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Uptake and modification of dietary polyprenols and dolichols in rat liver

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Short and long dolichols and polyprenols in free form or esterified with fatty acids were incorporated into liposomes and administered to rats through a gastric tube. The free alcohols were taken up by the liver to different extents. While uptake in other organs was less, it also involved the fatty acid esters. The use of systems other than liposomes did not increase the efficiency of uptake. Most of the administered lipids were recovered in the lysosomes. Exogenous dolichols and polyprenols were both partly esterified in the liver and, to some extent, also phosphorylated; a portion of the polyprenols was also α-saturated. These results indicate that various polyisoprenes are taken up, to a small extent, from the diet by tissues under normal conditions and in liver these dietary lipids undergo terminal modifications.

Dolichol; Polyprenol; Dietary uptake; Polyisoprenoid esterification; Dolichol phosphorylation

1. INTRODUCTION

The main products of the mevalonate pathway, i.e., cholesterol, ubiquinone and dolichol, are synthesized in most tissues so far investigated and these lipids are regarded as playing obligatory roles in basic cellular functions [1]. On the other hand, it is well established that a considerable portion of the cholesterol utilized in metabolic processes is not synthesized de novo in the cell, but is supplied in the diet. Concerning ubiquinone it was proposed that a few percent of this lipid in the tissues originates from the diet [2]. Uptake of polyisoprenoid lipids from the diet has been studied much less than the uptake of cholesterol or ubiquinone. Concerning α-saturated and α-unsaturated polyisoprenoids, it was found in previous experiments that undecaprenol (polyprenol-11) supplied orally is taken up through the gastrointestinal system to a small extent [3], while dolichol-19 administered orally to the rat did not appear in any appreciable amount in the liver [4]. Possibilities arise that the nature of the polyisoprenoid and the route of its administration may be critical to the uptake process and that these lipids are selectively utilized.

The normal diet contains a rich supply of both α-unsaturated and α-saturated polyprenols from both plant and animal sources. A small dietary uptake may not necessarily be unimportant, since the liver and other organs are capable of modifying these lipids to metabolically more active species. Such modification may involve α-saturation, phosphorylation by a specific kinase, dephosphorylation by a phosphatase, esterification with a fatty acid and/or elongation [5].

In the present study different polyprenols were administered to rats in different mixtures to determine whether the rates of uptake were different. It appears that the conditions used affect the entrance of these lipids into the body, but that the net uptake is in all cases quite limited.

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2. MATERIALS AND METHODS

Male Spraque-Dawley rats weighing 180 g were used in these experiments. The animals were fasted for 20 h before administering the lipid emulsion (1.5 ml) into the stomach through a gastric tube attached to a syringe.

The lipid emulsion was prepared by dissolving 30 mg egg lecithin (Lipid Products, South Nutfield, England) in chloroform/methanol, 2:1, and subsequently mixing with 1–2 mg labeled polyisoprenoids (14×10^6 dpm). After evaporation of the solvent, 3 ml of 0.9% NaCl was added and the tube vortexed for 3 min. This mixture was maintained in an ice-cold water bath and under a nitrogen atmosphere and sonicated in intervals for 6 min. The final mixture had a clear appearance.

Homogenization of the various organs in 0.25 M sucrose was performed with an Ultra-Turrax homogenizer. The mitochondrial-lysosomal, microsomal and supernatant fractions were prepared as described earlier [6]. The mitochondrial fraction isolated by differential centrifugation contains the majority of the lysosomes. Since upon gradient fractionation only a part of these lysosomes can be isolated and since polyisoprenols are present only in very low concentrations in mitochondria, the combined mitochondrial-lysosomal fraction is considered to be the most appropriate fraction to use for assaying lysosomal polyprenol content.

A mixture containing 12.5 ml methanol, 6.25 ml chloroform and 5 ml homogenate or particle suspension was incubated at 40°C for 1 h for lipid extraction. The protein precipitate was reextracted with 6.25 ml chloroform at 40°C for 30 min. The combined extracts were seperated into two phases by the addition of 6.5 ml water and the chloroform phase was washed twice with theoretical upper phase [7]. After evaporation of the solvent, radioactivity was determined by scintillation counting.

When dolichol, polyprenol and their esterified derivatives were to be separated, these lipids dissolved in methanol were purified on a C-18 cartridge (Supelco) and the free alcohols and esterified derivatives were isolated by high-performance liquid chromatography (HPLC) on a C-18 column [8]. For separation of the α-unsaturated and α-saturated forms, HPLC on a silica column was employed [9]. When polyprenyl-P or dolichyl-P were to be isolated, the tissue suspensions were first subjected to alkaline hydrolysis and then the lipid extracts were purified by DEAE-Sephadex chromatorgraphy.

Dolichyl-P and polyprenyl-P were isolated by HPLC using a C-18 column in the presence of 20 mM phosphoric acid [8].

Polyprenol-7 was isolated from *Betula verrucosa*, polyprenol-12 from *Pinus silvestris* and polyprenol-19 from *Gingko biloba* [10]. α-saturation was performed by the method of Mankowski et al. [10]. The labeling of polyisoprenols with tritium was performed using NaB^3H_4 (Amersham, England; spec. act. 16.5 Ci/mmol) according to Keenan and Kruczek [11]. Esterification of various polyprenols and dolichols was achieved with palmitoyl chloride [12].

3. RESULTS

Various types of labeled polyisoprenols incorporated into liposomes were introduced into the stomach through a gastric tube and the appearance of the lipids in tissues 20 h later was analyzed (table 1). The amount of radioactive lipid recovered in the liver homogenate was clearly dependent on the nature of the compound administered. Relatively high uptake was obtained with polyprenol-12, exceeding to some extent the uptake of polyprenol-19. Uptake of dolichol was less efficient than that of its α-unsaturated counterpart. The use of esterified derivatives of both α-unsaturated and α-saturated polyisoprenes eliminated completely their appearance in liver.

In stomach and intestine, uptake of both short and long polyprenols occurred and no major dif-

Table 1

Uptake of various dietary polyprenols into organs of the rat

	Uptake (dpm per g wet weight)							
Organ	Pol-19	Pol-19--FA	Dol-19	Dol-19--FA	Pol-12	Pol-12--FA	Dol-12	Dol-7
Liver	52 212	543	21 382	397	93 642	2303	69 368	8719
Kidney	5260	5731	2154	1017	4036	4274		
Thyroid	2070	4341	781	509	1219	3026		
Fat	736	1505	1010	1159	1024	1469		
Lung	6719	19 490	5099	2697	8911	7305		
Brain	707	2981	322	719	1724	3115		
Spleen	1007	1962	808	786	2188	3291		
Testis	820	2332	1127	517	1815	3740		
Stomach	23 875	49 724	39 118	40 188	30 870	31 416		
Intestine	8700	26 173	10 303	4002	4805	5240		
Muscle	1425	7031	661	1281	7067	2671		

The values represent the means of 5–7 experiments. For the sake of clarity, no SE are presented

Table 2

Influence of the form of administration on [^{3}H]polyisoprenol-12 uptake in liver

Form administered	Uptake (dpm/g wet weight)
Lipsomes	93 642 ± 7679
In ethanol	74 281 ± 7502
In bile	33 459 ± 3680
In corn oil	20 346 ± 1506

The preparation of lipsomes is described in section 2. The labeled polyprenol was suspended in 95% ethanol, rat bile or corn oil. The suspension with rat bile was sonicated for 3 min. The values are the means ± SE of 6 experiments

ferences were observed when α-saturated or α-unsaturated lipids were administered. In contrast to the findings with liver, equal amounts of the esterified forms were also recovered in the homogenates from stomach and intestine. In the other organs examined the amount of radioactive lipid varied, being relatively high in kidney and lung and lower in other tissues. In all of these organs no selection was apparent when the uptake of different alcohols and their esterified derivatives was compared.

The uptake of lipids from the intestine is regulated by a number of physical parameters and the possibility arose that the lipid mixture as supplied was in a form which was taken up inefficiently. When dolichol was sonicated in an anqueous medium together with egg lecithin, electron microscopy demonstrated the formation of single unilamellar vesicles. This mixture was suitable for studying the uptake of polyprenol-12 into the liver (table 2). Using an ethanol solution as carrier instead of lipsomes, the uptake was reduced. A mixture prepared by sonication in bile was even less effectively taken up than that in ethanol. When polyprenols were dispersed in corn oil, the uptake into liver was reduced to 20% of that seen in the case of liposomes. Clearly, using the procedures described above for the solubilization of dietary lipids, the extent of lipid entering into the body was not substantially increased in comparison to the use of liposomes as carriers.

The intracellular distributions of the dietary polyprenol-12 and dolichol-12 recovered in the

Table 3

Distribution of dietary polyisoprenols in liver fractions

Fraction	[^{3}H]Polyprenol-12 (dpm/g wet weight)	[^{3}H]Dolichol-12 (dpm/g wet weight)
2000 × *g* pellet	13 195 ± 1267	12 127 ± 1237
Mitochondria-lysosomes	62 861 ± 7040	47 651 ± 5242
Microsomes	9409 ± 734	1738 ± 143
Supernatant	913 ± 93	526 ± 52

The values are the means ± SE of 7 experiments

Table 4

Modification of dietary dolichol-12 and polyprenol-12 in the liver

Lipid administered	Lipids isolated	Amount (dpm/g wet weight)
Dolichol-12	dolichol-12	40 623 ± 4194
	dolichyl-12-ester	15 387 ± 1416
	dolichyl-12-P	5538 ± 620
Polyprenol-12	α-saturated polyprenol-12	35 846 ± 3226
	α-unsaturated polyprenol-12	13 940 ± 1631
	polyprenyl-12-ester	26 339 ± 3161
	polyprenyl-12-P	6832 ± 687

Dolichol-12 or polyprenol-12 was given to the rats via a gastric tube. 20 h later the liver homogenates were prepared and extracted, and radioactivity was determined in the isolated lipid fractions. The values are the means ± SE of 6 experiments

liver were determined by subfractionation (table 3). More than half of the polyisoprenols were found in the mitochondrial-lysosomal fraction. Also, a relatively high polyprenol content was observed in the 2000 × *g* pellet. In addition to debris, this pellet contains mainly nuclei, which appear to accumulate polyisoprenols to some extent. Both microsomes and the high-speed supernatant fraction contained only smaller amounts of labeled polyisoprenols.

The lipids recovered in the liver homogenate were analyzed by HPLC and the radioactivity present in individual fractions determined. The data in table 4 demonstrate that a portion of the polyisoprenols taken up was modified enzymatically. One-third of the dolichol-12 taken up by the liver appeared in esterified form and a smaller portion, about 10%, was phosphorylated. Part of the polyprenol-12 was recovered in α-saturated form and, similar to the case of dolichol-12, this lipid was also esterified and phosphorylated. Under the conditions used here and during the time period employed, no sign of elongation of polyisoprenoids was observed upon HPLC analysis.

4. DISCUSSION

The uptake of polyisoprenols from the intestine in rat occurs only to a limited extent (a few percent of total) and this uptake was not increased when the mixture administered was in ethanol solution, in bile or mixed with corn oil. The best results were obtained using unilamellar vesicles, but the question remains as to whether micellar forms which are taken up more effectively may exist. Specific lipids may interfer with hydrophobic interactions or surface charge. Alternatively, it may turn out that carrier proteins are involved and that the uptake of polyisoprenols proceeds via a receptor mechanism.

The non-esterified polyisoprenols administered to the rat appeared in alle the organs examined with the highest uptake in the liver. Previous studies have established that the liver contains metabolic systems catalyzing terminal modifications of polyisoprenoid lipids [5]. These systems include the NADH-dependent α-saturase, which uses various polyprenols are substrate, the CTP-dependent dolichol kinase and the acyl-CoA:dolichol acyltransferase. Clearly, these enzymes utilize not only endogenously synthesized polyisoprenols, but also exogenous lipid taken up from the diet, since α-saturation, limited phosphorylation and a relatively extensive esterification of the exogenous compounds were observed here. Since condensation with isopentenyl-PP requires the presence of a pyrophosphate leaving group, it is not surprising that in our system no elongation was observed.

Upon subfractionation of the liver, most of the polyisoprenoids were recovered in the mitochondrial-lysosomal fraction and was obviously mainly located in the lysosomes. This finding suggests that the uptake process is receptor-mediated and involves lipoproteins [13] in agreement with the fact that dolichol is associated with lipoproteins in the blood [14]. It also appears that most extrahepatic tissues utilize a mechanism for dolichol uptake which differs from that of the liver, since, in contrast to the liver, other tissues took esterified derivatives up to the same extent as the free alcohols.

It is possible that in the future one may be able to identify conditions and pathways by which the entry of dietary polyprenols into the liver, as well as into other organs, may be increased. Altered physiological conditions or pathological processes may enhance uptake, as they enhance metabolic or catabolic reactions. A number of drug treatments influencing peroxisomes and the endoplasmic reticulum affect not only membrane synthesis and/or metabolic reactions such as hydroxylation and β-oxidation of fatty acids, but also rapidly increase dolichol content [15]. Under such conditions dietary uptake may supplement endogenous synthesis or maintain basic levels when synthesis is damaged or inhibited.

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