Published in final edited form as:

Life Sci. 2007 July 19; 81(6): 500–508. doi:10.1016/j.lfs.2007.06.010.

Nicotine's attenuation of body weight involves the perifornical hypothalamus

Phillip R. Kramer¹, Guoqiang Guan¹, Paul J. Wellman², and Larry L. Bellinger¹

¹ Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System Health Science Center, Dallas, Texas 75246

² Behavior Neuroscience Program, Department of Psychology, Texas A&M University, College Station, TX 77843-4235

Abstract

Previously we showed that intermittent administration of nicotine (NIC) in the dark phase decreased food intake and body weight and this could be blocked when the NIC receptor antagonist mecamylamine (MEC) was infused into the fourth ventricle. Catecholaminergic neurons adjacent to the fourth ventricle contain NIC receptors and directly innervate the feeding regulatory neurons within the perifornical hypothalamus (PFH). This study explored whether NIC regulates feeding behavior by modulating catecholaminergic input to the PFH. Catecholaminergic neuronal input was ablated within the PFH by infusion of 6-hydroxydopamine hydrobromide, while bupropion was infused to protect dopaminergic neurons. After recovery of body weights to pre-surgery levels, food intake, meal size, meal number and body weight were measured after intermittent NIC injections. The results showed the PFH lesioned animals did not exhibit the typical prolonged drop in food intake, meal size and body weight normally associated with NIC administration. High performance liquid chromatography analyses demonstrated that compared to control rats, 6-OHDA administration significantly reduced PFH norepinephrine and epinephrine levels, but not dopamine levels. These results are consistent with NIC reducing food intake in part by acting through catecholaminergic neurons within or extending through the PFH.

Keywords

meal patterns; food intake; 6-hydroxydopamine; lesions; metabolism

Introduction

Previous studies show that 0.35 mg/kg of nicotine (NIC) free base administered intraperitoneal (i.p.) four times over the course of the dark phase significantly decreased food intake and body weight in both male and female rats for a period of six to seven days (Bellinger et al., 2003a; Bellinger et al., 2005). These reductions in food intake were due to a decrease in meal size. After approximately seven days, the food intake attains pre-NIC levels due to an increase in the number of meals. Even though food intake normalized in these rats, body weight continued

Corresponding Author: Phillip Kramer, PhD, Associate Professor, Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System, Health Sciences Center, 3302 Gaston Ave., Dallas, TX 75246, Email: pkramer@bcd.tamhsc.edu, Phone: Ph. 214-828-8162, Fax 214-828-8951.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

to be reduced and this attenuation lasted for up to 14 days after NIC treatment was terminated (Bellinger et al., 2003a; Bellinger et al., 2003b; Bellinger et al., 2005).

Infusion of the broad spectrum NIC receptor antagonist mecamylamine (MEC) into the fourth ventricle prevented the reduction in food intake normally caused by i.p. administration of NIC equivalent to 1.5–2 packs of cigarettes a day (Guan et al., 2004). Previous studies noted that cell bodies in the norepinephrine A2 and epinephrine C2 regions, adjacent to the fourth ventricle, contain NIC receptors (Wada et al., 1989). NIC induced high levels of neural activity, as demonstrated by c-Fos staining, in the A2 and C2 regions (Matta et al., 1993; Valentine et al., 1996). Mapping the location of these catecholaminergic neurons demonstrated neuronal tracts that course from the A2 and C2 regions through the medial forebrain bundle and that a subset of these fibers turn medially where some terminate in the PFH (Jacobowitz et al., 1974; Hokfelt et al., 1988; Palkovits et al., 1992; Pierret et al., 1994). Infusion of NIC into the fourth ventricle induced catecholamine release in the hypothalamus, an effect that was blocked by infusion of the NIC receptor antagonist MEC (Sharp et al., 1993; Matta et al., 1995; Valentine et al., 1996). The PFH area has been shown to influence meal size through the action of neuropeptides Y (Bivens et al., 1998) and has also been shown to be involved in the feeding response of orexin A (Dube et al., 1999). Thus, it is possible that a NIC-dependent pathway, acting through the PFH, could signal reduced feeding by subsequently effecting these downstream hypothalamic regions that are known to affect feeding behavior and body weight (Swanson et al., 1983; Allen et al., 1995; Champagne et al., 1998).

In this study we tested the hypothesis that NIC reduces food intake and body weight by influencing catecholaminergic input to the PFH. Accordingly, norepinephrine and epinephrine releasing neurons in the PFH were specifically ablated with 6-hydroxydopamine (6-OHDA) and food intake, meal size and meal number, as well as, body weight were measured following administration of NIC or saline.

Materials and Methods

Animals

The present studies were reviewed and approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee and complied with the principles of laboratory animal care as described in the ILAR Guide. Sprague-Dawley out-bred males (150g) rats (Harlan Industries, Houston, TX) were housed individually in a constant temperature room at 23° C environment with standard food pellets (Harland Industries, Rodent diet 7002) and water available ad libitum. All animals were maintained on a 12:12 L:D cycle (lights out at 0800 h). The rats were randomly assigned to one of four groups: sham PFH lesion and saline injected (SHAM/SALINE), n = 6, SHAM and NIC injected (SHAM/NICOTINE), n = 6, PFH lesion and SALINE injected (LESION/SALINE), n = 8 and LESION and NIC injected (LESION/NICOTINE), n = 10.

6-OHDA lesions

The PFH region at the level of the dorsomedial hypothalamic nucleus (DMN) contains many of the epinephrine satiety neurons; however, some epinephrine satiety fibers are found at the level of the PVN (Hokfelt et al., 1984). These two regions are far enough apart that they cannot be destroyed with a single infusion of 6-OHDA; therefore each LESIONED rat received two sets of bilateral infusions; one set at the level of the DMN and another set at the level of the PVN (i.e., 4 infusions per rat, see Fig. 1). To enhance the 6-OHDA lesion effect, the animals were pretreated i.p. with 50 mg/kg body weight of the monoamine oxidase inhibitor pargyline (Sigma, St Louis, MO) (Breese et al., 1973; Nobrega et al., 1986) and additionally were pretreated with 100 mg/kg (i.p.) of the dopamine re-uptake blocker bupropion HCl (Sigma) to

protect dopamine neurons from 6-OHDA destruction. Thirty minutes later, the PFH sites were bilaterally lesioned using 4 µg of 6-OHDA hydrobromide (free base, Sigma) infused in a volume of 0.2 µl per site (Sims et al., 1986). The 6-OHDA was dissolved in ice cold 0.9% saline, containing 0.02% ascorbic acid, under a nitrogen atmosphere and made fresh several times a day. SHAM animals were treated with pargyline and bupropion, but infused with 0.2 µl of vehicle per site of vehicle.

Specifically, the four infusions per rats were performed using a stereotaxic device from David Kopf Institute, Tujunga, CA equipped with an electronic micro-infusion apparatus from Stoelting Co. Wood Dale, IL, Model 780310S. First, for the bilateral PFH lesions at the level of the PVN, the 30 gauge needle was placed bilaterally at stereotaxic coordinates: anterior-posterior (AP) = 5.34 mm anterior to ear bar zero; 1.0 mm lateral from midline (ML); and at a depth of 1.7 mm below ear bar zero. Second, for the bilateral PFH lesions at the level of the DMN, the needle was placed at AP = 4.42 mm anterior to ear bar zero; ML = 1.0 mm; and height = 2.4 mm below ear bar zero (Konig et al., 1970). The 6-OHDA was infused over four minutes at each location and the injector was left in place for another four minutes before it was removed. Penicillin G (50,000 units) was administered subcutaneously daily for two days after surgery and the rats were given a wet powdered chow diet for several days. The rats were allowed a total of 14 days for recovery and then they were placed into the feeding modules.

Analysis of feeding behavior and body weight

Feeding behavior was measured using 16 sound-attenuated feeding modules equipped with photobeam computer-activated pellet feeders (Med Assoc. Inc., East Fairfield, VT) that dispense precision-made 45 mg grain based rodent chow pellets (Product No. FO 165, BioServ, Frenchtown, NJ). The chow pellets consisted of 55.3% carbohydrate, 20% protein, 4% fat and 4% fiber. The feeding modules are equipped with a photobeam that is blocked by a chow pellet and removal of the pellet causes the photobeam to signal the computer that a pellet was removed. The computer notes the time and dispenses another pellet. Thus, the number and the time the pellets were dispensed were recorded. From this information, meal patterns (i.e., meal size, meal number, meal duration) were calculated on a microcomputer using proprietary software (Bellinger et al., 1995; Bellinger et al., 1997). A meal was defined (Bellinger et al., 1986) using a ten-minute end of meal definition (i.e., no pellets consumed for ten minutes denotes the end of a meal) and the minimum meal size was set at 135 mg [i.e. 3 pellets]. Spillage is not a problem as the rats typically drop less than five pellets per day. Body weight was recorded daily.

Nicotine administration

During a three day acclimation period in the feeder, the rats were injected i.p. with 0.9% saline (1mg/kg) starting at the beginning of the dark phase and every three hours, thereafter, for a total of four injections. Following this, NIC was prepared by dissolving NIC hydrogen tartrate salt (Sigma, St. Louis, MO) in 0.9% NaCl at concentration of 0.35 mg/ml (free base). Following the acclimation period, the rats were divided and injected i.p. with NIC (total daily dose of 1.4 mg/kg, free base) or with saline in four equal volumes over the dark phase. The dose for each rat was based on the highest body weight for that rat during the injection period (Bellinger et al., 2003a). In a previous published (Bellinger et al., 2003a) study from our laboratory we completed a dose response study using saline (0), 0.75 mg/kg/day (NIC free base) and 1.40 mg/kg/day (NIC free base). Data from our previous study indicated there was a significant dose × day interaction only for the 1.4 mg/kg/day (free base) dose vs. saline and not for the 0.75 mg/kg/day (free base) dose (Bellinger et al., 2003a).

Our choice of a 1.40 mg/kg (free base) NIC dose was also based on a number of other considerations that are described below. Humans smoking one to three packs of cigarettes per

day take a total daily dose of approximately 0.3–0.5 mg/kg/day of NIC (free base) through their lungs (Benowitz et al., 1984; Perez-Stable et al., 1998). When rats self administer NIC they inject 0.18–1.38 mg/kg/day of NIC (free base) through jugular cannulas (Valentine et al., 1997). It should be recalled that 70–75% of NIC given by the i.p. route, as used in the present study, would be removed by the liver during a single pass (Svensson, 1987) and would not reach the brain. Therefore, the effective NIC dose used in the present study that would reach the brain would be about ~0.42 mg/kg/day (free base) and is in the range of that used spontaneously by both humans and rats. Moreover, a dose of NIC greater than 1.40 mg/kg (free base) would exceed the NIC levels taken in by heavy smokers (Murrin et al., 1987). Furthermore, those large doses additionally elicit stereotypic behavior (Li et al., 2000), which is atypical for the doses taken by rats and humans.

Dissection of PFH

The SHAM and LESION rats were sacrificed using CO₂ approximately six weeks post-surgery. Brains were rapidly removed and were immediately placed in an isopentane alcohol/dry ice bath. The PFH brain samples were taken by making a coronal section, cutting at AP –1.4 and –3.8 mm from Bregma (Paxinos et al., 1986) and then a 16.5 gauge needle (with a sharpened flat end) was used to take bilateral PFH tissue cores with a thickness of 2.4 mm each. Cores from the sections were centered at stereotaxic coordinates dorsal-ventral (DV) = 9.0 mm and ML = 1.0 mm. Core samples were ejected from the interior of the needle into an isopentane alcohol/dry ice bath. Tissue cores were stored at –80°C until analysis.

Quantitation of catecholamines

Tissue concentrations of epinephrine and norepinephrine were determined using high pressure liquid chromatography (Sved, 1989; Shah et al., 2005). Just prior to assay, the tissue samples were weighed to the nearest 0.001 g, transferred to a microcentrifuge tube, to which was added 0.5 ml volume of 0.1 N perchloric acid containing dihydrobenzylamine (DHBA; and an internal standard, 100 ng/ml: ESA, Chelmsford, MA)). The tissue samples were in turn disrupted by sonification for 10 sec (Model 300 Dismembrator, Fisher Scientific, Houston, TX) and then centrifuged (Model 5415: Eppendorf North America, New York, NY) in a refrigerated centrifuge at 10,000 g for 5 min. The amines were passed through a low volume 0.2 micron filter (Model 8110: Fisher Scientific, Houston, TX) and the resulting supernatant was injected onto a reversed phase C-18 column (Shiseido, 5 cm, Model # A3RE01176: ESA: Chelmsford, MA). The sample amines were eluted using a filtered and degassed mobile phase (Model MD-TM II: ESA, Chelmsford, MA) and then quantified by electrochemistry (Coulochem II: ESA Chelmsford, MA) using a microdialysis cell (Model 5014B: ESA: Chelmsford, MA). E1 was set at –75 mV, E2 was set at +250 mV and the guard potential was set at +250 mV. The mobile phase flow rate was maintained at 0.5 ml/min using a dual-piston pump (Model 582: ESA: Chelmsford, MA). Sample peak heights were compared with external standard peak heights (Sigma Chemical, St. Louis, Mo) that were processed in a manner similar to that of the samples. The DHBA internal standard was used to accommodate for changes in extraction efficiency and detector sensitivity. Sample values were expressed as amine concentrations (pg) per gram wet weight of sample tissue.

Statistics

Epinephrine, norepinephrine and dopamine concentrations were analyzed in SHAM and LESION rats using a t-test. The body weight of the SHAM and LESIONED group was compared on the day prior to the start of NIC and SALINE injections by t-test. The feeding data (food intake, meal size, meal number) from the two days prior to nicotine injection were analyzed using two-way analysis of variance (ANOVA) using the between-group factor of surgery (SHAM or LESION) with days being the repeated factor. Following NIC and SALINE

injections the feeding and body weight data were analyzed using a three-way ANOVA using the between-group factors of surgery (SHAM or LESION) and drug (SALINE or NIC) with the 14 days of injections being the repeated factor. Significant ANOVA effects were further analyzed using Duncan's multiple range test. Statistical significance was set at $p < 0.05$. Values are given as mean $\pm$ standard error of the mean (SEM).

Results

6-OHDA lesions of the PFH reduced ($P < 0.05$) the levels of epinephrine and norepinephrine in that region (Fig. 2). In contrast, there was no significant difference ($P > 0.98$) in the concentration of PFH dopamine in the SHAM (19.23 ± 2.2 pg dopamine/mg tissue) and LESION (19.37 ± 5.2 pg dopamine/mg tissue) groups. These data suggest that administration of the dopamine re-uptake blocker bupropion HCl protected the dopaminergic neurons from 6-OHDA-induced damage.

Food intake of the LESION and SHAM groups was similar prior to NIC treatment, $F(1,28) = 2.95$, not significant. NIC administration significantly reduced food intake (Fig. 3) in the SHAM/NICOTINE animals for seven days, but only for one day in the LESION/NICOTINE rats, drug effect $F(1,26) = 4.80$, $P < 0.05$. There was a significant, surgery effect, $F(1,26) = 4.78$, $P < 0.05$ and a significant surgery $\times$ day interaction, $F(13,338) = 2.03$, $P < 0.05$ and drug $\times$ day interaction, $F(13,338) = 2.47$, $P < 0.01$.

Meal size of the LESION/SALINE group was significantly reduced when compared to the SHAM/SALINE group prior to NIC administration (Fig. 4B), surgery effect $F(1,28) = 4.33$, $P < 0.05$ and after NIC administration, surgery effect $F(1,26) = 5.36$, $P < 0.05$. Starting on the first day of NIC treatment, meal size was reduced in the SHAM/NICOTINE group when compared to the SHAM/SALINE group (Fig. 4A), drug effect $F(1,26) = 6.05$, $P < 0.05$. Meal size of the SHAM/NICOTINE group remained attenuated during NIC administration (Fig. 4A). With exception of one day (Fig. 4C), administration of NIC did not reduce meal size in the LESION/NICOTINE group when it was compared to the LESION/SALINE group, surgery $\times$ drug $F(1,25) = 4.08$, $p < 0.05$. Meal size for the SHAM/NICOTINE and LESION/NICOTINE were not significantly different (Fig. 4D).

Prior to drug treatment the meal number of the LESION/SALINE groups was significantly increased when compared to the SHAM/SALINE group (Fig. 5B), surgery effect $F(1,56) = 15.83$, $P < 0.001$. After NIC injections, the meal number of the SHAM/NICOTINE group did not differ significantly from the SHAM/SALINE controls, drug effect $F(1,26) = 1.06$, not significant (Fig. 5A). NIC treatment also did not significantly alter the meal number of the LESION/NICOTINE group when compared to the LESION/SALINE group, drug $\times$ surgery interaction $F(1,26) = 0.10$, not significant (Fig. 5C). Interestingly, the meal number values of both PFH lesioned groups (LESION/SALINE and LESION/NICOTINE) were elevated compared to the non-lesioned groups (SHAM/SALINE and SHAM/NICOTINE), lesion effect $F(1,26) = 15.63$, $P < 0.001$ Fig. 5B and Fig. 5D, respectively. Both the surgery $\times$ days $F(15, 390) = 1.97$, $P < 0.02$ and the drug $\times$ days $F(15, 390) = 1.97$, $P < 0.02$ interactions were significant. The increase in food intake after seven days following NIC administration in the SHAM/NICOTINE group was likely due to a slight, but not significant, increase in meal number over time (Fig. 5A).

The above data show that 6-OHDA damage alone decreased meal size (Fig. 4B), but also resulted in an increase in meal number (Fig. 5B) causing food intake of the LESION/SALINE group to be similar to the SHAM/SALINE group (Fig. 3). NIC administration persistently lowered meal size in the SHAM/NICOTINE animals (Fig. 4A), but with the exception of one

day did not significantly reduce meal size in the LESION/NICOTINE group (Fig. 4D), possibly indicating that 6-OHDA lesioning caused a minimum meal size to be reached.

Body weight of the SHAM (265.4 ± 3.55 g) and LESION (260.9 ± 5.05 g) did not differ the day before NIC administration. Body weight gain was significantly reduced in both SHAM and LESION animals for at least 9 of the 14 days during NIC injection (Fig. 6), drug effect $F(1,26) = 31.85$, $P < 0.001$ and drug $\times$ days interaction $F(13, 338) = 4.43$, $P < 0.001$. However, this weight loss was significantly attenuated in the LESION/NICOTINE group when compared to the SHAM/NICOTINE group, lesion effect $F(1,26) = 4.36$, $P < 0.05$. After 6 days of NIC treatment the body weight in the LESION/NICOTINE group was significantly higher than the SHAM/NICOTINE group (Fig. 6).

Discussion

6-OHDA administration significantly reduced the levels of norepinephrine and epinephrine, but not of dopamine, within the PFH in comparison to non-lesioned animals. Lesions of the PFH attenuated the typical drop in food intake, meal size and body weight associated with NIC administration. Previous studies have shown that NIC administered in the dark phase reduces food intake by first reducing meal size (Bellinger et al., 2003a; Bellinger et al., 2005). Later food intake returns to normal when the rat increases its meal number to compensate for the continued NIC-induced reduction in meal size (Bellinger et al., 2003a; Bellinger et al., 2005).

In the present study, rats with PFH lesions had reduced meal size prior to NIC administration as compared to the SHAM/SALINE group. This decrease in meal size was concomitant with a higher number of meals that resulted in a normal daily food intake. Therefore, the PFH lesion alone affected the meal patterns. Since PFH area has been shown to influence meal size (Bivens et al., 1998) it is suggested, but not proven, that the PFH lesion may have first caused a permanent decrease in meal size followed by the rat compensating by increasing meal frequency to maintain a normal daily food intake.

Administration of NIC reduced meal size in the non-lesioned rats group, but NIC did not significantly reduce the meal size of the PFH lesioned rats. Because of the NIC induced decrease in meal size food intake was suppressed in the non-lesioned NIC treated group. Prior to giving NIC meal size was already attenuated in the PFH lesioned group and NIC administration did not significantly reduce the already attenuated meal size. Because meal size was reduced in the PFH lesioned NIC treated group the normal food intake suppression caused by NIC administration was attenuated in the PFH lesioned group. The data are consistent with the idea that NIC may alter meal size, in part, through a pathway that involves the PFH.

As noted above, prior to NIC administration, the food intake of the PFH lesioned animals was normal when compared to control animals. While the PFH lesions caused a reduction in the animals' meal size, to potentially a minimum level (i.e., floor effect), the rats used a strategy of increasing their meal number to normalize food intake. Animals normally defend their body weight by modulating feeding behavior or metabolism to attenuate the loss of stored energy (Keesey et al., 1984; Keesey et al., 1986; Keesey et al., 1997). Thus changes in meal size and meal number may be compensatory mechanisms to defend body weight.

After NIC, body weight gain was initially reduced in both the SHAM/NICOTINE and LESION/NICOTINE groups. Previous reports suggest that metabolism is not substantially increased by NIC to the degree that would induce body weight loss (Davis et al., 1971; Yoshida et al., 1990; Perez-Stable et al., 1998; Chen et al., 2007) supporting the idea that weight changes induced by NIC result from changes in food intake. In the SHAM/NICOTINE group NIC significantly reducing food intake for several days and this resulted in a decrease in body weight gain. In the LESION/NICOTINE food intake was significantly reduced for only one day and

this caused the animals to lose body weight. In both the SHAM/NICOTINE and LESION/NICOTINE groups body weight remained significantly attenuated compared to the control groups even after their food intake returned to normal. Importantly, weight loss was attenuated in the LESIONED/NICOTINE group, because of the inability of NIC to significantly reduce the already decreased meal size in that group. This was reflected by the food intake of the LESION/NICOTINE group returning to normal several days sooner than the SHAM/NICOTINE group. Because of this greater food intake in the LESIONED/NICOTINE group their body weight, while attenuated by NIC treatment, was significantly greater than the SHAM/NICOTINE group by the sixth day of treatment.

Under normal conditions one would expect the rats to defend their body weight and we would expect a compensatory increase in food intake (i.e., hyperphagia) above that measured in the saline treated group, such that, the body weight of NIC treated animals would catch up to saline treated group. This was not observed, in both groups food intake just returned to normal levels resulting in both NIC treated groups not obtaining the body weight of the saline treated groups. These results are consistent with NIC reducing food intake and subsequently body weight by acting, in part, through the PFH to reduce meal size. NIC could reduce meal size (SHAM/SALINE versus SHAM/NICOTINE) through a separate pathway from the pathway that modulates meal size in the PFH LESION animals (SHAM/SALINE versus LESION/SALINE), but both pathways would need to have a minimum meal size of approximately 1 gram a day as indicated by our results.

Infusion of norepinephrine directly into the PVN and the PFH elicits a feeding response (Leibowitz, 1978) by binding to α receptors (Margules, 1970; Wellman et al., 1993) to disinhibit satiety signals (Ritter et al., 1975; Leibowitz, 1988). Hypothalamic $\alpha 2$ receptor levels increase in rats at the onset of the dark phase, a period of increased feeding (Jhanwar-Uniyal et al., 1986; Morien et al., 1995). NIC has been shown to stimulate norepinephrine release in the hypothalamus (Sperlagh et al., 1998; O'Leary et al., 2006) thus, NIC acting through the PVN should increase norepinephrine levels and increase feeding rather than decreasing feeding as we observed. Zarrindast et. al., (1997) reported no effect of systemic administration of noradrenergic antagonists on NIC induced hypophagia although, the antagonist was given systemically and may not have reached the PFH. Moreover, the Zarrindast et al., 1997 study was a short-term study of NIC effects in contrast to a long-term analysis of NIC action after catecholamine ablation.

Release of another catecholamine, epinephrine, is also stimulated by NIC (Diana et al., 1990). Epinephrine has been shown to have suppressive effects on feeding, in contrast to norepinephrine that stimulates feeding behavior (Leibowitz et al., 1986; Wellman, 1992). Moreover, reduced food intake and body weight can be induced by central infusion of epinephrine into the PFH (Leibowitz et al., 1979), suggesting a key role for epinephrine in inhibiting feeding. The epinephrine component of the central tegmental tract contains axonal fibers that innervate the PFH and mediate catecholaminergic suppression of feeding behavior (Leibowitz et al., 1980). Epinephrine release in the PFH inhibits feeding through binding to $\beta 2$ -adrenoceptors (Lands et al., 1967; Wellman, 1992). It is possible that NIC induced epinephrine release in the PFH would reduce feeding and ablation of the PFH epinephrine input would attenuate NIC effects on feeding as observed in our studies.

Since the PFH lesions in this study greatly attenuated, but did not completely eliminate, NIC suppression of food intake and body weight gain other central pathways may be involved. Serotonergic and dopaminergic regulation of satiety and feeding control are alternative avenues for NIC's effects on hypophagia. The ventromedial nucleus and lateral hypothalamic area show increased dopamine and serotonin levels after NIC infusion (Yang et al., 1999; Miyata et al., 1999; Meguid et al., 2000; Ramos et al., 2004). Up-regulation of the dopamine and serotonin

pathways upon NIC infusion suggest a function of these pathways in NIC induced hypophagia (Yang et al., 1999; Miyata et al., 1999; Meguid et al., 2000; Ramos et al., 2004). Our laboratory findings do not rule out effects that are occurring due to dopamine and serotonin pathways, but we did not reduce dopamine input into the PFH nor are serotonergic neurons effected by 6-OHDA thus, these pathways were not tested in these studies.

Conclusion

NIC caused a reduction in food intake leading to reduced body weight. 6-OHDA induced damage to norepinephrine and epinephrine inputs into the PFH attenuated the action of NIC on both food intake and body weight. Prior to drug treatment base line meal size was reduced and meal number was increased by damage to the PFH. NIC no longer significantly reduced meal size in the PFH lesioned animals consistent with the view that NIC modulates food intake primarily through modulation of meal size.

Acknowledgments

We would like to thank Connie Tillberg, Priscilla Gillaspie and Gerald Hill for their expertise and assistance on this work. This work was supported by Tobacco Settlement Proceeds (TEF 2000-22) and a Texas A&M Health Science Center Research Development Grant.

References

- Allen GV, Cechetto DF. Neurotensin in the lateral hypothalamic area: origin and function. *Neuroscience* 1995;69(2):533–544. [PubMed: 8552247]
- Bellinger L, Cepeda-Benito A, Wellman PJ. Meal patterns in male rats during and after intermittent nicotine administration. *Pharmacology Biochemistry and Behavior* 2003a;74(2):495–504.
- Bellinger LL, Fabia R, Husberg BS. Meal patterns prior to and following liver transplantation in rats. *Physiology and Behavior* 1997;62(3):525–529. [PubMed: 9272659]
- Bellinger LL, Mendel VE. Blood profile and balance study of rats given the putative anorectic agent satietin. *American Journal of Physiology* 1995;268(1 Pt 2):R1–R7. [PubMed: 7840308]
- Bellinger LL, Mendel VE, Bernardis LL, Castonguay TW. Meal patterns of rats with dorsomedial hypothalamic nuclei lesions or sham operations. *Physiology and Behavior* 1986;36(4):693–698. [PubMed: 3520611]
- Bellinger LL, Wellman PJ, Cepeda-Benito A, Kramer PR, Guan G, Tillberg CM, Gillaspie PR, Hill EG. Meal patterns in female rats during and after intermittent nicotine administration. *Pharmacology Biochemistry and Behavior* 2005;80(3):437–444.
- Bellinger L, Cepeda-Benito A, Bullard RL, Wellman PJ. Effect of i.c.v. infusion of the [alpha]-MSH agonist MTII on meal patterns in male rats following nicotine withdrawal. *Life Sciences* 2003b;73(14):1861–1872. [PubMed: 12888124]
- Benowitz NL, Jacob P III. Daily intake of nicotine during cigarette smoking. *Clinical Pharmacology and Therapeutics* 1984;35(4):499–504. [PubMed: 6705448]
- Bivens CL, Thomas WJ, Stanley BG. Similar feeding patterns are induced by perifornical neuropeptide Y injection and by food deprivation. *Brain Research* 1998;782(1–2):271–280. [PubMed: 9519273]
- Breese GR, Smith RD, Cooper BR, Grant LD. Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. *Pharmacology Biochemistry and Behavior* 1973;1(3):319–328.
- Champagne D, Beaulieu J, Drolet G. CRFergic innervation of the paraventricular nucleus of the rat hypothalamus: a tract-tracing study. *Journal of Neuroendocrinology* 1998;10(2):119–131. [PubMed: 9535058]
- Chen H, Hansen MJ, Jones JE, Vlahos R, Bozinovski S, Anderson GP, Morris MJ. Regulation of hypothalamic NPY by diet and smoking. *Peptides* 2007;28(2):384–389. [PubMed: 17207894]
- Davis JR, Keesey RE. Norepinephrine-induced eating: its hypothalamic locus and an alternate interpretation of action. *Journal of Comparative Physiological Psychology* 1971;77(3):394–402.

- Diana JN, Qian SF, Heesch CM, Barron KW, Chien CY. Nicotine-induced skeletal muscle vasodilation is mediated by release of epinephrine from nerve terminals. *American Journal of Physiology* 1990;259(6 Pt 2):H1718–H1729.
- Dube MG, Kalra SP, Kalra PS. Food intake elicited by central administration of orexins/hypocretins; identification of hypothalamic sites of action. *Brain Research* 1999;842(2):473–477. [PubMed: 10526145]
- Guan G, Kramer SF, Bellinger LL, Wellman PJ, Kramer PR. Intermittent nicotine administration modulates food intake in rats by acting on nicotine receptors localized to the brainstem. *Life Science* 2004;74(22):2725–2737.
- Hokfelt, T.; Foster, GA.; Johansson, O.; Schultzberg, M.; Holets, V.; Ju, G.; Skagerberg, G.; Palkovits, M.; Skirboll, L.; Stolk, JM.; U'Prichard, DC.; Goldstein, M. Central phenylethanolamine-N-methyltransferase-immunoreactive neurons: distribution, projections, fine structure, ontogeny, and coexisting peptides. In: Stolk, JM.; U'Prichard, DC.; Fuxe, K., editors. *Morphology of Brain Epinephrine Systems*. Oxford University Press; New York: 1988. p. 10-32.
- Hokfelt, T.; Johansson, O.; Goldstein, M. Central catecholamine neurons as revealed by immunohistochemistry with special reference to adrenalin neurons. In: Bjorklund, A.; Hokfelt, T., editors. *Handbook of Chemical Neuroanatomy*. Elsevier Science Publishers B. V; Amsterdam: 1984. p. 157-276.
- Jacobowitz DM, Palkovits M. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). *Journal of Comparative Neurology* 1974;157(1):13–28. [PubMed: 4855220]
- Jhanwar-Uniyal M, Roland CR, Leibowitz SF. Diurnal rhythm of alpha 2-noradrenergic receptors in the paraventricular nucleus and other brain areas: relation to circulating corticosterone and feeding behavior. *Life Science* 1986;38(5):473–482.
- Keesey RE, Corbett SW. Metabolic defense of the body weight set-point. *Research Publications: Association of Research on Nervous and Mental Disorders* 1984;62:87–96.
- Keesey RE, Hirvonen MD. Body weight set-points: determination and adjustment. *Journal of Nutrition* 1997;127(9):1875S–1883S. [PubMed: 9278574]
- Keesey RE, Powley TL. The regulation of body weight. *Annual Review of Psychology* 1986;37:109–133.
- Konig, JFR.; Klippel, RA. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*. Robert E. Krieger Publishing Co. Inc; Huntington, New York: 1970.
- Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG Jr. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 1967;214(88):597–598. [PubMed: 6036174]
- Leibowitz SF. Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. *Pharmacology Biochemistry and Behavior* 1978;8(2):163–175.
- Leibowitz SF. Hypothalamic paraventricular nucleus: interaction between alpha 2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. *Neuroscience and Biobehavioral Reviews* 1988;12(2):101–109.
- Leibowitz SF, Brown LL. Histochemical and pharmacological analysis of catecholaminergic projections to the perifornical hypothalamus in relation to feeding inhibition. *Brain Research* 1980;201(2):315–345. [PubMed: 7417845]
- Leibowitz SF, Rossakis C. Mapping study of brain dopamine- and epinephrine-sensitive sites which cause feeding suppression in the rat. *Brain Research* 1979;172(1):101–113. [PubMed: 466454]
- Leibowitz SF, Shor-Posner G, Maclow C, Grinker JA. Amphetamine: effects on meal patterns and macronutrient selection. *Brain Research Bulletin* 1986;17(5):681–689. [PubMed: 3801930]
- Li MD, Kane JK, Parker SL, McAllen K, Matta SG, Sharp BM. Nicotine administration enhances NPY expression in the rat hypothalamus. *Brain Research* 2000;867(1–2):157–164. [PubMed: 10837809]
- Margules DL. Alpha-adrenergic receptors in hypothalamus for the suppression of feeding behavior by satiety. *Journal of Comparative Physiological Psychology* 1970;73(1):1–12.
- Matta SG, Foster CA, Sharp BM. Nicotine stimulates the expression of cFos protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions. *Endocrinology* 1993;132(5):2149–2156. [PubMed: 8386611]

- Matta SG, McCoy JG, Foster CA, Sharp BM. Nicotinic agonists administered into the fourth ventricle stimulate norepinephrine secretion in the hypothalamic paraventricular nucleus: an in vivo microdialysis study. *Neuroendocrinology* 1995;61(4):383–392. [PubMed: 7783852]
- Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A, Rossi-Fanelli F. Hypothalamic dopamine and serotonin in the regulation of food intake. *Nutrition* 2000;16(10):843–857. [PubMed: 11054589]
- Miyata G, Meguid MM, Fetissov SO, Torelli GF, Kim HJ. Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. *Surgery* 1999;126(2):255–263. [PubMed: 10455892]
- Morien A, Wellman PJ, Fojt J. Diurnal rhythms of paraventricular hypothalamic norepinephrine and food intake in rats. *Pharmacology Biochemistry and Behavior* 1995;52(1):169–174.
- Murrin LC, Ferrer JR, Zeng WY, Haley NJ. Nicotine administration to rats: methodological considerations. *Life Science* 1987;40(17):1699–1708.
- Nobrega JN, Coscina DV. Dopamine-norepinephrine interactions in the development of hyperphagia and obesity following medial hypothalamic lesions. *Pharmacology Biochemistry and Behavior* 1986;25(2):401–409.
- O'Leary KT, Leslie FM. Enhanced nicotinic acetylcholine receptor-mediated [3H]norepinephrine release from neonatal rat hypothalamus. *Neuropharmacology* 2006;50(1):81–88. [PubMed: 16213535]
- Palkovits M, Mezey E, Skirboll LR, Hokfelt T. Adrenergic projections from the lower brainstem to the hypothalamic paraventricular nucleus, the lateral hypothalamic area and the central nucleus of the amygdala in rats. *Journal of Chemical Neuroanatomy* 1992;5(5):407–415. [PubMed: 1418754]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Vol. 2. Orlando, Florida: Academic Press Inc.; 1986.
- Perez-Stable EJ, Herrera B, Jacob P III, Benowitz NL. Nicotine metabolism and intake in black and white smokers. *Journal of the American Medical Association* 1998;280(2):152–156. [PubMed: 9669788]
- Pierret P, Christolomme A, Bosler O, Perrin J, Orsini JC. Catecholaminergic projections from the solitary tract nucleus to the perifornical hypothalamus. *Brain Research Bulletin* 1994;35(2):153–159. [PubMed: 7525026]
- Ramos EJ, Meguid MM, Zhang L, Miyata G, Fetissov SO, Chen C, Suzuki S, Laviano A. Nicotine infusion into rat ventromedial nuclei and effects on monoaminergic system. *Neuroreport* 2004;15(14):2293–2297. [PubMed: 15371752]
- Ritter RC, Epstein AN. Control of meal size by central noradrenergic action. *Proceedings of the National Academy of Science (USA)* 1975;72(9):3740–3743.
- Shah ZA, Gilani RA, Sharma P, Vohora SB. Attenuation of stress-elicited brain catecholamines, serotonin and plasma corticosterone levels by calcined gold preparations used in Indian system of medicine. *Basic Clin Pharmacol Toxicol* 2005;96(6):469–474. [PubMed: 15910411]
- Sharp BM, Matta SG. Detection by in vivo microdialysis of nicotine-induced norepinephrine secretion from the hypothalamic paraventricular nucleus of freely moving rats: dose-dependency and desensitization. *Endocrinology* 1993;133(1):11–19. [PubMed: 8391419]
- Sims JS, Lorden JF. Effect of paraventricular nucleus lesions on body weight, food intake and insulin levels. *Behavioral Brain Research* 1986;22(3):265–281.
- Sperlgh B, Sershen H, Lajtha A, Vizi ES. Co-release of endogenous ATP and [3H]noradrenaline from rat hypothalamic slices: origin and modulation by alpha2-adrenoceptors. *Neuroscience* 1998;82(2):511–520. [PubMed: 9466457]
- Sved AF. PNMT-containing catecholaminergic neurons are not necessarily adrenergic. *Brain Res* 1989;481(1):113–118. [PubMed: 2706454]
- Svensson CK. Clinical pharmacokinetics of nicotine. *Clinical Pharmacokinetics* 1987;12(1):30–40. [PubMed: 3545615]
- Swanson LW, Sawchenko PE. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annual Review of Neuroscience* 1983;6:269–324.
- Valentine JD, Hokanson JS, Matta SG, Sharp BM. Self-administration in rats allowed unlimited access to nicotine. *Psychopharmacology (Berl)* 1997;133(3):300–304. [PubMed: 9361337]
- Valentine JD, Matta SG, Sharp BM. Nicotine-induced cFos expression in the hypothalamic paraventricular nucleus is dependent on brainstem effects: correlations with cFos in

- catecholaminergic and noncatecholaminergic neurons in the nucleus tractus solitarius. *Endocrinology* 1996;137(2):622–630. [PubMed: 8593811]
- Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW. Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *Journal of Comparative Neurology* 1989;284(2):314–335. [PubMed: 2754038]
- Wellman PJ. Overview of adrenergic anorectic agents. *American Journal of Clinical Nutrition* 1992;55 (1 Suppl):193S–198S. [PubMed: 1309478]
- Wellman PJ, Davies BT, Morien A, McMahon L. Modulation of feeding by hypothalamic paraventricular nucleus alpha 1- and alpha 2-adrenergic receptors. *Life Science* 1993;53(9):669–679.
- Yang ZJ, Blaha V, Meguid MM, Oler A, Miyata G. Infusion of nicotine into the LHA enhances dopamine and 5-HT release and suppresses food intake. *Pharmacology Biochemistry and Behavior* 1999;64(1): 155–159.
- Yoshida T, Yoshioka K, Hiraoka N, Kondo M. Effect of nicotine on norepinephrine turnover and thermogenesis in brown adipose tissue and metabolic rate in MSG obese mice. *Journal of Nutritional Science and Vitaminology (Tokyo)* 1990;36(2):123–130.
- Zarrindast MR, Oveisi M-R. Effect of monoamine receptor antagonists on nicotine-induced hypophagia in the rat. *European Journal of Pharmacology* 1997;321:157–162. [PubMed: 9063683]

Figure 1.

Panel A is a schematic drawing of parasagittal rat brain showing phenylethanolamine N-methyltransferase (PNMT) positive fibers (black lines and arrows) originating from PNMT positive cells (dots) in the C1, C2, and C3 regions extending into the hypothalamus (HY). PNMT fibers from the C1, C2, and C3 fasciculate to form part of the medial forebrain bundle (thick black line). Panel B shows a coronal section at the level of the paraventricular hypothalamic nucleus (PVN). The PVN extends anterior-posterior -1.3 to -2.3 mm from Bregma. Panel C shows a coronal section at the level of the dorsomedial hypothalamic nucleus (DMN). The DMN extends anterior-posterior -2.5 to -3.6 from Bregma. Thick straight lines in panels B and C indicate cannula placement (arrowhead). Density of PNMT fibers staining

was arbitrarily graded as +3 most intense, +2 less intense and +1 least intense as described in (Hokfelt et al., 1984). PNMT fibers in cross section are indicated by plus signs (axons cross) and PNMT fibers longitudinally visualized (see key =axons long.) were marked as thick wavy lines. F= fornix, FMT= fasciculus mamillothalamicus and PFH= perifornical hypothalamic area. Redrawn with permission from Elsevier Publishing.

Figure 2. Changes in tissue content of catecholamines measured in the perifornical hypothalamus (PFH) after lesions (LESION) were produced by infusing 4 μ g of 6-OHDA (0.2 μ l) per site into the PFH bilaterally in two anterior-posterior locations, one at the level of the PVN and the other at the level of the DMN. Sham lesioned animals (SHAM) had the same amount of vehicle infused. Values are given as the amount of catecholamine (pg) in an mg of brain tissue. Comparing SHAM (n = 8) and LESION (n = 10) groups an asterisk indicates p < 0.05. Means $\pm$ SEM. See Fig. 1 legend for abbreviations.

Figure 3.

Mean group 24 hour food intake. Food intake, in grams, was recorded for one (-1) and two days (-2) before nicotine injection and 14 days post injection in sham lesion and i.p. injected with saline (SHAM/SALINE, n = 6); sham lesioned and i.p. injected with nicotine (SHAM/NICOTINE, n = 6); PFH lesioned and i.p. injected with saline (LESIONED/SALINE, n = 8) and PFH lesioned and i.p. injected with nicotine (LESIONED/NICOTINE, n = 10) animals. Nicotine (1.4 mg/kg/day, free base.) or a similar volume of 0.9% saline was given i.p. in four equal increments throughout the dark phase. Values on the X-axis are represent days before (-2, -1) or after nicotine injection (1, 2, etc.). Lettering indicates a significant difference between groups of at least $p < 0.05$; a = SHAM/SALINE vs SHAM/NICOTINE; b = SHAM/SALINE vs LESION/NICOTINE; c = SHAM/NICOTINE vs LESION/NICOTINE, d = LESION/SALINE vs SHAM/NICOTINE. See Fig. 1 and 2 legends for abbreviations and explanations.

Figure 4.

Mean group 24 hour meal size. Lettering indicates a significant difference between groups of at least $p < 0.05$; a = SHAM/SALINE vs SHAM/NICOTINE; b = SHAM/SALINE vs LESION/SALINE; c = LESION/SALINE vs LESION/NICOTINE; d = SHAM/NICOTINE vs LESION/NICOTINE. See Fig. 1 and 2 legends for abbreviations and explanations.

Figure 5.

Mean group 24 hour meal number. Lettering indicates a significant difference between groups of at least $p < 0.05$; b = SHAM/SALINE vs LESION/SALINE; c = LESION/SALINE vs LESION/NICOTINE; d = SHAM/NICOTINE vs LESION/NICOTINE. See Fig. 1 and 2 legends for abbreviations and explanations.

Figure 6.

Mean group change in body weight (in g) for non-lesioned rats (SHAM) and lesioned rats (LESION). The change in body weight (GRAMS DIFFERENCE) on the 14 days post injection was calculated by subtracting the body weight of that day from the body weight of that animal one day before NIC injection (-1). Lettering indicates a significant difference between groups of at least $p < 0.05$; a = SHAM/SALINE vs SHAM/NICOTINE; b = SHAM/SALINE vs LESION/NICOTINE, c = LESION/SALINE vs LESION/NICOTINE; d = SHAM/NICOTINE vs LESION/NICOTINE. See Fig. 1 and 2 legends for abbreviations and explanations.