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

amount of the enzyme. Incubation mixture (310 µl) contained: enzyme, 100 µl; water 100 µl; 1 *M Tris*-HCl buffer, pH 6.0 10 µl; 40 m*M* ascorbic acid in the *Tris* buffer, 30 µl; 40 m*M* sodium fumarate, 30 µl; 6 m*M* pargyline, 10 µl; 1 m*M* $CuSO_4$, 10 µl; 30 m*M* tyramine, 10 µl; and catalase (5 mg crystals in 2 ml of water), 10 µl. Incubation was carried out at 37 °C for 20 min. After the incubation the following reaction mixtures were added: 1 *M Tris*-HCl buffer, pH 8.6, 80 µl; 0.5 m*M* EDTA, 20 µl; phenylethanolamine-N-methyltransferase partially purified from bovine adrenal medulla by the method of CONNETT and KIRSHNER[13] (0.17 nmoles/min/mg protein, 12 mg/ml), 10 µl; S-adenosylmethionine-(methyl-C^{14}), 10 µl (0.1 µCi, 1.8 nmoles). The incubation was continued further for 30 min at 37 °C. The radioactive N-methyl-octopamine formed was extracted into toluene-isoamyl alcohol (3:2, v/v) and counted. Boiled enzyme (95°C, 5 min) was used for the blank incubation. Octopamine, 0.4 nmole, was added into a reaction mixture as an internal standard.

The results are shown in the Table. Serum dopamine-β-hydroxylase activities of male or female SH rats were slightly lower than those of normotensive male or female Wistar rats, but the differences were not statistically significant. The enzyme activities of female rats of normotensive Wistar or SH strains were slightly lower than those of male rats of normotensive Wistar or SH strains, but the differences were not statistically significant. The results suggest that the increase in dopamine-β-hydroxylase in adrenal glands of SH rats[7] does not cause the elevation of the level of the serum enzyme and that the rate of secretion of the enzyme from the peripheral sympathetic nerves of SH rats may not be different from that of normotensive Wistar rats[14].

Serum dopamine-β-hydroxylase activity of normotensive wistar rats and spontaneously hypertensive rats

Enzyme activity	Normotensive wistar rats	Spontaneously hypertensive rats
Male (numbers)	(5)	(9)
*p*moles/min/ml serum±S.E.M.	76 ±17	52 ±12
*p*moles/min/mg protein±S.E.M.	1.7± 0.3	1.1± 0.3
Female (numbers)	(5)	(8)
*p*moles/min/ml serum±S.E.M.	58 ±22	47 ± 9
*p*moles/min/mg protein±S.E.M.	1.0± 0.4	1.0± 0.2

Zusammenfassung. Es wird gezeigt, dass die Dopamin-β-Hydroxylase-Aktivität im Serum bei spontan hypertonischen Ratten im Vergleich zu normotonen Kontrollen nicht verändert ist.

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The Affinity of Mitochondria for Ca^{++}

It has been shown that the transport of Ca^{++} and the phosphorylation of ADP in mitochondria are alternative processes[1]. It is thus reasonable to suggest that the former may be a means of regulating the concentration of Ca^{++} in the cytosol, and affecting a number of Ca^{++} dependent reactions. Among these, the contraction and relaxation of heart is particularly important. Indeed, whereas the role of the sarcoplasmic reticulum (SR) in the contraction and relaxation of fast skeletal muscle seems well established[2-4], several lines of evidence point to its inefficiency in cardiac muscle, and indicate that mitochondria could play a role in the process. In heart, SR is poorly developed, and has a very limited capability for Ca^{++} transport[5].

Mitochondria, on the other hand, are very abundant, and are capable of efficient Ca^{++} transport both in vitro[6-8] and in vivo[9]. An important factor in assessing their possible role in the relaxation and contraction of heart is their affinity for Ca^{++}. Since contraction begins when the free Ca^{++} concentration in the sarcoplasm approaches $10^{-7}M$, mitochondria must be able to take up Ca^{++} with reasonable efficiency at concentrations not far removed from this level. Most measurements of the mitochondrial K_m for Ca^{++}, based on the shift of the redox state of cytochrome b, or on the rate of O_2 consumption, have, however, yielded figures around $5 \times 10^{-5}M$[10], clearly inconsistent with a role in heart relaxation. However, since the uptake of Ca^{++} by mitochondria is