The metabolism of *N,N'*-dimethylclonidine by rat liver microsomes

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N,N'-Dimethylclonidine, 1,3-dimethyl-2-(2,6-dichlorophenylimino) imidazolidine, is a dimethylated analogue of the hypotensive agent clonidine. Previous studies on the metabolism of clonidine have shown the imidazolidine ring to be a major site of metabolism (Baillie, Davies, Davies, Hughes & Neill, 1978) with *p*-hydroxyclonidine the only metabolite of the aromatic ring which has been identified (Rehbinder & Deckers, 1969). The imidazolidine ring was shown to undergo ring cleavage to form 2,6-dichlorophenylguanidine (Hodges, 1976) and our investigations have led to the identification of two further metabolites 4,5-dehydroclonidine and 4-oxoclonidine (Baillie, *et al.*, 1978).

Initial studies using *N,N'*-dimethylclonidine led to the tentative identification of other metabolites and further investigations of the metabolism of the imidazolidine ring were carried out using this dimethyl derivative of clonidine. Metabolites formed during the incubation of *N,N'*-dimethylclonidine with rat liver microsomal preparations were extracted using Amberlite XAD-2 and subsequently purified by high pressure liquid chromatography. Identification of metabolites was carried out using gas chromatography-mass spectrometry.

In order to identify sites of metabolic attack, two deuterium labelled analogues, [3',4',5',-$^2H_3$]*N,N'*-dimethylclonidine and *N,N'*-di[$^2H_3$]methyl clonidine, were synthesised and used in our metabolic studies. From this work it was apparent that *N,N'*-dimethylclonidine was metabolised by a similar pathway to that of clonidine with the formation of all the corresponding metabolites *viz*, *p*-hydroxy-*N,N*-dimethylclonidine, *N,N'*-dimethylguanidine, 4,5-dehydro-*N,N'*-dimethylclonidine and 4-oxo-*N,N'*-dimethylclonidine. In addition to these metabolites two hydroxylated products, 4-hydroxy-*N,N'*-dimethylclonidine and 4,5-dihydroxy-*N,N'*-dimethylclonidine, were identified. The presence in the incubation medium of 4,5-dehydro-*N,N'*-dimethylclonidine and the 4,5-dihydroxy-*N,N'*-dimethylclonidine led us to propose an epoxide diol pathway. Further investigation of this pathway was carried out by incubating *N,N'*-dimethylclonidine in an atmosphere of N_2:$^{18}O_2$ (80:20) and analysing the hydroxylated metabolites. The $^{18}O_2$ content of the 4-hydroxy metabolite was found to be only 16%. This low incorporation of ^{18}O is probably due to a reversible hydration reaction to give the 4,5-dehydro derivative. In the 4,5-dihydroxy metabolite however, 47% incorporation of 2 atoms of ^{18}O had occurred indicating that molecular oxygen and not water was the source of both oxygen atoms. This is incompatible with hydration of an epoxide intermediate and thus for *N,N'*-dimethylclonidine epoxidation is not the only route to the 4,5-dihydroxy compound and may not operate at all.

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The effect of paraquat on the covalent binding of radio-labelled DOPA to liver and lung microsomal protein

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The mechanism whereby paraquat produces its toxic effects in the lung is unknown. Histological similarities to oxygen toxicity led to the suggestion that paraquat damages the lung by catalysing the generation of superoxide (O_2^-), a chemically reactive form of oxygen. Under normal circumstances superoxide is inactivated by the enzyme superoxide dismutase (EC.1.15.1.1) but it is suggested that in the presence of paraquat its capacity is exceeded. Superoxide has been shown to convert catechols including [^{3}H]-DOPA to reactive intermediates which can bind covalently with cellular macromolecules (Dybing, Nelson, Mitchell, Sasame & Gillette, 1976). Therefore the covalent binding of radioactivity following administration of radio-labelled DOPA may be a means of demonstrating the site of production of superoxide by paraquat *in vivo*. We report results of initial studies

using lung and liver microsomes in the presence of paraquat, to generate superoxide and covalently bind a metabolite of DOPA.

Male Wistar rats (150–250 g) were killed by decapitation and their lungs were perfused with ice cold normal saline. The lungs and livers were removed, homogenised and the microsomal fraction was isolated by differential centrifugation. The covalent binding of DOPA to washed microsomal protein was measured according to the method of Dybing *et al.* (1976).

In the absence of paraquat the covalent binding of radioactivity (expressed as nmol of DOPA) to microsomal protein was 0.200 ± 0.012 nmol mg protein^{-1} min^{-1} (mean ± s.e. mean, $n = 11$) for lung and 0.401 ± 0.012 nmol mg protein^{-1} min^{-1} ($n = 7$) for liver. However, the addition of paraquat (5 mM) caused a 132% increase in binding with lung microsomes with only a 22.4% increase with liver microsomes. Superoxide dismutase (30 μg/ml) in the absence of paraquat completely inhibited the covalent binding while in the presence of paraquat (5 mM) significant superoxide dismutase insensitive covalent binding remained. Preliminary studies to ascertain the involvement of species other than superoxide in the covalent binding were therefore initiated. Benzoate, a hydroxyl radical scavenger and the singlet oxygen scavenger, 1,4-diazabicyclo-2,2,2-octane in the absence or presence of paraquat had no effect on the covalent binding. Catalase in the absence of paraquat significantly reduced the binding whereas in the presence of paraquat it had no effect.

The addition of glutathione, which can form a conjugate with the reactive intermediate of DOPA, completely inhibited covalent binding in the absence and presence of paraquat. If glutathione plays a similar role in the intact cell then it may be necessary to deplete intracellular glutathione before this technique can be used *in vivo* to identify the cell type or types in the lung involved in paraquat toxicity.

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Environmental factors affecting monooxygenase activity of microsomal fractions of human liver biopsies

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Considerable attention has been focused on the influence of environmental factors on hepatic monooxygenase activity in man. Most studies have been performed *in vivo* and thus little is known about functional changes in the liver itself. As part of an investigation into *in vitro* monooxygenase activity in microsomal fractions of human liver we have accumulated clinical and biochemical information on 49 patients undergoing routine liver biopsies (26 needle, 23 wedge) in whom histology showed only non-specific changes with no evidence of established liver disease. The influence of alcohol consumption, caffeine intake, drug ingestion and cigarette smoking on *in vitro* monooxygenase activity was analysed. Samples were assayed for cytochrome P-450 content, NADPH-cytochrome c reductase and aryl hydrocarbon hydroxylase (AHH) activities. We have previously shown that in biopsies with preserved hepatic architecture the range of monooxygenase activities were similar in needle and wedge samples (Boobis, Brodie, Davies, Fletcher & Saunders, 1978). Therefore the two groups of biopsies were treated as a single population for statistical analysis.

The higher the weekly alcohol consumption, which varied from 0 to 350 units per week (equivalent to 20 pints of beer or 1 bottle of whisky per day), the lower the microsomal cytochrome P-450 content when expressed per mg microsomal protein ($P < 0.01$) and per g liver ($P < 0.05$). Cytochrome P-450 content decreased by up to 70% with increasing alcohol ingestion. There was no accompanying change in NADPH-cytochrome c reductase activity with alcohol ingestion. This suggests that changes in cytochrome P-450 content were not simply due to non-specific loss of protein following fatty infiltration. AHH activity per nmol cytochrome P-450 was positively correlated with alcohol intake ($P < 0.05$). Thus alcohol may selectively induce this monooxygenase.

Coffee and tea ingestion (1 cup tea = 0.6 cup coffee) had no significant effect on any of the parameters measured. However, there was a trend for AHH activity to increase ($P = 0.057$) with increasing coffee