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Nicotine administration effects on feeding and cocaine–amphetamine-regulated transcript (CART) expression in the hypothalamus

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Abstract

In previous studies food intake and meal size significantly decreased in rats two days after injecting 4 mg/kg/day nicotine tartrate. Food intake returned to normal after nine days of continued nicotine treatment, when reduced meal size is countered by an increase in meal number. Nicotine also reduced body weight after nicotine injection and body weight remained low after nine days. To begin characterizing the mechanism that modulates these changes in feeding behavior and/or body weight during nicotine exposure the transcript levels for agouti related protein (AGRP), cocaine–amphetamine-regulated transcript (CART), corticotropin releasing hormone receptor one (CRH-R1), melanocortin receptors three and four (MC3R/4R), neuropeptide Y (NPY), NPY Y1 and Y5 receptors and/or pro-opiomelanocortin (POMC) were analyzed in the arcuate (ARC), dorsomedial (DMN) and paraventricular (PVN)/periventricular (PE) hypothalamic nuclei on the second and ninth day of saline or nicotine treatment. Results show that the transcript levels of the anorexigenic molecule CART increased in the PVN and/or PE two days after nicotine treatment but after nine days CART levels equalize. In contrast, nine days of nicotine treatment reduced CART levels in the DMN as compared to saline controls. To investigate CART's role in regulating feeding, infusion of CART (55-102) into the third ventricle reduced food intake and meal size. These results are consistent with nicotine modulating feeding behavior and body weight, in part, by affecting CART transcript levels in the DMN, PVN and/or PE.

Keywords: CART; Hypothalamus; Food intake; Nicotine

1. Introduction

Using a model of intermittent nicotine administration during the rat's normal activity period, previous studies have shown that nicotine results in decreased food intake and decreased meal size [3]. However, after nine days of continued nicotine administration a significant compensatory increase in meal number results in an increase in food intake to control levels, but body weight continues to be reduced [3]. Currently, the mechanism by which this model of nicotine administration affects feeding and body weight is unknown.

To address the mechanism of nicotine action on feeding and body weight the expression of several genes was quantitated in the hypothalamus during the course of nicotine administration. Genes that nicotine could utilize to regulate feeding and body weight are agouti related protein (AGRP) with known orexigenic action [51] and cocaine–amphetamine-regulated transcript (CART), with known anorexigenic effects [33]. CART is found in several hypothalamic areas including the ARC, DMN, PVN and PE [11,30] and AGRP is known to be expressed in the ARC [45]. AGRP is a natural antagonist of the melanocortin receptors 3 and 4 (MC3R and MC4R), which modulates the effects of the anorexigenic peptide alpha-melanocyte stimulating hormone (α-MSH) [16,21,28,29,39,45]. α-MSH is derived from the precursor molecule pro-opiomelanocortin

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(POMC). A previous study in our lab showed that administration of nicotine attenuated the ability of the MC3R/4 receptor agonist, MTII, to suppress food intake [2] suggesting an involvement of AGRP, MC3R/4 receptors and the α-MSH pathway in modulating nicotine's effects on feeding. Corticotropin releasing hormone receptor-1 (CRH-R1) or often called corticotropin releasing factor receptor-1 (CRF-R1) mRNA has been localized to the PVN [42,52]. CRH-R1 has been shown to modulate feeding behavior in experiments using pharmacological agents and knockout mice [37,43]. Another gene, NPY has been shown to be modulated during nicotine administration at the transcript and protein level [18,34]. Infusion of neuropeptide Y (NPY) in the PVN has been shown to increase meal size and number [10]. NPY stimulation of feeding can occur through the Y5 receptor [19,22]. However, the Y1 receptor is also thought to be involved in NPY's stimulation of ingestion [15,25,27]. NPY is produced in the ARC and there is also NPY mRNA present in both the DMN [5] and PVN [12,26,32,36].

To begin to understand the mechanisms by which nicotine induced changes in feeding behavior and body weight we studied the transcript levels of multiple genes in the ARC, DMN and PVN plus PE. Often a correlation between transcript and neuropeptide levels can be detected in the hypothalamic nuclei suggesting that measurement of transcript levels reveals changes in gene expression [17,23,35,44,53] although this correlation is not always observed [14]. Gene expression was analyzed at time points where critical changes in meal patterns had occurred. One critical time point was at two days, where continued nicotine administration suppressed food intake by decreasing meal size and the second time point was at nine days where increased meal number led to normalized daily food intake [3].

2. Materials and/or methods

2.1. Animals and nicotine injections

The present study was reviewed and approved by the Baylor College of Dentistry Institutional Animal Care and Use Committee and complied with the principles of laboratory animal care.

Sprague–Dawley out-bred male (250 g) rats (Harlan Industries, Houston, TX) were housed individually in a constant room temperature at 23 °C with standard food pellets (Harland Industries, Rodent diet 7002) and water available *ad libitum*. All animals were maintained on a 12:12 light/dark cycle (lights out at 0800 h). The animals were left undisturbed for five days before experimentation and then injected intraperitoneally (i.p.) for at least four days with saline to desensitize the animals to the injection protocol.

2.2. Nicotine administration

Nicotine was prepared by dissolving nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) in 0.9% NaCl. Starting at the beginning of the dark phase the rats were injected i.p. over the entire dark phase with four equally spaced (approximately 3 h) doses of nicotine (total daily dose of 4 mg/kg) or the same volume of saline. The dose for each rat was based on the highest body weight for that rat during the injection period [3]. We previously completed a dose response study using saline 0, 2 and 4 mg/kg/day nicotine tartrate [3]. Data from our previous study indicated that there was a significant dose × day interaction only for 4 mg/kg/day nicotine tartrate versus saline and not for the 2 mg/kg/day dose [3]. A 2 mg/kg/day nicotine tartrate and 4 mg/kg/day nicotine tartrate dose are equivalent to 0.75 and 1.40 mg/kg/day (free base) dose, respectively. Our choice of a 1.40 mg/kg (free base) nicotine 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 nicotine (free base) through their lungs [9,41]. When rats self administer nicotine, 0.18–1.38 mg/kg/day of nicotine (free base) is injected through jugular cannulas [47], however not all this reaches the brain as much of this dose is removed by the liver. It should be recalled that 70–75% of nicotine given by the i.p. route, used in the present study, would be removed by the liver during a single pass [46] and would not reach the brain. Therefore, the effective nicotine 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 nicotine greater than 4 mg/kg/day (i.e., 1.4 mg/kg/day free base) exceeds nicotine levels taken in by heavy smokers [38] and additionally elicits stereotypic behavior [34], which is atypical for doses taken by rats and humans. In a subset of animals we observe a behavioral response such as a wobble in the animal's gait, extension of the limbs, slow movement and sometimes a shuddering response after the initial nicotine injection but these responses were not detectable after the first injection.

2.3. Dissection of the hypothalamic DMN, PVN plus PE and ARC

Rats were injected with either saline or nicotine for two or nine days and then sacrificed. After sacrificed the brains were rapidly removed and the hypothalamus dissected by making a horizontal cut 3 mm above the base of the brain, two coronal cuts, one 2 mm anterior to the decussation of the optic chiasm and another approximately 1 cm caudal to the first cut and two sagittal cuts immediately lateral of the optic tract. Further dissection was completed with the aid of a dissection microscope (Olympus, Japan) and a caliper. Coronal sections were made by cutting at the stereotaxic coordinates −1.3 mm and −2.3 mm (Bregma) for the PVN plus PE and −2.3 and −3.8 for the DMN and ARC [40]. A triangular shaped portion of tissue containing the PVN plus PE was obtained by first, making a horizontal cut on the coronal section 0.2 mm above the third ventricle; second, making a cut 2 mm below the top of the third ventricle and third, making two lateral cuts angled at 45° to the two previous cuts completing the dissection. A rectangular piece containing the DMN was dissected from the second section by making horizontal cuts at the top of the third ventricle and another 1.1 mm below this cut. Two lateral parallel cuts were then made