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INTERACTION OF COCAINE WITH CHLORDIAZEPOXIDE ASSESSED BY MOTOR ACTIVITY IN MICE

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1 Effects of a range of doses of cocaine and chlordiazepoxide given separately and as mixtures were determined on the spontaneous locomotor activity of mice.

2 Cocaine increased locomotor activity (walking) during 3 or 5 min trials in a dose-related manner.

3 Chlordiazepoxide had little effect on the total amount of locomotor activity except for depression at very high doses. A lower dose of chlordiazepoxide increased activity at the beginning of the trials only.

4 Mixtures containing certain doses of cocaine and chlordiazepoxide increased locomotor activity to a much greater extent than cocaine alone. This high level of activity was manifested throughout 5 min trials.

5 This action of cocaine is similar to that of amphetamine.

Introduction

The behavioural effects of cocaine have been studied less intensively than those of the synthetic central nervous system stimulants such as amphetamine. In common with amphetamines, cocaine can facilitate locomotor activity and induce stereotyped behaviours, can either increase or decrease rates of operant responding, and can serve as a powerful positive reinforcer (Woods & Downs, 1973). There is also evidence that cocaine can substitute for amphetamine as a discriminative stimulus in rats trained to respond differentially according to their drug state (Huang & Ho, 1974). However, cocaine has often been found to be less potent and to have a shorter duration of action than amphetamine, and it may also differ in its interactions with iproniazid, α-methyltyrosine and haloperidol (Smith, 1965; Simon, Sultan, Chermat & Boissier, 1972). Amphetamine can induce rotational behaviour in rats with unilateral lesions of the nigro-striatal dopamine pathway, but cocaine does so only after the administration of a monoamine oxidase inhibitor (Christie & Crow, 1973).

Experiments have been carried out to examine further the behavioural profile of action of cocaine by testing its interaction with chlordiazepoxide. When rodents are placed in an unfamiliar environment, mixtures of amphetamine with chlordiazepoxide or barbiturates can induce much more locomotor activity (walking) than the constituent drugs given separately (Rushton & Steinberg, 1963, 1966; Rushton, Steinberg & Tomkiewicz, 1973). A failure to find hyperactivity produced by mixtures of cocaine and chlordiazepoxide would add support to the view that there are significant differences between the actions of cocaine and amphetamine.

Methods

Animals

Female albino mice weighing 16–25 g were used throughout. They were housed in colony cages containing 8–10 mice and had unlimited access to food and water.

Locomotor activity

A mouse was placed in a rectangular chamber (26 × 18 × 10 cm) constructed of black Perspex, with a clear Perspex lid. Test chambers with dimensions of this order can elicit an activity level sufficiently low for further facilitation by drugs to be clearly demonstrable (Kršiak & Janků, 1971). The chamber was illuminated by a 60 W lamp at a height of 1 metre. The amount of time spent walking was recorded cumulatively on an electronic timer, directly controlled by an observer. Rearing onto the hind feet was not included. The duration of the trial was either 5 min (experiment 1) or 3 min (experiments 2 and 3) and each mouse was used once only. In experiment 1 only, the activity scores were also printed automatically every minute. Inter-observer correlations were determined on several occasions and were considered to indicate a satisfactory degree of reproducibility (e.g. $r=0.94$, d.f. 14, $P<0.001$).

Experiment 1

Thirty mice were allocated to 4 treatment groups by a randomization procedure ($n=7-8$). The groups were injected with either cocaine (20 mg/kg), chlordiazepoxide (15 mg/kg), a mixture of both drugs, or isotonic saline. These doses were selected on the basis of previous work with the drugs given separately (e.g. Rushton *et al.*, 1973; Christie & Crow, 1973).

Experiment 2

In order to test a range of doses of cocaine and chlordiazepoxide, 80 mice were allocated to 10 groups by a randomization procedure ($n=8$). Three groups received cocaine (7.5, 15.0 or 30.0 mg/kg), 3 groups received chlordiazepoxide (10.0, 20.0 or 40.0 mg/kg), and 3 groups received mixtures of both drugs such that the ratio of the doses (cocaine:chlordiazepoxide) was held constant at 0.75:1. The tenth group received isotonic saline.

Experiment 3

In order to test a wider range of doses and dose-ratios, 250 mice were allocated to 25 groups by a randomization procedure ($n=10$). A range of doses of cocaine (4.0, 8.0, 16.0 or 32.0 mg/kg) and chlordiazepoxide (4.0, 8.0, 16.0 or 32.0 mg/kg) were studied when administered separately and in all combinations. The ratio of cocaine:chlordiazepoxide in the mixtures studied varied therefore from 0.125:1 to 8.0:1. One group of mice received isotonic saline.

Drugs

Cocaine hydrochloride (B.P.) or chlordiazepoxide hydrochloride (Librium, Roche) were dissolved in saline and injected intraperitoneally in a volume of 0.2 ml per mouse 20 min before locomotor activity was assessed. Mixtures of cocaine and chlordiazepoxide were given in a single injection and all doses were calculated as salts.

Statistical analysis

The results were analysed by single factor and two factor analyses of variance, and by Dunnett's t test for multiple comparisons with a control group (Winer, 1971). Locomotor activity scores were subjected to square root transformation to stabilize variances (cf. Kršiak, Steinberg & Stolerman, 1970).

Results

Experiment 1

The mean motor activity scores for control mice receiving saline declined slightly but not significantly

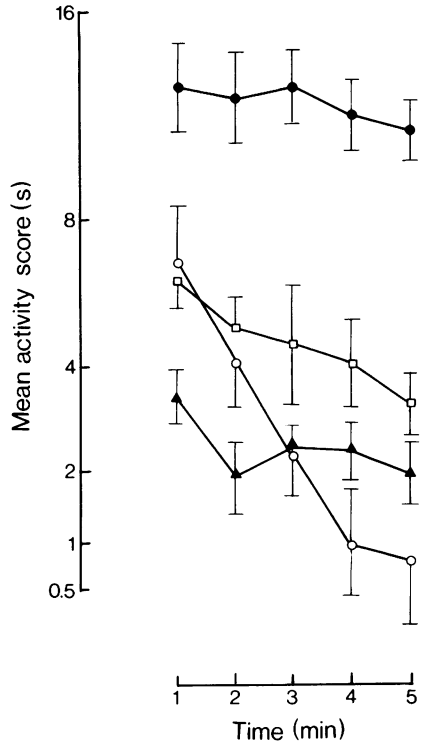

Figure 1 Activity of mice influenced by cocaine (20 mg/kg, □) and chlordiazepoxide (15 mg/kg, ○) given separately and as a mixture (●). Each activity score is the mean for 7–8 mice and the vertical bars indicate one standard error on each side of the mean (square root scale). Some bars have been omitted for clarity. Controls received saline (▲).

during the course of the 5 min trial. Cocaine (20 mg/kg) slightly increased the mean activity scores ($F=5.41$, d.f. 1,104, $P<0.05$) and from Figure 1 it can be seen that this effect was essentially constant throughout the trial. Chlordiazepoxide (15 mg/kg) increased activity significantly during the first minute only ($t=2.21$, d.f. 13, $P<0.05$); this was followed by a decline in activity at a rate which was significantly greater than that for saline controls ($t=3.91$, d.f. 13, $P<0.01$). Figure 1 also shows that the mean activity scores for mice receiving the mixture of cocaine and chlordiazepoxide were consistently higher than those for the mice receiving cocaine alone ($F=25.0$, d.f. 1,104, $P<0.001$). This high level of activity was maintained throughout the trial, but since the effect of the mixture was evident within the first 3 min, trials of this length were used subsequently.

Experiment 2

Administration of cocaine alone increased the mean activity scores ($F=3.29$, d.f. 3.28, $P<0.05$), although

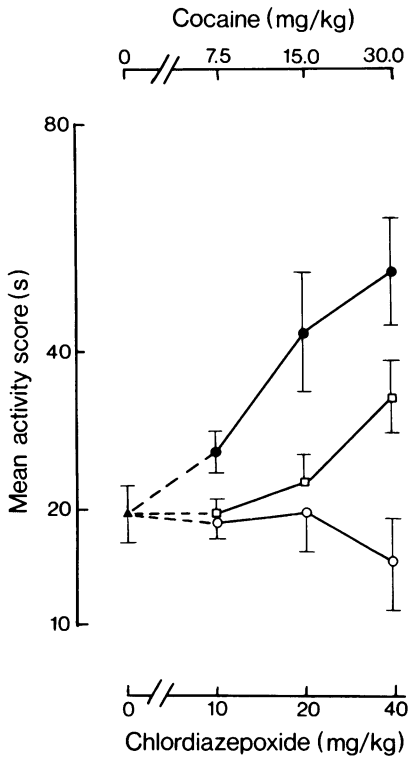

Figure 2 Activity of mice influenced by cocaine (□) and chlordiazepoxide (○) given separately at 3 doses of each, and in mixtures (●) of the same 3 doses in a constant ratio of 0.75:1 ($n=8$). (▲) Control mice.

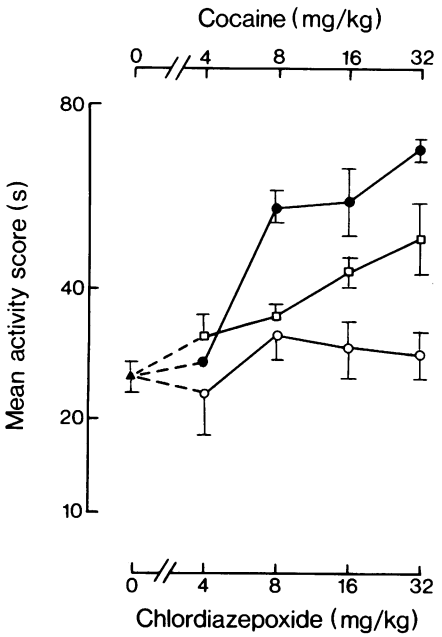

Figure 3 Activity of mice influenced by cocaine (□) and chlordiazepoxide (○) given separately at 4 doses of each, and in mixtures (●) of the same doses in a constant ratio of 1:1 ($n=10$). (▲) Control mice.

this effect was not marked except at the highest dose used (30 mg/kg). Chlordiazepoxide alone had no significant effects on the mean activity scores ($F<1$, d.f. 3,28); a depressant tendency at the highest dose was not statistically significant but it was noted that some mice showed characteristic periods of total immobility. Figure 2 shows that mixtures of cocaine and chlordiazepoxide significantly increased activity scores as compared with saline ($F=5.27$, d.f. 3,28, $P<0.01$). A two-factor (3 x 3) analysis of variance on the 9 groups of mice receiving drug treatments confirmed an overall difference between drugs ($F=24.6$, d.f. 2,63, $P<0.001$) and it was further shown that mixtures of cocaine and chlordiazepoxide yielded significantly greater mean activity than cocaine alone ($F=10.7$, d.f. 1,63, $P<0.01$), which in its turn yielded greater activity than chlordiazepoxide alone ($F=4.09$, d.f. 1,63, $P<0.05$). The overall differences between dose levels and the drug x doses interaction were not significant.

Experiment 3

The results for cocaine and chlordiazepoxide given separately and in mixtures at a dose ratio of 1:1 are shown in Figure 3 in a format which facilitates comparison with experiment 2 (Figure 2). Cocaine alone increased the mean activity scores in a dose-related manner ($F=5.65$, d.f. 4,45, $P<0.01$). Chlordiazepoxide alone had no significant effect ($F<1$, d.f. 4,45) nor was there a noteworthy trend across doses with this drug. Figure 3 also shows that in adequate doses, mixtures of the two drugs increased activity significantly above that after the administration of cocaine alone ($F=9.68$, d.f. 1,83, $P<0.01$). Cocaine yielded activity greater than that after chlordiazepoxide alone ($F=10.6$, d.f. 1,83, $P<0.01$). It may be noted that this pattern of results is essentially similar to that for experiment 2.

The complete results of experiment 3 are represented in Figure 4 as a response surface, and the following statistical comparisons refer to differences between mixtures and the constituent doses of cocaine given alone. Chlordiazepoxide administered in mixtures with the lowest dose of cocaine (4 mg/kg) had no significant effect. However, in mixtures with cocaine (8 mg/kg), chlordiazepoxide significantly

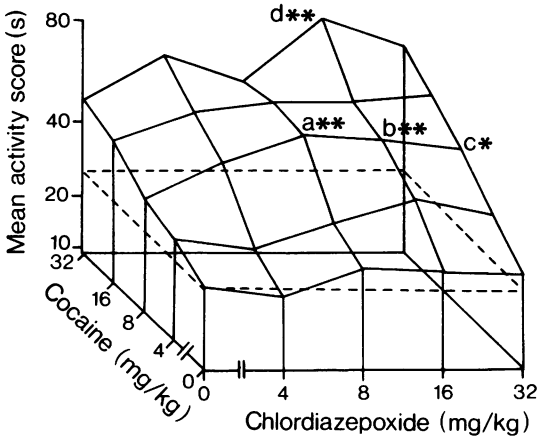

Figure 4 Response surface for doses of cocaine and chlordiazepoxide, showing the mean activity scores for groups of 10 mice given the drugs separately or in mixtures. Points are indicated where mice receiving mixtures were significantly more active than mice receiving the constituent dose of cocaine only (*$P<0.05$; **$P<0.01$). The dashed line represents the activity scores for mice receiving saline only.

increased the mean activity scores when given in doses of 8 mg/kg ($P<0.01$), 16 mg/kg ($P<0.01$) and 32 mg/kg ($P<0.05$). These effects can be seen as a peak approximately in the centre of the response surface and are marked as (a), (b) and (c) in Figure 4. Chlordiazepoxide did not increase activity significantly in mixtures with cocaine (16 mg/kg). The effects of chlordiazepoxide mixed with the highest dose (32 mg/kg) of cocaine did not show a good dose-response relationship, but chlordiazepoxide (16 mg/kg) yielded higher activity than cocaine alone ($P<0.01$). This can be seen as a second peak (d) in the response surface. The variance of the activity scores was greatest with the highest dose of cocaine, contributing to the lack of a good dose-response relationship.

Discussion

Mixtures of cocaine and chlordiazepoxide can greatly increase the coordinated locomotor activity of mice in an unfamiliar environment. Cocaine given alone also increases activity, but to a lesser extent. Earlier work has shown that mixing amphetamine with chlordiazepoxide can also produce very high activity in both rats (Rushton & Steinberg, 1966) and mice (Rushton *et al.*, 1973) and therefore, in this respect the behavioural effects of cocaine and amphetamine appear to be similar.

Increased activity scores due to cocaine (without chlordiazepoxide) are consistent with previous work (Rossum, 1964; Smith, 1965). Amphetamine does not usually produce very marked increases in walking when this component of activity is distinguished from stereotyped responses such as stepping on the spot and head-shaking (Rushton & Steinberg, 1963; Kršiak *et al.*, 1970). Typically, high doses of amphetamine tend to suppress walking whereas in the present experiments, even large amounts of cocaine increased walking, but directly comparable data for amphetamine would be required to differentiate the two drugs on this basis. It is known that both cocaine and amphetamine can suppress operant behaviour when administered in sufficiently high doses (Smith, 1964). The effect of chlordiazepoxide (without cocaine) on mice in the present experiments was strikingly similar to that reported previously in rats, in the sense that an initially increased level of activity was followed by a sharp decline accompanied by periods of total immobility (Rushton & Steinberg, 1966).

Mixtures of cocaine with chlordiazepoxide yielded higher levels of activity than those produced by either drug given separately and this effect was seen in three separate experiments. Sansone (1975) has found that cocaine (1.0–10.0 mg/kg) in mixtures with chlordiazepoxide (10.0 mg/kg) can facilitate avoidance responding of mice in shuttle-boxes. However, avoidance responding was increased by chlordiazepoxide given separately as well as in mixtures, whereas inter-trial responses were not increased by chlordiazepoxide alone. In detailed studies of amphetamine-barbiturate interactions, it has been reported that apparent potentiation or antagonism may occur depending on which aspect of the performance of a complex task is used to assess the effects of the drugs (Rutledge & Kelleher, 1965; Branch, 1974). How these different interactions may be mediated in the central nervous system remains unclear, especially since little information is available about ways in which the mixtures of drugs influence their distribution and metabolism. Nevertheless, testing for interactions of the type shown can provide an empirical method for characterizing the profile of action of central nervous system stimulants.

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