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Effects of Nicotine on Body Weight, Food Intake and Brown Adipose Tissue Thermogenesis[1]

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WELLMAN, P. J., M. M. MARMON, S. REICH AND J. RUDDLE. *Effects of nicotine on body weight, food intake and brown adipose tissue thermogenesis.* PHARMACOL BIOCHEM BEHAV 24(6) 1605–1609, 1986.—Chronic treatment with nicotine results in reduced body weight gain without a change in food intake. To evaluate the role of brown adipose tissue (BAT) thermogenesis in this effect of nicotine, male Sprague-Dawley rats were chronically treated (3× daily, IP) over a 14 day period with either saline, 0.8 mg/kg nicotine, 10 mg/kg caffeine or a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine and were pretreated (once daily) with either saline or 20 mg/kg nadolol, a long-acting beta-adrenergic receptor blocker. Nicotine significantly reduced body weight gain but not food intake and nadolol did not reverse the effect of nicotine on body weight gain. To evaluate whether nicotine induces BAT thermogenesis, rats were injected IP with either saline or 0.8, 1.2 or 1.6 mg/kg nicotine hydrogen tartrate, with 5 mg/kg dl-phenylpropanolamine (dl-PPA) or with a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine with interscapular BAT (IBAT) temperatures recorded for 30 minutes after injection. No dose of nicotine produced a change in IBAT temperature whereas a combination of caffeine and nicotine produced a temperature increase in IBAT (0.95 degree C) 63% of that induced by 5 mg/kg dl-PPA. These data suggest that changes in body weight gain induced by nicotine treatment are not the result of an action of nicotine on BAT thermogenesis.

Brown adipose tissue Thermogenesis Nicotine Caffeine Body weight Food intake

EPIDEMIOLOGICAL studies of smokers reveal that basal body weights of chronic smokers are consistently lower than matched non-smokers and that cessation of smoking results in a gain of weight to control levels. This inverse relationship has been attributed to the effect of smoking on variables such as food consumption, food palatability, gastric clearance of ingesta, or metabolism but none has received critical support and acceptance [18].

With regard to alterations of metabolism in smokers, Schechter and Cook [16] demonstrated that rats treated twice daily with either 0.4 or 0.8 mg/kg nicotine tartrate exhibited reduced feeding efficiency as evident in reduced body weight gain without a significant change in food intake. Similar observations were made recently by Grunberg, Bowen and Morse [8] using rats infused with nicotine via implanted miniosmotic pumps. Moreover, nicotine, a major component of cigarette smoke, is known to enhance oxygen consumption in human subjects [3, 5, 10]. These as yet unexplained observations are consistent with a potential effect of nicotine on brown adipose tissue (BAT) thermogenesis. BAT is a form of adipose tissue, controlled by the sympathetic nervous system and activated by catecholamines, that is capable of uncoupled oxidative phosphorylation and marked energy expenditure [9,15]. Enhanced BAT thermogenesis may underlie the action of sympathomimetic compounds on body weight [1, 2, 20, 21].

Given that nicotine administration, at moderate dose levels, results in autonomic nervous system activation and adrenal catecholamine release [12], it is reasonable to expect that nicotine treatment might induce BAT thermogenesis. Indeed, Wager-Srdar, Levine, Morley, Hoidal and Niewoehner [19] observed that chronic cigarette smoke exposure increased the mass of interscapular BAT (IBAT) but these authors did not assess the effect of nicotine on BAT thermogenesis.

The present experiment evaluated the role of BAT thermogenesis in weight changes induced by chronic nicotine treatment by (a) assessing the effect of beta-adenergic blockade on the chronic action of nicotine and (b) assessing the action of nicotine on BAT thermogenesis. In subexperiment A, rats were treated twice daily with either 0.8 mg/kg nicotine, 10 mg/kg caffeine or a combination of nicotine and caffeine. Wellman and Marmon [21] demonstrated that caffeine potentiates the effect of dl-phenylpropanolamine on BAT thermogenesis. Given that caffeine comsumption and cigarette smoking frequently co-exist in smokers, it was of some interest to determine the combined effect of these compounds on body weight. Because BAT heat production is primarily controlled by beta-adrenergic receptors [4, 6, 15], a long-acting beta-adrenergic receptor blocker, nadolol [7,17], was given daily to determine whether beta adrenoreceptor blockade would reverse the action of nicotine

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on weight gain. In subexperiment B, separate groups of rats were treated with various dosages of nicotine preceding 30 minutes measurement of interscapular BAT (IBAT) temperature to assess the action of nicotine on IBAT thermogenesis.

METHOD

Animals

The animals were 64 male albino Sprague-Dawley rats (Timco: Houston) 60 days of age weighing 200–240 grams at the beginning of the experiment. The rats were individually housed in plastic rodent cages (Lab Products) in a temperature-controlled room (23.0±1.0 degrees C) under continuous illumination. The rats were given continuous access to tap water and a high-fat diet consisting of 1 part melted vegetable oil (Crisco) and 2 parts ground Teklad chow. The high-fat diet was prepared fresh every 3 days and offered to each rat in a glass jar. In addition, a 10% sucrose solution as well as tap water was offered to each rat in calibrated drinking tubes.

Drugs

A 0.9% saline solution was prepared using sodium chloride dissolved into sterile distilled water. Nicotine solutions (0.8, 1.2 and 1.6 mg/ml) were prepared by dissolving nicotine hydrogen tartrate (Sigma Chemical) into sterile distilled water whereas caffeine (1,3,7-Trimethylxanthine; Sigma Chemical) was used to prepare a caffeine solution (10 mg/ml). A nadolol solution was prepared by dissolving 20 mg/ml nadolol (Squibb 11725, Batch No. 02-770-508076; kindly donated by S. J. Lucania) and then adjusting the solution pH to 7.38 using hydrochloric acid. A dl-phenypropanolamine (5 mg/ml) solution was prepared using dl-phenylpropanolamine hydrochloride (Aceto, Lot No. 2680).

Procedures

Subexperiment A. Food and water intake as well as body weight were recorded daily for each rat over a 21 day period. On Days 4–7, the rats were treated 3× (0700, 1500, 2300 hr) daily with 0.9% saline (IP) to accustom them to the injection protocol. Body weight and ingestive data collected during this period were used to form 8 groups (n=5 each) of comparable mean group high-fat, sucrose and water intake as well as comparable mean group body weight. On Days 8–21, each rat received an injection (IP) 3 times (as above) daily of either 0.9% saline, 0.8 mg/kg nictoine hydrogen tartrate, 10 mg/kg caffeine or a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine. An injection of either 0.9% saline or 20 mg/kg nadolol was given at 1100 hr daily. Food intake was measured to the nearest 0.1 gram at 1500 hr daily and was corrected for spillage collected on a paper pad (Deotized Animal Care Board, Ancare, Inc.) positioned beneath the wire floor of each cage. Fluid (water, sucrose) intakes were measured to the nearest ml using calibrated drinking tubes attached to the rear of each cage.

Subexperiment B. Surgery and temperature measurements were made under anesthesia induced by injection of urethane (Sigma; 1.2 grams/10 ml/kg, IP). For each rat, the skin over the shoulders was shaved and a 3 cm longitudinal incision made over the interscapular region. Each rat was placed on a suspended foam pad. A thermoprobe insulated with silicone (Strawberry Tree, 3 mm in length and 2 mm in diameter) was positioned between the major lobes of IBAT and the skin over IBAT closed around the thermoprobe cable using hemostats. The tip of a second probe was positioned 4 cm into the rectum to record rectal temperature. IBAT and rectal temperatures were recorded every minute to the nearest 0.1 degree Centigrade using a microcomputer (Apple-IIE) outfitted with a dual thermometer card (Strawberry Tree, Inc.).

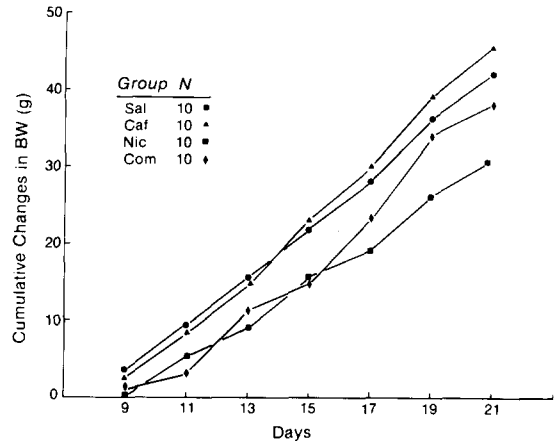

FIG. 1. Mean group changes in body weight (from Day 7) for rats treated (3× daily) with either 0.9% saline (Sal), 10 mg/kg caffeine (Caf), 0.8 mg/kg nicotine (Nic) and a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine (Com) on days 8–21. Data points are depicted every other day for simplicity of presentation and are collapsed across the nadolol pretreatment factor.

Baseline temperatures were recorded for a 10 minute period prior to drug injection in a room maintained at 24.0 (±1.0) degrees C. Separate groups of rats (n's=4, 4, 5, and 4) were treated (IP) with 0.9% saline or with either 0.8, 1.2 or 1.6 mg/kg nicotine and temperatures were recorded for a 30 minute period following injection. In addition, another group of rats (n=4) was simultaneously treated with 10 mg/kg caffeine and 0.8 mg/kg nicotine tartrate. This group was employed to determine the combined effect of nicotine and caffeine on BAT thermogenesis. A sixth group (n=3) was treated with 5 mg/kg dl-phenylpropanolamine to provide a comparison of the effects of the present drug treatments with the activational effect of phenylpropanolamine on in vivo IBAT thermogenesis.

Statistical Analyses

The design of subexperiment A represents a 4×2×17 factorial with between-group factors of treatment (saline, nicotine, caffeine and nicotine/caffeine) and blocker (saline, nadolol) and a within-group factor of days (3 baseline, 14 treatment). Food intake, water intake and body weight were analysed separately using ANOVA and were further analysed using a priori *t*-tests [11]. The design of subexperiment B represents a 4×9 split-plot factorial with dose (0.0, 0.8, 1.2 and 1.6 mg/kg nicotine) as the between-group factor and time (after injection: −10, −5, 0, 5, 10, 15, 20, 25 and 30 minutes) as the within-group factor. Additional analyses were computed comparing the saline and 5 mg/kg PPAgroups and comparing the 0.8 mg/kg nicotine and the combined caffeine/nicotine groups. Difference probabilities of less than 0.05 were deemed statistically significant.

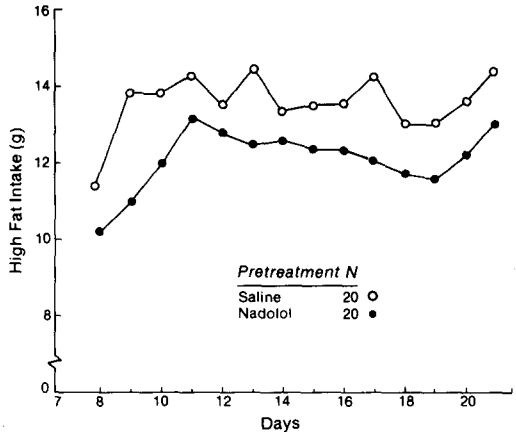

FIG. 2. Mean group daily high-fat diet intake (collapsed across the treatment factor) on Day 7 of baseline and Days 8–21 for rats pretreated (once daily) with either 0.9% saline or 20 mg/kg nadolol during days 8–21.

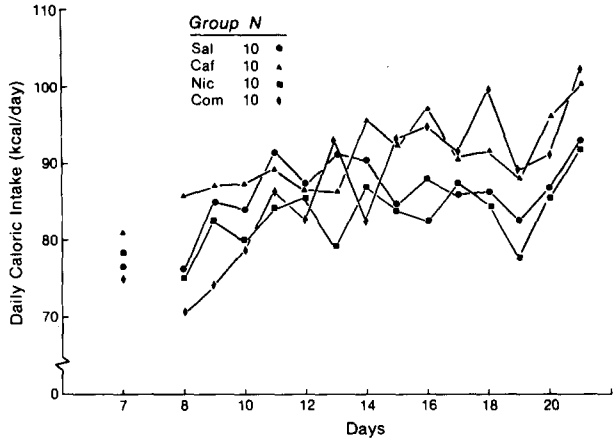

FIG. 3. Mean group daily caloric intake (kcal) on Day 7 of the baseline period and Days 8–21 for rats treated (3× daily) with either 0.9% saline, 0.8 mg/kg nicotine, 10 mg/kg caffeine and a combination of 0.8 mg/kg nicotine and 10 mg/kg caffeine.

RESULTS

Subexperiment A

Body weight. Figure 1 presents the changes in body weight induced by chronic treatment with nicotine, caffeine and a nicotine/caffeine combination. To provide an estimate of the changes in weight gain induced by nicotine and the other treatments, the body weight data were analysed as changes in body weight every other day during Days 8–21 from baseline weight on Day 7. There were no significant differences between the groups in body weight on Day 7. Rats treated with saline, 10 mg/kg caffeine or the combination of caffeine and nicotine exhibited comparable average daily weight gains (3.0, 3.3 and 2.8 grams/day respectively) whereas rats treated with 0.8 mg/kg nicotine exhibited an average weight gain of 2.2 grams/day. Analyses of variance revealed a significant treatment factor, $F(3,33)=3.5$, $p<0.026$, but the pretreatment factor was not statistically significant ($p<0.08$). In addition, the interaction between the factors of treatment and day was statistically significant, $F(18,192)=3.2$, $p<0.0001$, whereas the remaining interactions were not ($p<$at least 0.56). Thus the data are collapsed across the nadolol pretreatment conditon. Subsequent comparisons of final change in body weight (Days 21 minus Day 7) revealed that only the 0.8 mg/kg nicotine group exhibited a significantly smaller weight gain (30.7 g) than the weight gain (42.2 g) exhibited by the saline control group, $t(36)=3.5$ $p<0.001$.

Ingestive behavior. Figure 2 depicts mean group daily high-fat diet intake in rats treated (once daily) with either 0.9% saline or 20 mg/kg nadolol. Analyses of variance of these data indicated a significant effect of the nadolol pretreatment factor, $F(1,32)=4.7$, $p<0.04$, whereas the factors of treatment and day and the interactions among the three factors were not statistically significant. Rats treated with nadolol ate significantly less of the high-fat diet (12.2 g/day) than the saline-treated rats (13.6 g/day). Analyses of consumption of the 10% sucrose solution during days 8–21 revealed no significant factor or interaction among the factors of pretreatment, treatment or day (hence these data are not presented). Because the rats had access to 2 sources of calories (a high-fat diet, a 10% sucrose solution) in this experiment, daily high-fat diet and sucrose intakes were converted to caloric intakes and summed for each rat (Fig. 3). Whereas analyses of variance of the high-fat diet intake data revealed a significant effect of the nadolol pretreatment factor, analyses of total caloric intake revealed only a significant interaction between treatment and day, $F(39,416)=1.9$, $p<0.002$. This interaction reflected the gradual increase in caloric intake by the caffeine and the combined caffeine and nicotine groups. Subsequent comparisons of mean caloric intake, collapsed across Days 15–21, revealed that only the combined caffeine/nicotine group consumed significantly more calories than the saline control group, $t(32)=2.1$, $p<0.05$. Analyses of daily water intake during Days 8–21 revealed no significant factors or interactions (hence these data are not presented).

Saline-treated rats consumed a total of 1215 kcal during the 14 day treatment period and gained an average of 42.2 grams in body weight. Calculation of a feeding efficiency ratio (mg gained/cal consumed) revealed a control ratio of 34.7 mg/cal. Prediction of weight gain for the remaining groups using this ratio revealed a good fit between predicted and actual weight gain for the caffeine group (predict 44.3 grams gained, actually observed 45.7 grams). In contrast, overestimates of weight gain were obtained for the nicotine group (predict 40.6 grams, observed 30.7 grams) and for the combined caffeine/nicotine group (predict 43.7 grams, observed 36.1 grams). Analyses of variance of individual feeding efficiency ratios revealed a significant effect of the treatment factor, $F(3,32)=5.1$, $p<0.005$, but not of the pretreatment factor or of the interactions among the factors ($p<$at least 0.72). Subsequent comparisons of group feeding efficiency ratios revealed that only the nicotine group was significantly different from the saline group, $t(32)=2.9$, $p<0.01$.

Subexperiment B

The changes in brown adipose tissue thermogenesis (difference in temperature between baseline and 30 minutes postinjection) observed in the various treatment groups of subexperiment B are depicted in Fig. 4. Rats treated with saline exhibited a slight decline in IBAT temperature over

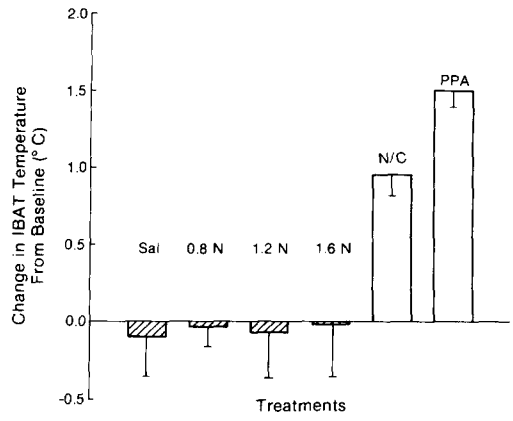

FIG. 4. Mean group changes in interscapular brown adipose tissue (IBAT) temperatures from time of injection and 30 minutes after injection with either 0.9% saline (Sal), 0.8, 1.2 or 1.6 mg/kg nicotine tartrate (N), 5.0 mg/kg dl-phenylpropanolamine (PPA), or a combination of 0.8 mg/kg nicotine tartrate and 10 mg/kg caffeine (N/C). The descending vertical bars indicate the magnitude of the S.E.M.

the 30 minute measurement period. No significant changes in IBAT temperature were observed after treatment with any dose of nicotine, $F(3,13)=0.8, p<0.5$. A combined treatment of 0.8 mg/kg nicotine and 10 mg/kg caffeine produced a modest but significant rise in IBAT temperature, $F(1,6)=14.5$, $p<0.009$. In contrast, 5 mg/kg dl-PPA induced a significant rise in IBAT termperature of approximately 1.5 degrees C over the 30 minute post-injection period, $F(1,5)=38.2, p<0.002$.

There were no significant changes in rectal temperature after any drug treatment, $F(5,17)=1.0, p<0.45$; hence, these data are not depicted.

GENERAL DISCUSSION

The results of this experiment document that nicotine (2.4 mg/kg/day) induces a significant reduction in weight gain in rats that is attenuated by caffeine treatment (30 mg/kg/day) but is unaffected by the long-acting beta adrenoreceptor blocker nadolol (20 mg/kg/day). This effect of nicotine is unlikely to be related to an action of nicotine on brown adipose tissue thermogenesis. In comparing the various effects on body weight gain and BAT thermogenesis noted in this experiment, no dose of nicotine alone increase BAT temperature whereas 0.8 mg/kg nicotine effectively reduced weight gain. A combination of caffeine and nicotine produced a slight increase in BAT temperature yet this combination resulted in increased rather than decreased body weight gain. Furthermore, were the action of nicotine on body weight gain to be mediated by an increase in BAT thermogenesis, chronic blockade of beta-adrenergic receptors via nadolol ought to have reversed the reduction in weight gain induced by nicotine. In the present experiment, nadolol had no significant impact on either body weight gain or total caloric intake. Nadolol is known to effectively block beta adrenoreceptors in the periphery [7,17]. Nadolol is 300–400% more active than propranolol and exhibits a half-life of 19 hours. By way of comparison, the dose of nadolol used by us in the present study is 4 times that used by Fregley [7] to block the effect of isoproterenol on tail temperature in the rat.

The failure to implicate BAT thermogenesis in the action of nicotine on weight gain was unexpected. Wager-Srdar *et al.* [19] noted that chronic exposure of rats to cigarette smoke induced greater mass within interscapular BAT whereas chronic nicotine (1 and 2 mg/kg/day) injections neither reduced food intake or body weight or altered IBAT mass. The action of nicotine on food intake is somewhat erratic in that some authors report that nicotine reduces food intake [13], while others report no change in food intake ([19]; the present study) or a selective effect on consumption of sweet solutions [8]. A consistent finding, also noted in the present study, is that nicotine reduces feeding efficiency ratios in that food intake alone cannot account for the weight loss or reduced weight gain induced by nicotine. The present study does not provide an explanation for the altered feeding efficiency observed in nicotine-treated rats. Although nicotine may increase oxygen consumption [3, 5, 10], this effect is unlikely to be related to sympathetically-mediated increases in brown adipose tissue heat production.

An unexpected observation in the present study was that caffeine not only failed to enhance the inhibitory effect of chronic nicotine on body weight gain but significantly increased food intake thereby reversing the nicotine effect on body weight gain. Examination of the literature, however, revealed that caffeine can increase or decrease food intake depending on the dose and experimental testing procedures. Merkel, Wayner, Jolicoeur and Mintz [13] observed that rats maintained on a 3 hour per day feeding schedule exhibited increased food intake after acute treatment with caffeine dose levels ranging from 3.12 mg/kg to 50 mg/kg whereas 100 mg/kg reliably reduced food intake. The increase in caloric intake observed in rats treated with 30 mg/kg/day caffeine are consistent with the data of Merkel *et al.* [13] and extend this finding to the free-feeding situation.

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