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Circulating leptin and pain perception among tobacco-dependent individuals

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ABSTRACT

Recent preclinical evidence suggests that leptin may modulate the stress response and may increase nociception. In this study, we examined for the first time the extent to which cigarette smoking is associated with leptin levels during an extended rest period and in response to noxious stimuli. Repeated blood samples were collected during a laboratory session from smokers and nonsmokers and assayed for leptin. Pain experiences, as well as neuroendocrine and cardiovascular measures, were collected across cold pressor and thermal heat pain tests. Both analysis of variance and correlations confirmed that smokers demonstrated dysregulations in leptin responsivity and association with pain relative to nonsmokers. The flat pattern of leptin release and the weak associations of this hormone with pain in smokers suggest a long-term effect of tobacco dependence on this regulatory hormone. In light of leptin's influence on reward pathways, further investigation of leptin's involvement in nicotine dependence is warranted.

1. Introduction

Leptin, a protein product of the *ob* gene produced primarily by adipocytes (Campfield, Smith, Guisez, Devos, & Burn, 1995), regulates hypothalamic centers involved in body weight, energy homeostasis, and gene expression of corticotrophin-releasing hormone and pro-opiomelanocortin (Cheung, Clifton, & Steiner, 1997; Enriori, Evans, Sinnayah, & Cowley, 2006; Mantzoros, 1999). Evidence also suggests an involvement of appetite hormones in the regulation of drug reward (Bruijnzeel, 2012; Dickson et al., 2011; Funahashi et al., 2000; Harris, Wimmer, & Aston-Jones, 2005; Hollander, Lu, Cameron, Kamenecka, & Kenny, 2008; Li et al., 2000; Opland, Leinninger, & Myers, 2010).

Basic research suggests a role for leptin in regulating nociception. Specifically, research on nociception has demonstrated leptin's involvement in the development of allodynia (high sensitivity to pain) and exacerbation of neuropathic pain (Maeda et al., 2009). Increased pain sensitivity has also been demonstrated in response to peripheral administration of leptin using multiple animal models (Kutlu et al., 2003; Tian et al., 2011). In addition, leptin modulates systems involved in the hypothalamic-pituitary-adrenocortical (HPA) stress response leading to reduced adrenocortical output, presumably by acting at the level of the hypothalamus (Ahima et al., 1996; Heiman et al., 1997). Consistent with this, research has shown that reduced cortisol levels are associated with increased pain sensitivity (Fries, Hesse, Hellhammer, & Hellhammer, 2005; Godfrey et al., 2014), although little is known about this in humans.

The extent to which leptin's associations with pain are affected by chronic smoking has not been investigated. Existing leptin research with smokers has addressed the effects of smoking or abstinence on changes in this hormone with some studies finding differences (Koc, Bulucu, Karadurmus, & Sahin, 2009; Reseland et al., 2005; Perkins & Fonte, 2002) and others finding no effect of short-term smoking abstinence on the hormone (Klein, Corwin, & Ceballos, 2004). However, there has been no systematic investigation of the differences between smokers and nonsmokers in baseline and repeated measures of this hormone as this relates to pain modulation. In light of recent findings showing disrupted endogenous pain modulation in smokers (Nakajima & al'Absi, 2014), manifested by increased pain and absence of stress-induced analgesia, it is important to examine the hormonal association with pain in this population. To that end, comparing smokers with nonsmokers allows for an examination of the impact of chronic

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smoking behavior on this hormone. The goal of this study was to examine the extent to which circulating leptin levels are associated with nicotine dependence during resting baseline and following exposure to noxious stimuli. We measured leptin during rest, before, and after completing two pain-induction procedures, and during an extended recovery period. We predicted that smoking would be associated with a disrupted pattern of leptin production across time, and that leptin levels would be associated with increased pain perception.

2. Methods

2.1. Participants

Participants were recruited from the community by posters and newspaper advertisements to participate in a larger study (al'Absi, Hatsukami, & Davis, 2005). Smokers were included if they had smoked at least 10 cigarettes per day for the past 2 years and were not interested in cessation at the time of the study. Nonsmokers were included if they had never smoked over the last 5 years or if they had smoked fewer than 100 cigarettes over their lifetime, but none over the previous year. All participants had to meet the following criteria: (1) no regular use of prescribed or over-the-counter medications except contraceptives; (2) no current or prior treatment for hypertension, renal, or hepatic disease, no current or history of chronic diseases (e.g., cardiovascular, respiratory, endocrine and neurological disorders, thyroid, and respiratory disorders); (3) no current or history of major psychiatric disorders (e.g., depression, schizophrenia, and alcohol and drug abuse); (4) no current opiate dependence, recent daily opiate use, or use of any narcotic medication within 3 days prior to the study; (5) nonpregnancy; and (6) weight within ± 30% of Metropolitan Life Insurance norms. Smokers were asked to maintain their normal smoking patterns and were to smoke one cigarette of their preferred brand 30 min prior to each laboratory session to minimize withdrawal effects. They did not smoke during the laboratory session (approximately 4 h). Data used in this study were collected in a larger project that was conducted to examine endogenous opioid blockade and pain sensitivity (al'Absi, Wittmers, Hatsukami, & Westra, 2008). We include here data from the placebo day (described below). Participants received a monetary incentive for participation (approximately $20 US per hour), and they signed a consent form approved by the Institutional Review Board of the University of Minnesota

2.2. Apparatus and measures

Pain Measures. The cold pressor test (CPT) apparatus consisted of a 1-gallon container that was filled with ice-water slurry (temperature range: 0–1 °C). Participants were asked to rate their pain at 15-s intervals throughout the 90-s hand immersion in ice-water slurry and the 90-s recovery period. The rating was based on a visual, numerical rating scale with a range from 0 (not at all painful) to 100 (extremely painful). Average ratings were calculated during and after the task, respectively. Subjective pain experience was also measured using the short form of the McGill pain questionnaire (MPQ; Melzack, 1987) after CPT.

For thermal pain, a computer-controlled 2 cm^2 Peltier contact thermode affixed in place with a Velcro strap was used to deliver thermal pain stimuli to the skin of the right volar forearm. Temperature was monitored by a contactor-contained thermistor (Medoc TSA 2001, Minneapolis, MN). The thermode was returned to the adapting temperature (32 °C) between trials by active cooling at a rate of 10 °C/s. Thermal pain threshold and tolerance were assessed using an ascending method of limits with a staircase ramp of 1 °C/s. Participants were instructed to press a button when the thermal stimulus first felt painful (i.e., pain threshold). For the tolerance assessment, subjects were instructed to press the button when the pain became intolerable. The assessment was repeated four times and the average of the last three trials was calculated to determine thermal pain threshold and tolerance. Each stimulus was presented four times in random order, and responses were averaged for each temperature. Participants rated their estimate of pain at each of the five levels between 45 and 49 °C using a visual analog scale of 0–100. Participants also completed the MPQ after the completion of the thermal pain test.

Hormonal and Cardiovascular Measure. Blood samples were collected during baseline rest (first sample), after 1 h rest (second sample), 30 min after exposure to the pain induction procedures (third sample), and after a 60-min recovery period (fourth sample). Blood was collected using a 20-gauge intravenous Teflon catheter inserted in a left forearm vein. The catheter was fitted with a rubber infusion plug through which samples were drawn. Sterile saline was used to flush the system. Each sample was collected in an 8 ml EDTA Vacutainer tube. At the end of the session, samples were centrifuged and stored at −70 °C. Plasma leptin was assayed using a direct sandwich ELISA (Linco, Missouri). Inter- and intra-assay coefficients of variance for these assays were below 8%. Adrenocorticotropic hormone (ACTH) was assayed using RIA kits with a lower sensitivity of 1 pg/ml. Plasma cortisol was assayed using EIA (DSL, Sinsheim, Germany) with a lower sensitivity of 0.1 μg/dl. Inter- and intra-assay coefficients of variance for these assays were below 10%. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were collected across the laboratory session prior to blood sampling using a Dinamap oscillometric monitor system (Critikon, Tampa, FL). Demographic information was collected from the entire sample. Smoking history and nicotine dependence levels (Fagerström Test of Nicotine Dependence; FTND; Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991) were assessed in smokers.

2.3. Procedures

All testing sessions started at approximately 12:00 PM to control for circadian rhythm. Women were tested during the follicular phase of their menstrual cycle (2–13 days after last menses) to avoid hormonal fluctuation. Prior to each session, participants were asked to abstain from alcohol or analgesic medication for 24 h and narcotic medication for 3 days. They were instructed to have a light lunch at least 1 h prior to the session and were provided specific suggestions for items to consume. Those who reported hunger at the beginning of the session were provided two oatmeal granola bars.

At the beginning of the session, an IV catheter was inserted, a blood pressure cuff was placed on the opposite arm, and participants were asked to sit quietly for 30 min prior to ingesting a placebo double-blind capsule that included placebo or naltrexone (data reported here included the placebo condition only). This was followed by a 60-min rest period; after which the two pain induction procedures were administered in a counterbalanced order, separated by a 20-min rest period. After the second pain test (CPT or thermal pain), participants rested for 60 min.

2.4. Dependent variables and data analyses

The primary dependent variables were leptin, ACTH, plasma cortisol, salivary cortisol, and pain measures. Smoking status was categorized as smoker or nonsmoker as described above. Bivariate correlation analysis indicated that BMI did not correlate with any of the appetite measures and was, therefore, not controlled for in the analyses. A series of one-way analysis of variance (ANOVA) were used to test differences as a function of smoking status on all demographic variables (e.g., age, body mass index (BMI), education) and smoking or other substance use history variables (caffeine, smoking rate, duration at that rate, age first smoked, and nicotine dependence score). We conducted a 2 (smoking status) × 4 (sample; 2 before and 2 after the pain assessment procedures) repeated measures of analysis of covariance (ANCOVA) with sex as a covariate to examine the extent to which leptin, ACTH, plasma, and salivary and cortisol changed acutely during the lab session. Sex was included as a covariate due to limited numbers of females in the study (9 nonsmokers; 6 smokers). Cardiovascular measures were analyzed by a series of 2 (smoking status) × 3 (sample: baseline, rest, and post-pain recovery) repeated measures ANCOVA (gender as the covariate). Greenhouse–Geisser correction of degrees of freedom was used in the event to violations of sphericity (Jennings, 1987). Separate one-way ANOVAs were conducted in each smoking group to examine significant interactions and simple comparisons with Bonferroni correction were used to examine significant time effects. Differences between smokers and nonsmokers in mean pain ratings during CPT, after CPT, thermal heat pain threshold and tolerance, and MPQ scores after each pain task were analyzed by one-way ANCOVAs. A 2 (smoking status) × 5 (temperature level) mixed ANCOVA was conducted using pain report to temperature at each degree (45–49 °C) as the within subjects factor and smoking status as the between subjects factor. Gender was again a covariate. Preliminary analyses found that the order of drug was not associated with hormonal and pain measures. This variable was not included in the subsequent analysis to save degrees of freedom. Finally, a series of bivariate correlation analysis was also conducted to examine association of appetite hormones with pain measures. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Participant characteristics

A total of 43 participants (23 smokers and 20 nonsmokers) had plasma samples available to be assayed for leptin. Due to missing data, variations exist in degrees of freedom for the reported variables, and only 39 participants (21 smokers) had the total set of blood samples for the entire session. Participants' characteristics are included in Table 1. Smokers and nonsmokers did not differ in age, BMI, or years of education (Fs < 1.3). Smokers reported consuming more daily caffeinated drinks than nonsmokers ($F(1, 41) = 9.19$, $p < 0.01$). Leptin levels from all four time points were not associated with demographic variables (i.e., age, length of education, and body mass index; correlation range = 0.20–0.24, ps > 0.10) or smoking variables (i.e., age when first smoked, cigarettes per day, years of smoking, and FTND scores; correlation range = −0.07 to 0.16, ps > 0.10).