Irreversible protein binding of [^{14}C]-practolol metabolite(s) to hamster liver microsomes

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The toxic effects of some chemically stable drugs, e.g. paracetamol, phenacetin and isoniazid, correlate with their metabolism by the microsomal mixed function oxidases via transient reactive intermediates which bind irreversibly to tissue proteins (Gillette, Mitchell & Brodie, 1974).

Practolol is a cardioselective β-blocking drug which can give rise, in a small proportion of patients, to characteristic skin and eye lesions and sclerosing peritonitis (Waal-Manning, 1975). As some mechanistic hypotheses have involved an immunological reaction (Amos, Brigden & McKerron, 1975; Behan, Behan, Zacharias & Nicholls, 1976), we have investigated the reactivity of practolol and its metabolites with tissue and serum proteins in order to evaluate its potential to form a possible antigenic complex.

[^{14}C]-practolol (nuclear labelled) was incubated in air with hamster liver microsomes in the presence of NADPH. The irreversible binding of reactive [^{14}C]-intermediates to microsomal proteins was determined by liquid scintillation counting after exhaustive solvent extraction of the microsomes (Potter, Davis, Mitchell, Jollow, Gillette & Brodie, 1973). Terminal metabolites were separated by thin layer chromatography and characterized by co-chromatography with authentic standards.

The characteristics of the binding reaction of [^{14}C]-practolol metabolite(s) to hamster liver microsomes is shown in Table 1. The binding was dependent on oxygen and NADPH and was sensitive to carbon monoxide. The binding was inhibited by glutathione and human serum albumin. [^{14}C]-practolol metabolite(s) were bound to the albumin. The rate of binding was linear up to 60 min (5 mg protein per incubation) and 10 mg protein (30 min reaction). The kinetic constants were $V_{max} = 0.08$ nmol bound mg microsomal protein^{-1} min^{-1} and $K_m = 0.06$ mM. The rate of microscomal binding was not altered following pretreatment of hamsters with practolol (50 mg/kg p.o. for 6 days) or phenobarbitone sodium (75 mg/kg i.p. for 3 days). Acetyl labelled [^{14}C]-practolol resulted in less binding than nuclear labelled [^{14}C]-practolol (0.71 nmol and 1.49 nmol bound mg protein^{-1} 30 min^{-1} respectively).

The major terminal metabolites formed *in vitro* were desacetylpractolol and 3-hydroxypractolol. The formation of 3-hydroxypractolol was NADPH dependent and the maximal rate was 6 nmol mg protein^{-1} 30 min^{-1}. [^{14}C]-desacetylpractolol *per se* did not combine irreversibly with liver microsomal protein or human serum albumin. Under the same reaction conditions (1 mM substrate, 5 mg protein, 30 min reaction) the rate of the NADPH dependent binding of [^{14}C]-desacetylpractolol to hamster liver microsomes was eight times greater than for [^{14}C]-practolol.

The possible relevance of the *in vitro* binding of practolol metabolite(s) to mammalian liver microsomes and human serum albumin to the adverse reactions found in man has not yet been established.

Table 1 Inhibition of the irreversible binding of practolol metabolite(s) to hamster liver microsomes *in vitro*

Reaction mixture	*Practolol bound nmol/mg protein/ 30 min*	*% control*
Complete	1.49 ± 0.04*	100
−NADPH	0.09 ± 0.01	6.0
−O_2 (100% N_2 atmosphere)	0.46 ± 0.03	30.8
+Carbon monoxide (90%, CO, 10% O_2)	0.26 ± 0.02	17.4
+Glutathione (10 mM)	0.32 ± 0.04	21.5
+Human Serum Albumin (12.5 mg)	0.05 ± 0.03	3.4

* Each value is the mean ± s.d. of 3 or more determinations.
Reaction mixtures consisting of ^{14}C-practolol (1 mM : 1 μCi), microsomes (5 mg protein), NADPH (0.8 mM) in 0.1 M phosphate buffer pH 7.4 were incubated at 37° for 30 minutes.

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The following demonstration, presented to the British Pharmacological Society Meeting at Mill Hill (5th–7th January, 1977), appeared as a title only in the March 1977 issue of the British Journal of Pharmacology.

Do 5-HT neurones support self-stimulation?

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With electrodes implanted in certain areas of the brain, rats can be trained to press a lever to deliver electrical stimulation through the implanted electrode (Olds & Milner, 1954). This 'self-stimulation' behaviour presumably indicates that the electrode activates neural systems which have rewarding effects. Previous pharmacological and biochemical evidence suggests that activation of catecholamine-containing systems plays a crucial role in reward mechanisms. In particular it has been found that there are foci of self-stimulation sites at the level of origin of a major dopamine-containing system in the ventral mesencephalon (Crow, 1972) and a noradrenergic system in the nucleus locus coeruleus (Crow, Spear & Arbuthnott, 1972).

More recently it has been found that self-stimulation can also be obtained with electrodes implanted in the region of the median and dorsal raphé nuclei which comprise 5-HT containing cell bodies giving rise to ascending fibres which innervate large areas of the forebrain with tryptaminergic terminals. This finding raises the question of whether the neurochemical mechanisms of reward are more complex than previously suggested. With electrodes on the median raphé nucleus, Miliaressis, Bouchard and Jacobowitz (1975) have claimed that self-stimulation responding is selectively suppressed by administration of the 5-HT synthesis inhibitor *p*-chlorophenylalanine. In these experiments we have attempted to replicate this effect and to study the time course of inhibition following *p*-chlorophenylalanine. We also investigated whether the inhibition is a function of responding by including a rest-pause of 30 min in each test session.

The results (Figure 1) show that there is a modest

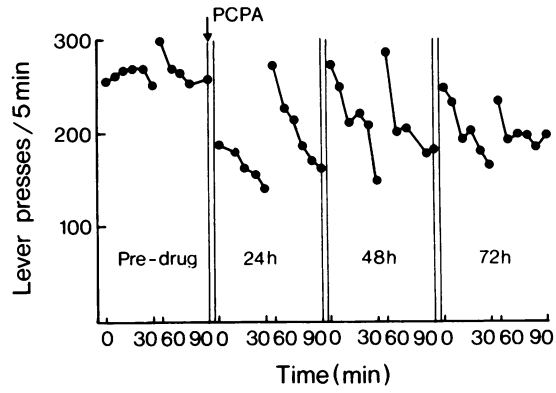

Figure 1

reduction in responding following *p*-chlorophenylalanine administration but that this effect is maximal at 24 h, when noradrenaline stores are known to be depleted, and that there is considerable recovery by 72 h when 5-HT depletion is greatest. The decline of responding during the test session, and recovery after the rest-pause are greater at 24 and 48 h than at 72 h, findings which also raise doubts as to whether 5-HT depletion is responsible for the inhibition of responding seen in these experiments.

Thus even though electrical self-stimulation can be obtained with electrodes located in the raphé nuclei the role of 5-HT neurones in reward processes appears doubtful.

J.F.W.D. is a Medical Research Council Fellow.

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