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Biochemical differentiation of amphetamine *vs* methylphenidate and nomifensine in rats

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Amphetamine-like stimulants were divided into two groups, one in which the stereotyped behaviour was not antagonized by reserpine [(+)-amphetamine, (−)-amphetamine, methamphetamine, phenmetrazine and phenethylamine] and another group in which the behavioural effects were blocked by reserpine (methylphenidate, nomifensine, pipradrol and amfonelic acid (NCA; Win 25978)). Both groups increased homovanillic acid (HVA) in whole brain 2 h after administration. The 'methylphenidate group' also increased brain 3,4-dihydroxyphenylacetic acid (DOPAC) in naive rats; whereas the '(+)-amphetamine group' decreased DOPAC in naive rats, as well as in reserpinized rats, α-methyl-*p*-tyrosine-treated rats, and after acute hemisection. The reserpine antagonism of the 'methylphenidate group'-induced stereotyped behaviour was partially reversed by type A monoamine oxidase inhibition. The '(+)-amphetamine group'-induced stereotyped behaviour was not blocked by short time pretreatment with α-methyltyrosine, only by longer pretreatment intervals. The mechanisms by which the two groups are differentiated biochemically is discussed with special attention to possible intra-neuronal inhibition of dopamine oxidation by the '(+)-amphetamine group'.

Recently it appeared that the new, apparently non-toxic antidepressant drug nomifensine (Angst, Koukkou & others, 1974; Eckmann, 1974; Pecknold, Ban & others, 1975; Taeuber, 1975) could be classified as a member of the 'methylphenidate-group'* of amphetamine-like stimulants by the antagonism of its behavioural effects by a high dose of reserpine (Braestrup & Scheel-Krüger, 1976). The possible therapeutic usefulness of a drug from a sub-group of amphetamine-like stimulants prompted a reinvestigation of their mechanisms of action as opposed to more classical amphetamines, such as (+)-amphetamine, methamphetamine and phenmetrazine, which readily cause euphoria and also psychosis (Kalant, 1966; Rylander, 1972).

Two sub-groups of amphetamines were therefore compared on biochemical and behavioural parameters associated with brain dopamine. The differentiation of the two groups of amphetamines by selective reserpine or α-methyltyrosine antagonism of behavioural effects (see Discussion) was confirmed. Moreover it appeared that the '(+)-amphetamine group' as a whole selectively decreased brain DOPAC while the 'methylphenidate group' surprisingly caused increases.

* The 'methylphenidate group' consists of methylphenidate, nomifensine, amfonelic acid and pipradrol; the 'amphetamine group' consists of (+)-amphetamine (−)-amphetamine, methamphetamine, phenmetrazine and phenethylamine.

Several possibilities for the biochemical differences were investigated. The effects of inhibition of dopamine uptake were investigated by comparison with the strong uptake inhibitor, benztropine, effects on impulse flow by acute hemisection, effects on newly synthesized dopamine by the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine methyl ester (α-MT) and effects of monoamine oxidase type A inhibition by the selective inhibitor clorgyline.

METHODS

Male Wistar rats 250 g were used except in stereotaxic experiments, where they were between 170–190 g. They were housed individually at 21–23° in coarse wire mesh cages. Food and water were withdrawn during behavioural observation in the home cage.

Stereotyped behaviour. The rats were observed continuously for the first 2 h after administration of the stimulants and then at various intervals. The behaviour was recorded every 10 or 20 min according to the following classification: 0: No observable effect of the stimulant compared to control rats. A: Increased activity, though not stereotyped. Occasional sniffing and slow head movements up and down and/or increased locomotor and rearing activities. B: Continuous sniffing at the cage. C: Licking or biting either continuous or interrupted by continuous sniffing. Stereotyped

behaviour was considered as present when either registration B or C was recorded.

Dopamine metabolites. Homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid were assayed by the g.l.c.-procedure of Braestrup, Andersen & Randrup (1975). Analyses were made on whole brains, including cerebellum, except in the experiment with α-MT or in stereotaxic experiments, where one or two striata were used. Groups of 4–5 animals were treated with stimulants and groups of 4–5 control animals (vehicle treated or antagonist + vehicle treated) were always analysed in parallel. Values are expressed in per cent of the control values. Statistical comparison was made by Student's *t*-test of the stimulant group *vs* the control group. The absolute control values in the Figures and Tables are all the actual control values used for that individual table or figure.

Surgery. Under light ether anaesthesia rats were mounted in a stereotaxic apparatus and a hole was drilled in the skull at 4·4 mm anterior to the inter aural line and lateral 0·2 to 2·2 mm. A knife with a 2 mm horizontal edge was lowered vertically through the hole to the floor of the cranium about 10·5 mm below the surface of the skull (coordinates according to König & Klippel, 1963). Thirty min after the unilateral hemisection the rats had almost recovered from anaesthesia and they were challenged with (+)amphetamine (10 mg kg^{-1}, s.c.).

The following drugs were used (drugs were dissolved in saline, except when the vehicle is indicated in brackets): (+)-amphetamine sulphate (Smith, Kline & French), methylphenidate hydrochloride (Ritaline, CIBA, Copenhagen), nomifensine (8-amino-2-methyl-4-phenyl-1,2,3,4-tetrahydroisoquinoline) hydrogen maleinate (HOE 984, Hoechst, Frankfurt), M_1 metabolite of nomifensine (8-amino-2-methyl-4-(4-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline fumarate, generously supplied by Hoechst) (0·1 N HCl + H_2O), cocaine hydrochloride, benztropine hydrobromide, reserpine (Serpasil ampoules, gift from CIBA, Copenhagen), α-methyl-*p*-tyrosine methyl ester (α-MT; 44/68, Hässle), haloperidol (Serenase, Janssen Pharmaceuticals), pipradrol, hydrochloride (Gerodyl, A/S GEA, Copenhagen) (propyleneglycol, 1 N HCl 9:1), amfonelic acid (NCA; Win 25978; 7-benzyl-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid; Winthrop) (propyleneglycol, 2·5 N K_2CO_3 9:1), phenethylamine (PEA) hydrochloride, methamphetamine hydrochloride (Mecobenzon, Copenhagen), (−)-amphetamine (gift from Ferrosan), phenmetrazine (Preludin, Boehringer Ingelheim), 1-deprenyl (gift from Dr Knoll, Budapest), clorgyline (May & Baker), baclofen (Lioresil, gift from CIBA, Copenhagen) (propyleneglycol, 1 N HCl 9:1). All drugs were injected subcutaneously except α-MT and cocaine which were injected intraperitoneally. Dose levels are expressed as the salt.

RESULTS

Behavioural results

All the stimulants used induce a similar quality of stereotyped behaviour. In the applied doses, all the drugs, except PEA, induced licking or biting stereotypies after about 1 h. PEA induced strong sniffing but never licking or biting. For detailed descriptions from this laboratory, which include dose-responses, see (Randrup, Munkvad & Udsen, 1963; Fog, 1969; Scheel-Krüger, 1971; Braestrup & Scheel-Krüger, 1976; Braestrup & Randrup, 1977).

On the basis of comprehensive studies from our laboratory, a dose of 7·5 mg kg^{-1} reserpine was selected for experiments on behavioural antagonism to distinguish between the two groups of amphetamines (Scheel-Krüger, 1972). In close agreement with these early studies the behavioural effects of the '(+)-amphetamine group' were not antagonized by reserpine, while the behavioural effect of the 'methylphenidate group' was completely inhibited.

The reserpine-induced inhibition of the 'methylphenidate-group' behavioural effects was partially

Table 1. *Stereotyped behaviour induced by two groups of amphetamine-like drugs.* Inhibition by reserpine (7·5 mg kg^{-1}, s.c., 22½ h before stimulant) of the 'methylphenidate group' and reversal by clorgyline (8 mg kg^{-1}, s.c., 4½ h before stimulant). Number of rats in brackets.

		% animals showing stereotyped behaviour		
			Pretreatment	
	mg kg^{-1}, s.c.	Saline	Reserpine	Reserpine + clorgyline
(+)-Amphetamine	10	100 (5)	100 (10)	
Phenmetrazine	100	100 (5)	100 (5)	not
Methamphetamine	10	100 (5)	100 (5)	tested
PEA+	40	100 (44)	100 (6)	
Methylphenidate	100	100 (5)	0 (5)	80 (5)
Nomifensine	30	100 (10)	0 (5)	100 (5)
M_1-metabolite	30	100 (5)	0 (4)	not tested
Amfonelic acid	10	100 (5)	0 (5)	87 (8)
Pipradrol	100	100 (5)	0 (4)	75 (4)
Saline		0 (10)	0 (10)	0 (10)

Stereotyped behaviour was considered as present when the rats engaged in continuous sniffing, licking or biting for prolonged periods.
+ Pretreatment 5 h before with 8 mg kg^{-1} subcutaneously l-deprenyl.

reversed by clorgyline (8 mg kg^{-1}, s.c.) either administered 4½ h before the stimulants (Table 1) or simultaneously with a stimulant (amfonelic acid, 10 mg kg^{-1}, n = 4). The stereotypies in the rats pretreated with reserpine and clorgyline never reached the same intensity (speed) as in unpretreated rats, the sniffing, licking or biting, however, still being clearly stereotyped. Of the total of 22 rats tested in the four stimulants, pretreated with reserpine + clorgyline, 15 exhibited licking or biting within the interval 1½ to 3½ h after the stimulants. After about 3½ h the stimulation subsided and the rats regained their reserpine postures.

Reserpine alone, reserpine + clorgyline (n = 10) or reserpine + deprenyl (8 mg kg^{-1}, s.c.) + amfonelic acid (n = 3) exhibited no signs of stimulation.

The monoamine oxidase-A inhibitor clorgyline thus partially reversed the reserpine-induced inhibition of the 'methylphenidate-group'. The onset was unchanged or delayed, the behavioural intensity was decreased and the duration was reduced.

Table 2. *Lack of blockade of the stereotyped behaviour by ½ h pretreatment with the catecholamine synthesis inhibitor α-MT (250 mg kg^{-1}, i.p.).* Number of rats in brackets.

	mg kg^{-1}, s.c.	% of animals showing stereotyped behaviour, α-MT at 5 h	1/2 h
(+)-Amphetamine	10	0 (5)	90 (9)
Phenmetrazine	100	40 (5)	100 (5)
Methamphetamine	10	0 (5)	80 (5)
PEA*	40	0 (6)	80 (5)
Methylphenidate	100	60 (5)	100 (5)
Nomifensine	30	25 (4)	100 (5)
Amfonelic acid	10	60 (5)	100 (5)
Pipradrol	100	80 (5)	100 (5)
Saline	0	0	0

Stereotyped behaviour was considered as present when the rats engaged in continuous sniffing, licking or biting for prolonged periods.

* Pretreatment 4½ h with 1-deprenyl 8 mg kg^{-1} subcutaneously.

Table 2 shows that, except for phenmetrazine, the stereotyped behaviour of the '(+)-amphetamine group' was completely inhibited by 5 h pretreatment with α-MT; the 'methylphenidate group' being less affected. The catecholamine synthesis is almost completely blocked ½ h after α-MT (Javoy & Glowinski, 1971), but at this time interval the stereotyped behaviour of the '(+)-amphetamine group' was not blocked (Table 2). The phase of continuous sniffing was a little delayed and appeared after 60 to 80 min. The period of continuous sniffing was interrupted by spells of licking/biting in 15 out of 24 rats treated with α-MT + the '(+)-amphetamine group' (note, however, that phenethylamine alone does not induce licking or biting but only sniffing). The intensity and the duration of (+)-amphetamine and methamphetamine stereotypies were reduced by α-MT, whereas the intensity and duration of the phenmetrazine and phenethylamine stereotypies were not reduced. This result shows that both groups of amphetamines can induce behavioural activation ½ h after α-MT, when the synthesis of catecholamines is maximally reduced. The rearing and locomotion normally produced 5–60 min after administration of the 'amphetamine group' was, however, much reduced by ½ h pretreatment with α-MT. At long time intervals (2 h as used by many investigators or 5 h, Table 2), when both the synthesis is reduced and the stores are partly depleted, the '(+)-amphetamine group' is much more sensitive to α-MT than the 'methylphenidate group'.

Biochemical results

HVA and DOPAC after amphetamine congeners. Fig. 1 shows the time-course and dose response of amphetamine on HVA and DOPAC in the whole

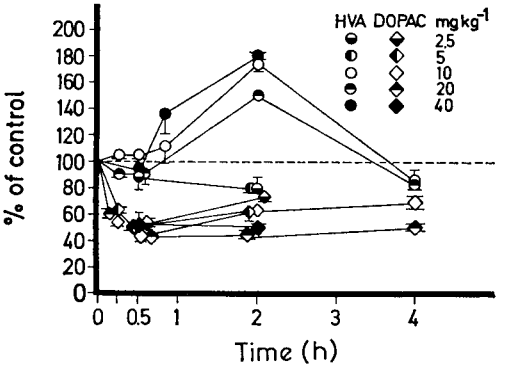

FIG. 1. Homovanillic acid (HVA, circles) and 3,4-dihydroxyphenylacetic acid (DOPAC, diamonds) in the whole rat brain after increasing doses of (+)-amphetamine sulphate (s.c.). The shortest time interval was 15 min. Shown are the mean ± s.e.m. of 4 to 10 values in % of appropriate controls. Control concentrations: HVA, 49·6 ± 1·5 ng g^{-1} (42 values), DOPAC, 58·6 ± 2·1 ng g^{-1} (36 values), not corrected for recovery. All values more than 20% different from control were actually significant, $P<0.05$, Student's *t*-test.

rat brain. HVA and DOPAC can be affected independently of each other. Already after 15 min DOPAC is maximally decreased to about 50 to 60% of controls by a wide range of dose levels, a reduction lasting for more than 4 h after 20 mg kg^{-1}. HVA showed a tendency to decrease after 15 to 30 min but then rose strongly about 1 h after administration of amphetamine at doses above 5 mg kg^{-1}.

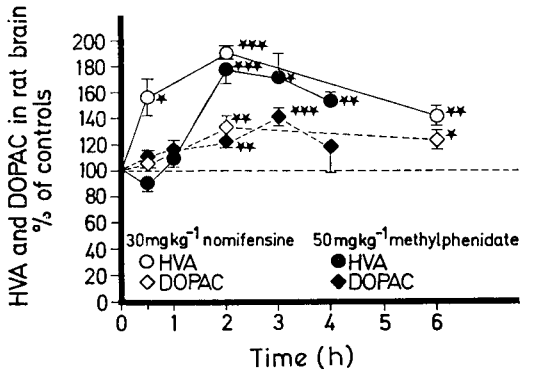

FIG. 2. HVA and DOPAC in whole rat brain after 30 mg kg^{-1} nomifensine or 50 mg kg^{-1} methylphenidate (s.c.). The shortest time interval was 30 min. Shown are the mean ± s.e.m. of 4 to 5 values in % of appropriate controls. Control concentrations: HVA, 53·0 ± 2·1 ng kg^{-1} (22 values); DOPAC, 57·7 ± 3·1 ng g^{-1} (21 values), not corrected for recovery.
*$P<0.05$; **$P<0.01$; ***$P<0.001$; Student's *t*-test.

Nomifensine and methylphenidate caused a long-lasting increase in HVA. In contrast to the amphetamine effect, DOPAC was increased (Fig. 2).

All the drugs in the '(+)-amphetamine group' caused the same biochemical pattern as (+)-amphetamine at the selected time intervals of 2 h: HVA was increased and DOPAC was decreased (Table 3). In sharp contrast, the 'methylphenidate group' caused an increase in DOPAC of about 20 to 40% as well as an increase in HVA (Table 3). Baclofen increased DOPAC after 2 h (Table 3), whereas DOPAC was unchanged (107 ± 9% of controls, n = 5, $P>0.25$) ½ h after 10 mg kg^{-1} baclofen.

Pretreatment of the rats with reserpine, which by itself caused an increase in HVA (to 180% of saline treatment, $P<0.001$) and DOPAC (to 154% of saline treatment, $P<0.001$), prevented or, in some cases reversed, the HVA-increasing effects of all the stimulants. The DOPAC decreasing effect of the '(+)-amphetamine group' was potentiated and the DOPAC-increase of the 'methylphenidate group' was reversed to a decrease. The two dopamine uptake inhibitors benztropine and cocaine

Table 3. *Increase in whole brain concentration of HVA and differential effect on DOPAC by two groups of amphetamine-like drugs.* The drugs were administered 2 h before decapitation and the values shown are the mean ±s.e.m. in % of controls analysed on the same day. Pooled control concentrations were: HVA, 57·3 ± 2·0 ng g^{-1} (32 values). DOPAC, 62·9 ± 2·5 ng g^{-1} (35 values), not corrected for recovery.

		Whole brain concn % of control	
	mg kg^{-1}, s.c.	HVA	DOPAC
(+)-Amphetamine	10	182·8 ± 6·4 (5)***	62·4 ± 5·8 (5)***
Phenmetrazine	50	162·5 ±13·5 (4)**	77·7 ± 1·6 (4)*
Methamphetamine	10	173·1 ±13·0 (4)***	79·8 ± 7·0 (4)*
Phenethylamine+	40	152·0 ± 6·0 (5)***	89·5 ± 1·3 (5)*
(−)-Amphetamine	50	163·1 ±21·2 (4)*	69·4 ± 2·8 (5)**
Methylphenidate	20	142·1 ±10·0 (4)*	109·7 ± 5·7 (4)
Methylphenidate	50	177·9 ±12·5 (4)***	121·0 ± 4·9 (4)**
Methylphenidate	100	154·2 ±10·6 (5)**	133·9 ±12·1 (5)*
Nomifensine	30	192·2 ± 6·5 (5)***	133·7 ± 7·8 (5)**
Amfonelic acid	5	188·5 ±11·9 (5)**	144·8 ± 2·3 (5)***
Pipradrol	50	136·7 ± 5·2 (4)**	121·0 ± 5 (3)*
Benztropine	25	100 ± 5 (4)	78 ± 3 (3)
Cocaine	15§	93 ± 4 (4)	68 ± 2 (4)**
Baclofen	10	149 ± 3 (4)***	136 ± 8 (8)*

+ Pretreated 5 h before PEA by 2 mg kg^{-1} 1-deprenyl subcutaneously.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$ compared to vehicle treated animals by Student's *t*-test.
§ 1 h pretreatment and intraperitoneal administration.

also reduced DOPAC somewhat in reserpinized rats (Table 4).

Table 4. *Reserpine-pretreated rats, effects of two groups of amphetamine-like drugs, cocaine and benztropine on HVA and DOPAC in the whole brain.* All rats received reserpine 7·5 mg kg^{-1}, s.c. 20 h and the stimulant 2 h before decapitation. Shown are the means ± s.e.m. in % of reserpine treatment alone analysed on the same day. The concentrations in reserpine treatment alone were: HVA 103·6 ± 3·6 ng g^{-1} (23 values) (180% of saline treatment, $P<0·001$); DOPAC 97·07 ± 3·5 ng g^{-1} (21 values) (154% of saline treatment, $P<0·001$).

	mg kg^{-1}, s.c.	Whole brain concn % of reserpine alone HVA	DOPAC
(+)-Amphetamine	10	50 ± 7 (5)***	24 ± 2 (5)***
Phenmetrazine	50	42 ± 2 (4)***	29 ± 3 (4)***
Methamphetamine	10	81 ± 8 (4)	32 ± 2 (4)***
Phenethylamine+	40	81 ± 3 (4)*	51 ± 2 (4)***
Methylphenidate	50	95 ±11 (4)	81 ± 5 (4)
Nomifensine	30	57, ± 3 (5)***	53 ± 2 (4)***
Amfonelic acid	5	74 ± 5 (5)**	68 ± 4 (5)***
Pipradrol	50	79 ± 9 (4)	74 ±11 (4)*
Benztropine	25	83 ±10 (4)	70 ± 6 (4)*
Cocaine	15, i.p. (1 h)	92 ± 7 (4)	69 ± 3 (4)*

*$P<0·05$; **$P<0·01$; ***$P<0·001$ compared with reserpine alone by Student's *t*-test.
+Pretreated 5 h before PEA by 2 mg kg^{-1} 1-deprenyl subcutaneously.

Pretreatment of rats with α-MT did not block the HVA-increase caused by (+)-amphetamine and phenmetrazine though the increase was less pronounced than for methylphenidate and nomifensine. The two latter drugs lost their DOPAC increasing potential after α-MT pretreatment, whereas (+)-amphetamine and phenmetrazine retained their DOPAC decreasing action (Table 5).

Table 5. *α-Methyltyrosine pretreated rats, effects of two groups of amphetamine-like drugs on HVA and DOPAC in the rat corpus striatum.* All animals received α-MT 250 mg kg^{-1}, intraperitoneally 2½ h and the stimulants 2 h before decapitation. Shown are the mean ±s.e.m. in % of α-MT treatment alone, analysed on the same day. The amounts in α-MT treated rats were HVA 20·5 ± 1·0 (8) ng/2 striata (about 90 mg); DOPAC 26·1 ± 1·4 (8) ng/2 striata, not corrected for recovery.

	dose mg kg^{-1}	% α-MT-treatment alone HVA	DOPAC
(+)-Amphetamine	10	129 ± 8 (4)*	49 ± 7 (4)***
Phenmetrazine	100	135 ± 8 (4)**	47 ± 7 (4)***
Methylphenidate	50	147 ± 3 (4)***	102 ± 4 (4)
Nomifensine	30	181 ± 10 (4)***	89 ± 7 (4)

* $P < 0·05$; ** $P < 0·01$; ***$P < 0·001$ compared to α-MT alone by Student's *t*-test.

Acute hemisection did not prevent the DOPAC-decreasing effect of amphetamine (Table 6). In another similar experiment, acute hemisection alone, at 3·5 mm anterior to the inter aural line, caused a strong increase in DOPAC; the reducing effect of (+)-amphetamine on this high DOPAC concentration was still present (n = 4, data not shown). *In vitro* uptake of [^{3}H]dopamine in striatal synaptosomes was reduced to less than 10% of control after 7 days (data not shown) indicating a profound degeneration of dopamine terminals in corpus striatum following hemisection.

Table 6. *Unilateral hemisection of rats, persistence of (+)-amphetamine effect on DOPAC.* Half an hour after unilateral hemisection at anterior 4·4 mm under ether anaesthesia, rats were treated with (+)-amphetamine (10 mg kg^{-1}, s.c.) and killed 2 h later. DOPAC was measured in each single striatum (about 45 mg) and the values expressed in ng total, mean ±s.e.m. of (n) values.

Surgery	ng in one striatum DOPAC
Sectioned + NaCl	47·7 ± 7·6 (4)
Sectioned + (+)-amphetamine	17·7 ± 0·9 (5)**
Unsectioned + NaCl	35·7 ± 3·7 (5)
Unsectioned + (+)-amphetamine	15·4 ± 1·5 (5)***
Without surgery	29·1 ± 2·5 (4)

** $P < 0·01$; *** $P < 0·001$ compared to appropriate NaCl treatment by Student's *t*-test.

DISCUSSION

The results in Tables 1 and 2 confirm that stereotypy-inducing amphetamine-like drugs can be classified into at least two groups according to whether they are inhibited or not by a high dose of reserpine and by the reverse sensitivity to inhibition by the synthesis inhibitor α-MT (van Rossum & Hurkmans, 1964; Aceto, Harris & others, 1967; Portoghese, Pazdernik & others, 1968; Scheel-Krüger, 1971; Sayers & Handley, 1973; Thornburg & Moore, 1973; Braestrup & Scheel-Krüger, 1976; Braestrup & Randrup, 1977). These results are currently believed to indicate that the 'methylphenidate group' releases dopamine from granular stores, while the '(+)-amphetamine group' releases newly synthesized dopamine. The finding, however, that α-MT does not block the stereotyped behaviour when administered only ½ h before amphetamine (Table 2), which is in agreement with similar results by Papeschi (1975), indicates that the effect of the '(+)-amphetamine group' are not specific for newly synthesized dopamine but that also granular stores of dopamine may be involved. This is also supported by the ability of (+)-amphetamine and phenmetrazine to increase HVA from granular stores after inhibition of dopamine synthesis (Table 6). (+)-Amphetamine effects on granular dopamine are also seen in other tests (Weisman & Koe, 1965; Stolk & Rech, 1970; Dorris & Shore, 1974; Moore & Chiueh, 1974). Nor does the 'methylphenidate group' action appear to be specific to granular stores of dopamine. In reserpinized rats, where granular stores are destroyed, the behavioural effects of the 'methylphenidate-group' were partially restored by monoamine oxidase type-A inhibition. This clearly shows that the 'methylphenidate group' can act independently of granular stores of dopamine.

The most clearcut biochemical difference found in the present study between the two stimulant groups is the decrease in DOPAC by the '(+)-amphetamine group', in contrast to the increase of DOPAC by the 'methylphenidate group' (Table 3). It is tempting to interpret the decrease in DOPAC as a monoamine oxidase inhibiting effect. The selective decrease in DOPAC compared with HVA (Table 3, Fig. 1; Maitre & Waldmeier, 1975) indicates that the MAO-inhibitory properties are not generalized like classical MAOI but may rather have a preferential intraneuronal localization.

However, several mechanisms of (+)-amphetamine other than MAO-inhibition might result in decreased DOPAC concentrations. 1. Uptake inhibition has been shown to cause extra- rather than intraneuronal oxidation of noradrenaline and dopamine (Rutledge, 1970; Tagliamonte, de Montis & others, 1975). In the dopamine system, real uptake inhibition by (+)-amphetamine has recently been disputed (Heikkila, Orlansky & Cohen, 1975) and further, the drugs of the 'methylphenidate group' are equally or more potent as uptake inhibitors than the '(+)-amphetamine group' (Ferris, Tang & Maxwell, 1972; Hunt, Kannengiesser & Raynand, 1974; Braestrup, unpublished) without causing a decrease in DOPAC (Table 3). Similarly, the two very potent uptake inhibitors benztropine and cocaine (Coyle & Snyder, 1969; Harris & Baldessarini, 1973) at most cause a weak decrease in DOPAC compared with (+)-amphetamine and phenmetrazine in reserpinized rats (Table 4). 2. (+)-Amphetamine inhibits impulse flow in dopaminergic neurons in the nigrostriatal tract (Bunney, Walters & others, 1973) and DOPAC might be decreased by a reduction in impulse flow (Roth, Murrin & Walters, 1976). The drug baclofen, however, also inhibits impulse flow in the nigral dopamine system (Bernard, Edwards & others, 1975), but without a substantial DOPAC decrease (this study). Furthermore, parenteral administration of methylphenidate in small doses also inhibit the impulse flow in the nigrostriatal dopamine system (B. S. Bunney, personal communication) and methylphenidate increased DOPAC (Table 3). (+)-Amphetamine is still able to reduce DOPAC in haloperidol treated rats (Tagliamonte & others, 1975; Braestrup, unpublished) irrespective of the fact that neuroleptics inhibit the (+)-amphetamine-induced decrease in impulse flow (Bunney & others, 1973; Bernard & others, 1975). Finally, even when the nigrostriatal dopamine tract was transected unilaterally, (+)-amphetamine still decreased DOPAC (Table 6), an effect obviously not mediated via impulse flow. 3. A decrease in DOPAC might be secondary to a decrease in dopamine synthesis. The reduced concentration of DOPAC is, however, already obtained 15 min (maybe even less) after (+)-amphetamine s.c. (see also Maitre & Waldmeier, 1975) and at this short time interval synthesis is not decreased (Costa, Gropetti & Naimzada, 1972; Walters & Roth, 1976). Furthermore, (+)-amphetamine retains its DOPAC- decreasing properties irrespective of synthesis inhibition with α-MT (Table 5). It appears therefore that the decrease in synthesis is developed later than the DOPAC-decrease and the decrease in synthesis may then be explained as feed-back inhibition on

tyrosine hydroxylase by extra-granularly accumulating dopamine in response to inhibition of dopamine oxidation (Glowinski, 1972). This explanation is consonant with the finding that (+)-amphetamine, but not methylphenidate, treatment *in vivo* reduces the *in vitro* dopamine synthesis, in brain synaptosomal or slice preparations (Kuczenski & Segal, 1975; Besson, Chermay & Glowinski, 1971; Braestrup, unpublished) and this action is apparently not secondary to dopamine receptor stimulation, since it is not inhibited by a high parenteral dose of haloperidol before amphetamine (Hudick, Wajda & Lajtha, 1976; Braestrup, unpublished) which blocks behavioural stimulation and the reduction in impulse flow induced by (+)-amphetamine.

The possible ability of (+)-amphetamine to act both as a dopamine releaser and as inhibitor of intraneuronal dopamine oxidation may account for some paradoxical effects, i.e. HVA may be either increased or decreased by amphetamine (Sayers & Handley, 1973; Maitre & Waldmeier, 1975); dopamine synthesis and turnover may be either increased or decreased (Littleton, 1967; Javoy, Hamon & Glowinski, 1970; Persson, 1970; Hitzemann, Loh & Domino, 1971; Costa & others, 1972; Javoy, Agid & others, 1972, 1974; Doris & Shore, 1974; Gerhards, Carenzi & Costa, 1974); endogenous dopamine may be either increased or decreased depending on the animal used and dose level or time schedule (Welch & Welch, 1970; Papeschi, 1975; Scheel-Krüger, unpublished). Clinically the '(+)-amphetamine group' is apparently more apt to cause euphoria, tolerance and psychosis than the 'methylphenidate group' in normal subjects (see references in Braestrup & Scheel-Krüger, 1976), the contribution of inhibitory effects on dopamine oxidation yet to be investigated.

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