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Distribution of Histamine-N-methyltransferase in Nature

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A survey has been made on the distribution of histamine-N-methyltransferase in various types of living material. The enzyme, which catalyzes the methyl group transfer from S-adenosylmethionine to histamine, has been found in the livers of all vertebrates investigated except rat. Other organs of the animals as brain, lung, and kidney also contain significant enzyme activity. By the methods used no enzyme activity could be found in the invertebrates, plants, or microorganisms studied.

Some supernatant fractions of liver were also tested for their possible ability to catalyze the N-methylation of tryptamine and serotonin. Of the animals tested only rat showed a slight ability to methylate both amines.

Enzyme preparations from fish liver have been shown to be much more heat labile than those from other vertebrates tested.

In previous communications from this laboratory a purification procedure as well as some mechanism studies of histamine-N-methyltransferase have been reported.[1,2] It is the purpose of this paper to give a survey of the histamine-N-methyltransferase activity in various types of living material. The enzyme has previously been detected in some mammals and in *Bufo marinus*.[3-5]

MATERIAL AND METHODS

Material of animal origin to be tested for enzyme activity was homogenized in a Potter-Elvehjem homogenizer or in a MSE ATOMIX. Some of the plant material and the microorganisms were crushed in a mortar containing sand and buffer. 0.1 M sodium acetate buffer, pH 5.6, or 0.05·M sodium phosphate buffer, pH 7.5, were used in all procedures. Cell debris and insoluble material were centrifuged off and the supernatants were used for testing enzyme activity.

Histamine-2(ring)-^{14}C (The Radiochemical Center, England) was used as substrate for the methylation reaction. S-Adenosylmethionine (AMe) was prepared from bakers' yeast as described by Schlenk *et al.*[6]

A reaction mixture consisting of 0.0164 μmole histamine-^{14}C, 0.163 μmole AMe, 500 μl supernatant and 100 μl Tris buffer, pH 8.1 was incubated for 1-5 h in test tubes. The incubation temperature was 37°C and in some cases 25°C. Methylhistamine obtained was determined as previously described.[1] The protein content of the different preparations was estimated according to Kalckar.[7]

RESULTS

Distribution studies. The results of the investigation of histamine-N-methyltransferase activity in various types of living material are summarized in Table 1. A pig liver supernatant fraction was always run as a control of the test system. Under the conditions used it cannot be excluded that the protein fractions contained activators or inhibitors. As can be seen in Table 1 significant activity was detected in the livers of all the vertebrates examined except rat. The most active tissues tested were fish livers. No enzyme activity could be found in the invertebrates or microorganisms studied by using the methods described.

Comparison of temperature stabilities of histamine-N-methyltransferases from different species. Enzyme preparations from pig, hen, frog, and cod were tested for their ability to withstand elevated temperatures for 5 min. Enzyme activity was measured with standard method, and the results are shown in Fig. 1. As can be seen in the figure the transferase of cod liver is much more sensitive to heat than those of pig, hen, and frog. The latter ones can withstand heat denaturation at 40°C for 5 min which may be used in the purification procedure of the enzyme from these animals.

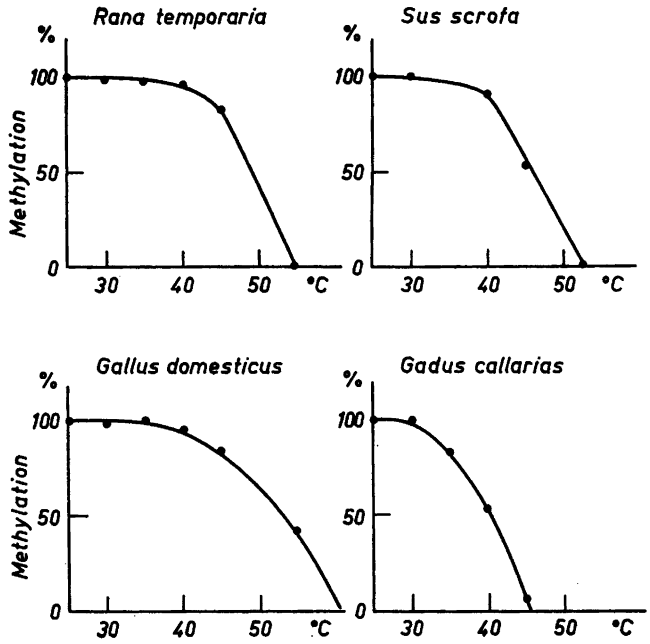

Fig. 1. The temperature stability of histamine-N-methyltransferase from different species. Enzyme solutions were kept for 5 min at the temperatures indicated, and enzyme activity was determined with standard method.[1]

Table 1. Distribution in nature of histamine-N-methyltransferase.

Name	Latin name	Material tested	pH of homogenate	Transferase activity*
Pig	*Sus domesticus*	liver	5.6	16.5
Rat	*Rattus norvegicus*	liver	5.6	0
		»	7.5	0
Hen	*Gallus domesticus*	liver	5.6	17.4
		brain	5.6	1.1
		heart	5.6	37.6
		lung	5.6	9.2
Viper	*Vipera berus*	liver	5.6	2.1
		»	7.5	2.3
		brain	5.6	1.0
Frog	*Rana temporaria*	liver	5.6	13.3
		lung	5.6	39.6
		muscle	5.6	2.8
Cod	*Gadus callarias*	liver	5.6	96.0
Plaice	*Pleuronectes platessa*	»	5.6	80.1
Perch	*Perca fluviatilis*	brain	5.6	34.4
		liver	5.6	43.6
		gills	5.6	6.6
Roach	*Leuciscus rutilis*	liver	5.6	13.7
Bream	*Abramis blicca*	liver	5.6	112.0
		kidney	5.6	9.4
Humble-bee	*Bombus terrestris*	whole	7.5	0
Crab	*Cancer pagurus*	gills	5.6	0
		»	7.5	0
		intestine	5.6	0
		»	7.5	0
		heart	5.6	0
		»	7.5	0
		muscle	5.6	0
		»	7.5	0
Slug	*Arion ater*	liver	7.5	0
		intestine	7.5	0
Pond mussel	*Anodonta cygnea*	liver	7.5	0
		all exept the shell	5.6	0
		»	7.5	0
Starfish	*Asterias sp.*	divertic.	7.5	0
Tomato	*Solanum lycopersicum*	leaves	7.5	0
Tobacco	*Nicotiana rustica*	leaves	7.5	0
		»	5.6	0
	Nicotiana tabacum	leaves	7.5	0
		»	5.6	0
Henbane	*Hyoscyamus niger*	leaves	7.5	0
Belladonna	*Atropa bella-donna*	leaves	7.5	0
Carrot	*Daucus carota*	whole	7.5	0
Pea	*Pisum sativum* var. *arvense*	leaves	7.5	0
Clover	*Trifolium repens*	whole	7.5	0
Hemp	*Cannabis sativa*	leaves	7.5	0
Nettle	*Urtica dioca*	leaves	7.5	0
		»	5.6	0
Poppy	*Papaver somniferum*	leaves	7.5	0
		fruit	7.5	0

Name	Latin name	Material tested	pH of homogenate	Transferase activity*
Chestnut	*Castanea sativa*	leaves	7.5	0
Onion	*Allium sativum*	bulb	7.5	0
	Allium cepa	whole	7.5	0
Barley	*Hordeum distichum*	leaves	7.5	0
		stem	7.5	0
		coleoptiles	7.5	0
		»	5.6	0
Oat	*Avena sativa*	seeds	7.5	0
Millet	*Paniceum miliaceum*	whole	7.5	0
Grass	*Dactylus glomerata*	whole	7.5	0
Larch	*Larix desidua*	leaves	7.5	0
Fern	*Dryopteris Filix-mas*	leaves	7.5	0
Seaweed	*Laminaria saccarina*	whole	7.5	0
Microorganisms	*Penicillium atrovenetum*	»	7.5	0
	Penicillium islandicum	»	7.5	0
	Streptomyses rimosus	»	7.5	0

* μmole methylhistamine formed per g and h $\times 10^5$

Methylation of tryptamine and serotonin. Some of the supernatant fractions were also tested for their possible ability to methylate two other amines, namely tryptamine and serotonin. In the former only N-methylation is possible, but serotonin may also be O-methylated.[8]

To 100 μl of the supernatant fractions were added 0.094 μmole (1.06 μC/μmole) AMe-$^{14}CH_3$, amines to a final concentration of 10^{-3} M and 100 μl 0.5 M Tris buffer, pH 8.1. The reaction mixture was incubated for 60 min at 37°C. The reaction was stopped by adding 0.5 ml of borate buffer, pH 10.0, and 2 ml of isoamylalcohol, previously saturated with water. Methylated products were extracted into the alcohol by shaking for 10 min. After centrifugation an 1-ml aliquot was transferred to a vial containing 2 ml of ethanol and 5 ml of phosphor solution, and the radioactivity measured in a liquid scintillation counter. Control samples, where the amines were omitted, were run at the same time to correct for the small amount of AMe-$^{14}CH_3$ that is extracted into the isoamylalcohol.

Supernatant fractions of liver from pig, hen, frog, cod, and crab were unable to methylate tryptamine or serotonin. Small amounts of methylated products were found when preparations from rat liver and viper liver were used. In the former both amines seemed to be methylated, in the latter only serotonin. No attempts have been made to investigate the products formed.

DISCUSSION

This work, and those of previous investigators, has revealed the presence of histamine-N-methyltransferase in the livers and other organs of a wide range of vertebrates. The absence of activity in rat liver is remarkable and has also been reported from other laboratories.[3-4]

The fact that histamine-N-methyltransferase could not be detected in invertebrates, plants, or microorganisms must not be taken as conclusive evidence for its non-existence in such materials. It may only show that the method used was unsuitable or that the selection of these species is not representative.

Ericson [9] has investigated the distribution of betaine-homocysteine-methyltransferase in nature and found enzymic activity in the livers of all vertebrates examined. Of the invertebrates tested only pond mussel (*Anodonta cygnea*) was found to contain the enzyme, and no significant activity could be detected in plants or microorganisms. Similar results have been reported by Maw, who studied the distribution of thetin-homocysteine transmethylase.[10]

Methyl group transfer from S-adenosylmethionine to various acceptors has been reported to take place both in plants and microorganisms (for survey see Ref.[11]). Tyramine-N-methyltransferase is till now the best investigated enzyme of plant origin.[12] This enzyme catalyzes the methyl group transfer from S-adenosylmethionine to tyramine, forming hordenine.

Histamine is widely distributed in nature.[13] Animals, sensitive to histamine, inactivate the amine by N-methylation or by oxidative deamination.[14] The metabolism of histamine in lower animals, plants and microorganisms is sparingly investigated and it is difficult to find any relation between the distribution of histamine and the occurrence of histamine-inactivating enzymes.

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