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-

Table 1 Comparison of different quantitative histochemical methods for the assessment of cytochrome oxidase activities in tissues from control rabbits and those receiving lethal doses of KCN (8 mg CN/kg, i.m.); results expressed as mean ± s.e.mean for 6 animals per group

Group	*Cytochrome oxidase activity measured by*			
	Kinetic microdensitometry (ΔOD)		*End-point histophotometry (OD)*	
	Myocardium	*Brain*	*Myocardium*	*Brain*
Control	0.060 ± 0.001	0.016 ± 0.001	0.46 ± 0.04	0.20 ± 0.03
Cyanide	0.033 ± 0.001	0.008 ± 0.001	0.31 ± 0.01	0.16 ± 0.01
*P**	0.001	0.001	0.001	0.001

* Significance of differences in enzyme activities between controls and cyanide injected animals (*t*-test).

amine (5 mg) and *p*-amino *p*-methoxydiphenylamine in 0.2 ml methanol. Fluid was poured over cover slips to a thickness of 1 mm and allowed to solidify. Cryostat sections of fresh-frozen tissues were applied to the gel films, immediately after which azine dye production was determined microdensitometrically by measuring the rate of change in optical density (ΔOD) with a Leitz MPV 2 microscope recording photometer equipped with a grating monochromator. Optimum results were obtained with 10 μm thick sections of 2–4 mm diameter and measurement of ΔOD at 550 nm.

Measurement of cytochrome oxidase activity by kinetic microdensitometry was compared with assessment of enzyme activity by conventional end-point histophotometry, in which tissue sections were incubated in histochemical substrate solutions (Burstone, 1960) and dye production estimated by a single measurement of optical density after 20 min incubation. Measurements were made on brain and myocardium from control (carbon dioxide euthanasia) and cyanide sacrificed rabbits (8 mg CN/kg, i.m.). Cytochrome oxidase activity (Table 1) was significantly lower in tissues from cyanide animals compared with controls, and measured enzyme inhibition was greater by kinetic microdensitometry (brain 50%; myocardium 45%) than by end-point histophotometry (brain 20%; myocardium 33%). With the latter approach spontaneous reactivation of the enzyme causes a progressively increased rate of substrate utilization, but using kinetic microdensitometry the initial reaction rate is measured before significant reactivation occurs.

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Profiles of α-adrenoceptor antagonists in the pithed rat

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There is evidence that presynaptic and postsynaptic α-adrenoceptors (Langer, 1974) differ in their sensitivity to antagonists (Starke, Borowski & Endo, 1975; Drew, 1976; Doxey, Smith & Walker, 1977). The profiles of 3 antagonists (yohimbine, phentolamine and prazosin) on pre- and post-synaptic α-adrenoceptors were studied in pithed rats. The ability of the 3 antagonists to inhibit electrically induced contractions of the vas deferens and anococcygeus muscle was used as an assessment of postsynaptic activity. Presynaptic activity was assessed by determining the ability of the antagonists to reverse the inhibitory effects of clonidine on sympathetic outflow to the vas deferens and anococcygeus muscle (Doxey & Everitt, 1977).