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Aspirin-caffeine interaction in the rat

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Both aspirin at a high dose (400 mg kg^{-1}) and caffeine (5 mg kg^{-1}) induced hyperactivity in the DA rat, but lower doses of aspirin were without effect. Caffeine-induced hyperactivity was brief (2 h) but that due to aspirin was evident from 1–6 h after dosing. Co-administration of the two drugs caused long-lasting hyperactivity, even with doses of aspirin which had no stimulant effects themselves. Absorptive and metabolic effects did not appear to play a major role in the interaction. The most likely effect is that of salicylate on catecholamine utilization in the central nervous system, which is compounded in the presence of a phosphodiesterase inhibitor.

Because analgesic mixtures (notably aspirin-phenacetin-caffeine) and not single substance formulations have accounted for most of the analgesic abuse within Australia (Stewart et al 1975), we have examined the interactive effects of the component drugs in an attempt to find an explanation.

Phenacetin and caffeine were shown (Collins et al 1977) to have complex interactive effects in rats, involving both absorption and metabolism as well as physiological antagonism. In this paper the interactive effects of aspirin and caffeine in the rat, are reported.

Plasma concentrations of caffeine and/or salicylate were determined after their oral administration to the rat, alone and in combination, and central nervous system (c.n.s.) effects were monitored by measuring locomotor activity.

MATERIALS AND METHODS

Drugs and animals. Drugs and mixtures of drugs were suspended in a 1 in 8 dilution of mucilage of tragacanth B.P.C. and the dose volume was 20 ml kg^{-1}. Caffeine was administered as the citrate. 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.

Locomotor activity studies. The apparatus and methodology used has been described previously (Collins et al 1977). A square root transformation of activity counts (Steel & Torrie 1960) was made and the significance of differences among the treatment means for each hourly period was assessed using Student's *t*-test.

Caffeine and salicylate plasma concentrations. Plasma caffeine concentrations were measured by the method of Collins et al (1977).

Total salicylate concentrations in brain or plasma were determined by methods based on those of Rowland & Riegelman (1967) and Levy & Procknal (1968). Groups of 5 rats were decapitated (0·5–8 h) after dosing and a 3 ml blood sample was collected from each rat in a beaker containing 50 μl of heparin solution B.P. (5000 units ml^{-1}) stored in an ice bath, centrifuged at 3000 rev min^{-1} and the plasma extracted within 5 min of sampling. Whole brain was homogenized in a 5 ml Potter homogenizer at $\gtrsim$4 °C with 1 ml 0·416 M sodium hydrogen sulphate solution for 1 min, the slurry was decanted and the process repeated with a second 1 ml aliquot. The combined brain homogenate or 0·5 ml plasma to which 1 ml of the sodium hydrogen sulphate solution had been added, was placed in a 15 ml glass stoppered Pyrex tube together with 7 ml of anaesthetic ether B.P.C. The tube was then agitated for 15 min, centrifuged at 3000 rev min^{-1} for 5 min after which the ether layer was removed, placed in another 15 ml Pyrex tube and evaporated to dryness under a stream of dry nitrogen at 40 °C. The residue was dissolved in 7 ml of carbon tetrachloride and 3 ml of dilute ferric nitrate reagent (Levy & Procknal 1968) was added. The tube was then agitated for 15 min and centrifuged for 5 min at 3000 rev min^{-1}. The absorbance of the aqueous layer was determined at 540 nm using a Varian Techtron u.v.-visible spectrophotometer (Model 635). Salicylate concentrations were read from a standard curve prepared from solutions of known salicylic acid concentration subjected to the above procedure.

The significance of differences in mean plasma caffeine and salicylate concentrations was assessed using Student's *t*-test.

RESULTS

Locomotor activity studies (Table 1). Caffeine (5 mg kg^{-1}) induced a significant ($P < 0.001$) increase in

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Table 1. The mean locomotor activity Δ (±s.e.) of female DA rats recorded during the first 8-hourly intervals after drug administration in mg kg^{-1}

	Mean activity count Δ (± s.e.) Time after drug administration (h)							
	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8
Suspending agent (n = 22)	36·86 ± 1·33	22·34 ± 0·90	19·48 ± 1·17	15·89 ± 1·02	14·96 ± 0·92	13·50 ± 0·91	14·18 ± 1·21	15·59 ± 1·02
Aspirin 50 (n = 15)	39·64 ± 1·25	21·57 ± 1·20	20·88 ± 1·29	17·43 ± 1·37	14·96 ± 1·06	13·38 ± 1·21	14·39 ± 1·51	15·20 ± 0·99
Aspirin 100 (n = 17)	39·32 ± 1·24	22·70 ± 1·10	20·35 ± 1·15	19·81 ± 1·21	14·63 ± 1·25	18·00 ± 1·13	12·70 ± 1·00	14·89 ± 1·55
Aspirin 200 (n = 15)	39·73 ± 1·62	23·64 ± 1·36	19·24 ± 1·33	18·49 ± 1·42	15·43 ± 1·63	14·07 ± 1·56	16·38 ± 1·20	12·74 ± 0·88
Aspirin 400 (n = 19)	41·21 ± 1·73	28·14 ± 1·96	24·99 ± 1·35	21·68 ± 1·42	18·28 ± 1·43	18·19 ± 1·67	15·03 ± 1·18	16·68 ± 1·40
Caffeine 5 (n = 24)	49·03 ± 1·84	30·66 ± 1·83	20·03 ± 1·33	14·93 ± 1·14	14·50 ± 1·06	13·87 ± 1·06	14·55 ± 1·02	16·41 ± 1·37
Aspirin 50 + caffeine 5 (n = 25)	47·67 ± 1·17	29·80 ± 1·22	23·34 ± 0·96	18·91 ± 1·19	16·53 ± 1·12	15·16 ± 0·97	14·78 ± 0·92	17·09 ± 1·36
Aspirin 100 + caffeine 5 (n = 17)	49·42 ± 2·28	32·73 ± 1·88	22·99 ± 1·13	19·66 ± 1·44	18·35 ± 1·37	18·44 ± 1·25	18·52 ± 1·54	17·88 ± 0·96
Aspirin 200 + caffeine 5 (n = 16)	50·16 ± 2·81	35·80 ± 2·69	25·10 ± 2·16	18·15 ± 1·21	18·11 ± 0·99	17·44 ± 1·54	17·78 ± 1·25	17·81 ± 1·25
Aspirin 400 + caffeine 5 (n = 17)	46·84 ± 2·00	35·55 ± 2·32	29·54 ± 2·04	24·00 ± 1·58	22·09 ± 1·57	20·45 ± 1·58	16·81 ± 1·29	16·01 ± 1·24

Δ expressed as the square root transformation.

locomotor activity only during the first 2 h after administration and differences from controls were non-significant thereafter.

The highest dose of aspirin used (400 mg kg^{-1}) induced stimulation compared with controls ($P < 0.01$ at 2–4 h; $P < 0.05$ at 1–2 and 5–6 h). Lower doses (200 and 50 mg kg^{-1}) had no consistent stimulant effect although after the 100 mg kg^{-1} dose increased activity was apparent at 3–4 h and 5–6 h ($P < 0.05$).

All the aspirin-caffeine mixtures induced hyperactivity for the first 2 h after administration ($P < 0.001$), an effect essentially similar to that seen after caffeine alone. Longer lasting and more marked locomotor stimulation was observed after aspirin (400 mg kg^{-1}) plus caffeine ($P < 0.001$ at 0–5 h; $P < 0.01$ at 5–6 h). Hyperactivity also resulted when lower doses of aspirin which had no significant effect when given alone, were co-administered with caffeine. Locomotor stimulation extended to the seventh hour with the 100 and 200 mg kg^{-1} doses of aspirin ($P < 0.05$) and to the third hour with the 50 mg kg^{-1} dose ($P < 0.05$).

Salicylate plasma concentrations (Table 2). Co-administration of caffeine (5 mg kg^{-1}) had no consistent effect on the plasma salicylate concentrations attained. In all cases, peak plasma values occurred within 2 h and declined thereafter. After both the 100 and 200 mg kg^{-1} doses of aspirin, plasma salicylate concentrations were significantly ($P < 0.01$) elevated at 4 h in animals which also received caffeine. But at 1 h, plasma salicylate concentrations were higher in animals which received aspirin (400 mg kg^{-1}) alone.

Table 2. Mean plasma salicylate concentration ± s.e. attained after oral administration of aspirin alone or in combination with caffeine (doses in mg kg^{-1}) to the DA rat. Each result is the mean from 5 animals.

	Mean plasma concentration (mg litre^{-1}) Time after drug administration (h) →					
	0·5	1	2	4	6	8
Aspirin (100)	235·07 ±12·63	250·48 ±10·95	181·45 ±16·25	180·32 ±11·74	152·52 ±12·55	131·83 ±11·59
Aspirin (200)	342·48 ±13·56	308·24 ±15·46	326·74 ±15·95	290·16 ± 4·87	317·58 ±13·42	268·55 ±14·80
Aspirin (400)	525·33 ±20·24	556·05 ±10·11	427·49 ±23·88	389·15 ± 9·89	311·76 ±12·42	359·20 ±18·15
Aspirin (100) + caffeine (5)	238·36 ± 8·95	279·72 ±15·89	200·07 ±10·86	253·67 ±17·11	157·36 ±16·18	117·94 ±10·91
Aspirin (200) + caffeine (5)	375·49 ±20·76	341·71 ±15·81	378·70 ± 8·38	358·41 ±17·83	315·20 ±13·93	237·33 ± 5·02
Aspirin (400) + caffeine (5)	485·99 ±19·23	506·92 ± 9·09	497·47 ±26·61	419·14 ±21·53	393·61 ±50·24	326·42 ±17·49

Brain salicylate concentrations. The mean whole brain plasma salicylate concentration attained 5·5 h after aspirin (200 mg kg^{-1}) was 22·81 ± 1·86 (s.e.; n = 10) μg g^{-1} of tissue and was not significantly modified by the co-administration of caffeine (23·63 ± 1·43 μg g^{-1}).

Caffeine plasma concentrations (Table 3). The concomitant administration of aspirin almost invariably lowered plasma caffeine concentrations. Differences from caffeine controls only reached significance ($P < 0.05$) however, with the two higher doses (200 and 400 mg kg^{-1}) of aspirin at 0·5 h and the lowest dose (100 mg kg^{-1}) at 4 h.

DISCUSSION

Previously (Collins et al 1977), it was shown that, in the rat, both absorptive and metabolic interactions occur between caffeine and phenacetin which were

Table 3. Mean plasma caffeine concentration ± s.e. attained after administration of caffeine alone and in combination with aspirin (doses in mg kg^{-1}) to the DA rat. Each result is the mean from five animals.

Mean plasma concentration (mg $^{1-1}$) ± s.e. Time after drug administration (h) →				
0·5	1	2	3	4
Caffeine (5)				
2·40 ±0·50	2·40 ±0·43	1·46 ±0·37	0·82 ±0·13	0·46 ±0·05
Caffeine (5) + aspirin (100)				
2·30 ±0·43	2·09 ±0·39	1·58 ±0·33	0·49 ±0·14	0·19 ±0·10
Caffeine (5) + aspirin (200)				
1·14 ±0·11	1·53 ±0·44	0·81 ±0·25	0·43 ±0·14	0·38 ±0·11
Caffeine (5) + aspirin (400)				
1·09 ±0·04	1·47 ±0·15	0·80 ±0·15	0·65 ±0·24	0·44 ±0·11

reflected in the locomotor effects of the drug combination. Here, evidence is presented that two other components of the APC mixture—aspirin and caffeine—also have a potential for interaction.

A short (2 h) period of hyperactivity was observed after caffeine (5 mg kg^{-1}) which is in accord with our previous experience with this strain of rat (Collins et al 1977). The lowest doses of aspirin (50, 100 and 200 mg kg^{-1}) had no marked effects on locomotor activity. When the dose was increased to 400 mg kg^{-1}, however, hyperactivity was apparent after the first hour and continued for a further 5 h. When aspirin and caffeine were given together, an initial stimulant effect, which was not significantly different from that induced by caffeine alone, was observed. Hyperactivity was prolonged in the presence of aspirin, however, even with doses which had no stimulant effect when given alone.

Neither absorptive nor metabolic interactions appear to provide an explanation for the effects of aspirin-caffeine combinations on locomotor activity. The peak concentration of caffeine was not increased by the co-administration of aspirin, nor was its presence prolonged. Moreover, although the two highest doses of aspirin (200 and 400 mg kg^{-1}) caused an initial delay in the absorption of caffeine, hyperactivity was not significantly reduced. The plasma salicylate concentrations were also largely unaffected by the addition of caffeine to the aspirin dose, a finding in good agreement with that of Vinegar et al (1976). Although both an increase in locomotor activity and elevated plasma salicylate concentrations were found 4 h after caffeine was given together with aspirin (100 and 200 mg kg^{-1}), it was considered unlikely to represent a cause-and-effect relationship since the salicylate concentrations attained were much lower than those shown to be associated with hyperactivity.

Aspirin-induced locomotor stimulation has not been reported previously. Since locomotor activity has been shown to involve central monoaminergic—especially dopaminergic—pathways (Svensson & Waldeck 1970), it is likely that here, the effects of aspirin will ultimately be exerted. Paalzow (1973) showed that salicylic acid, to which aspirin is rapidly metabolized (Levy & Leonards 1966), augmented the rate of disappearance of noradenaline and dopamine from mouse brain following the administration of α-methyl-*p*-tyrosine. This was considered to be suggestive of increased catecholamine utilization in the presence of salicylate and lends support to the above concept. If a phosphodiesterase inhibitor, such as caffeine, were also present, it is possible that a salicylate effect on catecholamine utilization might be augmented and appear with lower doses of aspirin, as was found in these experiments.

A delay of 1 h was observed before aspirin-induced hyperactivity became apparent which may be indicative of an indirect effect. Laborit et al (1975) have shown that arachidonic acid, which is the precursor of a number of prostaglandins, reduced the locomotor activity of mice. Pretreatment with aspirin, which is a prostaglandin synthetase inhibitor (Flower et al 1972), was found to antagonize this effect and it was proposed that prostaglandins might be modulators of central noradrenergic function. Thus, a high dose of aspirin might induce locomotor stimulation by such a mechanism.

Alternatively, it might be postulated that in the presence of caffeine, increased concentrations of salicylate might occur in the c.n.s. because of effects on the passive uptake and/or the active removal systems (Spector & Lorenzo 1973). However, since brain salicylate concentrations measured 5·5 h after drug administration (when hyperactivity was apparent) were not affected by caffeine co-administration, the former explanation is a more likely one.

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