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The differential effects of amphetamine and methylphenidate on the biosynthesis of [^{3}H]dopa from [^{3}H]tyrosine in mouse striata in vivo

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Recently, we found that EGTA, which decreases the stimulation of dopamine (DA) synthesis produced by amphetamine, inhibited the circling behaviour elicited by amphetamine but not that by methylphenidate in mice lesioned unilaterally in one striatum with 6-hydroxydopamine (Fung & Uretsky 1980a, b, c). While amphetamine stimulates DA synthesis in striatal slices and synaptosome preparations (Harris et al 1975; Kuczenski 1975; Patrick et al 1975; Uretsky & Snodgrass 1977; Fung & Uretsky 1980c), and in vivo (Costa et al 1972; Kuczenski 1978), the effects of methylphenidate on DA biosynthesis remain unclear. Kuczenski & Segal (1975) reported that methylphenidate increased tyrosine hydroxylase activity in a synaptosome preparation, while Snodgrass & Uretsky (1979) could not demonstrate a stimulatory role for this compound on DA formation in striatal slices. As the in vitro condition may not accurately reflect the biochemical effects of methylphenidate in vivo, and since in vivo effects have not received attention, we set out to clarify the role of methylphenidate on the striatal dopaminergic system in vivo by examining the effects of amphetamine and methylphenidate on the biosynthesis of [^{3}H]dopa from [^{3}H]tyrosine in mice pretreated with a dopa decarboxylase inhibitor, seryltrihydroxybenzylhydrazine (RO4-4062), using a dose and time in which we found both drugs to induce marked circling behaviour (Fung & Uretsky 1980b).

Material and methods

Male Swiss-Webster mice (Laboratory Supply), 23–29 g, were anaesthetized with chloral hydrate (430 mg kg^{-1} i.p.) and the skull exposed by a longitudinal incision. A hole was made on the right side (1·2 mm lateral to the bregma) for the intraventricular administration of [^{3}H]tyrosine. On the day of the experiment, mice received either 0·9% NaCl (saline), amphetamine (4 mg kg^{-1} i.p.) or methylphenidate (20 or 50 mg kg^{-1} i.p.). Ten min later RO4-4602 (800 mg kg^{-1} i.p.) was given. After another 15 min, the mice under halothane anaesthesia were given [^{3}H]tyrosine (5 μl, 12·5 μCi) intraventricularly, using a 10 μl Hamilton syringe fitted with a polyethylene cuff that allowed the distal 2·5 mm of the needle to be exposed. The injection was made over 20 s, and the needle held in place for an additional 10–15 s. The incision was then closed with a wound clip. Animals recovered from the halothane anaesthesia in 2–3 min, and 10 min after the injection they were killed and the levels of [^{3}H]dopa, [^{3}H]tyrosine and endogenous tyrosine in the striata analysed. The injection site was verified by examining the stain produced by a 10% methylene blue solution given intraventricularly. Drugs were dissolved in saline and administered intraperitoneally in a 0·1 ml/10 g weight. [^{3}H]Dopa was assayed according to Fung & Uretsky (1980c). [^{3}H]Tyrosine and endogenous tyrosine in the effluent and 1 ml water wash were collected from the alumina columns used for the dopa assay. The pH was adjusted to 2 with 1 M HCl, and the samples then applied to Dowex 50 × 4 columns. Tyrosine was eluted with 4 ml of 1 M NH_4OH, the eluate dried under vacuum, and the residue dissolved in 1·02 ml 5% trichloroacetic acid. An aliquot (20 μl) was taken for the determination of [^{3}H]tyrosine by liquid scintillation counting (Fung & Uretsky 1980c). The remaining solution was used for the spectrofluorimetric assay of endogenous tyrosine (Udenfriend 1962). The rate of formation of [^{3}H]dopa was expressed as a conversion index, i.e. the amount of [^{3}H]dopa formed divided by the specific activity of tyrosine in the tissue.

Results and discussion

[^{3}H]Dopa accumulation was used to measure DA biosynthesis instead of [^{3}H]DA, since [^{3}H]DA is rapidly released

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Table 1. The effect of amphetamine and methylphenidate on the biosynthesis of [^{3}H]dopa from [^{3}H]tyrosine in mouse striata in vivo. The control values were 1·4 ± 0·1 nmol g 10^{-1} min (n = 10). All values are presented as Mean ± s.e., n = 6–7 in other treatment groups.

Treatment groups	% of control
Amphetamine (4 mg kg^{-1} i.p.)	152 ± 15*
Methylphenidate (20 mg kg^{-1} i.p.)	83·± 5
Methylphenidate (50 mg kg^{-1} i.p.)	87 ± 4

*$P<0·05$ (Dunnett's t-test) when compared with saline controls.

and metabolized in the presence of amphetamine. After the intraventricular injection of [^{3}H]tyrosine, 15 min after RO-4-4062, the [^{3}H]catechols formed consisted almost totally of [^{3}H]dopa, since no [^{3}H]DA could be detected by cation exchange chromatography (Fung & Uretsky 1980c). At a dose which caused marked behavioural effects, amphetamine significantly stimulated the formation of [^{3}H]dopa. In contrast, methylphenidate at doses (20 and 50 mg kg^{-1}) which produced marked behavioural effects such as circling and stereotyped behaviour in animals (Von Voigtlander & Moore 1973; Costall & Naylor 1975; Fung & Uretsky 1980b), produced no significant change in [^{3}H]dopa formation compared with controls (Table 1).

Therefore, amphetamine but not methylphenidate can stimulate DA synthesis in vivo and these in vivo effects of amphetamine are the same as those reported with striatal slices or synaptosome preparations (Harris et al 1975; Kuczenski 1975; Patrick et al 1975; Uretsky & Snodgrass 1977). Amphetamine has been hypothesized to produce behavioural changes by releasing a pool of newly synthesized DA from nerve endings. The stimulation of DA synthesis by amphetamine would maintain the size of the newly synthesized DA pool despite the enhanced release of DA from this pool by amphetamine. In contrast, the inability of methylphenidate to stimulate [^{3}H]dopa accumulation agrees with in vitro studies using striatal slices (Snodgrass & Uretsky 1979) but not with synaptosome studies (Kuczenski & Segal 1975). Our results suggest that the behavioural effects of methylphenidate are not associated with the stimulation of DA biosynthesis. This is consistent with the observation that the behavioural effects of methylphenidate are not inhibited by EGTA or α-methyl-*p*-tyrosine which blocks ongoing DA synthesis. Thus, methylphenidate may produce behavioural effects in animals by inhibiting the re-uptake of DA into DA neurons as postulated by others (Scheel-Kruger 1971, 1972; Ross 1976, 1979). Our findings support the hypothesis that these two classes of central nervous stimulants may exert their effects via different mechanisms.

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