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Histochemical demonstration of an additional form of rat brain MAO

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Monoamine oxidase (MAO) is known to exist in a number of forms, and in the rat brain two types (A & B) have been described by Johnston (1968). Type A-MAO deaminates 5-HT and tyramine and is highly sensitive to inhibition by clorgyline, while the B form deaminates tyramine but not 5-HT and is relatively insensitive to clorgyline. These different forms may well be of considerable importance in the metabolism of monoamines in mammalian brain, and may well have differing distributions (Collins, Sandler, Williams & Youdim, 1970; Goridis & Neff, 1971).

A histochemical method has been developed which permits the demonstration of MAO in rat brain, using both 5-HT and tyramine as substrates. Using clorgyline as an inhibitor A- & B-MAO have been separately demonstrated. Both types are broadly distributed in the rat brain, with a basically similar distribution. Both are present in high amounts in areas known to be rich in monoamines.

A third type of MAO which readily utilizes 5-HT as substrate but is relatively clorgyline insensitive has also been demonstrated. It is predominantly circumventricular in distribution. It is suggested that this form, differing from A- & B-MAO not only in substrate and inhibitor characteristics, but also in distribution should be designated C-MAO. Its distribution suggests a potentially important role for this form of MAO in regulating the movement of biogenic amines between the CSF and the brain.

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Some studies on the purification of monoamine oxidase by affinity chromatography

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Partially purified preparations of monoamine oxidase (MAO) have been prepared by a number of workers (Youdim & Sourkes, 1966; Tipton, 1968; Oreland, 1971) using conventional purification procedures which utilize differences in the chemico-physical properties between MAO and other proteins. Over the past few years affinity chromatographic techniques have been developed using selective adsorbents which have biological affinity for a particular protein and this technique has been utilized to purify enzymes (Cautrecasas, Wilchek & Anfinsen, 1968; Wilchek & Gorecki, 1969).

In the present experiments a number of inhibitors of MAO were used as ligands and were attached to sepharose columns in an attempt to purify MAO by a single step experimental procedure.

An organomercurial-sepharose column was prepared by utilizing p-chloromercuribenzoate as the ligand. p-Chloromercuribenzoate was added to aminohexane sepharose suspended in 40% dimethyl formamide. 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide was added, the pH maintained at 4.8 and the mixture allowed to react

for 18 h at room temperature. The gel was then washed with 0.1 M $NaHCO_3$ followed by distilled water and was then equilibrated with 1 mM phosphate buffer pH 7.8. Sonicated soluble rat liver MAO was then applied to the gel and washed through with buffer. The MAO enzyme was eluted in the unbound fraction and was 5.6 times purer than the starting material with a 100% recovery of the enzyme. The bound protein was eluted from the organomercurial-sepharose column with 50 mM cysteine in 0.2 M phosphate buffer at pH 7.4 containing 0.01 M EDTA.

A tranylcypramine-sepharose column was prepared by attaching tranylcypramine to succinyl aminohexane sepharose. Soluble MAO applied to this column was not bound nor was there an increase in the specific activity of the eluate from the column.

A sepharose column was also prepared by attaching 1-meta-aminophenyl-2-cyclo-propyl-aminoethanol (AB-15), to succinyl amino hexane-sepharose. Soluble MAO was applied to this column and 10% of the enzyme together with 15% of the protein was bound to the column. Elution of the column with 0.2 M phosphate buffer, pH 7.6, recovered only 1% of the enzyme, but this fraction had a 40 fold purification over the original material.

The results suggest that affinity chromatographic techniques may provide a suitable procedure for preliminary purification of MAO.

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Behavioural and biochemical evidence for cerebral dopamine receptor blockade by metoclopramide in rodents

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Metoclopramide, a widely used anti-emetic agent, causes dyskinesias in a small percentage of patients (Borenstein & Bles, 1965; Casteels-van Daele, Jaeken, van der Schueren, Zimmerman & van der Bon, 1970); like other neuroleptic agents which cause similar dyskinesias, it may block cerebral dopamine receptors (Costall & Naylor, 1973; Janssen, Niemegeers, Schellekens & Lenaerts, 1967). These behavioural and biochemical studies described here indicate that metoclopramide is a cerebral dopamine receptor antagonist.

Metoclopramide administered i.p. 30 min before apomorphine (2 mg/kg) produced a dose-dependent inhibition of stereotopy (ED_{50} 1.5 mg/kg). The reversal of reserpine-induced suppression of locomotor activity by apomorphine (2 mg/kg) was significantly decreased by prior administration of metoclopramide (ED_{50} 17 mg/kg). Metoclopramide also antagonized the effect of apomorphine or amphetamine in producing turning behaviour in mice with unilateral lesions of the nigrostriatal pathway (ED_{50} 5.0 and 4.0 mg/kg respectively). Metoclopramide resembled pimozide in all these respects and appeared to be a relatively potent antagonist of striatal dopamine receptors in these behavioural models (Dolphin, Jenner, Marsden, Pycock & Tarsy, 1975).

Pretreatment of male mice (20-30 g) with metoclopramide (50 mg/kg i.p.) caused a time-dependent increase in homovanillic acid (HVA) in whole brain, which was maximal (740%) $1\frac{1}{2}$ h after injection and lasted some 5 hours. Metoclopramide (50 mg/kg) increased HVA concentrations to the same extent in the corpus striatum (450%) and the mesolimbic area (590%) (a slice containing corpora amygdala, nucleus accumbens and olfactory tubercle). On the other hand no change in whole brain concentrations of dopamine, noradrenaline (NA), 4-hydroxy-3-methoxyphenylglycolsulphate (MOPEG-SO4), 5-hydroxytryptamine (5HT), or 5-hydroxy-indoleacetic acid were observed after metoclopramide treatment. The biochemical results suggest that metoclopramide blocks cerebral dopamine receptors in both striatum and mesolimbic areas,