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Exposure of mitochondrial outer membranes to neuraminidase selectively destroys monoamine oxidase A activity

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The enzyme monoamine oxidase is involved in the catabolism of biogenic amines, and is the site of action of a number of antidepressant drugs (see Blaschko 1972; Knoll 1976).

The monoamine oxidase activity of rat liver mitochondrial outer membranes can be divided into two categories, MAO-A and MAO-B based upon their substrate specificity and sensitivity to irreversible and reversible inhibition (Houslay & Tipton 1974). The binding sites for the selective inhibitors, clorgyline and pargyline, appear to have a different localization in the membrane (Houslay 1977) and it has been suggested that the membrane environment may give rise to some of the features of enzyme multiplicity (Houslay & Tipton 1973a).

Recently Russell et al (1979a, b) have demonstrated that the active site of MAO-A faces the mitochondrial inner membrane and the active site of MAO-B faces the cytosol. The possibility exists that membrane components that are asymmetrically disposed could modulate the activity of MAO-A and MAO-B.

It is envisaged for biosynthetic purposes that the external surface of the mitochondrial outer membrane should correspond to the external surface of the endoplasmic reticulum (see Rothman & Lenard 1977). This may imply that carbohydrate residues attached to proteins exposed at the lumenal side of the endoplasmic reticulum should be found associated with the inner face of the mitochondrial outer membrane. We thus decided to investigate the effect of neuraminidase treatment on isolated mitochondrial outer membranes. This enzyme is known to remove substantial amounts of sialic acid from mitochondrial outer membranes (see Hughes 1976).

Mitochondrial outer membranes from male, Sprague Dawley rats were prepared as described before (Houslay & Tipton 1973b), except that K phosphate buffers were used instead of Tris buffers which have a selective inhibitory effect on the two enzymes (Fowler et al 1977). Also the final mitochondrial outer membrane fraction was obtained by diluting the fraction obtained from the sucrose gradient, tenfold with 10 mM K phosphate pH 7·2 and centrifuging it for 1 h at 100 000*g*. The pellet was resuspended at 3–4 mg ml^{-1} protein in the same buffer.

These membranes were treated with purified neuraminidase preparations from *Clostridium perfringens* obtained from either Boerhinger Corp. (U.K.), East Sussex or type VIII from Sigma (U.K.) Ltd., Kingston-Upon-Thames with similar results. Final concentrations of 1·3 mg ml^{-1} mitochondrial outer membranes, 1·1 mg ml^{-1} neuraminidase in 67 mM K phosphate buffer pH 6·0 were incubated for periods of up to 30 min at 30 °C. Any residual phospholipase activity of these enzyme preparations would be effectively inhibited by the absence of high [Ca^{2+}] and the presence of high phosphate concentrations in our incubations. At appropriate time intervals aliquots were taken for assay of monoamine oxidase activity. [1-^{14}C]β-phenethylamine (from NEN, Germany) and [^{3}H]-5-hydroxytryptamine (Amersham) were used as substrates, assayed as described by Callingham & Laverty (1973). Incubation of mitochondrial outer membranes for a

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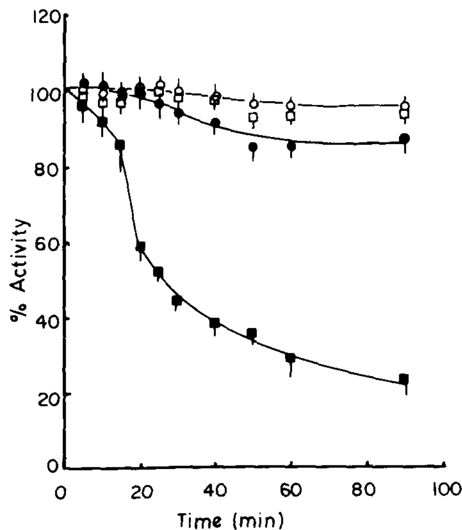

FIG. 1. The effect of neuraminidase treatment of mitochondrial outer membranes on MAO-A and MAO-B activity. Membranes were treated as stated in text either with (●, ■) or without (○, □) neuraminidase and assayed with β-phenethylamine (○, ●) or 5-hydroxytryptamine (□, ■) as substrates. Errors are given as s.d. on three experiments.

90 min period under these conditions in the absence of neuraminidase had little significant effect on β-phenethylamine oxidation (MAO-B) or 5-hydroxytryptamine oxidation (MAO-A). At pH values less than pH 6·0, or using acetate buffers, substrate selective inhibitory effects ensued. Although neuraminidase has a pH optimum around pH 5·0 (Burton 1963), it appears to efficiently remove (>95%) sialic acid residues from cell membranes at pH values around neutrality (Kraemer 1966), and therefore pH 6·0 was adopted for our investigation.

Incubation of mitochondrial outer membranes with neuraminidase under these conditions led to the abolition of 77% of MAO-A activity, whereas the loss of MAO-B activity was less than 14% (see Fig. 1). These results imply that sialic acid residues are involved in the functioning of MAO-A.

Interestingly it was MAO-A that Russell et al (1979a) had assigned an active site location on the face of the mitochondrial outer membrane facing the mitochondrion. This is precisely the side that one might have expected to be core glycosylated during protein biosynthesis (Rothman & Lenard 1977). Indeed purified preparations of monoamine oxidase appear to be glycoproteins (Gomes et al 1969; Oreland et al 1973; Salach 1979), and possess two closely associated subunits of similar or identical molecular weight, only one of which contains covalently bound flavin (Oreland et al 1973; Youdim 1976; Salach 1979). However, in this respect we should note that whilst core glycosylation of proteins during biosynthesis is presumed to occur only at those sites exposed to the lumen of the endoplasmic reticulum (see e.g. Rothman & Lenard 1977), there is evidence that isolated mitochondria can incorporate radioactive sugars, which may well lead to peripheral glycosylation of protein or lipid species on both sides of the membrane (see e.g. Hughes 1975).

Whether the action of neuraminidase is in fact due to the removal of sialic acids from the protein itself or from associated glycolipid is not clear from our studies, as this will need the isolation of a pure MAO-A species. The production of a pure MAO-A enzyme has so far proved elusive which lends support to the suggestion that membrane components are involved in the functioning of MAO-A (Houslay & Tipton 1973a).

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