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Effect of heparin on the utilization *in vitro* of labelled glycerides from triglyceride-rich lipoproteins in rat adipose tissue

BY

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Triglyceride-rich lipoproteins from adult rat plasma were labelled *in vivo* with ^{3}H in the esterified fatty acids and ^{14}C in the labelled glyceride glycerol of neutral lipids by injecting i.v. sodium 9-10 (n)-[^{3}H] palmitate and [U-^{14}C] glycerol, after which the prelabelled lipoproteins were purified by ultracentrifugation and dialysis. The lipoproteins were incubated *in vitro*, in the presence or not of heparin, with pieces of epididymal fat pads or isolated adipocytes from fed rats. The disappearance of both [^{3}H]- and [^{14}C] lipids from the media was greater when incubations were performed with adipocytes than with fat-pad pieces and it increased with heparin in both preparations. More ^{3}H-label than ^{14}C was found in the tissue lipids, a higher percentage being present in adipocytes than in fat-pad pieces, and the amount of label in tissue lipids was always enhanced by heparin. Some ^{14}C-label appeared as esterified fatty acids in both tissue preparations and it also was enhanced by the presence of heparin. These findings are in agreement with the recognized influence of heparin on the release of lipoprotein lipase and show the direct relationship between heparin action and tissue ability to take up products of lipoprotein triglyceride breakdown. They also demonstrate the ability of adipose tissue to metabolize glycerol coming from the hydrolysis of lipoprotein glycerides.

Introduction

Plasma lipoprotein triglycerides are hydrolysed by the action of lipoprotein lipase prior to the deposition of fatty acids in adipose tissue (AUSTIN & NESTEL, 1968; SCOW *et al.*, 1972; SMITH *et al.*, 1978). We have shown that a minor proportion of glycerol moiety released in the hydrolysis *in vitro* of lipoprotein triglycerides is also taken up by adipose tissue to be further metabolized (LASUNCIÓN, 1979). Both the disappearance from the incubation medium and the tissue uptake of the hydrolytic products of triglyceride-rich lipoproteins with adipose tissue *in vitro* were less with epididymal fat pad pieces than with isolated adipocytes (LASUNCIÓN, 1979) although it has been shown that lipoprotein lipase activity is lower in adipocytes than in fat pad pieces (NILSSON-EHLE *et al.*, 1976; VANHOVE *et al.*, 1978). This contradiction has been explained by the fact that, unlike intact tissue activity during incubation, isolated adipocytes secrete substantial amounts of lipoprotein lipase to the

medium (STEWART & SCHOTZ, 1974), making it more available for the substrate. Also, heparin is known to release lipoprotein lipase activity from incubated adipose tissue pieces and isolated fat cells (STEWART & SCHOTZ, 1974; HOLLENBERG, 1959). If this explanation is correct, heparin may enhance the hydrolysis of lipoprotein triglycerides when acting on either tissue preparation (isolated adipocytes or fat pad pieces). We have explored this possibility and also investigated the direct relation between the amount of lipoprotein triglyceride hydrolysed and the proportions of fatty-acids and glycerol moieties taken up by the tissue preparations.

Materials and Methods

Labelled triglyceride-rich lipoproteins were obtained from adult female rats by injecting i.v. 60 μCi of sodium [9-10(n)-^{3}H] palmitate (230 mCi/mmol) and 30 μCi of [U-^{14}C] glycerol (46 mCi/mmol) dissolved in 0.5 ml of 8 % bovine albumin, purified by the method of CHEN (1967). Animals were sacrificed 30 min after injection and blood was collected from the aorta into syringes containing 100 mg of Na_2-EDTA. After centrifugation at 1 000 × g for 30 min at 4 °C, plasma was placed under 0.15 M NaCl ($d = 1.006$) and centrifuged at 143 000 × g for 18 h at 15 °C in the 40.3 rotor of a Beckman L5-75 ultracentrifuge. The floating lipoproteins corresponded to triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins, VLDL), as determined by electronmicroscopic study (LASUNCIÓN, M. A., LLOBERA, M. & HERRERA, E., unpublished results). The lipoproteins were recovered by tube slicing and were further purified by dialysis against 0.9 % NaCl. Aliquots of these purified triglyceride-rich lipoproteins were used for lipid extraction (FOLCH *et al.*, 1957), purification and fractionation (KERPEL *et al.*, 1961; DOMINGUEZ & HERRERA, 1976; HERRERA & AYANZ, 1972). This procedure revealed that over 88 % of the ^{3}H in these lipoprotein lipids was in the esterified fatty acids while more than 97 % of the ^{14}C-lipids was found in the ^{14}C-labelled glyceride glycerol of neutral lipids (presumably triglycerides). Other aliquots (0.5 ml) of these labelled lipoproteins (coming from 0.4 ml of initial plasma) were incubated in a final volume of 1.25 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, with a suitable salt content to yield the desired final concentration (UMBREIT *et al.*, 1964), in the presence of glucose (4 mM), purified bovine albumin (0.8 %), and fasted rat serum (5 μl/vial). Incubations were performed with or without epididymal fat pad pieces (18.3 ± 1.3 mg, corresponding to 358 ± 29 μg protein/vial) or adipocytes from epididymal fat pads coming from 160-180 g fed male Wistar rats, sacrificed by cervical fracture. Adipocytes were isolated by a modification (BELLIDO & HERRERA, 1978) of the method of RODBELL (1964) in the presence of an ovomucoid trypsin inhibitor (Sigma) (2 mg/ml) and crude collagenase (Worthington Biochemical Co.) (2 mg/ml). Incubations were performed at 37 °C in sealed, siliconized glass vials gassed for 5 min with O_2-CO_2 (95 : 5) in a Dubnoff shaking incubator at 100 cycles/min. After 120 min, the incubations were terminated by centrifuging tissue preparations and media at 200 rev./min for 2 min at room temperature in plastic syringes sealed at the tip. Media were allowed to elute while tissue preparations were thoroughly washed with plain Krebs-Ringer bicarbonate buffer. Tissue preparations and aliquots of the media were placed in chloroform-methanol (2 : 1, v/v) for lipid extraction (FOLCH *et al.*, 1957) and fractionation (KERPEL *et al.*, 1961; HERRERA & AYANZ, 1972; DOMINGUEZ & HERRERA, 1976). In the fraction containing glycerides, phospholipids were eliminated by treatment with activated silicic acid in a chloroform medium. Aliquots of all lipid fractions were used for counting their radioactivity in a PPO/POPOP xylene/Triton X-100 based scintillation cocktail. Radioactivity values were adjusted to 1 × 10^4 dpm

TABLE I. *Effect of heparin on the disappearance of labelled glycerides from the medium of either epididymal fat-pad pieces or isolated adipocytes from fed rats, incubated in the presence of prelabelled triglyceride-rich lipoproteins.*

	Disappearance of ^{3}H-esterified fatty acids (dpm/100 μg proteins) = A	Disappearance of ^{14}C-labelled glyceride glycerol of neutral lipids (dpm/100 μg proteins) = B	Ratio of A/B
Fat-pad pieces			
Basal	732 ± 51	540 ± 42	1.38 ± 0.10
+ Heparin	2 084 ± 318	2 102 ± 227	0.98 ± 0.07
P	<0.001	<0.001	<0.05
Isolated adipocytes			
Basal	1 356 ± 79***	1 219 ± 76***	1.15 ± 0.12
+ Heparin	3 801 ± 162**	3 801 ± 128**	1.01 ± 0.08
P	<0.001	<0.001	NS

Incubations were performed for 120 min as indicated in the text in the presence or not of heparin (2 U/vial). Initial radioactivity was adjusted to 1×10^4 dpm per vial of each tracer, which corresponded to esterified fatty acids of the triglyceride-rich lipoproteins for ^{3}H and to ^{14}C-labelled glyceride-glycerol of neutral lipids of the same preparation for ^{14}C. Lipid parameters correspond to values in the neutral lipid fractions. Statistical comparisons between basal and heparin vials are shown by the *P* values while those between adipocytes and fat pad pieces are indicated by asterisks ** = $P < 0.01$, *** = $P < 0.001$. Values are means ± SEM of 8-4 rats/group.

for the ^{3}H- and ^{14}C-label contained in each vial before the incubation. When unlabelled glycerol was measured, incubations were stopped with $HClO_4$ and glycerol measured in aliquots of the medium after neutralization by the method of GARLAND & RANDLE (1962). Proteins were measured (WANG & SMITH, 1975) in aliquots of the tissue preparations. Statistical analysis of the data was performed by the Student t test.

Results

As shown in Table I, the presence of fat pad pieces in the incubations caused the disappearance of both ^{3}H-esterified fatty acids and ^{14}C-labelled glyceride glycerol of neutral lipids from the prelabelled triglyceride-rich lipoproteins in the medium. This effect was greater when the incubations were performed with isolated adipocytes than with the fat-pad pieces from the same animals. The presence of heparin in the incubation media produced marked significant increase in the disappearance of both labelled lipid fractions from the media, with the greater effect in incubations with isolated adipocytes. The ratio of disappearance of ^{3}H-esterified fatty acids/^{14}C-labelled glyceride glycerol of neutral lipids from the media was slightly higher with fat-pad pieces than with isolated adipocytes (Table I) and heparin caused a significant reduction of this parameter only in the fat-pad pieces preparation.

A certain proportion of the labelled lipids that left the media was taken up by the tissue preparations, appearing as lipid components. Their values are shown in Table II. Most of the ^{3}H-fatty acids taken up by the tissue preparations appeared

TABLE II. *Effect of heparin on the incorporation of labelled lipid products into the tissue lipids after the incubation* in vitro *of either epididymal fat pieces or isolated adipocytes from fed rats in the presence of prelabelled triglyceride-rich lipoproteins.*

	^{3}H			
	Total lipids dpm/100 µg protein	FFA dpm/100 µg protein	EFA dpm/100 µg protein	Disappearance of EFA from medium/tissue total lipids ratio
Fat-pad pieces				
Basal	139 ± 26	28 ± 12	125 ± 22	8.01 ± 2.69
+Heparin	394 ± 71	21 ± 8	361 ± 55	5.80 ± 0.46
P	<0.001	NS	<0.001	NS
Isolated adipocytes				
Basal	1 006 ± 97***	89 ± 29	911 ± 59***	1.43 ± 0.16*
+Heparin	2 897 ± 273***	202 ± 72*	2 772 ± 197***	1.33 ± 0.08***
P	<0.001	NS	<0.001	NS

Incubations were performed for 120 min, as indicated in the text, in the presence or not of heparin (2 U/vial). Initial radioactivity was adjusted to 1×10^{4} dpm per vial of each tracer, which corresponded to esterified fatty acids of the triglyceride-rich lipoproteins in the case of ^{3}H and to labelled glyceride glycerol of neutral lipids of the same preparation in the case of ^{14}C. Statistical comparisons between basal and heparin vials are shown by the P values while those between adipocytes and fat-pad pieces are shown by asterisks : * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. Values are means ±SEM of 8-4 rats/group.

as esterified fatty acids. Heparin produced an increase in ^{3}H-lipids in both fat-pad pieces and isolated adipocytes. In both basal conditions and in preparations treated with heparin, the isolated adipocytes incorporated more ^{3}H into their lipids than did the fat-pad pieces. This greater efficiency of adipocytes to take up lipids from the triglyceride-rich lipoproteins in the medium is evident not only in comparison with the proportion of esterified fatty acids that disappeared from the medium but also in absolute amounts. The ratio between the disappearance of ^{3}H-esterified fatty acids from the medium and the ^{3}H-tissue total lipids was much lower in the isolated adipocytes than in the fat-pad pieces preparations. In other words, a much greater proportion of the ^{3}H-esterified fatty acids that disappeared from the medium were incorporated into tissue preparations with isolated adipocytes than with fat-pad pieces. The presence of heparin in the media did not alter these relationships (Table II). A significant proportion of the ^{14}C-labelled glyceride glycerol of neutral lipids leaving the medium during incubations was also taken up and metabolized by the tissue preparations, being converted to ^{14}C-free fatty acids, ^{14}C-esterified fatty acids or ^{14}C-labelled glyceride glycerol of neutral lipids. Here again, the adipocytes were more active in taking up the ^{14}C-glycerol moiety than were the fat-pad pieces, and heparin augmented the formation of ^{14}C-total lipids in the tissues in both preparations. In all preparations studied, the proportion of ^{14}C-labelled glyceride glycerol of neutral lipids leaving the medium during incubation and appearing as tissue ^{14}C-lipids was less than the amount of ^{3}H-lipids taken up. The ratio between ^{14}C-labelled glyceride glycerol of neutral lipids that disappeared from the medium and

^{14}C				
Total lipids dpm/100 μg protein	FFA dpm/100 μg protein	EFA dpm/100 μg protein	^{14}C-GG dpm/100 μg protein	Disappearance of ^{14}C-GG from medium/tissue total lipids ratio
22 ± 2	5 ± 1	12 ± 2	5 ± 1	26.0 ± 2.3
53 ± 12	10 ± 5	28 ± 5	16 ± 9	42.7 ± 6.7
$P < 0.001$	NS	<0.01	NS	<0.05
94 ± 11***	19 ± 7	38 ± 10*	40 ± 9**	14.5 ± 2.1**
148 ± 22**	23 ± 7	84 ± 20*	52 ± 19	26.6 ± 4.3
<0.05	NS	<0.05	NS	<0.05

+ The ratios were calculated by using the disappearance values of Table I and the dpm in the total lipids of each individual tissue preparation.

FFA = Free fatty acids; EFA = Esterified fatty acids; ^{14}C-GG = ^{14}C-labelled glycierde glycerol of neutral lipids.

^{14}C-lipids appearing in the tissue was much greater than that observed with ^{3}H-lipids (Table II). That parameter is greater in fat-pad pieces than in adipocytes and in either preparation, heparin produced a significant increment.

In parallel experiments performed under the same conditions, glycerol production during the incubation period was also determined. The production of glycerol, expressed as nmol per 100 μg of protein, did not differ between fat-pad pieces (30.2 ± 4.8) and isolated adipocytes (24.0 ± 4.0) when incubated under basal conditions. Heparin produced a small but significant rise ($P < 0.05$) in the production of glycerol in fat-pad pieces (45.1 ± 4.5) but no change in the adipocytes (27.1 ± 4.5) when compared to preparations incubated without heparin.

Discussion

The hydrolysis *in vitro* of glycerides of prelabelled triglyceride-rich lipoproteins was found to be greater when incubations were performed in the presence of isolated adipocytes than with fat-pad pieces from the same animal. On the basis that hydrolysis is catalysed by lipoprotein lipase action, the present results are in agreement with the data of NILLSON-EHLE *et al.* (1976) showing that, with incubation, isolated adipocytes and not fat-pad pieces release the enzyme into the medium, making it more available to its substrate. The disappearance of labelled esterified fatty acids and ^{14}C-labelled glyceride glycerol of neutral lipids in both preparations is augmented by the presence of heparin. This may also be influenced by the increased action of lipoprotein lipase, as it is well known that heparin augments the release of this enzyme into the medium (STEWART & SCHOTZ, 1974; KORNHAUSER & VAUGHAN, 1975). The ratio between the disappearance of labelled esterified fatty acids and glyceride glycerol from the triglyceride-rich lipoproteins in the medium was close to 1 when the incubations were performed with adipocytes, indicating that the phenomenon corresponds to complete hydrolysis of the glyceride molecules. When incubations were performed with fat-pad pieces, that ratio was greater than 1, perhaps caused by incompletely hydrolysed products of triglyceride breakdown (presumably di- and mono-acyl-glycerides). This explanation would agree with the data of SCOW & OLIVECRONA (1977) who reported the formation of these products after the incubation of chylomicrons with purified lipoprotein lipase. Heparin reduces that ratio to 1 in the fat-pad pieces preparation, demonstrating that it not only augments the release of lipoprotein lipase to the medium but changes its activity, permitting the complete hydrolysis of glycerides. Suggestions that heparin stimulates lipoprotein lipase release and also acts on its intrinsic activation have also been previously proposed (STEWART & SCHOTZ, 1974).

A significant proportion of the labelled fatty acids of the triglyceride-rich lipoproteins leaving the medium during incubation appeared in the tissue preparations as esterified fatty acids, and a minimal proportion as free fatty acids. Similar findings have been reported by AUSTIN & NESTEL (1968). The efficiency of the uptake of these fatty acids by the tissue seems to be more dependent on the amount of free fatty acids available than on the actual lipoprotein lipase activity because it is affected by heparin precisely in proportion to the disappearance of labelled fatty acids from the media. Thus, as more free fatty acids are released by the lipoprotein lipase action on lipoprotein triglycerides, a greater proportion of them is taken up by the tissues. This uptake of labelled fatty acids was found to be lower in the fat-pad pieces than in the adipocytes, and further studies are required to explain this difference.

Our findings also demonstrated that part of the ^{14}C-labelled glyceride glycerol of neutral lipids leaving the medium during incubation was taken up by the tissue

preparations. This process may be caused by earlier complete hydrolysis of the lipoprotein triglycerides, since a considerable proportion of the labelled glycerol taken up by the tissue is used for the synthesis of fatty acids. The results are in agreement with our previous reports of the ability of adipose tissue to metabolize glycerol (HERRERA & LAMAS, 1970; HERRERA & AYANZ, 1972; DOMÍNGUEZ & HERRERA, 1976) following its phosphorylation by glycerokinase action. Although the amount of glyceride glycerol processed is small, it may be important in a condition like obesity, where there is hyperlipemia and increase of both glycerokinase and lipoprotein lipase in adipose tissue (TREBEL & MAYER, 1963; RATH *et al.*, 1974; JAILLARD *et al.*, 1976). The efficiency of glycerol uptake by the tissue preparations is much less than that of fatty acids and it is also less affected by heparin. This difference could be partially explained by the continuous dilution of labelled glycerol with unlabelled glycerol released by the tissue preparations. This release is slightly greater in tissue pieces than in adipocytes and is enhanced by heparin in the medium in tissue pieces. Although a similar explanation could apply to the uptake of fatty acids, while the tissue may not differentiate between free glycerol coming from the breakdown of endogenous triglycerides and that coming from hydrolysis of lipoprotein triglycerides in the medium, it may use the free fatty acids released by lipoprotein lipase action more effectively than those coming from lipolysis. Further experimental evidence is required to substantiate this possibility.

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