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Synergism Between Caffeine and dl-Phenylpropanolamine on Brown Adipose Tissue Thermogenesis in the Adult Rat[1]

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WELLMAN, P. J. AND M. M. MARMON. *Synergism between caffeine and dl-phenylpropanolamine on brown adipose tissue thermogenesis in the adult rat.* PHARMACOL BIOCHEM BEHAV 22(5) 781–785, 1985.—Caffeine produces enhanced oxygen consumption, an effect that may reflect an action of caffeine on brown adipose thermogenesis. In Experiment 1, adult male rats were anesthetized with 1.2 g/kg urethane and treated (IP) with either 0.9% saline or 10, 20 or 40 mg/kg caffeine (n=4 each group). Interscapular BAT (IBAT) and rectal temperatures were recorded every minute for 10 minutes prior to and 30 minutes following drug injection. Stable IBAT and rectal temperatures were observed prior to and after saline injection whereas rats treated with 20 and 40 mg/kg caffeine exhibited moderate increases in IBAT, but not rectal, temperature. In Experiment 2, adult male rats were treated with either 0.9% saline or 10 mg/kg caffeine, anesthetized with urethane (1.2 g/kg) and treated (30 minutes after pretreatment injections) with either 0.9% saline or 10 mg/kg dl-phenylpropanolamine (dl-PPA). A combination of caffeine and dl-PPA produced significantly greater BAT thermogenesis than just dl-PPA alone. The implications of these data for the inclusion of caffeine in over-the-counter diet-pills are discussed.

Caffeine Metabolism Brown adipose tissue Thermogenesis dl-Phenylpropanolamine Over-the-counter diet pills Drug synergism

CAFFEINE (1,3,7-trimethylxanthine) is a widely used stimulant that induces alertness, increased cognitive function and activity, presumably via stimulation of the central nervous system [3, 16, 25]. Caffeine also induces a variety of physiological and metabolic effects including hypertension, enhanced serum and urinary catecholamine levels, lipolysis, glycogenolysis and enhanced oxygen consumption [4–6, 9, 13, 18, 19, 22].

The effect of caffeine on oxygen consumption in fed and fasted humans [18] may reflect an indirect action of this drug on brown adipose tissue (BAT) thermogenesis. BAT is a heat-producing tissue, controlled by the sympathetic nervous system, that functions to warm mammals in the cold, an effect termed non-shivering thermogenesis [12] and to oxidize excess calories when mammals overeat, an effect termed diet-induced thermogenesis [23]. In particular, sympathomimetic drugs such as amphetamine, ephedrine, 4-hydroxyamphetamine, noradrenaline and dl-phenylpropanolamine induce BAT thermogenesis ([1, 2, 7, 11, 23, 26, 27], Wellman, 1984, manuscript under review). To evaluate the proposal that caffeine may stimulate metabolism, in part, via activation of BAT thermogenesis, Experiment 1 examined the effect of caffeine (0, 10, 20 or 40 mg/kg, IP) on in vivo interscapular brown adipose tissue (IBAT) and rectal temperature in adult male rats.

EXPERIMENT 1

METHOD

Animals

The animals were 16 adult (70 days old) male Sprague-Dawley albino rats (Timco; Houston, TX) weighing 182–199 grams. The rats were double-housed in plastic rodent cages (Lab Products) in a temperature-controlled colony room (23.0±0.5 degrees C) under continuous illumination. The rats were given continuous access to tap water and standard rat pellets (Purina Rat and Mouse Diet) throughout the experiment.

Drugs

A saline solution was prepared using 0.9% sodium chloride dissolved into sterile distilled water. Caffeine solutions (10, 20 and 40 mg/ml) were prepared by dissolving anhydrous caffeine (Sigma Chemical) into sterile distilled water. The caffeine solutions were calculated as the base [24] and were prepared just prior to injection.

Procedure

Surgery and temperature measurements were made under

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anesthesia induced by injection (IP) of urethane (Sigma Chemical: 1.2 grams/10 ml/kg). For each rat, the skin over the shoulders was shaved and a 3 cm longitudinal incision made over the interscapular region. After placement of the rat on a plastic base, covered with foam, 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 thermoprobe was positioned 4 cm into the rectum to record core 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 Computers, Inc.).

Baseline temperatures were recorded for a 10 minute period prior to drug injection in a room maintained at 24.0 (±0.5) degrees C. Rectal temperature, at this room temperature, was stable (mean of approximately 35.3 degrees C) during the 10 minute period prior to injection. Thus, an external heat source was not required in the present experiment. Separate groups of male rats (n=4 each) were treated (IP) with 0.9% saline or with one of the dose levels of caffeine and temperatures were recorded for a 30 minute period following injection. Rats that were treated with caffeine that exhibited no change in IBAT temperature were treated with 20 mg/ml/kg (IP) dl-phenylpropanolamine (Lot No. 3E7, obtained from H. Reisman Company) at 30 minutes with temperatures recorded for an additional 10 minutes. The latter served as a check on the thermogenic capacity of the surgical preparation. Wellman (1984, manuscript under review) demonstrated that this dosage of dl-PPA induces a rapid increase in IBAT temperature. Data from 3 caffeine-treated rats that failed to exhibit IBAT thermogenesis to both a caffeine dose and to 20 mg/kg dl-PPA were discarded and additional caffeine treatments carried out.

Statistical Analyses

The design of this experiment represented a split-plot factorial with Dose (0, 10, 20 and 40 mg/kg caffeine) 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. Between- and within-group temperature comparisons were made using a priori two-tailed *t*-tests [17] after separate ANOVA's of the IBAT and rectal temperature data.

RESULTS

Figure 1 depicts the effects of caffeine on interscapular BAT temperature in the adult male rat. Analyses of variance of these data revealed a significant interaction between Dose and Time, $F(24,96)=3.5$, $p<0.0001$, but no significant effect of either Dose, $F(3,12)=0.6$, $p<0.60$, or Time, $F(8,96)=1.6$, $p<0.13$. IBAT temperature did not change significantly in saline-treated rats (within-group comparison of temperatures at injection and 30 minutes after injection: $t(96)=1.7$, $p<0.10$. In contrast, rats treated with 20 mg/kg caffeine exhibited a significant increase in IBAT temperature at 20 and 25 minutes after injection relative to temperature at injection, $t(96)=3.0$, $p<0.01$. Rats treated with 40 mg/kg caffeine exhibited changes in IBAT temperature slightly larger than those of 20 mg/kg caffeine rats and the changes in IBAT temperature were more rapid in the 40 mg/kg group. Whereas significant increases in IBAT temperature were not observed in the 20 mg/kg group until 20 minutes after caffeine treatment, the 40 mg/kg caffeine group exhibited a significant increase in IBAT temperature at 5 minutes after injection, $t(96)=4.1$, $p<0.001$. No significant changes in IBAT temperature were observed in the 10 mg/kg caffeine group.

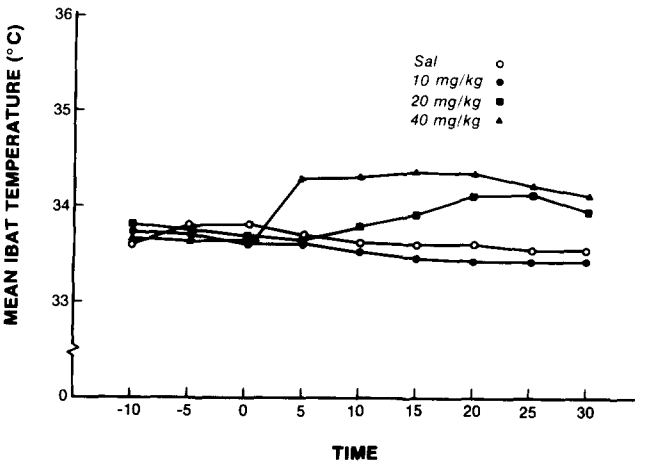

FIG. 1. Mean group interscapular brown adipose tissue (IBAT) temperatures (°C) plotted at 5 minute intervals prior to and after injection with either 0.9% saline (○), 10 mg/kg (●), 20 mg/kg (■) or 40 mg/kg (▲) caffeine.

The average rectal temperatures (collapsed over time) of the saline, 10 mg/kg, 20 mg/kg and 40 mg/kg caffeine groups were 35.3, 35.6, 35.3 and 35.6 degrees C, respectively. Analyses of variance of the effects of caffeine on rectal temperature (not depicted) revealed no significant effect of Dose, $F(3,12)=1.3$, $p<0.33$, Time, $F(8,96)=0.8$, $p<0.58$, or of the interaction between these factors, $F(24,96)=1.2$, $p<0.24$.

DISCUSSION

In the present experiment, modest increases in BAT temperature were recorded in rats treated with 20 mg/kg and 40 mg/kg caffeine. This effect is interesting because caffeine, in addition to its widespread use in beverages, was a common additive to over-the counter (OTC) diet preparations. Caffeine was recently excluded from inclusion in over-the-counter (OTC) diet aids that contain phenylpropanolamine (dl-PPA). Wellman (1984; manuscript under review) demonstrated that dl-PPA induces BAT thermogenesis in adult male and female rats. OTC diet preparations therefore contained 2 compounds that each can serve to induce BAT thermogenesis. The effect of combining caffeine and PPA on BAT thermogenesis, however, is not known. In order to evaluate a possible synergistic action between caffeine and dl-PPA, Experiment 2 assessed the effect of caffeine alone (10 mg/kg), dl-PPA alone (10 mg/kg) and combined caffeine/PPA (10 mg/kg: 10 mg/kg) on BAT thermogenesis. The 10 mg/kg dosage of caffeine was selected because this dose did not alter BAT thermogenesis in Experiment 1 whereas the 10 mg/kg dl-PPA dose produces a moderate stimulation of BAT thermogenesis (Wellman, 1984, manuscript under review).

EXPERIMENT 2

METHOD

Animals

The animals were 16 male albino Sprague-Dawley rats

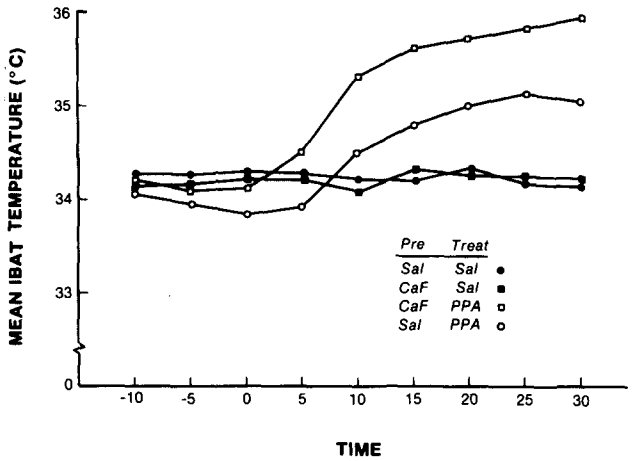

FIG. 2. Mean group IBAT temperatures (°C) plotted at 5 minute intervals prior to and after treatment injections. Pre-treatment/treatment designations are as follows: Saline/Saline (●), Caffeine/Saline (■), Saline/PPA (□), and Caffeine/PPA (○).

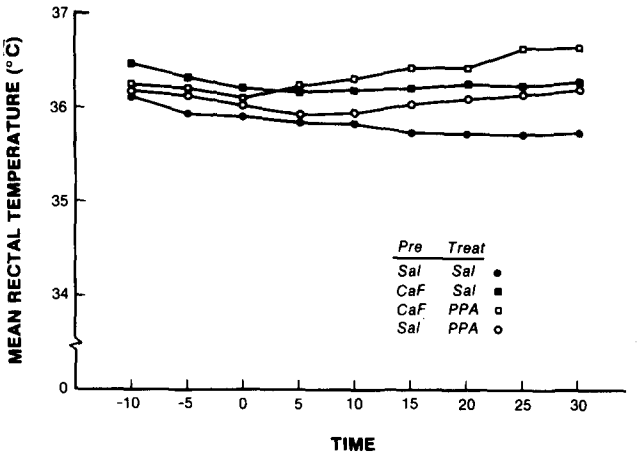

FIG. 3. Mean group rectal temperatures (°C) plotted at 5 minute intervals prior to and following treatment injections. Group designations are identical to those of Fig. 2.

(Timco: Houston, TX) 70 days old. The rats were housed and maintained as in Experiment 1.

Drugs

The saline and caffeine solutions were prepared as described in Experiment 1. A 10 mg/ml dl-PPA solution (concentration calculated as the salt) was prepared prior to injection by dissolving dl-PPA hydrochloride (Lot No. 3E7 obtained from H. Reisman Company) into sterile distilled water.

Procedure

The procedures of this experiment were identical to those of Experiment 1 except that different orders of injections were employed prior to anesthesia and to temperature measurement. Half of the rats were pre-treated with 0.9% saline (1.0 ml/kg, IP) 12 minutes prior to anesthesia with urethane (1.2 g/10 ml/kg, IP) whereas the remaining rats were pre-treated with 10 mg/kg caffeine (IP). Baseline IBAT and rectal temperatures were recorded for 10 minutes and then half of the rats in each pretreatment group were treated (IP) with either 0.9% saline (1.0 ml/kg,) or with 10 mg/kg dl-PPA. These pretreatment and treatment combinations resulted in the following groups: Saline/Saline, Saline/PPA, Caffeine/Saline and Caffeine/PPA (n=4 each). As in Experiment 1, IBAT and rectal temperatures were recorded for 30 minutes after treatment injection.

RESULTS

The effects of caffeine and dl-PPA on interscapular brown adipose tissue temperature are depicted in Fig. 2. There were no significant differences between the groups prior to treatment injections (−10, −5 or 0 minutes). Rats in the Sal/Sal and the Caf/Sal groups exhibited similar IBAT temperatures across the 30 minute period after treatment. The stable BAT temperature exhibited by the Caf/Sal group confirms the findings of Experiment 1 in which 10 mg/kg caffeine did not stimulate brown adipose tissue thermogenesis. In contrast, PPA induced significant increases in IBAT temperature. Analysis of variance of the IBAT temperature data at 30 minutes after injection revealed significant effects of the Pre-treatment factor, $F(1,12)=4.5$, $p<0.05$, and the Treatment factor, $F(1,12)=32.9$, $p<0.0001$. The interaction between the Pre-treatment and the Treatment factors approached statistical significance, $F(1,12)=4.1$, $p<0.06$. Subsequent comparisons between the groups revealed a significant difference between the temperature levels of the Sal/PPA group and the Sal/Sal group, $t(6)=2.5$, $p<0.05$. In contrast, the temperature level attained by the Caf/PPA group significantly exceeded the level of the Sal/Sal group, $t(6)=10.0$, $p<0.001$, and the Sal/PPA group, $t(6)=2.6$, $p<0.05$.

Figure 3 presents the effect of the caffeine and dl-PPA treatments on rectal temperature. Analyses of variance of the rectal temperature data at 30 minutes after injection revealed a significant effect of the Treatment factor, $F(1,12)=4.3$, $p<0.05$, whereas the Pre-treatment factor and the interaction between the factors were not significant, $F(1,12)=1.4$ and 0.2, $p<0.25$ and 0.68, respectively. Subsequent comparisons revealed that the rectal temperatures of the Caf/PPA group significantly exceeded that of the Sal/Sal group, $t(6)=4.7$, $p<0.01$. The other group rectal temperatures at 30 minutes were not significantly different from the Sal/Sal group temperature.

GENERAL DISCUSSION

Miller, Stock and Stuart [18] demonstrated that fasted male and female human subjects exhibited a 14% increase in oxygen consumption after consuming 250 mg caffeine. In contrast, a combination of caffeine and feeding (a 700 Kcal breakfast) induced a 25% increase in oxygen consumption whereas feeding alone induced only a 16% increase. The present data demonstrate that caffeine, at concentrations of 20 and 40 mg/kg, weakly stimulates brown adipose thermogenesis. The effect of caffeine on BAT thermogenesis may explain, in part, the finding of Miller *et al.* [18] in which caffeine increased oxygen consumption in humans. The activational property of 40 mg/kg caffeine on BAT thermogenesis is very weak; by way of comparison, 40 mg/kg dl-PPA induces 157% more thermogenesis over a 30 minute

period (Wellman, 1984, manuscript under review). The effects of caffeine on BAT thermogenesis may reflect the combined effects of this drug on several metabolic mechanisms. For example, caffeine stimulates the release of catecholamines that can activate BAT thermogenesis [5, 6, 15, 22]. In addition, caffeine may contribute to the activation of BAT thermogenesis, in part, by mobilizing fuel substrates via activation of lipolysis [4,9]. Finally, caffeine inhibits phosphodiesterase, thus increasing cellular cyclic AMP, a link between the activation of the brown fat cellular receptor and the oxidation of fatty acids within brown fat [20]. In a study that is relevant to the present results, Reed and Fain [21], using an in vitro procedure, demonstrated that caffeine enhances the thermogenic action of sub-optimal doses of norepinephrine. It should be noted that dl-PPA, at a dose of 10 mg/kg, does not induce maximal stimulation of BAT thermogensis.

The results of Experiment 2 document that the effects of caffeine and dl-PPA on BAT thermogenesis are synergistic for the doses used herein. Caffeine at 10 mg/kg had no effect on BAT thermogenesis (both Experiments 1 and 2) whereas dl-PPA alone (10 mg/kg) induced an increase in BAT temperature of approximately 1.4°C, a magnitude comparable to that described by Wellman (1984) for this same dose of dl-PPA. A combination of caffeine and PPA produced a larger thermogenic response than the sum of the separate drug effects. The synergistic action of caffeine on dl-PPA-stimulated BAT thermogenesis may reflect an action of caffeine on either lipolysis or cyclic-AMP (see above) or perhaps a central synergistic action. Mueller, Muller and Asdell [19] demonstrated that combined injections of caffeine (24 mg/kg) and PPA (6 mg/kg) produced hypertension and cerebral hemorrhage in elderly, food-deprived, stroke-prone/hypertensive rats maintained on tap water containing 1% salt, but these authors did not evaluate the separate effects of these drug doses so to determine whether there is a synergism between caffeine and PPA. Davis and Pinkerton [10] observed that atropine, a muscarinic cholinergic blocker, enhanced the lethality of 60 mg/kg dl-PPA in several strains of rats. The latter effect was interpreted, but not proven, as reflecting a central synergism between atropine and PPA. The parasympathetic nervous system has been reported to tonically inhibit BAT thermogenesis in that atropine treatment enhances BAT thermogenesis [23]. Although one might expect that atropine might synergize the effect of dl-PPA on BAT thermogenesis, no report to date has examined this possibility.

The present data may provide an additional rationale, albeit rather belated, for combining caffeine and dl-PPA in OTC diet aid preparations. Inasmuch as weight loss may accrue to either a reduction in food intake or nutrient absorption, an increase in exercise or an increase in BAT thermogenesis, the present results suggest that a portion of the effectiveness of OTC compounds in weight loss may reflect the effect of a caffeine/PPA combination on BAT thermogenesis. Indeed, a human clinical trial demonstrates a synergism between caffeine and PPA in that caffeine enhances the weight reducing property of PPA in obese outpatients [8]. Finally, the functional properties (e.g., anorexia and/or thermogenesis) of a drug that serve to reduce body weight are likely dissociable. Arch *et al.* [2] demonstrated that BRL 26830A, a beta-adrenoreceptor agonist, stimulated in vitro BAT thermogenesis and reduced body lipid content in lean and obese mice and rats but had no impact on feeding behavior. To the extent that a drug induces both anorexia and BAT thermogenesis, weight loss should be enhanced relative to the weight loss induced by a substance that induces only BAT thermogenesis. Whether dl-PPA induces greater weight loss than BRL 26830A remains to be determined.

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