Effects of cocaine and amphetamine on the metabolism of tryptophan and 5-hydroxytryptamine in mouse brain *in vivo*

The central stimulant drugs, cocaine and amphetamine, have both been shown to influence the metabolism of catecholamines in brain (Dengler, Spiegel & Titus, 1961; Baird & Lewis, 1964; Carlsson, Lindqvist & others, 1965; Corrodi, Fuxe & Hökfelt, 1967). Ross & Renyi (1969) found that cocaine is a potent inhibitor of 5-hydroxytryptamine (5-HT) uptake in brain slices *in vitro*, and Foote, Sheard & Aghajanian (1969) reported a stimulatory effect of amphetamine on midbrain raphe units, which presumably contain 5-HT. These results have prompted the present experiments on the effects of cocaine and amphetamine on rates of accumulation and disappearance of ^{3}H-5-HT in mouse brain *in vivo* after intravenous administration of [^{3}H]tryptophan.

Accumulation of ^{3}H-5-HT. When [^{3}H]tryptophan is administered to mice (male, NMRI, 18–22 g) by constant rate intravenous infusion, ^{3}H-5-HT accumulates in brain at an increasing rate (Schubert, Nybäck & Sedvall, 1970a). Mice were pretreated with cocaine hydrochloride (30 mg/kg, i.p.) or (±)-amphetamine sulphate (15 mg/kg, i.p.). After 10 min [^{3}H]tryptophan (40 μCi/animal, 6·0 Ci/mmol) was infused during 20 min. Cocaine and possibly also amphetamine reduced the accumulation of ^{3}H-5-HT in brain in comparison to saline-treated animals (Table 1). No significant effect of the drugs on the contents of [^{3}H]tryptophan, endogenous 5-HT or tryptophan was found after this relatively short period following drug administration.

Table 1. *Effects of cocaine and amphetamine on levels of labelled and endogenous tryptophan and* 5-HT *in mouse brain after infusion of* [^{3}H]*tryptophan,* [^{3}H]tryptophan (40 μCi) was infused i.v. for 20 min starting 10 min after i.p. injection of saline, cocaine hydrochloride (30 mg/kg) or (±)-amphetamine sulphate (15 mg/kg). Animals were killed immediately after the infusion. Figures represent mean value ± s.e. from 6–8 animals.

Treatment	[^{3}H]tryptophan counts/min. 10^3/g	Tryptophan μg/g	^{3}H-5-HT counts/min. 10^3/g	5-HT μg/g
Saline	86 ± 6	4·1 ± 0·29	1·59 ± 0·14	0·29 ± 0·02
Cocaine	84 ± 5	4·3 ± 0·39	1·00 ± 0·10*	0·25 ± 0·01
Amphetamine	81 ± 9	4·0 ± 0·38	1·14 ± 0·15†	0·28 ± 0·02

* Differs from saline group ($P < 0·01$)
† Differs from saline group ($P < 0·05$)

Disappearance of ^{3}H-5-HT. After an intravenous injection of [^{3}H]tryptophan to mice, the 5-HT store in brain is maximally labelled within 30 min. Between 1–3 h after administration of the labelled precursor, ^{3}H-5-HT disappears from brain at a rate which appears to be exponential and is not altered by treatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (Schubert & others, 1970a). Thus, in non-treated animals the disappearance of labelled 5-HT during the mentioned time interval is determined predominantly by the turnover rate of the amine. Saline, cocaine hydrochloride (30 mg/kg) or (±)-amphetamine sulphate (15 mg/kg) were administered intraperitoneally 1 and 2 h after the intravenous injection of [^{3}H]tryptophan (40 μCi/animal). Groups of animals were killed 1 or 3 h after precursor administration and the contents in brain of endogenous and labelled tryptophan and 5-HT were determined. The rates of disappearance of [^{3}H]tryptophan and ^{3}H-5-HT were significantly retarded by both drugs (Table 2). Cocaine caused a slight, but amphetamine a threefold increase of endogenous tryptophan content in brain. The level of endogenous 5-HT was possibly increased after amphetamine.

These experiments demonstrate that cocaine and amphetamine have effects on

Table 2. *Effects of cocaine and amphetamine on levels of labelled and endogenous tryptophan and* 5-HT *in mouse brain after injection of* [^{3}H]*tryptophan.* Saline, cocaine hydrochloride (30 mg/kg) or (±)-amphetamine sulphate (15 mg/kg) were administered i.p. 1 and 2 h after i.v. injection of [^{3}H]-tryptophan (40 μCi). Animals were killed 3 h after [^{3}H]tryptophan administration. Figures represent mean value ± s.e. from 8–9 animals.

Treatment	Time h	[^{3}H]tryptophan counts/min. 10^3/g	Tryptophan μg/g	^{3}H-5-HT counts/min. 10^3/g	5-HT μg/g
—	1	6·2 ± 0·4	4·8 ± 0·37	1·01 ± 0·07	0·39 ± 0·02
Saline ..	3	2·6 ± 0·2	4·1 ± 0·25	0·39 ± 0·02	0·38 ± 0·02
Cocaine ..	3	3·7 ± 0·2*	6·3 ± 0·33*	0·58 ± 0·03*	0·45 ± 0·02
Amphetamine	3	6·6 ± 0·4*	14·4 ± 1·03*	0·56 ± 0·03*	0·46 ± 0·02†

* Differs from saline group ($P < 0·001$)
† Differs from saline group ($P < 0·05$)

tryptophan and 5-HT metabolism in brain and display certain similarities. Both caused a significant increase of endogenous tryptophan levels in brain and retarded the rate of [^{3}H]tryptophan disappearance. This effect on tryptophan metabolism, which was much more pronounced for amphetamine than for cocaine, developed slowly. It was thus not significant in the accumulation experiment, i.e. 30 min after drug administration, but was prominent after 2 h in the disappearance experiment. The mechanism for the effect could be explained by an inhibitory effect of the drugs or their metabolites on tryptophan catabolism or binding, or both.

Cocaine, and possibly amphetamine decelerated rates of accumulation and disappearance of ^{3}H-5-HT in brain which could indicate a retardation of endogenous 5-HT synthesis and turnover rates. However, the effect of the drugs on 5-HT disappearance could be secondary in part to the drug-induced changes of tryptophan metabolism. Thus, the increased [^{3}H]tryptophan levels after cocaine and, above all, amphetamine treatment should accelerate ^{3}H-5-HT resynthesis, resulting in retardation of the ^{3}H-5-HT disappearance rate. However, it would seem difficult to explain the immediate inhibitory effect of the drugs on ^{3}H-5-HT accumulation as secondary to the slow but substantial increase of brain tryptophan levels. Therefore it is suggested from the present data that cocaine, and possibly also amphetamine, have effects on brain 5-HT metabolism which are partly secondary to changes of tryptophan metabolism, and partly direct. Cocaine is a potent inhibitor of *in vitro* 5-HT uptake in brain slices (Ross & Renyi, 1969), whereas amphetamine has no such effect (Pletscher & Bartolini, 1967). Both drugs are weak inhibitors of monoamine oxidase. The effect of cocaine on ^{3}H-5-HT metabolism is similar to that previously found for dimethylated tricyclic antidepressants (Schubert, Nybäck & Sedvall, 1970b), which also inhibit *in vitro* 5-HT uptake (Ross & Renyi, 1969). The influence of cocaine on 5-HT metabolism in brain may be interpreted as follows: reduction of 5-HT uptake or catabolism, or both, results in increased activation of 5-HT receptors. By a hypothetical negative feed-back mechanism, impulse activity and transmitter synthesis in 5-HT neurons are subsequently decelerated.

Regarding amphetamine our experiments demonstrate that this drug not only affects catecholamines but also influences transmitter metabolism in brain 5-HT neurons. The results could be explained by a decelerating effect of amphetamine on 5-HT synthesis, an effect which is difficult to relate to the stimulatory effect of the drug on activity of raphe units reported by Foote & others (1969). Since Glowinski, Axelrod & Iversen (1966) have presented evidence that the monoamine oxidase activity of tissues is reduced after treatment with amphetamine, at least some of the effects of amphetamine might follow from inhibition of monoamine oxidase activity.

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Departments of Psychiatry (S:t Göran's Hospital) and Pharmacology, Karolinska Institutet, S-104 01 Stockholm 60, Sweden.

JOHAN SCHUBERT
BENGT FYRÖ
HENRIK NYBÄCK
GÖRAN SEDVALL

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Restoration of blood pressure and heart rate responses to tyramine by infusion of 5-hydroxytryptamine in reserpine-treated pithed rats

In the pithed rat, pretreatment with reserpine abolishes the cardiovascular effects of tyramine due to depletion of intraneuronal stores of noradrenaline (Burn & Rand, 1960; Torchiana, Wenger & others, 1966; Clarke & Leach, 1968; Clarke, 1970). An infusion of noradrenaline or one of its precursors, by repleting the tissue stores, restores responses to tyramine (Burn & Rand, 1960; Torchiana & others, 1966). This is easier to demonstrate if deamination of the infused (or formed) amine is prevented by prior injection of drugs possessing monoamine oxidase activity (Clarke & Leach, 1968).

In the pithed reserpinized rat, there is a tissue uptake process for low doses of 5-hydroxytryptamine (5-HT) (Fozard, 1969) similar to that described previously for noradrenaline (Muscholl, 1961; Weiner & Trendelenburg, 1962; Van Zwieten, Widhalm & Hertting, 1965). It was tentatively suggested that 5-HT and noradrenaline shared a common uptake pathway into the sympathetic nerves (Fozard, 1969). The ability of infusions of 5-HT to restore responses to tyramine in reserpinized pithed rats would support such a suggestion.

A total of 21 female Wistar rats weighing 190–230 g were used. Those pretreated with reserpine were given 5 mg/kg intraperitoneally 18–22 h before the experiment. Rats were pithed under pentobarbitone anaesthesia and set up for femoral intravenous injection and recording of carotid blood pressure (Clarke & Leach, 1968). In most experiments heart rate was also recorded (Clarke, Hiscoe & others, 1966). Drugs, dissolved in saline, were given in volumes of 0·1 ml and washed into the animal with 0·2 ml of saline. Infusions were administered into a femoral vein by a Palmer slow injection apparatus at a rate of 2·5 ml/20 min. Test doses of tyramine were not given until 30 min after the heart rate had returned to pre-infusion levels.

The blood pressure and heart rate responses to a 25 μg dose of tyramine were abolished after pretreatment with reserpine (Fig. 1). Infusions of 5-HT (0·5 mg/kg in 20 min) routinely caused an increase in both blood pressure and heart rate, but failed to restore the response to tyramine when this was injected 30 and 60 min after the end