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EFFECTS OF ACUTE COCAINE TREATMENT ON THE TURNOVER OF 5-HYDROXYTRYPTAMINE IN THE RAT BRAIN

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1 The effects of cocaine (20 mg/kg s.c.) on 5-hydroxytryptamine (5-HT) turnover were examined in rats.

2 *In vivo* cocaine administration resulted in decreased turnover of 5-HT, as indicated by the decreased accumulation of 5-HT after pargyline administration and the decreased accumulation of 5-hydroxyindoleacetic acid (5-HIAA) following probenecid injection.

3 A time-related decrease in 5-HIAA concentrations and a small fall in 5-HT concentrations in the whole brain were observed following the acute administration of cocaine hydrochloride (20 mg/kg). Tryptophan levels were found to be slightly decreased in the brain.

4 Enhanced reactivity, but neither stereotypy nor hyperthermia, was observed following cocaine injection (20 mg/kg).

5 It is concluded that cocaine inhibits the turnover of brain 5-HT and that this action of cocaine may be responsible for the differences in a number of pharmacological effects between cocaine and amphetamine.

Introduction

Much of the recent work on the effects of cocaine on the central nervous system (CNS) has been concerned with catecholaminergic systems. Cocaine blocks the neuronal uptake of tritiated dopamine (Ross & Renyi, 1967; Snyder & Coyle, 1969) and noradrenaline (Carmichael & Israel, 1973); in field-stimulated brain slices, it increases the release of catecholamines (Farnebo & Hamberger, 1971; Starke & Montel, 1973). Evidence from behavioural studies also suggests an increased catecholamine release induced by cocaine (Scheel-Kruger, 1972). Moreover, on the basis of electroencephalographic data, Wallach & Gershon (1971) have demonstrated amphetamine-like effects mediated by catecholamines after cocaine administration. However, other work suggests that cocaine acts not only on catecholamines and that an amphetamine model may be inadequate to explain all of cocaine's actions.

Simon, Sultan, Chermat & Boissier (1972), in a behavioural study, have shown that, while cocaine has amphetamine-like activity, the cocaine-induced increased locomotor activity is neither blocked by inhibition of catecholamine synthesis with α-methyl-*p*-tyrosine (α-MPT) nor by blockade of dopamine receptors with non-depressant doses of haloperidol. In addition, Knapp & Mandell (1972) have recently shown that after acute cocaine administration particulate tryptophan hydroxylase activity is decreased and that this is attributable to blockade of the high affinity uptake of tryptophan. These findings suggest that the effects of cocaine on 5-hydroxytryptamine (5-HT) turnover be examined.

Methods

5-hydroxytryptamine and 5-hydroxyindoleacetic acid (5-HIAA)

Male Sprague-Dawley rats weighing between 200 and 250 g were injected subcutaneously (s.c.) with either cocaine hydrochloride 20 mg/kg or 0.9% w/v NaCl solution (saline). After 0, 30 and 60 min, the rats were stunned and decapitated. The brains were rapidly removed, rinsed in cold saline, weighed, and homogenized in 10 volumes of cold acidified butanol. 5-HT and 5-HIAA were assayed spectrophotofluorometrically by the method of Curzon & Green (1970). Recoveries of 5-HT and 5-HIAA were 83% and 89%, respectively. The reported values have not been corrected for recovery.

Tryptophan

Sixty minutes after saline or cocaine 20 or 40 mg/kg, rats were killed and blood and brains were removed. Free plasma tryptophan was estimated by assaying for tryptophan in ultrafiltrates obtained by centrifugation of 0.5 ml plasma at 900 x *g* for 30 min in Centriflo-50 membrane cones (Amicon Corp.). Tryptophan levels in both tissues were determined by the method of Denckla & Dewey (1967).

5-hydroxytryptamine turnover

Turnover of 5-HT was studied by a modification of the method of Tozer, Neff & Brodie (1966). Fifteen minutes after cocaine (20 mg/kg) or saline pretreatment, rats were injected intraperitoneally with either pargyline 75 mg/kg or probenecid 200 mg/kg and killed at various times thereafter. The effects of cocaine on turnover were evaluated by following the accumulation of 5-HT after inhibition of monoamine oxidase (MAO) with pargyline as well as the accumulation of 5-HIAA after probenecid (200 mg/kg i.p.)-induced blockade of 5-HIAA efflux from the brain.

Results

Effect of acute cocaine treatment on 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations in whole brain

Cocaine (20 mg/kg) caused a small decrease in brain 5-HT concentrations at all time intervals evaluated; the concentrations differed significantly from control values only at 30 min after treatment. Sixty minutes after treatment with cocaine, there was a significant ($P < 0.01$) lowering of the brain 5-HIAA concentrations. No change in 5-HIAA was seen at 30 min (Table 1).

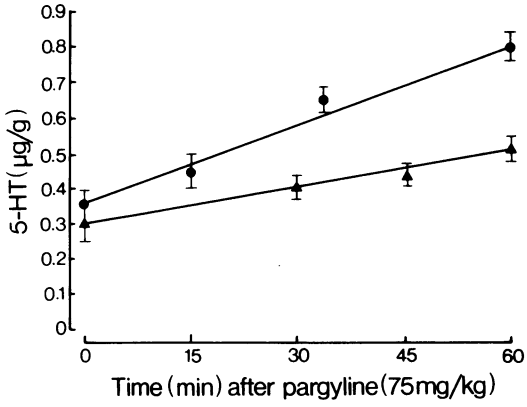

Figure 1 Effect of cocaine on the brain concentration of 5-hydroxytryptamine (5-HT) after pargyline administration. Cocaine 20 mg/kg (▲) or saline (●) was injected subcutaneously 15 min before pargyline 75 mg/kg. Each point represents the mean of five determinations. Vertical bars show s.e. mean.

5-hydroxytryptamine turnover

A net accumulation of 5-HT occurred over time after pargyline treatment. The rate of accumulation was decreased in the cocaine-pretreated group (Figure 1). A further indication of a reduced turnover rate in cocaine-treated rats is evident from the decreased accumulation of 5-HIAA after probenecid administration (Table 2).

Table 1 Effects of cocaine on 5-hydroxyindoleacetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) concentrations in whole brain

	Time (min)	*Saline*	*Cocaine*
5-HIAA (μg/g)	0	0.30 ± 0.02	0.30 ± 0.02
	30	0.29 ± 0.01	0.28 ± 0.02
	60	0.28 ± 0.01	0.22 ± 0.01*
5-HT (μg/g)	0	0.35 ± 0.02	0.33 ± 0.01
	30	0.39 ± 0.02	0.31 ± 0.02**
	60	0.36 ± 0.03	0.30 ± 0.03

Time is expressed as min after subcutaneous injection of cocaine (20 mg/kg). Each entry in the table represents the mean concentration of 5-HIAA or 5-HT with the corresponding standard error of the mean in a group of eight rats.
* $P < 0.01$ by two-tailed *t* test; ** $P < 0.02$.

Table 2 Effect of cocaine on accumulation of 5-hydroxyindoleacetic acid (5-HIAA) after probenecid administration

	Brain 5-HIAA contents (μg/g tissue)	
Time (min)	0	60
Saline	0.27 ± 0.02	0.54 ± 0.02
Cocaine	0.23 ± 0.02	0.33 ± 0.03*

Cocaine (20 mg/kg) was injected subcutaneously 15 min before probenecid (200 mg/kg). Values (μg/g) are the mean and s.e. mean of five determinations. Time (min) indicates time after probenecid administration.
* $P < 0.001$, saline vs. cocaine at 60 minutes.

Table 3 Effects of cocaine on blood and brain tryptophan concentrations

	Tryptophan concentrations		
	Plasma (μg/ml)		*Brain (μg/g)*
	Total	*Free*	
Saline	25.3 ± 0.19	0.596 ± 0.056	6.72 ± 0.11
Cocaine 20 mg/kg	26.3 ± 1.67	0.702 ± 0.066	6.28 ± 0.32
Cocaine 40 mg/kg	27.0 ± 1.41	0.470 ± 0.058	6.10 ± 0.10*

Tryptophan concentrations were determined 1 h after injection of cocaine hydrochloride. Each entry in the table represents the mean with the corresponding s.e. mean in a group of four rats.
* $P < 0.01$.

Tryptophan levels

A dose-related decrease in brain concentrations of tryptophan was observed 60 min after drug administration. However, the difference from control concentrations was significant only at the 40 mg/kg dose of cocaine. Cocaine treatment did not alter total and free plasma tryptophan concentrations (Table 3).

Behaviour

Enhanced reactivity to auditory and tactile stimulation was observed following cocaine (20 mg/kg) administration. No significant changes in rectal temperature were seen at 15, 30, or 60 min after this dose of cocaine and no evidence of motor activation or stereotyped sniffing was observed.

Discussion

The decreased rate of accumulation of 5-HT after MAO inhibition and decreased accumulation of 5-HIAA after probenecid indicate that cocaine inhibits the turnover of 5-HT. Knapp & Mandell (1972) have shown that cocaine *in vitro* decreased 5-HT synthesis by inhibition of tryptophan uptake and not by direct inhibition of tryptophan hydroxylase. However, inhibition of synthesis, especially in the presence of the blockade of 5-HT uptake known to be an effect of cocaine (Ross & Renyi, 1969), would be expected to result in a precipitous fall in brain 5-HT levels. Our finding of only a slight decrease in brain 5-HT concentrations suggests that cocaine also inhibits the release and/or metabolism of 5-HT. It is possible that cocaine stimulates 5-HT receptors directly and thus indirectly modifies release *in vivo* through a negative feedback system or that it directly inhibits 5-HT release. The effect of cocaine on 5-HT synthesis may also be partially mediated through a trans-synaptic feedback system (Andén, Corrodi & Fuxe, 1971), although tryptophan uptake appears to play a major role as shown by the *in vitro* studies (Knapp & Mandell, 1972). Our data are not sufficient to predict which, if any, of these mechanisms are involved.

The decrease in turnover as well as the postulated inhibition of release of 5-HT may be responsible for certain of the pharmacological differences between cocaine and amphetamine. Although similarities are abundant (each drug at some dose elicits stereotyped behaviour, increased locomotor activity, and similar electroencephalographic changes in mammals (Willner, Samach, Angrist, Wallach & Gershon, 1970; Wallach & Gershon, 1971; Simon *et al.*, 1972); in man, each produces euphoria, anorexia, and perceptual and affective changes at low doses and an hallucinatory psychosis that is frequently paranoid in nature at higher doses (Bejerot, 1970)), there is evidence that suggests that the action of cocaine on the catecholamines is insufficient to explain all of its effects. A study in our laboratory designed to distinguish between the effects of stimulants and the effects of psychotomimetics in chicks showed that cocaine could not be clearly classified (Wallach, Friedman & Gershon, 1972). Cocaine in low doses elicited behavioural and postural changes characteristic of amphetamine, whereas at higher doses its effects are characteristic of psychotomimetics. In addition, neither α-MPT nor haloperidol suppresses cocaine-induced hyperactivity in rats (Simon *et al.*, 1972), suggesting that the catecholamines are not responsible for these actions of the drug.

It has been proposed that certain of the psychotomimetic properties of lysergic acid diethylamide (LSD) are due to inhibition of 5-HT synthesis (Andén, Corrodi, Fuxe & Hökfelt, 1968) or to the inhibition of firing of 5-HT neurones (Aghajanian, Foote & Sheard, 1970). Snyder, Unger, Blatchley & Barfknecht (1974) have suggested that the strikingly similar clinical effects

of LSD and the psychotomimetic derivatives of amphetamine or phenethylamine are due to conformational factors of the drugs that permit similar specific receptor binding of chemically distinct compounds. If these two hypotheses are valid, and if turnover of 5-HT is indeed inhibited by cocaine as our results suggest, then it is likely that this action of cocaine is responsible for the inconsistencies cited above.

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