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

D.S. DAVIES & H. HUGHES

Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 0HS

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.

References

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

D.S. DAVIES & M.E. McMANUS

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS

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