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Nicotine Decreases Food Intake Through Activation of POMC Neurons

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Abstract

Smoking decreases appetite and smokers often report that they smoke to control their weight. Understanding the neurobiological mechanisms underlying the anorexic effects of smoking would facilitate the development of novel treatments to help with smoking cessation and to prevent or treat obesity. Using a combination of pharmacological, molecular genetic, electrophysiological and feeding studies, we found that activation of hypothalamic α3β4 nicotinic acetylcholine receptors (nAChRs) leads to activation of pro-opiomelanocortin (POMC) neurons. POMC neurons and subsequent activation of melanocortin 4 receptors were critical for nicotinic-induced decreases in food intake in mice. This study demonstrates that nicotine decreases food intake and bodyweight by influencing the hypothalamic melanocortin system and identifies critical molecular and synaptic mechanisms involved in nicotine-induced decreases in appetite.

Smoking remains the leading cause of preventable death in developed countries (1) and some smokers report that they smoke as a method of weight control (2, 3). Smokers have a significantly lower body mass index than non-smokers (4) and gain weight when they quit (5). These effects on body weight have been attributed to the nicotine in tobacco, because nicotine decreases feeding in animal models (6). Nicotine has some effects on peripheral energy metabolism (7–9), but little is known about potential central nervous system pathways mediating nicotine's effects on food intake and bodyweight. Identifying these pathways could help to determine a potential cholinergic modulation of appetite and weight control, but also lead to the development of novel appetite suppressants that might also aid in smoking cessation.

In a first step toward this goal, we determined that nicotine and the more selective drug cytisine (a full agonist at α3β4 nAChRs) with weaker effects at other nAChRs (10) could

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decrease weight gain over time (Fig. 1A; $F(72, 720) = 41.5$, $p < 0.0001$), body fat mass by about 15 to 20% (Fig. 1B; $F(2, 26) = 7.7$, $p < 0.001$) and food intake up to 50% (Fig. 1C; $F(1, 18) = 100.3$, $p < 0.001$) in mice, but did not affect water intake or tissue water content (Fig. S1). In addition, mecamylamine (a non-competitive nicotinic antagonist) had no effects on its own but prevented acute and chronic cytisine-induced hypophagia (all Fs <1), whereas the non-brain permeant nicotinic antagonist hexamethonium was ineffective in blocking these anorexic effects (Fig.1C), suggesting that activation of central nAChRs was essential for reduced food intake.

The pharmacological specificity of cytisine and the relatively low dose (1.5 mg/kg) needed to decrease food intake suggested that activation of central α3β4 nAChRs is essential for the anorexic effects of nicotinic compounds. We investigated this hypothesis by knocking down the expression of the β4 nAChR subunit using a neuron-specific adeno-associated virus (AAV-2) carrying specific shRNAs. Because the arcuate nucleus (ARC) is one of the most critical brain areas involved in feeding behavior and has been proposed as a potential site for the nicotinic modulation of appetite and food intake (8), we bilaterally infused 0.5 μl (1 μl/mouse) of high-titer AAV-shRNAs targeting the β4 nAChR subunit into the ventral hypothalamus (Fig. 2), and allowed at least 3 weeks for recovery (β4 KD). We first quantified the efficacy of the knockdown (~ 55 ± 8.4% in mRNA level compared to control (scrambled shRNA); Fig. S2A) and did not detect non-specific effects of the shRNA at other brain region and nAChRs (Fig. S2B, S2C, and S2D). Mice with β4KD in ARC were resistant to the effects of cytisine on food intake ($F(1, 14) = 0.01$, $p = 0.92$), whereas β2KD did not change the anorexic effects of cytisine (Fig. 2 and Fig. S3B, respectively).

The ability of β4 nAChR knockdown in the ARC to abolish the anorexic effect of cytisine suggested the involvement of the hypothalamic melanocortin system, an essential brain pathway involved in the regulation of energy balance and food intake (11), as a target for nicotinic drugs. In particular, activation of pro-opiomelanocortin (POMC) cells in the ARC decreases food intake and increases energy expenditure (12) and loss of function of the POMC gene leads to obesity in humans and animals (13, 14). We therefore hypothesized that activation of α3β4 nAChRs may induce POMC neuron firing, leading to the anorexogenic effects of nicotinic agonists. We first confirmed that the β4 nAChR is expressed in POMC neurons using laser-capture microscopy of neurons from transgenic mice expressing GFP under control of the POMC promoter (Fig. S4). We then performed double-labeling immunohistochemical experiments for Fos-like immunoreactivity (FOS-IR) and POMC (Fig. S5A) as a measure of neuronal activation in the ARC of mice treated with nicotinic drugs. There was a significant difference across treatments ($F(3,12) = 5.85$, $p = 0.0106$) and we found that chronic nicotine and cytisine, unlike mecamylamine, increased FOS-IR selectively in POMC neurons of the ARC by about 50% (Fig. 3A; nicotine: $F(1,6) = 8.60$, $p = 0.026$); cytisine ($F(1,6) = 7.68$, $p = 0.032$) with no detectable effects on other neuronal subtypes in this region (Fig. S5B), and the effect was even greater immediately after an acute injection (about 80%, $F(1,14) = 20.73$, $p < 0.001$; Fig. S5C). In contrast, there was no significant effect of mecamylamine treatment on c-fos or POMC immunoreactivity in the ARC overall (saline vs. mecamylamine, $F(3,12) = 0.619$, $p = 0.46$; c-fos: $F(3,12) = 1.39$, $p = 0.29$; POMC: $F(3,12) = 0.405$, $p = 0.75$, respectively). We then identified direct electrophysiological effects of nicotinic drugs on POMC neurons by recording excitatory post-synaptic potentials in the presence TTX (0.5μM) and the $GABA_A$ receptor antagonist picrotoxin (50μM), from identified, GFP-labeled, POMC neurons in slices from POMC-GFP transgenic mice. Application of nicotine (0.5, 5, 50 μM) for 1–2 min depolarized the membrane potential and strongly increased the spontaneous firing of POMC neurons to 289.0 ± 82.8% of baseline (Fig 3B; $F(2, 2) = 4.25$, $p < 0.05$). The nicotine effects were dose-dependent (Fig 3B, upper right panel). 0.5 and 50 μM nicotine increased the spike frequency to 173.4 ± 27.5% of baseline ($F(2, 17) = 4.1$, $p < 0.05$) and 456.3 ± 53.8% of baseline (F(2,

17) = 33.65, $p < 0.001$), respectively. Similarly, cytisine (10 μM) increased the firing rate of POMC neurons (Fig. 3C), with an average increase of action potential frequency of 186.2 ± 23.6% of baseline compared to baseline ($F(2, 29) = 11.3$, $p < 0.01$). Conversely, the nicotinic antagonist mecamylamine did not induce significant changes in the frequency of action potentials in POMC neurons (Fig. S6) and in the presence of TTX (0.5μM) and the $GABA_A$ receptor antagonist picrotoxin (50μM), neither nicotine nor cytisine had a significant effect on the frequency and amplitude of mEPSCs (data not shown).

To determine whether POMC neurons and melanocortin pathways were necessary for nicotinic-induced hypophagia, we treated POMC knockout (KO) mice with different doses of nicotine and cytisine and food intake was measured over 24 hours (Fig. 3D). POMC KO mice showed no significant difference in food intake in response to nicotine ($F(2, 16) = 0.56$, $p = 0.58$) or cytisine treatments ($F(2, 20) = 0.78$, $p = 0.46$), while cytisine-treated wild type mice showed a decrease in food intake at each of the concentrations tested (1.5 mg/kg: $F(1, 8) = 82.5$, $p < 0.001$; 3 mg/kg: $F(1, 8) = 57.1$, $p < 0.01$). Finally, we confirmed that release of melanocortin is critical for nicotinic-induced hypophagia by using AAV-shRNA delivery to knock down expression of the widely expressed melanocortin 4 receptor (Mc4r; Fig. S7) in the paraventricular nucleus (PVN), where efferents of POMC neurons are present (15). Chronic treatment induced a blunting of nicotine-induced hypophagia in mice with Mc4R knockdown in the PVN (knockdown vs. control: $F(10, 180) = 5.54$, $p < 0.0001$; Fig. 3E); a similar pattern was observed in response to acute cytisine (1.5 mg/kg) (Fig. S7; $F(1, 18) = 10.05$, $p = 0.005$).

Previous reports demonstrated that Mc4r activation by melanocortins is critical for the regulation of food intake and energy expenditure (see (16)), as confirmed by the trend for increased feeding at baseline following Mc4rKD in PVN. These data demonstrate that nicotinic drugs decrease food intake primarily through β4* nAChR-dependent activation of POMC neurons and melanocortin pathways. It has been demonstrated that POMC neurons express cholinergic markers (17) and that the naturally obese Tub/Tub strain of mice displays a decrease of perivascular cholinergic innervation in the arcuate nucleus (18). These observations underscore a possible role for acetylcholine in metabolic regulation though POMC neurons. It has also been suggested that cholinergic projections to the ventral hypothalamus could be provided by very localized groups of neurons found in the median eminence (19), a region harboring cells secreting hypophysiotropic hormones including corticotropin-releasing hormone, all known to affect metabolism. Post-synaptic modulation of POMC neurons could also occur through cholinergic projections emanating from the pedunculopontine tegmental and lateradorsal tegmental nuclei (Ch5 and Ch6), regions that can adapt rapidly to metabolic stimulation (20, 21), and that are also involved in feeding behavior (22). All these mechanisms could therefore alter activity of POMC neurons and neurotransmitter release from presynaptic terminals that could, in turn, affect energy expenditure and feeding patterns. Our results further suggest that α3β4 nAChRs are critical receptors mediating these effects. β4* agonists may therefore be useful for limiting weight gain following smoking cessation, and nicotinic drugs could also be useful to control obesity and related metabolic disorders.

One-sentence summary

Nicotinic agonists decrease food intake, body mass index and weight gain by activating the hypothalamic pro-opiomelanocortin neuron pathway through central activation of α3β4 nicotinic acetylcholine receptors*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

1. CDC. 2011. http://www.cdc.gov/chronicdisease/resources/publications/aag/osh.htm
2. Voorhees CC, Schreiber GB, Schumann BC, Biro F, Crawford PB. Early predictors of daily smoking in young women: the national heart, lung, and blood institute growth and health study. Prev Med. Jun.2002 34:616. [PubMed: 12052022]
3. Fulkerson JA, French SA. Cigarette smoking for weight loss or control among adolescents: gender and racial/ethnic differences. J Adolesc Health. Apr.2003 32:306. [PubMed: 12667735]
4. Albanes D, Jones DY, Micozzi MS, Mattson ME. Associations between smoking and body weight in the US population: analysis of NHANES II. American journal of public health. Apr.1987 77:439. [PubMed: 3493709]
5. Pistelli F, Aquilini F, Carrozzi L. Weight gain after smoking cessation. Monaldi Arch Chest Dis. Jun.2009 71:81. [PubMed: 19719041]
6. Grunberg NE, Winders SE, Popp KA. Sex differences in nicotine's effects on consummatory behavior and body weight in rats. Psychopharmacology. 1987; 91:221. [PubMed: 3107036]
7. Filozof C, Fernandez Pinilla MC, Fernandez-Cruz A. Smoking cessation and weight gain. Obes Rev. May.2004 5:95. [PubMed: 15086863]
8. Jo YH, Talmage DA, Role LW. Nicotinic receptor-mediated effects on appetite and food intake. Journal of neurobiology. Dec.2002 53:618. [PubMed: 12436425]
9. Wack JT, Rodin J. Smoking and its effects on body weight and the systems of caloric regulation. Am J Clin Nutr. Feb.1982 35:366. [PubMed: 7039293]
10. Picciotto MR, et al. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. Nature. Mar 2.1995 374:65. [PubMed: 7870173]
11. Garfield AS, Lam DD, Marston OJ, Przydzial MJ, Heisler LK. Role of central melanocortin pathways in energy homeostasis. Trends in endocrinology and metabolism. TEM. Jul.2009 20:203. [PubMed: 19541496]
12. Williams DL, Schwartz MW. The melanocortin system as a central integrator of direct and indirect controls of food intake. Am J Physiol Regul Integr Comp Physiol. Jul.2005 289:R2. [PubMed: 15956761]
13. Krude H, et al. Obesity due to proopiomelanocortin deficiency: three new cases and treatment trials with thyroid hormone and ACTH4–10. The Journal of clinical endocrinology and metabolism. Oct.2003 88:4633. [PubMed: 14557433]

14. Smart JL, Low MJ. Lack of proopiomelanocortin peptides results in obesity and defective adrenal function but normal melanocyte pigmentation in the murine C57BL/6 genetic background. Annals of the New York Academy of Sciences. Jun.2003 994:202. [PubMed: 12851317]

15. Freeman, ME.; Grattan, DR.; Houpt, TA. Neuroscience in Medicine. Michael Conn, P., editor. Humana Press; 2008. p. 301-358.

16. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocr Rev. Aug.2010 31:506. [PubMed: 20190196]

17. Meister B, et al. Hypothalamic proopiomelanocortin (POMC) neurons have a cholinergic phenotype. The European journal of neuroscience. Nov.2006 24:2731. [PubMed: 17156199]

18. Backberg M, Meister B. Abnormal cholinergic and GABAergic vascular innervation in the hypothalamic arcuate nucleus of obese tub/tub mice. Synapse. Jun 15.2004 52:245. [PubMed: 15103691]

19. Schafer MK, Eiden LE, Weihe E. Cholinergic neurons and terminal fields revealed by immunohistochemistry for the vesicular acetylcholine transporter. I. Central nervous system. Neuroscience. May.1998 84:331. [PubMed: 9539209]

20. Majkutewicz I, et al. Lesion and stimulation of the ventral tegmental area increases cholinergic activity in the rat brain. Acta Neurobiol Exp (Wars). 2010; 70:28. [PubMed: 20407484]

21. Woolf NJ. Cholinergic systems in mammalian brain and spinal cord. Progress in neurobiology. 1991; 37:475. [PubMed: 1763188]

22. Phillis JW. Acetylcholine release from the central nervous system: a 50-year retrospective. Crit Rev Neurobiol. 2005; 17:161. [PubMed: 17341198]

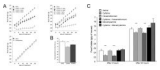

Fig.1. Change in weight, body fat content, and food intake following treatment with nicotinic drugs

Both nicotine and cytisine dose-dependently prevented weight gain in mice treated daily for 30 days, with more pronounced effects seen at higher doses (**A**, all Ps < 0.001). In contrast, the non-selective nicotinic antagonist mecamylamine had no significant effect ($F < 1$), suggesting that nAChR antagonism alone was not sufficient for the anorexic effects of nicotinic compounds. Body fat measured by MRI was also reduced in mice treated with cytisine (1.5 mg/kg; $F(1, 23) = 13.7$, $p = 0.006$) and nicotine (0.5 mg/kg; $F(1, 23) = 5.08$, $p = 0.03$; **B**). Acute injection of cytisine decreased food intake after 2 hr ($F(1, 18) = 100.3$, $p < 0.001$) and this effect was still observed after 24 hr ($F(1, 18) = 35.3$, $p < 0.001$). The effect of cytisine was not blocked by the peripherally-acting nAChR antagonist hexamethonium (2 hr: $F(1, 18) = 121.5$, $p < 0.001$); 24 hr: $F(1, 18) = 37.9$, $p < 0.001$) but was blocked by mecamylamine (Fs < 1; **C**) indicating cytisine acts at central nAChRs to exert its anorexic effects.

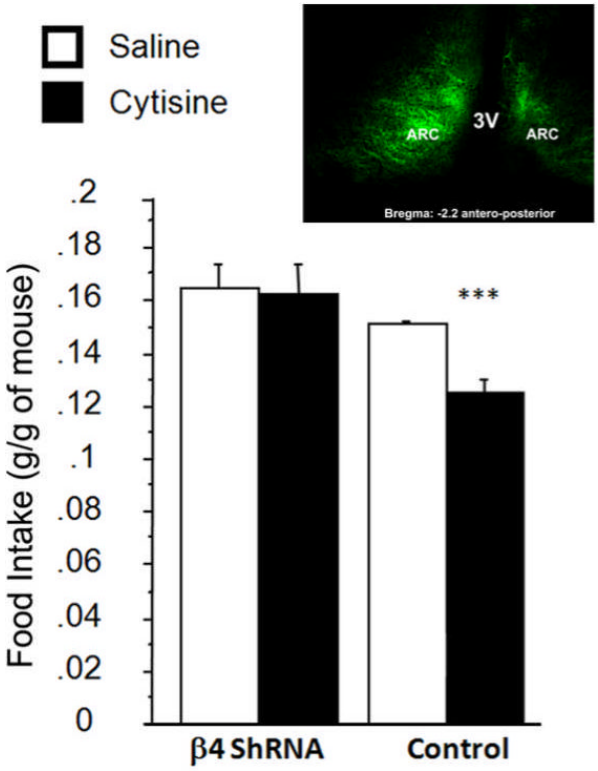

Fig.2. Knockdown of β4 nicotinic acetylcholine receptors in the ventral hypothalamus

We used AAV to deliver small hairpin RNAs to knockdown the β4 subunit in the ventral hypothalamus and sites of infusion were verified by green fluorescent protein (GFP) detection (Photograph, upper right; 3V = third ventricle; ARC = arcuate nucleus). Following recovery and knock down expression of β4 nAChRs, cytisine (1.5 mg/kg) was unable to decrease food intake contrary to the control group of animals. *** $p < 0.001$.

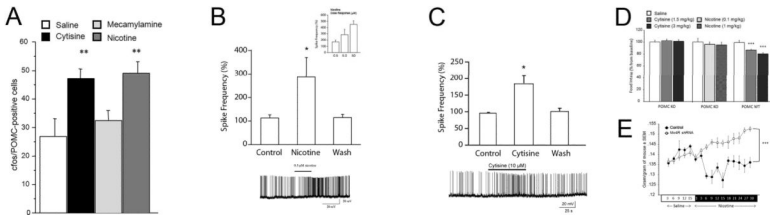

Fig. 3. POMC neuron activation by nicotinic drugs

Administration of nicotine (0.1 mg/kg) or cytisine (1.5 mg/kg), unlike mecamylamine (1 mg/kg), resulted in specific activation of POMC cells in the arcuate nucleus as measured by c-fos immunoreactivity (**A**) and electrophysiological studies further demonstrate a dose-dependent (see inset in **B**), reversible increase in the firing rate of identified POMC neurons in response to nicotine (**B**) or cytisine (**C**) application. Food intake was not significantly affected by nicotine or cytisine in POMC KO at two different doses (**D**). Furthermore, knock down of Mc4r by AAV-shRNAs in the PVN (detected by GFP fluorescence (Fig. S7)) significantly blunted the hypophagic response to nicotine over time, compared to mice injected with control virus and treated with nicotine. No signs of tolerance to nicotine were observed over 30 days, consistent with a role for MC4R signaling in the anorexic effects of nicotinic agents (**E**). ** $p < 0.01$; *** $p < 0.001$.

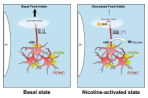

Fig. 4. Hypothetical model underlying the anorexogenic effect of nicotine in the arcuate nucleus

POMC neurons express nicotinic acetylcholine receptors (nAChRs) and therefore respond to nicotinic drugs. In the **Basal state** (i.e. in the absence of nicotine), POMC neurons project to second order neurons that decrease food intake. When nicotine reaches the arcuate nucleus (facilitated by its proximity to the third ventricle), activity of POMC neurons is increased (as measured by c-fos expression and neuronal activity measured in slices) through activation of α3β4 nAChRs and subsequent activation of MC4 receptors in the paraventricular nucleus of the hypothalamus (**Nicotine-activated state**).