The metabolism of clonidine and related imidazoline derivatives in rat liver

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Clonidine, 2-(2,6-dichlorophenylimino)-2-imidazolidine, is a potent hypotensive agent which is used clinically in the management of essential hypertension. Although clonidine has been reported to undergo extensive metabolism in several species, including man, rat and dog (Rehbinder, 1970), only two metabolites, *p*-hydroxy-clonidine (Rehbinder & Deckers, 1969) and 2,6-dichlorophenylguanidine, a product of imidazoline ring cleavage (Hodges, 1976), have hitherto been identified. In view of the steadily growing importance of imidazoline derivatives as therapeutic agents, we decided to carry out a detailed study of the metabolic fate of clonidine in an appropriate animal model. The similarity between the patterns of radioactive urinary metabolites obtained from rats and humans given [2-^{14}C]-clonidine (Rehbinder, 1970) led to the selection of the rat as the species of choice for the present investigation.

Metabolites formed during incubation of clonidine with rat liver microsomal preparations or present in the perfusate and bile from rat livers perfused with the drug 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.

The substituted imidazole, 2-(2,6-dichlorophenylamino)-imidazole, was the major metabolite produced in microsomal incubations; formation of this compound was shown to be dependent on the presence of both molecular oxygen and an NADPH-regenerating system. In the perfused liver, on the other hand, 2,6-dichlorophenylguanidine was the principle metabolite while 4-oxo-clonidine was also formed in significant amounts. Only trace quantities of *p*-hydroxy-clonidine were detected in either system.

In order to define the metabolic pathway by which the imidazoline ring system undergoes cleavage with loss of carbons 4 and 5, the following analogues of clonidine were synthesized and their metabolism studied: 4-methyl-clonidine, 4,4-dimethyl-clonidine, *N,N'*-dimethyl-clonidine, [4,4,5,5-$^{2}H_4$]-clonidine and [4,5-$^{14}C_2$]-clonidine. The findings from these experiments indicated that clonidine is first converted to the corresponding imidazole derivative which is metabolized, in turn, probably *via* the epoxide-diol pathway; spontaneous ring cleavage would then afford 2,6-dichlorophenylguanidine and glyoxal, both of which have been identified as clonidine metabolites in our liver perfusion system. Further metabolism of a glyoxal results in the formation of glyoxylic acid and related two-carbon fragments.

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Measurement of cytochrome oxidase activity by kinetic microdensitometry using substrate gel films

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With both biochemical and histochemical techniques, spontaneous reactivation of cyanide-inhibited cytochrome oxidase in tissue homogenates or sections may give spuriously low estimates for the *in vivo* inhibition of the enzyme by cyanides or cyanogenic compounds (Ballantyne & Bright, 1978; Camerino & King, 1966). A method is described for the more reliable assessment of enzyme inhibition, which is based on measuring the rate of utilization of amine substrates in gel films to which are applied tissue sections.

Films were prepared from a fluid containing agarose (0.3 g) in a mixture of 14 ml distilled water and 6 ml 0.2 M tris–maleate buffer of pH 7.4 to which was added a substrate solution of *p*-aminodiphenyl-