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Abstract

Some bile acids (dehydrocholic, cholic, chenodeoxycholic, ursodeoxycholic, and deoxycholic acids), and some hypocholesterolemic agents (22, 25 diazacholesterol, 20,25-diazacholesterol, triparanol, and SKF 525-A) are the inducers of isovalthinuria in guinea pig. Administration of methionine appears to increase the pool of sulfur compound which participates in the formation of isovalthine. Cholesterol appears to have no enhancing effect on the induction activity of isovalthinuria inducers. The mechanism of isovalthine formation and the role of sulfur amino acids in lowering blood cholesterol are discussed.

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EXPERIMENTAL ISOVALTHINURIA*

III. INDUCTION BY BILE ACIDS, AND HYPOCHOLESTEROLEMIC AGENTS

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For the elucidation of a question why some of the hypercholesterolemic patients excrete isovalthine^{1,2} in their urine, a series of research has been conducted on the problem how the isovalthinuria can be induced in some experimental animals. FUKUTOME³ has reported that oral or parenteral administration of isovaleric acid induces isovalthinuria in some animals which never excrete isovalthine in their normal urine. Isovaleric acid-1-C¹⁴ administered, however, has not significantly incorporated into urinary isovalthine in dog⁴.

Recently some other reproducible methods for the induction of isovalthinuria have been found in guinea pig by using bile acids or hypocholesterolemic agents.

1. Experimental Animal

Guinea pigs are used as the experimental animal throughout this work because of the following reasons. 1. They never excrete isovalthine in their normal urine. 2. They excrete proper volume of urine and the size of their body is suitable for the future isotopic experiments. 3. They contain much glutathione in the body as compared to other animals⁵, and S-(isopropylcarboxymethyl)-glutathione synthesized in liver is assumed to be a direct precursor of urinary isovalthine⁶.

Male guinea pigs of approximately 3 months of age weighing 300—400 gm were fed on RC-5 solid food (Oriental Yeast INC., Tokyo), and green vegetables. L-Ascorbic acid (2.5 mg per animal) was supplied every other day by mixing in drinking water. Temperature of the animal room was conditioned at around 25°C all the year round. Two guinea pigs were placed in a cage and the urine was collected once every week in a bottle containing toluene and hydrochloric acid. Average volume of urine was around 500 ml per two guinea pigs per week.

Bile acids used as inducers were dehydrocholic, cholic, chenodeoxycholic, ursodeoxycholic, and deoxycholic acids. Hypocholesterolemic agents used were

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22, 25-diazacholesterol (SC-11952), 20,25-diazacholesterol (SC-12937), triparanol (MER-29, 1-[p-(β -diethylaminoethoxy)-phenyl]-1-(p-tolyl)-2-(p-chlorophenyl)-ethanol), and β -diethylaminoethyl-diphenylpropylacetate hydrochloride (SKF 525-A). Five mg of each bile acid and two mg of each azasterol were orally administered with powdered RC-5 solid food per animal per day but not per Kg body weight, because body weight of each guinea pig gained about 100 gm every four weeks during experiments. Triparanol and SKF 525-A, however, were administered in 4.2 mg and 5 mg per Kg body weight per day respectively by weighing the animal every two days.

The influences of cholesterol (100 mg/animal/day) and of methionine (5 mg/animal/day) on isovalthineuria induced by several inducers were also tested in some cases.

2. Preparation of Urinary Acidic Amino Acid Fraction for the Determination of Isovalthine on Amino Acid Analyzer.

For the determination of urinary isovalthine on an amino acid analyzer, isovalthine in the urine must be concentrated beforehand.

Two hundred ml of urine is made weakly acidic with acetic acid or ammonia and filtered. The filtrate is transferred on a column containing 200 ml of Amberlite AG-45 (acetate-form). The column is washed with one liter of 0.15 N acetic acid and eluted 2 liters of 2 N acetic acid. The 2 N acetic acid eluate is directly transferred on a column containing 100 ml of Diaion SK-1 (H-form, strong cation exchanger, Mitsubishi Kasei Co. Ltd., Tokyo). The column is washed with deionized water and then eluted with 500 ml of 2 N ammonia. The ammonia solution is evaporated to dryness in vacuum under 40°C. The residue is dissolved in 8 ml of water and filtered. After isovalthine being identified in the filtrate according to the author's method⁷, two ml of the filtrate corresponding to 50 ml of original urine is analyzed on amino acid analyzer (Beckman Model 120 B). When the sample for the analyzer contains too much dirty substances, the dried residue above prepared is treated as follows. The residue is dissolved in about 10 ml of 0.15 N acetic acid and filtered. The filtrate is passed through a column containing 10 ml of Amberlite CG-50 (H-form), and the column is washed with 50 ml of 0.15 N acetic acid. The effluent and washings are combined and dried in vacuum. This procedure is useful for decolorization of the samples and the recovery of urinary isovalthine is not reduced.

Amino acid analyzer is operated with 0.2 N sodium citrate buffer (pH 3.24) at 30°C on 150 cm column throughout this work. The urinary isovalthine (L-isovalthine*) yields a single peak between serine and proline at around 196 efflu-

* Details on the configuration and synthesis of urinary isovalthine will be shortly reported elsewhere by S. Ohmori of this Laboratory.

ent ml and its HW-constant is 20.5. The preparation procedure of urinary acidic amino acid fraction described above never changes the configuration of urinary isovalthine and the recovery of urinary isovalthine is around 75% which is used for the calculation in this experiment.

3. Induction of Isovalthinuria by Bile Acids.

The results obtained by feeding with several bile acids are summarized in Table 1. Most of the guinea pigs received dehydrocholic acid begin to excrete isovalthine in 5th-7th day after the start of administration, and the maximum excretion is observed on the second week and decreased thereafter (Table 1. a, c, d). A group of guinea pigs receiving dehydrocholic acid (Table 1. b), however, showed the maximum excretion on the fourth week as in the cases of the other bile acids (Table 1. e, g, h, i). Since the amount of isovalthine excretion varies considerably from one animal to another, it is not certain at present whether cholesterol enhances the induction effect of bile acids or not (Table 1. d, f). Administration of cholesterol alone (100 mg/animal/day) has never induced isovalthinuria up to the end of fourth week. When the excretion of isovalthine became to zero after passing the maximum peak of excretion, the administration of methionine only on the sixth week induced again isovalthinuria (Table 1. c, e, h). After methionine administration on the sixth week, no isovalthinuria was observed by continued feeding with dehydrocholic acid up to the end of eleventh week (Table 1. c). But methionine administration on the twelfth week induced again isovalthinuria (Table 1. c). Isovalthine excretion on the fifth week following methionine administration is also considered to be the methionine effect (Table 1. b, g), because most animals having the maximum excretion on the fourth week do not excrete isovalthine on the fifth week unless methionine is given (Table 1. e, h, i).

Table 1 Experimental Isovalthinuria Induced By Bile Acids.

Inducers	Week							
	μ moles of isovalthine excreted by two guineapigs per week.							
	1st	2nd	3rd	4th	5th	6th	7-11th	12th
a. Dehydrocholic acid	1.56	3.24	2.50	2.11	1.48	—	—	—
b. Dehydrocholic acid	0.00	0.00	0.99	12.04	4.01*	—	—	—
c. Dehydrocholic acid	15.35	66.52	10.57	2.80	0.00	1.65*	0.00	4.40*
d. Dehydrocholic acid + Cholesterol	2.62	6.60	2.58	0.93	—	—	—	—
e. Cholic acid	0.00	0.00	0.00	2.80	0.00	28.15*	—	—
f. Cholic acid + Cholesterol	0.00	0.00	2.45	0.00	—	—	—	—
g. Chenodeoxycholic acid	0.00	0.00	0.99	11.25	1.80*	—	—	—
h. Ursodeoxycholic acid	0.00	0.00	3.44	14.40	0.00	37.44*	—	—
i. Deoxycholic acid	0.00	0.00	0.00	4.05	0.00	0.00*	—	—

* Methionine (5 mg/animal/day) is administered for seven days on the sited week only.

Since continued feeding with methionine alone (5 mg/animal/day) has not induced isovalthinuria at least up to the end of fourth week after the start of administration, the above phenomena may be considered to indicate that the feeding with bile acids is accompanied by the reduction of sulfur compounds in the animal body. No methionine effect observable in the case of deoxycholic acid cannot be explained at present (Table 1. i).

4. Induction of Isovalthinuria by Hypocholesterolemic Agents

The results obtained by feeding with hypocholesterolemic agents are summarized in Table 2. Triparanol and 22, 25-diazacholesterol can induce isovalthinuria having the maximum peak of excretion on the third week (Table 2. a, b, and d). Cholesterol appears to have no influence on the induction effect of azasterol (Table 2. b). Although induction effect of 20, 25-diazacholesterol seems to be quite weak, a large amount of isovalthine has been excreted following the administration of methionine on the sixth week (Table 2. c). This phenomenon may also indicate that the administration of azasterol is accompanied by the depression of sulfur compounds in the animal body. SKF 525-A exhibits very weak induction activity barely on the sixth week, and methionine administration on the seventh week has increased isovalthine excretion (Table 2, e).

Table 2 Experimental Isovalthinuria Induced By Hypocholesterolemic Agents.

Inducers	Week	μ moles of total isovalthine excreted by two guinea pigs per week.						
	1st	2nd	3rd	4th	5th	6th	7th	
a. 22, 25-Diazacholesterol	0.00	2.05	2.35	0.00	—	—	—	
b. 22, 25-Diazacholesterol + Cholesterol	0.00	1.08	1.28	0.00	—	—	—	
c. 20, 25-Diazacholesterol	0.00	0.00	0.00	0.60	0.00	20.30*	—	
d. Triparanol	0.00	1.71	20.40	0.00	—	—	—	
e. SKF 525-A	0.00	0.00	0.00	0.00	0.00	0.75	5.42*	

* Methionine (5 mg/animal/day) is administered for seven days on the sited week only.

DISCUSSION

It has been reported that bile acids inhibit the biosynthesis of cholesterol by a double feedback mechanism⁸. Chenodeoxycholic and 3-hydroxy-7-oxocholanic acids are known to be the main constituents of the bile of guinea pig, and cholic acids is said to be contained in the adult bile only⁹. But there appears to be no difference in the induction activity between constituents and non-constituents of the bile. The amount of urinary isovalthine following the administration of these bile acids may rather depend on the individual constitution of each animal.

Since hypocholesterolemic agents tested here are said to inhibit some enzymatic steps of cholesterol biosynthesis^{10,11,12,13} it may be considered that treatment of animals with these bile acids or those hypocholesterolemic agents will lead to the accumulation of cholesterol precursors such as isovaleric acid etc. and this accumulation of precursors will then lead to the formation of isovalthine by conjugating with sulfur compound. This consideration, however, is not certain at present, because some preparations of glycocorticoids and ACTH also induce isovalthinuria.* The latter findings might suggest also a correlation of isovalthine with stress. At any rate, further experiments will be needed for the elucidation of the exact meaning of isovalthine excretion.

The sulfur compound which conjugates directly with isovaleric acid or related compound is now considered to be glutathione from the experiment of KUWAKI et al.⁶. Methionine administered will fortify the concentration of glutathione in animal body or may also activate some SH enzymes which might participate in the synthetic reaction of isovalthine precursor (glutathione-isovaleric acid conjugate). Although it is only a single experiment in this laboratory, the experiment shows that an atherosclerotic patient having no isovalthinuria has excreted isovalthine with a concomitant decrease of blood cholesterol after receiving methionine. Thus isovalthine formation might have a correlation with some reports^{14,15} which show the blood cholesterol lowering effect of some sulfur amino acids.

SUMMARY

Some bile acids (dehydrocholic, cholic, chenodeoxycholic, ursodeoxycholic, and deoxycholic acids), and some hypocholesterolemic agents (22, 25-diazacholesterol, 20,25-diazacholesterol, triparanol, and SKF 525-A) are the inducers of isovalthinuria in guinea pig. Administration of methionine appears to increase the pool of sulfur compound which participates in the formation of isovalthine. Cholesterol appears to have no enhancing effect on the induction activity of isovalthinuria inducers. The mechanism of isovalthine formation and the role of sulfur amino acids in lowering blood cholesterol are discussed.

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