The selective inhibitory effect of EGTA on the circling behaviour produced by amphetamine but not methylphenidate in mice lesioned with 6-hydroxydopamine

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In response to amphetamine or methylphenidate administration, mice with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal dopaminergic pathway will rotate to the lesioned side (Ungerstedt 1968, 1971; Von Voigtlander & Moore 1973; Costall & Naylor 1975). Amphetamine is thought to produce this turning behaviour by releasing a reserpine-insensitive, newly synthesized pool of dopamine (DA) from the DA nerve endings in the intact striatum (Weissman et al 1966; Chiueh & Moore 1973). In contrast, methylphenidate is believed to act by releasing the reserpine-sensitive storage pool of DA from the striatal nerve endings or by inhibiting the uptake of DA into nerve terminals (Scheel-Kruger 1971, 1972; Ross 1976, 1979).

Recently we have found that intrastriatal injection of ethyleneglycol-bis-(β-aminoethyl ether)-*NN'*-tetra-acetic acid EGTA (2 nmol g^{-1}) into the intact striatum inhibited the turning behaviour produced by amphetamine in mice lesioned with 6-hydroxydopamine (Fung & Uretsky, unpublished observations). Furthermore, this inhibitory effect of EGTA was reversed by the addition of excess calcium to the EGTA solution. Since amphetamine and methylphenidate represent two different classes of central nervous system stimulants, with different mechanisms of presynaptic effects on striatal dopaminergic neurons (Scheel-Kruger 1971; Braestrup 1977; Clemens & Fuller 1979; Costall et al 1979; Fuller & Snoddy 1979), we sought to determine if intrastriatal administration of EGTA would also block the turning behaviour induced by methylphenidate.

Male Swiss-Webster mice (Laboratory Supply), 23–29 g, were anaesthetized with chloral hydrate (420 mg kg^{-1}) and an incision was made in a longitudinal direction through the skull. Four ml of chilled 0·9% NaCl (saline) containing 16 μg of 6-hydroxydopamine HBr and 24 μg ascorbic acid was injected stereotaxically into the right striatum of the mice over 4 min. The centre of the corpus striatum was determined to be 3·5 mm below the skull surface, 2·2 mm lateral to the midline and 5 mm anterior to the occipital suture. With the same co-ordinates, a hole was made in the left side of the skull for drug injection into the left striatum. Five days after the surgery, mice were tested with either amphetamine (5 mg kg^{-1}, i.p.) or methylphenidate (20 mg kg^{-1}, i.p.) and circling behaviour determined in a 2 litre round-bottom flask. Only mice that turned ipsilaterally at least 10 times in 2 min were used (approx. 95% of mice tested).

A modification of the method described by Pycock et al (1976) was used for 'free hand' intrastriatal drug injection into the left (intact) striatum. Under halothane anesthesia, EGTA (2 nmol g^{-1}) or vehicle in a volume of 2 μl was injected with a 10 μl Hamilton syringe, fitted with a polyethylene cuff so that only the distal 3·8 mm of the needle was exposed, into the left striatum of mice. Under these conditions, the orifice of the needle was 3·5 mm below the skull. This 'free hand' injection was made within 20–25 s and the incision was closed with a wound clip. All mice recovered from the halothane anaesthesia within 2–3 min of intrastriatal drug injection. The site of injection was confirmed histologically.

Ten min after the intrastriatal drug injection, either saline, amphetamine (4 mgkg^{-1}, i.p.), or methylphenidate (20 mg kg^{-1}, i.p.) was given to the mice. Total ipsilateral turns (toward the lesioned side) were recorded 10 min later in 2 min periods at various times for 70 min. EGTA was dissolved in 0·1 M sodium phosphate buffer and the pH adjusted to 7 with sodium hydroxide. Other drugs were dissolved in saline and the intraperitoneal drug injection was given in a volume of 0·1 ml/10 g body weight. Intrastriatal drug injection was expressed as nmol g^{-1} body weight.

Control animals that received EGTA or vehicle intrastriatally followed by saline (i.p.) showed little or

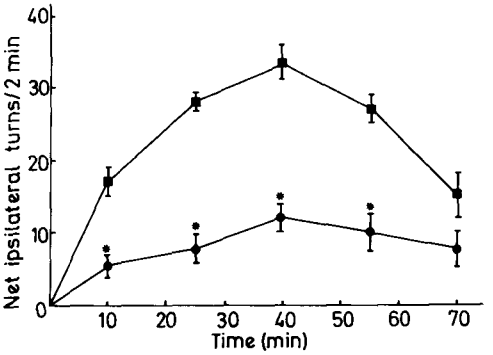

FIG. 1. Time-course of intrastriatal injection (left intact striatum) of EGTA (2 nmol g^{-1}) (circles) or the vehicle (squares) on amphetamine-induced turning in mice lesioned with 6-hydroxydopamine. Ten min after intrastriatal administration, amphetamine (4 mg kg^{-1}, i.p.) was given and the turning behaviour was determined. Each value is the mean ± s.e.m. of 6 animals. * $P < 0.05$ (Student's *t*-test).

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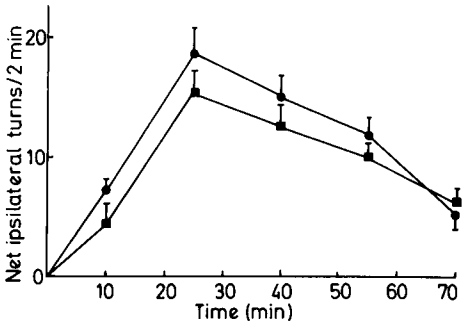

FIG. 2. Time-course of intrastriatal injection (left intact striatum) of EGTA (2 nmol g^{-1}) (circles) or the vehicle (squares) on methylphenidate-induced turning in mice lesioned with 6-hydroxydopamine. Ten min after intrastriatal administration, methylphenidate (20 mg kg^{-1}, i.p.) was given and the turning behaviour was determined. Each value is the mean ± s.e.m. of 5 animals.

no net ipsilateral turns. Most of the mice sat quietly at the bottom of the flask. Intrastriatal administration of EGTA (2 nmol g^{-1}) markedly inhibited amphetamine-induced circling for 55 min when compared with the controls which received intrastriatal injection of vehicle ($P < 0.05$) (Fig. 1). This dose of amphetamine was chosen because, under our conditions, it produced the maximum turning within a 2-min period. EGTA was also effective in reducing the circling produced by a higher dose of amphetamine (at 40–42 min after amphetamine, 8 mg kg^{-1}, i.p., the period of maximum turning, the turning of the saline-amphetamine group was 18 ± 2, n = 4, while the turning of the EGTA, amphetamine group was 3 ± 0·8, n = 4). However this dose of EGTA did not inhibit methylphenidate induced circling at any time ($P > 0.05$) (Fig. 2). We did not inject higher doses of EGTA into the striatum since these doses produced convulsions in approximately half of the mice injected.

These results show that EGTA, a chelator of extracellular calcium, has a differential effect on the turning behaviour produced by amphetamine and methylphenidate. Thus, the presence of calcium appears to be important in mediating the behavioural effects of amphetamine, but not that of methylphenidate. We have found that EGTA (2 nmol g^{-1}) did not block apomorphine-induced circling in mice lesioned with an electric current, suggesting that EGTA may exert its effects on amphetamine-induced turning via a presynaptic rather than a postsynaptic action (Fung & Uretsky, unpublished observations).

How EGTA acts to block the effect of amphetamine remains unclear. It may act by inhibiting the entry of amphetamine into the striatal DA nerve terminals, by decreasing the availability of DA for release, or by inhibiting the release process. EGTA has been shown to inhibit the stimulation of DA synthesis induced by amphetamine in vitro (Uretsky et al 1977; Uretsky et al 1979). If the release of newly synthesized DA is important in the functional effects of amphetamine, then EGTA by inhibiting the amphetamine-induced stimulation of DA synthesis should inhibit the release of DA by amphetamine. In contrast, the behavioural effects of methylphenidate are not dependent on newly synthesized dopamine (Scheel-Kruger 1971, 1972). Consequently, EGTA would not be expected to have an inhibitory effect on the turning induced by methylphenidate. Thus, the selective inhibitory effect of EGTA on amphetamine-induced but not methylphenidate-induced circling behaviour in striatal lesioned mice suggests that calcium plays an important role in mediating the behavioural effects of amphetamine, but not methylphenidate. Our results support the hypothesis that these two classes of c.n.s. stimulants may exert their effects via a different mechanism.

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