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Caffeine-phenacetin interaction in the rat: effects on absorption, metabolism and locomotor activity

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The interactive effects of caffeine and phenacetin on the locomotor activity of the DA rat involved changes in absorption and metabolism as well as effects possibly exerted at the cns level. Phenacetin initially retarded the absorption of caffeine when coadministered by gavage but not when caffeine was given intraperitoneally and phenacetin orally. Phenacetin also increased the time for the plasma/caffeine concentration to peak, increased its peak concentration and prolonged its presence in the plasma. Urinary excretion patterns, suggested a blockade of the *N*-demethylation of caffeine by phenacetin. In contrast, caffeine had only a minor influence on the absorption and metabolism of phenacetin. The locomotor effects of the caffeine-phenacetin combination reflected the absorptive and metabolic interactions which occurred. Caffeine-induced hyperactivity was initially masked by phenacetin in a dose-dependent manner but after 2 h, when the plasma phenacetin concentrations were much lower, its retarding influence on caffeine metabolism became apparent and hyperactivity consequent upon an elevated plasma caffeine concentration was seen. Phenacetin also antagonized the hyperactive effects of theophylline and of (+)-amphetamine.

Until comparatively recently, aspirin-phenacetin-caffeine preparations accounted for most of the analgesic abuse within Australia (Stewart, McCarthy & others, 1975) and as this abuse may have arisen from interactive effects of the component drugs, we have examined the interactions of caffeine and phenacetin since they have both been shown to have marked effects on the cns (Eade & Lasagna, 1967; Prescott, 1971; Goodman & Gilman, 1975).

Plasma concentrations of caffeine and/or phenacetin were determined after their oral administration alone and together to the rat and a comparison was made with results obtained when caffeine was given intraperitoneally and phenacetin orally. The effects of phenacetin on the urinary excretion pattern of caffeine were also investigated. The cns effects of phenacetin and of caffeine and of its metabolites, theophylline and theobromine, were monitored by measuring locomotor activity and the modifying influence of phenacetin on caffeine-induced hyperactivity was compared with its effects on hyperactivity due to theophylline and to (+)-amphetamine.

MATERIALS AND METHODS

Drugs and animals. Drugs and mixtures of drugs which were administered by gavage were suspended in a 1 in 8 dilution of mucilage of tragacanth B.P.C. and the dose volume was 20 ml kg^{-1}. Drugs given intraperitoneally were dissolved in normal saline and given in a dose volume of 1 ml kg^{-1}. Caffeine was administered as the citrate, theophylline and theobromine as base and (+)-amphetamine as sulphate. Female rats of the DA strain, 180 ± 20 g, which were allowed free access to food and water up to the time of drug administration, were used.

Caffeine and phenacetin plasma concentrations. Caffeine and phenacetin in plasma were determined by gas-liquid chromatography (Grab & Reinstein, 1968). Standards were prepared using B.P. caffeine, phenacetin and barbitone which were further purified by recrystallization, m.p.s 238°, 134° and 180° respectively. Reagents used were: phosphate buffer (0·5 M pH 8·0), freshly distilled chloroform (b.p. 61°) and heparin solution B.P. (5000 units ml^{-1}). G.l.c. conditions were: column 1·9 m × 6 mm glass U-tube packed with 3% OV-17 on Gas-Chrom Q (800–100 mesh); carrier gas nitrogen, flow rate 40 ml min^{-1}; hydrogen and air flow rates 40 and 500 ml min^{-1}; operating temperatures: oven 200°, injection port 240° and detector 260°. Standard curves of the peak-height ratios of caffeine/barbitone versus caffeine concentration and phenacetin/barbitone versus phenacetin concentration were obtained by assaying prepared solutions of caffeine (0–10 mg $litre^{-1}$) and phenacetin (0–50 mg $litre^{-1}$).

Groups of 5 rats were decapitated (0·25–5 h after dosing and a 3 ml blood sample from each rat was collected in a beaker containing 50 μl of heparin

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solution, stored in an ice bath, centrifuged at 3000 rev min^{-1} and the plasma extracted within 60 min of sampling.

1 ml of plasma was pipetted into a 15 ml glass-stoppered Pyrex tube and 1 ml of phosphate buffer and 7 ml of chloroform containing barbitone (1·5 mg l^{-1}) were added. The tubes were agitated for 15 min and centrifuged for 5 min at 2000 rev min^{-1}, 5 ml of the chloroform layer was transferred to a centrifuge tube and the extract was evaporated to dryness under nitrogen at 50°. After cooling, the residue was dissolved in 50 μl of chloroform and an aliquot (2 μl) was chromatographed.

Recovery was determined by the addition of known amounts (0·5–20 μg in a phosphate buffer) of caffeine and phenacetin to plasma which was assayed as above. Recovery of both drugs from plasma was essentially complete and identical to that from aqueous solution.

The significance of the differences in plasma caffeine and phenacetin concentrations was assessed using Student's t-test.

Determination of caffeine, theophylline and theobromine in rat urine. Groups of 5 rats were housed in a metabolism cage situated in a constant environment (21° ± 1°; 12 h day/night cycle; white noise) for 24 h before drug administration. Urine was collected 3, 6 and 24 h after dosage and analysed for caffeine, theophylline and theobromine as follows: 3 ml of urine, or of standard solutions of drug or metabolites, was pipetted into a glass-stoppered test tube containing 1 ml of phosphate buffer (0·5 M, pH 8·0) and 7 ml of chloroform. The tubes were then agitated for 15 min and centrifuged at 2000 rev min^{-1} for 5 min. 5 ml of the organic layer was evaporated to dryness under nitrogen at 50°, cooled, and the residue was dissolved in 50 μl of 95% ethanol and 30 μl aliquots were applied to thin-layer (t.l.c.) glass plates coated with silica gel (GF-254, type 60). The developing solvent was a mixture of ethyl acetate–acetone–chloroform–concentrated ammonium hydroxide solution 100:10:5:4·5). The compounds were located under ultraviolet light, scraped off the plates and eluted with absolute ethanol and the absorption determined at the absorption maximum for caffeine, theophylline or theobromine (273, 272 and 270 nm respectively). Calibration curves were obtained from standards run on the same t.l.c. plate (10, 30 and 50 μg ml^{-1}).

Locomotor activity studies. The activity area was a high-walled circular chamber 450 mm in diameter with four sets of photo cell/light beam circuits, operating in the near infrared, inset symmetrically into the wall 20 mm from the base of the chamber giving a square network of light beams crossing the base on 20 mm centres. Interruption of a beam initiated a 10 ms pulse which operated a summing switching circuit for each interruption in such a manner that co-incident breaks in beams were additive. The output of the summer was integrated and recordings were made on a polygraph. The activity chamber was situated in the same constant environment described for the metabolism studies.

Female rats in groups of 3 were housed together for at least one week before recordings were made. Immediately after receiving treatment the animals were placed in the chamber and their activity monitored for 20 h (except those rats receiving (+)-amphetamine + phenacetin or relevant controls, where activity was measured for only 1 h). The significance of differences among the mean activity counts for each hourly period was assessed using Student's t-test.

RESULTS

Caffeine and phenacetin plasma concentrations (Table 1). A peak plasma concentration occurred within 1 h of oral administration of caffeine (5 mg kg^{-1}), this declined to a low level within 4 h. When the same dose was given intraperitoneally the peak occurred within 0·5 h but the rate of decline was approximately the same.

Phenacetin was also absorbed readily from the gut, the highest plasma concentrations occurring within 1 h after the 50 and 200 mg kg^{-1} doses. At the lower dose, substantial elimination from the plasma took place within 2 h but an equivalent fall was not reached until 5 h after the higher dose.

When caffeine (5 mg kg^{-1}) and phenacetin (50 mg kg^{-1}) were given together by gavage, the plasma caffeine concentrations were slightly lower in the first hour than when the caffeine was given alone. After 2 h, however, the caffeine concentrations were significantly higher ($P<0·01$ at 2 and 3 h; $P<0·05$ at 4 h). With 200 mg kg^{-1} phenacetin the effect was more marked, a significant ($P<0·01$) reduction in plasma caffeine occuring at 0·25 h and a significant ($P<0·01$) elevation between 2 and 4 h. The peak was also significantly ($P<0·05$) higher. When caffeine was given intraperitoneally plasma caffeine concentrations were not reduced initially, but they rose later in the experiment.

The plasma phenacetin concentrations after 50 mg kg^{-1} were not affected by the co-administration of caffeine (5 mg kg^{-1}). At 200 mg kg^{-1}, how-

Table 1. *Mean plasma caffeine and/or phenacetin concentrations ± s.e. attained after administration of caffeine and phenacetin alone and in combination to the DA rat. Each result is the mean from 5 animals.*

Drug, dose (mg kg⁻¹) and route of administration	Mean plasma concn (mg litre⁻¹) ± s.e.												
	Caffeine							Phenacetin					
	0·25 h	0·5 h	1 h	2 h	3 h	4 h	5 h	0·5 h	1 h	2 h	3 h	4 h	5 h
Caffeine (5, oral)	2·60 ± 0·13	3·09 ± 0·23	2·66 ± 0·38	1·00 ± 0·10	1·25 ± 0·28	0·30 ± 0·15	0·60 ± 0·32	—	—	—	—	—	—
Caffeine (5, i.p.)	4·96 ± 0·88	3·11 ± 0·07	2·64 ± 0·32	1·27 ± 0·24	0·30 ± 0·94	—	—	—	—	—	—	—	—
Phenacetin (50, oral)	—	—	—	—	—	—	—	16·28 ± 2·76	9·73 ± 1·48	2·56 ± 0·61	—	—	—
Phenacetin (200, oral)	—	—	—	—	—	—	—	34·28 ± 1·85	29·78 ± 1·87	22·72 ± 3·94	17·04 ± 4·16	8·76 ± 2·49	2·40 ± 1·07
Caffeine (5, oral) + phenacetin (50, oral)	2·24 ± 0·34	2·42 ± 0·34	2·62 ± 0·17	2·86 ± 0·15	2·73 ± 0·21	1·19 ± 0·24	1·06 ± 0·15	13·70 ± 1·91	11·56 ± 1·88	1·80 ± 0·33	—	—	—
Caffeine (5, oral) + phenacetin (200, oral)	0·84 ± 0·08	2·84 ± 0·28	3·14 ± 0·28	3·76 ± 0·18	3·47 ± 0·28	1·73 ± 0·51	0·46 ± 0·03	30·12 ± 1·61	20·06 ± 3·93	19·44 ± 1·49	4·44 1·84	1·33 0·59	0·36 0·06
Caffeine (5, i.p.) + phenacetin (200, oral)	4·03 ± 0·27	4·24 ± 0·53	4·48 ± 0·34	4·28 ± 0·80	3·65 ± 0·49	—	—	—	—	—	—	—	—

ever, plasma phenacetin concentrations were lower 3 h after being given with caffeine ($P<0·05$ at 3 and 4 h; $P<0·1$ at 5 h).

Urinary excretion of caffeine, theophylline and theobromine (Table 2). When caffeine was given alone, only small quantities were detected as such in the urine but after co-administration with phenacetin (200 mg kg⁻¹), significantly ($P<0·01$) greater amounts were excreted during the first 6 h. The proportions of the caffeine dose excreted as theophylline and theobromine were concomitantly reduced ($P<0·05$) by phenacetin until after 6 h when an increase in the excretion of the metabolites occurred, so that their total recovery over 24 h was essentially similar to that from rats given caffeine alone.

Table 2. *Urinary excretion of caffeine, theophylline and theobromine by the female DA rat after oral administration of caffeine (5 mg kg⁻¹), with and without phenacetin (200 mg kg⁻¹).* Each result is the mean of 5 experiments on groups of 5 animals.

Urinary excretion of caffeine (µg ± s.e.)	Drug treatment	Caffeine	Caffeine + phenacetin
Caffeine	0–3 h	21 ± 6**	102 ± 17
	3–6 h	0 **	54 ± 8
	6–24 h	11 ± 7	45 ± 22
Theophylline	0–3 h	114 ± 12**	19 ± 8
	3–6 h	105 ± 15**	38 ± 10
	6–24 h	199 ± 45	336 ± 79
Theobromine	0–3 h	55 ± 6**	0
	3–6 h	85 ± 21*	14 ± 6
	6–24 h	112 ± 24	162 ± 23

* $P<0·05$. ** $P<0·01$.

Locomotor activity (Table 3). There was a non-significant tendency towards depression of activity after doses of phenacetin of 50 and 100 mg kg⁻¹ but at 200 mg kg⁻¹ a significant depression of activity occurred 0–1 h, $P<0·05$).

Caffeine (5 mg kg⁻¹) caused a significant ($P<0·01$) increase in locomotor activity only for the first 2 h. Theophylline (4·5 mg kg⁻¹) behaved similarly but theobromine (4·5 mg kg⁻¹) was devoid of cns stimulant activity.

The locomotor effects of caffeine were modified by the concurrent administration of phenacetin in two ways. Firstly, the initial 1 h period of stimulation was partially masked, the differences in activity between animals receiving only caffeine and those also given phenacetin at 100 and 200 mg kg⁻¹ being significant ($P<0·05$).

The second effect was a prolongation of the period of hyperactivity compared with controls. A comparison of the activity counts of groups treated with caffeine and caffeine plus phenacetin (200 mg kg⁻¹)

Table 3. *The mean locomotor activity* (± *s.e.*) *of female DA rats recorded during the first 5-hourly intervals after drug administration.*

Drug dose (mg kg^{-1})*	No. of experiments	Mean activity count (± s.e.) Time after drug administration (h) → 0–1	1–2	2–3	3–4	4–5
Suspending agent	15	1594 ± 143	485 ± 60	362 ± 64	257 ± 54	234 ± 47
Phenacetin 50	8	1572 ± 242	415 ± 105	366 ± 82	261 ± 64	281 ± 61
Phenacetin 100	7	1472 ± 106	447 ± 70	449 ± 70	312 ± 71	302 ± 74
Phenacetin 200	8	1227 ± 53	311 ± 62	358 ± 103	309 ± 68	210 ± 49
Caffeine 5	13	2684 ± 285	1097 ± 172	384 ± 64	238 ± 53	196 ± 49
Phenacetin 50 + caffeine 5	8	2371 ± 207	1508 ± 157	710 ± 164	446 ± 74	173 ± 52
Phenacetin 100 + caffeine 5	10	1913 ± 126	827 ± 94	920 ± 148	627 ± 181	348 ± 93
Phenacetin 200 + caffeine 5	9	1548 ± 99	812 ± 104	900 ± 138	652 ± 117	474 ± 83
Theophylline 4·5	6	2191 ± 218	901 ± 77	496 ± 92	332 ± 69	167 ± 36
Theobromine 4·75	3	1509 ± 18	399 ± 66	262 ± 43	387 ± 135	517 ± 219
Phenacetin 200 + theophylline 4·5	6	1100 ± 78	497 ● 87	433 ± 65	415 ± 86	349 ± 62
(+)-Amphetamine 0·25 i.p. + suspending agent	4	2034 ± 282				
(+)-Amphetamine 0·25 i.p. + phenacetin 200	4	1320 ± 231				
Saline i.p. + suspending agent	4	1150 ± 143				

* Oral route except where indicated.

showed that significant ($P<0{\cdot}01$) elevation occurred 2–5 h after dosage. These counts were also significantly ($P<0{\cdot}01$) elevated above control values. The combination of phenacetin 100 and 50 mg kg^{-1} with a fixed dose of caffeine produced similar but progressively less marked effects.

The increase in locomotor activity above control values induced by theophylline (4·5 mg kg^{-1}) was significantly ($P<0{\cdot}01$) depressed by phenacetin (200 mg kg^{-1}) during the first 2 h but significant ($P<0{\cdot}05$) hyperactivity was not apparent until the fourth hour. (+)-Amphetamine (0·25 mg kg^{-1}) hyperactivity was also significantly ($P<0{\cdot}05$) reduced by phenacetin (200 mg kg^{-1}) during the first h after their co-administration.

DISCUSSION

From the results it is apparent that the locomotor effects of phenacetin and caffeine in the rat are much modified when they are administered together but the interaction is not simple. Caffeine citrate was almost as rapidly absorbed from the gastrointestinal tract as from the peritoneum, and phenacetin was also absorbed readily after oral administration. When the drugs were given concurrently the plasma concentrations and the rates of accumulation and decline of both drugs were altered. Firstly, the plasma caffeine concentration attained 15 min after oral administration of caffeine with phenacetin (200 mg kg^{-1}) was significantly lower than when the same dose of caffeine was given alone. This difference did not occur when caffeine was given intraperitoneally, indicating an absorptive interaction.

The initial delay in absorption of caffeine when given with phenacetin may be the result of complexation in the stomach and/or an increase in gastric emptying time, since caffeine is known to form complexes with salicylic acid, aspirin (Goto, Takamatsu & others, 1968) and paracetamol (Chow & Repta, 1972). The effect of caffeine complexation on absorption, appears to be greatest on drugs which are well absorbed (Goto & others, 1968; Reuning & Levy, 1968). Both phenacetin (Coldwell, Solomonraj & Thomas, 1974) and caffeine (Siegers, 1973) delay the absorption of aspirin and paracetamol; effects attributed in each case to an increase in gastric emptying time.

A second and more marked effect was that the time taken for the plasma caffeine concentrations to reach their peak was increased from about 0·5 h to about 2 h when the drugs were given together, by gavage.

Thirdly, high concentrations of caffeine remained in the plasma up to 4 h after being given orally with phenacetin, whereas they had declined to a low level within 2 h when the same dose of caffeine was given alone. This effect of phenacetin appeared to be dose-dependent and to be post-absorptive since the route did not affect the results.

The overall effects of phenacetin may therefore be considered to both increase the concentration and prolong the presence of caffeine in the plasma. By contrast, caffeine appeared to have a relatively

minor influence on plasma phenacetin concentrations. That the post-absorptive effect of phenacetin may be due to competitive inhibition of the metabolism of caffeine was further confirmed by monitoring the urinary output of caffeine and two of its metabolites, theophylline and theobromine, in the rat (Khanna, Rao & Cornish, 1972). The urine excretion patterns were consistent with the plasma concentration data in that during the first 6 h significantly greater amounts of caffeine appeared in the urine after both drugs than when the same dose of caffeine was given alone. The urinary output of the metabolites was also significantly reduced during this period. After 6 h, however, when phenacetin had almost entirely disappeared from the plasma, excretion of the metabolites increased so that their total recovery over 24 h was essentially unchanged. There were no significant changes in urinary pH or flow (Collins, Richards & Starmer—in preparation). These results thus suggest a blockade of the *N*-demethylation of caffeine by phenacetin. Acute phenacetin hepatotoxicity was considered to be unlikely since paracetamol (200 mg kg^{-1}) had no effect on the metabolism of caffeine (Collins, Richards & Starmer—in preparation).

The locomotor effects of the caffeine-phenacetin combination reflected the absorptive and metabolic interactions which occurred. The cns-stimulant effects of a small dose of caffeine were clearly demonstrable and phenacetin alone caused some locomotor depression which reached significance at 200 mg kg^{-1} for the first h, a finding consistent with that of Eade & Lasagna (1967). Initially, phenacetin appeared to cause a dose-dependent antagonism of caffeine-induced hyperactivity. The initial stimulant effect of theophylline was also antagonized by phenacetin. A non-specific depressant effect was perhaps operative since phenacetin was also shown to significantly reduce the increase in locomotor activity due to (+)-amphetamine which is in conflict with the views cited by Eade & Lasagna (1967) that phenacetin has amphetamine-like effects.

After 2 h the locomotor activity of rats receiving caffeine and phenacetin was much higher than that of rats given caffeine or vehicle. This hyperactivity was dose-dependent for phenacetin and is consistent with an elevated plasma caffeine concentration and a much lowered plasma phenacetin concentration.

It was concluded that the initial effects are physiological antagonism between caffeine and phenacetin and possibly reduced caffeine absorption. After 2 h, however, when phenacetin plasma concentrations were much lower, the retarding influence of phenacetin on caffeine metabolism became apparent and the hyperactivity consequent upon an elevated plasma caffeine concentration was unmasked.

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