Control of Glucose Metabolism in Isolated Acini of the Lactating Mammary Gland of the Rat

EFFECTS OF OLEATE ON GLUCOSE UTILIZATION AND LIPOGENESIS

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Oleate (1 mM) had only small inhibitory effects on glucose utilization and lipogenesis in acini isolated from rat mammary gland. Esterification of [1-¹⁴C]oleate was unaffected by insulin but was decreased by 60% by acetoacetate (2 mM). Glycerol (1 mM), but not insulin, relieved this inhibition. These experiments provide further support for the role of acetoacetate in regulating substrate utilization by the gland.

In rat mammary gland glucose utilization is inhibited by acetoacetate (Robinson & Williamson, 1977*b,c*), as is found in heart, diaphragm, skeletal muscle and submaxillary gland (Randle *et al.*, 1964; Thompson & Williamson, 1975; Maizels *et al.*, 1977). Fatty acids are readily oxidized in heart and diaphragm (Fritz, 1961) and, like ketone bodies, inhibit glucose utilization in these tissues (Randle *et al.*, 1964). Studies of mammary gland of non-ruminants have shown that fatty acids are taken up by tissue preparations *in vitro* and are incorporated into triacylglycerol, with only a small proportion being oxidized (Hirsch *et al.*, 1956; Kinsella, 1973; Rao & Abraham, 1975). The present study is an investigation of whether long-chain fatty acids behave like ketone bodies and inhibit glucose utilization in acini isolated from rat mammary gland, and whether insulin can affect the esterification of fatty acids.

Experimental

Rats of the Wistar strain (250–340 g) with between 6 and 14 pups were used after a lactating period of between 10 and 14 days. Acini were prepared from the inguinal mammary gland as described by Robinson & Williamson (1977*a*). The acini (about 100 mg wet wt.) were incubated in a total volume of 4 ml of Krebs–Henseleit (1932) saline containing dialysed albumin (fatty acid-poor; final concentration 2.5%, w/v) and glucose (5 mM). The following concentrations of other compounds were used where indicated: oleate (1 mM); glycerol (1 mM); acetoacetate (2 mM); insulin (50 munits/ml). For further details see Robinson & Williamson (1977*a*). Fatty acid synthesis was measured by using ³H₂O as described by Robinson & Williamson (1977*c*). Incubations with [1-¹⁴C]oleate were performed in

duplicate to measure ¹⁴CO₂ production and incorporation of [1-¹⁴C]oleate into lipid, as described by Whitelaw & Williamson (1977). Non-esterified fatty acids were measured by the colorimetric method of Itaya & Ui (1965). Oleate uptake was determined by incorporation of radioactivity into acinar lipid after 20, 40 and 60 min of incubation. Rates were linear with time and agreed with measurements of disappearance of radioactivity from the medium. Measurements of the specific radioactivity of non-esterified fatty acids in the medium before and after 60 min incubations with different substrate combinations indicated that it decreased by less than 15%. This finding, together with the fact that measurements of the non-esterified fatty acid removed from the medium agreed well with the values calculated from the incorporation of radioactivity, suggests that dilution of the extracellular pool of fatty acids via tissue lipolysis is not an important potential source of error in these experiments. To test that the lipid extracted from the acini by chloroform was present as esterified fats the chloroform extract was analysed by t.l.c. on silica-gel plates with a solvent system of hexane/diethyl ether/acetic acid (80:20:1, by vol.).

All enzymes, including collagenase (grade II), and coenzymes were obtained from Boehringer Corp. (London) Ltd., London W.5, U.K. Crystalline insulin free of glucagon was a gift from Dr. N. Z. Lazarus (Wellcome Research Laboratories, Beckenham, Kent, U.K.). [1-¹⁴C]Oleate and ³H₂O were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Determination of metabolites, measurements of radioactivity and expression of results were carried out as described by Williamson *et al.* (1975) and Robinson & Williamson (1977*a*).

Table 1. *Effects of oleate on glucose metabolism in acini from mammary gland of fed lactating rats*

For experimental details see the text. Where indicated, glucose (5 mM), oleate (1 mM), glycerol (1 mM), acetoacetate (2 mM) or insulin (50 munits/ml) were added to the incubation medium. The results are mean values $\pm$ S.D. with the numbers of observations in parentheses. The units are: (a) μ mol/min per 100 mg defatted dry wt.; (b) μ mol of $^3\text{H}_2\text{O}$ /min per 100 mg defatted dry wt.; (c) nmol/min per 100 mg defatted dry wt.; (d) μ mol/g defatted dry wt. Values that are statistically different (Student's *t* test) from incubations with glucose or with glucose plus oleate are shown by * $P < 0.005$ and † $P < 0.005$ respectively.

	Glucose removed (a)	Lactate formed (a)	Pyruvate formed (a)	Fatty acid synthesis (b)	^{14}C from [$1\text{-}^{14}\text{C}$]oleate incorporated into		
					CO_2 (c)	Lipid (a)	Glycerol 3- phosphate (d)
Glucose	1.47 $\pm$ 0.26 (10)†	0.38 $\pm$ 0.14 (10)	0.088 $\pm$ 0.028 (10)	0.51 $\pm$ 0.12 (6)	—	—	1.99 $\pm$ 0.74 (9)
Glucose + oleate	1.08 $\pm$ 0.17 (15)*	0.31 $\pm$ 0.13 (15)	0.10 $\pm$ 0.03 (15)	0.40 $\pm$ 0.10 (6)	0.43 $\pm$ 0.17 (8)	0.26 $\pm$ 0.04 (10)	1.74 $\pm$ 0.58 (13)
Glucose + oleate + insulin	1.38 $\pm$ 0.26 (8)†	0.37 $\pm$ 0.12 (8)	0.082 $\pm$ 0.029 (8)	0.53 $\pm$ 0.12 (6)	0.28 $\pm$ 0.10 (4)	0.27 $\pm$ 0.03 (5)	2.34 $\pm$ 0.69 (7)
Glucose + oleate + acetoacetate	0.27 $\pm$ 0.08 (8)*†	0.079 $\pm$ 0.030 (8)*†	0.047 $\pm$ 0.025 (8)*†	—	0.60 $\pm$ 0.33 (4)	0.10 $\pm$ 0.02 (5)†	0.64 $\pm$ 0.62 (8)*
Glucose + oleate + acetoacetate + insulin	0.50 $\pm$ 0.27 (7)*†	0.18 $\pm$ 0.10 (7)*	0.12 $\pm$ 0.07 (7)	—	0.87, 1.02 (2)	0.11 $\pm$ 0.03 (4)†	0.81 $\pm$ 0.75 (7)*
Glucose + oleate + acetoacetate + glycerol	0.94 $\pm$ 0.26 (6)*	0.32 $\pm$ 0.12 (6)	0.12 $\pm$ 0.03 (6)	—	0.25 $\pm$ 0.06 (3)	0.28 $\pm$ 0.04 (3)	2.82 $\pm$ 1.42 (6)
Glucose + oleate + glycerol	0.64 $\pm$ 0.19 (7)*†	0.43 $\pm$ 0.15 (7)	0.045 $\pm$ 0.039 (7)†	0.062 $\pm$ 0.032 (6)*	0.38, 0.49 (2)	0.26 $\pm$ 0.03 (4)	24.0 $\pm$ 11.8 (7)*

Results and Discussion

The inclusion of albumin in the incubation medium did not greatly alter glucose uptake or lactate and pyruvate production by the acini (Table 1) when compared with rates measured previously in the absence of albumin (Robinson & Williamson, 1977a,c). In the presence of oleate, the glucose uptake was decreased by 26% (Table 1). This decrease is small in comparison with the inhibition of 75% found with acetoacetate (Robinson & Williamson, 1977c). Insulin increased glucose uptake in the presence of oleate, whereas acetoacetate and glycerol decreased glucose uptake to a similar extent to that in the absence of oleate (Robinson & Williamson, 1977c). Lactate and pyruvate formation was not altered by the presence of oleate (Table 1) and was decreased in the presence of acetoacetate, as might be expected from the decreased glucose uptake. The lack of any increase in lactate and pyruvate production in the presence of oleate suggests that, in contrast with muscle (Denton *et al.*, 1975), mammary-gland pyruvate dehydrogenase is not inhibited by long-chain fatty acids.

Fatty acid synthesis was not significantly affected by oleate (Table 1). However, the small decrease observed with oleate and the increase with insulin paralleled the changes in glucose uptake (Table 1), and measurements of incorporation of [1-¹⁴C]- and [6-¹⁴C]-glucose into lipid showed a similar decrease (about 30%) in the presence of oleate. This is less than the 50% decrease in the incorporation of [U-¹⁴C]glucose into fatty acids caused by palmitate and decanoate in mammary-gland slices from lactating mice (Rao & Abraham, 1975), but the mouse gland may not resemble the rat gland in all respects (Smith *et al.*, 1968, 1969; Rao & Abraham, 1975). Thus long-chain fatty acids seem to have a less-marked inhibitory effect on the glucose metabolism of acini when compared with acetoacetate, which dramatically decreases lipogenesis (Robinson & Williamson, 1977c).

Oleate was readily taken up by the acini and incorporated into lipid, with only 0.16% of that utilized being oxidized to ¹⁴CO₂ (Table 1). The oleate taken up by the acini did not accumulate as non-esterified fatty acid, and over 85% of the chloroform-soluble radioactivity was identified as triacylglycerol by t.l.c. No triacylglycerol was detected in the medium in the present studies, although Kinsella (1973) reported that after several hours esterified lipids were secreted into the medium by cultured rat mammary cells incubated with fatty acids.

Insulin and glycerol did not affect oleate esterification (Table 1), although both promoted acetoacetate incorporation into lipid (Robinson & Williamson, 1977c). Acetoacetate decreased oleate uptake by over 60%, and glycerol, but not insulin,

relieved this inhibition of oleate esterification caused by acetoacetate (Table 1). Since acetoacetate decreases [glycerol 3-phosphate] in acini (Robinson & Williamson, 1977c), oleate uptake may be limited by the availability of glycerol 3-phosphate for esterification. The results in Table 1 for [glycerol 3-phosphate] are for a 40 min incubation time; the concentration tended to increase with the time of incubation. [Glycerol 3-phosphate] was not decreased in the presence of oleate, but was greatly decreased when acetoacetate was present and oleate uptake was inhibited (Table 1). Insulin did not increase [glycerol 3-phosphate] or oleate uptake, but glycerol increased both [glycerol 3-phosphate] and oleate uptake in the presence of acetoacetate and oleate (Table 1). Oleate esterification was 0.049 ± 0.010 (4) $\mu\text{mol/min}$ per 100mg in the absence of any other exogenous substrate, but increased to 0.16 ± 0.02 (4) $\mu\text{mol/min}$ per 100mg with the addition of glycerol, when [glycerol 3-phosphate] increased from 0.75 ± 0.18 (3) $\mu\text{mol/g}$ with oleate alone to 4.32 ± 1.87 (3) $\mu\text{mol/g}$. Thus oleate uptake and esterification appear to be related to [glycerol 3-phosphate].

Fatty acids decrease fatty acid synthesis in adipocytes under some conditions (Sooranna & Saggerson, 1976; Saggerson *et al.*, 1977) and have been reported to inhibit lipogenesis in perfused rat liver (Mayes & Topping, 1974) and in isolated hepatocytes from neonatal chicks (Goodridge, 1973a). Goodridge (1973b) suggested that increased [fatty acyl-CoA], which inhibits acetyl-CoA carboxylase (Volpe & Vagelos, 1976), could be the cause of the inhibition of lipogenesis. Acetyl-CoA carboxylase from rat mammary gland is inhibited by long-chain fatty acids and their fatty acyl-CoA derivatives (Miller *et al.*, 1970) and would therefore be expected to be inhibited in incubations of acini with oleate. Since acetyl-CoA carboxylase may be rate-limiting for fatty acid synthesis in the gland (Baldwin & Yang, 1974), the lack of a marked inhibition of lipogenesis in the acini with oleate and in adipocytes with palmitate (Saggerson *et al.*, 1976; Evans & Denton, 1977) may be explained by [fatty acyl-CoA] not increasing to the value that inhibits the enzyme, perhaps as a result of protein binding and compartmentation of fatty acyl-CoA within the cell (Lunzer *et al.*, 1977).

Previously it was proposed that the inhibition of lipogenesis in rat mammary gland by acetoacetate involved inhibition of acetyl-CoA carboxylase by long-chain acyl-CoA, and the ability of glycerol and insulin to relieve this inhibition was postulated to require increased availability of glycerol 3-phosphate, which would promote esterification and decrease fatty acyl-CoA concentration (Robinson & Williamson, 1977c). This is consistent with decreased esterification of oleate in the presence of acetoacetate when [glycerol 3-phosphate] was decreased (Table 1).

Thus the failure of insulin to increase oleate esterification in the presence of glucose and acetoacetate may be related to its inability to increase glycerol 3-phosphate availability under these conditions. The maximal rate of oleate esterification appears to be $0.27 \mu\text{mol/min}$ per 100mg, and increased [glycerol 3-phosphate], as occurs in the presence of glycerol, cannot further increase this (Table 1). Since insulin cannot further increase this rate, and provided that other substrates and cofactors are not limiting for esterification, it seems unlikely that insulin acts on enzymes within the pathway of esterification, as has been suggested for adipose tissue (Jason *et al.*, 1976; Sooranna & Saggerson, 1976).

Fatty acids are made available to the gland *in vivo* by the action of lipoprotein lipase on circulating triacylglycerols (Scow *et al.*, 1977) or as non-esterified fatty acids in the plasma (Hawkins & Williamson, 1972). Evidence that the mammary gland has a large capacity to take up fatty acids is the finding that after 24h about half of the injected radioactivity was recovered as fatty acids in milk and mammary tissue of lactating rats injected intravenously with either [$1\text{-}^{14}\text{C}$]tripalmitin or [$1\text{-}^{14}\text{C}$]octanoate (Lossow & Chaikoff, 1958). The importance of fatty acids for the synthesis of milk is further emphasized by the changes occurring in lactation to favour their uptake by the gland. These include increased lipoprotein lipase activity in the gland (Hamosh *et al.*, 1970) associated with decreased lipoprotein lipase activity and increased lipolysis in adipose tissue (Smith & Walsh, 1976), and increased lipogenesis (Smith, 1973) and triacylglycerol synthesis in liver (Whitelaw & Williamson, 1977). From the present studies this increased availability of fatty acids would not appreciably decrease glucose utilization by the gland, which is physiologically desirable, since *in vivo* glucose is necessary to provide glycerol 3-phosphate for esterification of these fatty acids, as well as carbon, energy and reducing equivalents for the synthesis of milk constituents (Baldwin & Yang, 1974). Furthermore, the lack of sensitivity to fatty acids would be important to the gland in view of the report that the concentration of non-esterified fatty acids in rat milk is about 10mM (Miller *et al.*, 1970). The finding that the rate of lipogenesis from glucose or acetate in mouse mammary gland is not altered by feeding a high-fat diet (Smith *et al.*, 1969) suggests that even on a long-term basis fatty acids are not important regulators of lipogenesis in the lactating gland.

In contrast with the slight inhibitory effects of oleate, acetoacetate is a potent inhibitor of glucose uptake and lipogenesis in incubations of slices (Williamson *et al.*, 1975) and acini (Robinson & Williamson, 1977c) as well as *in vivo* in fed lactating rats injected with acetoacetate (Robinson & William-

son, 1977b). The novel finding in these studies is that acetoacetate decreases oleate esterification by the gland. Thus if acetoacetate may act as a 'signal' to the gland indicating decreased availability of carbohydrate (Whitelaw & Williamson, 1977), then in starvation, where ketone-body concentrations are increased, the consumption of fatty acids as well as of glucose by the gland will be decreased. This would be consistent with the decrease in lactose synthesis and hence milk synthesis after short-term (6h) withdrawal of food from lactating rats (Carrick & Kuhn, 1977). The net result of these changes in gland metabolism during starvation is to conserve substrates for the mother.

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