J. Pharm. Pharmacol. 1982, 34: 139–140
Received December 17 1981

0022–3573/82/020139–02 $02.50/0

Hydralazine is an irreversible inhibitor of the semicarbazide-sensitive, clorgyline-resistant amine oxidase in rat aorta homogenates

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Cardiovascular tissues of the rat, and other species, contain more than one amine oxidizing activity. The mitochondrial enzyme monoamine oxidase (MAO), which exists in two forms called MAO-A and MAO-B, has been studied extensively (see Fowler et al 1978 for review). However, a semicarbazide-sensitive and clorgyline-resistant amine oxidase (CRAO) activity, which is distinct from MAO has also been described in, for example, the rat heart and aorta (Coquil et al 1973; Lyles & Callingham 1975; Clarke et al 1982). In the rat, this enzyme is further characterized by its ability to metabolize benzylamine with a K_m value of around 5 μM, which is much lower than the K_m (>100 μM) for metabolism of benzylamine by MAO activities (Lyles & Callingham 1975). Thus at very low (e.g. 1 μM) concentrations of benzylamine in enzyme assays, the activity of CRAO can be measured in the absence of any significant contribution by MAO activities (Lyles & Callingham 1981).

We have previously reported that CRAO in the rat is sensitive to inhibition by a variety of substituted hydrazine-based compounds, including isoniazid (Lyles & Callingham 1975), phenelzine (Clarke et al 1982) and benserazide (Lyles 1982; Lyles & Callingham 1982). Here, we report that a similar type of compound, hydralazine, which is used clinically as a vasodilator drug, is an irreversible inhibitor of CRAO activity in rat aorta homogenates.

Materials and methods

Materials. Male Sprague-Dawley rats, about 200 g, were from A. J. Tuck and Sons, Rayleigh, U.K. [^{14}C]Benzylamine hydrochloride was purchased from the Radiochemical Centre, Amersham, U.K., and hydralazine hydrochloride from Sigma London, Poole, U.K. Benserazide hydrochloride (Ro 4-4602) was a gift from Roche Products Ltd, Welwyn Garden City, U.K. and phenelzine acid sulphate was kindly supplied by William Warner and Co Ltd, Pontypool, U.K.

Methods. Aortae from 4 rats were pooled and homogenized (1:40, w/v) in 1 mM potassium phosphate buffer, pH 7·8. The homogenate was centrifuged at 800 *g* for 10 min, and the supernatant decanted and stored frozen in several 0·5 ml aliquots (protein concentration of 2·3 mg ml^{-1}; Goa 1953) for subsequent use. After thawing, the samples were further diluted eight-fold with homogenization buffer and preincubated with inhibitors at 37 °C, followed by assay of remaining CRAO activity by the use of 1 μM [^{14}C]benzylamine, as described by Lyles & Callingham (1981). Mean specific activity (± s.e.m.) of CRAO (from three separate determinations) in these samples was 14·5 ± 0·8 nmole h^{-1} mg prot^{-1}. All inhibitor solutions were prepared in 0·2 M potassium phosphate buffer, pH 7·8, to prevent changes in pH when including their acid salts in the assay mixtures.

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Results and discussion

In preliminary experiments, preincubation of homogenates for 20 min with hydralazine, 1 μM, inhibited rat aorta CRAO activity almost completely. On the other hand, when hydralazine (1–100 μM) was added to control assay mixtures after the enzyme reaction had been stopped, the drug had no influence upon the subsequent extraction of the metabolites of benzylamine, indicating that inhibition of enzyme activity was a real property of hydralazine.

The potency of hydralazine was subsequently compared, in the same experiment, with that of phenelzine and of benserazide as inhibitors of CRAO after preincubation for 20 min (Fig. 1). Under these identical conditions, hydralazine (approx. IC50 of 2·5 × 10^{-8} M) was less active than phenelzine (IC50 of 1·6 × 10^{-9} M), but more potent than benserazide (IC50 of 3·5 × 10^{-7} M).

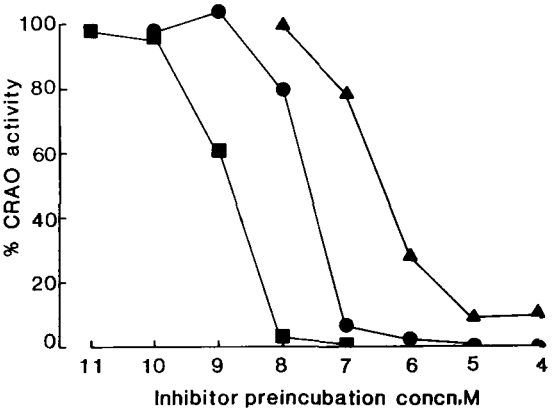

FIG. 1. Inhibition of rat aorta CRAO by phenelzine (■), hydralazine (●) and benserazide (▲). Homogenate samples were preincubated for 20 min at 37 °C with various drug concentrations. CRAO activities in inhibited samples were assayed with 1 μM benzylamine and expressed as a percentage of activities in control samples. Each point is the mean of quadruplicate determinations with s.e. ratio (not shown) less than 5%.

Fig. 2 shows the effect of varying preincubation time upon inhibition of CRAO by three different hydralazine concentrations. Some inhibition was apparent without preincubation particularly in the presence of 1 × 10^{-8} and

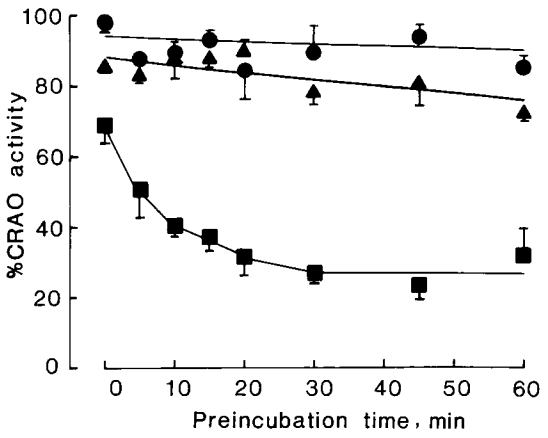

FIG. 2. Time-dependent inhibition of rat aorta CRAO by hydralazine. Homogenate samples were preincubated for various periods with 5×10^{-9} M (●), 1×10^{-8} M (▲) and 5×10^{-8} M (■) hydralazine. CRAO activites in inhibited samples were assyed with 1 μM benzylamine and expressed as a percentage of activites in corresponding control samples preincubated for identical periods. Each point is the mean ± s.e. ratio of triplicate determinations.

5×10^{-8} M hydralazine. With 5×10^{-8} M hydralazine the percentage inhibition increased with preincubation time up to a maximum at 30 min.

Duplicate samples containing 0·5 ml aorta homogenate and 0·5 ml hydralazine solutions (5×10^{-8} or 5×10^{-7} M preincubation concentrations) were preincubated for 1 h, along with control samples lacking the inhibitor. These samples were then dialysed for 21 h at 4 °C against 1 mM potassium phosphate buffer, pH 7·8. In these experiments, CRAO activity was found to be completely inhibited by both hydralazine concentrations in samples assayed without dialysis. After dialysis, enzyme activity remained totally inhibited compared with appropriate controls in those samples which had been preincubated with 5×10^{-7} M hydralazine, while only a very small recovery of activity (about 5%) was seen in samples containing 5×10^{-8} M. It thus appears that hydralazine is very largely an irreversible inhibitor under these circumstances. With these dialysis conditions, the inhibitory effects of benserazide, a reversible inhibitor of CRAO in vitro, can be completely removed (Lyles & Callingham 1982).

It is unclear at present whether these actions of hydralazine have any significance for its clinical use as a vasodilator agent since the physiological functions of CRAO, as well as its in vivo amine substrates, are currently unknown. An association of a similar enzyme, which has been called benzylamine oxidase, with smooth muscle in human tissues has been proposed (Ryder et al 1980). Other workers favour its localization in connective tissue (Andree & Clarke 1981). Recent evidence from subcellular fractionation studies on rat aorta and brown adipose tissue, suggests that CRAO may be a constituent of the cell plasmalemma (Wibo et al 1980; Barrand & Callingham 1982). During the current studies, Numata et al (1981) reported hydralazine to be an irreversible inhibitor in vitro (at μM concentrations) of lysyl oxidase prepared from chick aorta. However, benzylamine is not a substrate for lysyl oxidase (Shieh et al 1975), indicating that CRAO is a different enzyme, although its sensitivity towards inhibition by copper-chelating agents (Barrand & Callingham 1982) and carbonyl reagents, perhaps indicative of copper and pyridoxal phosphate as cofactors, would suggest that the properties of CRAO closely resemble those of the group of enzymes which includes benzylamine oxidase, diamine oxidase and lysyl oxidase. Our preliminary results (data not shown) also indicate *qualitatively* that hydralazine is able to inhibit the flavoprotein MAO-A and MAO-B activities in rat tissue homogenates at concentrations (10^{-5} to 10^{-3} M) much higher than those required for inhibition of CRAO, although the mode of inhibition has not yet been chracterized. Thus, hydralazine appears to inhibit a variety of different amine oxidase enzymes, with a selectivity possibly dependent upon the concentration used. Whether or not the inhibition of CRAO, or indeed other amine oxidases, may represent an important facet of this drug's pharmacological profile remains to be elucidated.

The support of the Wellcome Trust is gratefully acknowledged.

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