there are one and two tryptophan residues in HSA and BSA, respectively, and that one of the two tryptophan residues in the BSA molecule occupies the same position as the residue in the HSA molecule. The maximum binding number was approximately one in this experiment. The above results suggest that the hydrophobic region (intimately related to the binding site of TBM) on the albumin molecule is located in the same position in the HSA and BSA molecules. The possibility that conformational differences of the serum albumin molecule are induced by modification with the three chemicals must be considered. However, this was not investigated in the present study.

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Heterogeneity of Histamine N-Methyltransferase

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The distribution of histamine N-methyltransferase (HMT, EC 2.1.1.8) activity was studied in various tissues of the guinea-pig and rat. The properties of HMT in rat kidney were shown to differ from those of HMT in the brain and kidney of guinea-pig and the brain of rat. Quinacrine, a potent HMT inhibitor, inhibited the activity of HMT by 50% at concentrations of about 10^{-5} M in rat kidney and about 10^{-7} M in other tissues.

Keywords——histamine N-methyltransferase; guinea-pig and rat tissues; histamine methylation; quinacrine affinity; multiple form

Histamine N-methyltransferase (HMT, EC 2.1.1.8) occurs ubiquitously in the organs and tissues of animals,[2,3)] and its high degree of substrate specificity is well known.[2,4,5)] Studies on multiple forms of the enzyme and on the mechanism of histamine methylation, however, have yielded conflicting data, irrespective of species and tissue[3,6–11)] and therefore the properties of this enzyme are still uncertain. The purpose of the present work was to examine the heterogeneity of tissue HMT in common laboratory animals.

Results and Discussion

Histamine N-methyltransferase is a soluble enzyme which is not associated with any subcellular particles, and hence it is thought that non-HMT proteins can be conveniently removed by centrifugation.[2,6] Highly-purified HMT is unstable.[6] However, the ammonium sulfate fraction, containing large amounts of HMT in stable form, is suitable for comparative studies of the distribution and properties of HMT in various species and tissues and for the measurement of histamine levels in tissues.[2-4,8-11] Consistent data may easily be obtained by its use. In the present experiments, HMT was prepared and purified 7—8 times from the crude homogenates. The K_m value for histamine was 3×10^{-5} M for the partially purified enzyme from guinea-pig brain.

In addition to the high HMT activity in the brain of both animals, HMT was abundant in the stomach and lung of guinea-pig and the kidney of rat, whereas the liver of guinea-pig, and the stomach, lung, small intestine, heart, and liver of rat did not contain significant activity (Table I). These results are consistent with those of Brown *et al.*[2] In the guinea-pig, the brain has the highest activity of HMT[2] and in the rat, histamine methylation occurs mainly if not solely in the brain.[12] In the presence of guinea-pig brain HMT, 6×10^{-5}M histamine was 100% methylated. The methylation of histamine with S-adenosylmethionine seems to proceed almost completely in the presence of HMT.[4,15] However, there is no obvious association between histamine levels in tissues and enzyme activity.[2] Table I shows that guinea-pigs (sensitive to histamine) have higher HMT activity than rats (insensitive to it). Detoxification by N-methylation thus does not appear to correlate with histamine sensitivity. Interestingly, the activity in rat kidney was about twice that of the brain.

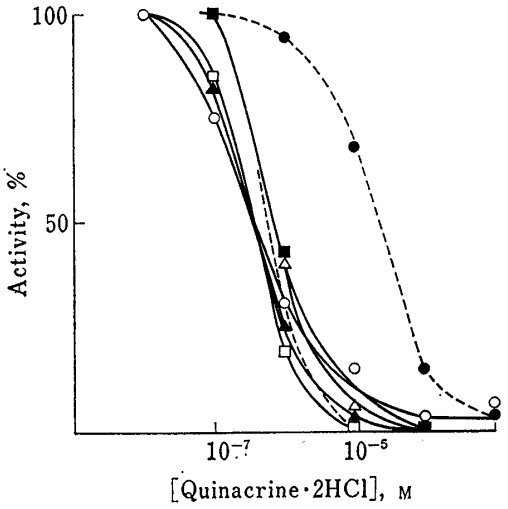

Fig. 1. Inhibitory Patterns of HMT Activities

Guinea-pig: ○——○ brain, ▲——▲ stomach, □——□ lung, △——△ kidney, ■——■ small intestine.
Rat: □----□ brain, ●----● kidney, (4 measurements).

Table I. Comparative HMT Activities

Tissues	Activities, in mg/min									
	Guinea-pig					Rat				
	dpm	%	%	%	%	dpm	%	%	%	%
Brain	2980	100	100[a]	100[b]	100[c]	1260	100	100[a]	100[b]	100[c]
Stomach	2760	93	92	—	—	206	16	0	—	—
Lung	2070	68	85	—	—	79	6	0	5120	—
Kidney	1305	44	63	300	32	2260	171	209	237	395
Small intestine	1290	43	Ileum 74	—	54	257	20	Ileum 131	—	138
Heart	970	33	50	2560	—	155	12	23	1870	—
Liver	71	2	21	—	15	240	19	0	1370	0

Values are given relative to those of the brain taken as 100%.
a) Ref. 2.
b) Histamine contents quoted from ref. 4.
c) Ref. 3.

In tissues containing large amounts of HMT, histamine methylation was exponentially inhibited by quinacrine, a potent HMT inhibitor (Fig. 1). The activities were 50% inhibited at $5—8\times10^{-7}$ M quinacrine in the brain, stomach, lung, kidney, and small intestine of guinea-pig, and the brain of rat and at 3.5×10^{-5} M in the kidney of rat. The data of Thithapandha *et al.*[6] imply that half-inhibition of the purified HMT of guinea-pig brain was seen when the concentration of quinacrine was of the order of 4×10^{-7} M. In the present experiments, the inhibitory patterns of HMT gave parallel plots without variation in V_{max}. This indicates. differences in the quinacrine affinity of HMT between tissues.

HMT shows species and tissue differences, and may be present in three to five different monomeric forms.[3,6,7] Thithapandha *et al.*[6] found five heterogeneous proteins by polyacrylamide gel electrophoresis of the enzyme purified from guinea-pig brain. Histamine methylation by HMT is said to function through a "sequential mechanism"[8–10] and/or "ping pong mechanism"[6] but the mechanism seems to vary with animal species. There are many theories on the regulation of histamine methylation:[8,10,11,13] methylation of histamine may be regulated by the ratio of substrate S-adenosylmethionine to product S-adenosylhomocysteine;[8] activation of HMT may represent a reversal of substrate inhibition;[10] an inhibitory site of HMT may be responsible for noncompetitive inhibition;[11] stimulation and inhibition of HMT may be induced by a regulatory site on the enzyme.[13] More than one of these mechanisms may operate, since the experimental data revealed species and tissue differences in the behavior of HMT, suggesting the multiplicity of the enzyme.

Experimental

HMT was partially purified from the brain, heart, lung, stomach, small intestine, liver, and kidney of the guinea-pig and rat, by the method of Brown *et al.*[2] HMT activity was assayed by a modification[14] of the procedure of Taylor and Snyder.[15] The substrate, methyl donor, and inhibitor were histamine, S-adenosylmethionine (New England Nuclear Co., SAMe-^{14}C), and quinacrine, respectively. The final concentrations of histamine and SAMe and the specific activity of radio-labeled SAMe were 6×10^{-5} M, 4×10^{-4} M, and 10 nCi/nmol, respectively. The volume ratio was enzyme 50, substrate 6, unlabeled SAMe 0.6, SAMe-^{14}C 12, and inhibitor 10, and 0.05 M Na-phosphate buffer (pH 7.5) was added to give a final volume of 150 μl. The reaction mixture was incubated for 15 min at 37°C and then made alkaline with 1 N NaOH under cooling. Methylhistamine was separated by $CHCl_3$-extraction at room temperature. The radioactivity of methylhistamine was measured in an Aloka liquid scintillation counter. The radioproduct was thin-layer chromatographically confirmed to be identical with biological methylhistamine obtained by the method of Brown *et al.*[2] A silica gel plate of micro slide glass (76×26 mm) showed one yellow spot with Pauly's reagent. The *Rf* value of methylhistamine was 0.64 with 8% NaCl and 0.6 with $CHCl_3$–CH_3OH–NH_4OH (12:7:1). Protein was determined by the method of Lowry *et al.*[16]

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