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# The inhibition of histamine formation *in vivo*

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Histamine formation was measured by the [$^{14}$C]histamine content of the stomach following injection of [$^{14}$C]histidine into rats. Carbonyl reagents (e.g. oxyamines) which inactivate pyridoxal phosphate, the co-enzyme of histidine decarboxylase, were the most effective inhibitors of histamine formation *in vivo*. Although the oximes prepared from imidazol-4(5)-ylmethoxyamine and pyridoxal or pyridoxal phosphate cannot inactivate the co-enzyme, they proved sufficiently potent to merit further study as inhibitors of histamine formation *in vivo*.

Many compounds of diverse chemical structure are active *in vitro* as histidine decarboxylase inhibitors. Of these only a few, mainly carbonyl reagents such as oxyamines and hydrazine derivatives which inactivate the co-enzyme pyridoxal phosphate (PLP), have been shown to inhibit histamine formation *in vivo* (Levine, Sato & Sjoerdsma, 1965; Johnston & Kahlson, 1967; Reilly & Schayer, 1968; Hakanson & Liedberg, 1972; Kobayashi, Kupelian & Maudsley, 1970; Menon, Clark & Aures, 1971). Carbonyl reagents are generally extremely potent inhibitors of histidine decarboxylase, but because of their action on the co-enzyme they lack specificity and may thus also inhibit other PLP-dependent enzymes.

A procedure for the screening of potential inhibitors of histidine decarboxylase *in vivo* is now described and applied to the evaluation of compounds selected from those previously studied *in vitro* (Mole & Shepherd, 1972).

## MATERIALS AND METHODS

### *Drugs*

We are indebted to Mr. D. Drain of Smith and Nephew Research for providing NSD-1055 [*O*-(*p*-bromo-*m*-hydroxybenzyl) hydroxylamine], to May and Baker Ltd., Dagenham, Essex for Phenobutiodil, and to Laboratoires Promedica, 41 rue Camille Pelletan, Lavallois-Perret, France for 554-L [7-amino-4,5,6-triethoxy3-(5,6,7,8--tetrahydro-4-methoxy-6-methyl-1,3-dioxolo(4,5-g)isoquinolin-5-yl)phthalide]. The remaining compounds were obtained from commercial sources or prepared by us as indicated below.

### *Oxime (PLPIMA) of pyridoxal phosphate and imidazol-4(5)-ylmethoxyamine*

Imidazol-4(5)-ylmethoxyamine dihydrochloride (IMA) (Mole & Shepherd, 1972) (1·86 g, 0·01 mol) was dissolved in 50 ml water and added to solid pyridoxal phosphate monohydrate (2·65g, 0·01 mol). The mixture was stirred until a clear solution was obtained (about 30 min) and set aside at 4° for 2 h. Sodium bicarbonate (1·68g, 0·02 mol) in 50 ml water was added during 30 min to the stirred solution which was then left overnight at 4°. The yellow solid which separated was removed by filtration and washed carefully with ice-cold water to give 2·80g (78%) of oxime, mp 178–183°.

Analyses for C, H, N and P were within expected limits. Traces of impurities were detected by paper chromatography, but there was no contamination by the reactants (PLP or IMA).

*Oxime (PLIMA) of pyridoxal and imidazol-4(5)-ylmethoxyamine*

This oxime was prepared as for PLPIMA (above) using pyridoxal (PL) hydrochloride (1·22g, 0·006 mol), imidazol-4(5)-ylmethoxyamine dihydrochloride (1·13g, 0·006 mol) and sodium bicarbonate (1·51g, 0·018 mol). The oxime (1·25g; 79%) had mp 188–189°. Calc. for $C_{12}H_{14}N_4O_3$: C, 54·95; H, 5·4; N, 21·35; Found; C, 52·35; H, 5·45; N, 21·2. Although the analytical figures indicate impurities to the extent of approximately 4%, unchanged reactants (IMA and PL) were shown to be absent by paper chromatography. Difficulty in purifying pyridoxal oximes has also been reported by Leinweber (1968).

*Paper chromatography*

Chromatograms were obtained by the ascending method using (a) Whatman No. 4 paper with n-butanol–acetic acid–water (12:3:5 by volume), as solvent and (b) Whatman P81 cellulose phosphate paper with 0·2M triethylamine acetate (adjusted to pH 6)– isopropyl alcohol (2:3 by volume) as solvent. The products PLPIMA and PLIMA were located by their fluorescence in ultraviolet light before spraying of the chromatograms. Imidazole derivatives were located by spraying with Pauly's reagent (red spots) and PL and PLP with a 1% aqueous solution of IMA (blue fluorescence in ultraviolet light).

*Animal studies*

As the inhibitors ranged from readily soluble to almost insoluble in water, they were administered by intraperitoneal injection as fine suspensions in corn oil.

Female Wistar rats (100–120g) were divided into two groups each containing 12 animals. One group was injected with the inhibitor suspended in corn oil (2 mg $kg^{-1}$, i.p.) at zero time and again at 10 h. The control group received corn oil alone. At 10 h both groups also received L-histidine-ring-2-$^{14}C$ (2 mg $kg^{-1}$, $4 \times 10^7$ d $min^{-1}$ $kg^{-1}$) in 0·1M sodium phosphate buffer pH 6·5 (4 ml $kg^{-1}$, i.p.). In one experiment the inhibitor 554-L was administered orally in aqueous suspension at zero time and again at 9 h, the L-histidine being administered intraperitoneally in the usual way at 10 h.

At 12 h, i.e. exactly 2 h after the injection of [$^{14}C$]histidine, the rats were killed, and the stomachs removed. The glandular portion of each stomach was washed, dried on filter paper and weighed. The tissue was placed in a mixture of 0·9% sodium chloride (5 ml), 1M perchloric acid (4 ml) and carrier solution (1 ml) containing 66·4 mg histamine dihydrochloride and 50 mg L-histidine, chopped with scissors and homogenized for 1 min. The homogenate was centrifuged at 10000 *g* for 5 min, the supernatant adjusted to pH 6·5 with 5M sodium hydroxide and the pH stabilized by addition of 0·1M sodium phosphate buffer pH 6·5 (10 ml).

The [$^{14}C$]histamine, after separation from [$^{14}C$]histidine on Dowex 50-WX4 (100–200 mesh) cation exchange resin (Kahlson, Rosengren & Thunberg, 1963), was converted to its dibenenesulphonyl derivative (Schayer, Rothschild & Bizony, 1959) which was recrystallized and the radioactivity measured in a liquid scintillation spectrometer.

## RESULTS AND DISCUSSION

The [$^{14}$C]histamine content of the rat stomach following administration of [$^{14}$C]-histidine provides a satisfactory index of histamine formation *in situ* (Kobayashi, & others, 1970). The results of the present *in vivo* studies utilizing this method are summarized in Table 1. For comparison, the relative *in vitro* potencies of the inhibitors towards the specific histidine decarboxylase of rat stomach as measured by the $C_{50}$ values (Mackay & Shepherd, 1960) are included in Table 1.

Table 1. *Relative potencies of histidine decarboxylase inhibitors* in vitro *and their effect on histamine formation by the rat stomach* in vivo.

| | | | [$^{14}$C]histamine formed per gram of rat stomach | | | |
|---|---|---|---|---|---|---|
| Inhibitor | $C_{50}$ (M) | Dose† | Control d min$^{-1}$ ± s.d. | Treated d min$^{-1}$ ± s.d. | % Inhibition | *P* |
| NSD-1055 | $7{\cdot}6\times10^{-7}$ | 2×150 | 3273±1360 | 285±153 | 91 | <0·001 |
| IMA | $1{\cdot}5\times10^{-7}$ | 2×150 | 2585±814 | 161±65 | 94 | <0·001 |
| PTSH | $3{\cdot}6\times10^{-7}$ | 2×50 | 3717±1442 | 149±97 | 96 | <0·001 |
| $B_{10}H_{14}$ | $2{\cdot}1\times10^{-8}$ | 2×15 | 2613±1485 | 900±435 | 66 | <0·001 |
| Rhodanine | $2{\cdot}2\times10^{-5}$ | 2×100 | 3092±1966 | 314±210 | 90 | <0·001 |
| PLPIMA | $5{\cdot}2\times10^{-7}$ | 2×150 | 3866±1089 | 2091±665 | 46 | <0·001 |
| PLIMA | $3{\cdot}4\times10^{-4}$ | 2×150 | 2958±638 | 1653±606 | 44 | <0·001 |
| Phenobutiodil | $2{\cdot}9\times10^{-4}$ | 2×250 | 3067±733 | 3949±995 | −29 | <0·025 |
| 554-L | $\geqslant1\times10^{-3}$ | 2×250 | 4107±1597 | 3825±1676 | 7 | NS |
| 554-L | | 2×250* | 3359±982 | 3692±1743 | −10 | NS |

† Unless otherwise stated dose in mg (of base) per kg i.p. in corn oil (2 ml kg$^{-1}$) at 12 and 2 h before killing.

* Dose in mg (of base) per kg orally in 0·5% Promulsin (2 ml kg$^{-1}$) at 12 and 3 h before killing.

NSD-1055 was used primarily to establish the conditions for screening *in vivo* the other inhibitors in this study, since it has already been widely used as an inhibitor of histidine decarboxylase *in vivo*. Its effectiveness in this respect has been questioned (Mesch & Sewing, 1971) and preliminary experiments in this laboratory showed that a high degree of inhibition was consistently achieved only when the inhibitor was injected on at least two separate occasions, usually 10 h apart. The results (Table 1) show that NSD-1055 administered in two such doses each of 150 mg kg$^{-1}$ produced 91% inhibition of histamine formation in the rat stomach.

Imidazol-4(5)-ylmethoxyamine (IMA), an imidazole analogue of NSD-1055, should have greater affinity than NSD-1055 for the apo-enzyme of histidine decarboxylase. IMA, has in fact, been shown to be more potent than NSD-1055 *in vitro* as an inhibitor of the specific histidine decarboxylase of a rat hepatoma (Reid & Shepherd, 1963): this is also true of the specific histidine decarboxylase of rat stomach (Table 1). *In vivo* IMA (2 × 150 mg kg$^{-1}$) was of similar potency to NSD-1055 as an inhibitor of histamine formation (Table 1).

Another carbonyl reagent, *p*-toluenesulphonylhydrazine (PTSH) has been reported to be a potent inhibitor of histidine decarboxylase *in vitro* and *in vivo* (Schayer, 1968). The results in Table 1 confirm these findings. Severe toxic effects, however, were seen with doses greater than 50 mg kg$^{-1}$.

Decaborane ($B_{10}H_{14}$), an extremely potent inhibitor of histidine decarboxylase

*in vitro*, also inactivates the co-enzyme PLP (Mole & Shepherd, 1972; Naeger & Leibman, 1972). *In vivo*, a relatively low dose ($2 \times 15$ mg kg$^{-1}$) reduced histamine formation in the rat stomach by 66%; there was no evidence of toxicity, though higher doses have been reported to be severely toxic (Henman, Mole & Shepherd, 1970).

Unlike most other carbonyl reagents, which strongly inhibit both aromatic L-amino acid decarboxylase and specific histidine decarboxylase *in vitro*, rhodanine shows considerable selectivity towards the latter enzyme (Mole & Shepherd, 1972). Although it was markedly less potent *in vitro* than the other PLP inactivators listed in Table 1, rhodanine ($2 \times 100$ mg kg$^{-1}$) was comparable with NSD-1055 and IMA as an inhibitor of histamine formation *in vivo*.

The *O*-benzyloxime of PLP inhibits histidine decarboxylase (Leinweber, 1968; Mole & Shepherd, 1972), but does not inactivate the co-enzyme; replacing the benzene ring by an imidazole ring should increase the affinity for histidine decarboxylase. Accordingly the imidazole analogue (PLPIMA) of this compound was prepared. PLPIMA ($2 \times 150$ mg kg$^{-1}$) produced a moderate, though highly significant degree of inhibition (46%) *in vivo*. Although PLPIMA is less effective than the parent oxyamine IMA, it is sufficiently potent, and possibly more selective than the carbonyl reagents, to render it worthy of further investigation. The closely related oxime (PLIMA), which lacks only the 5-phosphate group, was prepared in an attempt to increase the absorption/excretion ratio; it had virtually the same inhibitory activity *in vivo* as PLPIMA. It is possible that the PLIMA is readily phosphorylated *in vivo* to form the more potent PLPIMA; alternatively both PLIMA and PLPIMA could be converted *in vivo* to the parent oxyamine IMA.

Phenobutiodil, α-(2,4,6-tri-iodophenoxy)butyric acid, one of the most potent polyhalogenated carboxylic acids that we have studied as histidine decarboxylase inhibitors *in vitro* (Mole & Shepherd, to be published) was selected for evaluation *in vivo*. At $2 \times 250$ mg kg$^{-1}$ it did not inhibit histamine formation *in vivo*; in fact 29% stimulation was obtained.

In recent years, claims have been made that a tetrahydroisoquinoline derivative, known variously as Hypostamine, Tritoqualine or 554-L, possessed a variety of pharmacological properties including anti-allergic (Hahn, Teschendorf & others, 1970) and anti-inflammatory (Pelczarska, 1969) activity. Moreover, it has been inferred that these actions were due to inhibition of histidine decarboxylase (Pelczarska, 1969; Pena, 1966; Maslinski & Niedzielski, 1969).

When 554-L was tested *in vitro* for inhibitory activity, none could be detected (Table 1). Since the compound might be metabolized to an active histidine decarboxylase inhibitor, its potency was also measured *in vivo*, but even with a dosage of $2 \times 250$ mg kg$^{-1}$ (i.p.) there was no significant inhibition of histamine formation. As in much of the published work 554-L was given orally, we made a further series of experiments in which rats were dosed orally with the compound, the [$^{14}$C]histidine being injected as usual 2 h before killing, but there was no significant inhibition of histamine formation (Table 1). These findings suggest that, whatever pharmacological actions 554-L may possess, they are not mediated by inhibition of histamine synthesis. In at least one instance, the histamine formed was measured by bioassay (Pelczarska, 1969); it is possible that 554-L possesses antispasmodic activity on the guinea-pig ileum, leading to an underestimate of the histamine formed and thus suggesting that inhibition of histidine decarboxylase has occurred.

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