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INHIBITORY ACTIONS OF HYDRALAZINE UPON MONOAMINE OXIDIZING ENZYMES IN THE RAT

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Abstract—The inhibition by hydralazine of the clorgyline-resistant amine oxidase (CRAO) and monoamine oxidase (MAO) activities in various rat tissues has been studied. Hydralazine was a potent, time-dependent inhibitor of rat heart CRAO activity *in vitro*. The inhibition was not reversed by dialysis for 18 hr at 4°, and only partially reversed by dialysis at 37°. Dialysis at 4° in the presence of pyridoxal phosphate (10^{-4} M) also did not reverse the inhibition. *Ex vivo* inhibition of CRAO was found in heart and aorta homogenates in a dose-dependent manner after administration of hydralazine (1–40 mg/kg i.p.) to rats. In contrast, MAO-A activity was unaffected or, in some cases, significantly increased in these tissue homogenates from drug-treated animals. However, *in vitro* inhibition by hydralazine of both MAO-A and B activities of rat liver mitochondrial fractions was found, and these effects were fully reversible by dialysis for 18 hr at 4°. Inhibition of MAO-A was competitive (K_i of 2.5×10^{-6} M), while inhibition of MAO-B showed complex mixed non-competitive kinetics. These results indicate that hydralazine possesses different inhibitory properties towards the various amine oxidases in rat tissues, and these actions are discussed in relation to the clinical use of the drug as an anti-hypertensive agent.

It is now clear that the oxidative deamination of various monoamines can be brought about by a number of different amine oxidase enzymes in the rat and other species (see [1] for general review). The enzyme monoamine oxidase (monoamine O_2: oxidoreductase, EC 1.4.3.4, MAO), found mainly on the outer membrane of mitochondria from many animal sources, has been studied extensively and is believed to function in the control of intracellular concentrations of physiologically active amines.

Use of the selective acetylenic inhibitors clorgyline [2] and deprenyl [3] revealed that MAO exists as two enzymes forms, called MAO-A and -B, which differ in their relative sensitivities towards inhibition by these drugs. The use of these and other inhibitors to produce this binary classification has recently been reviewed [4, 5]. Furthermore, additional studies with these drugs have provided evidence that MAO-A and -B contain different electrophoretically-separable enzyme subunits, which may therefore be the molecular basis for some or all of the different biochemical and pharmacological properties of MAO-A and -B [6–8].

In addition to the MAO activities, many tissues of the rat, particularly those of the cardiovascular system, contain an amine oxidase with a substrate specificity and inhibitor profile different from that of MAO [9–11]. While awaiting a general consensus about nomenclature, we have found it convenient to call this enzyme clorgyline-resistant amine oxidase (CRAO) because of its relative insensitivity to inhibition by clorgyline at concentrations (around 10^{-4} M) which inhibit MAO-A and -B activities completely. Benzylamine is a useful substrate for assaying this enzymatic activity. The K_m (around 5 μM) for metabolism of this substrate by CRAO is much lower than the K_m values for its metabolism by MAO [10, 11]. However, in the rat at least, CRAO is also able to metabolize some other monoamines including tyramine, β-phenylethylamine and kynuramine (e.g. [9, 12–16]). CRAO is sensitive to inhibition by a number of carbonyl reagents including semicarbazide, and the lack of inhibition of MAO by this compound in particular provides a further means of differentiating these amine oxidases [9–11]. Other similar compounds possessing the 'substituted' hydrazine moiety, such as phenelzine and benserazide, are potent inhibitors of CRAO, although at higher concentrations they also inhibit MAO activities [11, 17–19].

Another therapeutically useful hydrazine-based compound is the vasodilator agent hydralazine. We recently reported this drug to be an irreversible inhibitor of CRAO in rat aorta homogenates [20]. In the present paper we have extended our observations to include the effects of hydralazine upon CRAO in rat heart homogenates, and have examined the influence of administration of the drug to rats upon *ex vivo* amine oxidase activities in the heart and aorta. The *in vitro* inhibition by hydralazine of MAO-A and -B in rat liver mitochondrial fractions has also been studied and these results are included here for comparative purposes.

MATERIALS AND METHODS

Male albino Sprague–Dawley rats weighing around 250 g were supplied by A. J. Tuck and Son (Rayleigh, U.K.). Radioactive substrates used for the assay of MAO activity were [G-^{3}H]-5-hydroxy-

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tryptamine (5-HT) creatinine sulphate and [methylene-^{14}C]benzylamine (BZ) hydrochloride from the Radiochemical Centre (Amersham, U.K.). Hydralazine hydrochloride was purchased from Sigma (London) Chemical Co. (Poole, U.K.).

Animals were weighed and then killed by cervical dislocation, followed by removal of the heart, abdominal aorta or liver. The tissue samples were blotted to remove blood, weighed and then stored at −20° until required for homogenization.

Tissue homogenates from heart and aorta were prepared in 1 mM potassium phosphate buffer, pH 7.8, as described in detail before [18]. For *in vitro* studies, pooled homogenates of hearts from 3–4 rats were prepared and subdivided into several suitable portions, which were stored frozen and then used as required. Tissues from animals treated *in vivo* with hydralazine were homogenized individually and CRAO activities of each tissue were assayed immediately after preparation of the homogenates. These were then frozen so that MAO-A activities could be measured together on the same set of thawed homogenates, usually on the following day.

Mitochondrial fractions from the livers of 3–6 rats were prepared in 0.25 M sucrose, 0.01 M potassium phosphate buffer, pH 7.8 and stored frozen in suitable aliquots as previously described [21].

Amine oxidase activities were assayed by the method of Callingham and Laverty [22] as described fully in [18]. MAO-A and MAO-B activities were measured by the use of [^{3}H]-5-HT (2 μCi/μmole) and [^{14}C]BZ (0.5 μCi/μmole), respectively, at 1 mM final concentrations unless otherwise specified. CRAO activity was assayed with [^{14}C]BZ (10 μCi/μmole) at a concentration of 1 μM (see [18]). All assay conditions were chosen to give linear metabolite production with both time and protein concentration. The method of Goa [23] was used to estimate protein concentrations of tissue homogenates, with bovine serum albumin as standard.

For *in vitro* studies involving hydralazine, drug solutions were prepared in 0.2 M potassium phosphate buffer, pH 7.8. Control assays contained a corresponding volume of drug-free buffer. For *in vivo* studies, hydralazine was dissolved in 0.9% saline (NaCl, w/v in distilled water) and rats were treated i.p. with appropriate doses given in injection volumes of 5 ml/kg. Control rats received injections of saline alone. All tissues were removed 24 hr after drug treatment.

Statistical significance of differences between mean values of control and drug-treatment groups was tested by the non-parametric rank-sum method of Wilcoxon (two-tailed analysis).

RESULTS

In vitro *inhibition of rat heart CRAO by hydralazine*

In preliminary experiments, samples of rat heart homogenates were preincubated for 20 min at 37° with hydralazine at preincubation concentrations from 2×10^{-9} to 2×10^{-5} M. Remaining CRAO activity was then assayed by the addition of [^{14}C]BZ to give a final substrate concentration of 1 μM. (Preincubation volumes were half those of the final assay volume [18]). Under these conditions, approximately 50% inhibition of enzyme activity was found at 2×10^{-8} M and complete inhibition was produced by 2×10^{-6} M hydralazine (Fig. 1).

Further studies revealed that inhibition of CRAO activity by hydralazine was partially time-dependent. In this experiment, 7.5×10^{-8} M hydralazine produced around 20% inhibition of enzyme activity when no preincubation was employed; inhibition increased to about 50% after 10 min preincubation and was unchanged thereafter up to 60 min preincubation. A higher concentration of hydralazine (10^{-6} M) resulted in complete inhibition of CRAO activity, even without preincubation being required.

The possibility that irreversible inhibition was involved was examined by dialysis. Heart samples (0.5 ml) were preincubated for 20 min at 37° with 0.5 ml solutions of hydralazine (2×10^{-7} M final preincubation concentration). Control samples contained drug-free buffer. These samples were then dialysed for 18 h against 1 l of 1 mM potassium phosphate buffer, pH 7.8, at either 4° or 25°, with changes of buffer after 2 and 4 hr of dialysis. CRAO activities in these samples were assayed after dialysis and were compared with activities in corresponding samples subjected to the preincubation step, but which had then been stored for 18 h at 4° without dialysis. These results are summarized as Experiment 1 in Table 1. Hydralazine-treated samples that were not dialysed were found to possess about 10% of the CRAO activity of non-dialysed control samples. Activities of control samples dialysed at 4° or 25° were almost identical to those of undialysed controls, showing that CRAO activity was stable under these experimental conditions. Dialysis of hydralazine-treated samples at 4° resulted in no reversal of inhibition. On the other hand, a slight recovery of enzyme activity to about 18% of control was obtained upon dialysis at 25°.

In a second experiment (Experiment 2, Table 1) the effects of (i) dialysis at 37° and (ii) dialysis at 4°

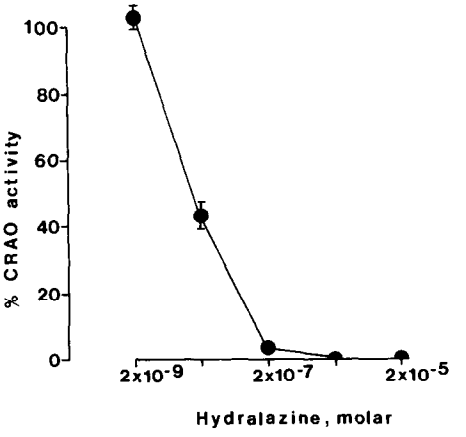

Fig. 1. Inhibition of rat heart CRAO by hydralazine. Homogenate samples were preincubated for 20 min at 37° with various drug concentrations. CRAO activities in inhibited samples were assayed with 1 μM benzylamine, and are expressed as percentages of activities in control samples. Each point is the mean (±S.E. of the ratio when exceeding symbol size) of five determinations on a pooled homogenate from three rats.

Table 1. Effects of dialysis upon inhibition of rat heart CRAO by hydralazine

Sample	CRAO specific activity (pmole/hr per mg protein)		
Experiment 1	Undial. at 4°	Dial. at 4°	Dial. at 25°
Control	470 ± 5 (100)	487 ± 3 (100)	480 ± 44 (100)
Hydralazine (2×10^{-7} M)	47 ± 3 (10)	50 (10)	86 ± 3 (18)
Experiment 2	Undial. at 4°	Dial. at 37°	Dial. at 4° vs pyridoxal phosphate
Control	479 ± 2 (100)	409 ± 21 (100)	189 ± 6 (100)
Hydralazine (2×10^{-7} M)	51 ± 5 (11)	132 ± 5 (32)	35 ± 2 (19)

Samples from the same pooled heart homogenate were preincubated for 20 min at 37° without (control) and with 2×10^{-7} M hydralazine. Mixtures were then dialysed for 18 hr at various temperatures against 1 mM potassium phosphate, pH 7.8 (in some experiments containing 10^{-4} M pyridoxal phosphate). Undialysed mixtures were kept at 4°. Specific activity of CRAO in each sample was then assayed (in triplicate) with 1 μM BZ. Results are expressed as means ± S.E. of determinations on three samples within each category. Figures in parentheses represent percentage activities of samples expressed relative to the appropriate controls subjected to the same experimental protocol.

against 1 mM potassium phosphate buffer, pH 7.8, containing 10^{-4} M pyridoxal phosphate were investigated. It was found that CRAO activity in control samples was not totally stable to dialysis at 37° and a loss of approximately 15% of the activity of undialysed controls kept at 4° was noted. On the other hand, dialysis of hydralazine-treated homogenates at 37° did result in a somewhat greater recovery of CRAO activity (to about 32% of control) than dialysis at 4° or 25° had produced.

Dialysis of control samples at 4° against pyridoxal phosphate produced a considerable reduction in activity to 40% of that of undialysed controls stored at 4°. Hydralazine-treated samples dialysed against pyridoxal phosphate also possessed lower CRAO activities than non-dialysed samples containing hydralazine. However, when expressed as percentages of the appropriate controls, the inhibition produced by hydralazine was slightly reduced after dialysis against pyridoxal phosphate.

In vivo *inhibition of rat heart and aorta CRAO by hydralazine*

CRAO activities were assayed in homogenates of hearts and aortae removed from groups of rats treated 24 hr previously with doses of hydralazine up to 40 mg/kg. These results (Fig. 2; left panel) indicated that dose-dependent inhibition of enzyme

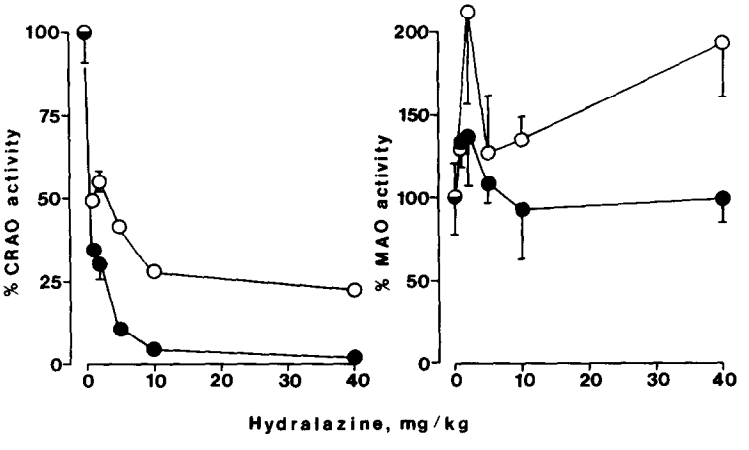

Fig. 2. *Ex vivo* inhibition of CRAO and MAO-A activities. Rats were treated i.p. with hydralazine, and hearts (○) and aortae (●) removed and homogenized 24 hr later. CRAO and MAO-A activities were assayed in tissue homogenates with benzylamine (1 μM) and 5-HT (1 mM) as substrates, respectively. Each point represents the mean ±S.E. of duplicate assays on five rats in each category, expressed as a percentage of the enzyme activity in a control group of rats. Specific enzyme activities (nmole/hr per mg protein) of control tissues were: heart: CRAO, 0.82 ± 0.02; MAO-A, 67 ± 15; aorta: CRAO, 24 ± 2; MAO-A, 25 ±6. All CRAO activities of hydralazine-treated groups were significantly ($P < 0.05$) lower than corresponding controls. Heart MAO-A activities of 2 and 40 mg/kg treated groups were significantly higher ($P < 0.05$) than controls.

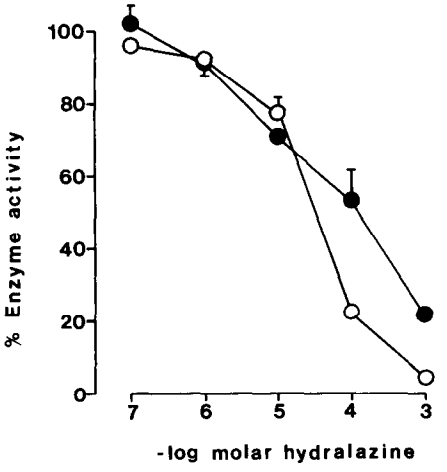

Fig. 3. Inhibition of rat liver MAO-A and -B activities by hydralazine. Rat liver mitochondrial fractions were incubated for 20 min at 37° with various drug concentrations. MAO-A (○) and MAO-B (●) activities were assayed with 1 mM 5-HT and benzylamine, respectively, and are expressed as percentages of activities in control samples. Each point is the mean (±S.E. of the ratio when exceeding symbol size) of three determinations.

activity occurred in these tissues, with mean activities in each group being significantly lower than control activities.

MAO-A activities of these tissue homogenates are also shown in Fig. 2 (right panel). No evidence for inhibition of MAO-A by hydralazine was found in these experiments. On the contrary, enzyme activities in heart homogenates from the 2 and 40 mg/kg groups were significantly higher than those of control rats.

In order to test whether or not the inhibition of CRAO in homogenates of heart and aorta from hydralazine-treated rats was reversible, the activity of 1 ml pooled samples from the 40 mg/kg treated group was compared with that of the 0 mg/kg group (i.e. controls), before and after dialysis for 20 hr at 4° against several 1 l. changes of 1 mM potassium phosphate buffer, pH 7.8. Before dialysis , CRAO activity (assayed in triplicate) of the hydralazine-treated sample was 22% (heart) and 2% (aorta) of the appropriate control sample. After dialysis, activities were 29% (heart) and 0% (aorta) of the corresponding dialysed control sample.

In vitro *inhibition of rat liver mitochondrial MAO-A and MAO-B by hydralazine*

In preliminary experiments, samples of rat liver mitochondria were preincubated for 20 min at 37° with hydralazine at concentrations between 10^{-8} and 10^{-3} M. Remaining MAO-A and MAO-B activities were then assayed by the addition of [^{3}H]-5-HT or [^{14}C]BZ at final concentrations of 1 mM, respectively. Inhibition of both enzymes occurred over the range 10^{-6} to 10^{-3} M hydralazine, with MAO-A appearing to be slightly more sensitive to inhibition than MAO-B under these conditions (Fig. 3).

In subsequent experiments it was found that inhibition of MAO-A activity (by 5×10^{-5} M hydralazine) was independent of preincubation time. On the other hand, inhibition of MAO-B by the same concentration of hydralazine increased from about 15% (no preincubation) to around 50% after 60 min preincubation. Further preincubation (up to 90 min) resulted in no additional inhibition.

In order to test for reversibility of inhibition, 0.5 ml samples of mitochondria were mixed with 0.5 ml solutions of hydralazine (in 0.2 M potassium phosphate, pH 7.8) to give preincubation drug concentrations of 2×10^{-4} and 5×10^{-4} M in the mixtures. Control samples contained no hydralazine. All samples were preincubated for 60 min at 37° and then dialysed for 20 hr at 4° against 1 l. 0.125 M sucrose, 0.105 M potassium phosphate buffer, pH 7.8, changed once after 4 hr. Non-dialysed samples were stored at 4°. MAO-A and MAO-B activities in these samples were assayed and are shown in Table 2. These results indicated that the

Table 2. Effects of dialysis upon inhibition of rat liver mitochondrial MAO-A and -B by hydralazine

	Specific activity (nmole/hr per mg protein)	
Sample	MAO-A	MAO-B
Undialysed:		
Control	367 ± 3 (100)	423 ± 2 (100)
2×10^{-4} M hyd.	48 ± 1 (13)	189 ± 5 (45)
5×10^{-4} M hyd.	11 ± 1 (3)	114 ± 2 (27)
Dialysed:		
Control	320 ± 24 (100)	389 ± 27 (100)
2×10^{-4} M hyd.	332 ± 18 (104)	372 ± 25 (96)
5×10^{-4} M hyd.	375 ± 8 (117)	412 ± 2 (106)

Mitochondrial samples were preincubated at 37° for 60 min without (control) and with hydralazine. Mixtures were then dialysed for 18 hr at 4° against 0.125 M sucrose, 0.105 M potassium phosphate buffer, pH 7.8. Undialysed samples were kept at 4°. Specific activity of MAO-A and MAO-B in each sample was then assayed (in triplicate) with 1 mM 5-HT or BZ, respectively. Results are expressed as means ± S.E. of determinations on three samples within each category. Figures in parentheses represent percentage activities of samples expressed relative to the appropriate controls subjected to the same experimental protocol.

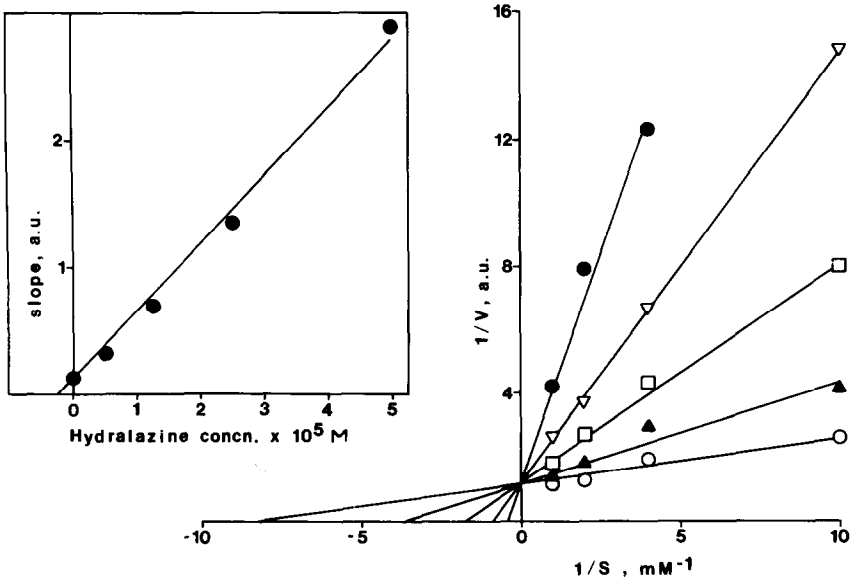

Fig. 4. Lineweaver–Burk plot showing inhibition of rat liver mitochondrial MAO-A activity by hydralazine. 5-HT was used as substrate at concentrations (S) of 0.1, 0.25, 0.5 and 1.0 mM. Reaction velocities (V) in arbitrary units (a.u.) were measured after preincubation of mitochondrial samples for 60 min at 37° in the presence of the following hydralazine concentrations: zero (○), 10^{-5} M (▲), 2.5×10^{-5} M (□), 5×10^{-5} M (▽), 10^{-4} M (●). Inset shows slope replot of the data.

inhibition of MAO-A and -B produced by these concentrations of hydralazine was completely reversed by dialysis.

The type of inhibition of MAO-A and -B produced by hydralazine was analysed in greater detail by the method of Lineweaver–Burk in which mitochondrial samples were preincubated for 60 min with various hydralazine concentrations, followed by assay of enzyme activity with 100–1000 μM of the appropriate substrate. Inhibition of MAO-A was competitive (Fig. 4) and a slope replot of this data indicated a K_i of about 2.5×10^{-6} M. Inhibition of MAO-B was rather more complex, showing mixed non-competitive kinetics, but without a common intersection point for the inhibition plots (Fig. 5). Although a representative experiment is shown in Fig. 5, this pattern was also obtained in other experiments. In addition, further studies confirmed that initial reaction velocities were being measured accurately at all substrate concentrations and assay times used here, and thus non-linear reaction conditions could not be responsible for the pattern observed.

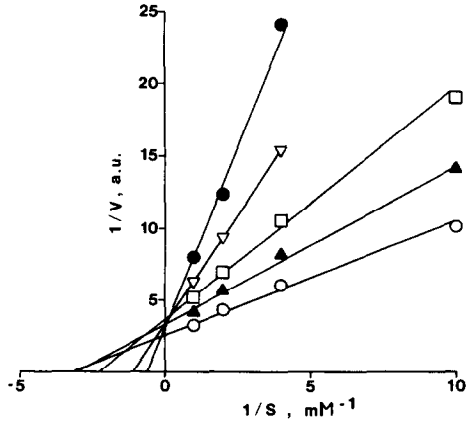

Fig. 5. Lineweaver–Burk plot showing inhibition of rat liver mitochondrial MAO-B activity by hydralazine. Benzylamine was used as substrate at concentrations (S) of 0.1, 0.25, 0.5 and 1.0 mM. Reaction velocities (V) in arbitrary units (a.u.) were measured after preincubation of mitochondrial samples for 60 min at 37° in the presence of the following hydralazine concentrations: zero (○), 5×10^{-6} M (▲), 1.25×10^{-5} M (□), 5×10^{-5} M (▽), 10^{-4} M (●).

DISCUSSION

The present results with the rat heart have confirmed and extended our previous findings with rat aorta homogenates showing that hydralazine is a potent inhibitor of CRAO [20]. The inhibition showed some dependence upon preincubation time when a low concentration (7.5×10^{-8} M) of hydralazine was employed, whereas inhibition by a higher concentration (10^{-6} M) was rapid, giving essentially complete inhibition of CRAO, even without preincubation. It would appear that these effects of hydralazine are largely irreversible since dialysis at 4° produced no detectable recovery of enzyme activity. However, in view of the findings of Andree and Clarke [19] that phenelzine also appeared irreversible as an inhibitor of CRAO in rat lung and skull after dialysis at 4°, and yet proved to be totally reversible if dialysis at 37° was employed, we carried out similar experiments here. A very small apparent reversal of inhibition occurred at 25°, and a slightly greater recovery was found at 37°. However, a considerable degree of inhibition still remained, and we failed to reverse completely the inhibitory effects of hydralazine under these conditions. Andree and Clarke [19] have suggested that a possible explanation for the reversal of phenelzine's actions at 37° may involve metabolism of the inhibitor by the

enzyme to an inactive metabolite. Although similar considerations may apply to the present studies, and irreversible covalent binding may not necessarily be involved, the interaction of hydralazine with the enzyme would appear to be of a tight-binding nature, with little or no metabolism by CRAO. It is worth remarking that we, also, have noted the general stability of CRAO in tissue homogenates subjected to prolonged dialysis at these higher temperatures, although unlike previous findings [19] we did detect some deterioration at 37°.

Further support for a tight-binding interaction between hydralazine and CRAO was provided by the fact that considerable inhibition of enzyme activity could be detected in heart and aorta homogenates prepared from the tissues of rats treated 24 hr earlier with various doses of hydralazine. In addition, little or no increase in activity was found after dialysis (at 4°) of these tissue homogenates, indicating that easily reversible interactions did not survive to any great extent. MAO-A activities were also measured in these same homogenates; no inhibition of enzyme activity was found, and in fact, significant increases in rat heart MAO-A activities were found in the 2 and 40 mg/kg groups, a result for which we can offer no obvious explanation. It should, of course, be pointed out that the inability to detect inhibition of MAO-A activity under these conditions is not proof that inhibition does not take place *in vivo*; rather that any reversible interactions which occur do not survive the period between drug treatment and killing of animals, and the subsequent preparation of dilute tissue homogenates.

In fact, *in vitro* experiments with rat liver mitochondria as a source of MAO-A and -B indicated that hydralazine can, indeed, inhibit these enzymes, with apparently some slight selectivity for MAO-A (Fig. 3). Huszti *et al.* [24] recently reported the almost complete inhibition of 5-HT deamination by 10^{-4} M hydralazine with rat hypothalamic extracts as enzyme source, whereas dopamine metabolism was less affected. In qualitative terms, our results indicated that much higher concentrations of hydralazine were required for inhibition of MAO activities than for CRAO. Inhibition of MAO-A was independent of preincubation time, whereas MAO-B required preincubation for 60 min with hydralazine (5×10^{-5} M) to reach its final level of inhibition. Under these conditions, inhibition of both MAO-A and -B could be completely reversed by dialysis for 18 hr at 4°. In addition, detailed kinetic analysis revealed a simpler linear competitive inhibition of MAO-A (K_i approximately 2.5×10^{-6} M). In contrast, the inhibition of MAO-B appeared to be a non-intersecting mixed non-competitive type of inhibition. Somewhat similar results were obtained by Hellerman and Erwin [25] who found hydralazine to be a reversible inhibitor of benzylamine and kynuramine metabolism by solubilised MAO from bovine kidney, although solely competitive kinetics (K_i of 2×10^{-5} M) were reported. In view of our more complex pattern of inhibition for MAO-B, we have not reported an apparent K_i value from our data since this will vary with the inhibitor concentration employed.

In summary, we have shown that hydralazine inhibits some amine oxidase enzymes, and that, furthermore, the characteristics of inhibition of mitochondrial MAO are different from those of CRAO. Effects on the latter enzyme are much less easily reversible and *in vivo* inhibition survives for long periods after drug administration. These different properties of hydralazine may be useful in future studies attempting to investigate the poorly understood role of CRAO in physiological function. It has been proposed that this enzyme is found on the cell plasmalemma [16, 26] as well as the microsomal fraction [16] in some rat tissues, while histochemical and biochemical studies have suggested a localization to smooth muscle of a similar enzyme, called benzylamine oxidase, found in human tissues by Lewinsohn and co-workers [27, 28]. Hydralazine is known to inhibit diamine oxidase [29] and lysyl oxidase [30], the latter enzyme being essential for connective tissue function. Although the susceptibility of these enzymes and CRAO to carbonyl-trapping reagents may indicate that they belong to the family of amine oxidases believed to contain pyridoxal phosphate as cofactor [1], nevertheless dialysis against pyridoxal phosphate in our experiments or those of Numata *et al.* [30] produced little or no reversal of inhibition by hydralazine. In fact, we found CRAO activity in control homogenates to be considerably reduced after overnight dialysis (at 4°) against 10^{-4} M pyridoxal phosphate, followed by assay in a reaction volume which reduced this concentration by one-half. In view of the fact that doubt now exists about pyridoxal phosphate being the cofactor for plasma amine oxidase [31, 32] it would appear that the situation with other amine oxidases may need reassessing.

Hydralazine is also effective as a chelator of cupric ions and this appears to be responsible for the inhibition of dopamine β-hydroxylase by the drug [33]. However, it has not yet been determined directly whether or not CRAO is a copper-containing enzyme. Although inhibition by the copper-chelating agent cuprizone has been reported for the enzyme in rat brown adipose tissue [16], recent experiments with rat aorta CRAO failed to find any inhibition by another chelator, D,L-penicillamine, used at concentrations up to 1 mM *in vitro* (G. A. Lyles, unpublished results). Thus, a possible role of copper in regulating the function and inhibition of this enzyme requires further investigation.

It is unclear whether or not inhibition of amine oxidases is of any significance for the therapeutic use of hydralazine as a vasodilator agent. It should be noted that the lower doses of hydralazine used here for administration to rats are similar to those used by other workers for producing antihypertensive effects in this species (e.g. [34, 35]), and they are also comparable with clinical doses (about 0.5–3.0 mg/kg) given orally to patients [36]. Although the vasodilating properties of hydralazine are generally ascribed to a direct effect on vascular smooth muscle, possibly by interfering with calcium mobilization for contraction [37], nevertheless it will be of interest in future studies to consider whether the effects on amine oxidases reported here may have any modifying influence on the drug's overall pharmacological activity.

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