Monoamine oxidase active site; the binding to and titration of monoamine oxidase with [^{14}C]-selective inhibitors

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Liver monoamine oxidase (MAO) (see Youdim, 1975 for review) and the brain enzyme (Salach, Yasunobu, Minamura & Youdim, 1975) contain 1 mol of covalently bound flavin adenine dinucleotide (FAD) as a cofactor. The isolated FAD is associated with a peptide, having the following amino acid sequence, Ser-Gly-Gly-Cys-Tyr, the flavin being attached via the 8α-carbon of riboflavin in a thio-ether linkage with the cysteine residue (Salach *et al.*, 1975).

Enzyme absorption spectra studies with selective [^{14}C]-labelled irreversible inhibitors have shown that the action of clorgyline (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy) propylamine hydrochloride), deprenil (phenyl-isopropylmethyl-propinylamine hydrochloride) and phenylethylhydrazine (Collins & Youdim, 1975) may involve the binding of the inhibitors with the flavin cofactor. The inhibition of enzyme by these compounds is prevented by monoamine substrates, is time-dependent and is a function of the amount of inhibitor bound to the enzyme. When MAO is fully inhibited 1 mol of the inhibitor deprenil or phenylethylhydrazine combines irreversibly and covalently to 1 g equivalent of purified enzyme of molecular weight 120,000-150,000 (Youdim & Sourkes, 1966) in a stoichiometric fashion.

Isolation of the flavin peptide adduct from [^{14}C]-inhibitor treated liver enzyme showed that 1 mol of [^{14}C]-inhibitor is bound to 1 mol of flavin. • Fluorescence and spectral absorption studies suggest that deprenil may bind to the nitrogen on the flavin at position 5, a result which is similar to that reported for the inhibitor 3-dimethylamino-1-propyne (Maycock, Abeles, Salach & Singer, 1975).

Certain physiological factors influence the activity of MAO, which in turn may affect monoamine metabolism. The changes in enzyme activity could be due to a change in MAO protein synthesis or degradation. In experimental studies and disease states in the human where MAO activity fluctuates it is desirable to know the exact amount of enzyme present. The results presented in this communication suggest the use of labelled selective inhibitors as agents for titration of MAO and its multiple forms because the inhibition is stoichiometric and thus the titration end-point can be used to estimate the enzyme concentration.

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Influence of etorphine acepromazine and diprenorphine on cardiovascular function in ponies

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The neuroleptanalgesic drug combination of etorphine and acepromazine (Large Animal Immobilon; Reckitt & Colman Ltd.) was administered i.v. at the recommended dose rate (24 μg/kg etorphine and 100 μg/kg acepromazine) to twelve Welsh Mountain ponies of 185 to 336 kg bodyweight. Cardiovascular measurements were made before and at pre-determined times up to 30 min after the injection. The etorphine antagonist, diprenorphine (Revivon; Reckitt & Colman Ltd.), was then injected i.v. (30 μg/kg) and further measurements were obtained.

Pronounced increases in heart rate, moderate increases in cardiac output and significant reductions in stroke volume occurred throughout the period of neuroleptanalgesia (Table 1). Mean