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EFFECTS OF CAFFEINE, THEOPHYLLINE AND THEOBROMINE ON SCHEDULED CONTROLLED RESPONDING IN RATS

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1 Rats were trained to respond under a variable interval 30 s (VI 30) schedule of food reinforcement.

2 Caffeine (0.32–32 mg/kg), theophylline (1.0–56 mg/kg) and theobromine (10–320 mg/kg) in general produced dose-related decreases in operant responding. At relatively low doses, caffeine (1.0 mg/kg) and theophylline (3.2 mg/kg) produced slight but nonsignificant increases in VI 30 responding.

3 The rank order of potency for producing decreases in responding was caffeine > theophylline > theobromine.

4 Daily caffeine injections (32 mg/kg, i.p.) resulted in the development of caffeine tolerance. This tolerance was characterized by a 6 fold shift to the right in the caffeine dose-effect curve. Saline substitution for the 32.0 mg/kg caffeine maintenance dose resulted in a substantial decrease in responding.

Introduction

Caffeine is probably the most widely used of the psychoactive drugs self-administered by humans (Gilbert, 1976). The biochemical effects of methylxanthines have been extensively studied and a number of mechanisms of action have been proposed. The proposed mechanisms of caffeine's effects fall into four categories: (1) caffeine produces its effects by preventing cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) destruction via brain cyclic AMP phosphodiesterase (Cheung, 1967); (2) caffeine causes the release of brain catecholamines which then stimulate the receptor-enzyme adenylate cyclase complex, resulting in increased cyclic AMP (Berkowitz, Tarver & Spector, 1970); (3) caffeine is a competitive antagonist of brain adenosine receptors (Bruns, Daly & Snyder, 1980); (4) caffeine may increase the availability of intracellular calcium in some tissues (Ritchie, 1970). With regard to the effects of xanthines on brain calcium, Diamond & Goldberg (1971) found that neither caffeine nor theophylline had an effect on calcium uptake in brain vesicles. Depending on the dose tested, it is reasonable to assume that the behavioural effects of caffeine involve one or more of these, and possibly other, mechanisms.

The behavioural pharmacology of methylxanthines has not been studied as extensively as have the other types of psychomotor stimulants. Caffeine has been reported to produce increases in the rate of responding under both fixed-interval schedules (Skinner & Heron, 1937; Mechner & Latranyi, 1963; McMillan, 1968; Davis, Kensler & Dews, 1973) and under differential reinforcement of low rates (DRL) schedules (Ando, 1973; Webb & Levine, 1978; Sanger, 1980). Under fixed ratio schedules, caffeine produces a dose-related decrease in responding (Davis *et al.*, 1973; Harris, Snell & Loh, 1978). While caffeine and related methylxanthines increased responding under interval schedules, both the pattern and magnitude of the effect differed from that produced by amphetamines (Harris *et al.*, 1978). In addition, drug discrimination studies indicate that the stimulus properties produced by amphetamine are qualitatively different from those produced by both caffeine and theophylline (Modrow, Holloway & Carney, 1980). Thus, it appears that the behavioural effects of methylxanthines may be due to a mechanism of action different from the amphetamine-like psychomotor stimulants.

The present study was designed to characterize the behavioural effects of the three commonly consumed methylxanthines: caffeine, theophylline and theobromine. These three methylxanthines were selected not only because they are directly consumed by humans, but also because theophylline and theobromine are active metabolites of caffeine which have been identified in all species studied. In addition to its relative potency, the rate and extent of caffeine tolerance development following chronic caffeine injections was determined.

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Methods

The animals used were 6 male Sprague-Dawley rats, weighing 250–275 g at the start of the study, which were obtained from the OUHSC breeding colony. Rats were housed singly and maintained in the OUHSC animal facility under a 12 h light, 12 h dark cycle at 22°C. Water was freely available in the home cage. Rats were housed under these conditions for 14 days before the start of the study. All rats were experimentally naive. All experiments were conducted during the light phase of the light/dark cycle.

Procedures

Before training each rat was deprived of food to 80% of its *ad libitum* feeding weight. Rats were trained to lever press for 45 mg food pellets (P.J. Noyes, Lancaster, N.H.) by reinforcing successive approximations of the lever press response. Once the rats began to respond reliably, the schedule was changed from continuous reinforcement (CRF) to a variable interval schedule (VI). Under this schedule, food pellets were delivered contingent upon the first lever press response after a variable time since the last reinforcement. The VI was gradually increased from 5 s to 30 s and then the rats were allowed to stabilize. The variable interval ranged between 5 and 150 s with an average value of 30 s. The duration of the daily session was 30 min. After 3 to 4 weeks under the VI 30 schedule, rats were tested with saline (0.9% w/v NaCl solution) and drug injections. Drugs or saline were injected (i.p.) 45 min before the start of the session. This time interval was selected so that the xanthine plasma and brain levels would be at or near their peak values following intraperitoneal dosing. Drugs were tested in the following order: caffeine, theophylline, theobromine. For each of the xanthines the doses were tested in an unsystematic order.

After completion of the initial dose-effect curve determinations, rats were not given any drug injections for 14 consecutive days before the start of chronic caffeine exposure. This was done to ensure that there would be no significant amounts of residual xanthine before the start of chronic caffeine. Caffeine tolerance was induced by daily pre-session 32 mg/kg (165 μmol/kg) caffeine injections which were administered 45 min before the start of each daily session. Once tolerance had developed to the effect of 32 mg/kg caffeine, the dose-effect curve for caffeine was redetermined. On test days the 32 mg/kg caffeine pre-session maintenance dose was omitted and saline vehicle or one of a series of caffeine doses was substituted. At the end of the 30 min session, rats received a supplementary dose of caffeine if the test dose was less than 32 mg/kg. All drugs were tested in an unsystematic order of doses. Caffeine doses were tested no more frequently than every three days.

Drugs

Caffeine, theophylline and theobromine were purchased as the free base from commercial sources. For the purpose of molar comparison, the molecular weights of the xanthines are: caffeine (194.19), theophylline (180.17) and theobromine (180.17). They were dissolved in warm saline. The pH of the saline was adjusted with NaOh to improve solubility. Because of the poor solubility of theobromine, both the concentration and the volume of the injection were increased as the dose was increased. The basic saline vehicle (pH 8.0) used was the same as was used for the highest xanthine concentrations.

Results

Control responding under the VI 30 schedule of reinforcement was characterized by a constant rate of responding throughout the 30 min session. The average rate of VI 30 responding was 0.76 responses/s. The rate and pattern of responding within the 30 min session was quite stable for each rat throughout the acute drug testing portion of the study. The rate of VI responding for each individual rat was quite stable and did not vary more than 10% from day to day.

Caffeine, theophylline and theobromine all produced dose-related decreases in responding (Figure 1). The order of relative potencies for disruption of responding was caffeine > theophylline > theobromine. Caffeine and theophylline appeared to produce a slight increase in VI responding at 1.0 and

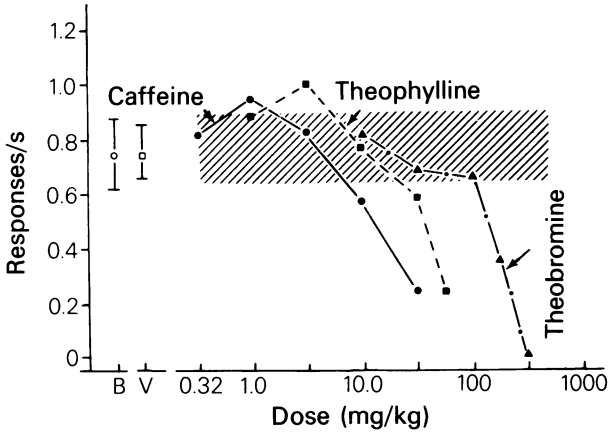

Figure 1 Effects of methylxanthines on responding under a VI 30 schedule. Each point for the methylxanthines is the mean of a single observation in six rats. B represents the mean (± 2 s.e.mean) of 6 sessions in each of the 6 rats for the non-injection baseline responding. The point at V represents the mean (± range) for the vehicle injections (2 observations for each of the 6 rats). Shaded bar also represents the baseline variability.

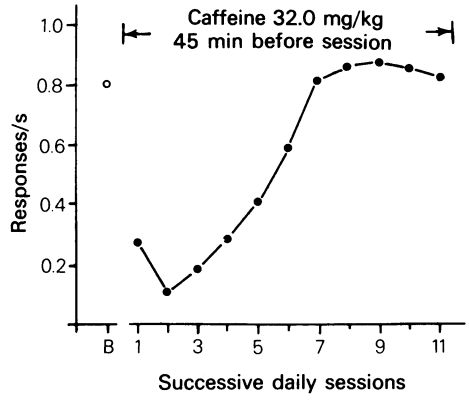

Figure 2 Caffeine tolerance development under a daily 32 mg/kg caffeine dosing regimen. The point at B represents the mean response rate of the group on the day before the start of chronic caffeine. Each point is the mean response rate for the group of 6 rats.

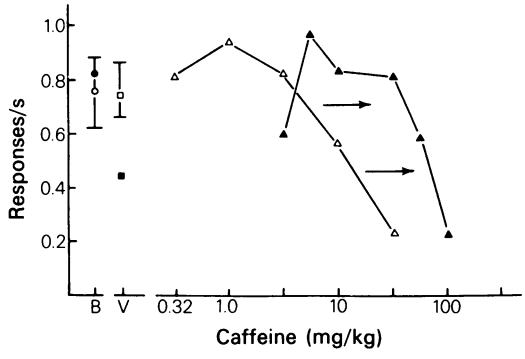

Figure 3 Reduced sensitivity to caffeine due to chronic 32 mg/kg caffeine injection. Open symbols represent the prechronic caffeine data and solid symbols represent the post chronic caffeine data. Open symbols at B and V are the same as in Figure 2. Solid symbols at B and V represent the rate of responding under the 32 mg/kg caffeine baseline condition and the effects of saline substitution for the 32 mg/kg caffeine maintenance dose.

3.2 mg/kg respectively. Theobromine did not produce any increases in VI responding at doses between 10 and 320 mg/kg.

Chronic administration of 32 mg/kg caffeine resulted in the development of tolerance (Figure 2). The effects of 32 mg/kg caffeine on the first day of chronic dosing was similar to that observed when the acute dose-effect curve for caffeine was determined. Thus, it does not appear that any caffeine tolerance had developed due to the periodic xanthine injections which were used to determine the non-tolerance dose-effect curves. Following an initial decrease in responding on day 2 of chronic caffeine, responding progressively (days 2–7) returned to control values. By day 7 responding had returned to the baseline, pre-chronic caffeine response rates. There was no indication of any stimulation of responding or any loss of tolerance to the rate-decreasing effects of 32 mg/kg caffeine from day 8 until the end of the study. Thus, a stable level of caffeine tolerance appears to have developed by the end of the first week of chronic caffeine.

Redetermination of the caffeine dose-effect curve in the rats maintained on 32 mg/kg caffeine per day, indicated that the caffeine dose-effect curve was shifted to the right. Probit analysis of the non-tolerant and the tolerant dose-effect curves indicated that there was a 6 fold shift to the right ($P<0.05$) in the caffeine dose-effect curve following chronic caffeine. In addition, there was a 50% reduction in responding when the saline vehicle was substituted for the 32 mg/kg caffeine maintenance dose.

Discussion

The rank order of xanthine potency for disruption of VI 30 s responding in the present study (caffeine > theophylline > theobromine) is similar to the traditional order of xanthine potency on the CNS. However, there are very few animal studies which directly assess the relative behavioural potencies of xanthines. Thithapandha, Boling & Gillette (1972) found that caffeine and theophylline were essentially equipotent at relatively low doses (5–20 mg/kg). Peak stimulation of spontaneous locomotor activity occurred at 20 mg/kg caffeine and at 30 mg/kg theophylline. In addition, theophylline appeared to produce a greater amount of locomotor activity than was achieved at any caffeine dose. Berkowitz *et al.* (1971) found that caffeine and theophylline had similar D_{50}s in the rat. McKim (1980) recently compared the effects of caffeine, theophylline and amphetamine responding under a multiple fixed interval, fixed ratio schedule of food reinforcement in C57B2 mice. Caffeine was approximately twice as potent as theophylline, both in producing slight increases in FI responding at low doses and in producing dose-related decreases at higher doses. These data agree with the relative potency estimates obtained in the present study.

In the study by McKim (1980), caffeine and theophylline produced only modest increases (140–160%) in FI responding, while amphetamine produced substantially greater increases in FI responding (270% of control).

The discriminative stimulus properties of caffeine and other compounds were recently reported by Modrow *et al.* (1981). In that study, theophylline generalized to the 32 mg/kg caffeine cue, but was slightly less potent. In contrast to theophylline, methylphenidate, (+)-amphetamine, nicotine, and thyrotropin releasing hormone (TRH), all failed to generalize to the caffeine cue. These data support the

hypothesis that the effects of methylxanthines on the CNS are due to a pharmacologically distinct mechanism of action from that of the phenethylamine and other psychomotor stimulants. One likely mechanism of xanthine action is the blockade of brain adenosine receptors (Bruns *et al.*, 1980; Snyder, Katims, Annau, Bruns & Daly, 1981).

Chronic daily injections of 32 mg/kg caffeine resulted in the development of tolerance. This tolerance was characterized by a 6 fold shift to the right in the caffeine dose-effect curve. There have been few studies on the development of caffeine tolerance in laboratory animals. Wayner, Jolicoeur, Rondeu & Barone (1976) reported that substantial tolerance developed to the effects of 100 mg/kg caffeine under a FI 1 min schedule of food reinforcement. Using a differential reinforcement of low rate schedule, McGuire, Snyder & Annau (1980) found that while tolerance may develop to the response rate decreasing effects of caffeine, little or no tolerance develops to the caffeine-induced changes in the number of reinforcements. This apparently selective tolerance to the response rate decreasing, as compared to the rate increasing effects, is in contrast to the behavioural tolerance observed with other psychomotor stimulants (Schuster, Dockens & Woods, 1966).

References

ANDO, K. (1973). Profile of drug effects on temporally spaced responding in rat. *Pharmac. Biochem. Behav.*, **3**, 833–841.

BERKOWITZ, B.A., TARVER, J.H. & SPECTOR, S. (1970). Release of norepinephrine in the central nervous system by theophylline and caffeine. *Eur. J. Pharmac.*, **10**, 64–71.

BRUNS, R.G., DALY, J.W. & SNYDER, S.H. (1980). Adenosine receptors in brain membranes: binding of ^{3}H-N^{6}-cyclohesyladenosine and ^{3}H-1,3-diethyl-8-phenylxanthine. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 5547–5555.

CHEUNG, W.Y. (1967). Properties of cyclic-3′,5′nucleotide phosphodiesterase from rat brain. *Biochemistry*, **6**, 1079–1087.

DAVIS, T.R.A., KENSLER, C.J. & DEWS, P.B. (1973). Comparison of behavioral effects of nicotine, d-amphetamine, caffeine and dimethylheptyltetrahydorcannabinol. *Psychopharmacologia (Berl.)*, **32**, 51–65.

DIAMOND, I. & GOLDBERG, A.L. (1971). Uptake and release of ^{45}Ca by brain microsomes, synaptosomes, and synaptic vesicles. *J. Neurochem.*, **18**, 1419–1431.

GILBERT, R.M. (1976). Caffeine as a drug of abuse. In *Recent Advances in Alcohol and Drug Problems*, vol. 3. ed. Gibbins, R.J., Israel, Y., Kalant, H., Pophan, R.E., Schmidt, W. & Smart, R.G. New York: Plenum Press.

HARRIS, R.A., SNELL, D. & LOH, H.H. (1978). Effects of stimulants, anorectics and related drugs on schedule-controlled behavior. *Psychopharmacology*, **56**, 49–55.

McGUIRE, P.S., SNYDER, S.H. & ANNAU, Z. (1980). Selective effects of acute and chronic caffeine on schedule performance in rats. *Neurosci. Abs.*, **6**, 364.

McKIM, W.A. (1980). The effects of caffeine, theophylline and amphetamine on operant responding of the mouse. *Psychopharmacology*, **68**, 135–138.

McMILLAN, D.E. (1968). Some interactions between sympathomimetic amines and amine depleting agents on the schedule-controlled behavior of the pigeon and the squirrel monkey. *J. Pharmac. exp. Ther.*, **163**, 172–187.

MECHNER, F. & LATRANYI, M. (1963). Behavioral effects of caffeine, methamphetamine and methylphenidate in the rat. *J. exp. anal. Behav.*, **6**, 331–342.

MODROW, H.E., HOLLOWAY, F.A. & CARNEY, J.M. (1981). Caffeine discrimination in the rat. *Pharmac. Biochem. Behav.*, **14**, 683–688.

RITCHIE, J.M. (1970). Central nervous system stimulants. II The xanthines. In *The Pharmacological Basis of Therapeutics*. ed. Goodman, L.S. & Gillman, A.L. New York: MacMillan Co.

SANGER, D.J. (1980). The effects of caffeine on timing behavior in rodents: Comparisons with chlordiazepoxide. *Psychopharmacology*, **68**, 305–309.

SCHUSTER, C.R., DOCKENS, W.S. & WOODS, J.H. (1966). Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia, (Berl.)*, **9**, 170–182.

SNYDER, S.H., KATIMS, J.J., ANNAU, Z., BRUNS, R.F. & DALY, J.W. (1981). Adenosine receptors and behavioral actions of methylxanthines. *Proc. natn. Acad. Sci. U.S.A.*, **78**, 3260–3264.

SKINNER, B.F. & HERON, W.I. (1937). Effects of caffeine and benzedrine upon conditioning and extinction. *Psychol. Rec.*, **10**, 340–346.

THITHAPANDHA, A., BOLING, H.M. & GILLETTE, J.R. (1972). Effects of caffeine and theophylline on activity of rats in relation to brain xanthine concentrations. *Proc. Soc. exp. Biol. Med.*, **139**, 528–536.

WAYNER, M.L., JOLICOEUR, F.B., RONDEU, D.B. & BARONE, F.C. (1976). Effects of acute and chronic administration of caffeine on schedule dependent and schedule induced behavior. *Pharmac. Biochem. Behav.*, **5**, 343–348.

WEBB, D. & LEVINE, T.E. (1978). Effects of caffeine on DRL performance in the mouse. *Pharmac. Biochem. Behav.*, **9**, 7–10.

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