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GUANYLHYDRAZONES, ANOTHER CLASS OF MONOAMINE OXIDASE INHIBITORS*

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Abstract—Guanylhydrazones of several aromatic carbonyl compounds were shown to be competitive inhibitors of monoamine oxidase (MAO) from guinea pig liver mitochondria *in vitro*. They are two to ten times as active as iproniazid. The activity of this new type of MAO inhibitors is closely correlated with a system of conjugated π-bonds in the side chain.

A LARGE number of substances with different chemical structures inhibit monoamine oxidase [E C 1.4.3.4. monoamine: O_2 oxidoreductase (deaminating)] *in vitro* and some of them also *in vivo*. Monoamine oxidase itself is not a homogeneous enzyme but is supposed to consist of at least two or even a group of different enzymes (review by Pletscher *et al.*[1]). These are involved in the oxidative deamination of biogenic amines and are located—irrespective of the soluble MAO—in the mitochondria of several tissues. The different chemical structures of inhibitors like amidines, aminopyrazines, cyclopropylamines, indolalkylamines, hydrazines, propargylamines, harmala alkaloids (for review see Pletscher *et al.*,[1] Biel *et al.*,[2] Pletscher[3]), styrylquinolinium compounds (Taylor *et al.*[4]) and many others show that the active center of the enzyme (or enzymes) tolerates a wide range of modification in the inhibitor molecule.

Only little is known about the potency and mode of inhibition of hydrazones as MAO inhibitors (Pletscher *et al.*,[1] McGrath and Horita,[5] Palm *et al.*[6]). This study deals with the inhibitory action of a series of monoguanylhydrazones on MAO of liver mitochondria *in vitro*. Chemically these compounds can be considered as a combination of hydrazones and amidines. The synthesis, pharmacological and physicochemical properties of these compounds has been described earlier (Voss and Wassermann,[7] Mutschler *et al.*[8]).

EXPERIMENTAL

Mitochondria from guinea pig livers were used in this study as MAO preparation. The mitochondria were isolated according to the methods of Hawkins[9] and Davison.[10] The freeze-dried mitochondria were stored at — 20°.

Using the standard Warburg technique we determined the MAO activity by measuring the oxygen consumption during the oxidative deamination of the substrate. Due to the heterogenity of MAO (Hardegg and Heilbronn,[11] Pisano *et al.*[12]) we used in

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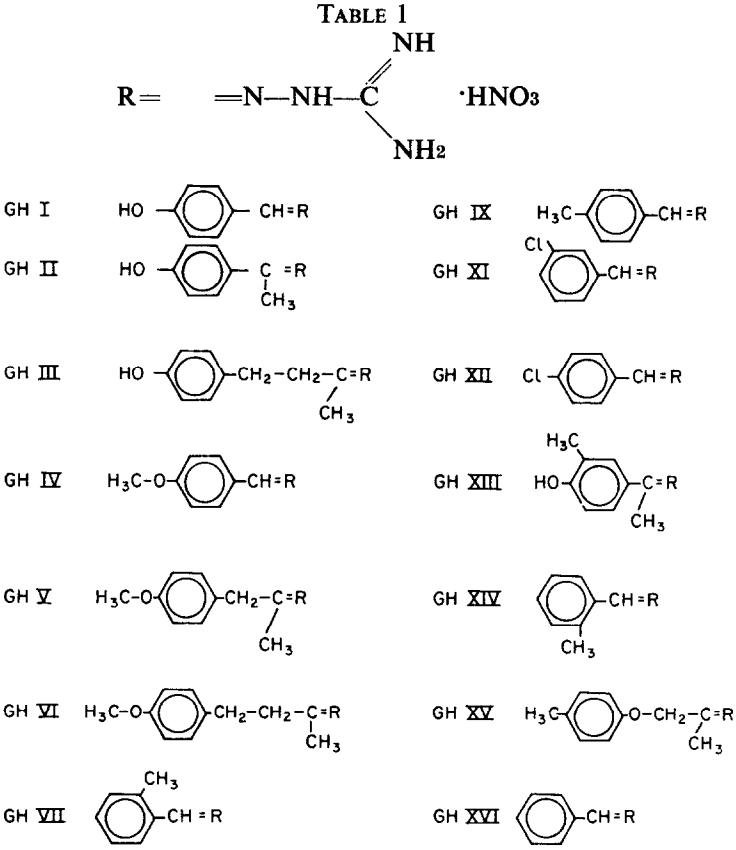
two different series of experiments either tyramine or 5-hydroxy-tryptamine (5-HT) as substrates (both from E. Merck AG, Darmstadt, Germany). The concentration of the substrates was 10^{-2}M. In experiments studying the mode of inhibition of the guanylhydrazones the concentrations were: tyramine 10^{-2} and 3×10^{-3}M; 5-HT 10^{-2} and 5×10^{-3} M. Inhibitors and substrates were dissolved in distilled water. In the main vessel of the Warburg manometer 30 mg mitochondria (= 1 g liver) were suspended in 1·6 ml Sörensen phosphate buffer, in the central cup a small strip of filter paper with 0·2 ml 10 % KOH was placed. According to Creasey[13] this enzyme preparation does not contain either aldehyde oxidase or active cytochromes, the addition of cyanide or semicarbazide is therefore not necessary. In the side bulb there were 0·2 ml substrate solution and 0·2 ml distilled water (controls) or 0·2 ml substrate solution and 0·2 ml inhibitor solution. The vessels were gassed for 30 sec with oxygen and equilibrated at 37° for 15 min. All experiments were carried out at 37°. Substrate and inhibitor were added to the enzyme preparation at the same time, and the oxygen consumption was checked every 5 min during 40 min. As a standard inhibitor we used iproniazid (Marsilid®, kindly supplied by Deutsche Hoffmann-La Roche AG, Grenzach, Germany). The fourteen guanylhydrazones studied are listed in Table 1.

TABLE 1



RESULTS

Figures 1a and 1b show some of the concentration–inhibition curves obtained with guanylhydrazones and iproniazid using tyramine and 5-HT as substrates. The I_{50}-

values (molar inhibitior concentrations, which depress the enzyme activity to 50 %) and the inhibitor constants (K_i) of the compounds studied are summarized in Table 2. Additionally GH XIV, the compound with the partially saturated ring and—as a control—aminoguanidine were checked. Both substances did not possess any inhibitory activity on MAO of guinea pig liver mitochondria.

As it is shown by the Figs. 1a and 1b and Table 2 most of the guanylhydrazones are two to ten times as active as iproniazid. The common feature of these active compounds is the short guanylhydrazone-side chain. The different substituents at the aromatic ring give rise to further quantitative differences in inhibitory activity. In the less active members of this series (GH I, III, V, VI, XIII, XV) either the guanylhydrazone group R is separated by methylene, *O*-methylene or ethylene groups from the benzene ring, or the introduction of a phenolic hydroxy group into the aromatic ring decreases the MAO inhibiting activity.

In order to obtain information about the mode of inhibition of these substances we plotted according to Dixon[16] the reciprocal of reaction velocity (1/v) against the inhibitor concentration [I] at two different substrate concentrations S_1 and S_2. Figure 2 demonstrates—as an example—the mode of inhibition of the reaction of MAO and tyramine by GH IX. The inhibitor constant is $K_i = 9 \times 10^{-5}$M, the inhibition is competitive. The K_i-values of the guanylhydrazones and iproniazid and the Michaelis–Menten constants for tyramine and 5-HT are given in Table 2. All these compounds are competitive inhibitors of MAO (except for GH XIV and aminoguanidine), using tyramine or 5-HT as substrates.

DISCUSSION

Guanylhydrazones of aromatic or aromatic-aliphatic carbonyl compounds prove to be competitive inhibitors of MAO from guinea pig liver mitochondria. Originally related to hydrazine this new type of MAO inhibitors does not possess a hydrogen atom at the nitrogen atom of the hydrazine group carrying the aralkyl radicals, as it should be necessary for MAO inhibitors of the hydrazine type according to Zeller *et al.*[17]

Palm *et al.*[6] report MAO inhibiting properties of furazolidone, a hydrazone derivative, and suggest a metabolic transformation from the inactive hydrazone into the active hydrazine. The immediate onset of the MAO inhibition by guanylhydrazones in addition to thin-layer chromatographic studies about a possible metabolic transformation *in vitro* makes it unlikely that these compounds first have to be activated metabolically in order to become inhibitors.

Under the experimental conditions we used both tyramine and 5-HT possess nearly the same Michaelis–Menten constant K_m (tyramine 4×10^{-3}M, 5-HT $3{\cdot}2 \times 10^{-3}$M), even if 5-HT shows a slightly —but not significant—higher affinity towards the active center(s) of the enzyme preparation. There is also practically no difference between the inhibitor constants K_i (Table 2) of the guanylhydrazones obtained with different substrates.

The inhibiting activity of this series shows considerable differences between the single compounds (Table 2). In general the compounds GH II, IV, VII, IX, XI, XII, and XVI are 2–10 times as active as iproniazid, which was used as a standard. Obviously necessary for the reaction with the enzyme is the aromatic nucleus—an already well-known fact for the majority of pharmacologically active drugs. Hydrogenation

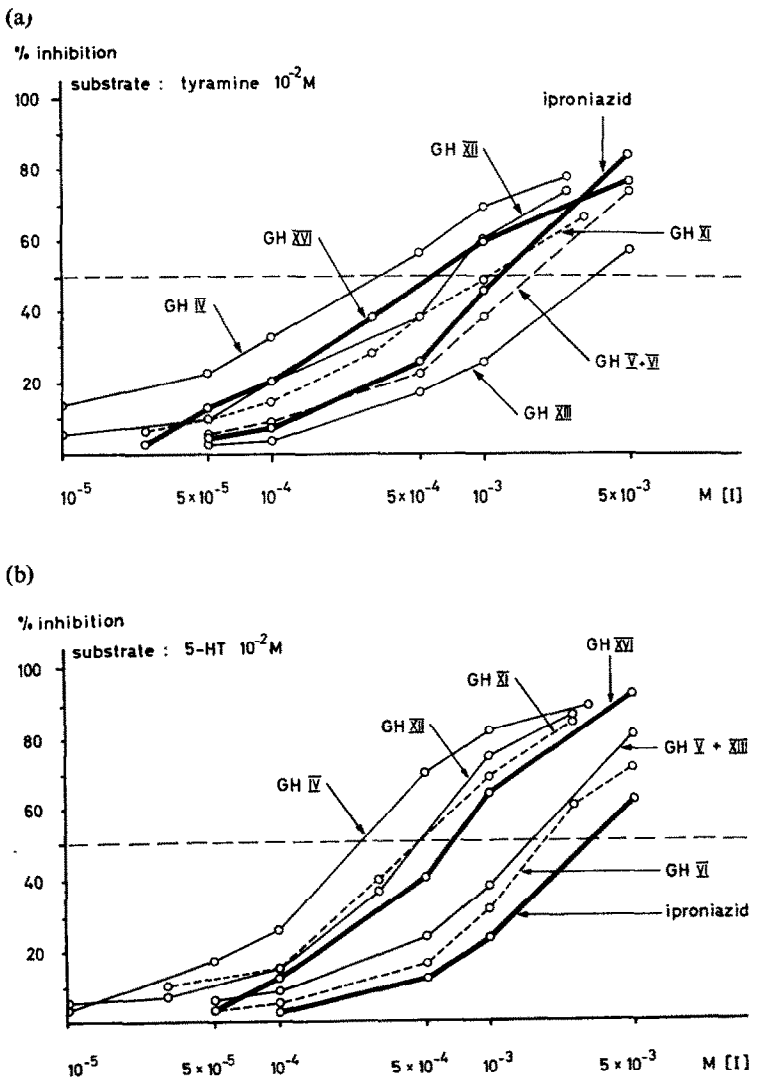


FIG. 1a and 1b. Concentration–inhibition curves of some guanylhydrazones and iproniazid using tyramine (10^{-2}M, above) or 5-HT (10^{-2}M, below) as substrates. Ordinate: % inhibition; abscissa: inhibitor concentration (M). The standard error was calculated for each point and is in all cases < 5 % ($n = 6$–10).

TABLE 2. CONCENTRATIONS FOR 50 PER CENT INHIBITION (I_{50}) AND INHIBITOR CONSTANTS* (K_i) OF THE GUANYLHYDRAZONES STUDIED AND IPRONIAZID USING TYRAMINE AND 5-HT AS SUBSTRATES

Inhibitor		Tyramine		5-HT	
		I_{50}	K_i	I_{50}	K_i
GH	I	16	4·6	16	3·9
	II	9·0	2·6	3·0	0·7
	III	16	4·6	15	3·6
	IV	3·5	1·0	2·5	0·6
	V	17	4·8	17	4·1
	VI	17	4·8	17	4·1
	VII	4·0	1·1	2·0	0·5
	IX	3·0	0·9	4·0	1·0
	XI	10	2·9	5·0	1·2
	XII	6·0	1·7	4·5	1·1
	XIII	35	10·0	17	4·1
	XIV	—	—	—	—
	XV	12	3·4	12	2·9
	XVI	6·5	1·9	6·5	1·6
aminoguanidine		—	—	—	—
iproniazid		12	3·4	30	7·3

*K_i obtained according to Massart[14] and Kolbezen *et al.*[15]: $K_i = (I_{50} \times K_m) / (K_m + S)$, where K_m is the Michaelis–Menten constant of the substrate and S the substrate concentration.

(All values $\times 10^{-4}$M, *KN* tyramine = 4×10^{-3}M, K_m 5-HT = $3{\cdot}2 \times 10^{-3}$M.)

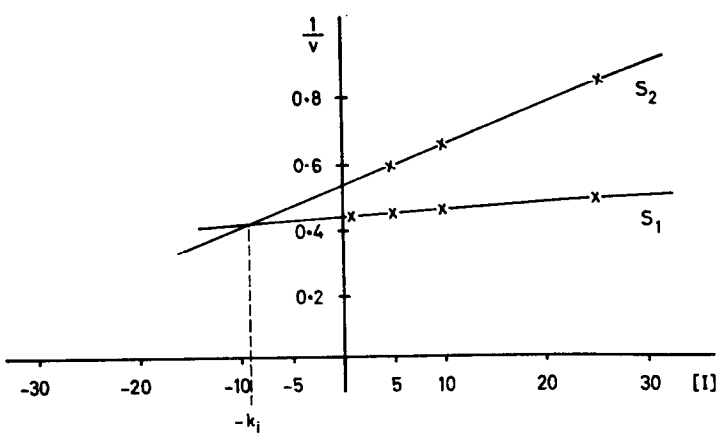
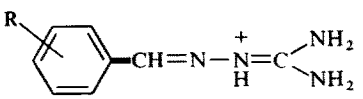


FIG. 2. Reaction of MAO and tyramine, inhibited by GH IX. Ordinate: 1/v; abscissa: inhibitor concentration [I] $\times 10^{-5}$M; K_i GH IX : 9×10^{-5}M.

of the ring (GH XIV) destroys the affinity for the enzyme as much as the total absence of the ring (aminoguanidine). Compared with the unsubstituted derivative XVI the substituents CH_3-, CH_3-O- or Cl- at the aromatic ring increase the activity. Introduction of the phenolic hydroxy group, however, reduces the activity. This group is around (or less than) 10 % ionized in these compounds at physiological pH.[8] In spite of the fact, that most of the natural substrates of MAO possess one or two phenolic OH groups, no potent MAO inhibitor is known with phenolic hydroxy groups. This substituent decreases the affinity of the inhibitor molecule towards the enzyme MAO. An exception to this rule is compound GH II. In comparison to the other derivatives (GH IV, VII, IX, XII, XVI), however, this substance possesses one methyl group in the side chain. This substituent seems to increase the MAO inhibiting activity, because the unsubstituted derivative GH I belongs to the less active members of this series. An explanation for this phenomenon could be, that as a result of the + I-effect of the methyl group the electron density in the side chain is increased, a fact, which is confirmed by an increase of basicity (GH I $pK_2 = 9{\cdot}77$, GH II $pK_2 = 10{\cdot}12$, Mutschler *et al.*[8] An additional methyl group at the aromatic ring in the vicinity of the OH group (GH XIII) gives rise to a considerable loss of inhibitory activity.

One important finding in these experiments is, that MAO inhibiting potency proved closely correlated to a short side chain. In the most active compounds the guanylhydrazone group is attached to the aromatic ring only over one (the former carbonyl) carbon atom. In this structure the + M-effect of the phenyl ring causes a system of conjugated double bonds, as already described earlier:[8]



The positive inductive (+ I) effect of a methyl group in the side chain enhances electron density and biological activity of these compounds.

At our experimental conditions the interruption of this system of conjugated π-bonds by oxymethylene, one or more methylene groups (GH XV, V, VI) gives rise to a remarkable decrease of inhibitory activity. This finding strongly supports the suggested (Belleau and Moran[18]) necessity of a double bond for example between α- and β-carbon of substrates or on corresponding sites in inhibitor molecules for an interaction between substrate (or inhibitor) and MAO. Further evidence for this possibility comes from observations of Taylor *et al.*[4] with styryl-quinolinium compounds as inhibitors of MAO.

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