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Effect of Epinephrine on the Synthesis of Glyceride Glycerol in Adipose Tissue *in vitro* *

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In order to study the effect of epinephrine on the rate of esterification of fatty acids in adipose tissue, pieces of epididymal fat pad were incubated in KRB in the presence of purified albumin, glucose and either 1-^{14}C-glycerol, 1-^{14}C-glucose or 6-^{14}C-glucose. Epinephrine enhances the production of glycerol but reduces the uptake of 1-^{14}C-glycerol by the tissue and its conversion to $^{14}CO_2$, ^{14}C-fatty acids and ^{14}C-glyceride glycerol. When the change in specific activity of the tracer is taken into account the effect of epinephrine on the utilization of glycerol by the tissue is only observed in the reduction of glyceride glycerol synthesis. When ^{14}C-labelled glucose was used as tracer, epinephrine enhances both the production of $^{14}CO_2$ from 6-^{14}C-glucose and the synthesis of ^{14}C-glyceride glycerol from 1-^{14}C and 6-^{14}C-glucose. The contrasting effects of epinephrine on the glyceride glycerol formation from glycerol and from glucose can explain the difficulties found in observing any change in the net rate of esterification of fatty acids by adipose tissue.

Althoug the effect of epinephrine stimulating adipose tissue lipolysis is well known, mediated by its action on the adenyl-cyclase system (for a review see ref. 7), the effect of this hormone on the esterification of fatty acids remains to be clarified. It has been shown that epinephrine stimulates the incorporation of ^{14}C-glucose into glyceride-glycerol (2, 3, 21) but decrease the incorporation of ^{14}C-labelled palmitate into glycerides (15, 22). DOLE (5), on the basis of studies on 1-^{14}C-palmitate incorporation, concluded that epinephrine did not alter the rate of fatty acid esterification. Using the measurements of net changes in glycerol and free fatty by epididymal fat pads incubated *in vitro* for the estimation of the rate of fatty acid esterification, VAUGHAN

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and STEINBERG (24) concluded that this parameter was stimulated by epinephrine. Using the same procedure, we were unable to demonstrate any change in the rate of esterification in adipose tissue incubated in the presence of epinephrine (16). As recently demonstrated, adipose tissue is able to utilize a considerable amount of glycerol for the synthesis of glycerides (6, 10-12, 19), via its phosphorylation (1, 20). The possibility exists then that, the effect of epinephrine on the esterification of fatty acids may be influenced by the reutilization of the glycerol released to the incubation medium via lipolysis. To check this possibility, the present study has determined the effect of epinephrine on the *in vitro* utilization of 1-^{14}C-glycerol by adipose tissue. To relate the results with the effect of this hormone on the metabolism of glucose, the utilization of 1-^{14}C and 6-^{14}C-glucose was also determined.

Materials and Methods

Male rats of the Wistar strain, weighing 161-195 g and fed *ad libitum* a Purina show diet were used. The animals were killed by cervical fracture without anesthesia and pieces of epididymal fat pads were removed and incubated in Krebs Ringer bicarbonate buffer, pH 7.4 (23) as previously described (11, 12). The final media always contained 10 mg of bovine albumin defatted by the method of CHEN (4) and 5 mM glucose. Where indicated, the medium was supplemented with 2.8 μM epinephrine bitartrate, 10 μM 1-^{14}C-glycerol (0.5 μCi/ml), 5 mM 1-^{14}C-glucose (0.5 μCi/ml) or 5 mM 6-^{14}C-glucose (0.5 μCi/ml). At the end of the incubation, the $^{14}CO_2$ evolved was trapped in Hyamine 10 $\times$ hydroxide and the media were processed as previously described (11, 12). Glycerol in the medium was assayed by an enzymatic method (9) and the lipids were extracted from the incubated tissue in chloroform-methanol (8) and purified and fractionated as elsewhere (11).

Results

The effects of epinephrine on the net production of glycerol and on the utilization of 1-^{14}C-glycerol by pieces of epididymal fat pads incubated for 180 minutes are shown in (table I). Epinephrine significantly enhacend the production of glycerol while reducing the uptake of 1-^{14}C-glycerol and its further metabolization to $^{14}CO_2$, ^{14}C-fatty acid and ^{14}-C glyceride-glycerol (table I). As some of these effects could be due to the dilution of the tracer by the higher concentration of glycerol in the media coming from the vials incubated in the presence of epinephrine, we decided to make the correction for this factor. Experiments were carried out in the same conditions as those described above but using different incuba-

Table I. *Effect of epinephrine on the metabolism of glycerol by pieces of epididymal fat pads from fed rats incubated for 180 minutes in Krebs Ringer bicarbonate containing purified albumin, 5 mM glucose and 1-^{14}C-glycerol.*

Additions to the media:	None	P	Epinephrine
Glycerol production (μmoles/100 mg)	0.64$\pm$0.13 *	$<$ 0.001	3.53$\pm$0.43
Uptake of 1-^{14}C-glycerol (%/100 mg) **	10.97$\pm$0.69	$<$ 0.001	3.13$\pm$0.66
$^{14}CO_2$ (%/100 mg)	4.50$\pm$0.55	$<$ 0.001	1.23$\pm$0.27
^{14}C-fatty acids (%/100 mg)	2.49$\pm$0.61	$<$ 0.01	0.47$\pm$0.14
^{14}C-glyceride glycerol (%/100 mg)	3.86$\pm$0.70	$<$ 0.005	1.45$\pm$0.31

* Means $\pm$ SEM of 8 rats/group.
** %/100 mg = percentage of initial radioactivity in the medium per 100 mg wet weight of tissue.

tion times (40, 60, 120 and 180 minutes). With the observed values, the rates of glycerol utilization were calculated after correcting by the specific activity changes of the tracer, as previously described (10). These corrected results are summarized in figure 1 where it can be seen that epinephrine actually inhibited the uptake of glycerol by the tissue and this inhibition was only concerned with the rate of synthesis of glyceride glycerol which was intensely decreased by the hormone. Epinephrine, however, did not affect the rate of formation of CO_2 nor that of fatty acids from glycerol (fig. 1).

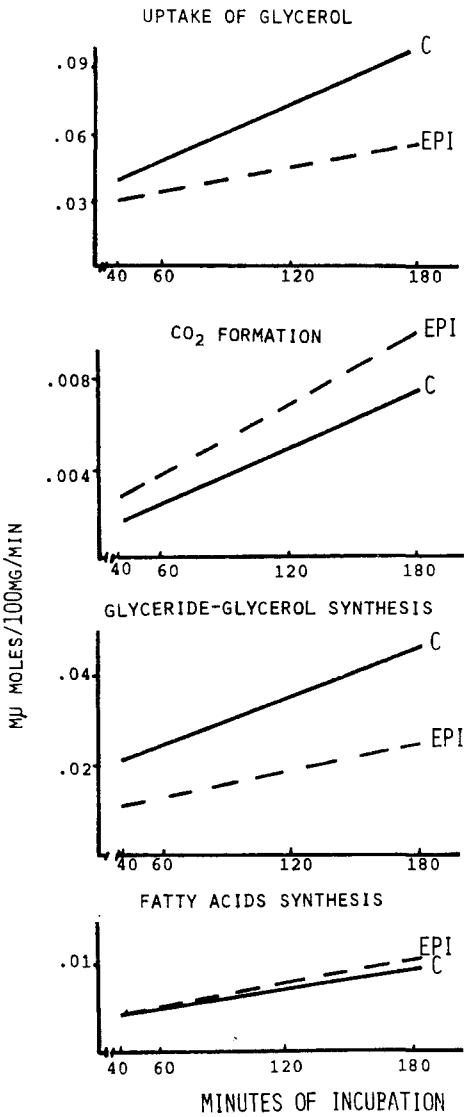

Fig. 1. *Effect of epinephrine on the rates of glycerol utilization by pieces of epididymal fat pads from fed rats* in vitro.
The values have been calculated as function of the specific activity changes of 1-^{14}C-glycerol at different times of incubation.

With the purpose of studying the effects of the hormone on the metabolism of glucose, pieces of epididymal fat pads were incubated for 180 minutes in the presence of 1-^{14}C and 6-^{14}C-glucose (table II). Epinephrine did not affect the uptake of either substrate nor their conversion to ^{14}C-fatty acids. Epinephrine did not affect the formation of $^{14}CO_2$ from 1-^{14}C-glucose but significantly enhanced the production of $^{14}CO_2$ from 6-^{14}C-glucose and the synthesis of ^{14}C-glyceride glycerol from both 1-^{14}C and 6^{14}C-glucose.

Discussion

The present study has shown that epinephrine inhibits the *in vitro* utilization of glycerol by adipose tissue for the synthesis of glyceride glycerol. These results are in agreement with those of other authors (17, 18) but are in variance with those of HUBBARD *et al.* (13) who found an increase in the conversion of 2-^{14}C-glycerol into labelled glyceride glycerol by norepinephrine in the presence of glucose. HUBBARD *et al.* use very high concentrations of cold glycerol in the media to compensate for changes in the dilution of the tracer by the glycerol coming from lipolysis. Previous studies have shown that high initial concentrations of cold glycerol in the media produce elevated rates of glycerol utilization by the tissue during the incubation (DOMÍNGUEZ and HERRERA, unpublished observations). To avoid this possible artefact, trace concentrations of

Table II. *Effect of epinephrine on the metabolism of glucose by pieces of epididymal fat pads from fed rats incubated in the presence of 1-^{14}C-glucose and 6-^{14}C-glucose for 180 minutes.*

Additions to the media:	1-^{14}C-glucose			6-^{14}C-glucose		
	None	P	Epinephrine	None	P	Epinephrine
Uptake (%/100 mg)*	22.27±5.81**	N.S.	25.45±4.14	14.26±2.46	N.S.	20.55±3.05
^{14}C-fatty acids (%/100 mg)	4.69±1.48	N.S.	3.71±1.14	6.86±1.33	N.S.	4.17±1.48
$^{14}CO_2$ (%/100 mg)	7.52±1.27	N.S.	8.29±1.34	2.00±0.41	< 0.001	7.64±0.96
^{14}C-glyceride glycerol (%/100 mg)	4.82±1.02	< 0.5	11.63±2.86	4.43±0.73	< 0.01	8.25±0.94

* %/100 mg = percentage of initial radioactivity in the medium per 100 mg wet weight of tissue.
** Means ± SEM of 8 rats/group.

1-^{14}C-glycerol with high specific activity have been used as substrate in this study. The changes in the dilution of the radioactivity during the incubations have been corrected to obtain the actual rates of glycerol utilization by the tissue, using the methods already described (10). Using this system, it was observed that the only parameter of the metabolization of glycerol affected by epinephrine in the presence of glucose was the synthesis of glyceride glycerol, which is inhibited by the hormone. Two possible explanations could be found to this phenomenon: *a*) It is known that epinephrine produces a reduction in the intracellular concentrations of ATP in adipose tissue (2, 14). It is required the ATP dependent phosphorylation of glycerol in order to be utilized by the tissue and it was previously demonstrated that the metabolization of glycerol in adipose tissue is very much dependent on the availability of energy (6). Thus, a reduction in the ATP content into the adipocyte due to the epinephrine could explain the effect of this hormone on the decrease of glyceride glycerol synthesis from glycerol. *b*) The second possible explanation can be that in the presence of glucose, the α-glycerophosphate synthesized from the glycolitic pathway could compete for the esterification of fatty acids with the one coming directly from glycerol phosphorylation. We have seen here that epinephrine enhances the formation of glyceride-glycerol from both 1-^{14}C and 6-^{14}C-glucose. The effect of epinephrine on glucose metabolism is exerted by enhancing the glycolitic pathway but not the hexose monophosphate shunt, as the ratios of $^{14}CO_2$ produced are reduced by epinephrine. These results agree with those of others (18). Most probably, both mechanisms (the decreased phosphorylation of glycerol due to decreased availability of ATP and the enhanced esterification of the α-glycerophosphate produced from glucose) are working concomitantly when epinephrine is present in the medium together with glucose. This opposite effects could explain the difficulties in observing any change in the net rate of esterification of fatty acids by adipose tissue incubated under these conditions (5, 16).

References

1. ANTONY, G., WHITE, L. W. and LANDAU, B. R.: *J. Lipid Res.*, **10**, 521, 1969.
2. BIHLER, I. and JEANRENAUD, B.: *Biochem. Biophys. Acta*, **202**, 496, 1970.

3. CAHILL, G. F., JR., LEBOEUF, B. and FLINN, R. B.: *Abstracts Endocrine Soc. 41st Meeting*, 1959, 62.
4. CHEN, R. F.: *J. Biol. Chem.*, **242**, 173, 1967.
5. DOLE, V. P.: *J. Biol. Chem.*, **236**, 3121, 1961.
6. DOMÍNGUEZ, M. C. and HERRERA, E.: *Horm. Metab. Res.* (In press).
7. FAIN, J. N.: *Pharmacological Reviews*, **25**, 67, 1973.
8. FOLCH, J., LEES, M. and SLOANE-STANLEY, G. H.: *J. Biol. Chem.*, **226**, 497, 1957.
9. GARLAND, P. B. and RADLE, P. J.: *Nature*, **196**, 987, 1962.
10. HERRERA, E.: *Rev. esp. Fisiol.*, **29**, 155, 1973.
11 HERRERA, E. and AYANZ, A.: *J. Lipid Res.*, **13**, 802, 1972.
12. HERRERA, E. and LAMAS, L.: *Biochem. J.*, **120**, 433, 1970.
13. HUBBARD, R. W., VOORHEIS, H. P. and THERRIAULT, D. G.: *Lipids*, **5**, 114, 1969.
14. JEANRENAUD, B.: *Diabetologia*, **7**, 209, 1971.
15. KERPEL, S., SHAFRIR, E. and SHAPIRO, B.: *Bull. Res. Council Israel*, **9A**, 90, 1960.
16. KNOPP, R. H., HERRERA, E. and FREINKEL, N.: *J. Clin. Invest.*, **49**, 1438, 1970.
17. LOCHAYA, S., HAMILTON, J. C. and MAYER, J.: *Nature*, **197**, 182, 1963.
18. LYNN, W. S., MACLEOD, R. M. and BROWN, R. H.: *J. Biol. Chem.*, **235**, 1904, 1960.
19. MONTOYA, E. and HERRERA, E.: *Hormone Res.*, **5**, 129, 1974.
20. ROBINSON, J. and NEWSHOLME, E. A.: *Biochem. J.*, **104**, 2c, 1967.
21. RODBELL, M.: *J. Biol. Chem.*, **239**, 375, 1964.
22. STEINBERG, D., VAUGHAN, M. and MARGOLIS, S.: *J. Biol. Chem.*, **235**, PC 38, 1960.
23. UMBREIT, W. W., BURRIS, R. H. and STAUFFER, S. F.: Manometric Techniques (4th ed.), Burgess Publishing Co., Minneapolis, 1964, p. 123.
24. VAUGHAN, M. and STEINBERG, D.: *J. Lipid Res.*, **4**, 193, 1963.