Monoamine oxidase inhibitors and histamine metabolism

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Summary. In the rat, histamine metabolism is altered by some nonspecific inhibitors of monoamine oxidase (MAO) such as iproniazid, and, to a lesser extent, tranylcypromine. Type A MAO inhibitors, such as clorgyline and MD780515, do not seem to interfere with the metabolism of histamine. Deprenyl, a type B MAO inhibitor, shows some inhibition which is, however, much lower than that observed with iproniazid. The strong effect of iproniazid is probably due to its DAO inhibiting properties.

Histamine is metabolized through 2 major pathways[2-5]: a) oxidation, catalyzed by diamine oxidase (DAO), to imidazole acetic acid, partly excreted as a conjugate with ribose. This pathway is almost completely inhibited by aminoguanidine, semi-carbazide and hydrazide derivatives, such as isoniazid and iproniazid[6-8]; b) methylation by N-methyltransferase to form tele-methylhistamine (τ-Me) according to the nomenclature introduced by Black and Ganellin[9]; the latter is mainly oxidized to methyl-imidazole acetic acid by monoamine oxidase (MAO). Iproniazid, an MAO inhibitor, inhibits oxidation of τ-Me in vivo[6].

As histamine is one of the amines present at high concentrations in some common food stuffs, particularly in yeast extracts, sauerkraut juice, and in canned, dried, salted and smoked fish[10], it is important to know whether MAO inhibitors other than those of the hydrazide type interfere with the metabolism of histamine. Adverse reactions to hydrazide derivatives have in fact been observed on ingestion of fish with a high histamine content[11] and on ingestion of cheese[12]. According to Blackwell and Marley[13], histamine-like effects in the cat could be attributed to the absorption of a histamine-like substance from yeast extracts in the intestine, which was facilitated by inhibition of MAO by nialamide and tranylcypromine. Moreover, as τ-Me seems to be selectively degraded by MAO B in the rat brain[14-17] and in the liver[18], it is also interesting to study whether MAO A or B inhibitors have different effects on histamine metabolism. The purpose of this work was therefore to study to what extent nonspecific MAO inhibitors (iproniazid, tranylcypromine) and specific MAO A (clorgyline, MD780515) or MAO B (deprenyl) inhibitors may affect the urinary levels of histamine and τ-Me after oral administration of histamine in the rat. Aminoguanidine has also been studied either alone or in association with MAO inhibitors.

Materials and methods. Histamine-2-^{14}C dihydrochloride, sp.act. 40-50 mCi/mmole, was purchased from CEA (Saclay), France. τ-Me dihydrochloride, iproniazid phosphate and tranylcypromine [trans-(±)-2-phenylcyclopropylamine] hydrochloride were purchased from Sigma and aminoguanidine bicarbonate from Koch-Light Laboratories. Clorgyline hydrochloride, dl-deprenyl hydrochloride and MD780515 [3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone] were synthesized in Delalande Research Centre. The thin layer plates used were Silicagel Merck 60 F_{254}. Autoradiography of the plates has been carried out using Kodirex films (Kodak). Liquid scintillation counting systems used were Unisolve (Koch-Light Laboratories) for urine samples and Unisolve/methanol (v/v = 10/2) for silicagel samples scraped from the plates.

Animal treatment. 85 male Sprague Dawley rats (average body weight 157.5 ± 1.6 g, SEM) have been used (Charles River CD®, France). Groups of 5 animals received orally

Urinary elimination of histamine and tele-methylhistamine after administration of MAO inhibitors

Compound	Radioactivity eliminated in 24-h urine (% of the dose ± SEM)	Histamine (% of the dose ± SEM)	Telemethylhistamine (% of the dose ± SEM)
Controls	79 ± 4	0.23 ± 0.03	2.46 ± 0.32
MD780515 10 mg/kg	82 ± 5	0.20 ± 0.02 (n.s., 1)	2.94 ± 0.38 (n.s., 1)
Controls	66 ± 5	0.15 ± 0.01	1.74 ± 0.19
Clorgyline 10 mg/kg	75 ± 3	0.39 ± 0.03 (s., 1)	2.98 ± 0.53 (n.s., 1)
Tranylcypromine 5 mg/kg	66 ± 2	0.28 ± 0.03 (s., 1)	3.29 ± 0.50 (s., 1)
Aminoguanidine 20 mg/kg	71 ± 4	1.28 ± 0.12	23.49 ± 2.80
Aminoguanidine 20 mg/kg + clorgyline 10 mg/kg	76 ± 3	1.43 ± 0.13 (n.s., 1)	28.15 ± 2.17 (n.s., 1)
Aminoguanidine 20 mg/kg + deprenyl 45 mg/kg	76 ± 3	2.80 ± 0.16 (s., 1)	28.96 ± 2.61 (n.s., 1)
Controls	76 ± 3	0.18 ± 0.03	2.73 ± 0.39
Deprenyl 45 mg/kg	67 ± 4	0.74 ± 0.12 (s., 2)	5.89 ± 0.98 (s., 1)
Aminoguanidine 20 mg/kg**	69 ± 4	1.29 ± 0.25	36.14 ± 2.67
Aminoguanidine 20 mg/kg + tranylcypromine 5 mg/kg	68 ± 2	1.20 ± 0.14 (n.s., 1)	49.69 ± 3.78 (s., 1)
Aminoguanidine 20 mg/kg + MD780515 10 mg/kg	72 ± 4	1.91 ± 0.26 (n.s., 1)	35.75 ± 3.42 (n.s., 1)
Controls	80 ± 4	0.31 ± 0.02	3.93 ± 0.47
Iproniazid 100 mg/kg	77 ± 4	1.00 ± 0.07 (s., 2)	48.52 ± 3.11 (s., 2)
MD780515 10 mg/kg	84 ± 1	0.34 ± 0.02 (n.s., 2)	5.08 ± 0.58 (n.s., 2)
Deprenyl 45 mg/kg	80 ± 4	0.80 ± 0.10 (s., 2)	3.29 ± 0.25 (n.s., 2)

** Comparison between aminoguanidine and controls is significant, both for histamine (2) and telemethylhistamine (2). s. = significantly different; n.s. = not significantly different; 1, Dunnett-test; 2, Steel-test.

the drug (in 0.5% aqueous solution of methylcellulose or in water); the doses administered are indicated in the table, and expressed as base. The doses of all the MAO inhibitors except iproniazid were equal or superior to the ED_{50} values for the potentiation of the behavioural syndrome induced by DL-5-hydroxytryptophan in the rat[19]. The dose of iproniazid used in the present study was lower than its ED^{50} value in this test, producing potentiation in 20% of the animals tested. For MD780515, at the dose studied, the compound can be considered to be a specific MAO type A inhibitor[20,21]. 30 min later, histamine was administered orally at the dose (base) of 1.25 mg/kg (5 µCi/rat); urine was collected during 24 h after histamine administration. Control groups received the aqueous solution of methylcellulose or water, and 30 min later histamine.

Extraction of τ-Me and chromatographic separation. Urine was extracted as described by Shore et al.[22] with minor modifications. Briefly, 5 ml urine were saturated with 2 g NaCl, then 100 µl of 5 N NaOH were added. Extraction was carried out into 12.5 ml n-butanol by shaking for 10 min. After 10 min centrifugation at 3000 × g, 10 ml of the organic phase were evaporated to dryness under nitrogen. The residue was dissolved in 0.5 ml of 0.005 N HCl and 20 µl spotted on thin layer plates, together with standard samples of histamine and τ-Me; the plates were then chromatographed in the solvent system 1: chloroform/benzene/methanol/ammonia 20% (15/15/17/2. v/v/v/v)[23], and the solvent system 2: t-butanol/methylethylketone/ammonia 20%/H_2O (40/30/10/20, v/v/v/v). The plates were put in contact with the films for 5 days, then a ninhydrin reagent was sprayed[24], which detected both histamine and τ-Me. Once localized on plates, each amine was scraped off and the radioactivity of the corresponding area measured by liquid scintillation counting.

Mass spectra of τ-Me isolated from urine. 24-h urine from 20 rats pretreated with iproniazid and histamine were collected and extracted as described previously. After evaporation of the organic phase, the residue was dissolved in 25 ml 0.06 N HCl. The solution was passed over a column of Dowex® 50 W resin (X4 200–400 mesh H^+-Form), which was then repeatedly washed with water. After elution with 10 N ammonia and evaporation to dryness, 1 ml of 0.005 N HCl was added, the solution was spotted on thin layer plates, chromatographed in solvent system 2 and put in contact with the radiographic film. The radioactive band having the Rf of τ-Me was scraped from the plates, the silica washed with water/methanol (v/v, 50/50) and the radioactivity extracted with methanol/ammonia 20% (v/v, 85/15) followed by centrifugation. The supernatant was evaporated to dryness, 0.5 ml of 0.005 N HCl was added, and the solution spotted on thin layer plates to be chromatographed in solvent system 1. The radioactive band having the R_f of τ-Me was scraped from the plates, the silica washed. The radioactivity was extracted from the silica, and filtered on a Millipore filter (Mitex, 5 µm, LS-WP). The filtrate was evaporated to dryness, and 0.5 ml of 0.005 N HCl was added. The solution was filtered on Sephadex G-10 and eluted with ammonia (2% aqueous solution). The mass spectrum was run with an AEI MS50 instrument equipped with a DS50 data system under the following conditions: ionizing energy 50 eV, filament current 500 µA, source temperature 150 °C (sample introduced using a direct insertion probe).

Statistical analysis. Comparisons were made with controls or aminoguanidine-treated rats when appropriate. The results were analyzed for statistical significance using the Dunnett-test[25] when the variances were homogenous, and the Steel-test[26] when significant heterogeneity of variances [Bartlett-test[27]] was present. In all cases the significance level chosen was $p \leq 0.05$.

Efficiency of the extraction procedure. A known amount of ^{14}C-τ-Me isolated from urine of iproniazid and ^{14}C-histamine treated rats was added to the urine of untreated animals, the urine extracted as described above, and the efficiency of τ-Me extraction determined. The extraction procedure used for τ-Me extracted also histamine; the efficiency of histamine extraction has also been established by adding known amount of labelled histamine to urine of untreated rats.

Results and discussion. Extraction efficiency for histamine was 78.0% ± 0.4% SD (n = 4) and for τ-Me 59.7% ± 1.9% SD (n = 4). The R_f values for histamine and τ-Me in solvent system 1 are 0.16 and 0.33 respectively, and in solvent system 2, 0.59 and 0.48. The mass spectrum of the radioactive area having the R_f of τ-Me in the 2 solvent systems used showed a low intensity molecular ion m/e = 125 (2%) and a base peak m/e = 96 resulting from β cleavage with concomitant hydrogen rearrangement. Other diagnostic ions were found at m/e = 95 (15%), 81 (48%), 68 (10%) and 30 (16%), in accordance with published data[28]. Results obtained for urinary histamine and τ-Me are presented in the table. The data in the table are corrected for recovery.

At the dose studied, iproniazid, a nonspecific MAO inhibitor, which is also an inhibitor of DAO[8,29], increased significantly the levels of τ-Me and, to a lesser extent, of histamine in urine; aminoguanidine, an inhibitor of DAO but not of MAO, increased also significantly the levels of τ-Me and, to a lesser extent, histamine. It is possible that, as soon as histamine is accumulated by iproniazid or aminoguanidine treatment, it is extensively methylated by the high levels of histamine N-methyltransferase found in several tissues, particularly liver[29] and kidney[30]. Among the other MAO inhibitors tested, clorgyline and MD780515 did not increase significantly the τ-Me levels, whereas tranylcypromine induced a small but statistically significant increase. Deprenyl produced a significant increase only in 1 of the 2 experiments carried out with this compound. The levels of τ-Me were also not significantly increased when clorgyline, MD780515 and deprenyl were associated with aminoguanidine, compared to the levels obtained in the group treated with aminoguanidine alone. When tranylcypromine was associated with aminoguanidine, the levels of τ-Me were significantly increased. Similar results were obtained by Kilgallon et al.[31]: these authors found an increase in the levels of τ-Me in urine of rats treated with pargyline and aminoguanidine compared to rats treated with aminoguanidine alone.

Concerning the effects of MAO inhibitors on histamine levels, MD780515 had no effect, whereas deprenyl, tranylcypromine and clorgyline showed a statistically significant increase. When the MAO inhibitors were associated with aminoguanidine, the levels of histamine were significantly increased only in the case of deprenyl.

The fact that tranylcypromine and deprenyl have an effect on both histamine and τ-Me, and that clorgyline increases histamine levels might indicate, by comparison with iproniazid and aminoguanidine, some inhibitory properties of these compounds on DAO as well. MD780515, at least at the dose studied, seems to be devoid of any effect.

Methylation is a major route of inactivation of histamine in most species, as τ-Me is devoid of histamine-like activity[18,32,33]. However, an increase of τ-Me in urine following MAO inhibitors might indicate that the compounds are also inhibitors of DAO and therefore that histamine levels might be higher than usual in some target tissues, even if temporarily, with subsequent local histamine-effects. It is also useful to remember that τ-Me is an inhibitor of histamine methylation in vivo[29], therefore an accumulation of τ-Me might produce as a secondary effect an increase of histamine.

Further work must be carried out on histamine metabolism using additional A, B or mixed type MAO inhibitors and different doses of histamine, as the dose of the amine may be an important parameter[34]. However, results of previous work[12,30], strengthened by these data, suggest that type A MAO inhibitors should interfere less with the metabolism of histamine than other MAO inhibitors.

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The effect of pH and methylation on the interaction of deoxycholate with rat liver alcohol dehydrogenase[1]

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Summary. The activation of rat liver alcohol dehydrogenase by deoxycholate depends on the anionic form of the steroid. Methylation of the enzyme protein leads to an increase of both turnover number and K_m for ethanol and to a change in the effect of deoxycholate, which behaves as an inhibitor. It is suggested that the steroid and methylation effects depend on the same basic mechanism, in which one or more Lys groups are involved.

Deoxycholate is a modifier of liver alcohol dehydrogenase and its effect is species-dependent[2-4]. The modification of the rat liver enzyme has been specially studied with reference to its kinetic features[5]. In this case the overall effect is an activation, and the kinetic changes caused by the steroid are very similar to those observed for the horse enzyme after chemical modification of Lys residues[6,7]. The present work aims at elucidating the nature of the enzyme-steroid interaction, by studying the effects of deoxycholate on rat liver alcohol dehydrogenase at different pH's and after methylation of Lys groups.

Materials and methods. Alcohol dehydrogenase was purified from the liver of Sprague-Dawley COBS male albino rats as described previously[2,5]. The assay of the enzyme was carried out at 30 °C by recording the change of optical density at 340 nm with a Gilford 2400 spectrophotometer, in a test mixture with the following composition: a) 50 mM triethanolamine buffer, pH 7.6, 5 mM EDTA, 0.2 mM NADH, 2 mM propionaldehyde; b) 50 mM sodium pyrophosphate buffer, pH 8.5, 1 mM NAD^+, 9 mM ethanol. The assay of the enzyme at different pH values was carried out using a 80 mM sodium phosphate-Tris-glycine buffer. 1 unit (U) of the enzyme corresponds to the enzymatic activity that transforms 1 µmole substrate per min, under the test conditions. Proteins were determined by the absorbance at 280 nm[8]. Methylation of alcohol dehydrogenase with formaldehyde in the presence of sodium borohydride was carried out at 0 °C as described by Means and Feeney[9], in 100 mM pyrophosphate buffer pH 9.0, with a final ratio of 6 µmoles formaldehyde/mg protein. After methylation the sample was supplemented with 0.4 mg/ml dithioerythritol and dialyzed overnight against the same buffer.

Results and discussion. Figure 1 shows the effect of pH on the activity of rat liver alcohol dehydrogenase. The initial reaction rate in the absence of bile acids declines sharply between pH 6.3 and 8.7. The activity in the presence of deoxycholate reaches a maximum between pH 6.9 and 7.2. At higher pH values the enzyme is always activated by deoxycholate, while at lower pH values this activation effect decreases and it is reversed into inhibition below pH 6.5. These data suggest that the activation effect on the enzyme could depend on the anionic form of the deoxy-