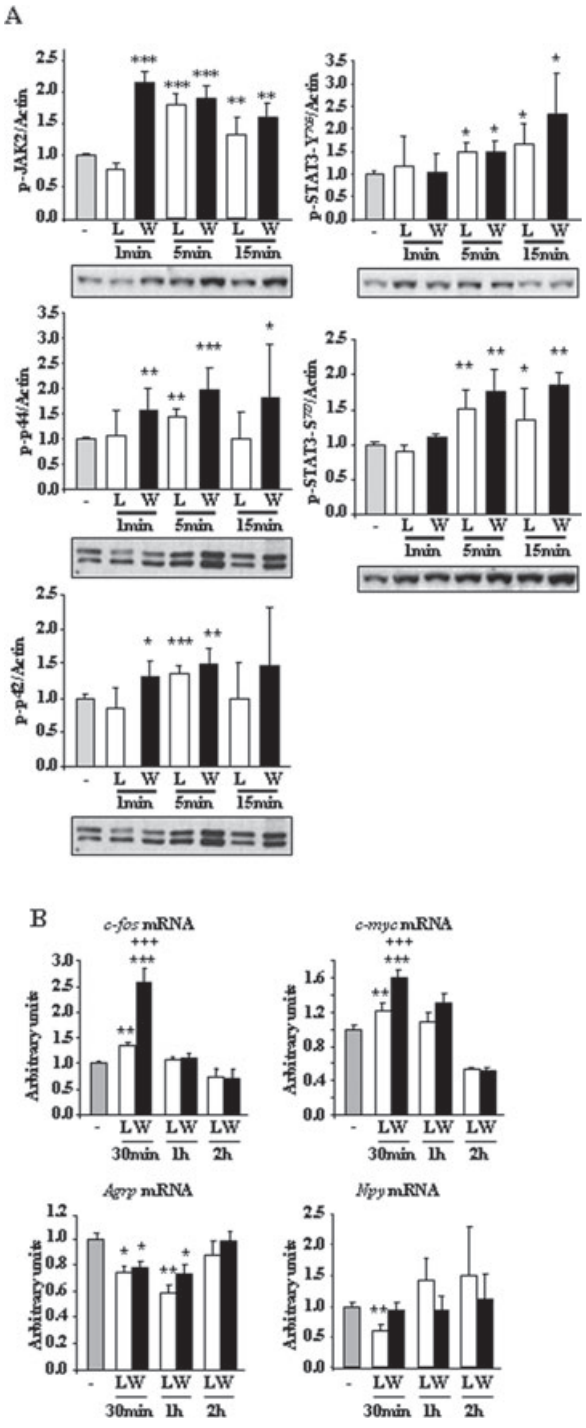

Figure 3. *In vitro* effects of sodium tungstate compared with leptin. N29/4 cells were treated at the indicated time points with 1 nM leptin or 100 µM sodium tungstate. (A) Sodium tungstate increases the phosphorylation state of janus kinase-2 (JAK2), signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinase 1/2 (ERK1/2), as soon as 1 min after treatment, although maximum effects were seen at 5 min (three independent experiments). Both forms of phosphorylated ERK, p-p44 and p-p42 were independently quantified. (B) mRNA expression of *c-fos, c-myc,* agouti-related peptide (*AgRP*) and neuropeptide Y (*NPY*) of treated cells (four independent experiments). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. untreated cells. $^{+}p < 0.05$; $^{+++}p < 0.001$ vs. leptin-treated cells. L, leptin; W, sodium tungstate.

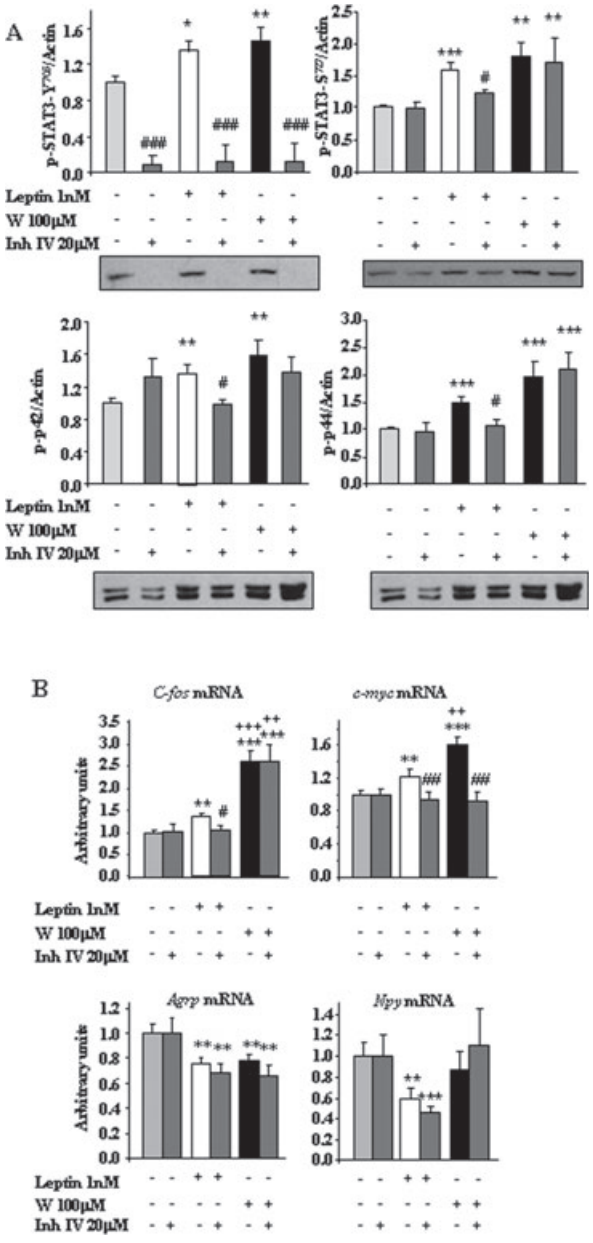

Figure 4. Sodium tungstate activates the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. N29/4 cells were treated with the JAK2 inhibitor IV (20 µM) for 1 h before being treated with leptin or sodium tungstate. (A) Phosphorylation state of extracellular signal-regulated kinase 1/2 (ERK1/2; p-p44 and p-p42) and STAT3 (three independent experiments). (B) Sodium tungstate-dependent induction of *c-myc* gene expression (30 min) was blocked with 20 µM JAK2 inhibitor IV, but not *c-fos* and agouti-related peptide (*AgRP*) mRNA expression (four independent experiments). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. untreated cells; $^{++}p < 0.01$; $^{+++}p < 0.001$ vs. leptin-treated cells; $^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$ vs. each treatment without the inhibitor. W, sodium tungstate.

When the ERK1/2 system was blocked using the MEK inhibitor U0126, pSTAT3-S^{727} decreased considerably. This inhibition did not affect the phosphorylation state of pSTAT3-Y^{705} regardless of the treatment applied (figure 5A). In this case, the rise in *c-fos* mRNA expression was completely

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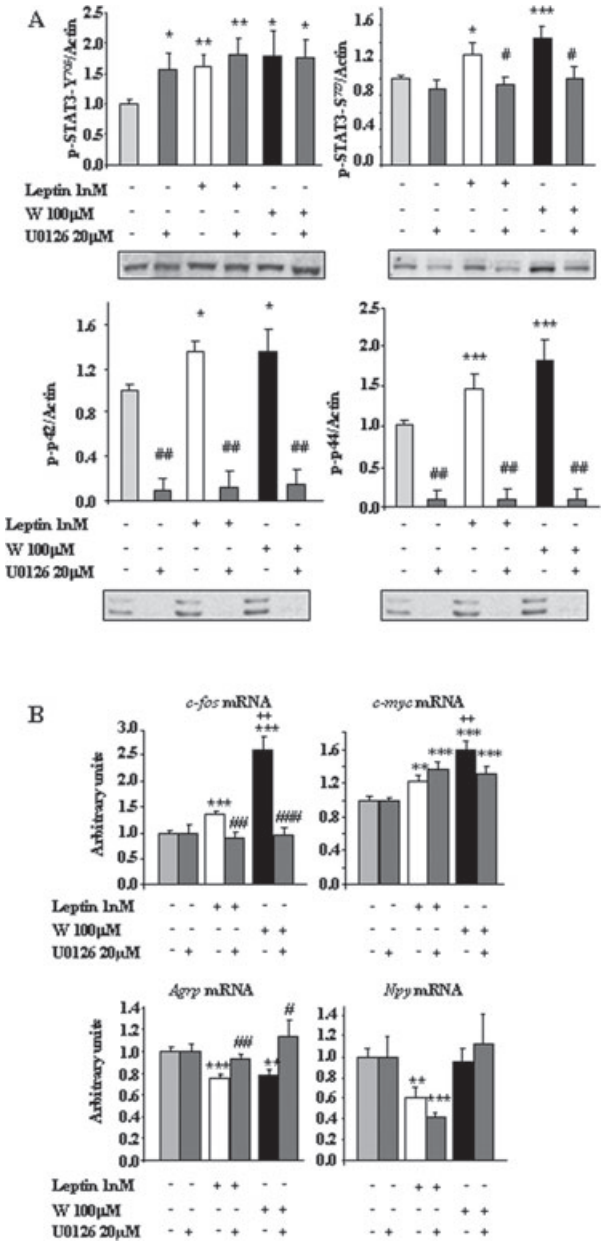

Figure 5. Sodium tungstate activates extracellular signal-regulated kinase 1/2 (ERK1/2). N29/4 cells were treated with the mitogen-activated protein kinase 1/2 (MEK1/2) inhibitor U0126 (20 µM) for 1 h before being treated with leptin or sodium tungstate. (A) Phosphorylation states of ERK1/2 (p-p44 and p-p42) and signal transducer and activator of transcription 3 (STAT3; three independent experiments). (B) Sodium tungstate-dependent induction of *c-fos* and agouti-related peptide (*AgRP*) gene expression (30 min) was blocked with 20 µM U0126 (four independent experiments). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. untreated cells; $^{++}p < 0,01$ vs. leptin-treated cells; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ vs. each treatment without the inhibitor. W, sodium tungstate.

abolished in treated cells, but not that of *c-myc* (figure 5B). The mRNA expression of *AgRP*, which decreased with either leptin or sodium tungstate treatment, reverted to normal levels when U0126 was used. *NPY* mRNA levels were not modified when ERK1/2 system was blocked (figure 5B). These results suggested that sodium tungstate could activate the ERK1/2 system in a way similar to how leptin does.

Altogether, these data suggested that, despite having similar effects on protein phosphorylation, leptin and sodium tungstate show slight differences in their ways of action, with sodium tungstate being only partially dependent on JAK2 or ERK1/2 activation.

Discussion

Previous results from our group suggested that sodium tungstate could have direct effects on the CNS, which would be responsible in part for its anti-obesity effects [24]. In this report, we summarize the neural effects of sodium tungstate *in vivo* and *in vitro*. Sodium tungstate is able to cross the BBB, probably reaches the CNS and then activates the leptin-signalling pathway of neural cells. This regulates food intake and body weight, two major components of energy homeostasis, in part through the regulation of neuropeptide gene expression (present results and Canals et al. [24]). Some anti-obesity drugs target the CNS, acting through the serotoninergic/noradrenergic [30,31] or cannabinoid systems [32]; recently, it has been shown that the combination of leptin and amylin elicits a higher effect in reducing body weight in both animals and humans through direct modulation of the leptin pathway [33].

Despite acting in a similar way, sodium tungstate exhibits differences when compared with leptin. Sodium tungstate is a phosphatase inhibitor [34], that is, it can independently act on several phosphatases, and, as a result, the phosphorylation state of the regulated kinases may increase, as well as their activities. This means that its activity would not rely on a cascade in which the signalling of one kinase depends on the action of the previous one. In fact, this is the case when leptin binds to its receptor: the activation of a signalling cascade of kinases, each of them dependent on the activity of the previous one.

This perfectly explains the differences observed in gene expression. Thus, sodium tungstate turns on JAK2 and ERK1/2, independently and simultaneously. When one of them is inhibited, the other can continue acting on gene expression. This is clearly reflected in the case of *c-fos* mRNA. This gene is directly regulated by ERK1/2 [16,17]. When JAK2 is blocked, leptin is unable to effectively activate ERK1/2 and increase *c-fos* gene expression. On the contrary, sodium tungstate, as acting on ERK1/2 independently of JAK2, can trigger *c-fos* gene expression.

The expression of *c-myc* depends exclusively on STAT3 [35,36], activated through JAK2 (pSTAT3-Y^{705}) and ERK1/2 (pSTAT3-S^{727}). The pSTAT3-Y^{705} is absolutely necessary for full STAT3 activation [13,37]. Thus, when JAK2 is inhibited, despite activating ERK1/2 and then pSTAT3-S^{727}, sodium tungstate is unable to raise *c-myc* mRNA levels.

The regulation of *AgRP* gene expression is more complex. It has been shown to be regulated by different kinases, depending on the system studied [8,38–40]. Thus, *in vivo*, it can be down-regulated through STAT3 [8] or phosphatidylinositol 3-kinase (PI3K) [38,40], and, *in vitro*, through ERK1/2 activation [39]. In the N29/4 cells, *AgRP* gene expression seems to be modulated in part through ERK1/2, but surprisingly not through JAK2.

Further analysis should be performed to exactly determine *AgRP* gene expression regulation in N29/4 cells.

NPY regulation seems to be completely different, and we were not able to clarify the way in which leptin acts on this gene. Since sodium tungstate does not affect *NPY* gene expression, we can assume that none of the kinases studied are involved in its regulation. Nevertheless, leptin may act through previously unknown mechanisms to downregulate *NPY* gene expression in N29/4 cells. Although modulated through ERK1/2 [39] in some cell types, other kinases reported to regulate *NPY* gene expression cannot be excluded, such as PI3K/AKT (protein kinase B) [8,37], protein kinase C [41] or AMP-activated protein kinase [42].

Obesity, especially in humans, is characterized by increased leptin serum levels, a situation considered to be a leptin-resistant state in which leptin is unable to decrease body weight and fat mass in order to prevent or ameliorate the pathology. Several mechanisms have been suggested as responsible for this insensitivity, with these two being most widely accepted [43]: (i) a defective transport of peripheral leptin across the BBB and (ii) an impaired response to central leptin signalling, because of the desensitization of its receptor. In our study, we show that sodium tungstate crosses the BBB and independently activates at least two kinases involved in the leptin signalling pathway. Although only part of its neural mechanism of action has been described, these special features make it a suitable and promising candidate as a leptin-mimetic compound in order to finally manage obesity.

Acknowledgements

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Conflict of Interest

M. A.-C., S. B.-B., A. B. and M. C. C. designed the study. M. A.-C., S. B.-B., R. G., M. C. C. and R. G. analysed the data. M. A.-C., S. P., M. S., G. P. and M. C. C. helped in conduct/data collection. M. A.-C., M. C. C. and R. G. wrote the manuscript. No competing interest exists for any author.

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