Identification of 2,6-dichlorophenylguanidine as a metabolite of clonidine

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This communication reports the discovery of a novel metabolic reaction of a 2-aryl-amino-imidazoline, *viz.* a net bis-*N*-dealkylation to give an aryl-guanidine.

Clonidine (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride) has anti-hypertensive activity in animals and in man (Hoefke & Kobinger, 1966; Bock, Heimsoth & others, 1966; Knoblock & Morr, 1966). Metabolic studies by Rehbinder & Deckers (1966, 1969) showed that after oral administration to the rat and the dog, drug-related material was excreted mainly in urine. In urine from the rat, unchanged drug was the major drug-related component and 4-hydroxyclonidine and its conjugates were also identified. At least two other metabolites were detected but not identified. In urine from the dog, unchanged drug constituted only a small proportion of the drug-related material. Several metabolites were detected but not identified. The present communication describes the identification of 2,6-dichlorophenylguanidine as a metabolite of clonidine in urine from the rat and the dog.

Clonidine was administered orally to two rats (Charles River CD strain, male, 250 g) as an aqueous solution at a dose level of 5 mg kg^{-1}. For oral administration to a dog (Pfizer beagle, male, 11·4 kg) the clonidine was blended with lactose in the proportions 1:1000 and given in gelatine capsules at a dose level of 0·5 mg kg^{-1}. Urine was collected for periods of 24 h before and after administration of clonidine. The urines were adjusted to pH 9 with saturated aqueous sodium carbonate solution and extracted with ethyl acetate (3 × equal volume). The extracts were evaporated under vacuum and the residue dissolved in methanol for application to t.l.c. plates (Merck $60F_{254}$). After development in the solvent system chloroform–methanol–ammonium hydroxide s.g. 0·880 (80:30:1) the plates were viewed under ultraviolet light (254 nm). Urine from rats which had been treated with clonidine contained two substances (R_F values 0·42 and 0·82) which were not present in predose urine. Urine collected from the dog after treatment with clonidine showed one substance (R_F 0·42) which was not present in predose urine.

The drug-related material in rat and dog urine with R_F 0·42 was eluted from the t.l.c. plate with methanol, and the mass spectra of both substances were determined with an LKB 9000S instrument. These had the same mass spectrum as an authentic

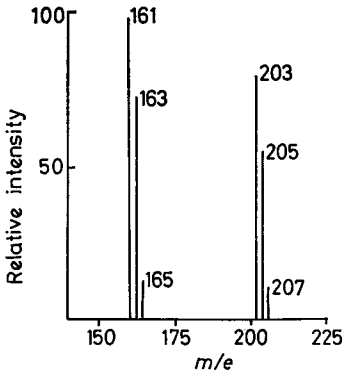

FIG. 1. Mass spectrum of 2,6-dichlorophenylguanidine.

sample of 2,6-dichlorophenylguanidine (Fig. 1) with molecular ions at *m/e* 203, 205, 207 (relative intensities 9:6:1) and fragment ions at *m/e* 161, 163, 165 (relative intensities 9:6:1). This pattern of three ions at 2 mass unit intervals with relative intensities 9:6:1 is characteristic of compounds containing two chlorine atoms. The authentic 2,6-dichlorophenylguanidine which was prepared by ammonolysis of 2,6-dichlorophenylimidocarbonyl chloride in boiling dioxan (Appleby, private communication) had identical chromatographic properties to those of the metabolite. Although quantitation was not possible, the intensity of fluorescence quenching on t.l.c. plates viewed under ultraviolet light suggests that 2,6-dichlorophenylguanidine may be a major metabolite of clonidine in dog urine. This compound is known to possess only weak hypotensive activity in the dog and the rat (Jen, van Hoeven & others, 1975).

This is believed to be the first observation of the metabolism of a 2-aminoimidazoline compound by a net bis-*N*-dealkylation although a similar type of ring cleavage has been reported for the piperazine ring in the drug perazine (Breyer, Krauss & Jochims, 1972; Gaertner & Breyer, 1972).

The substance from rat urine with the R_F value 0·82 was identified as unchanged clonidine by its behaviour on t.l.c. and by mass spectrometry of the material isolated from t.l.c.

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On the biological half-life of amitriptyline

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Little has so far been published about the biological half-life of amitriptyline. In 1971, Braithwaite & Widdop obtained figures of 41 and 45 h with two volunteers, in whom the biological half-life was determined from the declining plasma concentration curves after cessation of dosing to steady state (amitriptyline 3×50 mg day^{-1}). Hucker, Stauffer & others (1975) recorded an apparent plasma half-life of approximately 8 h. But this was obtained from the period 9–12 h after administration of a sustained release formulation of amitriptyline (Hucker, personal communication), a period which is not purely elimination phase (β-phase). Since part of the decline in plasma concentration in this period is due to distribution from plasma into the tissues this figure cannot be considered an estimate of the biological half-life of amitriptyline.

Based on a study with once a day administration of amitriptyline to eight patients we have been able to estimate the biological half-life of amitriptyline from data from