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.

References

COLLINS, G.G.S. & YOUDIM, M.B.H. (1975). The binding of (^{14}C)-phenylethylhydrazine to rat liver monoamine oxidase. *Biochem. Pharmac.*, **24**, 703-706.

MAYCOCK, A.L., ABELES, R.H., SALACH, J.I. & SINGER, T.P. (1975). Structure of the adduct derived from the inhibition of monoamine oxidase by 3-dimethylamino-1-propyne. In *Flavins and Flavoproteins*, ed. T.P. Singer. Amsterdam: Elsevier, (in press).

SALACH, J.I., YASHUNOBU, K.T., MINAMURA, M. & YOUDIM, M.B.H. (1975). Is there covalently bound flavin in monoamine oxidase from brain? In *Flavins and Flavoproteins*, ed. T.P. Singer. Amsterdam: Elsevier, (in press).

YOUDIM, M.B.H. (1975). Monoamine deaminating system in mammalian tissues. In *M.T.P. International Review of Science*, ed. H. Blaschko, pp. 169-211. Oxford Press: Butterworth Ltd.

YOUDIM, M.B.H. & SOURKES, T.L. (1966). Properties of purified, soluble monoamine oxidase. *Can. J. Biochem.*, **44**, 1397-1400.

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

Table 1 Effects of etorphine, acepromazine and diprenorphine on cardiovascular function

Time (min)	*Heart rate (beats/min)*	*Stroke volume (ml kg^{-1})*	*Cardiac output (ml kg^{-1} min^{-1})*	*T P R (dyn s cm^{-5})*	*M A P (mm Hg)*	*Hb concentration (g/100 ml)*
control	42 ± 2	1.66 ± 0.07	66 ± 3	656 ± 41	123 ± 3	11.7 ± 0.3
E + 5	134 ± 10**	0.77 ± 0.07**	102 ± 13**	608 ± 65	161 ± 8**	14.5 ± 0.3**
E + 15	117 ± 8**	0.76 ± 0.04**	87 ± 6**	474 ± 44**	117 ± 7	13.3 ± 0.3**
E + 30	122 ± 12**	0.83 ± 0.11**	93 ± 7**	503 ± 62**	126 ± 6	12.7 ± 0.3
D + 5	93 ± 12**	1.03 ± 0.07**	91 ± 10*	426 ± 60**	96 ± 4**	12.0 ± 0.4
D + 15	66 ± 6**	1.23 ± 0.08**	77 ± 6	434 ± 48**	90 ± 4**	10.7 ± 0.3**
D + 30	52 ± 4*	1.32 ± 0.07**	66 ± 5	546 ± 59*	90 ± 5**	9.8 ± 0.2**
D + 60	47 ± 3	1.37 ± 0.07**	62 ± 3	539 ± 50**	93 ± 4**	9.3 ± 0.2**

Values are means ± s.e. mean for twelve ponies. Times refer to the i.v. administration of etorphine and acepromazine (*E*) or the i.v. injection of diprenorphine (*D*). The significance of differences from control values was assessed by paired *t*-tests and is indicated by asterisks: *, $P < 0.05$; **, $P < 0.01$.

arterial blood pressure (MAP) and arterial HB concentration were increased after 5 min but had returned to the control ranges by 15 min (MAP) and 30 min (Hb concentration). Total peripheral resistance (TPR) was initially unchanged and then reduced at 15 and 30 minutes. Following diprenorphine administration, TPR decreased further and MAP and Hb concentration also fell below control levels, while heart rate and cardiac output initially remained above controls before returning to the normal ranges by 60 and 15 min, respectively.

It is concluded that etorphine causes sympathoadrenal stimulation as a result of its respiratory depressant effects (Hillidge & Lees, 1975) and possibly by other mechanisms and that some of the sympathetic effects are partially offset by an α-adrenoceptor blocking action of acepromazine. This action of acepromazine probably accounted also for the reductions in MAP, TPR and Hb concentration which occurred after the actions of etorphine had been antagonized with diprenorphine.

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References

HILLIDGE, C.J. & LEES, P. (1975). Influence of the neuroleptanalgesic combination of etorphine and acepromazine on the horse: blood gases and acid-base balance. *Equine vet. J.*, , 7, 148-154.

The effect of inhibitors of alcohol metabolism on the changes in the hepatic microsomal metabolism of foreign compounds produced by a single dose of ethanol in the rat

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The administration to rats of a single oral dose of ethanol has been found to produce a selective induction of hepatic microsomal aniline hydroxylation, a decrease in aminopyrine demethylation and no change in the activity of the components of the hepatic microsomal mixed function oxidase (Powis, 1975). The present work is an attempt to distinguish between the direct effects of ethanol and the effects resulting from the metabolism of the ethanol, on the hepatic microsomal metabolism of foreign compounds.

Ethanol or acetaldehyde were administered to unanaesthetized rats by stomach tube and inhibitors of alcohol and aldehyde dehydrogenase were administered by i.p. injection. The hepatic microsomal fraction was prepared, 24 h after the administration of the various compounds, by the method of Ernster, Siekevitz & Palade (1962) in 0.25 M sucrose containing 0.05 M Tris buffer pH 7.4. The metabolism of foreign compounds was measured over 30 min at 37°C using the