Inhibition of Liver-Derived Monoamine Oxidase by Several Isopropyl Hydrazides

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Six aromatic hydrazide derivatives were examined for their ability to inhibit enzymatic oxidation of tyramine *in vitro* utilizing the Warburg technique. Two compounds, 1-*p*-chloro- and 1-*p*-hydroxybenzoyl-2-isopropyl hydrazide, were included in this study in order to compare their *in vitro* activities with their reported MAO inhibiting actions *in vivo*. Four of the acyl isopropyl hydrazines examined were recently synthesized substances whose biologically-relevant activities had not been previously investigated. Iproniazid served as a reference standard against which the other hydrazides were compared. Dose-dependent inhibitory effects were demonstrated for all compounds and activities were expressed in terms of interpolated ID_{50}'s, micromolar concentrations of drugs required to reduce the rate of oxygen uptake to 50 percent of the control rate. None of the substances examined exceeded iproniazid in potency although the 1-*p*-chlorobenzoyl analog proved to be equivalent to the standard in activity.

SINCE 1952 when Zeller *et al.* (1, 2) described the monoamine oxidase (MAO) inhibiting action of iproniazid, a large variety of acyl hydrazine derivatives have been fabricated and examined for their ability to suppress amine oxidase activity. On theoretical grounds, at least, there seemed to be great medicinal potential for substances having the ability to influence the metabolism of biogenic amines. The enormous interest in such substances is reflected in the comprehensively documented monograph on MAO inhibitors by Pletscher *et al.* (3) which includes 1389 references.

The isopropyl hydrazides whose *in vitro* activities are described in this report are all known in the chemical literature. Four of these compounds were originally synthesized by one of the authors (M.H.W.) but had never been screened for their ability to inhibit MAO (4). 1-*p*-Chlorobenzoyl-2-isopropyl hydrazide, a comparatively potent *in vivo* MAO inhibitor (5) and 1-*p*-hydroxybenzoyl-2-isopropylhydrazide, a comparatively weak *in vivo* MAO inhibitor (5) were included in this study in order to compare their *in vitro* activities with their reported actions *in vivo*. Iproniazid served as a reference standard inhibitor.

METHOD

Preparation of the Hydrazides—The six isopropyl hydrazides were completely dissolved in small volumes of 0.1 *N* hydrochloric acid and these stock solutions were subsequently diluted with Sorensen's 0.067 *M* phosphate buffer, pH 7.2.[1] Iproniazid, available as the phosphate salt, was immediately soluble in buffer.

Substrate—A stock solution of tyramine HCl was prepared using phosphate buffer as a vehicle.

Enzyme Preparation—Adult male albino rats were killed by cervical dislocation and their livers were quickly removed, washed with cold buffer, minced with a scissors, and homogenized with a Potter-Elvehjem tissue grinder. The homogenate, containing about 20% w/v of fresh liver, was centrifuged for 2 min. at 4000 $\times g$, the supernatant decanted and recentrifuged for 10 min. at 8500 $\times g$ in a refrigerated centrifuge. The mitochondrial pellet was resuspended in buffer so that each ml. of preparation contained the mitochondria in 300 mg. (wet weight) of liver. Monoamine oxidase activity was determined by the Warburg respirometer technique as described by Davison (6). Each reaction flask contained 1 ml. of mitochondrial preparation and 2.2 ml. of buffer. Tyramine HCl and inhibitor dissolved in 0.3 and 0.5 ml. of buffer, respectively, were each placed in side arms of the flasks. Inhibitors of varying concentrations were incubated with mitochondrial suspensions for 30 min. prior to substrate addition. Final tyramine concentration in all flasks was 0.01 *M*.

Duplicate determinations were made at each inhibitor concentration. The mean 1-hr. oxygen uptake data were expressed as percent of control (no inhibitor) and plotted against final inhibitor concentration. Lines of best fit were constructed by the method of least squares and median inhibitory doses (ID_{50}'s) were interpolated from the regression equations. Relative potencies were computed as ratios of ID_{50}'s (or alternatively, ratios of line slopes) using iproniazid as the standard.

RESULTS AND DISCUSSION

Four or five concentrations of each inhibitor provided sufficient data to reliably establish dose-effect relationships. Duplicate determinations made at each inhibitor concentration did not vary by more than $\pm$ 5% of the mean of duplicates.

The relative *in vitro* activities of the compounds are presented in Table I. With the exception of 1-*p*-chlorobenzoyl-2-isopropyl hydrazide, all of the acyl hydrazines were inferior to iproniazid in potency. Differences in the ID_{50} values for the chlorobenzoyl derivative and iproniazid were not significant and thus the compounds were judged to be approximately equivalent in terms of their influence on rat-derived MAO. Zeller *et al.* (5) reported this compound to have about 1.34 times the activity of iproniazid as determined by its *in vivo* effect on rat brain 5-hydroxytryptamine. 1-*p*-Hydroxybenzoyl-2-isopropylhydrazide had a relative *in vitro* activity about 0.6 that of iproniazid; considerably greater than its *in vivo* effect on rat brain-derived

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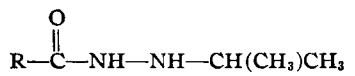
[1] In subsequent portions of this report "buffer" always refers to Sorensen's 0.067 *M* phosphate buffer, pH 7.2.

TABLE I—INHIBITION OF OXIDATIVE DEAMINATION OF TYRAMINE BY HYDRAZINE DERIVATIVES



Inhibitor	R	Slope of Regression Line[a]	$ID_{50} \pm SE$,[b] μmoles	Relative Activity
Iproniazid		−0.060	5.03 ± 0.28	1.00
1-*p*-Cl-benzoyl-2-IPH[c]		−0.054	5.59 ± 0.48	0.89[d]
1-*p*-OH-benzoyl-2-IPH		−0.038	7.95 ± 0.41	0.63
1-Benzoyl-2-IPH		−0.019	15.9 ± 1.9	0.31
1-α-Naphthylacetyl-2-IPH		−0.005	60.4 ± 2.0	0.08
1-Phenylacetyl-2-IPH		−0.003	100.7 ± 13.6	0.05
1-α-Naphthoyl-2-IPH		−0.002	151.0 ± 12.5	0.03

[a] In units of log percent (of control)/μmole of inhibitor base. The regression lines can be reconstructed by using a *y* intercept of 2.00 for all compounds. [b] Dose of inhibitor required to diminish oxygen uptake to 50% of control ± standard error. [c] Isopropylhydrazide. [d] Not significantly different from iproniazid standard.

enzyme which Zeller *et al.* (5) estimated to be about 0.11 that of iproniazid. 1-Benzoyl-2-isopropylhydrazide was approximately one-third as potent as the standard while the remaining compounds exhibited unimpressive degrees of activity.

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Keyphrases

Monoamine oxidase—liver derived

Isopropyl hydrazides—monoamine oxidase inhibition

Iproniazid—MOA inhibition standard

Tyramine HCl oxidation—isopropyl hydrazide inhibition