J. Pharm. Pharmacol. 1981, 33: 803–804
Communicated April 24, 1981

0022–3573/81/120803–02 $02.50/0

Differential effects of (+)-amphetamine, methylphenidate, and amfonelic acid on catecholamine synthesis in selected regions of the rat brain

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(+)-Amphetamine, methylphenidate and amfonelic acid increase motor activity and cause stereotypies in experimental animals and in man (Aceto et al 1967; Wallach 1974; Moore 1978). All of these drugs facilitate neuronal release of dopamine (DA) although there are differences in the mechanisms by which this release is effected (Besson et al 1971; Von Voigtlander & Moore 1973; Chiueh & Moore 1975; Shore 1976). The ability of amphetamine to cause central nervous stimulation appears to be dependent upon the availability of 'newly synthesized' DA; its effects can be blocked by the administration of α-methyl-*p*-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase (Weissman et al 1966), but not by reserpine, which depletes stores of DA (Aceto et al 1967). On the other hand, methylphenidate and amfonelic acid require the integrity of catecholaminergic stores; their central stimulant effects are blocked by pretreatment with reserpine, but not with AMPT (Aceto et al 1967; Dominic & Moore 1969; Scheel-Kruger 1971; Thornburg & Moore 1973; Braestrup 1977).

Therefore, although all three drugs facilitate DA release, the mechanism by which they do this differs. We have compared the actions of (+)-amphetamine, amfonelic acid and methylphenidate on the synthesis of catecholamines in selected regions of the rat brain. The results reveal that these drugs differ in their abilities to alter the in vivo synthesis of these amines, as estimated by the rate of dopa accumulation following the inhibition of dopa decarboxylase.

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI), 200–300 g, were housed in plastic cages in groups of 4 with food and water freely available. 3-Hydroxybenzylhydrazine dihydrochloride (NSD 1015; Sigma Chemical Co., St. Louis, MO), (+)-amphetamine sulphate (Smith Kline and French Laboratories, Philadelphia, PA) and methylphenidate hydrochloride (Ciba-Geigy Co., Summit, NJ) were dissolved in 0·9% NaCl (saline). Amfonelic acid (Sterling-Winthrop Research Inst., Rensselaer, NY) was dissolved in saline made basic with 1 M NaOH. Doses refer to the salts listed above.

Animals were injected s.c. with various doses of the central nervous system stimulants or their vehicles and then injected i.p. with NSD 1015 (100 mg kg^{-1}) 30 min before decapitation after which brains were quickly removed and placed on a cold plate. The striatum, hippocampus, nucleus accumbens and olfactory tubercle were dissected as previously described (Glowinski & Iversen 1966; Horn et al 1974) and homogenized in 10 volumes of 0·2 M perchloric acid containing 10 mg% EGTA and frozen at −20 °C until analysed. The samples were thawed, centrifuged, and 10 μl aliquots of the supernatant were analysed for dopa (Demarest & Moore 1980). Precipitated pellets were analysed for protein as described by Lowry et al (1951).

In the absence of a decarboxylase inhibitor the concentration of dopa in the brain regions examined is essentially zero, i.e. less than the sensitivity (50 pg) of assay. Following the injection of NSD 1015, dopa accumulates in brain regions linearly with time for more than 30 min (Demarest & Moore 1980). Since the concentration of noradrenaline in the striatum is negligible, the rate of dopa accumulation in this region represents the synthesis of DA in the terminals of nigrostriatal neurons and reflects the activity of these neurons (Roth et al 1975). Similarly, the concentrations and rates of turnover of DA in the olfactory tubercle and nucleus accumbens are greater than those of noradrenaline so that dopa accumulation in these regions also primarily reflects the synthesis of DA in terminals of mesolimbic neurons. On the other hand, the concentration of DA in the hippocampus is negligible so that dopa accumulation in this region reflects noradrenaline synthesis and the activity of these neurons (Salzman & Roth 1980).

The effects of (+)-amphetamine, methylphenidate and amfonelic acid on dopa accumulation in the striatum, nucleus accumbens, olfactory tubercle and hippocampus of the rat are summarized in Table 1. Control rates of dopa accumulation in these regions are consistent with previously reported values (e.g. Walters & Roth 1974; Andén & Grabowska-Andén 1978; Lyness et al 1980). (+)-Amphetamine caused a dose-related increase in dopa accumulation in the striatum, but not in the other brain regions. On the other hand, methylphenidate reduced dopa accumulation in all regions except the hippocampus, while amfonelic acid did not significantly alter the rate of catecholamine synthesis in any of the regions examined. The rather selective action of amphetamine in the striatum is consistent with previous reports (Pearl & Seiden 1979; Lyness et al 1980; Kehr et al 1977). The reasons for the regionally specific effects are not understood but they may reflect differences in mechanisms by which the synthesis of DA is controlled (i.e. end-product inhibition, autoreceptor density or sensitivity, neuronal feedback circuits, etc). Methylphenidate resembles (+)-amphetamine in that it

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Table 1. Effects of (+)-amphetamine, methylphenidate and amfonelic acid on the rate of dopa accumulation in selected regions of the rat brain. (+)-Amphetamine, methylphenidate or their respective vehicles were injected s.c. 45 min before decapitation while amfonelic acid or its vehicle were injected 90 min before. NSD 1015 (100 mg kg^{-1}) was injected i.p. 30 min before decapitation. Each value represents the mean dopa accumulation (ng dopa mg^{-1} protein/30 min) ± 1 s.e. as determined from 8 animals, except for the (+)-amphetamine controls (zero dose) which represents 32 animals in all regions except the hippocampus which represents 16 animals. * Values that are significantly different ($P < 0.05$) from appropriate vehicle controls as determined by a one-way analysis of variance and the Student-Newman-Keuls' test (Steele & Torrie 1960).

Treatment	Striatum	Nucleus accumbens	Olfactory tubercle	Hippocampus
(+)-Amphetamine				
0	12·3 ± 1·3	14·6 ± 1·3	13·3 ± 1·0	0·80 ± 0·09
0·125	13·5 ± 0·5	14·4 ± 1·2	11·4 ± 1·3	—
0·25	16·5 ± 0·5*	19·0 ± 1·9	18·1 ± 1·5	—
0·5	23·2 ± 1·1*	17·1 ± 0·9	14·1 ± 1·3	—
1·0	23·3 ± 1·8*	18·0 ± 2·3	16·1 ± 0·9	0·74 ± 0·07
Methylphenidate				
0	11·2 ± 0·4	11·1 ± 0·7	10·2 ± 0·4	0·81 ± 0·05
2	7·4 ± 0·3*	8·3 ± 0·5*	8·1 ± 0·4*	0·63 ± 0·05
4	8·1 ± 0·3*	8·1 ± 0·3*	8·2 ± 0·4*	0·62 ± 0·02
8	5·1 ± 0·4*	7·8 ± 0·5*	7·2 ± 0·7*	0·59 ± 0·04*
Amfonelic acid				
0	13·9 ± 0·9	14·4 ± 2·2	9·7 ± 1·1	0·77 ± 0·10
1	15·1 ± 0·7	12·7 ± 1·1	7·2 ± 1·0	0·71 ± 0·06
4	13·2 ± 1·3	13·7 ± 1·0	11·0 ± 0·6	0·54 ± 0·05

stimulates locomotor activity and increases the in vivo release of DA from the brain (Chiueh & Moore 1975). It was surprising, therefore, that methylphenidate differs markedly from (+)-amphetamine in that it depressed rather than stimulated the rate of dopa accumulation in all brain regions analysed. These two drugs also differ in that methylphenidate appears to exert its central stimulant effects by releasing DA from a reserpine-sensitive storage pool while (+)-amphetamine releases DA from a newly synthesized, AMPT-sensitive pool (see review by Moore 1978). Amfonelic acid, which resembles methylphenidate in that it also appears to exert its central stimulant action by releasing DA from a reserpine-sensitive pool, differs from that drug in that it does not alter the rate of dopa accumulation. Thus, although (+)-amphetamine, methylphenidate and amfonelic acid produce similar behavioural effects they have different actions on catecholaminergic synthetic processes.

This work was supported by USPHS grant NS 15911.

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