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# THE EFFECT OF DIFFERENT SUBSTRATES ON THE INHIBITION OF RAT BRAIN AND LIVER MONOAMINE OXIDASE BY ARYLALKYLHYDRAZINES

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**Abstract**—The structure-dependent action of arylalkylhydrazines on brain and liver MAO using benzylamine, tyramine, dopamine, tryptamine and serotonin as substrates was studied.

For all the substrates used the inhibitory power of unsubstituted arylalkylhydrazines with an alkyl chain ranging from —$CH_2$— to (—$CH_2$—)$_5$ decreased with increasing carbon chain length up to four methylene groups. The substance with five carbon alkyl chain exerted approximately the same action as the compound with three carbon alkyl chain.

All analogues of these substances methylated on the carbon adjacent to the hydrazine group showed potent inhibitory properties.

The deamination of dopamine, serotonin and tyramine was affected more strongly by the majority of MAO inhibitors, than that of benzylamine and tryptamine.

WE RECENTLY observed that under certain conditions MAO* inhibitors of the arylalkylhydrazine and arylalkylamine class exerted a different action (alone or in combination with reserpine and dopa) on the content in the brain of serotonin, noradrenaline and dopamine,[1–3] from that on the content in the brain of tryptamine using the tryptamine convulsion test[4] and on the brain $\gamma$-aminobutyric acid system.[5–8]

In order to throw some light on the structure-related differences in the MAO inhibiting properties of arylalkylhydrazines a number of unsubstituted and substituted compounds with one carbon to five carbon alkyl chains were synthesized and tested in their effect on MAO preparations of rat brain and liver, using benzylamine, tyramine, dopamine, tryptamine and serotonin as substrates.

## EXPERIMENTAL

All the work was done with male rats of Wistar origin (100–160 g) from our breeding stock. The animals were provided with Küstner's standard pellets and water *ad lib.*

Phenelzine sulfate and *N*-methyl-*N*-benzyl-propinylamine hydrochloride (pargyline) was kindly supplied by VEB Arzneimittelwerk Dresden. Iproniazid phosphate (Marsilid) and nialamide were generously donated by Deutsche Hoffmann-LaRoche A.G., Grenzach, Baden and Charles Pfizer Research Laboratories, Croton, Conn. respectively. The remaining MAO inhibitors were synthesized by Dr. E. Jassmann,

* Abbreviations used: MAO = monoamine:$O_2$ oxidoreductase (deaminating), (EC 1.4.3.4).

VEB Fahlberg-List, Magdeburg. All compounds are given as the salt forms. The rats were injected intraperitoneally with MAO inhibitors dissolved in 0·5 ml/100 g body wt. of saline. Saline injections of the same volume were administered to the corresponding daily control animals.

The MAO assay procedure was followed by measuring the optical density of aldehyde semicarbazone formed from the corresponding amine.[9] In the present series 25% homogenate of brain and liver tissue in 0·25 M-sucrose were used. The assay mixture contained in a total volume of 3·0 ml, 180 μmoles of sodium phosphate buffer (pH 7·4), 25 μmoles semicarbazide hydrochloride and 0·2 ml enzyme preparation (approximately 50 mg tissue, e.g. 4–5 mg protein). This mixture was incubated for 5 min at 37° and the reaction was started by the addition of 0·5 ml of the corresponding amine solution (15 μmoles benzylamine hydrochloride, 3 μmoles tyramine, 9 μmoles dopamine hydrochloride, 3 μmoles tryptamine hydrochloride and 3 μmoles serotonin creatinine-sulfate respectively). The tissue blanks were incubated without substrates; the corresponding amine solution was added at the end of incubation. The reaction was stopped after 20 min incubation at 37° by addition of 1·0 ml of 0·6 N perchloric acid. The optical density of the corresponding aldehyde semicarbazone formed were measured in a Beckman DK-2 Ratio Recording Spectrophotometer against the tissue blank with the same substrate (in the assay with benzylamine as substrate at 278 mμ, the remaining semicarbazones in the wavelength range from 240 to 250 mμ). The enzyme activity was expressed as mμmoles aldehyde semicarbazone formed per milligram protein per 20 min at 37°.

The determination of protein content was carried out by the method of Lowry *et al.*[10] with bovine serum albumin as standard.

## RESULTS

Table 1 shows the mean values of protein content as well as the MAO activities in controls 2 hr after intraperitoneal administration of saline solution.

The MAO inhibitors under investigation were tested usually 2 hr after intraperitoneal administration at a dose of between 2 and 600 μmoles/kg body wt. (phenylbutylhydrazine and iproniazid up to 2000 μmoles/kg). The percent inhibition against the corresponding daily saline controls was plotted against the logarithm of the dose using at least four animals per point. Because of differences in the slope of the curves 80 per cent ($ID_{80}$) and 50 per cent ($ID_{50}$) inhibition was recorded in Tables 2 and 3.

## DISCUSSION

Table 2 shows that for all substrates used, the inhibitory activity ($ID_{80}$ as well as $ID_{50}$) of arylalkylhydrazines without substitution of the alkyl chain decreased with increasing the carbon chain length up to four carbons. The substance with five carbon chain exerted approximately the same action as the compound with three carbon chain (see Fig. 1 with tyramine as substrate). Qualitatively the same picture was seen with the other four substrates studied. In contrast to the unsubstituted compounds, the methylated analogues showed no striking dependence on structure. The present findings agreed with previous investigations using the manometrique technique[3,11] on arylalkylhydrazines with one- to three-carbon alkyl chain. In the present experiments MAO activity was measured by aldehyde semicarbazone production because this is more sensitive.

TABLE 1. PROTEIN CONTENT AND MONOAMINE OXIDASE ACTIVITY IN BRAIN AND LIVER HOMOGENATES OF 80 CONTROL RATS (ADMINISTRATION OF SALINE SOLUTION)

| Material | Protein content (mg/g tissue) | Monoamine oxidase activity | | | | |
|---|---|---|---|---|---|---|
| | | Benzylamine | Tyramine | Dopamine | Tryptamine | Serotonin |
| Brain homogenate | 85·00 ± 0·14 | 4·87 ± 0·15 | 10·31 ± 0·42 | 15·85 ± 0·33 | 5·97 ± 0·36 | 5·43 ± 0·21 |
| Liver homogenate | 103·80 ± 0·13 | 10·10 ± 0·26 | 13·21 ± 0·54 | 15·85 ± 0·54 | 8·92 ± 0·33 | 6·86 ± 0·25 |

Enzyme activity is expressed as millimicromoles of aldehyde semicarbazone formed from corresponding amine per milligram protein per 20 min ± S.E.M.

TABLE 2. INHIBITION OF MONOAMINE OXIDASE ACTIVITY BY ARYLALKYLHYDRAZINES 2 hr AFTER INTRAPERITONEAL ADMINISTRATION OF DRUGS

| Compound no. | Drugs | Benzylamine | | | | Tyramine | | | | Dopamine | | | | Tryptamine | | | | Serotonin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | |
| | | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ |
| *Unsubstituted compounds* | | | | | | | | | | | | | | | | | | | | | |
| 1 | $C_6H_5$—$CH_2$—$NHNH_2$·HCl (Benzylhydrazine) | 11 | 2 | 22 | 6 | 21 | 4 | 50 | 14 | 13 | 3 | 40 | 12 | 30 | 8 | 70 | 12 | 20 | 3 | 36 | 7 |
| 2 | $C_6H_5$—$(CH_2)_2$—$NHNH_2$·Sulf. (Phenetzine) | 65 | 7 | 85 | 16 | 40 | 16 | 65 | 28 | 35 | 8 | 130 | 65 | 80 | 16 | 340 | 75 | 23 | 8 | 90 | 30 |
| 3 | $C_6H_5$—$(CH_2)_3$—$NHNH_2$·Oxal. | 400 | 100 | 280 | 120 | 240 | 110 | 220 | 90 | 75 | 55 | 140 | 75 | 260 | 100 | 260 | 125 | 140 | 85 | 155 | 90 |
| 4 | $C_6H_5$—$(CH_2)_4$—$NHNH_2$·Oxal. | 850 | 270 | 360 | 100 | 420 | 160 | 300 | 35 | 140 | 80 | 180 | 36 | 850 | 160 | 900 | 170 | 240 | 120 | 130 | 45 |
| 5 | $C_6H_5$—$(CH_2)_5$—$NHNH_2$·Oxal. | 230 | 90 | 155 | 65 | 220 | 100 | 140 | 28 | 180 | 50 | 195 | 40 | 220 | 100 | 650 | 115 | 200 | 100 | 210 | 50 |
| *Substituted compounds* | | | | | | | | | | | | | | | | | | | | | |
| 6 | $C_6H_5$—CH·$CH_3$—$NHNH_2$·Oxal. | 20 | 7 | 26 | 9 | 30 | 9 | 50 | 10 | 20 | 5 | 26 | 8 | 65 | 28 | 140 | 36 | 26 | 8 | 35 | 16 |
| 7 | $C_6H_5$—$CH_2$—CH·$CH_2$—$NHNH_2$·HCl (Phenylisopropylhydrazine, PIH) | 13 | 6 | 24 | 7 | 16 | 7 | 11 | 3 | 9 | 4 | 21 | 7 | 17 | 6 | 60 | 17 | 8 | 3 | 19 | 11 |
| 8 | $C_6H_5$—$(CH_2)_2$—CH·$CH_3$—$NHNH_2$·Oxal. | 8 | 2 | 10 | 5 | 12 | 6 | 7 | 4 | 6 | 2 | 9 | 4 | 13 | 4 | 19 | 6 | 10 | 4 | 20 | 8 |
| 9 | $C_6H_5$—$(CH_2)_3$—CH·$CH_3$—$NHNH_2$·Oxal. | 40 | 14 | 45 | 17 | 75 | 15 | 38 | 10 | 30 | 6 | 80 | 17 | 90 | 28 | 200 | 24 | 60 | 22 | 140 | 48 |
| 10 | $C_6H_5$—$(CH_2)_4$—CH·$CH_3$—$NHNH_2$·Oxal. | 30 | 11 | 45 | 14 | 38 | 15 | 45 | 28 | 22 | 8 | 23 | 7 | 70 | 45 | 140 | 55 | 6 | 22 | 60 | 24 |

Inhibitory doses (80 and 50 per cent inhibition plotted against saline controls) are expressed as micromoles per kilogram body weight.

TABLE 3. INHIBITION OF MONOAMINE OXIDASE ACTIVITY 2 AND 16 hr AFTER INTRAPERITONEAL ADMINISTRATION OF DRUGS

| Compound No. | Drugs | Benzylamine | | | | Tyramine | | | | Dopamine | | | | Tryptamine | | | | Serotonin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | | Brain | | Liver | |
| | | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ | $ID_{80}$ | $ID_{50}$ |
| *Treatment* 2 hr | | | | | | | | | | | | | | | | | | | | | |
| 11 | Tranylcypromin-Sulf. | 18 | 8 | 12 | 5 | 12 | 4 | 9 | 4 | 16 | 6 | 10 | 3 | 7 | 2 | 9 | 4 | 9 | 1 | 8 | 5 |
| 12 | Pargyline-HCl | 100 | 15 | 320 | 90 | 45 | 8 | 75 | 25 | 75 | 20 | 70 | 15 | 110 | 32 | 230 | 90 | 80 | 36 | 250 | 75 |
| 13 | Nialamide | 670 | 300 | 500 | 300 | 500 | 300 | 500 | 300 | 550 | 300 | 600 | 360 | 450 | 260 | 300 | 140 | 410 | 250 | 250 | 140 |
| 14 | Iproniazid-Phosphate | 1250 | 600 | 1000 | 650 | 1000 | 700 | 900 | 570 | 850 | 500 | 550 | 360 | 1800 | 550 | 1000 | 500 | 850 | 550 | 700 | 400 |
| *Treatment* 16 hr | | | | | | | | | | | | | | | | | | | | | |
| 12 | Pargyline-HCl | 40 | 3 | 170 | 40 | 28 | 2 | 130 | 20 | 26 | 2 | 140 | 22 | 65 | 7 | 140 | 24 | 40 | 7 | 340 | 33 |
| 13 | Nialamide | 440 | 190 | 420 | 190 | 280 | 110 | 270 | 85 | 220 | 75 | 280 | 90 | 250 | 110 | 240 | 80 | 170 | 45 | 220 | 65 |
| 14 | Iproniazid-Phosphate | 500 | 130 | 1200 | 200 | 550 | 190 | 800 | 160 | 550 | 190 | 800 | 110 | 700 | 250 | 1200 | 150 | 500 | 160 | 900 | 160 |

Inhibitory doses (80 and 50 per cent inhibition plotted against saline controls) are expressed as micromoles per kilogram body weight.

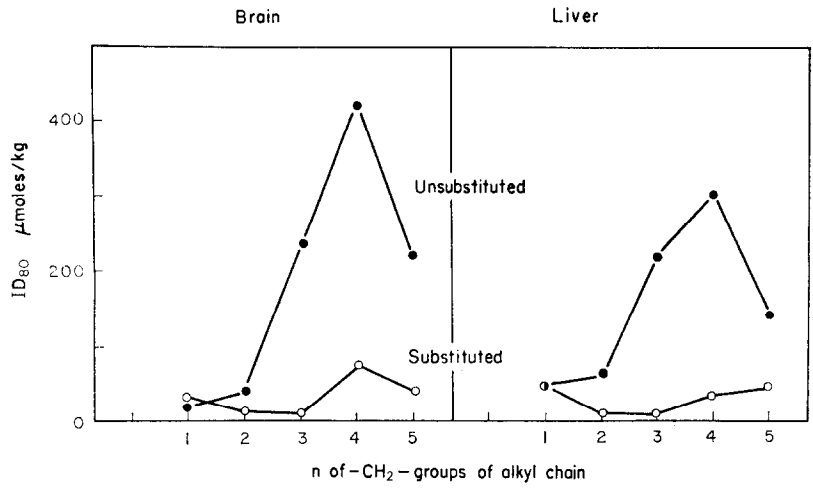


FIG. 1. Comparison of MAO inhibiting potency of unsubstituted (●—●) and substituted (○—○) arylalkylhydrazines in dependence on the carbon number of the alkyl chain. MAO activity with tyramine as substrate. The structure formulas are given in Table 2.

A possible explanation of the structure-dependent activity of MAO inhibitors might be provided by the physico-chemical considerations, mentioned by Bloom[12] and Belleau and Moran.[13]

Some workers found that the activity of MAO inhibitors depended on the substrate used. Thus, in experiments with MAO preparations from rat liver Gorkin and coworkers[14–16] found that harmine inhibits both *in vitro* and *in vivo* deamination of serotonin more than tyramine. In an earlier paper[17] we also reported that 15 mg/kg harmine hydrochloride caused a stronger inhibition of rat brain MAO with serotonin and noradrenaline as substrates than with dopamine and tyramine as substrates.

Gorkin *et al.*[18] found that pargyline produced *in vitro* and *in vivo* a much pronounced inhibition of oxidative deamination of tyramine than of serotonin in liver and brain tissue. Phenelzine, under similar conditions, showed a stronger inhibition of deamination of serotonin than of tyramine in the liver; no significant differences could be detected in the degree of inhibition of tyramine and serotonin deamination by phenelzine in brain tissue. Nialamide showed *in vitro* more pronounced inhibition of deamination of serotonin than of tyramine.[19] On the contrary, iproniazid, tranylcypromine and phenylisopropylhydrazine inhibited MAO activity independently to the substrates used.[19]

In the present series it has been found that the deamination of dopamine was inhibited to the same degree by arylalkylhydrazines as the deamination of serotonin. The 2-hr-treatment was chosen because most of arylalkylhydrazines and arylalkylamines exerted their MAO inhibiting action during the first hour after application and the inhibition continued unchanged for at least 24 hr.[11] Pargyline, nialamide and iproniazide, however, developed maximum inhibition only several hours after administration. As can be seen in Table 3, 2 hr after administration pargyline inhibited deamination of tyramine more than dopamine and serotonin, but at 16 hr after treatment the deamination of all substrates was strongly inhibited. Nialamide

inhibited deamination of serotonin in brain and liver preparations already at 2 hr after treatment. In all experiments the inhibition of deamination in brain and liver was similar.

Physiologically occurring amines (dopamine, serotonin, tyramine) as well as tryptamine and benzylamine were good substrates for MAO both in brain and liver homogenates. However, the strongest inhibition of deamination was found with dopamine and serotonin as substrates. Substituted arylalkylhydrazines exerted inhibition of deamination of all substrates without essential differences among substrates, while unsubstituted arylalkylhydrazines caused a less pronounced inhibition of deamination of benzylamine and tryptamine.

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