Nicotine Induces Negative Energy Balance Through Hypothalamic AMP-Activated Protein Kinase

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Smokers around the world commonly report increased body weight after smoking cessation as a major factor that interferes with their attempts to quit. Numerous controlled studies in both humans and rodents have reported that nicotine exerts a marked anorectic action. The effects of nicotine on energy homeostasis have been mostly pinpointed in the central nervous system, but the molecular mechanisms controlling its action are still not fully understood. The aim of this study was to investigate the effect of nicotine on hypothalamic AMP-activated protein kinase (AMPK) and its effect on energy balance. Here we demonstrate that nicotine-induced weight loss is associated with inactivation of hypothalamic AMPK, decreased orexigenic signaling in the hypothalamus, increased energy expenditure as a result of increased locomotor activity, increased thermogenesis in brown adipose tissue (BAT), and alterations in fuel substrate utilization. Conversely, nicotine withdrawal or genetic activation of hypothalamic AMPK in the ventromedial nucleus of the hypothalamus reversed nicotine-induced negative energy balance. Overall these data demonstrate that the effects of nicotine on energy balance involve specific modulation of the hypothalamic AMPK-BAT axis. These targets may be relevant for the development of new therapies for human obesity.

Nicotine, the main addictive component of tobacco, shows anorexigenic properties and promotes body weight reduction in humans and rodents (1–4). In keeping with these points, smoking cessation is associated with hyperphagia and weight gain in over 70% of people who attempt to do so. In fact, in many cases, the prospect of weight gain itself has acted as an incentive for persevering in the smoking habit (1,2,4). Therefore, it is clear that smoking leads to a state of negative energy balance and one that might be exploited for therapeutic purposes if its molecular mechanisms were better understood.

Maintaining energy balance depends on the efficiency of tightly regulated processes involved in 1) energy intake (feeding); 2) energy expenditure (EE) comprising basal metabolism, adaptive thermogenesis, and physical (locomotor) activity (LA); and 3) nutrient partitioning (5,6). The hypothalamus is a region within the central nervous system that plays a major role in the modulation of energy balance. The hypothalamic arcuate nucleus (ARC) expresses the orexigenic (feeding-promoting) neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY) and the anorexigenic precursors proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) (5,6). In addition to controlling food intake, the hypothalamus modulates EE in brown adipose tissue (BAT) (7,8), as well as glucose (9,10) and lipid metabolism in peripheral tissues (11,12) through the autonomic nervous system (7,8,12,13).

An important mediator of all of these effects is hypothalamic AMP-activated protein kinase (AMPK), a cellular gauge that controls whole-body energy balance and is activated in conditions of low energy (14,15). When activated, AMPK optimizes energy utilization by increasing energy-producing and reducing energy-wasting processes at the whole-body level. In the hypothalamus, AMPK activation drives feeding by modulating mitochondrial fatty acid oxidation and intracellular levels of reactive oxygen species. This untimely regulates neuropeptide expression in the ARC (16–18) and also suppresses EE and thyroid-induced thermogenesis in the BAT (7,8). Activated AMPK is also an important regulator of fatty acid biosynthesis, suppressing de novo lipogenesis by phosphorylation and inactivation of acetyl-CoA carboxylase (ACC) (7,8,14,15,17,18).

Whether the effects of nicotine on energy balance are mediated by modulation of hypothalamic AMPK is currently unknown. However, there are some clues that might indicate the existence of such an action. Nicotine conjugates most of the processes controlled by AMPK. For instance, chronic nicotine exposure in rats reduces the expression of NPY (19) and consistently blunts NPY-induced hyperphagia (20). Nicotine withdrawal also increases hypothalamic NPY and AgRP expression, mechanisms that contribute to increased feeding after nicotine cessation (20,21). Current evidence has demonstrated that nicotine also upregulates the expression of POMC (22) and decreases feeding through activation of POMC neurons (3). Besides its actions on hypothalamic feeding circuits, nicotine also modulates peripheral metabolism in rats, increasing BAT thermogenesis through activation of the sympathetic nervous system (SNS) and increased expression of uncoupling protein 1 (UCP1) (23,24).

Based on this evidence, we hypothesized that the effect of nicotine on energy homeostasis could be mediated at least in part by hypothalamic AMPK. The aim of this study was to investigate the effect of nicotine on hypothalamic AMPK and its impact on feeding and physiological parameters modulating energy balance such as EE, LA, BAT function, and nutrient partitioning. Our data confirm the
existence of a previously unknown link between nicotine-induced negative energy balance and hypothalamic AMPK.

**RESEARCH DESIGN AND METHODS**

**Animals.** We used adult male Sprague-Dawley rats (9–11-weeks-old; Animalario-Generals, USA). Rats were housed in a temperature-controlled (22°C) room, with a 12-h light/dark cycle (7:00 to 19:00). The experiments were performed in accordance with the *International Law on Animal Experimentation* and approved by the USC Biosafety Committee and the Ministry of Science and Innovation of Spain (PS09/01880). For all of the experimental groups and analyses, 9–16 rats were assigned to each experimental group in a random manner.

**Nicotine treatment.** Nicotine (nicotine hydrogen tartrate salt; Sigma-Aldrich, St. Louis, MO) or saline (PBS) was given subcutaneously (2 mg/kg per 12 h) in a total volume of 100 μL (25). Rats received a nicotine or vehicle injection every 12 h in both subchronic (48 h) and chronic (17 days) experiments. To validate the EE data, all rats were matched for body weight before treatment began. To control nicotine-induced respiratory depression (RD), rats were treated with nicotine and symptoms of RD were visibly controlled from 2 min before to 12 h after treatment. For the antagonism of nicotinic acetylcholine receptors, rats were treated previously (30 min) intraperitoneally with mecamylamine (mecamylamine hydrochloride, 1 mg/kg; Sigma-Aldrich) as described recently (3) and subcutaneously with nicotine during 48 h.

**Intracerebroventricular treatment.** Intracerebroventricular cannulation was stereotaxically implanted under ketamine/xylazine anesthesia in the lateral ventricle (coordinates 1.2 mm lateral to sagittal suture and 1.0 mm posterior to bregma), as described previously (8,18,26), and demonstrated to be correctly located by methylene blue staining. Rats received either a single administration of the AMPK activator AICAR (Sigma-Aldrich; 5 μg, dissolved in 5 μL of saline) or vehicle (5 μL saline) (8) at 19:00.

**Stereotoxic microinjection of adenoviral expression vectors.** Rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) under ketamine/xylazine anesthesia. The ventromedial nucleus of the hypothalamus (VMH) was targeted bilaterally using a 25-gauge needle (Hamilton, Reno, NV), and adenoviral vectors (green fluorescent protein (GFP) or AMPKα2; Virasquest, North Liberty, IA) were delivered at a rate of 200 nL/min for 5 min (1 μL/injection site) (8,18).

**Conditioned taste aversion.** Five days before the test, rats were allowed 24 h/day access to water. On the day of the experiment, rats were given access to 0.15% sodium saccharin rather than water for 30 min. Next, one experimental group was injected intraperitoneally with 0.15 mol/L lithium chloride (LiCl) in saline and simultaneously with saline (vehicle). The second experimental group was injected intraperitoneally with saline and subcutaneously with nicotine. A third experimental group was injected intraperitoneally and subcutaneously with saline. Twenty-four hours later, rats were given a 2-h access to a two-bottle choice test of 0.15% saccharin versus water. Conditioned taste aversion (CTA) was expressed as percent saccharin preference ratio ([100 × saccharin consumption + water intake] / water intake)]. A saccharin preference ratio <50% was considered as a signal of CTA (20).

**Temperature measurements.** Body temperature was recorded with a rectal probe connected to digital thermometer (BAT-12 Microprobe-Thermometer; Physitemp, Clifton, NJ). Skin temperature surrounding BAT was recorded with an infrared camera (Flir; Compact-Infrared-Thermal Imaging-Camera; FLIR, West Malling, Kent, U.K.) and analyzed with a specific software package (FLIR-Tools-Software; FLIR). We used eight animals per group, and for each animal, three or four pictures were analyzed. The skin temperature surrounding BAT for one particular animal was calculated as the average temperature recorded by analyzing those pictures.

**In situ hybridization.** Coronal brain sections (16 μm) were probed as published (8,18,26). We used specific oligonucleotides for AgRP, CART, fatty acid synthase (FAS), NPY, and POMC detection (Supplementary Table 1). Anatomical regions were analyzed using the rat brain atlas (27). Sections were scanned, and the hybridization signal was quantified by densitometry using ImageJ-1.33 (National Institutes of Health, Bethesda, MD) (8,18,26). We used between 16 and 20 sections for each animal (4 to 5 slides with sections per slide). The mean of these 16–20 values was used as the densitometry value for each animal.

**Enzymatic assays.** FAS activity was performed as described previously (8,18).

**Western blotting.** The hypothalamus, defined by the posterior margin of the optic chiasm and the anterior margin of the mammillary bodies (to the depth of ~2 mm), was dissected out and immediately homogenated on ice to preserve phosphorlated protein levels (8,18,26). Hypothalamic total protein lysates were subjected to SDS-PAGE, electrophoresed on a polyvinylidene fluoride membrane, and probed with the following antibodies: ACC, pACCSer79, AMPKα1, and AMPKα2 (Upstate Biotechnology, Temecula, CA); FAS (BD Biosciences, Franklin Lakes, NJ), pAMPK-Thr172, and phospho-liver kinase B1 (pLKB1)-Ser428 (Cell Signaling, Danvers, MA); P2PPco and Ca2+/calmodulin-dependent protein kinase kinase α and β (CamKKks and CamKKβ) (Santa Cruz Biotechnology, Santa Cruz, CA); and β-actin (Sigma-Aldrich) as described previously (8,18,26). Values were expressed relative to β-actin level.

**Real-time quantitative PCR.** We performed real-time PCR (TaqMan; Applied Biosystems, Carlsbad, CA) as described previously (8,26) using specific sets of primer probes (Supplementary Table 2). Values were expressed in relation to hypoxanthine-guanine phosphoribosyltransferase levels.

**EE, LA, respiratory quotient, and nuclear magnetic resonance analysis.** During the 48-h experiment and the 17-day experiment, rats were analyzed for EE, respiratory quotient (RQ), and LA using a calorimetric system (LabMaster; TSE Systems, Bad Homburg, Germany). This system is an open-circuit instrument that determines O2 consumption, CO2 production, and RQ (VCO2/V02) (12,28). Rats were placed for adaptation for 1 week before starting the measurements. For the measurement of body composition, we used the nuclear magnetic resonance imaging (Whole Body Composition Analyzer; EchoMRI, Houston, TX) (8,12,28).

**Statistical analysis.** Data are expressed as mean ± SEM. Statistical significance was determined by Student t tests or ANOVAs and post hoc two-tailed Bonferroni test. P < 0.05 was considered significant.

**RESULTS**

Nicotine treatment induced negative energy balance by decreasing feeding and increasing EE. Nicotine-treated rats exhibited a marked reduction in body weight (Fig. 1A) associated with reduced food intake over 24 and 48 h of treatment (Fig. 1B). We evaluated whether the anorectic effect was a genuine action of nicotine or a secondary effect of the drug. It has been reported that nicotine induces RD in neonatal and anesthetized rats (29,30). Nicotine-treated rats showed a significant short-term RD after the injection (RD-vehicle-treated; nondetected; RD-nicotine-treated; 5.0 ± 1.4 min; P < 0.001). Ten minutes after nicotine administration just 5% of the rats showed symptoms of RD; 15 min after injection those symptoms were completely abolished (Fig. 1C). These data suggest that an effect of RD on 12-h feeding patterns is unlikely.

We further evaluated whether the anorectic effect of nicotine was associated with an aversive response by performing a CTA experiment. In this type of experiment a saccharin preference ratio <50% was considered a marker of CTA, since it indicates that rats prefer to drink water after an aversive response to saccharine (26). LiCl was used as a positive control, and as expected, LiCl-treated rats drank significantly less saccharin solution (LiCl paired flavor; Fig. 1D), indicating that they had developed CTA (26). Vehicle and nicotine-treated rats had a similar saccharin preference consumption ratio, and it is noteworthy that this was >50% (Fig. 1D). Supporting the absence of stress, illness, or malaise associated with the nicotine treatments, we did not observe reduced LA (Fig. 1G), changes in skin aspect, stool consistency, or obvious abnormal behavior such as was evident in the LiCl-treated animals (data not shown) (26).

We next evaluated whether nicotine treatment induced a change in the other side of the energy balance equation. Nicotine-treated rats showed a significant increase in body temperature (Fig. 1E) associated with higher EE (Fig. 1F), area under the curve: vehicle: 100 ± 2.4; nicotine: 132 ± 9.1; P < 0.01), increased LA (Fig. 1G), and reduced RQ (Fig. 1H) compared with vehicle-treated rats. Overall, these data indicate that nicotine treatment induced negative energy balance, including decreased feeding, increased EE, and increased lipid oxidation.

Nicotine treatment reduced AgRP and NPY and increased POMC expression in the ARC and decreased hypothalamic AMPK activation. Consistent with their hypophagic state, expression of orexigenic neuropeptides...
FIG. 1. Effects of nicotine administration on energy balance. A: Body weight change. B: Food intake. C: RD. D: CTA. E: Body temperature change. F: EE; cumulative in left panel and total in right panel. G: LA, cumulative in left panel and total in right panel. H: RQ (cumulative in left panel and total in right panel) of vehicle (Veh) and nicotine-treated (Nic) rats for 48 h are shown. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle; ###P < 0.001 nicotine vs. LiCl. In the right panels of F and G, the asterisk above the lines refers to the daily (diurnal + nocturnal) EE or LA. All data are expressed as mean ± SEM.
AgRP and NPY decreased and POMC expression increased (Fig. 2A and B) in the ARC of nicotine-treated rats. No significant changes were detected in CART mRNA expression in the ARC (Fig. 2B). Nicotine treatment also reduced FAS mRNA expression (Fig. 2C) and activity (Fig. 2D) in the VMH. Recent data have demonstrated that inhibition of this enzyme in this hypothalamic nucleus induces anorexia (26). In keeping with the mRNA and activity data, FAS protein expression was reduced in the hypothalamus of nicotine-treated rats (Fig. 2E).

We next focused on other central components that could mediate the effects of nicotine on energy balance. Nicotine-treated rats showed a marked decrease in phosphorylation of hypothalamic AMPKα (pAMPKα) and its molecular target ACCα (pACCα) compared with vehicle-treated rats (Fig. 2E). The reduction in pAMPKα levels was associated with unaltered protein concentrations of the nonphosphorylated isoforms of AMPKα1 and AMPKα2. ACCα levels were reduced in the hypothalamus of nicotine-treated rats (Fig. 2E). AMPK is regulated in a tissue-specific fashion, normally opposite between brain and peripheral tissues. For example, AMPK activation promotes fatty acid oxidation and glucose uptake in skeletal muscle, whereas conversely, hepatic AMPK activity inhibits fatty acid and cholesterol synthesis. In the hypothalamus AMPK activation elicits feeding and inhibits EE (8,11,14–18). Thus, we analyzed the effect of nicotine on the AMPK pathway in liver and skeletal muscle. Our data show that 48-h nicotine treatment did not induce changes in this metabolic pathway either in liver (Supplementary Fig. 1A) or muscle (Supplementary Fig. 1B).

AMPK is activated by phosphorylation of upstream kinases. The main upstream AMPK kinases are the tumor suppressor liver kinase B1 (LKB1) and Ca2+/calmodulin-dependent protein kinase kinase α and β (CaMKKα and CaMKKβ), which phosphorylate AMPK at Thr72 (14,15). AMPK phosphorylation levels are also regulated by protein phosphatase-2Co (PP2Co) (14,15). Our data show that CaMKKβ (but not CaMKKα) protein levels were reduced and PP2Co increased in the hypothalamus of nicotine-treated rats (Fig. 2E), whereas no changes were detected in the phosphorylation levels of LKB1 (pLKB1; Fig. 2E).

**Nicotine administration increased BAT temperature and the expression of thermogenic markers in BAT.** Recent evidence has linked inhibition of hypothalamic AMPK to activation of BAT thermogenesis (7,8). To further characterize the mechanisms mediating nicotine-induced negative energy balance, we examined the effects of nicotine on BAT thermogenesis. Our data showed that nicotine-treated rats displayed a marked and significant increase in skin temperature surrounding interscapular BAT (Fig. 2F). In keeping with this effect, nicotine treatment for 48 h increased the expression of thermogenic markers such as UCP1 and peroxisome proliferator–activated receptor γ coactivator 1α and -β in BAT (Fig. 2G).

**Nicotine action on hypothalamic AMPK pathway is mediated by α3β4-nicotinic acetylcholine receptors.** Current data have demonstrated that nicotine-induced activation of hypothalamic α3β4-nicotinic acetylcholine receptors leads to activation of POMC neurons and decreased feeding (3). Therefore, we aimed to investigate whether these receptors were also mediating the actions of nicotine on AMPK. Our data show that cotreatment with mecamylamine, an antagonist of α3β4 receptors (3,30,31), blunted nicotine-induced weight loss (Fig. 3A) anorexia (Fig. 3B) and inhibition of AMPK (Fig. 3C). Overall, these results indicated that α3β4 receptors mediated the effects of nicotine on hypothalamic AMPK.

**Nicotine withdrawal restored energy balance, hypothalamic AMPK pathway, and the thermogenic program in BAT.** We next investigated the effect of nicotine withdrawal on energy balance. First, we confirmed that chronic nicotine administration for 8 days resulted in marked negative energy balance, as demonstrated by reduced body weight (Fig. 4A), decreased feeding (Fig. 4B), and lowered fat mass (Fig. 4C; without affecting lean mass). At the same time we observed increased EE (Fig. 4D; area under the curve: vehicle: 100 ± 41; nicotine: 121 ± 6; withdrawal: 103.9 ± 2.1; P < 0.05 vehicle vs. nicotine; P < 0.05 nicotine vs. withdrawal), elevated LA (Fig. 4E), and decreased RQ (Fig. 4F). Withdrawal of nicotine treatment shifted all of the above parameters toward the level of vehicle-treated rats.

In keeping with restoration of normal feeding and EE, nicotine withdrawal also elevated the decreased hypothalamic protein levels of the AMPK pathway (Fig. 5A) and reversed the elevated mRNA expression of BAT thermogenic markers (Fig. 5B). Overall, these data suggest that nicotine withdrawal restored energy balance by normalizing feeding, EE/thermogenesis, and lipid mobilization, potentially via normalization of AMPK activity.

**Activation of hypothalamic AMPK reversed nicotine-induced negative energy balance.** To establish the dependence of the actions of nicotine on AMPK, we investigated whether pharmacological or genetic activation of hypothalamic AMPK could reverse nicotine-induced effects on food intake and EE. First, intracerebroventricular administration of the AMPK activator AICAR reversed the hypophagia observed in rats treated with nicotine for 48 h (Fig. 6A). Second, we aimed to identify the hypothalamic nucleus where nicotine exerted its actions on AMPK. To elucidate the contribution of AMPK in the VMH to the effects of nicotine on neuropeptide expression and the thermogenesis in BAT, adenoviruses encoding either constitutively active AMPKα (AMPKα-CA) with GFP, or control virus expressing GFP alone (8,18), were injected stereotaxically into the VMH of nicotine-treated rats. Infection efficiency was assessed by 1) expression of GFP in the VMH, 2) increased hypothalamic protein concentration of pACCα, and 3) decreased hypothalamic malonyl-CoA levels (data not shown) (8,18). Overexpression of AMPKα-CA in the VMH was accompanied by increased body weight (Fig. 6B) and food intake (Fig. 6C) in nicotine-treated rats. AMPKα-CA administration also restored expression levels of AgRP, NPY, and POMC in the ARC (Fig. 6D and E) and mRNA expression of BAT thermogenic markers (Fig. 6F). These results are in agreement with the initial observation that nicotine impairs the function of AMPK and that this is associated with decreased orexigenic signaling (AgRP and NPY) and increased anorexigenic signaling (POMC) in the hypothalamus and reduced activation of the thermogenic program in BAT.

**DISCUSSION** This study identifies a previously unknown link between nicotine-induced negative energy balance and hypothalamic AMPK. More specifically, the inactivation of hypothalamic AMPK caused by nicotine alters the physiological parameters of energy balance. This ultimately decreases feeding behavior, stimulates EE (by triggering both physical activity and thermogenesis in BAT), and drives an increase in lipid oxidation.
FIG. 2. Effects of nicotine administration on hypothalamic neuropeptides, AMPK, and BAT thermogenic program. In situ hybridization autoradiographic images (A) and AgRP, NPY, POMC, and CART mRNA levels in the ARC (B), FAS mRNA levels in the VMH (C), hypothalamic FAS activity (D). Western blot autoradiographic images (left panel) and hypothalamic protein levels of the different proteins of the AMPK pathway (right panel) (E), and infrared thermal images (left panel) with quantification of temperature (Temp; right panel) (F) and thermogenic markers (G) in the BAT of vehicle (Veh) and nicotine-treated (Nic) rats for 48 h are shown. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle. 3V, third ventricle. PGC1, peroxisome proliferator-activated receptor γ coactivator 1. All data are expressed as mean ± SEM. (A high-quality digital representation of this figure is available in the online issue.)

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The current therapeutic arsenal to treat obesity is very limited. Besides lifestyle interventions focusing on diet, exercise, or behavioral changes (32,33) there is a paucity of available pharmacological treatments (34). For this reason, and given the urgency of the problem, we decided to focus on current situations where there is evidence of interventions leading to weight loss. One of these is smoking, such that there is no doubt that nicotine promotes weight loss and, conversely, those individuals who stop smoking gain weight. Extensive epidemiological studies have shown a strong relationship between smoking and body weight, with nonsmokers weighing more than smokers at any age (1,4,35,36). In keeping with this, nicotine replacement therapy and in particular the nicotine gum, has proved to be effective in delaying postcessation weight gain (1,37).

It is well known that nicotine, the main addictive constituent of tobacco, is the major component linking smoking to negative energy balance. Nicotine exerts anorectic actions and elicits weight loss in humans and rodents (1,2,4,35,36). In keeping with this, nicotine replacement therapy and in particular the nicotine gum, has proved to be effective in delaying postcessation weight gain (1,37). The main hypothalamic effects of nicotine are exerted on NPY (19–21,39,40) and POMC neurons (3,22) in the ARC. In light of the aforementioned findings we decided to focus our attention in the VMH, since data have involved this nucleus in the context of AMPK-driven biological effects (7,8,18).

In this study we aimed to investigate whether nicotine-induced negative energy balance was mediated by specific modulation of the hypothalamic AMPK pathway. Our findings show that nicotine decreases body weight through its combined effects of hypophagia and increased thermogenesis in BAT, in addition to driving elevated locomotor activity and increased lipid mobilization (demonstrated by a lower RQ). In keeping with former reports (3,19–22,39,40), nicotine also decreased the expression of ARC orexigenic neuropeptides such as AgRP and NPY and increased POMC expression. Our data indicate that at the same dose that caused anorexia, nicotine inhibited the hypothalamic AMPK pathway. This effect, as well as the aforementioned actions, was reversed by nicotine cessation and antagonism of α3β4 nicotinic acetylcholine receptors, which have recently been demonstrated to be involved in the action of nicotine in the hypothalamus (3). Together, these results suggest the existence of a mechanistic link between hypothalamic AMPK function and nicotine-induced changes in energy homeostasis. To specifically

![Figure 3](image-url)
FIG. 4. Effects of nicotine withdrawal on energy balance. Body weight (BW) change (A), food intake (B), fat and lean mass (C), EE (D; cumulative in left panel and total in right panel), LA (E; cumulative in left panel and total in right panel), and RQ (F; cumulative in left panel and total in right panel) of vehicle (Veh), nicotine (Nic), and nicotine withdrawal (With) rats are shown. EE, LA, and RQ data are from nicotine and nicotine withdrawal rats during the last 72 h of nicotine cessation. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle; #P < 0.05, ##P < 0.01, ###P < 0.001 nicotine vs. withdrawal; in the right panels of D and E, the symbols above the lines refer to the daily (diurnal + nocturnal) EE or LA. All data are expressed as mean ± SEM.
address this hypothesis we investigated whether activation of hypothalamic AMPK could prevent the anorexigenic and thermogenic effects of nicotine. We first showed that intracerebroventricular administration of the AMPK activator AICAR reversed nicotine-induced anorexia. Secondly, overexpression of AMPKα-CA in the VMH reversed the anorectic and thermogenic effects observed in nicotine-treated rats. The VMH is considered as a key hypothalamic nucleus controlling both sides of the energy balance equation (feeding and EE) (41–43), and recent evidence has demonstrated that AMPK in this nucleus plays a major role in its ability to do so (8,18,41). In addition, our data demonstrate that genetic overactivation of AMPK in the VMH totally reversed the nicotine-induced anorexia and weight loss. AMPK-mediated normalization of energy balance was associated with normalized mRNA levels of AgRP, NPY, and POMC in the ARC, as well as reduced or restored levels of thermogenic markers in BAT increased previously by nicotine treatment. Overall, our results indicate that nicotine impairs AMPK function in the hypothalamus and that this effect is essential for the weight loss, hypophagia, and increased BAT-mediated energy dissipation. The mechanism connecting the nicotine-induced changes in the hypothalamus that stimulates the thermogenic program in the BAT is likely mediated via activation of the α3β4-nicotinic acetylcholine receptors (3) and through the SNS. This is supported by recent evidence showing that AMPK inhibition stimulates thermogenic program in the BAT through the SNS (7,8,41) and that nicotine increases BAT thermogenesis and UCP1 expression in rats through SNS activation (7,8,23,24,38,41).

Our data support the pathophysiological relevance of the hypothalamic AMPK-BAT axis in the control of energy balance (7,8,41) and demonstrate that the well-established effects of nicotine on body weight are mediated through this mechanism. These results also raise the question of its suitability as a therapeutic target for the treatment of human obesity. Up to now this was considered unlikely because of the well-known addictive actions of nicotine. Our data, however, show that its effects on increasing EE are mediated by a specific hypothalamic nuclei, the VMH (8). VMH neurons play a minor role in the rewarding properties of nicotine (31), indicating that by targeting this nicotine-activated pathway it may be feasible to preserve nicotine’s effect on body weight without developing addiction. Further work addressing this issue is clearly merited, since it has also been demonstrated that peripheral AMPK is a suitable target for human disease using, for example, metformin and thiazolidinediones to improve insulin sensitivity (14,15). Current evidence has highlighted the importance of BAT in adult humans and its potential as an alternative to the existing strategies to solely target food intake (44–47), which have seen limited success (34). Taken together, our data support drug-induced BAT activation as a robust strategy to promote weight loss.

In summary, we show for the first time that nicotine inactivates hypothalamic AMPK and this effect mediates decreased food intake and BAT activation, likely via the SNS (23,24,38). Whether nicotine administration or smoking activates the AMPK-BAT axis in humans remains to be determined, but it is well known that EE is increased in smokers and that this effect may be mediated in part by the SNS (48). Thus, it is tempting to speculate that negative energy balance induced by cigarette smoking may be mediated by the specific activation of the hypothalamic AMPK-BAT axis, a possibility that will require further investigation.

**FIG. 5.** Effects of nicotine withdrawal hypothalamic AMPK pathway and BAT thermogenic program. Western blot autoradiographic images (left panel) and hypothalamic protein levels of the different proteins of the AMPK pathway (right panel) (A) and thermogenic markers (B) in the BAT of vehicle, nicotine, and nicotine withdrawal rats. Dividing lines show spliced bands. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle; ##P < 0.01, ###P < 0.001 nicotine vs. withdrawal. HPRT, hypoxanthine-guanine phosphoribosyltransferase; PGC1, peroxisome proliferator–activated receptor γ coactivator 1. All data are expressed as mean ± SEM.
FIG. 6. Effects of hypothalamic AMPK activation on nicotine actions on energy balance, neuropeptides, and BAT thermogenic program. A: Food intake in rats treated with nicotine and the AMPK activator AICAR. B: Daily food intake (C); in situ hybridization autoradiographic images (D); AgRP, NPY, and POMC mRNA levels in the ARC (E); and thermogenic markers (F) in the BAT of rats treated with vehicle or nicotine and stereotaxically treated with a green fluorescent protein (GFP)-expressing adenoviruses or GFP plus AMPK constitutively active (AMPKα-CA) adenoviruses are shown. *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle or vehicle GFP; #P < 0.05, ##P < 0.01, ###P < 0.001 nicotine vehicle vs. nicotine AICAR or nicotine GFP vs. nicotine AMPKα-CA. 3V, third ventricle; PGC1, peroxisome proliferator-activated receptor γ coactivator 1; HPRT, hypoxanthine-guanine phosphoribosyltransferase. All data are expressed as mean ± SEM.
Although nicotine is not considered the most harmful component of cigarette smoke, and in fact has been reported to have potentially beneficial effects on mental health (49,50), our results should not be construed as supporting continued tobacco use as a treatment option. It is noteworthy that our observation provides new insights into the molecular actions of nicotine and suggest that evaluating candidate drugs modulating the hypothalamic AMPK-BAT axis (7,8,41) may provide a more targeted approach to develop new therapeutic targets. These targets may be relevant not just for the treatment of obesity, but also for smoking cessation in humans.

ACKNOWLEDGMENTS

The research leading to these results was funded by the European Community’s Seventh Framework Programme (FP7/2007-2013) under Grant Agreements (No. 281584-the ObEStrress project [M.L.], No. 245009-the Neurofast project [R.N., C.D., and M.L.], and No. 018734-the Hepadip project [A.V.-P.]); Dr. Elmar Martens Fund (J.F.); Norwegian Council for Mental Health; ExtraStiftelsen Helse og Rehabilitering (J.F.); Xunta de Galicia (ML:10PXIB208164PR, RN:2010/14); FIS (MPS08/1880); and MICINN (RN:RYC-2008-02219 and SAF2008-07049, CD:BFU2008-02001, ML:RYC-2007-0211). CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of ISCIII. A.J.W. was supported by RyC-2007-00211). CIBER de Fisiopatología de la Obesidad y Nutrición is an initiative of ISCIII. A.J.W. was supported by the Biotechnology and Biological Sciences Research Council.

No potential conflicts of interest relevant to this article were reported.

P.B.M.M., A.J.W., and R.N. performed the in vivo experiments and analytical methods (in situ hybridization, Western blotting), EE, RQ, LA, and nuclear magnetic resonance analyses and collected the data. P.B.M.M., A.J.W., J.F., R.N., C.D., A.V.-P., and M.L. designed the experiments, analyzed and interpreted the data, and discussed, reviewed, and edited the manuscript. P.B.M.M. and M.L. developed the hypothesis. M.L. wrote the manuscript. All authors had final approval of the submitted manuscript. M.L. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank David Carling (Imperial College London) for reagents and advice.

REFERENCES

2. Grunberg NE. A neurobiological basis for nicotine withdrawal. Proc Natl Acad Sci USA 2007;104:17901–17902
22. Hur YN, Hong GH, Choi SH, Shin KH, Chun BG. High fat diet altered the mechanism of energy homeostasis induced by nicotine and withdrawal in C57BL/6 mice. Mol Cells 2010;30:218–226