Effects of alkali-metal ions on phospholipid and triglyceride synthesis in rat liver slices

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SUMMARY The influence of alkali-metal ions on the incorporation of labeled precursors into phospholipids and triglycerides was studied in rat liver slices. By use of acetate-1-C^{14} and palmitate-1-C^{14} as lipid precursors, a comparison was made of the pathway from acetate to phospholipid and the pathway from palmitate to phospholipid. While the rate of incorporation of palmitate-1-C^{14} into phospholipids and triglyceride fatty acids was virtually independent of the alkali-metal ion present in the medium, the rate of incorporation of acetate-1-C^{14} into the same compounds was strongly influenced by the alkali-metal ions, the highest incorporations being obtained with potassium and rubidium. Under the same conditions lithium appears to have an inhibitory effect. The effects of the alkali-metal ions on phospholipid and triglyceride synthesis in liver are located on the pathway between acetate and long-chain fatty acids.

KEY WORDS fatty acid biosynthesis · phospholipid biosynthesis · triglyceride biosynthesis · rat · liver slices · alkali-metal ions

SOME ALKALI-METAL IONS are known to affect lipid metabolism in several organs. Both lithium and potassium ions have been shown to influence fatty acid oxidation in liver slices (1, 2). Cholesterol synthesis, as studied by acetate incorporation in rat liver slices, also appears to be greatly depressed when lithium is present in the incubation medium (3). On the other hand, Curran and Clute (4) demonstrated that cholesterogenesis from acetate by rat liver is increased by the addition of potassium to the incubation medium and Ashmore, Weber, and Landau (5) found that the same ion stimulated fatty acid synthesis from glucose in liver slices. Nevertheless, Kline and DeLuca (6) were unable to obtain a consistent effect of potassium ion on the rate of incorporation of acetate into phospholipids. More recently, Minard and Davis (7) found a stimulatory effect of potassium on lipogenesis from acetate in rat liver slices and Yoshida and Nukada (8) showed that the incorporation of orthophosphate-P^{32} into the phospholipids of brain slices, but not of liver slices, was increased by potassium ion, provided that sodium was also present in the incubation medium.

In the present work the effects exerted by the group of elements known as alkali metals on phospholipid and triglyceride synthesis in rat liver are studied and a first attempt is made to localize their action in the scheme leading to phospholipid and triglyceride synthesis, by comparing their effects on the incorporations of acetate-1-C^{14} and palmitate-1-C^{14} into the phospholipids and glyceride fatty acids of rat liver slices.

It has been established that the alkali-metal ions affect the incorporation of acetate into long-chain fatty acids.

MATERIALS AND METHODS

Animals

Adult female rats of the hooded Norwegian strain aged about 5–6 months and weighing 180–200 g were fed ad lib. on diet 41 of Bruce and Parkes (9). The animals were killed by cervical dislocation and the livers were rapidly removed, rinsed, and placed in ice-cold isotonic sucrose.

Incubation Procedure

Slices approximately 0.4 mm thick were cut from the livers with a Stadie-Riggs (10) microtome and 500 mg

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of them were suspended in 5 ml of a modified Krebs-Ringer phosphate buffer (11), pH 7.4, in which sodium and potassium chlorides had been replaced by the chloride of the alkali metal to be investigated. The solution contained either acetate-1-C^{14} or albumin-bound palmitate-1-C^{14} at a final concentration of 0.2 μc/ml. The acetate had a specific activity of 12.1 mc/mmole and the palmitate, 30.8 mc/mmole. Both substrates were obtained from the Radiochemical Centre, Amersham, U.K. No carrier was added. The palmitic acid-1-C^{14} was complexed with crystallized bovine albumin (British Drug Houses, Ltd., Poole, Dorset, U. K.,) by the technique described by Glenn et al. (12).

The flasks were gassed with oxygen and were incubated at 37° with shaking. The time of incubation varied from 30 min to 3 hr.

Extraction and Purification of Lipids

At the end of the incubation period the slices were collected by centrifugation, washed twice with ice-cold 0.1 M sodium phosphate buffer, pH 7.0, and then suspended and homogenized in 7 ml of cold 10% trichloroacetic acid in an all-glass Potter-Elvehjem (13) homogenizer. The suspension was centrifuged and the residue reextracted with 7 ml of cold 5% trichloroacetic acid. The trichloroacetic acid extracts were rejected. The solid residue was extracted once with 5 ml of warm 80% ethanol, twice with 4-ml portions of absolute ethanol, and finally once with 4 ml of warm ether. The alcohol and ether extracts were combined, the solvents were removed by evaporation in vacuo, and the residue was extracted three times with 4-ml portions of light petroleum (bp 60–80°). From the combined extracts the phospholipids were isolated by precipitation as their magnesium complexes from acetone solution, purified by redissolving in 0.5 ml of chloroform and reprecipitating by the addition of acetone, and counted at infinite thinness in a windowless gas-flow counter, as described previously (14).

Saponification and Radioactivity Measurements

The nonphospholipids remaining in the acetone solution were saponified in 4 N aqueous potassium hydroxide for 3 hr at 80°. After extraction of the unsaponified fraction by light petroleum the aqueous layer was acidified with 5 N H_2SO_4 and fatty acids were extracted with light petroleum. These fatty acids are derived mainly from triglycerides and are referred to as "triglyceride fatty acids" in this paper.

In the experiments on the incorporation of albumin-bound palmitate into triglyceride fatty acids, before the saponification procedure, the phospholipid-free extract was evaporated to dryness in vacuo, the residue was dissolved in petroleum ether (bp 60–80°) and shaken in a separating funnel with cold aqueous 0.02 N potassium hydroxide solution. This procedure was repeated twice and appeared to remove adequately any free palmitic acid, as tested in experiments where palmitic acid-1-C^{14} was added to unlabeled liver triglycerides and the triglyceride fraction tested for radioactivity. Samples of fatty acids were plated at infinite thinness on nickel planchets and weighed, and the radioactivity was measured in a 20th Century Electronics WF2 windowless gas-flow counter with a conventional scaler.

TABLE 1 EFFECT OF ALKALI-METAL IONS ON THE INCORPORATION OF ACETATE-1-C^{14} INTO THE PHOSPHOLIPIDS OF RAT LIVER SLICES

Time	Specific Radioactivity				
	Lithium	Sodium	Potassium	Rubidium	Cesium
min			*cpm/mg*		
30	63	224	447	363	251
60	116	329	679	591	350
90	191	542	799	692	518
120	241	640	971	883	611
180	288	731	1061	950	750

The liver slices for a given time interval for all alkali-metal ions were taken from the same liver.

Each flask contained initially in a total volume of 5 ml: 78 μmoles of sodium phosphate buffer pH 7.4, 6.0 μmoles of $MgSO_4$, 12.8 μmoles of $CaCl_2$, 625 μmoles of the metal to be investigated as its chloride, 1.0 μc of acetate-1-C^{14}, and 500 mg of rat liver slices. Gas phase: oxygen.

RESULTS AND DISCUSSION

The results showing the incorporation of carboxyl-labeled acetate into the liver phospholipids after various periods of incubation are given in Table 1. It may be seen that the rate of incorporation of the precursor used into the phospholipid molecule is strongly influenced by the alkali-metal ions, the highest activity being obtained when potassium is present in the incubation medium. Lithium, on the other hand, appears to have an inhibitory effect on phospholipid synthesis from acetate. It can also be seen that cesium resembles sodium closely in its influence on the rate of incorporation of the precursor used into the phospholipid fraction. Under the same conditions, rubidium appears to be slightly less

TABLE 2 EFFECT OF ALKALI-METAL IONS ON THE INCORPORATION OF ACETATE-1-C^{14} INTO THE TRIGLYCERIDE FATTY ACIDS OF RAT LIVER SLICES

Time	Specific Radioactivity				
	Lithium	Sodium	Potassium	Rubidium	Cesium
min			*cpm/mg*		
30	164	572	1117	1001	677
60	336	987	1833	1605	980
90	553	1409	2391	2007	1398
120	747	1728	2621	2401	1699
180	892	2189	2900	2398	2005

Conditions of incubation were as described in Table 1.

TABLE 3 EFFECT OF ALKALI-METAL IONS ON THE INCORPORATION OF PALMITATE-1-C^{14} INTO THE PHOSPHOLIPIDS OF RAT LIVER SLICES

Time	Specific Radioactivity				
	Lithium	Sodium	Potassium	Rubidium	Cesium
min			*cpm/mg*		
30	301	290	265	293	304
60	690	661	522	580	501
90	705	700	654	723	695
120	746	675	600	724	781
150	735	780	664	704	746

Conditions as for Table 1, except that 0.9 μc of palmitate-1-C^{14}, as its albumin complex, was substituted for 1 μc of acetate-1-C^{14}.

active than potassium in its ability to stimulate phospholipid synthesis from acetate, but it still can maintain lipid synthesis at a rate higher than that of the other three ions of the same group.

The results showing the incorporation of acetate-1-C^{14} into the triglyceride fraction are shown in Table 2. It will be seen that, even at the earliest incubation period studied (30 min), the alkali-metal ions appear to alter greatly the rate of incorporation of the labeled precursor into the lipid molecule. It can also be seen that the overall picture of the radioactivities incorporated into the triglyceride fatty acids under the influence of the alkali-metal ions appears to be roughly the same as for the phospholipid fraction. This would suggest that the pathway is affected by those ions at some point before the formation of diglycerides, which are common precursors for both phospholipids and triglycerides (15).

To try to find in what part of the metabolic process the alkali-metal ions affect the rate of lipid synthesis in liver, a comparison was made between the pathway from acetate to phospholipid or triglyceride and that from palmitate to phospholipid or triglyceride. Tables 3 and 4 show the results of these experiments. It will be seen that the incorporation of the labeled fatty acid into phospholipids and triglycerides is virtually independent of the alkali-metal ion added to the medium. Thus the site of activity of the metal ions must be between acetate and palmitate.

TABLE 4 EFFECT OF ALKALI-METAL IONS ON THE INCORPORATION OF PALMITATE-1-C^{14} INTO THE TRIGLYCERIDE FATTY ACIDS OF RAT LIVER SLICES

Time	Specific Radioactivity				
	Lithium	Sodium	Potassium	Rubidium	Cesium
min			*cpm/mg*		
30	1116	998	908	1003	1237
60	2560	2403	2007	2302	2001
90	3001	2983	2501	2899	2781
120	3009	2912	2500	2987	3002
150	2977	3128	2729	2850	2666

Conditions of incubation as for Table 3.

The main reactions in the synthesis of long-chain fatty acids from acetate are the activation of acetate to acetyl CoA, the carboxylation of acetyl CoA to malonyl CoA, and the reaction of acetyl CoA with 7 moles of malonyl CoA to form palmitate. From the data presented here, however, it is not possible to conclude at which of these steps the pathway of fatty acid synthesis is affected by the alkali-metal ions.

It must also be emphasized that the effect of the alkali-metal ions on lipogenesis may not be a direct one: the possibility exists that they can influence fatty acid synthesis indirectly by their action on carbohydrate metabolism. Potassium ion, for instance, is known to be involved in glucose metabolism in rat liver slices (16) and homogenates (17), and it has also been shown to affect glycogen synthesis in liver (18), which would tend to influence lipid metabolism.

It is also worthy of mention that a similar effect of the alkali-metal ions, on another synthetic pathway, has been reported. Walwick and Main have found (19) a marked effect of the alkali metals on nucleic acid synthesis in a rat thymus system, and the order of increasing effectiveness of the monovalent cations on DNA synthesis was the same as reported here for phospholipid and triglyceride synthesis from acetate in rat liver slices.

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