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BRIEF COMMUNICATION

Effect of Caffeine on Cocaine Locomotor Stimulant Activity in Rats

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MISRA, A L , N L VADLAMANI AND R B PONTANI *Effect of caffeine on cocaine locomotor stimulant activity in rats* PHARMACOL BIOCHEM BEHAV **24**(3) 761–764, 1986 —The effect of caffeine on the locomotor stimulant activity induced by intravenous cocaine in rats was investigated Low doses of caffeine (20 mg/kg IP) potentiated the locomotor activity induced by 1, 2 5 mg/kg intravenous doses of cocaine and higher doses of caffeine (50, 100 mg/kg IP) had no significant effect The locomotor stimulant effect of 20 mg/kg IP dose of caffeine *per se* in vehicle was significantly higher and that with 100 mg/kg dose significantly lower than that of the vehicle control Thus caffeine produced dose-dependent effects on cocaine-induced locomotor stimulant activity, with low dose potentiating and higher doses having no significant effect on such activity Pharmacokinetic or dispositional factors did not appear to play a role in potentiation of cocaine locomotor stimulant activity by caffeine

Cocaine-caffeine interaction Locomotor stimulant activity Dose-dependent effects Potentiation
[^{3}H] Cocaine disposition Male Wistar rats

CAFFEINE is a widely used central nervous system (CNS) stimulant Caffeine produces a number of behavioural and biochemical changes in laboratory animals and human subjects In human subjects, low non-toxic doses of caffeine increase cortical excitation, and higher doses cause anxiety, nervousness, impaired thinking, sleep disturbance, heart palpitations, stomach irritation and toxic doses provoke seizures [18] In rodents, caffeine produces marked dose-dependent increases in locomotor activity in a biphasic manner [12], biphasic effects on operant behaviour [29] and dose-related decreases in operant responding [4] Chronic administration produces tolerance to the caffeine-induced stimulation of locomotor activity that is complete, pharmacologically specific and fully reversible on cessation of drug intake [4, 12] Drug discrimination studies have shown [14] that the stimulus produced by amphetamine-like psychomotor stimulants is qualitatively different from that of caffeine, implying that the effects of caffeine are due to a pharmacological mechanism distinct from that of psychomotor stimulants

Multiple sites in the CNS appear to mediate the complex spectrum of the pharmacological effects of caffeine These effects include the inhibition of phosphodiesterases [3], blockade of adenosine receptors [8], increases in the rate of turnover of norepinephrine and dopamine [1,27], increases in the sensitivity of post-synaptic central catecholamine receptors [28], increases in the brain content but not synthesis of 5-hydroxytryptamine [9] and translocation of intracellular Ca^{2+} [2,13]

Combinations of caffeine with stimulants, e g , phenylpropanolamine and ephedrine, are particularly hazardous [17,20] and have recently been banned by the Food and Drug Administration In view of the marked increase in the recreational use of cocaine in recent years and because of the presence of caffeine in "street cocaine" samples (in addition to local anesthetics and sugars), the interaction of caffeine with cocaine may produce systemic effects not preferred by drug abusers This investigation was undertaken to study the effect of caffeine on cocaine locomotor stimulant activity in rats

METHOD

Drugs

Caffeine injection solutions were prepared in warm, 40% aqueous propylene glycol Cocaine solutions were prepared by dissolving cocaine hydrochloride in 0 9% NaCl (saline) Doses are expressed as free base

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TABLE 1

EFFECT OF PRETREATMENT WITH DIFFERENT DOSES OF CAFFEINE ON COCAINE INDUCED LOCOMOTOR ACTIVITY IN RATS

Group	Treatment	Mean cumulative photocell counts ± S E M
A	Vehicle	874 ± 114
	Caffeine (20 mg/kg)	1512 ± 264*
	Caffeine (50 mg/kg)	725 ± 66
	Caffeine (100 mg/kg)	386 ± 75†
B	Vehicle + cocaine (2 5 mg/kg)	1169 ± 142
	Caffeine (20 mg/kg) + cocaine (2 5 mg/kg)	3712 ± 621†
C	Vehicle + cocaine (2 5 mg/kg)	2093 ± 351
	Caffeine (50 mg/kg) + cocaine (2 5 mg/kg)	2744 ± 463
	Caffeine (100 mg/kg) + cocaine (2 5 mg/kg)	1507 ± 288
D	Vehicle + cocaine (1 mg/kg)	1510 ± 214
	Caffeine (20 mg/kg) + cocaine (1 mg/kg)	2632 ± 451*
E	Vehicle + cocaine (5 mg/kg)	1622 ± 613
	Caffeine (20 mg/kg) + cocaine (5 mg/kg)	2716 ± 760
	Caffeine (100 mg/kg) + cocaine (5 mg/kg)	1388 ± 376

Male Wistar rats (n=6 and 5 in groups A to C and groups D and E respectively) were pretreated IP either with the vehicle (40% aqueous propylene glycol) or different doses of caffeine in vehicle and 45 min later injected IV with different doses of cocaine Cumulative photocell counts were determined during the 30 min period after cocaine injection

*†Denote significant differences from the corresponding controls at $p<0$ 05 and $p<0$ 01 respectively

TABLE 2

COCAINE CONCENTRATIONS* IN PLASMA AND SELECTED TISSUES OF CONTROL (VEHICLE-TREATED) AND CAFFEINE-PRETREATED† RATS AFTER A 2 5 MG/KG DOSE OF [^{3}H] COCAINE BY BOLUS IV INJECTION

Biofluid tissue	Cocaine concentration	
	Control	Caffeine-pretreated
Plasma	258 ± 16	274 ± 16
Brain	3199 ± 197	4024 ± 323
Liver	492 ± 29	606 ± 44
Heart	1129 ± 59	1166 ± 45
Lung	3805 ± 200	4376 ± 203

*Data represent mean ± s e m (ng/g tissue or ml fluid) from 5 animals in each group

†The rats were pretreated IP either with the control vehicle (40% aqueous propylene glycol) or a 20 mg/kg dose of caffeine in vehicle and 45 min later injected IV with a 2 5 mg/kg bolus dose of [^{3}H] cocaine The animals were killed 10 min after cocaine injection and plasma and tissues obtained for analyses

Animals

Male Wistar rats (230–300 g) were randomly housed in groups of 5 rats in suspended stainless steel cages equipped with an automatic watering system under the standard laboratory conditions of 12 hr light/dark cycles Ambient room temperature was 25°C Rats were deprived of food 18 hr prior to the experiment and water was available ad lib throughout the study All experimental sessions were conducted in rooms maintained at 25°C during 1000 to 1600 hours

Locomotor Activity

Locomotor activity (ambulation) was determined in BRS/LVE (Model 1497) activity counting chambers 61 cm diameter, 43 cm height (Tech Serv Corp , Beltsville, MD) which utilize six infra red light beams and six photocells Digital counters recorded each time the beam of light was interrupted The activity chambers were dark during the experiment After a familiarization period of 1 hr, rats were injected IP either with the control vehicle or the appropriate dose of caffeine (20, 50 or 100 mg/kg) in vehicle and 45 min later injected IV with a 2 5 mg/kg dose of cocaine Locomotor activity of each rat was recorded as cumulative photocell counts during 30 min post-injection of cocaine Locomotor activity of rats injected IP with the vehicle and different doses of caffeine (20, 50, 100 mg/kg) in vehicle was also determined

In a separate group of experiments, the effect of pretreatment with low and high doses (20, 100 mg/kg IP) of caffeine on the locomotor activity induced by different doses of cocaine (1, 5 mg/kg IV) injected 45 min after the vehicle or caffeine pretreatment in rats (150–200 g) was determined during 30 min post-injection of cocaine

Estimation of [^{3}H] Cocaine in Biological Materials

[^{3}H] Ring-labeled cocaine as prepared by previous method [15] was diluted with nonradioactive cocaine hydrochloride in 0 9% saline to provide a specific activity of 11 17 μCi/mg Doses are expressed as the free base Male Wistar rats (175–220 g) were injected IP either with the vehicle or caffeine (20 mg/kg) and 45 min later injected IV with a 2 5 mg/kg dose of [^{3}H] cocaine Animals were killed 10 min after the cocaine injection and plasma and tissues obtained for analyses

Aliquots (2 ml) of plasma (diluted 1 5 with distilled water) or tissue homogenates (10% w/v in 0 5 M HCl) containing nonradioactive cocaine hydrochloride as carrier (500 μg/ml) were adjusted to pH 9 with 1 5 M ammonia, and the solution buffered with 1 ml 20% w/v K_2HPO_4 and extracted with 15 ml cyclohexane as previously described [16] Benzoylecgonine, benzoylnorecgonine, ecgonine methyl ester and ecgonine, the metabolites of cocaine [16] were not extracted by this procedure and the method was specific for cocaine *In vitro* percent recoveries of cocaine in the conc range 10–1000 ng/ml from diluted plasma and tissue homogenates were 99±1 (m±s e m) The comparative concentrations of total

cocaine metabolites [16] in the plasma and tissues of the vehicle and caffeine pretreated rats was also determined to ascertain whether the caffeine pretreatment altered the metabolism of cocaine Total radioactivity in diluted samples of plasma (0 2 ml) was determined by direct counting with 10 ml Aquasol solution (New England Nuclear, Boston, MA) Aliquots of tissue homogenates were mixed with an equal volume of NCS solubiliser (Amersham/Searle Corp Arlington Heights, IL) and the radioactivity counted with Aquasol Appropriate corrections for quenching were applied using [^{3}H] toluene as an internal standard The total conc of the cocaine metabolites given above (as cocaine conc equivalents) were obtained by subtracting the conc of extractable cocaine per g tissue or ml fluid from the corresponding total radioactivity values

Statistical Analysis

Statistical significance of all data on the comparative cumulative photocell counts or comparative cocaine concentrations in the vehicle and caffeine-pretreated groups after cocaine injections was evaluated by Student's *t*-test

RESULTS

Effect of Different Doses of Caffeine Per Se on its Locomotor Activity

Data on the cumulative photocell counts in rats pretreated IP either with the vehicle or 20, 50 and 100 mg/kg doses of caffeine *per se* in vehicle appear in Table 1A Locomotor activity of rats treated with 20 mg/kg dose of caffeine was significantly higher and that with 100 mg/kg dose significantly lower than that in the vehicle control group The activity of rats treated with 50 mg/kg dose of caffeine was not significantly different from that of the vehicle control

Effect of Pretreatment With Low Dose of Caffeine on Cocaine Locomotor Activity

Data on the cumulative photocell counts in rats pretreated IP either with the control vehicle or a 20 mg/kg dose of caffeine in vehicle after a 2 5 mg/kg IV injection of cocaine appear in Table 1B Cumulative counts in caffeine pretreated animals were significantly higher than those in the vehicle treated group

Effect of Pretreatment With Higher Doses of Caffeine on Cocaine Locomotor Activity

Data on the cumulative photocell counts in a separate group of animals pretreated IP either with the vehicle or 50 and 100 mg/kg doses of caffeine after a 2 5 mg/kg IV injection of cocaine appear in Table 1C Cumulative counts in caffeine pretreated animals were not significantly different from those in the vehicle control group Counts in 100 mg/kg caffeine pretreated group were significantly lower ($p<0$ 05) than those in the 50 mg/kg caffeine group

Effect of Low and High Dose Caffeine Pretreatment on Cocaine Locomotor Activity

Data on the cumulative photocell counts in rats pretreated IP with 20 mg/kg dose of caffeine after a 1 or 5 mg/kg IV injection of cocaine appear in Table 1, D and E Cumulative counts in caffeine pretreated rats after a 1 mg/kg IV injection of cocaine were significantly higher than those in the vehicle group Cumulative counts in the caffeine pretreated group after a 5 mg/kg IV injection of cocaine were not significantly different from those in the vehicle group Cumulative counts in animals pretreated with a 100 mg/kg IP dose of caffeine after a 5 mg/kg IV injection of cocaine were not significantly different from those in the corresponding vehicle-treated group

Comparative Concentrations of Cocaine in Plasma and Tissues of the Vehicle and Caffeine Pretreated Rats

Concentrations of cocaine in plasma and selected tissues of animals pretreated IP either with the vehicle or a 20 mg/kg dose of caffeine 10 min after a 2 5 mg/kg IV injection of [^{3}H] cocaine appear in Table 2 The concentrations of cocaine in plasma, brain, liver, heart and lung of caffeine pretreated animals were not significantly different from those in the control group Brain to plasma cocaine concentration ratio (m±s e m) in the caffeine pretreated group (14 98±0 96) was significantly higher ($p<0$ 05) than that in the controls (12 50±0 35) No significant differences were observed in liver to plasma cocaine concentration ratio, the ratio of total concentration of the cocaine metabolites to the concentration of unmetabolized drug in the liver or the total concentration of the cocaine metabolites in plasma and different tissues in the two groups

DISCUSSION

This study shows that a low dose of caffeine (20 mg/kg IP) potentiated the locomotor stimulant activity of different doses of cocaine (1, 2 5 mg/kg IV) and higher doses of caffeine (50, 100 mg/kg IP) had no significant effect on this activity The locomotor activity of low dose of caffeine (20 mg/kg IP) *per se* in vehicle was significantly higher and that with 100 mg/kg dose significantly lower than that in the vehicle group Thus, a low dose of caffeine potentiated and higher doses had no significant effect on cocaine locomotor stimulant activity Previous work [11] has shown that lower doses (20 mg/kg) of caffeine produced maximal motor stimulation and this effect either decreased or disappeared with higher doses which were still below the toxic levels Medial thalamus and brain stem reticular formation have been suggested as the important sites for the stimulant effects of caffeine, but the basic mechanisms underlying such effects of caffeine and amphetamine-like psychomotor stimulants appear to be quite different [5,14]

The mechanism involved in the potentiation of the cocaine-induced locomotor stimulant effect by caffeine is unclear at this time A variety of drugs have been shown to affect the cocaine locomotor stimulant effect, e g , atropine and monamine oxidase inhibitors potentiated it [6, 10, 19], reserpine, chlorpromazine, pimozide, phenoxybenzamine reduced or blocked it [19, 21, 22, 24] and α-methyl para tyrosine (tyrosine hydroxylase inhibitor) and iproniazide had no effect [19, 23, 26] Although no clear-cut neurochemical mechanisms for the cocaine locomotor stimulant effects have emerged from these studies, it is generally believed that the reserve pools of catecholamines rather than the newly synthesized pools mediate such cocaine effects [19,30] Increased turnover of striatal dopamine rather than the telencephalic norepinephrine correlated better with the cocaine stimulant effect [7,25] Caffeine increases the turnover of norepinephrine and dopamine [1,27] and the sensitivity of the post-synaptic central catecholamine receptors [28] Consequently it is conceivable that the possible increases in the

turnover of striatal dopamine by caffeine-cocaine combination may play a role in the potentiation of cocaine-induced locomotor activity by caffeine However, pretreatment of rats with a moderate non-depressant dose of haloperidol (0 15 mg/kg IP), a dopamine antagonist 1 hr before cocaine (2 5 mg/kg IV) did not significantly reduce the potentiated locomotor activity in caffeine pretreated (20 mg/kg IP) rats (doses of haloperidol 0 5 mg/kg IP or higher produced catalepsy in rats)

It is important to determine the potential role of pharmacokinetic and dispositional factors in drug interactions, where the effect of one drug can be modified by the concurrent administration of another drug Experiments on kinetics were done 10 min after the cocaine injection, because a significant ($p<0$ 05) potentiation of locomotor activity occurred even at this time The cumulative photocell counts (m±s e m) 10 min after IV cocaine injection (2 5 mg/kg, in the control and caffeine pretreated groups were 626±110 and 1266±352 respectively Although the measures of locomotor activity and pharmacokinetics were not taken at the same point in time, our data show that pharmacokinetic or dispositional factors do not appear to play a role in the potentiation of cocaine induced locomotor stimulant effects by caffeine

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