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Effects of amphetamine on the turnover rate of brain catecholamines and motor activity

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Summary

1. Rats receiving (+)-amphetamine (either 0·3 or 0·2 mg/kg, i.v.) are anorexic. Only the former dose increases their motor activity. Both doses fail to change dopamine (DM) and noradrenaline (NA) concentrations in striatum and tel-diencephalon. The turnover rate of striatal DM is increased only by 0·3 mg/kg of (+)-amphetamine; neither dose changes NA turnover rate in tel-diencephalon.

2. (−)-Amphetamine (1 mg/kg, i.v.) causes anorexia and hyperthermia in rats but it changes neither the steady-state concentration nor the turnover rate of striatal DM and tel-diencephalic NA. Motor activity is not increased by this dose of (−)-amphetamine.

3. Cocaine (3 mg/kg, i.v.) increases motor activity and accelerates the turnover rate of striatal DM. This drug neither accelerates turnover rate of tel-diencephalic NA nor causes anorexia.

4. These observations suggest that the acceleration of striatal DM turnover rate elicited by (+)-amphetamine and cocaine may be associated with an effect on motor activity. In contrast, the increase of motor activity seems unrelated to the effects of these drugs on noradrenergic tracts of the tel-diencephalon.

Introduction

Weissman, Koe & Tenen (1966) have suggested that the increase of locomotor activity caused by (+)-amphetamine can be observed only if the biosynthesis of brain catecholamines is unimpaired. The view that catecholamines mediate the central actions of (+)-amphetamine is supported by other reports in the literature (Hanson, 1966, 1967; Randrup & Munkvad, 1966). However, the data available fail to indicate clearly whether noradrenaline (NA) or dopamine (DM) or both are involved in the action of (+)-amphetamine. Randrup & Scheel-Krüger (1966) suggested that an action on dopaminergic neurones may explain the stereotyped behaviour elicited by high doses of (+)-amphetamine whereas the increase in motility also elicited by these high doses may be associated with an action on noradrenergic neurones. In interpreting the action of large doses of (+)-amphetamine on brain NA turnover rate, one must take into account possible interference with uptake, storage and release of NA by *p*-hydroxynorephedrine, a metabolite of (+)-amphetamine which accumulates in the brains of rats receiving several mg/kg

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of the drug (Groppetti & Costa, 1969 ; Costa & Groppetti, 1970 ; Brodie, Cho & Gessa, 1970).

Ungerstedt & Arbuthnott (1970) have shown that small doses of (+)-amphetamine act on brain dopaminergic axons. However, these experiments were not designed to test whether brain noradrenergic axons are also affected by these doses of (+)-amphetamine. Carlsson (1970) proposed that the stimulation of motor activity caused by (+)-amphetamine in mice is mediated through a release of brain DM.

The results described in this report suggest that (+)-amphetamine (0·3 mg/kg, i.v.) increases motor activity and enhances the turnover rate of striatal DM in rats. This dose neither changes the turnover rate of tel-diencephalic NA nor causes hyperthermia but it does produce anorexia.

Methods

Sprague Dawley male rats, 180–200 g body weight, obtained from Zivic Miller, Pittsburg, Penna., were used throughout these experiments. Upon arrival in the laboratory, the animals were housed at 20° C for about five days before beginning the experiments.

Estimation of brain catecholamine turnover rate

3,5-^{3}H-Tyrosine (31·6 Ci/mmol) dissolved in 50% ethanol was freeze dried and the residue dissolved in saline so that 1 ml injected into the tail vein of rats weighing 200 g contained 1 mCi/kg of 3,5-^{3}H-L-tyrosine. The animals were decapitated 25 min after the injection of 3,5-^{3}H-L-tyrosine. They received intravenously, 15 min before decapitation, either (+)- or (−)-amphetamine sulphate or cocaine hydrochloride or saline. In each experiment a group of rats was killed 10 min after injection of the labelled tyrosine. Immediately after decapitation, the brains were removed from the skull and the cerebellum was discarded. The remaining brain tissue was placed on its dorsal surface and, with a scalpel blade, the brain tissue caudal to a section through the anterior border of the pons was dissected from the tel-diencephalon. The two hemispheres were separated and the corpus callosum was severed to expose the two lateral ventricles. The corpus striatum was located and dissected from the tel-diencephalon. The tel-diencephalon and corpus striatum were frozen and kept at −20° C until assayed. The specific radioactivity of tyrosine, NA and DM was assayed in the same tissue sample by the procedure described by Neff, Spano, Groppetti, Wang & Costa (1971). In this procedure tyrosine is purified by ion exchange chromatography with two Dowex 50 W × 4 (200–400 mesh) columns buffered at pH 6·5 ; NA and DM are isolated by ion exchange chromatography with Dowex 50 W × 4 (200–400 mesh) and adsorption on aluminium oxide Woelm Neutral (Alupharm Chemicals, New Orleans, La.). The radioactivity of deaminated catecholamine metabolites was assayed in the combined effluent fraction and pH 4·5 buffer wash from the first Dowex column. Both fractions containing tyrosine and other amino acids were collected in a 13 ml centrifuge tube containing about 500 mg of aluminium oxide. The effluent was brought to about pH 8·3 by adding 0·75 ml of 3 M ethylenediamine tetraacetic acid disodium salt (EDTA). For catecholamine assays we prepared the aluminium oxide as described by Crout (1961), and washed with 5 ml of 0·1 M EDTA prior

to use. The tubes were shaken manually for 10 min and then centrifuged. The supernatant was discarded and the alumina washed twice with 5 ml water. Then 3 ml of 0·4 N hydrochloric acid was added to the aluminium oxide and the mixture shaken manually for 10 minutes. After centrifugation, an aliquot of the final solution was added to a Triton × 100 solution (Triton × 100, 1 litre; toluene, 2 litres; 2-5 diphenyloxazol, 23 g) and the radioactivity was measured with a Beckman LS 250 liquid scintillation spectrometer with automatic quench correction. An approximate estimate of the rate of synthesis of tissue catecholamines can be calculated from equation (1). This approximation does not take into account the efflux of unlabelled catecholamines from tissue stores during the time interval between the injection of the labelled precursor and the termination of the experiment, and cannot be used to obtain physiological data. It is useful, however, in the assessment of the comparative pharmacological effects of drugs.

$$\text{nmol CA/g tissue} \times t^{-1} = \frac{\text{dpm CA/g tissue}}{\text{S.A. tyrosine}} \quad (1)$$

In equation (1) t is the time interval between labelling and killing the rats and the specific radioactivity (S.A.) of tyrosine refers to the S.A. of tyrosine assayed in the same tissue as the catecholamine (CA). The validity of the approximation is greater when, at the time of measurement, the S.A. of tissue tyrosine is greater than the S.A. of tissue catecholamines.

The data in Table 1 show that the times selected for our study conform with this principle for at 40 min after injection of the labelled tyrosine, the specific radioactivity of tyrosine is almost three fold that of tel-diencephalic NA and twice that of striatal DM.

Pharmacological studies

Measurement of anorexic effects. Rats, placed one per cage (25 × 33 cm) were trained to consume their daily food intake (Purina chow) between 10 and 12 a.m. The daily food consumption was measured by weighing the food before and after feeding time. On the tenth day of the experiment, in the two hours the rats were observed to consume about 80% of their normal daily food intake. On the 16th day of the experiment, ten minutes before feeding time, the rats were injected in the tail vein with either saline (5 ml/kg) or one of the drugs. The anorexic effect of the drugs was estimated by comparing the average food intake on the previous seven days (g/2 h) and that consumed on the day in which the drug was tested. Statistical significance of differences in this and in other experiments was calculated by Student's t test.

TABLE 1. *The specific activity of noradrenaline (NA) in the tel-diencephalon and dopamine (DM) in the striatum at time intervals after injection of* 3,5-^{3}*H-L-tyrosine*

Minutes after 3,5-^{3}H-L-tyrosine	Tel-diencephalic tyrosine dpm/nmol±S.E.	Tel-diencephalic NA dpm/nmol±S.E.	Striatal DM dpm/nmol±S.E.
10 (4)	7,869±447	542±85	790± 80
25 (4)	4,226±140	729±45	1,064±128
40 (3)	2,860±320	980±84	1,395±130

Rats were injected with 3,5-^{3}H-L-tyrosine (1 mCi/kg, i.v.) and killed at 10, 25 and 40 min after injection. Number of animals in parentheses.

Measurement of body temperature. Body temperature was measured rectally, at an ambient temperature of 22° C, with a tele-thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA) at 15 min intervals during the hour preceding and following the intravenous injection of either the various drugs or saline.

Measurement of motor activity. Rats were housed one to each of eight compartments (21 × 33 cm) of an I.R. Electronic Motility Meter (Motron Co., Sweden). Locomotor activity was measured by 40 infrared photocells equally spaced 4 cm apart in the floor of the cages. The floor of the cages was illuminated by a red light. The space between the sensors allowed detection not only of locomotor activity but also of grooming activity. Rearing on the hind legs was detected by 8 photocells placed 7 cm from the floor on one side of the cage. The activity is reported in events/min which are calculated from cumulative counts monitored from 0 to 15 min, from 15 to 30 min, and from 30 to 60 min, respectively. The photocells of each compartment are connected to a separate meter. Each run included two control rats injected with saline and another six rats injected with various drugs. Rats were kept in the motility meter for 30 min before injection and were placed in the same compartment immediately after injection.

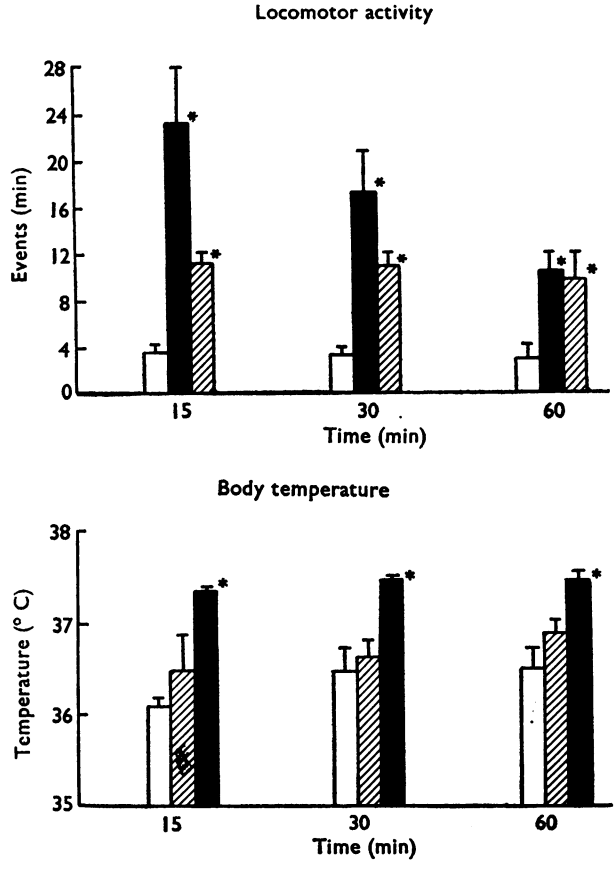

FIG. 1. Effect of (+)-amphetamine and cocaine on locomotor activity and body temperature in groups of five rats. Vertical bars represent standard error of the mean. □, Saline; ■, cocaine, 3 mg/kg, i.v.; ▩, (+)-amphetamine 0·3 mg/kg, i.v. *$P<0.01$.

Drugs. (−)-Amphetamine sulphate and (+)-amphetamine sulphate were gifts of Smith, Kline & French Laboratories, Philadelphia, Pa. Cocaine hydrochloride was purchased from S. B. Penick & Co., New York, N.Y., and 3,5-^{3}H-L-tyrosine from New England Nuclear Co., Boston, Mass.

Results

Motor activity

Figure 1 shows that (+)-amphetamine (0·3 mg/kg, i.v.) and cocaine (3 mg/kg, i.v.) increased the locomotor activity of the rat. Both drugs increased motor activity for at least 60 minutes. The increase in motor activity was already evident 15 min after the injection of both drugs. During the 60 min following (+)-amphetamine injection we did not observe gnawing, biting or other stereotyped behaviour.

A dose of (−)-amphetamine (Fig. 2) three times greater than that of (+)-amphetamine (Fig. 1) did not increase motor activity. These rats also failed to show stereotyped behaviour.

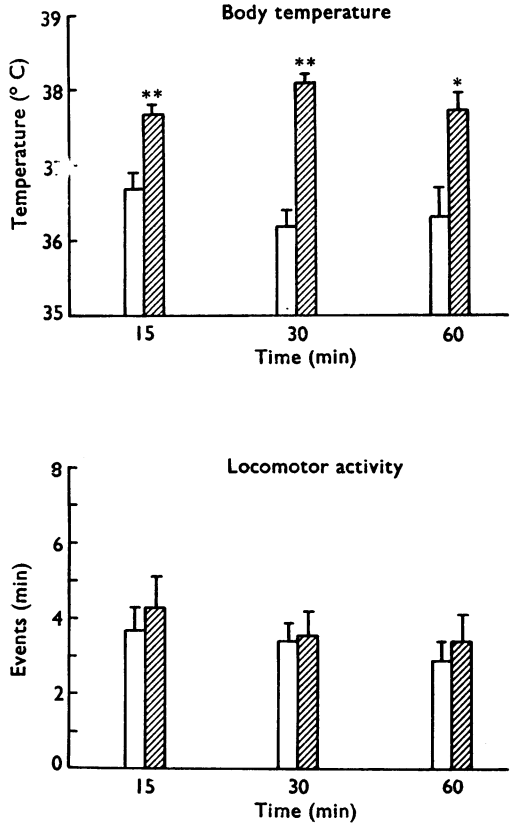

FIG. 2. Effect of (−)-amphetamine on the locomotor activity and body temperature in groups of five rats. Vertical bars represent standard error of the mean. □, Saline; ▨, (−)-amphetamine 1 mg/kg, i.v. *$P<0.05$, **$P<0.01$.

Hyperthermia

The two isomers of amphetamine differ in their ability to produce hyperthermia (Figs 1 and 2). At the doses tested only (−)-amphetamine and cocaine caused hyperthermia (Figs. 1 and 2).

Anorexia

The results of experiments carried out to compare the anorexic effect of cocaine and (+)-and (−)-amphetamine are shown in Figure 3. Cocaine (3 mg/kg, i.v.) or saline failed to reduce the food intake when injected 10 min before feeding. In contrast, the injection of either (+)-amphetamine (0·3 mg/kg, i.v.) or (−)-amphetamine (1 mg/kg, i.v.) significantly reduced the food consumed during feeding time. We also tested the effect of 0·2 mg/kg, i.v. of (+)-amphetamine and found that this dose also caused anorexia.

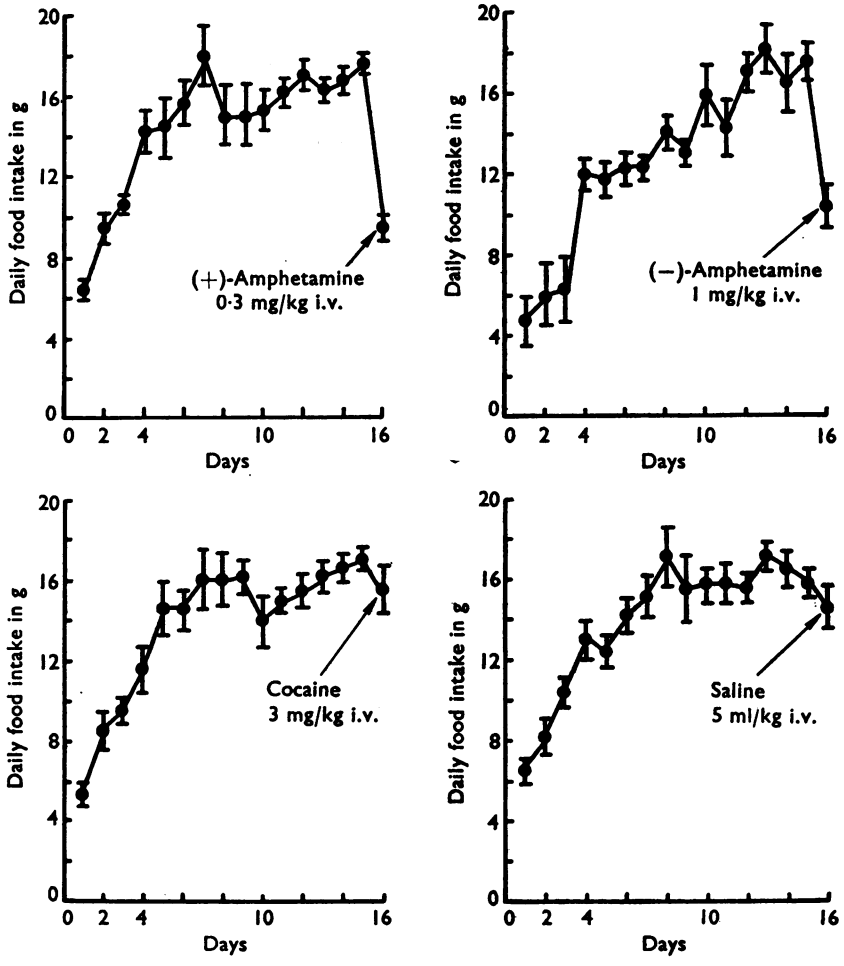

FIG. 3. Effect of (+)- and (−)-amphetamine and cocaine on food intake in groups of four rats. The food intake after (+)- and (−)-amphetamine is significantly smaller ($P<0·01$) than the average food intake measured in each of the seven days before (+)- or (−)-amphetamine administration. Cocaine and saline do not change the average food intake. Vertical bars represent standard error of the mean.

Turnover of brain catecholamines

In a preliminary study brain DM and NA concentrations were measured 2 h after intravenous injections of various doses of (+)- and (−)-amphetamine. We found that 0·3 mg/kg of (+)-amphetamine failed to change the concentrations of DM in the striatum and of NA in the tel-diencephalon whereas 0·6 mg/kg significantly decreased NA concentrations in this tissue ($P<0·05$, $n=4$). After 1 mg/kg intravenously of (−)-amphetamine, the catecholamine content of the brain was not changed but the concentration of NA in the tel-diencephalon was significantly reduced after 2 mg/kg intravenously ($P<0·05$, $n=4$).

DM turnover in striatum. The data listed in Table 2 show that the specific radioactivity of striatal DM was significantly higher in rats receiving (+)-amphetamine (0·3 mg/kg, i.v.) than in rats receiving saline alone. Since the specific radioactivity of tyrosine in the brain tissue and the concentration of DM in the corpus striatum was unchanged in the two groups of animals, the conversion rate of 3,5-^{3}H-L-tyrosine into striatal DM was greater in rats injected with (+)-amphetamine than in those receiving saline. Table 2 also includes data showing the effects of cocaine (3 mg/kg, i.v.) on the conversion of 3,5-^{3}H-L-tyrosine into striatal DM. This drug, like (+)-amphetamine, increased the conversion index of tel-diencephalic tyrosine into striatal DM. In contrast, (−)-amphetamine (1 mg/kg, i.v.) failed to change the specific radioactivity of striatal DM and the conversion index of tel-diencephalic tyrosine into striatal DM. In the striatum of rats receiving (−)-amphetamine, (+)-amphetamine or cocaine the concentrations of tritiated deaminated catecholamine metabolites were similar to those of saline-treated rats used as controls (Table 2).

The effects of two doses of (+)-amphetamine on the locomotor activity and conversion index of tissue tyrosine into striatal DM are compared in Table 3. It appears that 0·3 mg/kg, i.v. increased motor activity during the first 15 min after injection and enhanced the conversion index of striatal tyrosine into DM; the smaller dose (0·2 mg/kg, i.v.) failed to change the motor activity and the conversion index of striatal tyrosine into DM.

NA turnover in tel-diencephalon. The effects of two doses of (+)-amphetamine on the conversion index of tel-diencephalic tyrosine into NA are compared in Table 3. Neither dose of (+)-amphetamine changed this conversion index.

TABLE 2. *Effect of (+)-amphetamine, cocaine and (−)-amphetamine on the conversion of 3,5-^{3}H-L-tyrosine into dopamine (DM) in the corpus striatum*

Drug injected		Tel-diencephalic tyrosine dpm/nmol ±S.E.	Striatal DM dpm/nmol ±S.E.	Striatal DM nmol/g ±S.E.	Conversion index (nmol/g)/25 min ±S.E.	Deaminated catecholamine metabolites in striatum dpm×10^{-3}/g ±S.E.
Saline	(3)	4,024±379	1,264±158	53±9	15±2	14·8±1·6
(+)-Amphetamine	(3)	4,229±536	1,716±36†	60±3	27±2*	15·7±0·58
Saline	(9)	4,094±145	1,105±44	58±6	15±1	14·9±0·53
Cocaine	(6)	3,553±155*	1,364±126*	62±4	24±2†	13·5±0·52
Saline	(11)	4,104±120	1,301±130	53±5	17±1	14·9±0·53
(−)-Amphetamine	(8)	3,796±205	1,492±75	54±2	21±2	14·8±1·3

Rats received 1 mCi/kg, i.v. of 3,5-^{3}H-L-tyrosine and 10 min later either saline (5 ml/kg, i.v.) or one of the following drugs ((+)-amphetamine, 0·3 mg/kg, i.v.; cocaine, 3 mg/kg, i.v.; or (−)-amphetamine, 1 mg/kg, i.v.). The rats were decapitated 15 min after the drug injection. Number of animals in parentheses. * $P<0·05$. † $P<0·01$.

TABLE 3. *The effect of two doses of (+)-amphetamine on locomotor activity and turnover rate of brain catecholamines*

		Striatum				Tel-diencephalon			
(+)-Amphetamine mg/kg i.v.	Motor activity (events/min) during 15 min	Tyrosine dpm/nmol ±S.E.	Dopamine nmol/g±S.E.	Dopamine dpm/nmol ±S.E.	Conversion Index (nmol/g)/25 min±S.E.	Tyrosine (dpm/nmol)/g ±S.E.	Noradrenaline nmol/g±S.E.	Noradrenaline dpm/nmol ±S.E.	Conversion Index (nmol/g)/25 min±S.E.
– (5)	4·53±1·71	2,537±307	59±3	640±70	16±3	2,923±157	3·1±0·2	830±84	0·87±0·04
0·3 (5)	24·56±7·44†	2,089±351	59±8	1,135±104*	32±5*	2,805±178	3·3±0·06	858±54	0·98±0·15
– (4)	2·53±0·71	2,157±296	63±6	848±76	12±0·9	2,847±111	2·8±0·08	687±66	0·52±0·08
0·2 (4)	5·78±1·25	2,927±214	55±2	929±58	10±1·3	3,282±211	2·7±0·05	833±47	0·70±0·09

Rats were injected initially with 0·8 mCi/kg, i.v. of 3,5-^{3}H-L-tyrosine and 10 min later with either saline (5 ml/kg, i.v.) or one of the doses of (+)-amphetamine and killed 15 min later. Motor activity was recorded for 15 min after the injection of either saline or one of the two doses of (+)-amphetamine in other groups of animals run in parallel. Number of animals in parentheses.

*$P<0·05$; †$P<0·01$.

TABLE 4. *The effect of cocaine and (+)- and (−)-amphetamine on the conversion of 3,5-^{3}H-L-tyrosine to noradrenaline in the tel-diencephalon*

Drug injected		Tel-diencephalic tyrosine dpm/nmol±S.E.	Tel-diencephalic noradrenaline dpm/nmol±S.E.	Tel-diencephalic noradrenaline nmol/g±S.E.	Conversion index (nmol/g)/25 min±S.E.
Saline	(11)	4,104±120	735±48	2·2±0·14	0·38±0·05
(+)-Amphetamine	(6)	3,925±302	731±60	2·5±0·15	0·41±0·04
(−)-Amphetamine	(11)	3,796±205	716±25	2·8±0·16	0·39±0·04
Cocaine	(10)	3,756±146	763±43	2·6±0·09	0·43±0·04

Rats were injected with 1 mCi/kg, i.v. of 3,5-^{3}H-L-tyrosine and 10 min later with saline (5 ml/kg, i.v.), (+)-amphetamine (0·3 mg/kg, i.v.), or (−)-amphetamine (1 mg/kg, i.v.) or cocaine (3 mg/kg, i.v.) and killed 15 min later. Number of animals in parentheses.

The specific radioactivity of tel-diencephalic NA in rats receiving (+)-amphetamine (0·3 mg/kg, i.v.), (−)-amphetamine (1 mg/kg, i.v.) or cocaine (3 mg/kg, i.v.) is equal to that of rats injected with only saline (Table 4). These drugs do not change the conversion index of tyrosine into tel-diencephalic NA.

Discussion

The results of our study differentiate between the pharmacological and neurochemical effects of the two doses of (+)-amphetamine we selected. This drug at a dose of 0·3 mg/kg, intravenously, stimulates motor activity and causes anorexia but not hyperthermia. In contrast, 0·2 mg/kg, intravenously, of (+)-amphetamine fails to enhance motor activity during the 15 min following injection but still causes anorexia. Since both doses of (+)-amphetamine neither change the steady state concentration of tel-diencephalic NA nor increase the incorporation of 3,5-^{3}H-L-tyrosine into NA, we can conclude that the anorexia and increase of motor activity elicited by (+)-amphetamine are not related to a change in the turnover rate of tel-diencephalic NA. This view is supported by the results obtained with cocaine.

The dose of (−)-amphetamine selected (1 mg/kg, i.v.) causes anorexia and hyperthermia but does not increase motor activity. Since this drug does not change NA turnover rate we can suggest that these two pharmacological responses are unrelated to an action on brain NA. However, we cannot exclude the possibility that in some discrete areas of the tel-diencephalon the turnover rate of NA is increased and that this change may be obscured in our experiments by the size of the tissue sample.

Our data suggest that an accelerated conversion of tyrosine into striatal DM may be involved in the increase of motor activity elicited by 0·3 mg/kg intravenously of (+)-amphetamine and by cocaine (3 mg/kg, i.v.). The increase in the turnover rate of striatal DM did not occur after 1 mg/kg intravenously of (−)-amphetamine or after 0·2 mg/kg intravenously of (+)-amphetamine which failed to increase motor activity. However, our experimental design does not allow us to state that an increase of striatal DM turnover rate is instrumental in determining the effects of (+)-amphetamine on motor activity. The results in Table 1 indicate that in our experiments a change in the turnover rate of tel-diencephalic NA would be revealed as promptly as a change in the turnover rate of striatal DM. Thus, it seems valid to conclude that both doses of (+)-amphetamine and the dose of (−)-amphetamine and cocaine we have tested do not change the turnover rate of tel- diencephalic NA (Tables 3 and 4). Moreover, our experimental design ensures that the drug treatment does not interfere with the uptake and distribution of the radioactive amino acid for both phenomena occur prior to the drug injection.

Glowinski, Iversen & Axelrod (1966) reported that high doses of (+)-amphetamine can inhibit brain monoamine oxidase. The effects on the concentration of radioactive deaminated catecholamine metabolites shown in Tables 2 and 3 suggest that an inhibition of monoamine oxidase does not occur to a great extent in rats receiving either (+)-amphetamine (up to 0·3 mg/kg, i.v.) or cocaine (3 mg/kg, i.v.) because neither drug reduced the level of these radioactive metabolites. Since the steady-state concentration remains unchanged and the tritiated catecholamine deaminated metabolites are not increased we suggest that (+)-amphetamine increases the extraneuronal release of striatal DM. This possibility would be in keeping with reports by Weissman *et al.* (1966), Hanson (1966, 1967) and Randrup & Munkvad (1966) on the indirect action of (+)-amphetamine on catecholaminergic neurones and would suggest that a site of action of (+)-amphetamine might reside in brain dopaminergic neurones rather than in brain noradrenergic neurones. In conclusion, minimal effective doses of (+)-amphetamine increase motor activity without increasing the turnover rate of brain NA. This view is in agreement with proposals made by Van Rossum, Van der Schoot & Hurkmans (1962) and Smith (1963, 1965).

REFERENCES

BRODIE, B. B., CHO, A. K. & GESSA, G. L. (1970). Possible role of *p*-hydroxynorephedrine in the depletion of norepinephrine induced by (+)-amphetamine and in tolerance to this drug. In: *International Symposium on Amphetamines and Related Compounds*, Proceedings of the Mario Negri Institute for Pharmacological Research, ed. Costa, E. and Garattini, S., pp. 217–230. New York: Raven Press.

CARLSSON, A. (1970). Amphetamine and brain catecholamines. In: *International Symposium on Amphetamines and Related Compounds*, Proceedings of the Mario Negri Institute for Pharmacological Research, ed. Costa, E. and Garattini, S., pp. 289–300. New York: Raven Press.

COSTA, E. & GROPPETTI, A. (1970). Biosynthesis and storage of catecholamines in tissues of rats injected with various doses of (+)-amphetamine. In: *International Symposium on Amphetamines and Related Compounds*, Proceedings of the Mario Negri Institute for Pharmacological Research, ed. Costa, E. and Garattini, S., pp. 217–230. New York: Raven Press.

CROUT, R. J. (1961). Catecholamines in urine. In: *Standard Methods of Clinical Chemistry*, Vol. 3, ed. Seligson, D., pp. 62–80. New York: Academic Press.

GLOWINSKI, J., IVERSEN, L. L. & AXELROD, J. (1966). Regional studies on catecholamines in rat brain. IV. Effects of drugs on the disposition and metabolism of ^{3}H norepinephrine and ^{3}H dopamine. *J. Pharmac. exp. Ther.*, **153**, 30–41.

GROPPETTI, A. & COSTA, E. (1969). Tissue concentrations of *p*-hydroxynorephedrine in rats injected with (+)-amphetamine: effect of pretreatment with desipramine. *Life Sci.*, **8**, 653–665.

HANSON, L. C. F. (1966). Evidence that the central action of amphetamine is mediated via catecholamines. *Psychopharmacologia*, **9**, 78–80.

HANSON, L. C. F. (1967). Evidence that the central action of (+)-amphetamine is mediated via catecholamines. *Psychopharmacologia*, **10**, 289–297.

NEFF, N. H., SPANO, P. F., GROPPETTI, A., WANG, C. T. & COSTA, E. (1971). A simple procedure for calculating the synthesis rate of norepinephrine, dopamine and serotonin in rat brain. *J. Pharmac. exp. Ther.*, **176**, 701–710.

RANDRUP, A. & MUNKVAD, J. (1966). Role of catecholamines in the amphetamine excitatory response. *Nature, Lond.*, **211**, 540.

RANDRUP, A. & SCHEEL-KRÜGER, J. (1966). Diethyldithiocarbamate and amphetamine stereotype behaviour. *J. Pharm. Pharmac.*, **18**, 752.

SMITH, C. B. (1963). Enhancement by reserpine and α-methyldopa of the effects of (+)-amphetamine upon the locomotor activity of mice. *J. Pharmac. exp. Ther.*, **142**, 343–347.

SMITH, C. B. (1965). Effects of (+)-amphetamine upon brain amine and locomotor activity of mice. *J. Pharmac. exp. Ther.*, **147**, 96–102.

UNGERSTEDT, U. & ARBUTHNOTT, G. W. (1970). Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of the nigrostriatal dopamine system. *Brain Res.*, **24**, 485–493.

VAN ROSSUM, J. M., VAN DER SCHOOT, J. B. & HURKMANS, J. A. (1962). Mechanism of action of cocaine and amphetamine in the brain. *Experientia, Basel*, **18**, 229–231.

WEISSMAN, A., KOE, B. K. & TENEN, S. (1966). Anti-amphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmac. exp. Ther.*, **151**, 339–352.

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