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The effects of two histidine decarboxylase inhibitors in guinea pig anaphylaxis*

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SEVERAL methods of investigation have attested to the role that histamine plays in guinea pig anaphylaxis. Concentration of histamine in blood and plasma increases during anaphylaxis;[1–3] chopped lung from sensitized guinea pigs releases histamine on the addition of antigen;[4] and antihistaminics protect guinea pigs from anaphylactic death.[5] Because histamine is formed by the enzymatic decarboxylation of histidine,[7] inhibition of histidine decarboxylase should result in a decrease in the amount of histamine available for anaphylactic release and hence might modify the anaphylactic reaction. These experiments were performed to see whether α-methyl-5-hydroxytryptophan and α-methyl-3,4-dihydroxyphenylalanine, which are moderately effective *in vitro* histidine decarboxylase inhibitors,[7] would alter either blood, plasma, or tissue histamine concentrations or the anaphylactic reaction.

MATERIALS AND METHODS

Guinea pigs were sensitized with ovalbumin subcutaneously and assigned to treatment or control groups. α-Methyl-5-hydroxytryptophan† (Me5HTP) in 0·9% saline was injected i.p. twice daily for 15 to 20 days in amounts of 0·1 to 0·9 g/kg/per day to 17 animals. α-Methyl-3,4-dihydroxyphenylalanine‡ (methyldopa) in 0·1 N HCl was given through an oral gastric tube twice daily for 30 days in amounts of 200 mg/kg/ per day to 6 animals. Five control animals received intraperitoneal saline and six received 0·1 N HCl by gastric intubation.

After the treatment period, the animals were subjected to active anaphylaxis by i.v. injection of 5 mg ovalbumin. Fatal anaphylaxis was judged to have been present when death, preceded by respiratory distress and restlessness, occurred within 5 min of antigen administration. All animals which survived the antigenic challenge were exsanguinated. Specimens of the tissues were obtained for microscopic study. Fluorometric assays of tissue histamine content were performed in most of the animals given Me5HTP.[8]

Paper chromatography with techniques appropriate for indoles[9] was performed with urine from three animals receiving Me5HTP and three animals receiving saline.

RESULTS AND DISCUSSION

Table 1 summarizes the results of drug treatment and antigen challenge. A number of animals did not survive the treatment period to be challenged; 26 guinea pigs were challenged with the antigen,

TABLE 1. EFFECT OF HISTIDINE DECARBOXYLASE INHIBITORS ON ANAPHYLAXIS IN GUINEA PIGS

Group	Maximal daily dose (g/kg)	Initial no. of animals	Died during study	Anaphylaxis	
				Attempted	Fatal
Me5HTP–I	0·10	5	0	5	5
Me5HTP–II	0·45	6	2	4	3
Me5HTP–III	0·90	6	4	2	2
Me5HTP control	NaCl	5	0	5	5
Methyldopa	0·20	6	2	4	3
Methyldopa control	0·1 NHCl	6	0	6	4

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‡ Lot C-2993; kindly supplied by Merck, Sharpe & Dohme Research Laboratories, West Point, Pa.

and all but 4 succumbed to anaphylaxis. Hyperinflated pale lungs were found at autopsy in all 26 of these animals and examination of tissue sections showed bronchiolar constriction, alveolar dilation, and ruptured alveoli. An acute inflammatory exudate was found in the lungs of many of the methyldopa-treated animals and their controls. No pathologic alterations were found that could be specifically attributed to the drugs.

Histamine concentrations of the major organs from most of the animals given Me5HTP are shown in Fig. 1. All the animals given large doses of Me5HTP (Table 1, groups II and III) were so studied. It is clear that histamine concentrations of the tissues of the experimental animals coincided closely with controls, and no significant depletion of tissue histamine stores followed the use of this potent histidine decarboxylase inhibitor *in vitro*.

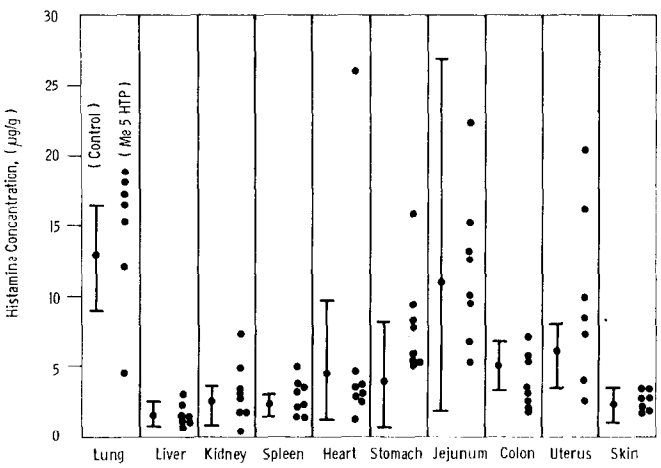

FIG. 1. Tissue histamine concentrations after anaphylaxis. In each compartment the range and mean for three control animals are indicated on the left; each dot on the right represents a determination from an organ of an animal treated with Me5HTP.

On paper chromatography Me5HTP was easily detected as a pink fluorescent spot under u.v light, which gave an initially purple color with Ehrlich's reagent that later changed to brown or dark blue. A smaller secondary spot of green fluorescence under u.v. light gave no Ehrlich reaction. Thirty min after i.p. Me5HTP, the urine contained a large amount of unchanged Me5HTP. Identification of the compound in the urine was made by comparison of color reactions and migration in two solvent pairs. Two other Ehrlich-reacting substances which were not specifically identified also appeared in the urine 30 min after Me5HTP injection. One was probably kynurenine, and the other had staining and mobility characteristics similar to those of 5-hydroxytryptamine, suggesting that it might be α-methyl-5-hydroxytryptamine, the expected product of Me5HTP decarboxylation. The amounts of Me5HTP and the two other Ehrlich reactors in the urine reached a maximum 1 hr after injection. No indoles taking an Ehrlich stain were found in the urine of saline-treated guinea pigs or in the urine of Me5HTP-treated animals 18 hr after injection.

Histamine concentrations in tissue were not altered by methyldopa or Me5HTP despite the previously described inhibitory effects *in vitro* of these compounds on histidine decarboxylase. This may be explained as due to inadequate inhibition *in vivo* by the drugs, or persistence of previously formed histamine, or a combination of these factors.

Two different decarboxylases are active in the formation of histamine. Nonspecific amino acid decarboxylase such as that found in guinea pig kidney[10] is more efficiently inhibited by Me5HTP and methyldopa than is the specific histidine decarboxylase found in mast cells, some rapidly growing tissues, and the transplantable rat hepatoma.[7] Me5HTP is a reasonably active inhibitor of the latter enzyme, but methyldopa had little inhibitory effect in the studies cited above. Schayer[6] estimated that

the tissue half-life of bound histamine was in excess of 50 days; consequently, prolonged administration of a potential histidine decarboxylase inhibitor would be necessary to deplete tissue histamine stores. We attempted to anticipate this problem by giving large amounts of drug for several weeks. Failure to detect any depletion of tissue histamine in this study makes it unlikely that longer treatment with higher doses of these drugs would significantly change the results.

Me5HTP was rapidly absorbed and excreted; it appeared in the urine 30 min after i.p. administration and was completely absent 18 hr later. Since the drug was in the DL form, there might have been rapid excretion of nonmetabolized *dextro*-form. Nevertheless, the large amount excreted and the paucity of metabolites suggested that the *levo*-form was also little metabolized. Two other indoles appeared in the urine after Me5HTP administration. We suspect that one was α-methyl-5-hydroxytryptamine, the expected product of Me5HTP decarboxylation.

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The effect of homogenization conditions on sub-cellular distribution in brain

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THE purpose of this communication is to draw attention to the effect which relatively small changes in homogenization conditions may have on the distribution of bound acetylcholine, and therefore possibly of other components in subcellular fractions derived from brain tissue.

When brain tissue is homogenized in eserine-free 0·32 M sucrose according to our usual procedure,[1,2] about 70–75% of the total acetylcholine of the tissue survives the action of the powerful cholinesterases present in the preparation and is recovered mainly in the crude mitochondrial (P_2) fraction and in a subfraction (B) derived from it, consisting largely of pinched-off presynaptic nerve terminals (synaptosomes). The acetylcholine content of brain fractions prepared in the absence of cholinesterase inhibitors such as eserine may therefore be used as a measure of the survival in the fractions of these organized neuronal elements.