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Stress-Like Adrenocorticotropin Responses to Caffeine in Young Healthy Men

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LOVALLO, W. R., M. AL'ABSI, K. BLICK, T. L. WHITSETT AND M. F. WILSON. *Stress-like adrenocorticotropin responses to caffeine in young healthy men.* PHARMACOL BIOCHEM BEHAV **55**(3) 365–369, 1996.—The effects of oral caffeine (3.3 mg/kg, equivalent to 2–3 cups of coffee) on plasma adrenocorticotropin (ACTH) and cortisol (CORT) were tested in 47 healthy young men at rest in a double-blind, placebo-controlled, crossover study. Following caffeine, ACTH was significantly elevated at all times from 30 min to 180 min, and CORT was elevated from 60 min to 120 min ($Fs \geq 8.4$, $ps < 0.01$). Peak increases relative to placebo were: ACTH, 33% (+ 5.2 pg/ml) and CORT, 30% (+ 2.7 μg/dl) at 60 min postcaffeine. The results suggest that caffeine can activate important components of the pituitary–adrenocortical response in humans during the resting state. Caffeine's known ability to increase CORT production appears at least partly due to an increase in ACTH release at the pituitary. **Copyright © 1996 Elsevier Science Inc.**

ACTH Cortisol Caffeine

CAFFEINE is a widely used pharmacological agent, having significant effects on the central nervous system and endocrine output. Among the most important effects of caffeine is its ability to alter the activity of the hypothalamic–pituitary–adrenocortical axis (HPAC). We and others have noted that caffeine may elevate CORT production in humans at rest and during mental stress (9,11), suggesting important interactions between this central nervous system stimulant and endocrine components of the stress response. Persons at risk for hypertension and who are borderline hypertensive may be especially sensitive to caffeine's effects on the HPAC (4,5,11).

Caffeine intake results in beta endorphin release by the pituitary (6), implicating effects of caffeine at the hypothalamus or pituitary. However, ACTH has not been studied in vivo following caffeine intake, and therefore, caffeine's ability to affect CORT production by actions above the adrenal cortex has not been established. We have recently compared men at low and high risk for hypertension for ACTH and CORT responses following a moderate oral dose of caffeine (3.3 mg/kg, equivalent to 2–3 cups of coffee) and report here the results of those observations.

METHOD

Subjects were 47 healthy men, 21 to 35 years of age, recruited from the community. All had weight within 20% of normal, blood pressure less than 140/90 mmHg, good health by history and physical exam, no functional impairment on treadmill testing (8), no current use of medication, usual caffeine intake equivalent to 1 to 5 cups of coffee per day with no reported intolerance, smoking ≤10 cigarettes per day, alcohol use ≤ 2 drinks per day, no reported history of alcohol or drug abuse, and a normal nighttime sleep pattern. Based on reports that persons at risk for hypertension are more sensitive to the effects of caffeine (11), we examined the contribution of parental history of hypertension to the responses in question. Twenty-three subjects had a positive parental history as documented by physician report. Volunteers signed a consent form approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center and the Veterans Affairs Medical Center and were paid for participation.

Each subject was tested twice, 2 or more days apart in a double-blind, randomized, placebo-controlled study. Sessions began at 0730 h and lasted 5 h. Subjects abstained from medica-

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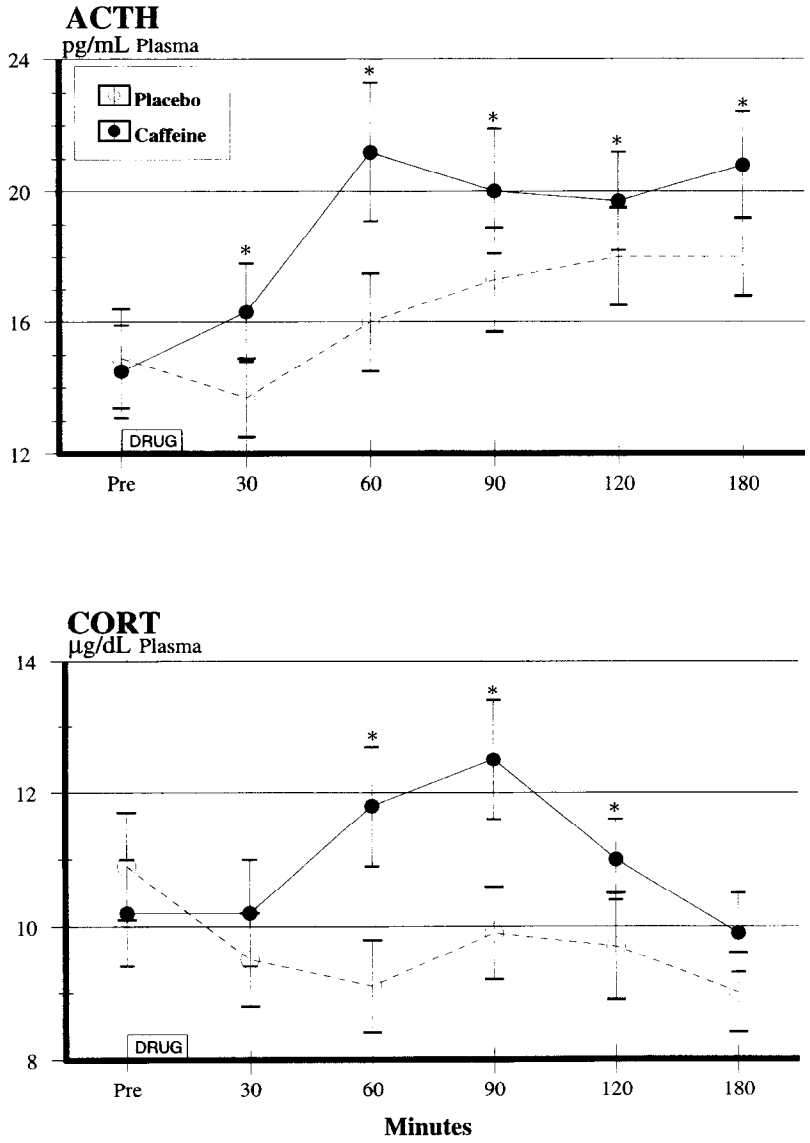

FIG. 1. Plasma ACTH and cortisol at baseline and over 180 min following administration of caffeine or placebo.

tions for 48 h, caffeine, alcohol, and tobacco for 12 h. They consumed a low-fat dinner the evening before and a low-fat breakfast on mornings of study days. During testing, the subject rested semirecumbent and wore a 20-gauge saline-filled catheter in a left forearm vein fitted with an infusion plug. The protocol included quiet rest (45 min), caffeine or placebo administration (5 min), and rest (180 min). A 5-min rest room break occurred 60 min postadministration.

Caffeine (3.3 mg/kg; USP, anhydrous; Amend Drug and Chemical Company, Irvington, NJ) was given with 180 ml unsweetened grapefruit juice (Texsun, Weslaco, TX). Placebo consisted of grapefruit juice alone.

Blood was collected by syringe every 15 min. The frequency of sampling was predicated on the desire to capture secretory bursts of both hormones. In order provide time-integrated samples, equal aliquots from the two 15-min specimens in each half hour were placed into a 5 ml EDTA Vacutainer (Becton Dickinson, Rutherford, NJ) to provide a single specimen for each 30-min period. The two 30 min combined specimens from 120–180 min were also combined into a single specimen representing the third hour postcaffeine. Plasma was separated and stored at −70°C. Plasma taken pre drug and at 45, 120, and 180 min was also assayed for caffeine.

ACTH concentrations were measured by radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) having a minimum sensitivity of 1 pg/ml and an upper range of 1500 pg/ml. Interassay coefficient of variation is 7.8%, and intraassay coefficient of variation is 3.0% (22). CORT levels were assayed by a competitive binding immunoassay in combination with fluorescence polarization (Abbott Laboratories, North Chicago, IL). Caffeine concentrations were quantified by high-performance liquid chromatography (7).

Predrug values of ACTH and CORT were compared using a 2 drug (caffeine, placebo) × 2 groups (positive, negative

family history of hypertension) repeated measures ANOVA. Postdrug data were analyzed using a 2 drug × 2 groups × 6 periods (0–30, 30–60, 60–90, 90–120, and 120–180 min postdosing) ANOVA with repeated measures on the periods and drug factors. Placebo vs. caffeine values were tested at each postdrug point by simple effects tests (31). A value of $p < 0.05$ was used for statistical significance for all tests.

RESULTS

Plasma caffeine concentrations were uniformly low on placebo days and before drug administration on caffeine days (mean ± standard deviation = 0.2 ± 0.28 μg/ml). Postdrug caffeine day values were: 4.6 ± 1.7 μg/ml, 4.1 ± 1.2 μg/ml, and 3.6 ± 1.5 μg/ml, at 45, 120, and 180 min, respectively, in agreement with published values (11,23). Parental history of hypertension did not account for differences in ACTH or CORT among subjects on either placebo or caffeine days (Fs < 1.3).

Predrug values for ACTH and CORT, as shown in Fig. 1, were comparable on caffeine and placebo days (Fs < 1). Placebo day CORT concentrations declined from the first period, as suggested by a significant main effect of period, $F(5, 41) = 6.4$, $p < 0.01$. ACTH postdrug showed an upward trend over the periods resulting in a significant main effect of period, $F(5, 41) = 6.0$, $p < 0.01$.

In examining the effect of caffeine on ACTH, we noted that caffeine caused a significant increase in ACTH levels across postdrug periods as evidenced by a significant drug × period interaction, $F(5, 41) = 4.1$, $p = 0.004$. The change in ACTH secretion induced by caffeine was tested by comparing the caffeine and placebo day values at each time point using simple effect tests on this interaction. We observed significantly greater ACTH levels after caffeine ingestion at each postdrug period, Fs(1, 225) > 4.3, ps < 0.05). The greatest rise occurred at 60 min, $F(1, 225) = 40$, $p < 0.0001$.

CORT secretion was also significantly altered by caffeine as indicated in a significant drug period interaction, $F(5, 41) = 5.7$, $p < 0.001$. Simple effect tests at each time point indicated that CORT was significantly elevated at 60, 90, and 120 min following caffeine, Fs(1, 225) > 8.4, ps < 0.001. The peak rise was seen at 60 min, $F(1, 225) = 33$, $p < 0.0001$.

DISCUSSION

The results suggest two pharmacological and biochemical points of interest. First, the increased ACTH secretion to caffeine was established soon after administration and sustained for the remaining 3 h of the protocol. Second, the ACTH rise preceded the CORT increase. This suggests that caffeine acted on the HPAC at or above the pituitary and that the CORT rise was in response to the rise in ACTH.

Caffeine's pharmacological effects on the central nervous system are due to blockade of the adenosine receptor and to interference with cyclic adenosine monophosphate (cAMP) phosphodiesterase (19,22,25). Each of these can result in increased availability of cAMP, which stimulates expression of the corticotropin releasing factor (CRF) gene (27). CRF secretion at the median eminence of the hypothalamus, in turn, stimulates ACTH release by the pituitary, resulting in increased CORT production by the adrenal cortex (22). Caffeine may, therefore increase CRF, ACTH, and CORT through cAMP accumulation in the median eminence or pituitary, as has been shown in rat (15,30). Caffeine may also contribute indirectly to increased ACTH release through stimulation of epinephrine secretion (20), which can enhance cAMP production in relevant neuronal systems. In addition, caffeine blocks adenosine in the mesopontine reticular formation resulting in activation of this system (24), contributing to increased generalized arousal, and in turn, enhancing HPAC activity.

A third issue raised by the present results concerns caffeine's effects on pituitary–adrenocortical function in relation to stress responses. It is noteworthy that CORT and ACTH are core components of the endocrine response to a variety of stressors (17) including mental stress (18). Threatening or aversive challenges increase CORT production in preference to nonaversive challenges (10,14). Studies of mental stress show that peak ACTH increases may range from 3.5 pg/ml during routine psychometric testing (18) to 4.8 pg/ml during mental arithmetic challenge (1) to 5.1 pg/ml during simulated public speaking (26) to 13 pg/ml during an actual oral examination in front of a committee (16). The maximum difference from placebo seen in the present study was 5.2 pg/ml, placing the changes we observed to caffeine alone well within the range reported to psychological stressors. In the same vein, epinephrine secretion, another important element in the endocrine response to stress, is increased by caffeine in resting persons (28). Therefore, caffeine alone may evoke both adrenocortical and adrenomedullary components of the fight flight response in persons at rest and in the absence of explicit challenge.

In addition to caffeine's effects on persons at rest, the same dose as used here may enhance the adrenocortical response to behavioral stress in the laboratory (5,11). Caffeine may also potentiate cardiovascular responses during mental stress or bicycle exercise (5,9,12,23,28). These considerations lead us to suggest that caffeine in dietary doses is capable of initiating and enhancing a pattern of responses associated with mental stress.

A related issue concerns caffeine's ability to alter adrenocortical activity in persons at high risk for hypertension. In the absence of caffeine, borderline hypertensives show enhanced responses to a variety of psychological stressors indicative of enhanced central nervous system activation (13). For example, when at rest in a novel experimental environment, borderline hypertensives show adrenocortical activation relative to low-risk controls (2) and they have larger responses during work mental arithmetic and psychomotor stress (3). These tendencies are exaggerated the presence of caffeine. The challenge of an impending mental stressor may produce a cortisol response in borderline hypertensives, although this anticipatory response is absent in controls (2,3). We have previously shown that compared to low-risk controls, caffeine can differentially increase cortisol secretion in normotensive men at high risk for hypertension during work on a demanding psychomotor task (11). However, the present study did not indicate that caffeine differentially enhances ACTH or CORT secretion, even in hypertension-prone persons. The observations presented here were made in subjects adapted to the laboratory, and they were aware that no behavioral demands would be placed on them during the day's study. This suggests that persons at elevated risk of hypertension may be differentially sensitive to caffeine's pituitary adrenocortical effects when the situation has significant behavioral demand characteristics or invokes the anticipation associated with a novel environment.

Inspection of Fig. 1 indicates that the ACTH values increased over the rest period on the placebo day while cortisol values declined. In humans, glucocorticoid secretion normally declines from about 0600 h to about 1130 h, and so the rise in ACTH in our observations initially appears to be paradoxical.

However, ACTH is negatively regulated by cortisol feedback, and we would expect a decline in peripheral cortisol concentration to disinhibit ACTH secretion allowing its levels to rise. Given the normal, declining trend in cortisol concentration over this period, the observed rise in ACTH is not unexpected.

In relation to caffeine's enhancement of HPAC function, there is evidence that caffeine can counteract the effect of drugs that normally suppress pituitary secretion. Chronic prednisolone administration suppresses corticosterone secretion in rats, and caffeine can increase the rate of recovery of the HPAC following discontinuation of the prednisolone (15). In humans, rises in ACTH and CORT following morning or evening coffee consumption may produce a false positive response to the dexamethasone suppression test. A single dose of 480 mg of caffeine produced a significant escape from dexamethasone suppression in 34% of one sample (29), suggesting that dietary caffeine intake should be examined as a possible confounding factor in diagnostic tests of HPAC axis function.

The observations reported here suggest further study concerning the scope and mechanism of caffeine's influence on the HPAC. The minimum effective dose and duration of action of caffeine on ACTH secretion remains to be determined. At present, we do not know if ACTH is elevated beyond 3 h following an acute dose of caffeine. Interestingly, CORT had declined by the end of the observation period while ACTH remained elevated. Whether this reflects a change in receptor function at the adrenal cortex or some other phenomenon remains to be determined in mechanistically based studies.

The present results are limited to the effects of a single dose in regular users of caffeine who had abstained overnight. The acute and chronic tolerance effects of caffeine on the ACTH response to a challenge dose remain to be tested. Similarly, possible tolerance effects should be evaluated for their ability to modify caffeine's potentiation of HPAC responses to stressors. The present results are further limited by the exclusive use of men in the test sample, the relative infrequency of blood sampling, and the use of a single dose of caffeine. Future studies could profitably address these issues.

The present study is the first that we know of to observe ACTH following caffeine intake. The results compliment previous findings that caffeine in moderate doses is able to elicit significant components of the stress response in persons at rest or to enhance such response components during behavioral stress. Caffeine's ACTH increase should be examined systematically for its effects on diagnostic tests in subject groups varying in susceptibility to its effects.

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