서울醫大學術誌 제22권 제 1 호
The Seoul Journal of Medicine
Vol. 22, No. 1, March 1981

Activity Distribution of Guanine Aminohydrolase in Rat Tissues

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INTRODUCTION

Enzyme activities are the most frequent examples of tissue components used in characterizing tissue functions; and standardization of relative enzyme activities, therefore, among tissues in health and disease has relevance of biochemical studies to particular biological problems of any kind.

The bias in favor of enzymes with this regards, however, seems critical in any case of enzyme because the size of the rate units of enzymes is mostly determined by various degrees of subtleties of the reaction conditions as used by each reporter (Knox, 1976). It seems not to be exceptional in the case of guanine aminohydrolase (EC 3.5.4.3), while it exerts an important rọle in purine catabolism which not only involves interconversions between purines at the level of the monophosphates, the ribosides, and the free bases, but also gives rise to uric acid as the end product in man and other primates as well as in birds and terrestial reptiles and to allantoin in mammals other than the primates (Kimm, 1973).

The enzyme has been under continuous investigation during the last several years by the authors' laboratory which has proved its isoenzymic nature (Kimm *et al.*, 1967: Cha *et al.*, 1980), its significances in health and disease (Kimm and Lee, 1970; Kimm and Han, 1971), proposing a new colorimetric method for the determination of its activity (Park and Kimm, 1976) and a staining method for it on the electrophoretic gel bed (Kimm, 1976).

During this period it attracted the authors' attention that observations on activity patterns of this enzyme among tissues were so scanty and inconsistent to compare for the purpose of characterizing tissue functions pertinent to this particular enzyme, not to mention little progress made in the purification of the enzyme since the initial works (Schmidt, 1932: Kalckar, 1947) as well as in the study of its properties (Kumar *et al.*, 1965: Currie., 1967: Kumar *et al.*, 1973).

Fostered by interest in the regulation of purine catabolism, it came into the authors' consideration to undertake analyses of activities of guanine aminohydrolase distributed among tissues of rats because we thought it to be a burgeoning trial prior to any of the further investigation on this enzyme, of which clinical application, for instance, would cast a clue to pathological tissue diagnoses together with its biochemical implications regarding purine catabolism.

MATERIALS AND METHODS

Animals: Adult rats of Sprague Dawley strain, weighing 200 through 400g, were used throughout the present study disregarding the sex, through the courtesy of the Animal Laboratory, College of Medicine, Seoul National University.

Tissue Preparation: Decapitating the animals, tissues were removed immediately and chilled in ice-cold 0.25M sucrose solution, foll-

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owed by homogenization of the tissues carried out in the same sucrose solution with the Potter Elvehjem homogenizer to have 20%(w/v) total tissue homogenates.

Chemicals: Guanine, xanthine and bovine serum albumin were purchased from the Sigma Co. (St. Louis, Mo., U.S.A.) and other chemicals from the Kanto Co. (Tokyo, Japan).

Assay of guanine aminohydolase activity: The assay procedure was based on the reaction that guanine is converted by the enzyme to xanthine with the concomitant decrease in the absorbance of the substrate at 245 nm after Roush and Norris (1950) with modifications in composition of the reaction mixture.

The assay system was consisted of 150μ moles of Tris buffer, pH 8.0, 1.5μ moles of the substrate, and 0.1ml of the tissue preparation as an enzyme source in a final volume of 2.0 ml, starting the reaction by the addition of the substrate at 30°C. Incubating the reaction mixture at the same temperature for 30min., 1.0ml of 10%(w/v) perchloric acid solution was added to stop the reaction.

The acidic reaction mixture was centrifuged for deproteinization, the supernatant of which was diluted to 20 fold by distilled water, and the absorbance at 245nm was measured with the UV spectrophotometer (Pye Unicam Co., England). The reference assay of the enzyme activity for control was performed with the same mixture, adding the perchloric acid solution prior to the addition of the substrate in an identical treatment thereafter.

Difference in the molar extinction coefficient was obtained from the respective absorption profiles of guanine and xanthine at 245nm, 7.20×10^3, by which molar concentrations of guanine converted to xanthine were calculated, which is based on the method reported elsewhere (Knight *et al.*, 1965).

A unit of the enzyme activity is defined as one μ mole of the substrate which is converted during the incubation of one minute at 30°C.

Protein determination: Protein concentrations of enzyme samples were monitored after the method of Lowry *et al.* (1951), using the bovine serum albumin as the standard, the nitrogen content of which was previously estimated by micro-kjeldahlometry, in order to calculated the specific activities of the enzyme.

RESULTS AND DISCUSSION

It has been reported that guanine aminohydrolase appeared to be quite stable over a broad pH range and that incubation of the enzyme in a series of buffers from pH 5.6 to 9.4, followed by assay in Tris buffer at pH 8.0, as adopted in the present study, indicated at least 75% retention of activity over the entire range. Therefore, the present condition regarding the pH optimum seems to offer excellent results as discussed by Fogle(1974).

Optical densities, however, of guanine and xanthine vary with great magnitudes as pH's and wavelengths vary when, in the present paper, the action of guanine aminohdrolase on guanine was followed by the changes in absorption at 245nm of the substrate after Kumar *et al.* (1965).

Fig. 1 gives the absorption spectra for 25μ moles guanine and xanthine respectively at pH 1-2, because the enzyme, though incubated at pH 8.0 based on the previous reports (Kalckar, 1947; Fogle, 1974), was observed presently through its activity exerted on guanine and xanthine; the absorption of the former being decreased and of the latter increased at their specific peaks of wavelength.

Under these conditions, the extinction coefficients of both guanine and xanthine based on the ΔE's of them at 245nm were 10.8×10^3 and 3.6×10^3 respectively, the difference of which

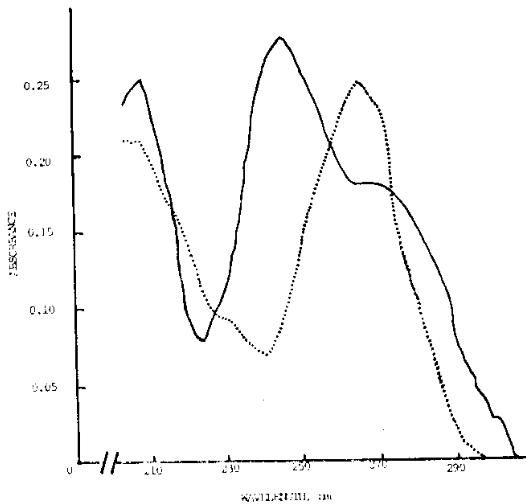

Fig. 1. UV-absorption profiles of guanine (——) and xanthine (⋯⋯). The concentrations of each were 25uM respectively in 05% $HClO_4$, pH 1-2.

is, therefore, a maximum possible conversion of guanine to xanthine.

Earlier publications from this department dealt with the assay method and properties including isoenzymic nature of guanine aminohydrolase in brain and liver of rats (Kimm and Lee, 1970; Kimm and Han, 1971; Kim *et al.*, 1975; Park and Kimm, 1976; Kimm, 1975; Cha *et al.*, 1980), since it was reported that the activity of the enzyme in normal human tissue shows high levels in the liver, kidney, and brain and little or no activity in other organs, so as to evaluate its diagnostic significances in liver (Knight *et al.*, 1965; Hue and Free, 1965) and brain diseases (Kimm and Han, 1971). The enzyme was utilized even in a study to assess portal venous blood to observe if it can alone maintain the function of the heterotrophic auxiliary liver graft in rats (Kim *et al.*, 1975).

Taking into consideration a pivotal position of guanine aminohydrolase in guanine metabolism, either anabolic or catabolic, the authors attempted to show its rather ubiquitous distribution of activity among tissues, where as far as purine regulatory metabolism is necessitated, since it has long been known not only that salvage pathways allow guanine to incorporate into nucleic acids by means of either phosphoribosyl transferase or nucleoside phosphorylase, but that the intracellular guanine is converted by guanine aminohydrolase to xanthine for further degradation in competition with the former reaction.

As is apparent from Table I, activities of the enzyme showed rather ubiquitous distribution among the tissues studied, though the magnitudes of differences are prominent.

It is noteworthy from Table I that the enzyme activity per g. of wet tissue weight was the highest in lung among other tissues examined,

Table 1. Comparison of guanine aminohydrolase activities among tissues of adult rats

Tissue	Animals No's	GDA activity U/g. of tissue	Protein content mg/g. of tissue	Specific activity mU/mg of protein
Brain	21	1.08±0.06	81.94± 9.00	13.18±1.54
Cardiac muscle	21	0.46±0.05	62.16±12.80	7.40±1.18
Kidney	20	0.81±0.06	131.49±13.00	6.16±0.69
Liver	21	1.47±0.09	148.48±12.20	9.90±1.23
Lung	20	2.15±0.13	91.37±12.75	23.53±2.31
Pancreas	20	0.74±0.09	83.42±18.25	8.87±2.05
Skeletal muscle	20	0.50±0.15	70.62± 9.15	7.08±1.63
Skin	20	0.40±0.06	18.94± 1.30	21.12±3.57
Small intestine	20	1.01±0.12	70.09±12.30	14.41±2.09
Spleen	21	1.18±0.10	121.40±18.25	9.72±1.23

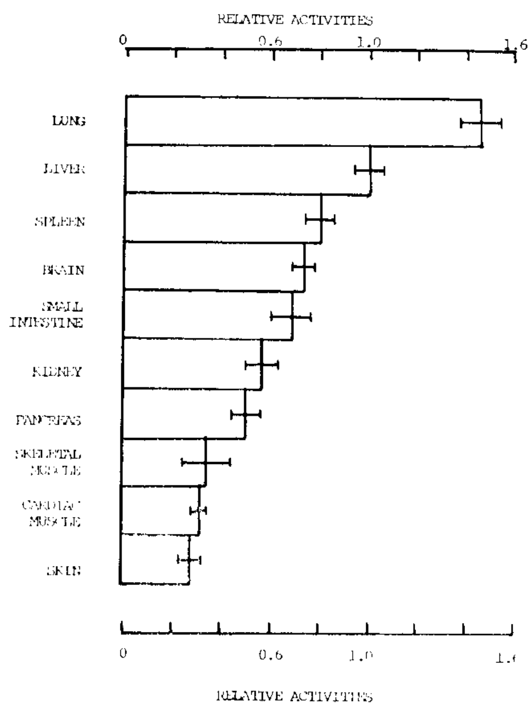

Fig. 2. Relative activities of guanine aminohydrolase of rat tissues are compared taking the activity of liver as an unitary parameter.

followed by liver, spleen, and small intestine in decreasing order, forming a group of higher souces of the enzyme. Intermediate sources of the activity were kidney and pancreas, while lower ones were cardiac and skeltal muscles as well as skin, the relative orders of its contents among tissues are compared in Fig. 2.

Since the discovery of the presence of the enzyme in rabbit liver (Schmidt, 1932), bacteria (Rakosky and Beck, 1955; Rakosky *et al.*, 1955), yeast (Roush, 1954), cockroaches (Pierre, 1965), fish (Roy, 1966), and various mammals (Talwar *et al.*, 1961; Kumar *et al.*, 1965; Kumar *et al.*, 1966; Currie *et al.*, 1967; Kumar *et al.*, 1967; Kumar et al., 1972), significant levels of the enzyme activity have been known to be associated with liver, brain and kidney in mammals, while, on the other hand, lower levels of it with spleen, skin, muscle, intestines and serum.

A slight discrepancy was noted regarding the relative order of richness in the enzyme activity between the previous reports mentioned above and the present paper. Particularly the highest level of the enzyme found in lung by the authors seems to cast a problem of interest, disclosing an active catabolic rate of purine in this organ, which has not been reported so far. As is apparent from Fig. 2, the relatively higher levels of the enzyme were found in lung and liver, intermittent levels in spleen, brain, small intestine, kidney, and pancreas, and the relatively lower levels of it in the rest of tissues observed.

The general tendency of relative order of specific activities among the tissues examined is shown in Fig. 3, which seems to be roughly divided into two categories; one forming a higher group of organs in their specific activities, another forming a comparable or lower group of organs than liver. Comparing with that of liver as a unit, the former tissues were lung, skin, small intestine, and brain; the latter were

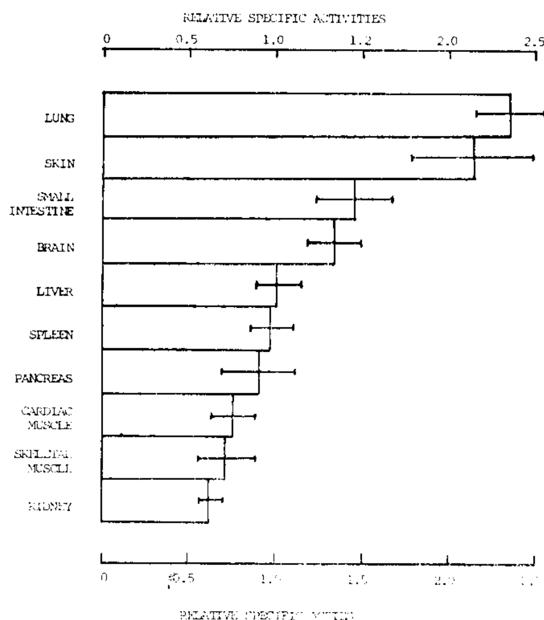

Fig. 3. Relative specific activities of guanine aminohydrolase of rat tissues are compared taking the specific activity of liver as an unitary parameter.

spleen, pancreas, cardiac muscle, skeletal muscle, and kidney.

It is, therefore, easily assumable to think the enzyme in serum would not necessarily be a specific liver function parameter as reported earlier (Knight *et al.*, 1965), whereas its serum activity could reveal a variety of pathological tissues including, of course, lung and liver as major sources.

In addition to the above finding, one must bear it in mind that elevated serum activity of the enzyme could be a reflection of overall variate of the enzymes distributed among various tissues. It is, therefore, hopeful to discriminate a particular species of the enzyme in a given tissue from another species of the same enzyme in another tissue, because guanine aminohydrolases from different sources do not appear to be identical as Currie *et al.* (1967) discussed regarding peaks of optimum activity over a wide range of pH's.

Moreover, as is apparent from the present results, the distribution pattern or rather the order of activity among tissues examined is not necessarily correlated with the germinal origins of the tissues. Lung and liver, the entodermal origins, showed the higher level of activity, while small intestine and pancreas, the same origins, disclosed the lower level of activity; and even if the mesodermal organs including kidney and muscle contained lower activities of the enzyme, spleen, for example, of the same germinal origin, contained relatively higher activity.

Though no correlation with each germinal origin is as apparent from the present results as discussed above, tissues undergoing atypical growth has shown appreciable variations (Levine *et al.*, 1963: Greengard *et al.*, 1980), the morphological alterations of which seems to be correlated with (Musseer *et al.*, 1966) and reflected by the elevated activities in cerebrospinal fluid and serum (Kimm and Han, 1971). And the guanine aminohydrlase activity in tissues appears to serve as a good index of growth rate of the tissue when observed on g. tissue basis, because it represents catabolic rate of purines, which, underlies active nucleic acid metabolism (Kimm and Lee, 1970).

It was of noteworthy to observe that the specific activity of the enzyme in the skin was quite remarkably high in the present study ever since the presence of this enzyme was reported in human epidermis (de Bersaques, 1967). In short, distribution of the enzyme activity among tissues of rats appeared to be rather ubiqitous, no matter what differences and variations are disclosed.

CONCLUSION

Distribution of guanine aminohydrolase activities among rat tissues was analyzed with the following conclusions.

1. Guanine aminohdrolase is rather ubiquitous among tissues, characterized by variable distribution patterns.

2. With regard to the enzyme activity per g. tissue, there are the higher sources of organs, lung, liver, spleen, and small intestine; the intermittent, kidney and pancreas; and the lower sources, muscles and skin.

3. Comparing with the specific activity of liver as a reference; organs showing the higher specific activity are lung, skin, small intestine, and brain, while organs showing the lower specific activity are spleen, pancreas, muscles, and kidney.

=國文抄錄=

Guanine Aminohydrolase의 活性分布

서울대학교 의과대학 생화학교실
金昇元 · 金正子 · 朴鏘培

흰쥐조직내 guanine aminohydrolase의 활성분포를

측정한 결과, 다음과 같은 결론을 얻었다.

1. 흰쥐조직내의 guanine aminohydrolase는 그 활성의 정도는 다르지만 여러 조직에 범재해 있다.

2. 조직 1gm당 guanine aminohydrolase의 활성은 폐, 간, 비장 및 소장조직에서 높고, 신장과 췌장조직이 중간정도이며, 근육과 피부조직에서는 낮다.

3. 각 조직의 比활성을 간조직의 比활성과 비교하여보면 폐, 피부, 소장 및 뇌조직은 높은 반면 비장, 췌장, 근육 및 신장조직은 낮다.

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