Br. J. clin. Pharmac. (1986), 22, 81–87

The psychopharmacological and electrophysiological effects of single doses of caffeine in healthy human subjects

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1 The effects of single doses of anhydrous caffeine (250 mg and 500 mg) and placebo on physiological, psychological measures and subjective feelings were studied in a double-blind, cross-over study in nine healthy subjects who had abstained from caffeine-containing beverages for 24 h before each occasion.

2 Caffeine and caffeine metabolites in plasma and urine were assayed. Peak plasma concentrations were observed at 1 to 2 h with an approximate half-life of 5 h. The concentrations of the metabolite 1,7-dimethylxanthine increased during the 5 h. The major urine metabolite was 1-methyluric acid.

3 The EEG showed a dose-related decrease in log 'theta' power and a decrease in log 'alpha' power. Other dose-related effects were an increase in skin conductance level (sweat-gland activity) and self rating of alertness. Ratings of headache and tiredness were decreased by the caffeine.

4 The study illustrates the complexities of studying a drug which is widely taken and which is often associated with withdrawal effects.

Keywords caffeine metabolites psychophysiology cognitive tests psychomotor tests

Introduction

Caffeine, a central nervous system stimulant (Rall, 1980), is widely consumed (Dews, 1982). Studies in America show a daily intake of approximately 200 mg per adult (Gilbert, 1976; Wells, 1984). The acute administration of this amount increases alertness, stimulates attention and restores performances degraded by factors such as fatigue and boredom (Weiss & Laties, 1962). However, a wide variation in the behavioural effects of caffeine has been demonstrated in humans receiving doses of this magnitude (Gilbert, 1976).

Interest in caffeine has revived recently with some observations suggesting that it might induce a state indistinguishable from Generalized Anxiety Disorder, so-called 'caffeinism' (Greden, 1974). Administration of caffeine might thus provide a model for anxiety in normal subjects and a possible provocation test in anxious patients. As a group, anxiety disorder patients consume no more caffeine than controls (Boulenger & Uhde, 1982). However, it might be that they are more sensitive than controls to caffeine for pharmacokinetic (Levy & Zylber-Katz, 1983) and/or pharmacodynamic reasons.

To test these hypotheses, sensitive chemical assays of caffeine concentrations and practical pharmacological measures of caffeine's effects are needed. We report the results of a small-scale study evaluating such techniques with

particular emphasis on drug assays and quantitative EEG recordings respectively.

Methods

The Ethics Committee (Research) of the Institute of Psychiatry approved the study and subjects gave informed consent. They were told that the experiment would evaluate the effects of caffeine, the maximum dose being equivalent to eight cups of instant coffee. Nine normal healthy adult volunteers, four males and five females, aged 18–40 years, took part. All were habitual caffeine users, and the daily mean caffeine consumption as assessed by retrospective questionnaire was 428 mg ± 146 mg (s.d.), range 230–670 mg. Each subject was tested on three different occasions, at least 1 week apart. On each test day, they were given either 250 mg or 500 mg of anhydrous caffeine or a placebo in matching capsules, using a randomised design. For 24 h prior to each test day subjects were asked to abstain from caffeine and alcohol and to have only a light breakfast.

On arrival in the laboratory at about 12.00 h, a cannula was inserted into a forearm vein and kept patent with heparin. Electrodes were applied, and the pre-drug test sequence commenced which lasted about 30 min. The subject was then asked to provide a sample of urine. Subsequently all urine passed during the experiment was collected. The capsule was finally taken and the tests repeated 1, 3 and 5 h later. At hourly intervals throughout the test period, 5 ml venous blood samples were taken for caffeine and caffeine metabolite analysis.

Blood and urine samples

The blood samples were assayed for caffeine and a few dimethylxanthine metabolites using high performance liquid chromatography (Scott *et al.*, 1984). The urine samples collected before and after the 5 h test period were assayed for methylxanthine metabolites (see Figures 2 and 3) by ion-pair extraction followed by gradient elution high performance liquid chromatography (Scott *et al.*, 1986). Excretion patterns were compared with results obtained from other normal subjects who had undergone a controlled dietary caffeine intake of 450 mg 24 h^{-1}.

Physiological measures

Electroencephalogram The EEG was recorded from vertex and left temporal electrodes (Cz and T3). It was amplified and passed through a 2–32 Hz bandpass filter before undergoing a Fourier Transform and power-spectral density analysis. Thirty-two samples each of 4.8 s, were taken during the reaction time task (commencing at least 3 s after each click), subject's eyes open and the spectral data pooled. The spectra were divided for convenience into the clinical wavebands: 'theta' 4–7.5; 'alpha' 8.0–13.5; and 'beta' 14.0–32 Hz. The mean log power in each waveband was computed.

Skin conductance level Skin conductance was measured from a marked area on the left thumb. The mean level and number of fluctuations/minute were measured during the reaction time task.

Pulse rate and blood pressure These were measured using a 'Copal' auto-inflation digital sphygmomanometer with counter.

Tremor This was measured using an accelerometer attached to the middle finger, extended horizontally with the arm supported from the elbow to the wrist. The output was amplified and analysed in the computer using a Fourier power-spectral density programme.

Critical flicker fusion Subjects held a monocular device with Maxwellian view optics to the dominant eye. The stimulus was a red light-emitting diode activated by the computer. Background illumination was excluded. Three runs from flicker to fusion alternated with three from fusion to flicker to allow computation of a mean of the six values.

Psychological measures

Tapping rate The subject tapped a key with two fingers of his preferred hand as quickly as possible for 60s. The mean inter-tap-interval was calculated.

Reaction time Thirty-two auditory clicks were presented to which the subjects were asked to respond as fast as possible.

Digit-symbol substitution task (DSST) This is a coding task in which the subject has to code symbols for digits. The score is the number completed in 90s.

Symbol copying task (SCT) The same symbols are used as for the DSST but the subject has to copy and not code them. It is scored similarly to the DSST.

Number cancellation task (CT) This involves and measures attention, the subject cancelling 4s in a series of numbers. The time to complete the task and the number of errors were noted.

Parallel versions of the DSST, SCT, and CT were used on each occasion.

Self-ratings

Mood rating scale Feeling at the time of each testing was measured on a series of sixteen analogue scales (Bond & Lader, 1974). This mood rating scale has been subjected to a principal component analysis which yielded three factors. The first factor is one of alertness and consists of nine scales; alert–drowsy, strong–feeble, muzzy–clear-headed, well coordinated–clumsy, lethargic–energetic, mentally slow–quick-witted, attentive–dreamy, incompetent–proficient and interested–bored. The second factor measures contentedness and the five scales which load on it are; contented–discontented, troubled–tranquil, happy–sad, antagonistic–amicable and withdrawn–gregarious. The third factor, calmness, is composed of two scales; calm–excited and tense–relaxed. On each scale, the subject marked the point along a 100 mm line that represented how they felt.

Bodily symptom scale A similar scale has been constructed to measure bodily symptoms. It has 13 items which have been used in the investigation of anxiety; anxiety, sweating, shaking or trembling, palpitations, nausea or sickness, dizziness, irritability, loss of appetite, muscular tension, indigestion or stomach trouble, physical tiredness, headache and concentration. The subject rated them along a 100 mm line between absent and severe.

State anxiety inventory (Spielberger, 1970) This consists of a 20-item self-report scale designed to measure the current level of tension and apprehension. Widely used in a variety of research settings, the STAI is easily administered and scored.

Analysis of data

A four-way analysis of variance was calculated, the main sources of variance being subjects, drugs, occasions and times. Differences between drugs were obtained from the drugs × times interaction. Differences between means were assessed using the 0.05 critical difference.

Results

Chemical analyses

Blood methylxanthine concentrations Pre-drug blood samples contained almost no methylxanthines, confirming that each subject was adequately 'decaffeinated'. Following the ingestion of the caffeine capsules, peak blood concentrations were observed at 1–2 h (Figure 1). Of the dimethylxanthine metabolites, theo-

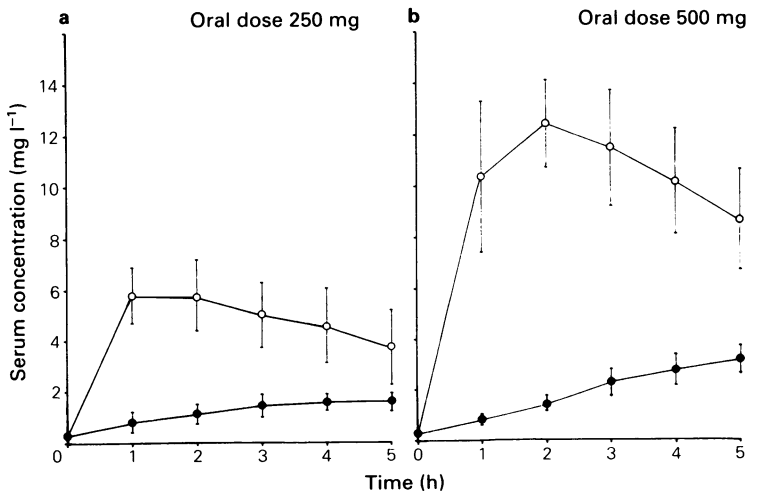

Figure 1 Mean ± s.e. mean serum caffeine (○) and 1,7-dimethylxanthine (●) levels, following the oral ingestion of (a) 250 mg caffeine and (b) 500 mg caffeine in nine subjects.

bromine and theophylline hardly increased, but 1,7-demethylxanthine rose steadily to the 5 h point.

Urinary methylxanthine levels Methylxanthine levels in urine samples collected for the 5 h period following ingestion of 500 mg caffeine showed in comparison with pre-drug levels a significant increase (mg g^{-1} creatinine) in 1-methylxanthine, caffeine, 1,7-dimethylxanthine, 1-methyluric acid, 1,7-dimethyluric acid, 1,3,7-trimethyluric acid, 1,3-dimethyluric acid, theobromine and theophylline (Figure 2). The excretion pattern resembled that in 24 h urine collections from normal subjects receiving a controlled dietary caffeine intake of 450 mg 24 h^{-1} (Figure 3).

Physiological measures

Electroencephalogram Log 'theta' power was decreased by caffeine in a dose-related manner (Figure 4a), with the effect persisting to 5 h, especially with the high dose. Log 'alpha' power was also decreased (Figure 4b), the doses initially having similar effects but the lower dose wearing off quicker. Log 'beta' showed paradoxical effects, the 500 mg dose having less effect than the 250 mg dose (Figure 4c). The total power however, reflects drug effects on the slower frequencies (Figure 4d).

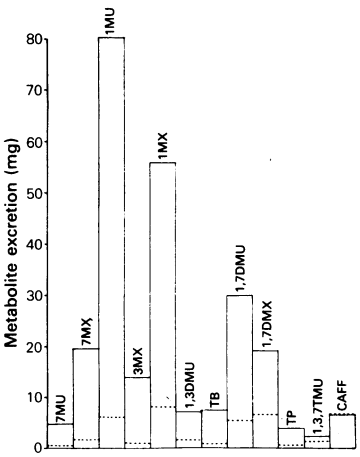

Figure 2 Mean methylxanthine metabolite value (mg g^{-1} creatinine) in the basal urine samples (-----) and in samples collected for a 5 h period following the oral ingestion of 500 mg caffeine (———).
7MU = 7-methyluric acid, 7 MX = 7-methylxanthine, 1MU = 1-methyluric acid, 3MX = 3-methylxanthine, 1,3DMU = 1,3-dimethyluric acid, TB = theobromine, 1MX = 1-methylxanthine, 1,7DMU = 1,7-dimethyluric acid, 1,7DMX = 1,7-dimethylxanthine, TP = theophylline, 1,3,7TMU = 1,3,7-trimethyluric acid, CAFF = Caffeine.

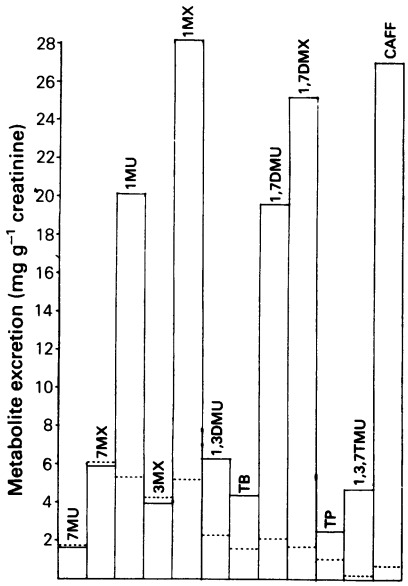

Figure 3 Mean methylxanthine metabolite output in urine samples (-----) collected for a 5 h period (mg 5 h^{-1} vol.) following the oral ingestion of 500 mg caffeine from normal subjects. This is compared with normal subjects (mg 24 h^{-1} vol.) receiving a controlled dietary intake of 450 mg caffeine in 24 h (———). Abbreviations as in Figure 2.

Skin conductance Caffeine was associated with a significant dose-related increase in skin conductance level (sweat gland activity) (Figure 5), the effects persisting over the 5 h period. There was no drug effect on the number of fluctuations.

Pulse and blood pressure were unaffected by either dose of caffeine.

Critical flicker fusion Caffeine tended to increase c.f.f. as compared with placebo but this effect marginally failed to reach significance ($P = 0.052$).

Tremor Caffeine produced a non-significant increase in the amount of tremor.

Psychological measures Tapping rate, reaction time, DSST, SCT and CT all failed to show significant differences between the drug and placebo.

Self ratings

Mood rating scale Caffeine was associated with a significant dose-related increase in alertness which persisted for 5 h (Figure 6). The significant sub scales were: alert–drowsy ($P < 0.01$);

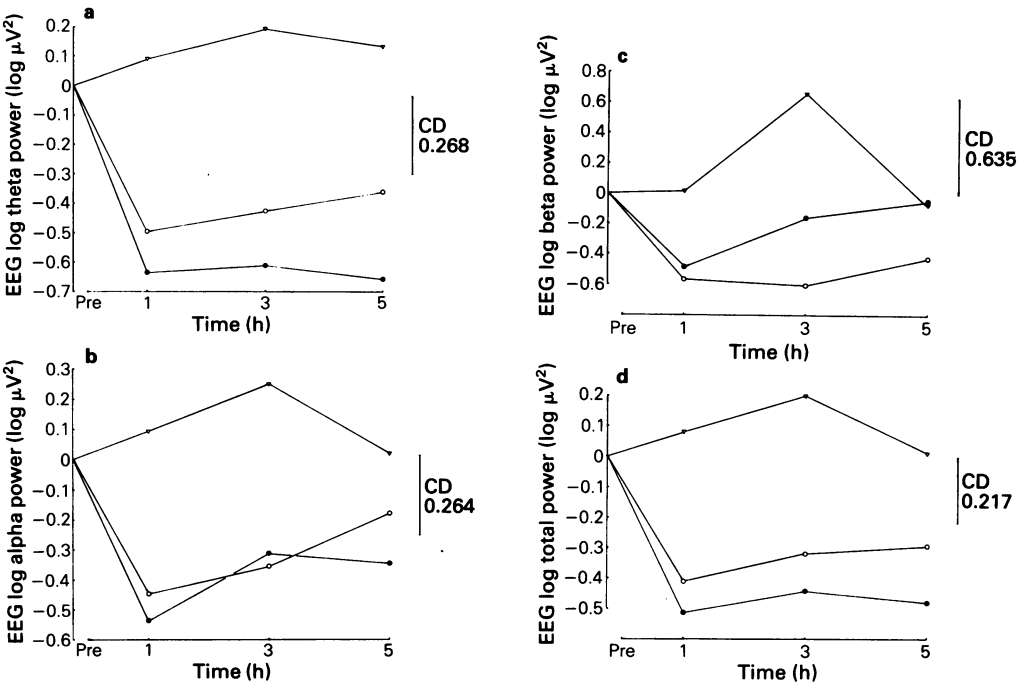

Figure 4 (a) Mean EEG log power in 4–7.5 Hz waveband in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The vertical bar is the 0.05 critical difference. That means that points further apart than that shown by the bar are significantly different at the 0.05 level of probability. (b) Mean EEG log power in 8.0–13.5 Hz waveband in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**). (c) Mean EEG log power in 14.0–25 Hz waveband in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**). (d) Mean EEG log total power across wave bands in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**).

energetic–lethargic ($P < 0.02$); quick witted–mentally slow ($P < 0.03$); and attentive–dreamy ($P < 0.04$). In general, the effects were dose-related, the effect began within the first hour, and tended to diminish thereafter, except on the alert–drowsy sub scale where the effect persisted. Other mood factors did not significantly distinguish drug from placebo.

Bodily symptoms The subjects reported more severe shaking and trembling (Figure 7), maximal at 1 h, and diminishing rapidly thereafter. There were also fewer complaints of headache ($P < 0.05$) and less tiredness ($P < 0.01$ after caffeine than after placebo.

State anxiety inventory failed to show any difference between drug and placebo effects.

Discussion

High caffeine levels were obtained with rapid absorption of the drug. Random plasma caffeine concentrations in a clinical biochemistry outpatient group taken for another biochemical investigation showed that 5% of that population had levels greater than 5.6 μg ml^{-1} (Smith *et al.*, 1982). This was the same as the average peak concentration after the 250 mg capsule. The mean caffeine half-life could not be calculated precisely due to the small number of subjects and samples per subject, lack of control of relevant factors such as previous caffeine consumption, amount of smoking, and female menstrual status (Kalow, 1985). However, the value of about 5 h which we found is consistent with previous studies (Zylber-Katz *et al.*, 1984; Spindel *et al.*, 1984).

The limited period of urine collection resulted in a low recovery of methylxanthine metabolites. However, the pattern of excretion after 500 mg resembled closely the methylxanthine metabolite output estimated in normal subjects receiving a controlled dietary caffeine intake of 450 mg 24 h^{-1}.

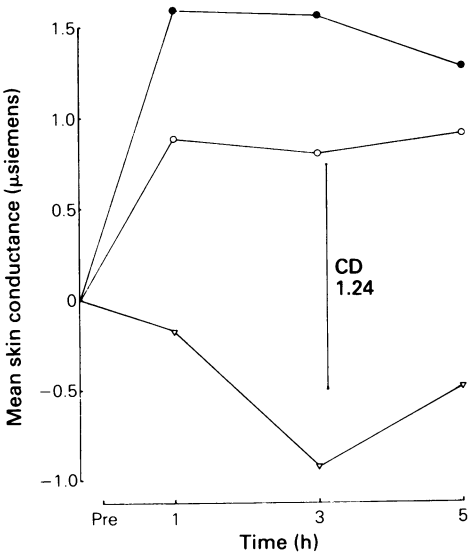

Figure 5 Mean skin conductance levels in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**).

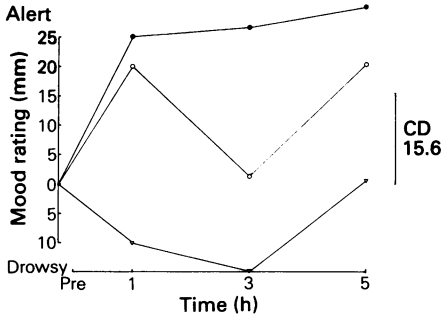

Figure 6 Mean mood rating for 'alert–drowsy' in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**).

The metabolite values observed in blood and urine samples agree with data from previous studies (Callahan, 1980; Arnaud & Welsch, 1980; Bonati *et al.*, 1982) which indicate that the primary step in the metabolism of caffeine is a 3 *N*-demethylation yielding 1,7-dimethylxanthine (paraxanthine). The principal urinary product of caffeine is 1-methyluric acid resulting from 7 *N*-demethylation of paraxanthine followed by 8 oxidation; unchanged caffeine accounts for only 1–3% of the total administered dose.

One of the objectives of the study was to evaluate the quantitative EEG as an empirical

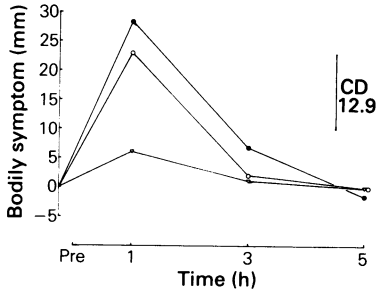

Figure 7 Mean bodily symptom rating for 'shaking or trembling' in nine subjects after placebo (▽), 250 mg caffeine (○), and 500 mg caffeine (●). The 0.05 critical difference is shown (see **Figure 4a**).

dose- and time-marker of the putative stimulant effects of caffeine. The slow-wave (theta) activity showed a highly significant dose-related decrease, according with a stimulant action. The alpha decrease, although again consistent with a stimulant action was not dose related, and the fast-wave (beta) activity showed consistently paradoxical effects. It could be postulated that two effects are impinging on fast-wave activity, a direct (but dose-limited) effect of caffeine lowering EEG power as with the other wave-bands, and an indirect (dose-related) effect related to a stimulant action which would increase beta. Similar complex effects are sometimes seen in the opposite directions with sedatives (e.g. premazepam, Golombok & Lader, 1984). In practical terms, the theta waveband provides the best empirical concomitant of caffeine's action on the brain.

The failure to show a change in the blood pressure contrasts with Robertson *et al.* (1978), who showed a rise of 14/10 mm Hg 1 h after a single dose of 250 mg of caffeine. However Robertsons' subjects did not normally take caffeine, and the mean plasma caffeine concentration at 1 h after ingestion was approximately 12 $\mu g\ ml^{-1}$, in excess of levels obtained in this experiment with twice the dose.

The accelerometer measures of tremor, and the body symptom analogue of degree of severe shaking and trembling, were both increased, but only the symptom scale showed significant changes. This suggests that the tremulousness is mainly subjective in nature.

None of the psychological tests showed significant effects. They are well-established tests, with known sensitivity to the depressant effects of a wide range of psychotropic drugs. However, our normal subjects are usually well-motivated and are performing the tests at their maximum capabilities, leaving no room for drug-induced

improvement. Caffeine will counter the fall-off in performance in repetitive tasks, and longer term tasks such as a standard vigilance task should be much more sensitive.

An increase in 'arousal' was indicated by the mean skin conductance measures in accord with caffeine's stimulant effects (Veleber & Templer, 1984). The self rating mood scale also demonstrated an alerting effect with caffeine, subjects feeling more alert, energetic, quick witted, and attentive. Self rating bodily symptoms also indicated less tiredness. This finding illustrates the complexities of studying a drug which is so widely taken in effective and sometimes almost toxic doses (Wells, 1984). The prior withdrawal must be prolonged before true base-line readings can be attained. After shorter (but practicable) withdrawal periods, any effects may be a composite of suppression of withdrawal and superadded direct effects. The present study, essentially of a pilot nature, establishes the suitability of the test battery for further investigation of this complex drug-subject interaction.

The state anxiety inventory in normal subjects has previously been shown not to distinguish high caffeine consumption (Boulenger & Uhde, 1982), and this study supports that finding.

The decrease in self rating bodily symptom of headache was expected. The subjects had abstained from caffeine for 24 h. The previously reported caffeine withdrawal headache (Greden *et al.*, 1980), was beginning to be experienced, and was relieved in those given caffeine.

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(Received 30 October 1985, accepted 4 March 1986)