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HISTAMINE INHIBITION OF MIXED FUNCTION OXIDASE ACTIVITY IN RAT AND HUMAN LIVER MICROSOMES AND IN THE ISOLATED PERFUSED RAT LIVER

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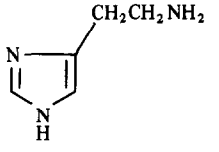
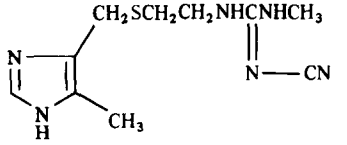
Abstract—The imidazole ring is a common structural feature of some xenobiotics that inhibit cytochrome P-450-catalysed reactions. Histamine is a 4-substituted imidazole and a preliminary study has shown it to be an inhibitor of rat liver microsomal drug oxidation. This work has now been extended. Histamine appears to be a competitive inhibitor of the α-hydroxylation (HM) (K_i = 164 μM; IC_{50} at 20 μM = 308 μM) and O-demethylation (ODM) (K_i = 243 μm; IC_{50} at 20 μM = 400 μM) of metoprolol in rat liver microsomes. Of the metabolites of histamine only *N*-acetylhistamine showed comparable inhibitory potency to that of the parent compound. Histamine impaired the disappearance of lignocaine when incubated with rat liver microsomes. This was accompanied by a corresponding inhibition of 3-hydroxylignocaine appearance. Histamine produced a type II spectral interaction with rat liver microsomes (λ_{max} = 432 nm, λ_{min} = 408 nm; K_s = 0.11 mM). When histamine was incubated alone with rat liver microsomes no loss of substrate was observed. The oxidation of metroprolol by human liver microsomes was impaired by histamine (IC_{50} values for ODM appearance at 25 μM: liver HL1 > 10, HL3 = 3.8 and HL4 = 3.7 mM). In comparison, cimetidine had an IC_{50} value of 1.5 mM using microsomes from liver HL3. Addition of histamine impaired the elimination of metoprolol by the isolated perfused rat liver in a dose-dependent manner ($P < 0.001$, one-way analysis of variance). These data demonstrate that histamine can enter hepatocytes, interact with cytochrome P-450 and inhibit some drug oxidation reactions. The physiological relevance of inhibition of drug metabolism by histamine remains to be determined.

The imidazole ring is a common structural feature of several drugs and other xenobiotics that inhibit mixed function oxidation reactions. The binding of imidazoles to cytochrome P-450 and the subsequent inhibition of metabolism depend on a sterically unhindered nitrogen atom at the 3-position on the ring [1]. Thus, imidazoles substituted at the 1- or 4-position are inhibitory, whereas this property is lost on substitution at the 2-position [2].

Cimetidine, a 4-substituted imidazole derivative (Fig. 1), is an inhibitor of drug oxidation and has been implicated, as a causative agent, in some drug–drug interations [3]. Cimetidine was developed as a specific antagonist of histamine at H_2-receptors. As the imidazole ring of histamine is also substituted at the 4-position (Fig. 1), it was thought that histamine itself may be capable of inhibiting cytochrome P-450-catalysed reactions. Preliminary *in vitro* experiments have suggested this to be a possibility [4]. Metabolic and spectral studies extending that work using rat and human liver microsomes and the isolated perfused rat liver are described here.



Histamine



Cimetidine

Fig. 1. Chemical structures of histamine and cimetidine.

MATERIALS AND METHODS

Chemicals and drugs. Metoprolol tartrate, α-hydroxymetoprolol *p*-hydroxybenzoate, *O*-demethylmetoprolol base and pamatolol hydrochloride were gifts from AB Hassle (Molndal, Sweden), nadolol base from Squibb & Sons Ltd (Hounslow, Middlesex) and cimetidine from Dr D. W. Holt (Poisons Unit, New Cross Hospital, London). Histamine hydrochloride, its metabolites and 3-methylhistamine were purchased from the Sigma Chemical Co. (Poole, Dorset) and *O*-phthaldehyde reagent, "Fluoraldehyde", from Pierce & Warriner (U.K.) Ltd (Chester, U.K.).

Animals. An outbred strain of Wistar rats from the University of Sheffield Breeding Colony was used. Male (200–220 g) rats maintained on a standard laboratory small animal diet (Labsure Foods, Poole,

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U.K.) and allowed access to tap water *ad lib.* were used. For the preparation of microsomes the rats were stunned and killed by cervical dislocation and the livers removed immediately. The tissue was then frozen in liquid nitrogen and stored at −80° until use.

Source of human liver. Small samples of three human livers were obtained from male Caucasian renal transplant donors. The samples were taken as soon as possible after the kidneys were removed, cut into 2 cm cubes, frozen in liquid nitrogen and stored at −80° until use. The period between cessation of organ perfusion and cooling of the tissue was about 30 min. The drugs given to the donors prior to and during organ removal are described elsewhere [5].

Studies with liver microsomes. Liver microsomes were prepared as described previously [6]. Microsomal protein concentration was measured by the method of Lowry *et al.* [7] using bovine serum albumin as the standard. The cytochrome P-450 content was assayed by the method of Omura and Sato [8]. Difference spectra for the interaction of histamine with cytochrome P-450 were measured by the method of Schenkman *et al.* [9] between 350 and 500 nm (Model 219 Cary double beam scanning spectrophotometer, Varian Associates Ltd, Walton-on-Thames, U.K.) at a protein concentration of 1 mg/ml.

Liver microsomal suspensions (0.2 ml containing either 0.4–0.6 mg rat liver protein or 2–4 mg human liver protein) were incubated at 37° and pH 7.4 with metoprolol or lignocaine dissolved in 1.15% (w/v) KCl solution (0.2 ml), histamine or its metabolites in 1.15% (w/v) KCl solution (0.2 ml) and an NADPH generating system containing 4 μmol G6P, 0.4 μmol NADP, 0.4 units G6PD and 2 μmol $MgCl_2$ in 0.2 M phosphate buffer (0.4 ml). Earlier studies had established that the rates of appearance of metabolites were linear with respect to time and protein concentration under these experimental conditions. Histamine solutions were prepared freshly on the day of the experiment. The reaction was stopped by transferring 0.4 ml of the incubate in duplicate to capped plastic vials containing 6% (v/v) perchloric acid solution (50 μl). Pamatolol hydrochloride (20 μl of a 10 μg/ml solution) was added as the internal standard. The acid precipitated samples could then be stored at 4° for at least 6 months without detectable degradation of metoprolol metabolites.

The results of incubations with the NADPH-generating system alone indicated that the formation of NADPH was virtually unaffected (<5%) by the presence of histamine (0.1–10 mM) (unpublished observations).

Liver perfusion. Livers were perfused *in situ* as described by Lennard *et al.* [10]. The perfusion medium consisted of Krebs–Henseleit bicarbonate buffer, dialysed bovine serum albumin fraction V (2.6% w/v) and washed, aged human red cells (2.5% w/v haemoglobin). Livers were perfused at a flow rate of 15 ml/min in the recirculation mode. Drug and inhibitor were introduced by bolus injection into the reservoir (150 ml) from which serial samples were taken. Samples were stored at −20° until assayed. Preliminary studies had established that hepatic blood flow was unaffected by histamine when livers were perfused under conditions of constant pressure.

Drug analysis. All analyses were performed using high performance liquid chromatography.

Liver perfusate samples were assayed for metoprolol by the method of Lennard and Silas [11] using 1-(4-butyramido-2-butyrylphenoxy)-2-hydroxy-3-isopropylaminopropane·HCl as the internal standard.

A modification of the method of Lennard [12] was used to analyse α-hydroxymetoprolol and *O*-demethylmetoprolol. Sample, NaOH (1.2 M, 50 μl) and water (50 μl) were gently mixed with dichloromethane (5 ml) for 10 min. After centrifugation (3000 rpm, 5 min) the organic layer was transferred to a conical glass tube and evaporated to dryness using a Buchler vortex evaporator (Baird & Tatlock, Romford, U.K.). The residue was reconstituted in mobile phase and an aliquot was injected onto the HPLC column. HPLC was performed using a Model 6000A pump (Waters Associates, Northwich, U.K.), a Model 7125 Rheodyne injector (HPLC Technology, Macclesfield, U.K.), a Z-Module column system containing a cartridge packed with Nova-Pak C_{18} reversed phase packing material (Waters Associates) and a Model 970FS Kratos Fluorescence Detector (Applied Biosystems, Warrington, U.K.). A water–acetonitrile mixture (90:10) containing 1% (w/v) triethylamine and adjusted to pH 3 with orthophosphoric acid was used as the mobile phase. Chromatography was performed isocratically at a flow rate of 3 ml/min and at ambient temperature. The detector was operated at an excitation wavelength of 193 nm and no emission filter was used.

Lignocaine and 3-hydroxylignocaine were assayed by the method of Al-Asady *et al.* [13]. Microsomal incubation mixture (1.0 ml), the internal standard ethylmethylglycine xylidide (0.3 μg) and NaOH solution (200 μl, 1.2 M) were vortex mixed for 2 min with ethyl acetate (3.0 ml). After centrifugation (900 *g* for 3 min) the organic layer was removed and vortex mixed with sulphuric acid (200 μl, 0.1 M, pH 2.2) for 2 min. After centrifugation (900 *g* for 3 min) the acid layer was separated and evaporated to dryness using a Buchler vortex evaporator at 55–60°. The residue was dissolved in distilled water (200 μl) and an aliquot (20–40 μl) was injected onto the HPLC column. Chromatographic conditions were the same as those described for the analysis of metoprolol metabolites with the exception that the ratio of water–acetonitrile was 88:12 and UV detection was at 205 nm.

Histamine was analysed following derivatization with *O*-phthaldehyde. Sample (250 μl) was mixed with the internal standard (3-methylhistamine; 50 μl of a 2.5 μg/ml solution) and distilled water (50 μl). One minute prior to injection onto the HPLC column the *O*-phthaldehyde reagent (60 μl) was added and the contents of the tube thoroughly mixed. An aliquot (50 μl) was immediately injected onto the HPLC column. The HPLC conditions were the same as those described for the analysis of metoprolol metabolites except that the ratio of water–acetonitrile in the mobile phase was 80:20, the fluorescence excitation wavelength was 211 nm and a 340 nm emission filter was used. Intra-assay coefficients of variation were less than 5% for all compounds.

Table 1. The effect of histamine and its metabolites on metoprolol oxidation by rat liver microsomes

	α-Hydroxylation		O-Demethylation	
	K_i (mM)	IC_{50} (mM)	K_i (mM)	IC_{50} (mM)
Histamine	0.16	0.31	0.24	0.40
N-Acetylhistamine	—	0.60	—	0.65
Imidazolylacetic acid	—	6.3	—	6.5
1-Methylhistamine	—	4.7	—	4.8
Methylimidazolylacetic acid	—	9.5	—	>10

Apparent K_i and IC_{50} values for histamine are the mean data from three experiments. IC_{50} values for the histamine metabolites are mean data from single experiments. K_i values were estimated using the method of Dixon [16]. The substrate concentration was 20 μM and incubations were performed over 5 min. Mean values for apparent K_m and V_{max} are 18 μM and 0.29 nmol/min/mg protein, respectively, for α-hydroxylation and 18 μM and 0.17 nmol/min/mg protein, respectively, for O-demethylation [14].

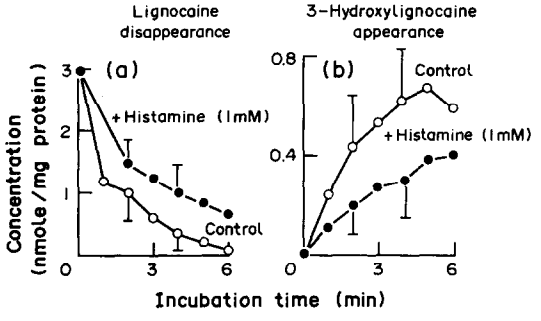

Fig. 2. The effect of histamine (1 mM) on (a) the disappearance of lignocaine (4.27 μM) and (b) the appearance of 3-hydroxylignocaine in rat liver microsomes. Mean data from six livers. Each liver acted as its own control. Vertical bars indicate SD.

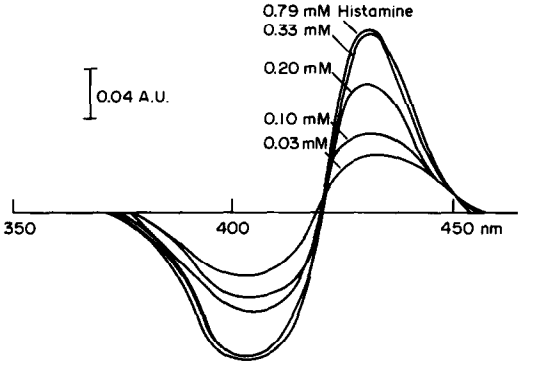

Fig. 3. The spectral interaction of histamine (0.03–0.79 mM) with rat liver microsomes.

RESULTS

Histamine inhibited the α-hydroxylation and *O*-demethylation of metoprolol by rat liver microsomes in an apparently competitive manner (Table 1). Known metabolites of histamine, namely *N*-acetylhistamine, 1-methylhistamine, imidazolyl acetic acid and methylimidazolyl acetic acid were also tested for their effect on metoprolol oxidation. All of these compounds caused some inhibition, but only *N*-acetylhistamine showed comparable potency to that of histamine (Table 1).

Histamine caused a dose-dependent impairment of lignocaine metabolism by rat liver microsomes, increasing the mean areas under the drug concentration–time curves (AUC) by 47 ± 13(SD)% (N = 4 livers, P = 0.017, Mann–Whitney test) at 0.25 mM and by 109 ± 35(SD)% (N = 6 livers, P < 0.001) at 1 mM histamine concentration (Fig. 2a). This was accompanied by a corresponding inhibition of 3-hydroxylignocaine appearance [mean decrease in AUC = 16 ± 8(SD)% at 0.25 mM (P = 0.046) and 49 ± 17(SD)% at 1 mM histamine (P = 0.001)] (Fig. 2b).

The results of spectral studies indicated a type II interaction between histamine and rat liver microsomes (Fig. 3). Values for the wavelengths of maximum and minimum absorption were 432 and 408 nm, respectively. The reversibility of this binding was demonstrated by a loss of spectral absorption following centrifugation of the incubation mixture and resuspension of the microsomal pellet in fresh buffer solution. Further addition of histamine to the sample cuvette resulted in the reappearance of the type II spectrum. Hanes plots of histamine concentration (0.03–0.08 mM)/the difference in absorption between maximum and minimum wavelengths against histamine concentration were linear and gave a value for the apparent spectral dissociation coefficient (K_s) of 0.11 mM. In comparison, a K_s value of 0.04 mM was obtained for cimetidine.

When histamine was incubated with rat liver microsomes and an NADPH-generating system, its concentration in the mixture did not decrease with time at the two doses used (8.3 and 1.7 μM).

Histamine inhibited the O-demethylation of metoprolol in human liver microsomes. IC_{50} values were >10 mM (liver HL1), 3.8 mM (liver HL3) and 3.7 mM (liver HL4) (Fig. 4). In comparison, an IC_{50} value of 1.5 mM was obtained for cimetidine when incubated with microsomes from liver HL3.

Addition of histamine to the reservoir of the isolated perfused rat liver preparation caused a dose-dependent impairment of metoprolol elimination

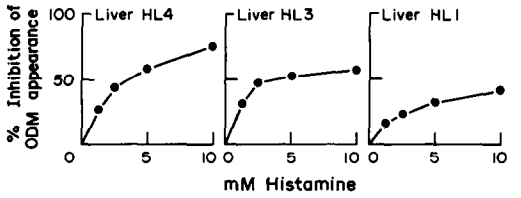

Fig. 4. The effect of histamine (1.25–10 mM) on the O-demethylation of metoprolol (25 μM) by human liver microsomes. ODM, *O*-demethylmetoprolol. Incubations were performed over 30 min. Each data point is the mean of duplicate analyses. Mean apparent K_m and V_{max} values for the high affinity site of O-demethylation are 26 μM and 0.14 nmol/min/mg protein, respectively [6].

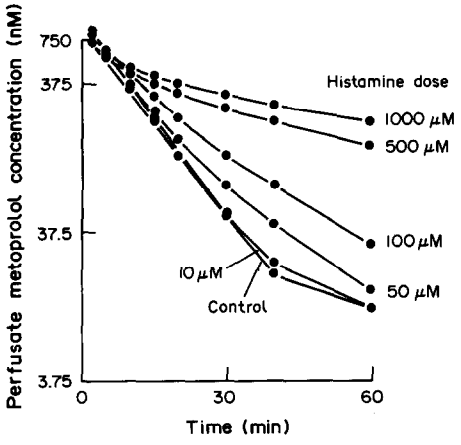

Fig. 5. Semi-logarithmic plots of perfusate drug concentration vs time showing the effect of histamine (0.1–1.0 mM) on the elimination of metoprolol (1 μM) in the isolated perfused rat liver. Drugs were injected into the reservoir (volume = 150 ml) of the preparation, which was set at a hepatic flow rate of 15 ml/min. Each data point is the mean value of each series of experiments. Ranges of $AUC_{0\text{-}60\,min}$ (nmol/l × min) values: control = 8662–10,950, N = 14; 10 μM histamine = 10,022–10,306, N = 4; 50 μM = 10,411–12,123, N = 5; 100 μM = 12,302–15,478, N = 3; 500 μM = 17,013–19,556, N = 4; 1000 μM = 19,437–22,991, N = 4.

($P < 0.001$, one-way analysis of variance) (Fig. 5). The mean AUC value at each dose of histamine was significantly higher than that of the controls (P values, control vs treatment: 0.05, 10 μM; 0.004, 50 μM; 0.01, 100 μM; 0.004, 500 μM; 0.004, 1000 μM histamine—Student's *t*-test).

DISCUSSION

Previous findings that histamine inhibited the α-hydroxylation and O-demethylation of metoprolol in rat liver microsomes [4] have been confirmed. In this regard histamine was about ten times less potent than cimetidine [14]. The stronger basicity of histamine (pK_a 9.8) [15] compared to that of cimetidine (pK_a 6.8) [3] and differences in lipophilicity and/or structure may explain this observation.

Convergence of the lines of Dixon plots and the parallel nature of s/v vs i plots suggested a competitive mechanism of inhibition [16] for cimetidine [14] and histamine [4] in rat liver microsomes. This is consistent with a direct interaction of the inhibitor with the catalytic site(s) of oxidation. However, in the present work values of apparent V_{max} decreased with increasing histamine concentration in two of the three experiments. This is at variance with predictions for pure competitive inhibition and indicates that the mechanism of inhibition of metoprolol oxidation by histamine is more complex. To study this further, we characterized the spectral interaction of histamine with rat liver microsomes. Histamine produced a type II spectral change, an interaction which is documented for cimetidine and other imidazoles [17, 18]. Type II changes depend upon the presence of accessible, non-bonded electrons on the nitrogen atom at the 3-position, which bind to the ferric form of the haemoprotein as a sixth ligand. However, there is evidence that imidazoles interact with two cytochrome P-450 binding sites [19, 20], one of low and one of high affinity. Therefore, histamine may bind to both the haem and catalytic site of cytochrome P-450.

Histamine is metabolized mainly by three enzymes, imidazolyl-*N*-methyl transferase, histaminase and histamine acetylase [15]. There is evidence to suggest that histamine metabolism occurs in hepatocytes [21]. Accordingly, the ability of metabolites of histamine to inhibit metoprolol oxidation in rat liver microsomes was studied. All metabolites tested caused some inhibition of metabolism but only *N*-acetylhistamine had comparable potency to that of histamine. Histamine is thought to be acetylated mainly by bacterial enzymes in the gut [15]. The product *N*-acetylhistamine is then absorbed and excreted as a minor product in the urine. Thus, it is possible that inhibitory concentrations of this metabolite are attained in the liver *in vivo*.

Despite the observation that histamine binds to rat liver cytochrome P-450, we found no evidence that it was a substrate for this enzyme system.

Lignocaine is metabolized mainly by aromatic hydroxylation and N-dealkylation [10]. Histamine impaired the metabolism of lignocaine as indicated by a slowing of the disappearance of the drug from microsomal incubates, and of the appearance of 3-hydroxylignocaine, the product of aromatic hydroxylation. Since it is not known whether the oxidations of lignocaine and metoprolol are catalysed by common forms of cytochrome P-450, no conclusions were possible regarding the selectivity of inhibition by histamine.

The oxidation of metoprolol in man exhibits genetic polymorphism of the debrisoquine-type [22, 23]. Poor metabolizers possess anomalous forms of one of the P-450 gene family designated P-450IID1 [24]. There is evidence that the rat and mouse orthologues of this isozyme have markedly different catalytic properties to those of the human enzyme [6, 25, 26]. The present studies in the rat were thus extended to examine the effect of histamine on metoprolol oxidation in human liver microsomes. O-Demethylation is the major route of metoprolol metabolism in man [23]. In microsomes from the three livers studied the rate of *O*-demethylmetoprolol appear-

ance was decreased significantly by histamine. The inhibitory potency was about 10 times less than that observed in rat liver microsomes. In contrast to findings with rat liver microsomes [14], the difference between the inhibitory action of histamine and cimetidine was small in human liver microsomes. Thus, cimetidine, which has been shown to impair the clearance of metoprolol in man *in vivo* [27], was only 2–3 times more potent than histamine *in vitro*. Reilly *et al.* [28] have also shown that histamine and cimetidine have similar inhibitory actions on the human microsomal O-deethylation of phenacetin, another substrate whose metabolism may be related to the polymorphic oxidation of debrisoquine [29].

A dose-dependent impairment of metoprolol elimination by histamine in the isolated perfused rat liver was observed. These data indicate that, although histamine is a relative polar compound (99.6% ionized at pH 7.4 [15]), it can enter hepatocytes and attain concentrations sufficient to cause inhibition of drug metabolism.

The physiological relevance of our findings remains to be determined. Hepatocytes are exposed to histamine through the uptake from the portal vein and hepatic artery and through degranulation of hepatic mast cells. The mesenteries of animals and man are rich in mast cells and this tissue may, therefore, be a major source of hepatic histamine [30]. It has been shown that histamine is taken up avidly by the liver after infusion into the portal veins of pigs and dogs [31]. Furthermore, the intact mast cell is probably impervious to histamine [32], suggesting that histamine entering the liver from extrahepatic sources is concentrated in hepatocytes. Although total liver concentrations of histamine are of the order of 5 μg per gram tissue (0.045 mM) [32], concentrations in the hepatic mast cell may be as high as 200 mM*. Thus, *in vivo* release of histamine from mast cells may give rise to concentrations sufficient to cause inhibition of cytochrome P-450-catalysed metabolism.

Many compounds including some drugs cause the release of histamine *in vivo* [33]. Furthermore, it is established that certain pathophysiological states lead to elevated concentrations of histamine. Whether drug- or disease-induced histamine release is associated with inhibition of drug metabolism is not known.

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* This value has been estimated from published data for the histamine content (34 pg per cell; Ref. 34) and volume (1440 μm^3; Ref. 35) of mast cells.

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