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# 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMINE: A NEW POTENT INHIBITOR OF HISTIDINE DECARBOXYLASE*

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**Abstract**—Histidine decarboxylase inhibiting and histamine lowering effects of 2-hydroxy-5-carbomethoxybenzyloxyamine have been studied in detail. The compound proved to be a potent inhibitor of both rat stomach pyloric histidine decarboxylase and guinea-pig liver aromatic L-amino acid decarboxylase, showing a more pronounced effect on the former enzyme. The type of inhibitions were ascribed as reversible and competitive with respect to substrate and coenzyme in both cases. The $K_i$ values for histidine and pyridoxal phosphate were $1 \times 10^{-7}$ and $2{\cdot}5 \times 10^{-7}$ M; for 5-hydroxytryptamine and pyridoxal phosphate, $1 \times 10^{-6}$ and $5 \times 10^{-6}$ M. Studies with the pyridoxal phosphate oxim of this substance showed no remarkable inhibition of the nonspecific enzyme, and substantially less effect on specific decarboxylase than that obtained with 2-hydroxy-5-carbomethoxybenzyloxyamine itself. These results suggested that the inhibiting effect of the compound is partly directly on the coenzyme, forming a pyridoxal phosphate inactivator complex, but mainly on the apoenzyme, displacing pyridoxal phosphate from the apoenzyme. The fact that the inhibitory activity of the compound mainly depended on the relative strength of binding of pyridoxal phosphate to apoenzyme, showing substantially less effect on aromatic L-amino acid decarboxylase and on diamine oxidase than that on histidine decarboxylase, confirmed this assumption.

Administration of the inhibitor to male rats resulted in lower levels of histamine in stomach, lungs, heart and skin. The minimum effective dose of the compound was 15 mg/kg; maximal effects were obtained with doses of 45 mg/kg in lungs, heart and skin and 135 mg/kg in stomach. The maximal effects were observed 1–2 hr after the treatment and were significantly less at 5 hr. Repeated administration resulted in lower histamine levels than after single treatments. No toxic effects were apparent during repeated and prolonged administrations of the inhibitor.

THE DECARBOXYLASE inhibiting properties of meta and para substituted benzyloxyamines and pyridylmethoxyamines have been studied in detail.[1] Further investigations were carried out with *ortho* substituted benzyloxyamine derivatives, and 2-hydroxy-5-carbomethoxybenzyloxyamine was selected as the most potent and specific inhibitor in this series.

Histidine decarboxylase inhibiting properties and histamine lowering effects of this compound are presented in this paper.

## MATERIALS AND METHODS

2-Hydroxy-5-carbomethoxybenzyloxyamine was used as the hydrochloride or hydrobromide salt and was dissolved in water immediately before use. The synthesis

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Abbreviations used: HD, histidine decarboxylase; CD, aromatic L-amino acid decarboxylase; DAO, diamine oxidase; MAO, monoamine oxidase.

of the compound was described previously.[2] The pyridoxal phosphate oxim of the inhibitor was obtained by the method of Leinweber,[3] and proved to be homogeneous in analytical measurements. The sources of other chemicals, used in experiments, were previously described.[1]

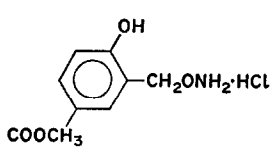
For *in vitro* inhibition studies, histidine decarboxylase was obtained from rat stomach (pyrolus) according to Hakanson and Owman[4] and diamine oxidase was prepared from hog kidney, using the method of Okuyama and Kobayashi.[5] Guinea-pig liver homogenate was used as enzyme source for aromatic L-amino acid decarboxylase measurements. The fundic histidine decarboxylase of rat stomach was obtained according to Leinweber and Braun.[6]

Aromatic L-amino acid decarboxylase activity was measured with 5-hydroxytryptophan as substrate, at pH 8·1, using the method of Udenfriend *et al.*[7] Histidine decarboxylase inhibition studies were made according to Hakanson and Owman,[4] using L-histidine as substrate and with pyloric of fundic histidine decarboxylase from rat stomach, as enzyme source. Histamine formed during enzymatic decarboxylation was measured using the method of Shore *et al.*[8] Diamine oxidase activity and monoamine oxidase activity measurements were carried out with putrescine and 5-hydroxytryptamine as substrates and with partially purified hog kidney DAO and rat liver homogenate as enzyme sources according to Holmstedt and Tham,[9] and to Udenfriend *et al.*[10] respectively. Protein was determined using the method of Palladin,[11] and tissue histamine levels, by the method of Shore *et al.*[8]

For *in vivo* studies male, Sprague–Dawley rats, (170–180 g), and male guinea-pigs (300–350 g) were used. Animals were fed *ad lib.* until 12 hr before treatment. During this period animals received only water. Tissues removed immediately after the animals were decapitated were washed with physiological saline and then placed on ice for less than 15 min before being used for assay.

## RESULTS

*Chemical structure of the inhibitor.* Decarboxylase inhibiting studies were carried out with the compound: 2-hydroxy-5-carbomethoxybenzyloxyamine, obtained as hydrochloride, hydrobromide or tartrate salts. The compound showed the highest solubility as the hydrochloride and, therefore, in most of the experiments, this salt was used. Its chemical structure is shown in Fig. 1.




2-hydroxy-5-carbomethoxybenzyloxyamine · hydrochloride

FIG. 1. Chemical structure of the inhibitor.

*Histidine dicarboxylase, aromatic* L-*amino acid decarboxylase, diamine oxidase and monoamine oxidase inhibiting effects.* Preliminary studies were carried out using the compound, at $10^{-3}$, $10^{-4}$ and $10^{-5}$ M final concentrations. Inhibitor was incubated for 15 min without the addition of substrate, and subsequently for 30, 60 or 120 min

in the presence of substrate. Substrates were added in the following final concentrations to mixtures: L-histidine $3 \times 10^{-3}$ M; DL-5-hydroxytryptophan $5 \times 10^{-3}$ M; putrescine $5 \times 10^{-4}$ M; 5-hydroxytryptamine $1 \times 10^{-3}$ M; and enzyme activity was measured as described in "Methods". The compound inhibited rat stomach histidine decarboxylase completely, and guinea-pig liver decarboxylase by 12 per cent at $10^{-5}$ M concn. With $10^{-4}$ M concn there was 100 per cent inhibition of guinea-pig liver decarboxylase and about 40 per cent inhibition of hog kidney diamine oxidase and of rat liver monoamine oxidase. The $I_{50}$ values for the latter two enzymes were $5 \times 10^{-4}$ M and $10^{-4}$ M.

Further experiments were carried out on the two kinds of histidine decarboxylases obtained from the pylorus and fundus of rat stomach.[6] The inhibitor, $3{\cdot}3 \times 10^{-5}$ M final concn, was incubated with the reaction mixtures for 15 min before and for 120 min after the addition of the substrate at pH 6·8 and 5·5, according to Leinweber and Braun.[6] Complete inhibition of the former, and 36 per cent inhibition of the latter enzyme was observed showing significantly less affinity for fundic histidine decarboxylase.

*Kinetics of inhibitions of histidine- and aromatic* L-*amino acid decarboxylase.* The effect of the inhibitor on histidine and aromatic L-amino acid decarboxylases were studied in more detail to obtain information on the kinetics of inhibition. In these experiments a wide range of concentration of substrate and pyridoxal phosphate was used, and inhibitor was added to the mixture to give a final concentration of $10^{-5}$–$10^{-6}$ M. Results, obtained from these experiments, were plotted according to Reiner,[12] and to Lineweaver and Burk,[13] and shown in Fig. 2 and in Figs. 3–6.

The Reiner diagram suggested reversible inhibition in the case of histidine decarboxylase and the Lineweaver–Burk plots showed competitive character of inhibition

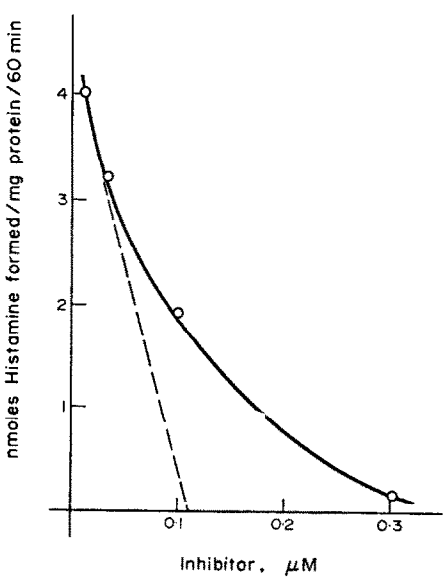



FIG. 2. Reiner diagram of histidine decarboxylase inhibition by 2-hydroxy-5-carbomethoxybenzyloxyamine.

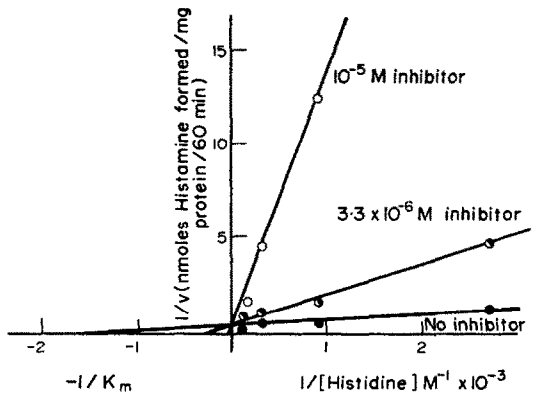



FIG. 3. Lineweaver–Burk plot of histidine decarboxylase inhibition by 2-hydroxy-5- carbomethoxybenzyloxyamine with respect to histidine. Enzyme source: rate stomach (pyloric).

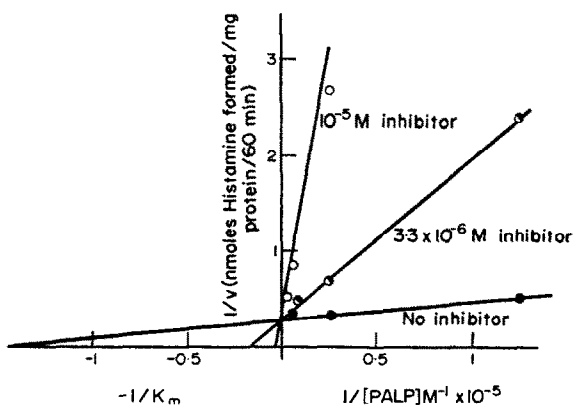



Fig. 4. Lineweaver–Burk plot of histidine decarboxylase inhibition by 2-hydroxy-5-carbomethoxybenzyloxyamine with respect to pyridoxal phosphate. Enzyme source: rat stomach (pyloric).

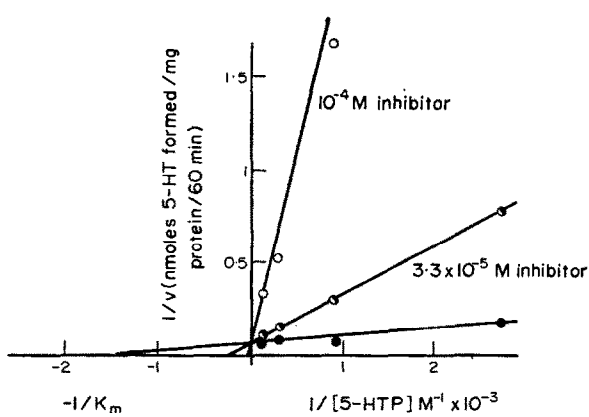



FIG. 5. Lineweaver–Burk plot of aromatic L-amino acid decarboxylase inhibition by 2-hydroxy-5-carbomethoxybenzyloxyamine with respect to 5-hydroxytryptophan. Enzyme source: guinea-pig liver, homogenate.

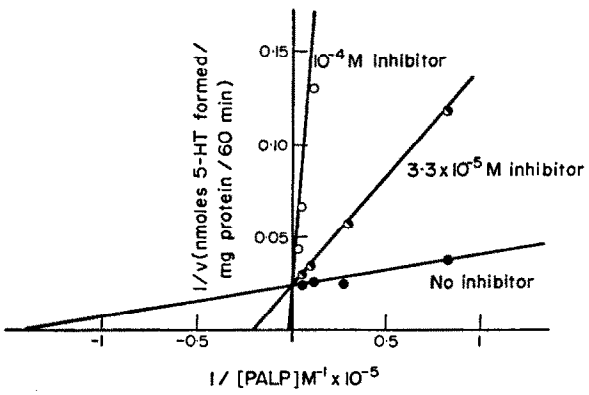



FIG. 6. Lineweaver–Burk plot of aromatic L-amino acid decarboxylase inhibition by 2-hydroxy-5-carbomethoxybenzyloxyamine with respect to pyridoxal phosphate. Enzyme source: guinea-pig liver, homogenate.

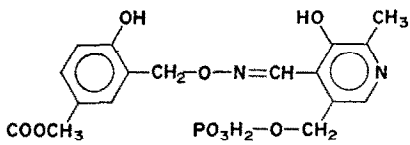
with respect to both substrate and coenzyme both on rat stomach histidine decarboxylase (Figs. 3 and 4) and guinea-pig liver aromatic L-amino acid decarboxylase (Figs. 5 and 6).

Inhibitor constants, obtained from the Lineweaver–Burk equation, described for competitive inhibitions, are listed in Table 1. These values showed a marked inhibitory effect on specific histidine decarboxylase, and significantly lower effect on non-specific decarboxylase.

TABLE 1. INHIBITOR DISSOCIATION CONSTANTS OF 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMINE

| Enzyme | Variable | $\tilde{K}_i$(M) |
|---|---|---|
| Rat stomach (pyloric) Histidine-decarboxylase | Histidine | $1 \times 10^{-7}$ |
| | PALP | $2{\cdot}5 \times 10^{-7}$ |
| Guinea-pig liver Aromatic L-amino acid Decarboxylase | 5-hydroxytryptophan | $5 \times 10^{-6}$ |
| | PALP | $1 \times 10^{-6}$ |

*Inhibitor-coenzyme interaction studies.* In order to clarify whether the inhibitor acts directly, binding to the active site of the apoenzyme, or indirectly forming a complex with the coenzyme in the first step of inhibiting action, the pyridoxal phosphate oxim of the inhibitor was prepared and its inhibiting properties were studied in detail. The chemical structure of this complex was shown in Fig. 7. In preliminary experiments the complex was incubated at a final concn of $10^{-3}$ M with guinea-pig liver homogenate (source of aromatic L-amino acid decarboxylase) and with rat stomach (pyloric) histidine decarboxylase at pH 6·8 and 8·2. The complex produced a complete (100 per cent) inhibition of specific histidine decarboxylase, while it had little effect on aromatic L-amino acid decarboxylase. Kinetic studies suggested competitive inhibition of the former enzyme, with respect to both substrate and coenzyme (Figs. 8 and 9). The




[2-Hydroxy-5-carbomethoxybenzyloxyamine] - [Pyridoxal P]

FIG. 7. Chemical structure of the pyridoxal phosphate complex of the inhibitor.

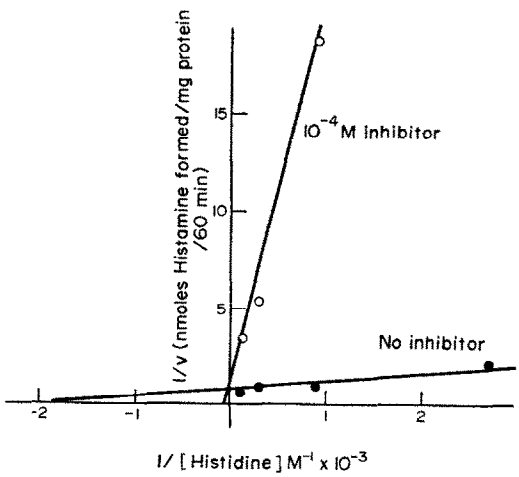



FIG. 8. Lineweaver–Burk plot of histidine decarboxylase inhibition by the pyridoxal phosphate complex of the inhibitor, with respect to histidine. Enzyme source: rat stomach (pyloric).

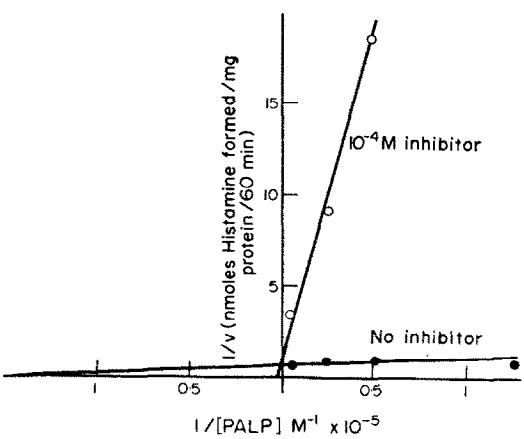



FIG. 9. Lineweaver–Burk plot of histidine decarboxylase inhibition by the pyridoxal phosphate complex of the inhibitor, with respect to pyridoxal phosphate. Enzyme source: rat stomach (pyloric).

inhibitor constants obtained in these experiments are listed in Table 2, and show significantly less inhibition in the case of the complex than with the inhibitor itself.

TABLE 2. 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMIDE PYRIDOXALPHOSPHATE DECARBOXYLASE INHIBITING PROPERTIES

| Enzyme | Variable | $\tilde{K}_i$(M) | Type of inhibition |
|---|---|---|---|
| Rat stomach (pyloric) Histidine decarboxylase | Histidine | $3{\cdot}3 \times 10^{-6}$ | Competitive |
| | PALP | $1{\cdot}1 \times 10^{-5}$ | Competitive |
| Guinea-pig liver Aromatic L-amino acid Decarboxylase | No inhibition | | |

In vivo *effects*. The non-specific decarboxylase inhibiting effect of the compound was also studied *in vivo*. In these experiments, the inhibitor was administered orally to guinea-pigs in a dosage of 100 mg/kg. The animals were decapitated 1 hr after the treatment, livers removed, homogenized, and their aromatic L-amino acid decarboxylase activity was measured with 5-hydroxytrytophan as substrate, at pH 8·1. Results, obtained in these studies, are summarized in Table 3. The compound inhibited the non-speciffc enzyme by 30 per cent, and this was less than was expected on the basis of *in vitro* studies. However, *in vivo* measurements indicated that the compound is a weak inhibitor of this enzyme.

TABLE 3. *In vivo* AROMATIC L-AMINO ACID DECARBOXYLASE INHIBITING EFFECT OF 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMINE

| Treatment | Dose (mg/kg i.p.) | Sp. act. ($\mu$moles serotonin formed/mg protein/hr) | Inhibition (%) |
|---|---|---|---|
| Untreated | — | $0{\cdot}0172 \pm 0{\cdot}0018$ | — |
| Treated | 100 | $0{\cdot}0121 \pm 0{\cdot}005$ | 30 |

Groups of seven guinea-pigs were injected peritoneally with 100 mg/kg of the inhibitor. One hour later animalswere decapitated, the livers removed, homogenized in 0·25 Msucrose and decarboxylase activity determined using 5-hydroxytryptophan as substrate.

The histamine lowering effect of the inhibitor was studied in male rats by single oral and by repeated intraperitoneal and oral treatments. The inhibitor was administered in a single dose of 15, 45 and 135 mg/kg. Animals were decapitated 3 hr after the treatments, and the level of histamine in the stomach, lungs, heart and abdominal skin was assayed (Table 4).

Results, obtained with 45 mg/kg oral dosage, showed significant decreases in histamine levels in stomach (25 per cent), lungs (35 per cent), heart (27 per cent) and skin (20 per cent). The minimum oral dose of inhibitor required to lower histamine levels in stomach and in lungs was 15 mg/kg, producing 14 and 25 per cent diminutions respectively. Maximal effects (40–45 per cent decrease in histamine levels) were obtained with the dose of 45 mg/kg in the lungs and 135 mg/kg in the stomach.

TABLE 4. HISTAMINE LOWERING EFFECTS OF A SINGLE DOSE OF 2-HYDROXY-5-CARBOMETHOXYBENZYLOXYAMINE

| Tissues | Control | Treated | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 15 mg/kg | | | 45 mg/kg | | | 135 mg/kg | | |
| | Histamine* | Histamine | Δ (%) | P | Histamine | Δ (%) | P | Histamine | Δ (%) | P |
| Stomach | 20·54 ± 1·76 | 17·66 ± 1·64 | −14 | 0·20 | 15·40 ± 0·94 | −25 | 0·10 | 12·03 ± 0·79 | −42 | 0·01 |
| Lungs | 6·23 ± 0·95 | 4·73 ± 0·32 | −24 | 0·10 | 4·04 ± 0·51 | −35 | 0·01 | 6·00 ± 0·51 | −4 | N.S. |
| Heart | 4·20 ± 0·39 | 4·85 ± 0·40† | +15 | N.S. | 3·07 ± 0·14† | −27 | 0·05 | 3·80 ± 0·043† | −10 | N.S. |
| Abdominal skin | 28·75 ± 1·40 | 27·31 ± 2·10† | −5 | N.S. | 23·00 ± 1·60† | −20 | 0·05 | 25·36 ± 1·24† | −12 | 0·20 |

Groups of five rats were treated with various doses of inhibitor. Three hours after the treatment animals were decapitated, organs were immediately removed, homogenized and levels of histamine determined.

* Histamine levels were expressed in μg/g tissue.

† Values were obtained with the hydrobromide salt of the compound.

N.S.—non significant.

Duration of histamine lowering effect was studied after 45 mg/kg dosage of the compound was given orally to rats, and the histamine levels in the stomach and lungs were assayed at ½, 1, 2, 3, 5, 16 and 24 hr after the treatment. Results obtained are presented in Fig. 10.

Maximal diminutions in histamine levels were detected at 1–2 hr in both tissues. Histamine levels returned to normal within 6–8 hr, and no remarkable diminution in histamine levels could be observed at 16 and 24 hr after drug administration. The results of resorption studies were comparable with these experiments,* and suggested that the inhibitor acts directly *in vivo*. The compound showed a slower elimination, if

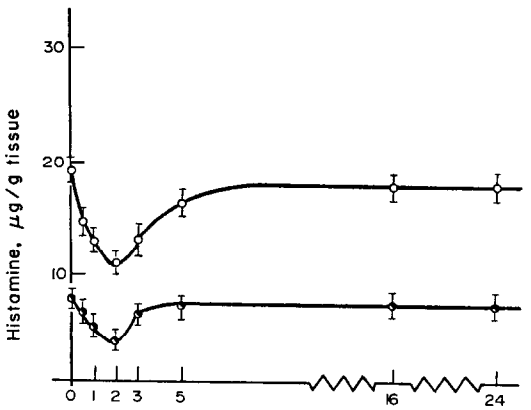



FIG. 10. Duration of the histamine lowering effect of 2-hydroxy-5-carbomethoxybenzyloxyamine. 45 mg/kg was administered orally to groups of five rats. Animals were killed at 0·5, 1, 2, 3, 5, 16 and 24 hr after the treatment. The histamine levels in stomach and in lungs were assayed. (○) Histamine level in stomach. (◔) Histamine level in lungs.

it was given to rats as the hydrobromide salt, and also if the animals were fed freely during treatments.* Repeated administrations produced more marked decrease in histamine levels than that observed by single treatments (Table 5). These values agreed with those obtained by single oral treatment with 45 mg/kg.

TABLE 5. HISTAMINE LOWERING EFFECT OF REPEATED TREATMENT WITH 2-HYDROXY-5-CARBOMETHOXY-BENZYLOXYAMINE

| Organs | Treatment | Dose (mg/kg) | Histamine (μg/g tissue) Control | Treated | Δ(%) | P |
|---|---|---|---|---|---|---|
| Stomach | Intraperitoneal | 3 × 15 | 21·55 ± 0·81 | 16·81 ± 0·50 | −22 | 0·05 |
| | Oral | | | 15·73 ± 0·80 | −27 | 0·01 |
| Lungs | Intraperitoneal | 3 × 15 | 6·72 ± 0·80 | 4·80 ± 0·28 | −28 | 0·10 |
| | Oral | | | 4·40 ± 0·51 | −34 | 0·05 |

Groups of five rats were treated with the inhibitor intraperitoneally and orally three times daily for 3 days with a dose of 15 mg/kg. Three hours after the last treatment, animals were decapitated, organs removed and the levels of histamine measured.

* I. Elekes, personal communication.

For prolonged administration the compound was given to rats in an oral dose of 45 mg/kg, once daily for 28 days, and histamine levels in stomach, lungs and abdominal skin were assayed 1 hr after the last administration (Table 6). No toxic effects were observed during these treatments. These single daily administrations of the compound

TABLE 6. HISTAMINE LOWERING EFFECT OF PROLONGED TREATMENT WITH 2-HYDROXY-5-CARBOMETHOXY-BENZYLOXYAMINE

| Organs | Treatment | Dose (mg/kg) | Histamine ($\mu$g/g tissue) | | | |
|---|---|---|---|---|---|---|
| | | | Untreated | Treated | $\Delta$(%) | P |
| Stomach | 1 × daily | 45 | 21·20 ± 1·9 | 16·11 ± 0·30 | −24 | 0·10 |
| Lungs | 1 × daily | 45 | 7·33 ± 0·39 | 4·84 ± 0·17 | −34 | 0·05 |
| Abdominal skin | 1 × daily | 45 | 31·13 ± 2·4 | 24·90 ± 1·30 | −20 | 0·10 |

Groups of ten rats were treated orally and once daily with the inhibitor, for 28 days. Animals were decapitated 3 hr after the last treatment and the levels of histamine measured.

for nearly a month, however, did not give any more marked effects. Prolonged treatment resulted in a decrease in histamine levels similar to the single treatment. The results of repeated administrations suggest that more pronounced decrease in histamine levels could be achieved, if animals are treated three times daily for a longer period.

*Toxicity studies.* Acute toxicological studies were carried out on rats and mice, by intravenous, intraperitoneal and oral administration of the inhibitor. The acute lethal dose of the compound was determined for 24 hr and for 7 days, using the method of Lichfield and Wilcoxon.[14] Results are summarized in Table 7. The compound given in doses of 100 and 300 mg/kg to rats appeared to be non-toxic in acute and also in subacute examinations.

TABLE 7. ACUTE LETHAL DOSES OF 2-HYDROXY-5-CARBOMETHOXYBENZYL-OXYAMINE HCl IN MICE AND RATS BY ORAL, INTRAPERITONEAL AND INTRAVENOUS ADMINISTRATION

| Treatments | $LD_{50}$ (mg/kg) | | | |
|---|---|---|---|---|
| | Mice | | Rats | |
| | 24 hr | 7 days | 24 hr | 7 days |
| Oral | 2400 | 2400 | 2500 | 2500 |
| Intraperitoneal | 500 | 500 | 270 | 270 |
| Intravenous | 100 | 100 | — | — |

## DISCUSSION

Present results indicate that 2-hydroxy-5-carbomethoxybenzyloxyamine is a potent and specific inhibitor of histidine decarboxylase. The compound showed more pronounced effect on the specific enzyme of rat stomach, than on the non-specific

decarboxylase of guinea-pig liver, especially in the *in vivo* experiments. This relatively low aromatic L-amino acid decarboxylase inhibiting effect of the compound confirmed earlier results,[1] showing special importance of the 3-hydroxyl group in benzyloxyamine molecule relating to the non-specific decarboxylase inhibition, and showing significantly less effectiveness, if the hydroxyl is in the 2 position. The diamine and monoamine oxidase inhibiting effects of the compound were much lower than that of NSD–1055[15] and that of the previously described inhibitor, pyridyl-3-methoxyamine.[1] According to the measurement of endogenous histamine the compound decreased the levels of histamine in lower doses than most inhibitors of histidine decarboxylase, such as NSD-1055,[16] alpha-methylhistidine[16] and thiazol-4-methoxyamine[17] but in somewhat higher doses than pyridyl-3-methoxyamine.[1] The minimum effective oral dose of the compound was 15 mg/kg in lungs and in stomach, and maximum effects were achieved with doses of 45 mg/kg in lungs, and 135 mg/kg in stomach. In the cases of NSD-1055 and alpha-methylhistidine, the minimum effective intraperitoneal doses were found to be 25 mg/kg,[16] and for thiazol-4-methoxyamine it was described as 50 mg/kg.[17] The minimum dose of pyridyl-3-methoxyamine, required to lower histamine levels after oral administration, was established as 5 mg/kg.[1] The maximum effective dose of these compounds was 100 mg/kg and 200 mg/kg.[1,16–19] According to Johnson and Kahlson[20] 100, 200 and 400 mg/kg of intraperitoneal doses of NSD-1055 and alpha-methylhistidine did not alter the endogenous levels of histamine, but if animals received L-histidine, the higher levels of histamine were significantly decreased by these compounds.

Repeated administration seemed more advantageous than single treatment for 2-hydroxy-5-carbomethoxybenzyloxiamine as well as for alpha-methylhistidine.[16] Repeated treatment with NSD-1055 proved to be toxic, killing the rats on the third day of the experiment.[16] Decaboran, the most active inhibitor of histidine decarboxylase *in vivo*,[21] showed serious, while pyridyl-3-methoxyamine,[1] showed minor toxic effects during prolonged treatments.* This compound appeared to be a non-toxic inhibitor of specific decarboxylase, given in effective doses, repeatedly for 1, or for several day periods. The reason, in general, why repeated administration is more advantageous than single treatment is presumably because of the rapid turnover of the enzyme.[15] In the case of 2-hydroxy-5-carbomethoxybenzyloxyamine, such treatments are especially reasonable because of its relative rapid elimination.† The relatively short duration of its effect, however, does not alter the chance of possible therapeutic use. In this case repeated administration is essential for all inhibitors.[22]

Kinetic studies showed reversible and competitive inhibition relative to both substrate and coenzyme. According to Westheimer,[23] the pyridoxal phosphate attaches to the apoenzyme by the phosphate, phenolic and aldehyde groups, aldehyde group forming a Schiff base by interaction with an amino group of the apoenzyme molecule. The substrate presumably attaches to the Schiff base linkage between pyridoxal phosphate and apoenzyme, forming another Schiff base with the aldehyde group of pyridoxal.[24] If it is true, a competition between substrate and inhibitor for the aldehyde group of pyridoxal phosphate, has to be assumed in the cases of substrate-competitive inhibitors. Such competition was suggested between 2-hydroxy-5-carbomethoxybenzyloyamine, and histidine, or 5-hydroxytryptophan.

* R. Schayer, personal communication.

† I. Elekes, personal communication.

The pyridoxal phosphate oxim of this inhibitor, assumed to be formed during inhibition, showed, however, much lower competitive effect on histidine decarboxylase than the inhibitor itself, and absolutely no effect on aromatic L-amino acid decarboxylase. The pyridoxal phosphate complex formation seemed to explain only partly the mechanism of inhibition of decarboxylases in this case. A more complete explanation is given if we assume that the inhibiting effect of 2-hydroxy-5-carbomethoxybenzyloxyamine is partly directly on the coenzyme, and partly on the apoenzyme, as was suggested by Hansson *et al.* for benzylhydrazines and benzyloxyamines.[25] If we consider that inhibitors are competitive with respect to both substrate and coenzyme, and also that the effect was more pronounced on the specific than on the non-specific decarboxylase, showing a much greater affinity of the compound for the enzyme which is attached relatively loosely to coenzyme,[24] this later explanation seemed more reliable. According to Levine,[16] and also to Leinweber,[3] in the mechanism of inhibition produced by NSD-1055, the formation of the pyridoxal phosphate oxim plays absolute role.

For therapeutic use, the histamine lowering potency of inhibitors has to be considered. 2-Hydroxy-5-carbomethoxybenzyloxyamine proved to be highly active in decreasing the levels of histamine in various tissues, especially in stomach and lungs, and to be non-toxic in repeated treatments. Preliminary pharmacological studies showed that the compound was effective in diminishing the primary and secondary reactions in adjuvant induced rheumatoid arthritis[26] and in lowering the sensitivity against histamine in bronchus spasm.* These favourable effects promote further pharmacological studies with the compound and also offer some hope for its therapeutic application.

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