AMPHETAMINE AND COCAINE ON AMINE TURNOVER*

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ALTHOUGH it is generally accepted that brain catecholamines are implicated in the central action of *d*-amphetamine (GLOWINSKI and AXELROD, 1965, 1966; STEIN and WISE, 1969; CARR and MOORE, 1969; HANSON, 1966; RANDRUP and MUNKVAD, 1966; MCLEAN and MCCARTNEY, 1961; SANAN and VOGT, 1962; MOORE, 1963) conflicting reports exist whether norepinephrine (NE) or dopamine (DM) or both are involved in modulating single behavioural changes induced by this drug.

Recently it has been suggested that stereotyped behaviour elicited by *d*-amphetamine depends on the availability of DM, whereas NE appears to be required for locomotor stimulation (RANDRUP and SCHEEL-KRUGER, 1966; COYLE and SNYDER, 1969). On the other hand Carlsson (CARLSSON, 1970) has proposed that in mice, hypermotility caused by *d*-amphetamine is mediated through a release of brain DM. This hypothesis is consistent with studies by VAN ROSSUM *et al.*, 1962 and SMITH, 1963 in disputing that *d*-amphetamine effect on motor activity involves a release of brain NE.

The role of brain catecholamine in the regulation of food intake is also controversial.

It has been reported that the noradrenergic terminals in the lateral hypothalamus are associated with satiety (MARGULES, 1970). The anorexia produced by amphetamine, with particular reference to the indirect action on the central noradrenergic system of this drug, is brought forward in support of this theory (MARGULES, 1969).

However in apparent contradiction to the noradrenergic satiety theory, other investigators have shown that direct application of NE in the lateral area of the hypothalamus produces eating in satiated animals (GROSSMAN, 1960, 1962, 1968). This effect can be suppressed by contemporary administration of α-blocking agents and potentiated by desmethylimipramine (LEIBOWITZ, 1970; BERGER *et al.*, 1971). According to these investigators amphetamine anorexia does not depend on the release of brain NE but derives from a direct interaction of this drug with brain receptors.

Recently UNGERSTED (1971) has given evidences that the nigrostriatum dopaminergic system plays an important role in regulating food intake, suggesting that amphetamine anorexia may be mediated by brain DM.

The purpose of the present investigation was to further explore the individual roles of NE and DM in amphetamine induced anorexia, hypermotility and hyperthermia.

In these studies changes of turnover rates of brain norepinephrine and dopamine have been chosen as indices of functional status of the two neuronal systems at the presynaptic site.

In order to establish whether the experimental data concerning behavioural and

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neurochemical parameters were related to each other and, if so, whether their relationship was unique, the effects of cocaine, fenfluramine and *l*-amphetamine on brain catecholamine turnover rate and animal behaviour were also investigated.

The experimental evidence resulting from this study seems to suggest:

(1) an indirect action on the noradrenergic neuronal system in the hypothalamus and telediencephalon does not appear to be required in eliciting anorexia or hypermotility.
(2) *d*-amphetamine and cocaine may elicit psychomotor stimulation by releasing dopamine from striatum nerve terminals.
(3) brain DM may play some role in regulating food intake in *d*-amphetamine treated rats.

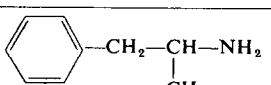
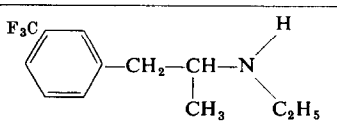
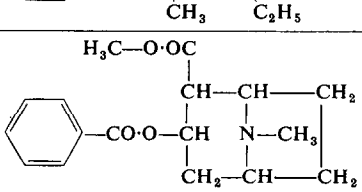
BEHAVIOURAL EFFECTS

Table 1 reports the structures and the behavioural effects of *d*-amphetamine, *l*-amphetamine, fenfluramine and cocaine. The dose for each drug has been chosen in order to have comparable and as selective as possible effects on food intake.

It is known that high doses of *d*-amphetamine decrease food intake and increase body temperature and motor activity. However, at smaller doses, behavioural effects of *d*-amphetamine are more selective. In fact doses of 0·3 mg/kg i.v. do not change body temperature while they still increase motor activity and give anorexia (Table 1).

The optical isomer, *l*-amphetamine, has much less central stimulant effect than *d*-amphetamine (PRINZMETAL and ALLES, 1940; STEIN, 1964). In non striatal brain areas *l*-amphetamine is 10 fold less potent than *d*-isomer in inhibiting NE uptake by synaptosomes, whereas in the corpus striatum the two isomers are almost equally active (COYLE and SNYDER, 1969).

TABLE 1. BEHAVIOURAL EFFECTS OF *d*-AMPHETAMINE, *l*-AMPHETAMINE, FENFLURAMINE AND COCAINE IN RAT

Drug	Structure	Dose (mg/kg i.v.)	Food* intake	Motor† activity	Body‡ temperature
Saline		—	2·3 ± 0·3 (5)	103 ± 15 (14)	36·2 ± 0·2 (5)
d-Amphetamine		0·3	1·2 ± 0·3§ (5)	335 ± 35§ (4)	36·5 ± 0·2 (5)
l-Amphetamine		1·0	1·3 ± 0·6§ (5)	107 ± 19 (12)	38·1 ± 0·1 (5)
Fenfluramine		1·0	0·76 ± 0·2§ (5)	132 ± 30 (4)	36·0 ± 0·2 (5)
Cocaine		3·0	0·80 ± 0·1§ (5)	515 ± 130§ (4)	37·4 ± 0·1§ (5)

* Food intake: g of food eaten/100 g of body weight ± S.E. during the first 30 min after drug injection. The rats were trained for two weeks to eat for 4 hr/day. Food was presented immediately after drug injection.

† Motor activity: events ± S.E. during the first 30 min after drug injection. Rats were singularly housed in compartments of an I.R. Electronic Motility Meter. Locomotor activity was measured by infrared photocells equally spaced in the floor of the cages.

‡ Body temperature: °C ± S.E. measured rectally 30 min after drug injection.

§ $P < 0·01$ Number of animals in parentheses

The administration of 1 mg/kg i.v. of *l*-amphetamine to rats, decreases food intake without affecting motor activity (Table 1). Body temperature was also increased.

Fenfluramine, a compound chemically related to *d*-amphetamine (Table 1), also causes anorexia but, unlike amphetamine, fails to change motor activity and body temperature in the rat (ALPHIN *et al.*, 1964). Only at high doses this drug decreases motor activity (COLMORE and MOORE, 1966). Fenfluramine also reduces brain 5-HT and NE concentrations (DUCE and GESSA, 1967; OPITZ, 1967).

Cocaine, although having an entirely different structure than amphetamine, shows similar behavioural effects. This drug, given at 3 mg/kg i.v., increases body temperature and motor activity and decreases food intake (Table 1).

NEUROCHEMICAL EFFECTS

The turnover rates of the two catecholamines were estimated "*in vivo*" from the conversion of labeled tyrosine into NE and DM respectively. In these experiments the following procedure was adopted: At 0 time 3,5^{3}H-tyrosine (S.A. 30 Ci/mM) was injected (1 mCi/kg i.v.). After 10 min drugs or saline were given intravenously. 25 min after tyrosine injections the rats were sacrificed and the brains dissected. Concentrations of labeled as well as endogenous tyrosine, NE and DM were determined by a method previously described by NEFF *et al.* (1971).

As a model to calculate the incorporation rate of tyrosine into NE or DM, a two compartments closed system has been selected. In this system, first proposed by SEDVALL *et al.* (1968), the amount of catecholamine formed can be calculated from the ratio between the radioactivity incorporated in the amines and the specific activity of the aminoacid precursor, in this case tyrosine. The quotient of this equation is called "conversion index" to indicate that this value is not an absolute measurement of incorporation rate. This model can be used for comparative estimation of incorporation rate of aminoacids into amines providing:

(1) aminoacid and amine concentrations have reached steady state; (2) specific activity of amino acid precursor are higher than specific activity of tissue amines.

The data listed in Table 2, some of which have been previously reported (COSTA *et al.*, 1972), indicate that conversion index of tyrosine into striatal DM was significantly higher in rats receiving *d*-amphetamine and cocaine. Since neither steady state concentrations of tyrosine and DM nor the specific activity of striatum tyrosine were changed by these two drugs, it follows that the turnover time of striatal DM in *d*-amphetamine and cocaine treated rats is faster than in rats injected with saline.

These results are consistent with "*in vivo*" studies by MACKENZIE and SZERB (1968) indicating that amphetamine applied directly into the caudate nucleus enhances the release of DM from dopaminergic terminals. JORI and BERNARDI (1969) have also observed an increase of brain homovanillic acid in amphetamine treated rats.

This effect is restricted to these two drugs. In fact both fenfluramine and *l*-amphetamine fail to change the incorporation rate of tyrosine into striatal dopamine. Table 2 also includes data showing the effects of *d*- and *l*-amphetamine, cocaine and fenfluramine on the conversion index of tyrosine into telediencephalic NE.

None of these drugs seems to modify the steady state or the incorporation rate of tyrosine into NE.

Previous reports (COSTA *et al.*, 1971; GROPPETTI *et al.*, 1972) have indicated that fenfluramine may increase the turnover rate of both NE and DM in the rat brain.

TABLE 2. EFFECT OF *d*-AMPHETAMINE, *l*-AMPHETAMINE, COCAINE AND FENFLURAMINE ON CONVERSION OF 3,5^{3}H-TYROSINE INTO TELDIENCEPHALIC NOREPINEPHRINE (NE) AND STRIATAL DOPAMINE (DM).*

Treatment	Dose (mg/kg i.v.)	Teldiencephalon			Striatum	
		Tyrosine (dpm/mμmol ± SE × 10^3)	NE mμmol/g ± SE	Conversion index mμmol NE/g/25 min. ± SE	DM mμmol/g ± SE	Conversion index mμmol DM/g/25 min ± SE
Saline	—	4·1 ± 0·1 (11)	2·2 ± 0·1 —	0·38 ± 0·05 —	71 ± 13 (6)	15 ± 2 —
d-Amphetamine	0·3	3·9 ± 0·3 (6)	2·5 ± 0·1 —	0·41 ± 0·04 —	83 ± 3 (6)	27 ± 2† —
Saline	—	—	—	—	53 ± 5 (11)	17 ± 1 —
l-Amphetamine	1·0	3·8 ± 0·2 (11)	2·8 ± 0·2 —	0·39 ± 0·04 —	54 ± 2 (8)	21 ± 2 —
Saline	—	—	—	—	58 ± 6 (9)	15 ± 1
Cocaine	3·0	3·7 ± 0·1 (10)	2·6 ± 0·1 —	0·43 ± 0·04 —	62 ± 4 (6)	24 ± 2†
Saline	—	2·9 ± 0·2 (5)	2·8 ± 0·1	0·91 ± 0·09	81 ± 5 (6)	24 ± 1
Fenfluramine	1·0	3·4 ± 0·3 (5)	3·3 ± 0·1	0·89 ± 0·08	87 ± 6 (5)	28 ± 3

* Part of the data reported in this table were taken from COSTA *et al.*, *Brit. J. Pharmacol.* (1972) **44,** 742
† $P < 0·01$ Number of animals in parentheses

However the apparent discrepancy may be due to the higher doses used in those experiments.

The present findings suggest that the receptors that control feeding behaviour, motor activity and body temperature may be influenced without affecting the biochemistry of presynaptic noradrenergic neurons in the rat telediencephalon.

However it is not possible to exclude that in some more discrete areas of the brain the turnover rate of NE is changed and this change may be masked in our experiments by the size of tissue sample.

Since the hypothalamus is generally considered as the brain area where the processes regulating food intake mostly occur (ANAND *et al.*, 1955), a study of how anorexic doses of *d*- and *l*-amphetamine, fenfluramine and cocaine affect NE turnover rate in this brain area was thought more suitable.

In order to increase the sensitivity of the method, otherwise insufficient, 3,5^{3}H tyrosine had to be injected intraventricularly instead of intravenously. Two permanent cannulas of poliethylene were placed one for each lateral brain ventricle of rats (ROBINSON *et al.*, 1969) 48 hr before injecting labeled tyrosine.

On the day of the experiment 7 μCi of 3,5^{3}H-tyrosine (S,A. 30 Ci/mM) were injected in each ventricle. Interferences with uptake and distribution of labeled tyrosine were minimised by injecting the drugs, intravenously, 12 min after labeling. Twenty-five min after the injection of 3,5^{3}H-tyrosine the rats were sacrificed and the hypothalamus separated from the rest of the brain. Chemical determinations and conversion index have been calculated as indicated above.

The method offers the following advantages:

(1) minimal stress to the animal at the time of injection,
(2) avoidance of anesthesia,
(3) accumulation of sufficient radioactivity in the hypothalamus to permit measurements of specific activity of tyrosine and norepinephrine in individual animals.

Figure 1 shows the changes, as a function of time, of the specific activity of tyrosine and NE in brain and hypothalamus of rats treated with 3,5^{3}H-tyrosine, intravenously (1·0 mCi/kg) or intraventricularly (7 μCi/ventricle) respectively.

K_m values were calculated according to the equation proposed by NEFF *et al.*, (1971). (Fig. 1)

For this calculation each graph was divided into consecutive 5 min intervals. As shown in Fig. 1 while the individual K_m values calculated in the brain after intravenous injection of labeled tyrosine were uniform during the entire 40 min. period of investigation, after intraventricular injection 10–15 min must elapse before K_m values in the hypothalamus become relatively constant. These results suggest that even after intraventricular injection the equilibrium between the injected labeled and the endogenous tyrosine is rapidly reached but that it takes at least 10–15 min before being complete.

The data listed in Table 3 show that of *d*- and *l*-amphetamine, fenfluramine and cocaine change neither the endogenous levels of NE nor the S.A. of tyrosine nor the incorporation rate of this aminoacid into NE in rat hypothalamus.

These results are consistent with the findings indicating that when given in moderate pharmacological doses *d*-amphetamine does not significantly increase the release of stored H^3–NE into the perfusate from the hypothalamus *in vivo* (STRADA and SULSER 1970).

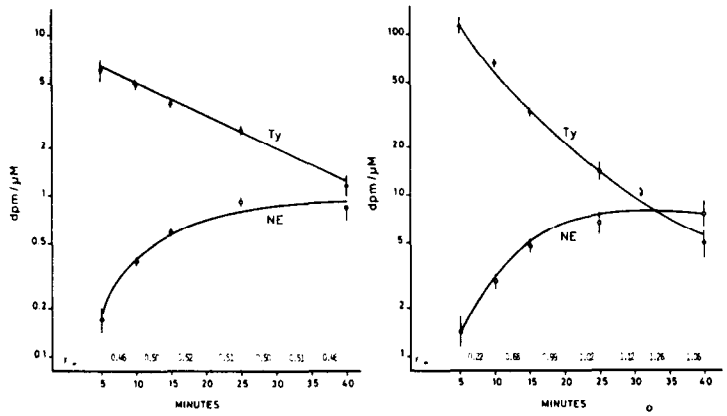


FIG. 1.—Specific activity of brain (left) and hypothalmic (right) tyrosine and norepinephrine after intravenous (1 mCi/kg) or intraventricular (7 μCi/ventricle) injection of 3,5-^{3}H-tyrosine in the rat.

K_m values were calculated from the formula

$$K_m \approx \frac{\dfrac{M_{t_2} - M_{t_1}}{t_2 - t_1}}{\dfrac{[A - M]t_2 - [A - M]t_1}{2}}$$

Where: M = specific activity of the amine
A = specific activity of the aminoacid

RELATIONSHIP BETWEEN BEHAVIOURAL AND NEUROCHEMICAL EFFECTS

The data reported in Tables 1, 2 and 3 tend to exclude the possibility that anorexia as well as hyperthermia and hypermotility induced by *d*-amphetamine, *l*-amphetamine, fenfluramine and cocaine occur together with an increase of the extraneuronal release of telediencephalic or hypothalamic NE.

TABLE 3. EFFECT OF *d*-AMPHETAMINE, *l*-AMPHETAMINE, COCAINE AND FENFLURAMINE ON CONVERSION INDEX OF TYROSINE INTO NOREPINEPHRINE (NE) IN RAT HYPOTHALAMUS AFTER INTRAVENTRICULAR INJECTION OF 3,5^{3}H-TYROSINE.*

Treatment	Dose mg/kg i.v.	Tyrosine dpm/mμmol ± SE × 10^3	NE mμmol/g ± SE	Conversion index mμmol NE/g ± SE
Saline	—	14·43 ± 1·27 (6)	11·48 ± 0·62 (6)	5·15 ± 0·60 (6)
d-Amphetamine	0·3	16·90 ± 1·03 (7)	10·61 ± 0·53 (7)	5·58 ± 0·77 (7)
l-Amphetamine	1·0	18·09 ± 1·14 (7)	9·35 ± 0·53 (7)	3·84 ± 0·26 (7)
Saline	—	16·64 ± 1·65 (5)	8·3 ± 0·91 (5)	2·26 ± 0·03 (5)
Cocaine	3·0	21·42 ± 4·97 (6)	9·3 ± 0·28 (7)	2·76 ± 0·50 (6)
Fenfluramine	1·0	22·22 ± 1·58 (6)	8·1 ± 0·47 (7)	2·05 ± 0·30 (6)

* 3,5^{3}H-tyrosine (7 μCi/ventricle)
Number of rats in parentheses

These results support and extend to amphetamine congeners the proposal made by other investigators (LEIBOWITZ, 1970; BERGER *et al.*,1971; GROPPETTI *et al.*, 1972) that the central action of minimal effective doses of *d*-amphetamine does not involve a release of brain NE.

Changes in DM turnover rates do not seem to be related to hyperthermia that follows some of these drug treatments. So that a dose of *d*-amphetamine (0·3 mg/kg i.v.) that increases incorporation rate of tyrosine into striatal DM does not affect body temperature,while on the other hand an hyperthermic dose of *l*-amphetamine (1 mg/kg i.v.) does not change striatal DM turnover.

The increase of DM turnover seems instead to be more related to changes in motor activity. Both, *d*-amphetamine (0·3 mg/kg i.v.) and cocaine (3mg/kg i.v.) stimulate motor activity and accelerate the incorporation rate of tyrosine into striatum DM. On the other hand fenfluramine and *l*-amphetamine at doses that do not change motor activity failed to affect DM turnover rate. COSTA *et al.* (1971) have already suggested that dopaminergic axons could be involved in the motor stimulation elicited by aminorex and *p*-chloroamphetamine.

The role of brain DM in regulating food intake is still unresolved. While *d*-amphetamine and cocaine accelerate DM turnover and also produce anorexia, suggesting that the increase of striatal DM turnover may be related to the anorexic effect of these drugs, on the other hand it is possible to reduce food intake without affecting striatal DM conversion index by administration of *l*-amphetamine and fenfluramine.

WEISSMAN *et al.* (1966) have reported that α-methyltyrosine (α-MT), an inhibitor of catecholamine synthesis (SPECTOR *et al.*, 1965), antagonizes the anorexic effect of amphetamine. This observation has been generally interpreted as evidence that amphetamine anorexia is due to an indirect effect of this drug on NE neurons. However brain DM as well as other non specific mechanisms entirely unrelated to the inhibition of catecholamine synthesis may also be involved, but the data reported in Table 4 seem to negate the possibility that α-MT may block the *d*-amphetamine induced anorexia by these non specific mechanisms.

TABLE 4. EFFECT OF α-METHYLTYROSINE (α-MT) AND PIMOZIDE (PZ) PRETREATMENT ON *d*-AMPHETAMINE, FENFLURAMINE AND APOMORPHINE INDUCED ANOREXIA IN RAT.

Treatment*	Food intake†		
	Saline	α-MT‡	PZ§
Saline	3·0 ± 0·28 (5)	3·71 ± 0·45 (5)	3·14 ± 0·52 (5)
d-amphetamine 1·5 i.p.	0·90 ± 0·20 (5)	2·1 ± 0·15 (5)	2·1 ± 0·40 (5)
Fenfluramine 3 mg/kg i.p.	0·96 ± 0·27 (5)	0·36 ± 0·10 (5)	0·34 ± 0·16 (5)
Apomorphine 1 mg/kg s.c.	0·56 ± 0·16 (5)	0·75 ± 0·12 (5)	1·44 ± 0·44 (5)

* Drugs were given 15 minutes before presentation
† g of food/100 g of Body Weight ± SE measured 1 hr after food presentation
‡ α-MT 50 mg/kg i.p. 2 hrs. before food presentation
§ PZ 0·25 mg/kg i.p. 4 hrs. before food presentation

In fact α-MT selectively antagonizes *d*-amphetamine anorexia while it does not block but eventually potentiates the fenfluramine effect on food intake.

On the other hand the fact that dopaminergic system may play some role in amphetamine anorexia is suggested by the findings that pimozide, a drug indicated to block dopaminergic receptors (ANDÉN *et al.*, 1970), antagonizes the depressive effect of amphetamine on food intake. This antagonism appears to be selective because fenfluramine anorexia was not inhibited but potentiated by pimozide pretreatment. (Table 4).

In this context it is interesting to note that apomorphine, an agonist of dopaminergic receptors (ERNST and SMELIK, 1966) also causes anorexia and that this effect is antagonized by pimozide and not by α-MT. (Table 4)

This observation gives further support to the hypothesis that activation of the brain dopaminergic system may be responsible, at least in part, for the reduction of food intake by *d*-amphetamine. This correlation seems to be restricted to this drug because fenfluramine anorexia is not antagonized either by α-MT or pimozide pretreatment.

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