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Takehira Shimomura

Abstract

1. For the elucidation of the mechanism of isovalthinuria induction, some precursors of cholesterol biosynthesis and some other compounds were tested in guinea pigs to know whether they induced isovalthinuria or not. 2. Among the cholesterol precursors tested, isovaleric, β -methylcrotonic, and β -methylglutaconic acids were found to be strong inducers of isovalthinuria, but acetic acid, acetoacetic acid, β -hydroxy- β -methylglutaric acid, squalene, α -ketoisocaproic acid, and leucine were not. 3. Among the other compounds tested, palmitic acid and L-cysteine were found to be weak inducers of isovalthinuria. 4. Some antagonistic phenomena were found to occur. The inducing effect of isovaleric acid was inhibited strongly by dexamethasone or dehydrocholic acid, and weakly by insulin. The inducing effect of dexamethasone was inhibited by dehydrocholic acid or thyroxine. The inducing effect of dehydrocholic acid was inhibited by insulin or dexamethasone. 5. Although ethionine was not an inducer in itself, it acted as a substitute for methionine or cysteine in the case of the sulfur deficiency produced by prolonged feeding on bile acid. 6. Isovalthinuria was observed by the administration of bromural, but this effect may be a detoxication but not induction,

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EXPERIMENTAL ISOVALTHINURIA*

VI. INDUCTION BY CHOLESTEROL PRECURSORS AND SOME OTHER COMPOUNDS

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Isovalthine is a new amino acid which is found in the urine of cats and some hypercholesterolemic patients¹. In the previous papers on experimental isovalthinuria, it has been reported that isovaleric acid², bile acids³, some hypocholesterolemic agents³, ACTH⁴, glucocorticoids⁴, methionine^{4,5}, and Δ^4 -cholestenone⁴ are the inducers of isovalthinuria in guinea pigs.

Isovaleric acid is known to be a precursor of cholesterol biosynthesis⁶, so some other precursors are tested and it is found that β -methylcrotonic acid and β -methylglutaconic acid are strong inducers of isovalthinuria. For understanding the mechanism of isovalthinuria induction, some other data related to experimental isovalthinuria are also presented in this paper.

MATERIALS AND METHODS

Male guinea pigs weighing around 400 g were used as the experimental animals. The methods of breeding, urine collection, and the determination of urinary isovalthine were the same as described in a previous paper⁴.

Acetoacetic acid⁷, β -methylcrotonic acid⁸, β -methyl-glutaconic acid^{9,10}, β -hydroxy- β -methylglutaric acid⁹, and 3-methylpentanoic acid¹¹ were prepared by S. OHMORI and T. UBUKA of laboratory according to the method of each reference cited. α -Keto-isocaproic acid was a generous gift of Dr. M. IGARASHI of the Institute of Physical and Chemical Research (Tokyo)¹². The other compounds used in this experiment were the commercial products.

The dose and the administration procedure of these compounds were described in each column of Tables 1 and 2. Organic acids were all administered in the form of sodium salts.

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RESULTS

I. Induction of Isovalthineria by Cholesterol Precursors:

Isovaleric acid has already been reported to be an isovalthineria inducer in some animals², but the data were not quantitative. It was therefore reexamined quantitatively, and the influences of some compounds on the inducing effect of isovaleric acid were also tested. As shown in Table 1, when administered orally, 50 mg per animal per day of isovaleric acid induced strong isovalthineria in the first week, but 25 mg per animal per day of isovaleric acid did not induce isovalthineria in guinea pigs. Intraperitoneal injection of isovaleric acid (50 mg/animal/day) showed a very weak inducing effect. Subcutaneous injection of ACTH or epinephrine induced isovalthineria, as shown in a previous paper⁴, but parenteral administration of some strong inducers such as dexamethasone or dehydrocholic acid did not induce isovalthineria as shown in Table 2. The discrepancy of the different effects between oral and parenteral administration is unintelligible at present.

Insulin counteracted the inducing effect of dexamethasone⁴, ACTH⁴, or dehydrocholic acid (Table 2), but the inducing effect of isovaleric acid was weakly counteracted by insulin and completely inhibited by dexamethasone or dehydrocholic acid. It is quite a comprehensive fact that a strong isovalthineria inducer inhibits the inducing effect of another strong inducer. As shown in Table 2, the inducing effect of dehydrocholic acid is also inhibited by dexamethasone, and *vice versa*.

Methionine was an inducer⁴ and did not disturb the inducing effect of isovaleric acid, but it somewhat delayed the maximum peak of isovalthine excretion.

Leucine was once reported to enhance isovalthine excretion in cats¹³, but the data were not quantitative. Reexamination in guinea pigs showed that leucine was not an inducer at the dose of 150 mg per animal per day and weakly inhibited the inducing effect of isovaleric acid.

Acetic acid was not an isovalthineria inducer and appeared to disturb the inducing effect of isovaleric acid. Isobutyric acid and 3-methylpentanoic acid were the non-inducers at the dose of 50 mg per animal per day (Table 2), and they completely inhibited the inducing effect of isovaleric acid.

In all the above experiments, isovalthine excreted was the mixture of L- and L-allo-isovalthine¹⁴.

α -Ketoisocaproic acid showed no induction effect of isovalthineria.

By the administration of β -methylcrotonic acid, guinea pigs excreted the mixture of L- and L-allo-isovalthine and also S-(1, 1-dimethyl-2-carboxyethyl)-L-cysteine (called β -isovalthine). The formation of β -isovalthine may be a non-

Table 1 Experimental Isovalthinnria by Cholesterol Precursors

	dose per animal per day	method of administration	μ moles of isovalthine excreted per two guinea pigs per week					
			1st	2nd	3rd	4th	5th	6th
Isovaleric Acid	25.0 mg	p. o.	0	0	0	0	0	0
Isovaleric Acid	50.0 mg	i. p.	0	0	+	0		
Isovaleric Acid	50.0 mg	p. o.	80.2	+	+	+		
Isovaleric Acid	50.0 mg	p. o.						
+ Dexamethasone	0.1 mg	p. o.	0	0	0	0	0	
+ Dehydrocholic Acid	5.0 mg	p. o.	0	0	0	0	0	
+ Insulin	0.5 unit	s. c.	0	0	21.9	25.9	0	
+ Insulin	2.0 unit	s. c.	0	0	+	0		
+ Methionine	5.0 mg	p. o.	2.0	12.1	+	+		
+ Methionine	5.0 mg	p. o.	+	0	30.4	0		
+ Leucine	25.0 mg	p. o.	0	+	+	0		
+ Acetic Acid	30.0 mg	p. o.	+	+	+	+		
+ Isobutyric Acid	50.0 mg	p. o.	0	0	0	0		
+ 3-Methylpentanoic Acid	50.0 mg	p. o.	0	0	0	0		
L-Leucine	150.0 mg	p. o.	0	0	0	0	0	
α -Ketoisocaproic Acid	100.0 mg	p. o.	0	0	0	0		
β -Methylcrotonic Acid	50.0 mg	p. o.	0	29.5	0	0		
β -Methylcrotonic Acid	50.0 mg	p. o.						
+ Methionine	5.0 mg	p. o.	0	0	+	0		
β -Methylglutaconic Acid (cis+trans)	100.0 mg	p. o.	0	29.6	5.9	15.6*	+	0
β -Methylglutaconic Acid (trans)	100.0 mg	p. o.	0	23.8	0	0*		
β -Methylglutaconic Acid (cis+trans)	100.0 mg	s. c.	0	0	0	0		
β -Methylglutaconic Acid (cis)	100.0 mg	i. p.	0	0	0	0		
β -Hydroxy- β -methylglutaric Acid	50.0 mg	p. o.	0	0	0	0		
β -Hydroxy- β -methylglutaric Acid	100.0 mg	p. o.	0	0	0	0		
Acetic Acid	30.0 mg	p. o.	0	0	0	0		
Acetoacetic Acid	200.0 mg	p. o.	0	0	0	0		
Acetoacetic Acid	100.0 mg	i. p.	0	0	0	0		
Acetoacetic Acid	500.0 mg	i. p.	0	0	0	0		
Squalene	100.0 mg	p. o.	0	0	0	0	0	

+ Positive on paper electropherogram but negative on amino acid analyzer.

| Stop the administration of inducer at the week end cited.

* Methionine (5mg/animal/day) is administered for 7 days on the cited week only.

p. o.: per os i. p.: intraperitoneal s. c.: subcutaneous.

enzymic reaction. It has not been found in any normal animal urine. Synthesis of β -isovalthine has already been reported elsewhere¹⁵. Methionine did not enhance the inducing effect of β -methylcrotonic acid.

β -Methylglutaconic acid was also a strong isovalthinnria inducer when

Table 2 Supplementary Data on Experimental Isovalthineuria

	dose per animal per day	method of administration	μ moles of isovalthine excreted per two guinea pigs per week							
			1st	2nd	3rd	4th	5th	6th	7th	8th
Palmitic Acid	1.0 gm	p. o.	0	0	0	+	17.5	0	0	0*
Palmitic Acid	1.0 gm	p. o.								
+ Dexamethasone	0.1 mg	p. o.	0	0	0	0	28.3	0	0	
Octanoic Acid	7.5 mg	p. o.	0	0	0	0	0	0	0	
Butyric Acid	50.0 mg	p. o.	0	0	0	0	0			
Isobutyric Acid	50.0 mg	p. o.	0	0	0	0	0			
3-Methylpentanoic Acid	50.0 mg	p. o.	0	0	0	0	0			
L-Cysteine	5.0 mg	p. o.	0	0	0	0	12.8	20.4	1.5	0
S-Methylcysteine Sulfoxide	10.0 mg	p. o.	0	0	0	0	0	0	0	0
Methionine Sulfoxide	20.0 mg	p. o.	0	0	0	0	0	0	0	0
Methionine Sulfoxide	50.0 mg	p. o.	0	0	0	0	0	0	0	0
DL-Ethionine	20.0 mg	p. o.	0	0	0	0	0	0	0	0
Taurine	10.0 mg	p. o.	0	0	0	0	0	0		
L-Valine	200.0 mg	p. o.	0	0	0	0	0			
Norepinephrine	0.1 mg	s. c.	0	0	0	0				
Thyroxine	0.1 mg	p. o.	0	0	0	0	0	0		
Dexamethasone	0.1 mg	i. p.	0	0	0	0				
Dexamethasone	0.1 mg	p. o.								
+ Thyroxine	0.1 mg	p. o.	0	0	0	0	0	0		
+ Dehydrocholic Acid	5.0 mg	p. o.	0	0	0	0	0	0	0	
+ Acetic Acid	10.0 mg	p. o.	0	0	0	5.7	8.8	19.6	0	
+ L-Leucine	5.0 mg	p. o.	0	0	0	7.8	6.0	+		
+ L-Methionine	5.0 mg	p. o.	0	11.9	7.3	27.1	8.7	+		
+ Leu (5 mg) + Met (2 mg)		p. o.	0	30.5	14.0	13.5	7.2	+		
Glycoursodeoxycholic Acid	6.0 mg	p. o.	0	0	0	0	0	0*		
Dehydrocholic Acid	5.0 mg	i. p.	0	0	0	0	0			
Dehydrocholic Acid	5.0 mg	p. o.								
+ Dexamethasone	0.1 mg	p. o.	0	0	0	0	0	0	0	
+ Insulin	0.5 unit	s. c.	0	0	0	0	0	0	0	0
+ L-Methionine	5.0 mg	p. o.	0	4.6	5.9	14.5	10.7	4.4	1.3	3.9
+ Insulin (0.5 unit) + Met (2 mg)		s.c. & p.o.	0	0	0	0	+	0	0	
Cholic Acid	5.0 mg	p. o.	0	0	17.3	25.4	18.6	18.7		
+ DL-Ethionine	10.0 mg	p. o.					40.8	2.9		
+ Cystathionine	20.0 mg	p. o.					19.0	+		
+ Sodium Thiosulphate	25.0 mg	p. o.					0	0		
Bromural	60.0 mg	p. o.	18.2	15.2	20.7					

+ Positive on paper electropherogram but negative on amino acid analyzer.

| Stop the administration of inducer at the week end cited.

* Methionine (5 mg/animal/day) is administered for 7 days on the cited week only.

p. o.: per os i. p.: intraperitoneal s. c.: subcutaneous.

administered per os, but parenteral administration was not effective at the dose cited in Table 1.

β -Hydroxy- β -methylglutaric acid (HMG), unexpectedly however, was not an inducer. To our surprise, HMG showed a strong diuretic effect. The volume of urine of guinea pigs fed HMG was about 5—7 times that of control animals. Acetoacetic acid and squalene were not inducers of isovalthinnria. Δ^4 -Cholestenone³ has already been reported to be an isovalthinnria inducer, but it has not been accepted as a precursor of cholesterol biosynthesis.

II. *Supplementary Data on Experimental Isovalthinnria:*

The supplementary data on experimental isovalthinnria are summarized in Table 2.

Among the fatty acids tested, only palmitic acid acted as an isovalthinnria inducer. Dexamethasone did not disturb the inducing effect of palmitic acid. Isovalthine excreted in these cases was a mixture of L- and L-allo-isovalthine, and L-allo was more in quantity than L-form.

By the administration of L-cysteine to guinea pigs, a mixture of L- and L-allo-isovalthine was excreted at the 5th—7th week after the start of its administration. S-Methylcysteine sulfoxide, methionine sulfoxide, ethionine, taurine, and L-valine were not inducers of isovalthinnria.

Although epinephrine was a inducer⁴, norepinephrine was not. The inducing effect of dexamethasone⁴ was inhibited by thyroxine or dehydrocholic acid, but not by acetic acid, leucine, and methionine. Intraperitoneal injection of dexamethasone was not effective.

Ursodeoxycholic acid has been reported to be an inducer³ but glycourso-deoxycholic acid was not. The inducing effect of dehydrocholic acid³ was inhibited by insulin or dexamethasone, but not by methionine. In the latter case, L-isovalthine was excreted much more than L-allo. Intraperitoneal injection of dehydrocholic acid was not effective.

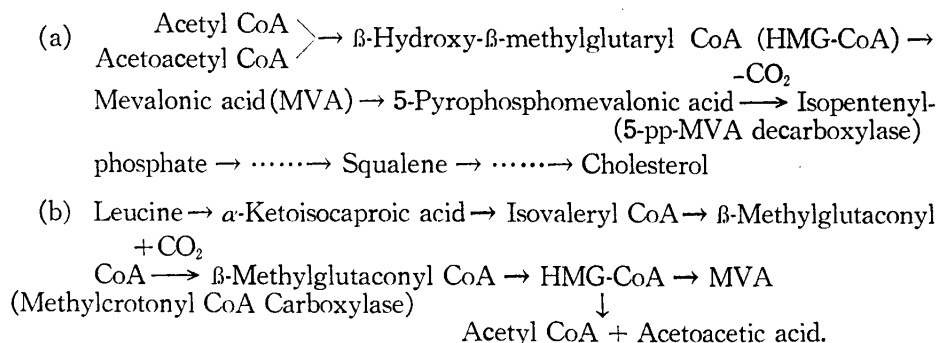
It has been shown in the previous papers^{3,5} that, when guinea pigs were fed on cholic acid, the maximum excretion of isovalthine was usually observed in the 4th week and then isovalthine disappeared from urine when the feeding on cholic acid was continued. At such a time, when methionine or cysteine was administered for 7 days, a remarkable isovalthinnria was again observed. Taking advantage of this phenomenon, ethionine, cystathionine, and sodium thio-sulphate ($\text{Na}_2\text{S}_2\text{O}_3$) were tested in place of methionine or cysteine. Eight male guinea pigs weighing about 450 g were fed on cholic acid (5 mg/animal/day) in a cage for 4 weeks, and urinary isovalthine was measured every week, and one fourth of the amount of urinary isovalthine is cited in the Table 2. On the 5th week, each two guinea pigs was separated in 4 cages and fed on cholic acid, cholic + ethionine, cholic + cystathionine, and cholic + $\text{Na}_2\text{S}_2\text{O}_3$ respec-

tively. Although ethionine alone had no induction effect as described above, it showed the same enhancing effect of isovalthine excretion as methionine or cysteine. This finding might be related to the observation previously reported by SEIDEL *et al.* that ethionine had a lowering effect of serum cholesterol level in rats¹⁶. Cystathionine appears to have a weak or no enhancing effect, but thiosulphate inhibits the effect of cholic acid.

Isovalthine has been isolated from the urine of some people who took bromural (α -bromoisovalerylurea)¹⁷. This phenomenon was reexamined quantitatively in guinea pigs. As shown in Table 2, nearly the same amount of isovalthine (only L-alloisovalthine) was excreted every week. The mechanism of isovalthine formation by the administration of bromural may be different from that by isovaleric acid, etc. The former will be a detoxication (direct conjugation) and the latter an induction.

DISCUSSION

The main biosynthetic pathway of cholesterol from acetate (a) and an alternate pathway (b) have been outlined as follows:



The above scheme, however, seems to be unsatisfactory to explain the following findings. ZABIN and BLOCH⁶ have reported that isopropyl group of isovaleric acid incorporates much more efficiently into cholesterol than acetate or carboxyl group of isovaleric acid does, and that the incorporation of carbon dioxide into cholesterol is more markedly increased in the presence of isovaleric acid than in the absence of the acid. According to the above scheme, however, the incorporation efficiency of isopropyl group and carboxyl group of isovaleric acid should be equal, and the carbon dioxide which is fixed in the methylcrotonyl CoA carboxylase reaction should be eliminated in the 5-pyrophosphomevalonate decarboxylase reaction and never incorporates into cholesterol. Furthermore, the data obtained in this laboratory show that isovaleric acid is a strong isovalthinuria inducer but isovaleric acid-4-C¹⁴ or -1-C¹⁴ does not incorporate

into urinary isovalthine^{18,19}, and that several compounds on the alternate pathway of cholesterol biosynthesis are the inducers of isovalthinnria but some on the main pathway are not. These data may represent another alternate pathway of cholesterol biosynthesis from isovaleric acid.

Fairly numerous data on isovalthinnria induction have now been obtained, but its exact mechanism is still far from clear. Several curious phenomena encountered in these experiments will be given as follows: 1. A strong inducer inhibits the inducing effect of another strong inducer. 2. Some of non-inducers inhibit the inducing effect of isovaleric acid. 3. The effectiveness of inducers is different according to the methods of administration.

Further work will be needed for the elucidation of the mechanism of isovalthinnria induction.

SUMMARY

1. For the elucidation of the mechanism of isovalthinnria induction, some precursors of cholesterol biosynthesis and some other compounds were tested in guinea pigs to know whether they induced isovalthinnria or not.

2. Among the cholesterol precursors tested, isovaleric, β -methylcrotonic, and β -methylglutaconic acids were found to be strong inducers of isovalthinnria, but acetic acid, acetoacetic acid, β -hydroxy- β -methylglutaric acid, squalene, α -ketoisocaproic acid, and leucine were not.

3. Among the other compounds tested, palmitic acid and L-cysteine were found to be weak inducers of isovalthinnria.

4. Some antagonistic phenomena were found to occur. The inducing effect of isovaleric acid was inhibited strongly by dexamethasone or dehydrocholic acid, and weakly by insulin. The inducing effect of dexamethasone was inhibited by dehydrocholic acid or thyroxine. The inducing effect of dehydrocholic acid was inhibited by insulin or dexamethasone.

5. Although ethionine was not an inducer in itself, it acted as a substitute for methionine or cysteine in the case of the sulfur deficiency produced by prolonged feeding on bile acid.

6. Isovalthinnria was observed by the administration of bromural, but this effect may be a detoxication but not induction,

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