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Distribution of Tryptophan Metabolites in the Organs of Mice

MAKIKO SUZUKI* and MITSUO WATANABE

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

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After intravenous injection of tryptophan-U-^{14}C into mice, the radioactivities of carcinogenic tryptophan metabolites in the organs were determined. Kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and xanthurenic acid in the liver, pancreas, kidneys and bladder were separated by thin layer chromatography and the radioactivity of each metabolite was counted. The radioactivities of xanthurenic acid were 25—80, 50—115, 200—480 and 170—370 nCi/g in the pancreas, liver, kidneys and bladder, respectively. The radioactivities were in the order xanthurenic acid>kynurenine>3-hydroxykynurenine and 3-hydroxyanthranilic acid in every organ. The radioactivity of xanthurenic acid was especially high in the kidneys and bladder.

Keywords——xanthurenic acid; kynurenine; 3-hydroxykynurenine; 3-hydroxyanthranilic acid; tryptophan metabolite; distribution; radiometry; TLC

Introduction

Some metabolites of tryptophan in the kynurenine pathway are suspected to be carcinogenic, because when cholesterol pellets containing 3-hydroxykynurenine, 3-hydroxyanthranilic acid, xanthurenic acid, kynurenine and quinaldic acid were implanted in the bladder of mice, bladder cancer was induced,[1,2] and leukemia, sarcoma or pancreas carcinoma was induced by subcutaneous injection of 3-hydroxykynurenine into mice.[3] In the preceding paper,[4] Watanabe and Sugimori studied the affinity of 3-hydroxykynurenine to the organs of mice and suggested that this compound has an affinity for the pancreas. In this paper, the radioactivities of the metabolites in the organs after administration of tryptophan-U-^{14}C were determined in order to clarify the distribution of tryptophan metabolites in the organs.

Materials and Methods

Animals——Ten-week-old male BALB/c×DBA/2 F mice (23—28 g, Shizuoka Laboratory Animal Center) were used.

Reagents——L-Tryptophan-U-^{14}C (556 mCi/mmol) purchased from New England Nuclear (NEN 628) was evaporated to dryness and diluted appropriately in saline. L-Tryptophan (Trp), L-kynurenine (KY), 3-hydroxykynurenine (3-OHKY) and 3-hydroxyanthranilic acid (3-OHAA) were obtained from Wako Pure Chemical Co., respectively. Xanthurenic acid (XA) was obtained from Sigma Chemical Co.

Procedures——At 30 min after intravenous injection of 30 μCi of L-tryptophan-U-^{14}C in 0.3 ml of saline into mice, the mice were decapitated. In each case, the liver, pancreas, kidneys and bladder were immediately removed and frozen by placing them between 2 stainless steel plates cooled with dry ice. Each organ was homogenized in 10 ml of methanol containing 0.2 ml of 1 mM Trp, KY, 3-OHKY, 3-OHAA and XA. The homogenate was centrifuged at 3000 rpm for 10 min, the pellet was washed twice with 2 ml of methanol, and the supernatant and washings were combined and concentrated under a vacuum. The concentrate was applied to a cellulose powder plate (No. 5577 Merck) and developed with benzene–*n*-butanol–methanol–H_2O (1: 1: 2: 1).[5] Fluorescent bands of Trp, KY, 3-OHKY, 3-OHAA and XA were extracted with methanol. Each extract was concentrated and developed again on a cellulose powder plate with the solvents described below. Trp, KY and 3-OHKY: *n*-butanol–acetic acid–H_2O (4: 1: 1) and H_2O (2 dimensional chromatography). 3-OHAA: benzene–ethanol–H_2O (50: 10: 1).[6] XA: benzene–*n*-butanol–28% NH_4OH (1: 1: 2: 0.1).[7] Trp, 3-OHKY, 3-OHAA and XA were extracted with methanol and KY with H_2O.

Trp, 3-OHAA and XA in the extracts were directly determined by fluorescence measurement (Trp: ex.288 nm, em.340 nm. 3-OHAA: ex.330 nm, em.417 nm. XA: ex.345 nm, em.402 nm). KY in the extracts was determined by fluorescence measurement (ex.368 nm, em.455 nm) after dilution with 9 volumes

of acetone. 3-OHKY was determined by the method reported by Watanabe *et al.*[8] First, 0.4 ml of the methanol extract, 0.4 ml of 0.5% solution of *p*-toluenesulfonylchloride in acetone and 0.2 ml of 0.2 M Tris buffer (pH 9.0) were mixed and kept for 30 min, then the fluorescence (ex.367 nm, em.455 nm) was measured. All the extracts from thin layer chromatography (TLC) were put into vials, and the radioactivity was measured in dioxane scintillator. The radioactivity of the metabolites in the organs was calculated by the method described below. The radioactivity of the metabolites = the radioactivity of the extracts from TLC × the quantity of the metabolites added before homogenation ÷ the quantity of the metabolites in the extracts from TLC.

Results

Thin Layer Chromatography

Table I shows the *Rf* values of authentic samples of tryptophan metabolites. Each metabolite was separated by two-dimensional chromatography.

Radioactivities of the Organs

The radioactivities of supernatants of the organs are shown in Table II. The supernatant

TABLE I. *Rf* Values of Authentic Compounds

Compounds	*Rf* values in various solvent systems				
	1[a]	2[b]	3[c]	4[d]	5[e]
Anthranilic acid[f]	0.92		0.93		
3-Hydroxyanthranilic acid[f]	0.86		0.56		
Quinaldic acid[f]	0.84		0.84		
Nicotinic acid	0.78	0.63			
Nicotinamide	0.76	0.76		0.74	
Xanthurenic acid 8-methyl ether[f]	0.75	0.79		0.54	
Xanthurenic acid[f]	0.66	0.47			
Kynurenic acid[f]	0.65	0.62			
Nicotinuric acid	0.62	0.58			
Tryptophan[f]	0.40			0.41	0.52
Kynurenine[f]	0.39			0.38	0.60
Alanine[g]	0.39			0.24	0.86
Nicotinic acid mononucleotide	0.37			0.05	
3-Hydroxykynurenine[f]	0.27			0.27	0.48
NAD	0.26			0.00	
Nicotinamide mononucleotide	0.21			0.05	
Serotonin	0.17			0.40	0.34

a) Benzene:*n*-butanol:methanol:H_2O=1:1:2:1.
b) Benzene:*n*-butanol:methanol:H_2O:28% NH_4OH=1:1:2:1:0.1.
c) Benzene: ethanol:H_2O=50:10:1.
d) *n*-Butanol:acetic acid:H_2O=4:1:1.
e) H_2O.
f) Authentic samples were detected by fluorescence.
g) Alanine was detected by ninhydrin reagent. Other metabolites were detected as quenching spots under ultraviolet light.
TLC was carried out on cellulose.

TABLE II. Radioactivities of the Supernatants of Homogenates in Methanol

No. of mice	Radioactivity of supernatants (nCi/g wet weight)			
	Bladder	Kidney	Pancreas	Liver
1	321	724	532	416
2	131	1294	578	502
3	329	1221	385	346
Mean	260	1080	498	421

of the kidneys had the highest radioactivity. Those of the pancreas and liver had lower but roughly equal radioactivities and that of the bladder had the lowest radioactivity.

Radioactivities of Tryptophan Metabolites

Radioactivities of Trp, KY, 3-OHKY, 3-OHAA and XA in the organs of 3 mice are shown in Fig. 1. In the kidneys and bladder, the radioactivity of XA was highest, that of KY was the next highest, and those of 3-OHKY and 3-OHAA were low. In the liver and pancreas, the radioactivity of XA was lower than that in the kidneys or bladder, but the patterns were similar.

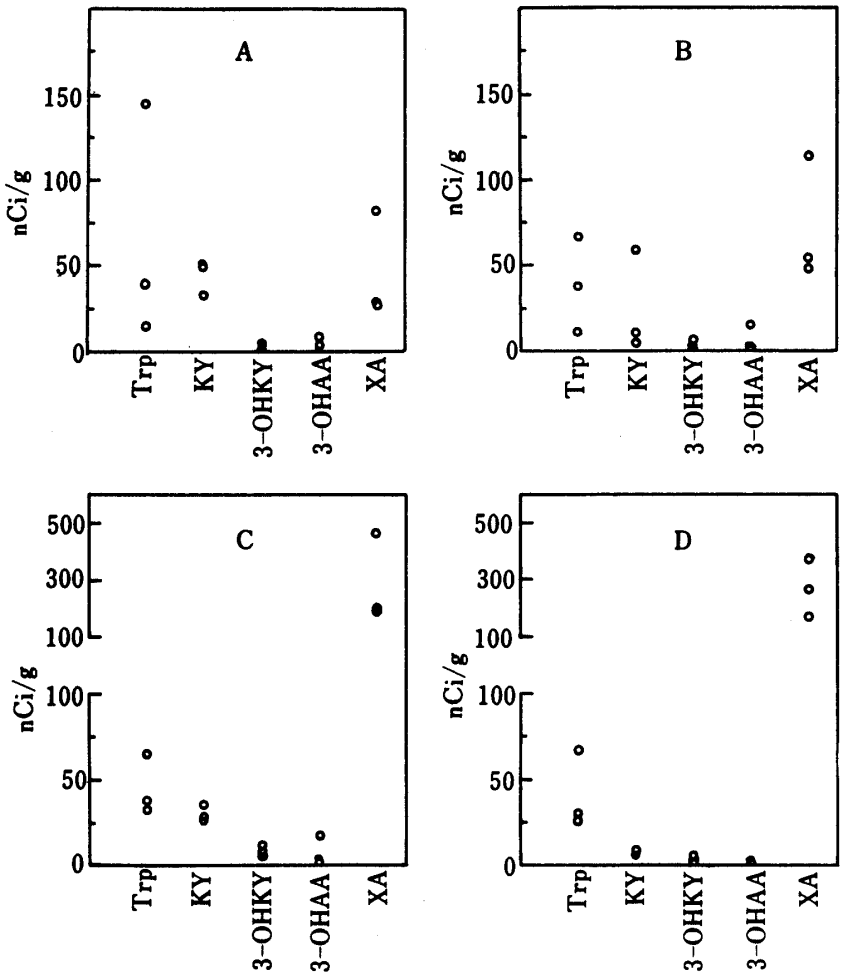

Fig. 1. Distribution of Tryptophan Metabolites in the Organs

Each mouse was decapitated at 30 min after *i.v.* injection of 30 μCi of L-tryptophan-U-^{14}C. The liver, pancreas, kidneys and bladder were removed and homogenized in methanol. The tryptophan metabolites of the methanol-soluble fraction in each organ were separated on cellulose powder plates and the radioactivities were determined.
A, pancreas; B, liver; C, kidney; D, bladder.

Discussion

Some metabolites of tryptophan are suspected to be related to certain diseases, for instance, cancer[13] or diabetes.[9] The distribution of such metabolites in the organs can provide useful information for studies on the action of these compounds on each organ. However, full information is not yet available, although urinary excretion of these metabolites has been studied by many researchers.[6,8,10,12] In the present work, the radioactivities of carcinogenic

metabolites of tryptophan in the organs of mice were measured at 30 min after injection of labelled tryptophan, because it was reported that 30 min was the time at which evolution of $^{14}CO_2$ was the highest,[13,14] and the radioactivities of the metabolites were expected to be high enough for determination.

It was found that the amounts of labelled metabolites were in the order XA>KY>3-OHKY and 3-OHAA in every organ. The amounts of labelled XA were especially high in the kidneys and bladder. These data show that XA is the predominant metabolite derived from tryptophan under these conditions.

Watanabe and Sugimori reported that radioactivity was remarkably concentrated in the pancreas and kidneys of mice after injection of labelled 3-OHKY, and 70% of the radioactivity was due to unchanged 3-OHKY in the pancreas, whereas 17% was due to unchanged 3-OHKY in the kidneys.[4] These data suggest that 3-OHKY has affinity for the pancreas. However, our results show that the radioactivity of 3-OHKY in the pancreas after injection of L-tryptophan-U-^{14}C was at the same level as that in the liver, indicating that 3-OHKY is not concentrated in the pancreas.

The similarity of the metabolite patterns in the various organs suggests that L-tryptophan may be metabolized in all the organs. It is known that many organs contain indoleamine 2,3-dioxygenase while L-tryptophan 2,3-dioxygenase is present in the liver.[15] L-Tryptophan might be metabolized by the former enzyme in organs other than the liver.

References and Notes

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