In vitro INHIBITION OF BRAIN MITOCHONDRIAL MONOAMINE OXIDASE BY 6-HYDROXYDOPAMINE

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6-Hydroxydopamine (6-OHDA) inhibits rat brain mitochondrial monoamine oxidase (MAO) when kynuramine or dopamine are used as substrates. The effect is competitive and reversible giving *Ki* values of 74 and 176 μM with the respective substrates. At high concentrations (5 mM) of each substrate, inhibition of MAO was not observed.

Introduction 6-Hydroxydopamine (6-OHDA) produces a relatively selective degeneration of adrenergic nerve terminals (see Kostrzewa & Jacobowitz, 1974 for review). Although the precise mechanism by which it acts is not known, the highly reactive products e.g. hydrogen peroxide, super-oxide and guinones generated during the reaction may react nonspecifically with neuronal structures, eventually destroying them (Heikkila & Cohen, 1971, 1972, 1973). 6-OHDA is a substrate for monoamine oxidase (MAO) (Malmfors & Sachs, 1968; Malmfors, 1971; Jonsson, Malmfors & Sachs, 1972). This enzyme, together with granule storage, may thus serve as protective mechanisms (Kostrzewa & Jacobowitz, 1974). Recently Agid, Javoy & Youdim (1973) showed that injection of 6-OHDA (20 μg) directly into the substantia nigra brings about a decrease in activity of MAO as well as of aldehyde dehydrogenase, although such an effect may be nonspecific (Duncan, Sourkes, Duborovsky & Quik, 1975). The present study was undertaken to examine whether 6-OHDA inhibits MAO activity *in vitro*.

Methods Male Charles River rats (250-300 g body weight) were killed by decapitation. The brains were rapidly removed and homogenized in ice-cold 0.32 M sucrose solution with a Teflon pestle homogenizer to give a final 10% (w/v) preparation. Crude brain mitochondria were prepared by the method of Hawkins (1952). MAO activity was assayed using kynuramine as substrate by the spectrofluorimetric procedure of Kraml (1965). The enzyme mixture in a total volume of 1 ml contained 100 μl of 10% (w/v) crude brain mitochondria, 500 μl 0.10 M phosphate buffer (pH 7.4), 100 μl of 1 mM kynuramine and water. The reaction which was linear for the first 20 min was terminated by the addition of 500 μl 10% (w/v) trichloroacetic acid, and the 4-hydroxyquinoline formed estimated fluorimetrically (Kraml, 1965). When dopamine was the substrate, MAO activity was measured by the procedure of Robinson, Lovenberg, Keiser & Sjoerdsma (1968).

Results When kynuramine concentration was 0.1 mM, the addition of 6-OHDA caused inhibition of MAO activity. The degree of inhibition was 15, 30, 42 and 82% when 6-OHDA concentrations were 0.001, 0.01, 0.1 and 1.0 mM respectively. Preincubation of enzyme preparation for 30 min at 37°C with these concentrations of 6-OHDA prior to the addition of kynuramine resulted in a slight increase in inhibitory effect, the values being 25, 42, 50 and 87% respectively.

When these results were plotted according to the method of Lineweaver & Burk (1934) and Dixon (1953) the results shown in Figure 1a and b were obtained. The graphs demonstrate a reversible and competitive inhibition of oxidative deamination of kynuramine by 6-OHDA with a *Ki* value of 74 μM which is similar to the *Km* for kynuramine oxidation by MAO (35-85 μM) (Youdim, Collins & Sandler, 1969). The deamination of dopamine was inhibited in a similar way by 6-OHDA, the *Ki* value being 176 μM which is close to the *Km* (160 μM) for dopamine (Achee, Togulga & Gabay, 1974).

At high concentrations of substrate (5 mM) there was no inhibition of MAO by 0.1 mM 6-OHDA as would be expected from the nature of the inhibition. This concentration is 10 times lower than that used by Wagner & Trendelenburg (1971).

Discussion The present study clearly demonstrates inhibition of MAO by 6-OHDA when kynuramine or dopamine are used as substrates. This effect is reversible and competitive with a *Ki* value similar to the *Km* for its substrates. Wagner & Trendelenburg (1971) reported that there was no inhibition of liver homogenate MAO by 6-OHDA. Their results can be simply explained by the fact that they used substrate concentrations of

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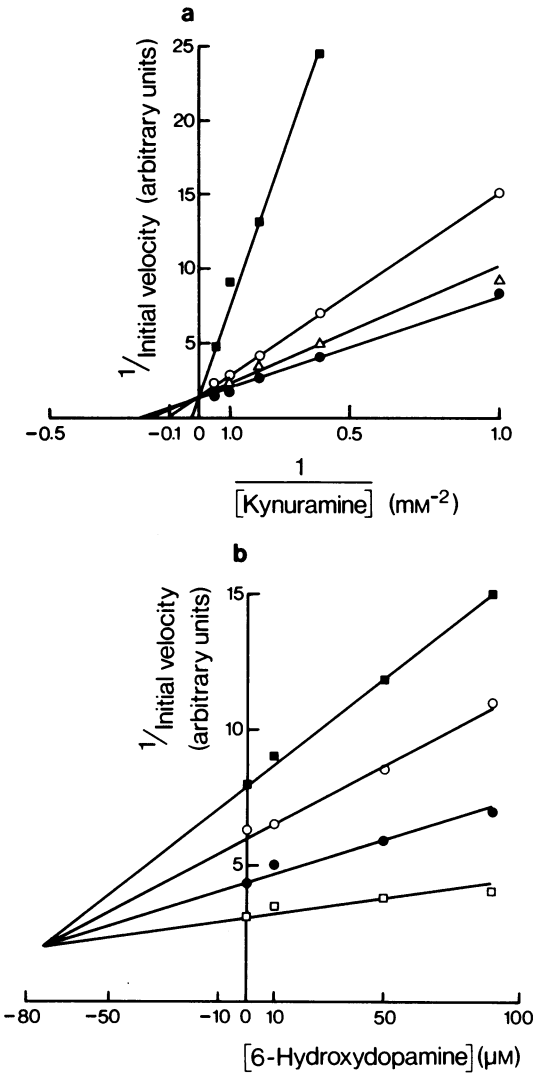

Figure 1 (a) Reciprocal plots of the inhibition of monoamine oxidase (MAO) by 6-hydroxydopamine (6-OHDA). Reciprocal plots of initial velocities against kynuramine concentration at different 6-OHDA concentrations: zero (●), 0.01 mM (△), 0.1 mM (○) and 1 mM (■). (b) Dixon (1953) plots of the inhibition of MAO by 6-OHDA. Reciprocal plots of initial velocities against inhibitor concentrations at a series of kynuramine concentrations: 0.01 mM (■), 0.025 mM (○), 0.05 mM (●) and 0.1 mM (□).

50 mM, some five hundred times greater than the enzyme *Km* (see Tipton, 1968; Tipton & Spires, 1971).

Previous studies have indicated that 6-OHDA is actively accumulated by catecholaminergic neurones where it is localized in granules, eventually producing degenerative effects (Kostrzewa & Jacobowitz, 1974). The initial action of 6-OHDA is not known, although it does uncouple oxidative phosphorylation in concentrations which have been calculated to occur intraneuronally (Wagner & Trendelenburg, 1971) and cause a loss in ability to conduct action potentials (Haeusler, 1971). 6-OHDA is a substrate for MAO (Senoh, Creveling, Udenfriend & Witkop, 1959; Malmfors, 1971), and MAO inhibitor pretreatment potentiates its effect on adrenergic neurones (Malmfors, 1971; Jonsson *et al.*, 1972). At concentrations (0.05 mM) sufficient to uncouple oxidative phosphorylation (Wagner & Trendelenburg, 1971), it inhibits MAO by about 60%. The inhibition which is competitive can be produced by 6-OHDA itself, by the hydrogen peroxide generated from its action or by both (Heikkila & Cohen, 1971, 1973).

It has already been shown that hydrogen peroxide is able to inhibit brain MAO activity competitively (Tipton, 1968; Tipton & Spires, 1971). Thus it is possible that concentrations of 6-OHDA used for selective degeneration of adrenergic neurones in the central nervous system would be high enough to inhibit intraneuronal as well as extraneuronal MAO reversibly. Furthermore, it is probable that the degree of MAO inhibition is even greater intraneuronally since 6-OHDA can be taken up and accumulated within the adrenergic neurone. It is tempting to speculate that initial reversible inhibition of MAO may be involved in the events leading to the degeneration of adrenergic nerve endings after administration of 6-OHDA.

This work was partly supported by a Travelling Fellowship from the Wellcome Trust and INSERM which is gratefully acknowledged. I thank Dr J. Glowinski in whose laboratory this work was performed.

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(Received March 18, 1975)