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LIPOLYSIS IN HEART AND ADIPOSE TISSUE; EFFECTS OF INHIBITION OF GLYCOGENOLYSIS AND UNCOUPLING OF OXIDATIVE PHOSPHORYLATION

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SUMMARY. In the Langendorff heart, lipolysis is arrested when glycogenolysis is inhibited by the addition of 5-gluconolactone. Glucose partially overcomes the inhibition as well as uncoupling of oxidative phosphorylation by dinitrophenol. In isolated fat cells hormone-sensitive lipolysis is also inhibited by glycogenolysis inhibition and in these cells also, glucose addition overcomes the inhibition. In fat cells, uncoupling of oxidative phosphorylation does not stimulate lipolysis, probably because of the relatively low concentration of mitochondria in white adipose tissue. The data are interpreted that both in heart and adipose tissue cells, the removal of fatty acids produced by the endogenous lipase is the main stimulus for lipolysis. Attempts to generate in fat cells glycerol-3-phosphate by glycerogenesis from pyruvate or lactate led to the observation that not only these latter anions, but also propionate and acetate strongly stimulate lipolysis. It suggests that long-chain fatty acid removal from fat cells may be stimulated by anion exchange.

We have previously reported[1] that hormone-sensitive lipolysis is probably of lysosomal origin, since chloroquine, a lysosomal inhibitor, inhibits this process in heart and adipose tissue. Further work[2] in heart revealed the presence of fat-filled lysosomes (comp. also ref. 3), as judged by the electron-microscopical demonstration of acid phosphatase in fat-filled particles 3 days after rapeseed-oil feeding to rats. We also found that in the presence of glucose insulin had an anti-lipolytic effect and that glucocorticoid was required as a "permissive factor" to elicit glucagon stimulation of lipolysis[2]. Hence the similarities between lipolysis in adipose tissue and in fat-loaded hearts[1] were extended. An important further similarity between the two tissues may be the (product) inhibition of hormone-stimulated lipolysis by fatty acids. This has been documented for both tissues[4-6]. Ca^{++} is an important cofactor in the regulation of lipolysis in both heart[7,8] and adipose tissue[9-11]. This has led us to perform experiments in the isolated perfused rat heart to investigate whether hormone-sensitivity of lipolysis is secondary to the influence of lipolytic hormones on fatty acid removal. It was found, as will be published elsewhere, that in heart the response of glycerol release to glucagon addition parallelled the glyco(geno)lytic response to the hormone, suggestive for an important role of glycerol-3-phosphate generation in hormone-sensitive lipolysis. The observed stimulatory effect of Ca^{++} on hormone-stimulated lipolysis in arrested hearts suggested Ca^{++} as an intracellular factor

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involved in the release of lipolysis inhibited by product fatty acids. That on the other hand fatty acids may promote Ca^{++} translocation in heart cells has been shown by us before[12,13]. The present paper reveals that inhibition of glycogenolysis during substrate-free perfusion of fat-loaded hearts strongly inhibits lipolysis and that glucose addition partially restored lipolysis, indicating again that removal of inhibitory fatty acids by reesterification to a product of glyco-(geno)lysis, probably glycerol-3-phosphate, stimulates lipolysis. This also holds for lipolysis in isolated fat cells: 5-gluconolactone, an inhibitor of phosphorylase a[14], inhibits hormone-stimulated lipolysis, whereas the addition of glucose stimulates. Pyruvate and lactate were also tried. Since uncoupling of the oxidative phosphorylation did not affect the stimulatory effect of lactate and pyruvate on lipolysis, it is not likely that these substances act by conversion to glycerol-3-phosphate, as energy is required in the pyruvate carboxylase and phosphoenol pyruvate carboxykinase reactions. Moreover, propionate and acetate also stimulated lipolysis, suggesting that long-chain fatty acid release to the medium is stimulated by exchange with other organic anions.

METHODS

For the heart perfusion experiments male Wistar rats of 240+20 g were fed for 3 days with a diet containing 50 cal% of a 2:1 mixture of trierucate and sunflower-oil to enrich the lipid depôt of heart (comp. refs. 1 and 2). Perfusion was carried out by the Langendorff technique, as described in detail elsewhere[15]. Glycerol and lactate released from the heart were determined fluorometrically[16]. Calibration of fluorescence was carried out in each experiment by adding a known amount of NADH.

Adipocytes were isolated from epididymal fat pads with collagenase according to Rodbell[17]. They were incubated in Nalgene^{R} tubes under shaking at 37°C in Krebs-Ringer bicarbonate buffer, containing 3% (w/v) bovine serum albumin (made fatty acid-free according to Chen[18]). The atmosphere consisted of 95% O_2 and 5% CO_2 (v/v). The final pH was 7.5. The reactions were stopped after 10 min by decanting the cell suspensions in cold glass centrifuge tubes, followed by centrifugation, deproteinization with perchlorid acid and neutralization with KOH. The 5-gluconolactone (Sigma, St. Louis, MO, U.S.A.) was dissolved immediately before use, because of its hydrolysis to gluconic acid ($T_{\frac{1}{2}}$=30 min). Results are given in mean values ($\bar{X}$) ± standard error of the mean (SEM). n is the number of the observations. Statistical analysis was performed according to Student's t-test. P>0.05 was considered to be not significant.

RESULTS

Lipolysis during in vitro perfusion of rat hearts. It can be seen from Fig. 1A that during substrate-free perfusion the rate of glycerol release both in the absence and presence of glucagon is strongly inhibited by the presence of 5-gluconolactone, an inhibitor of glycogenolysis[14]. Partial restoration of lipolysis is achieved by the addition of glucose. Fig. 1B shows that 5-gluconolactone indeed inhibits the endogenous lactate production both in the absence and presence of glucagon. These results suggest a connection between lipolysis and glycogenolysis, probably by the synthesis of glycerol-3-phosphate, which as a fatty acid acceptor will help to remove fatty acids formed during

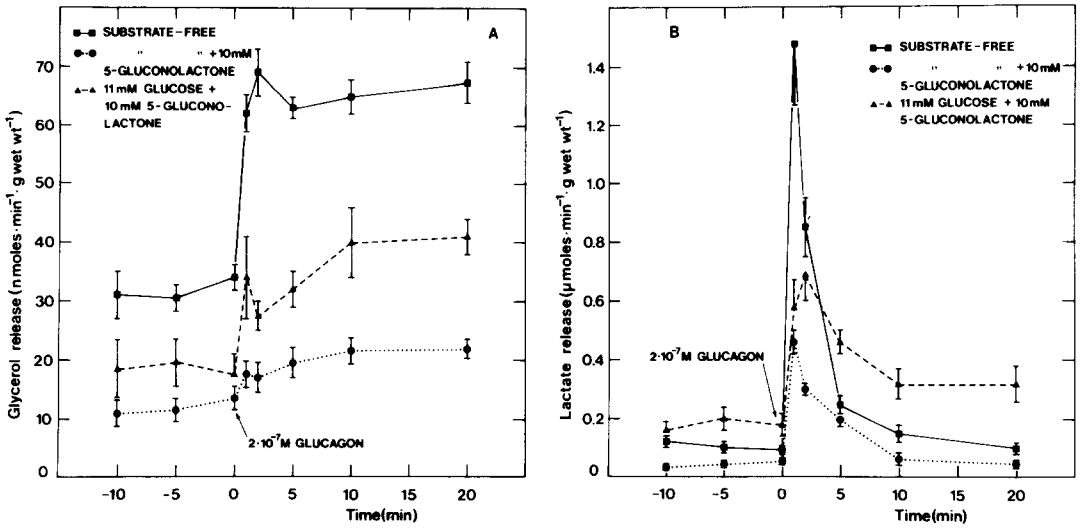

Fig. 1 A+B. The effect of 5-gluconolactone ± glucose upon basal and glucagon-stimulated glycerol and lactate release from isolated, perfused hearts from trierucate fed rats ($\bar{X}$ ± SEM, n=5). 5-Gluconolactone was added to the perfusion medium immediately prior to preperfusion and prior to perfusion in the presence of glucagon.

lipolysis. That lipolysis is not fully restored by glucose addition may be due to the formation of excess pyruvate which serves as an alternative substrate to the mitochondria besides fatty acids. If pyruvate is preferentially oxidized then fatty acid accumulation will be expected, resulting again in product inhibition of lipolysis. This will be discussed in detail elsewhere.

The important role of mitochondria in product fatty acid removal is reflected in Fig. 2. When during substrate-free perfusion 5-gluconolactone is

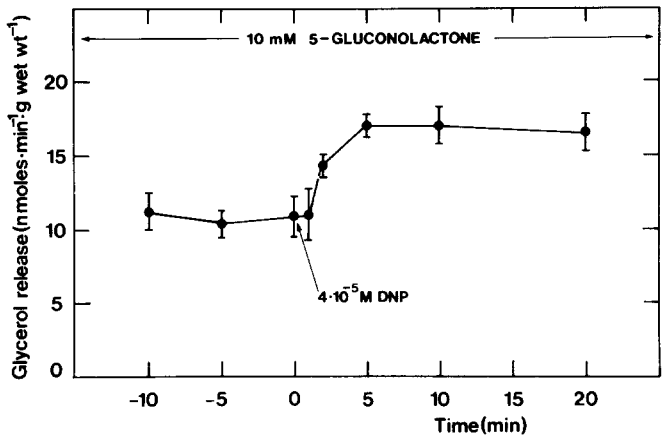

Fig. 2. The effect of 2,4-dinitrophenol (DNP) upon 5-gluconolactone-inhibited glycerol release from isolated, substrate-free, perfused hearts of trierucate fed rats ($\bar{X}$ ± SEM, n=4). 5-Gluconolactone was added to the perfusion medium immediately prior to preperfusion and prior to perfusion in the presence of DNP.

TABLE I. EFFECT OF VARIOUS ADDITIONS ON HORMONE-SENSITIVE LIPOLYSIS IN ADIPOCYTES

Condition	% of norepinephrine-stimulated activity*			
	no norepinephrine		0.5 μM norepinephrine	
No additions	8.0±2.9	(n=9)	100	(n=10)
10 mM gluconolactone	6.3±5.9	(n=3)	68.0±4.4	(n=6)
10 mM glucose	5.9±2.8	(n=5)	226.1±38.5	(n=5)
0.5 mM 2,4-dinitrophenol (DNP)	-		106.6±5.1	(n=5)
10 mM glucose + DNP	-		255.6±86.5	(n=3)
10 mM lactate	-		175.4±6.7	(n=3)
10 mM lactate + DNP	-		183.3	(n=1)
10 mM pyruvate	14.4	(n=2)	273.8±72.2	(n=5)
10 mM pyruvate + DNP	-		317.5±67.0	(n=4)
10 mM propionate	33.8±14.0	(n=4)	227.0±32.7	(n=5)
10 mM acetate	27.7±18.8	(n=3)	163.3±21.4	(n=4)

* Measured as glycerol production; the activity in the presence of norepinephrine (between 230 and 330 nmol formed/min x mg fat cell lipid) was made 100% in each individual experiment. The values are significantly different ($P<0.05$) from the reference value.

present, the rate of lipolysis is stimulated when respiration is stimulated by the addition of the uncoupler of oxidative phosphorylation 2,4-dinitrophenol (DNP) both in the absence (Fig. 2) or presence (not shown) of glucagon. In the absence of the hormone the rate of glycerol production is reduced, but the fact that lipolysis may be stimulated by an uncoupler instead of a lipolytic hormone is of interest.

Lipolysis by isolated fat cells. It can be seen from TABLE I that 5-gluconolactone inhibits hormone-stimulated lipolysis in fat cells significantly ($P<0.001$), whereas glucose stimulates (observed in all separate experiments). Here the addition of dinitrophenol has no effect, possibly because of the relatively low concentration of mitochondria in white adipose tissue. Lactate and pyruvate were also tested, since these compounds may be converted to glycerol-3-phosphate as well[19]. TABLE I shows that neither stimulation is sensitive to uncoupling of oxidative phosphorylation, suggesting that stimulation is not accomplished by glycerol-3-phosphate formation. Moreover, propionate and acetate, in each separate experiment, have also been found to stimulate hormone-sensitive lipolysis. The data also suggest that the low lipolytic activity in the absence of norepinephrine is also stimulated by the organic anions ($P<0.025$ for propionate-stimulated basal lipolysis and $P<0.1$ for acetate-stimulated basal lipolysis). From these experiments then it is most likely that the presence of a glycerol-3-phosphate precursor, to stimulate fatty acid re-esterification, or an organic anion to stimulate long-chain fatty acid secretion by exchange, will relieve product inhibition and stimulate lipolysis.

DISCUSSION

The presented data reveal that both in heart and adipose tissue inhibition of glycogenolysis, by the addition of 5-gluconolactone[14], results in inhibition of hormone-sensitive tissue lipolysis. The addition of glucose, allowing glyceride synthesis, partially restores hormone-sensitive lipolysis in heart (Fig. 1) and stimulates this process more than 2-fold in isolated fat cells (TABLE I). Uncoupling of oxidative phosphorylation by the addition of 2,4-dinitrophenol results in a strong stimulation of lipolysis during inhibited glycogenolysis in heart (Fig. 2), by stimulating fatty acid oxidation. This is not seen in isolated fat cells (TABLE I), probably because white adipose tissue is relatively poor in mitochondria. In heart, the stimulation of lipolysis by dinitrophenol is seen both in the absence and presence of the lipolytic hormone glucagon (comp. Fig. 2). Apparently the vicinity of the mitochondria to the sites of lipolysis[2] (probably the fat-filled autophagosomes[1,2]) does not absolutely require the operation of a hormone-induced fatty acid shuttle, which is likely to be Ca^{++} (see Introduction and below).

Recent studies in our laboratory[13] revealed that inhibition of lipolysis in heart by chloroquine addition severely depressed contractility, which could be restored by adding fatty acids, prostaglandin E_1, the Ca^{++}/Mg^{2+} ionophore X-537A or more Ca^{++} to the perfusion fluid. Hence, using contractility as a bioassay principle, we were able to demonstrate the Ca^{++}-ionophoric properties of long-chain fatty acids in heart (comp. also ref. 12). As quoted before[12], Clark already stated in 1913 that fatty acids, by forming insoluble calcium soaps, may stimulate the hypodynamic heart[20]. Ca^{++} then may remove the fatty acid ion probably also from the site of lipolysis, which will relieve product inhibition. A role of Ca^{++} in hormone-stimulated lipolysis in fat cells has been related to stimulation of Ca^{++} (ef)flux[21-24]. Therefore it is possible that also in adipose tissue Ca^{++} availability, induced by hormone addition, may help to remove inhibitory fatty acids during lipolysis, so that they may be re-esterified at the endoplasmic reticulum or exported at the cell surface. The latter process probably requires an electroneutral mechanism: either fatty acid export together with a cation or in exchange for an anion such as lactate, pyruvate, propionate or acetate - comp. TABLE I. Carboxylate anion exchange involving fatty acids has been observed in other tissues before[25].

In conclusion: Stimulation of lipolysis in tissues by hormones might, at least partially, be explained by preventing product inhibition by facilitating transport of fatty acids from the site of formation[1] to mitochondria, endoplasmic reticulum and cell surface membrane. When in adipose tissue in the catabolic state the availability of glycerol-3-phosphate for reesterification

decreases[19] fatty acid export is increased. Renold[26] suggested that the rate of albumin delivery through intravascular spaces may be an important determinant in the in vivo rate of lipolysis. Fatty acid-induced vasodilation[12] may contribute to this phenomenon.

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