An Alternative Route for Biosynthesis of Methylhistamine: In vitro and in vivo Formation Through Decarboxylation of L-3-Methylhistidine

Methylhistamine (MHA) (1-methyl-4 (β-aminoethyl imidazole) is known as one of the main metabolites of histamine (HA), resulting from the action of histamine-N-methyltransferase[1,2]. The present work offers evidence for an alternative route for the biosynthesis of MHA which consists in the decarboxylation of the naturally-occuring amino acid, L-3-methylhistidine (Mehis). The enzyme involved appears to be histidine decarboxylase (HD).

Methods. Several preparations known to posses high HD activities in vitro were tested for their ability to decarboxylate L-histidine (His) or Mehis: 1. The supernatant fluid obtained by homogenization of rat glandular stomach in 5 volumes of 0.08 *M* phosphate buffer pH 7, followed by centrifugation for 20 min at 10,000 *g*[3]. 2. Mast-cells isolated from rat peritoneal fluid according to the procedure of JOHNSON and MORAN[4]. 3. A soluble preparation of bacterial HD obtained from an acetone-dried powder of *Cl. welchii*[5,6].

Incubations were performed in the presence of tracer amounts of labelled amino acids, i.e. 1–1.5 μCi/ml of ^{3}H-His (The radiochemical Center, Amersham; sp. act. 9.1 Ci/mmole) or ^{3}H-Mehis (New England Nuclear Corp; sp. act. 4.34 Ci/mmole). Both the chemicals have previously been purified by passage onto columns of Amberlite CG 50 followed by evaporation under reduced pressure in order to remove traces of ^{3}H-amines and ^{3}H-water; their purity (⩾ 98%) was checked by thin-layer chromatography in several solvent systems.

At the end of 1 h- or 2 h-incubations, the ^{3}H-histamine formed was assayed by isotope dilution[7]. ^{3}H-methylhistamine was isolated by passage onto an Amberlite CG 50 column followed by solvent extraction[8]. In this two-step purification procedure, the recovery for ^{3}H-MHA was 45–55% while contamination by ^{3}H-Mehis was less than 0.01%. Accordingly, ^{3}H-MHA levels were corrected for these values. The identity of the labelled chemical isolated in this way, at the end of a 2 h-incubation with the rat stomach HD preparation, was assessed by chromatography on cellulose thin-layers: in the 2 solvent systems, i.e. chloroform - methanol - ammonia (12:7:1) and *n*-butanol-acetone-diethylamine-water (70:70:14:35), used either mono-or bi-dimensionally, there was only one radioactive peak, representing at least 80% of the total ^{3}H, which migrated into the same area as authentic MHA. Additionally, extraction from an alkaline medium into chloroform and re-extraction into 0.1 *N* HCl were performed and they gave the same recovery for authentic MHA and for the presumed ^{3}H-MHA.

Results. With all the 3 preparations decarboxylation of Mehis was apparent but occurred more slowly than that of His, especially with the bacterial enzyme (Table I). That HD (EC 4.1.1.22) is probably involved was indicated by the fact that addition of N.S.D. 1055, a potent inhibitor of this enzyme[9], completely prevented at 10^{-4} *M* the formation of ^{3}H-MHA by the rat stomach preparation, although α-methyl DOPA, an inhibitor of L-aromatic aminoacid decarboxylase (EC 4.1.1.26) had no effect at the same concentration.

It was also observed that decarboxylation of Mehis occurred in vivo. Male rats were pretreated with tranylcypromine (10 mg/kg, i.p.) in order to prevent the deamination of MHA by monoamine oxidase[10]. Two h later, 50 μCi of ^{3}H-Mehis were injected i.v. and the animals were sacrificed 30 min or 90 min thereafter. ^{3}H-MHA was assayed in 0.4 *N* ClO_4H extracts from several organs: levels found in liver and urine were not significantly higher than the blanks but were appreciable in the brain and especially in the stomach (Table II) which is known to be by far the richest rat organ for HD ^{3}H-MHA was clearly identified on bi-dimensional thin-layer chromatograms of stomach extracts.

Discussion. Our results show that ^{3}H-MHA can be synthetized in vitro as well as in vivo through decarboxylation of ^{3}H-Mehis. There is evidence for this metabolic pathway in mammalian tissues known for their high HA forming capacity; moreover, this reaction was blocked by an HD inhibitor. Hence, it could be inferred that HD is probably responsible for Mehis decarboxylation.

In vitro, decarboxylation of tracer amounts of Mehis in rat stomach and mast cells was approximately 5 times slower than that of His, suggesting that this reaction could be only of minor biological importance. Nevertheless, free Mehis is known to be present in mammalian tissues, plasma and urine and, in some cases, its levels appeared to be only slightly inferior to those of His[11].

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Table I. Decarboxylation of ^{3}H-His and ^{3}H-Mehis in vitro

Preparation	Incubation time (h)	Decarboxylation of ^{3}H-His (%)	Decarboxylation of ^{3}H-Mehis (%)
Rat stomach supernatant	2	1.74±0.06	0.32±0.05
Rat mast-cells (from peritoneal fluid)	1	0.42±0.05	0.09±0.01
Bacterial HD (from *Cl. welchii*)	1	100±7	0.15±0.02

Incubations were performed at 37°C in the presence of 1–1,5 μCi/ml of either ^{3}H-His or ^{3}H-Mehis. Results are expressed as percentages of the total radioactivity recovered as the corresponding ^{3}H-amines. Means±S.E.M. of 5–7 experiments.

Table II. Formation of ^{3}H-MHA after administration of ^{3}H-Mehis in tranylcypromine-treated rats

Organ	Time (min)	Total ^{3}H (cpm $\times 10^{-3}$)	^{3}H-MHA (cpm)	^{3}H-MHA/Total ^{3}H (%)
Stomach	30	174±13	4,293±356	2.49±0.13
	90	118±25	3,590± 1,263	3.38±1.52
Brain	30	190±15	201± 16	0.11±0.01
	90	168± 8	155± 13	0.09±0.01

Male rats (150—200 g) received an i.p. injection of tranylcypromine (10 mg/kg). 2 h later, they were injected with 50 μCi of ^{3}H-Mehis (i.v.). Means ± S.E.M. from 4 experiments.

Therefore, it seems likely that MHA formation in vivo occurs, at least to a certain extent, by decarboxylation of Mehis, i.e. by-passing the histamine step. The relative contribution of this pathway in the overall biosynthesis of MHA would presumably be the greatest in species, such as the rat, in which HA methylation is known to be low[1,2].

This finding may explain why the ratio of endogenous MHA/HA in the urine of several species has been repeatedly found to be much higher than the corresponding ratio of the radioactive amines evaluated after injection of ^{14}C-HA[12,13]. Thus it may be inferred that methylhistamine (or methylimidazolacetic acid) level in urine is not a specific index of histamine release.

Although MHA is known to exhibit a lower biological activity than HA in most organs, on iontophoretic application to cerebral neurones, it modified their firing rate as did HA[14]. Moreover, brain MHA was found to be stored in the same subcellular fractions as the putative neurotransmitter, HA[15]. In conformity with the above finding, preliminary experiments in this laboratory indicated that ^{3}H-MHA synthetized from ^{3}H-Mehis could be released from several tissues under the same conditions as HA.

Taken together, these observations suggest that methylhistamine should not be discarded as a mere inactivation product of histamine.

Résumé. On a mis en évidence chez le Rat une formation de méthylhistamine-^{3}H par décarboxylation de la L-3-méthylhistidine-^{3}H. Cette réaction se produit in vitro et in vivo dans des tissus riches en histidine décarboxylase et elle est prévenue par un inhibiteur de cette enzyme. Les implications biologiques de l'existence de cette nouvelle voie métabolique sont envisagées.

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Serum Dopamine-β-Hydroxylase in Spontaneously Hypertensive Rats

Spontaneously hypertensive (SH) rats (a Wistar strain, which had been developed by Okamoto and Aoki[1]) seem to be a good model for human essential hypertension. The relationship of essential hypertension to abnormal adrenergic function is not yet clear, but there are several indications that SH rats may have abnormality in the enzymes of norepinephrine biosynthesis and metabolism. SH rats were more sensitive to the hypotensive effect of a tyrosine hydroxylase inhibitor, oudenone[2–4], and a dopamine-β-hydroxylase inhibitor, fusaric acid[5,6], and had 2-fold increase in tyrosine hydroxylase and dopamine-β-hydroxylase activities and about 1.3-fold increase in DOPA decarboxylase and phenylethanolamine-N-methyltransferase activities in adrenal glands[7,8]. In contrast, tyrosine hydroxylase activity in mesenteric activity was decreased[9], that in brainstem and heart was normal[7,9,10], and DOPA decarboxylase activity in brainstem was decreased[10].

Dopamine-β-hydroxylase has been found in rat serum[11,12]. The enzyme is supposed to be secreted from sympathetic nerves and adrenal medulla together with norepinephrine or epinephrine and could be an indicator of the activity of peripheral sympathetic nerves. To investigate wheter there is any change in the activity of peripheral sympathetic nerves in SH rats, the activity of serum dopamine-β-hydroxylase of SH rats has been compared to that of normotensive Wistar rats.

The SH rats were 16 weeks old, with blood pressures between 160 and 200 mmHg. Normotensive Wistar rats of the same age raised in the same conditions were used as controls. Serum dopamine-β-hydroxylase activity was measured by the radioassay of Weinshilboum and Axelrod[11]. Blood samples were obtained from the rats by decapitating and exsanguinating them into a test tube kept on ice. Serum was removed after centrifuging the blood at 10,000 $\times g$ for 10 min. To 0.5 ml of serum were added 2 ml of a saturated ammonium sulphate solution (pH 6.7). The mixture was centrifuged at 15,000 $\times g$ for 15 min. The precipitate was dissolved in 0.5 ml of 20 mM potassium phosphate buffer (pH 6.8) and dialyzed against 1 l of the same buffer for 15 h. The dialyzed solution was centrifuged at 15,000 $\times g$ for 15 min, and the supernatant was adjusted to 1.0 ml with the buffer. This partial isolation procedure of dopamine-β-hydroxylase eliminates some endogenous inhibitors and permits the determination of the enzyme activity which is proportional to the