Inhibition of histamine methylation *in vivo* by drugs

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COMPOUNDS inhibiting specific pathways of histamine formation and catabolism *in vivo* have been useful tools in studies of histamine transformation. The major pathway of histamine metabolism in many species involves ring N-methylation. It has been previously shown that drugs such as chlorpromazine, serotonin, and brom-lysergic acid diethylamide inhibit histamine N-methyl transferase *in vitro*.[1] Employing large doses of chlorpromazine, White[2] was able to attain a small inhibition of histamine methylation in perfused cat brains. This report will describe the effects of various drugs on histamine metabolism *in vivo*. The action of drugs on histamine metabolism was examined by measuring their effect on the disappearance of administered ^{14}C-histamine and the appearance of ^{14}C-methylhistamine in the whole mouse.

METHODS

Drug dosages are given in Table 1. All drugs were administered intraperitoneally 15 min before the intravenous injection of 10 μg of histamine-2-^{14}C-HCl (40 μc/mg), except for chlorpromazine, imipramine, reserpine, and β-phenylisopropylhydrazine which were given intraperitoneally 1 hr before ^{14}C-histamine. Ten mice were used in each group. Animals were decapitated 10 min after intravenous ^{14}C-histamine and homogenized in a Waring blender with 100 ml of ice-cold 0·4 N perchloric acid. Aliquots were assayed for ^{14}C-histamine and ^{14}C-methylhistamine by the following method which involved extraction into two solvent systems, butanol : chloroform (3 : 2) and chloroform, at an alkaline pH. Both histamine and methylhistamine are extracted in the former solvent while in the latter only methylhistamine is extracted. 2-ml aliquots of the perchloric acid extracts of the whole mouse in each of two glass-stoppered centrifuge tubes were made alkaline with 0·3 ml of 5 N NaOH, saturated with sodium chloride, and extracted respectively with 6 ml of a 3 : 2 mixture of *n*-butanol and chloroform or with 6 ml of chloroform. 3-ml aliquots of the organic phases were transferred to counting vials and evaporated to dryness in a stream of hot air. 3 ml of ethanol and 10 ml of phosphor were added, and the radioactivity was measured in a liquid scintillation spectrometer. By this extraction procedure about 90% of both histamine and methylhistamine was extracted into the butanol : chloroform mixture, while 80% of methylhistamine and 6% of histamine were extracted into chloroform. With this information it was possible to calculate, from differential extractions of whole mouse extracts into the two solvents, the percentages of ^{14}C-histamine and ^{14}C-methylhistamine in each sample. Paper chromatograms of butanol : chloroform and chloroform extracts of the whole mouse after ^{14}C-histamine were developed in ethyl acetate : butanol : acetic acid : H_2O (1 : 1 : 1 : 1) or ethanol : 0·1 N HCl (95 : 5). Both of these solvents afford a clear-cut separation of histamine from methylhistamine. The proportion of radioactivity present in the peaks corresponding to histamine and methylhistamine, in all cases, was the same as that obtained by the differential extraction procedure.

RESULTS AND DISCUSSION

Ten minutes after its injection, 14–24% of the circulating ^{14}C-histamine remained in the whole mouse (Table 1). At this time 10–22% of the metabolized histamine was present as methylhistamine. In the mice treated with chlorpromazine, serotonin, bufotenine, and aminoguanidine, the disappearance of histamine was markedly slowed. The amount of methylhistamine found was reduced when chlorpromazine, serotonin, and bufotenine were given and elevated when aminoguanidine and β-phenylisopropylhydrazine were administered. Chlorpromazine, serotonin, and bufotenine have been previously shown to be inhibitors of histamine-N-methyl transferase *in vitro*.[1, 3] The effects of these drugs on histamine metabolism indicated that these compounds inhibit histamine methylation *in vivo*. Aminoguanidine, a known inhibitor of diamine oxidase, would be expected to block the disappearance of administered histamine. The elevation of methylhistamine after aminoguanidine treatment could represent a diversion of histamine metabolism into the methylation pathway or may indicate inhibition of methylhistamine catabolism. β-Phenylisopropylhydrazine, a potent monoamine oxidase inhibitor, had no effect on histamine disappearance but slowed the disappearance of methylhistamine, indicating that monoamine oxidase is involved in the metabolism of methylhistamine but not of histamine. The extent to which methylhistamine is metabolized by diamine oxidase and monoamine oxidase is under investigation in this laboratory. Rothschild and Schayer[4] administered

^{14}C-methylhistamine to mice pretreated with aminoguanidine or 1-isobutyl-2-isonicotinylhydrazine (IBINH), a monoamine oxidase inhibitor. Urinary ^{14}C-methylimidazoleacetic acid was depressed in animals treated with IBINH and was at normal levels in animals given aminoguanidine. These authors concluded that methylhistamine and histamine are oxidized by different enzymes in mice.

TABLE 1. EFFECT OF DRUGS ON THE METABOLISM OF ^{14}C-HISTAMINE *in vivo*

Drug	Dose (mg/kg)	^{14}C-Histamine remaining Control (% of injected dose ± S.E.M.)	^{14}C-Histamine remaining Drug-treated (% of injected dose ± S.E.M.)	^{14}C-Methylhistamine found Control (% of injected dose ± S.E.M.)	^{14}C-Methylhistamine found Drug-treated (% of injected dose ± S.E.M.)
Chlorpromazine	40	24·1 (± 2·9)	32·7 (± 2·3)*	15·3 (± 0·9)	7·8 (± 0·9)†
Serotonin	10	24·1 (± 2·9)	39·4 (± 2·5)†	15·3 (± 0·9)	8·1 (± 1·8)†
Bufotenine	20	14·0 (± 4·2)	37·5 (± 7·8)*	17·5 (± 4·1)	2·7 (± 2·7)†
Aminoguanidine	10	19·3 (± 3·2)	40·0 (± 3·9)†	23·6 (± 0·6)	36·9 (± 4·3)*
β-Phenylisopropyl-hydrazine	25	20·6 (± 3·1)	25·6 (± 5·0)	10·4 (± 1·4)	17·2 (± 3·0)*
Imipramine	40	20·6 (± 3·1)	26·8 (± 6·0)	10·4 (± 1·4)	9·8 (± 1·1)
Compound 48-80	2	21·4 (± 4·3)	28·0 (± 7·9)	22·0 (± 2·7)	22·3 (± 5·6)
Reserpine	5	21·4 (± 4·3)	23·4 (± 4·7)	22·0 (± 2·7)	19·6 (± 4·5)
Pyribenzamine	5	21·4 (± 4·3)	23·4 (± 4·7)	22·0 (± 2·7)	19·6 (± 4·5)
5-Methoxytryptamine	25	20·6 (± 3·1)	27·0 (± 7·0)	10·4 (± 1·4)	6·3 (± 2·3)

Mice were given drugs followed by 10 μc ^{14}C-histamine. After 10 min the mice were killed and assayed for ^{14}C-histamine remaining and ^{14}C-methylhistamine formed.

* $p > 0.05$.

† $p > 0.01$.

Kapeller-Adler and MacFarlane[5] have reported the purification of a single enzyme from hog kidney which is active in oxidizing both histamine and methylhistamine.

The histamine liberator Compound 48-80, reserpine, imipramine, the antihistaminic pyribenzamine, and 5-methoxytryptamine had no effect on histamine metabolism in the intact mouse.

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Interaction of plasma protein with related 1,3-propanediol dicarbamates

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ALTHOUGH many drugs are known to bind to plasma protein,[1-5] relatively few studies have been reported comparing the protein-binding properties of a series of chemically related compounds. This report summarizes the results of such an investigation, in which four pharmacologically active 1,3-propanediol dicarbamates were used. The compounds examined for their ability to bind with human plasma were: carisoprodol, N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate; mebutamate, 2-methyl-2-*sec*-butyl-1,3-propanediol dicarbamate; meprobamate, 2-methyl-2-propyl-1,3-propanediol dicarbamate; tybamate, N-*n*-butyl-2-methyl-2-propyl-1,3-propanediol dicarbamate.