Stimulation by Epinephrine of the Membrane Transport of Long Chain Fatty Acid in the Adipocyte*

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In isolated rat adipocytes, epinephrine rapidly stimulates the transport of long chain fatty acid across the plasma membrane. At a concentration of unbound oleate of 0.1 μM ([fatty acid]/[albumin] = 1) and 5 min exposure to the hormone, the minimal effective concentration of epinephrine is 0.03 and the optimal concentration 0.3 μM (0.01 and 0.1 μg/ml). The stimulated rates are 5–10-fold the basal rate of influx or efflux. The hormone effect is on the transport process specifically as shown by isolation of the product of transport in either direction as unesterified fatty acid and inhibition by the transport inhibitors phloretin and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid. This effect of epinephrine on transport coordinates physiologically with lipase activation to bring about fatty acid release from adipose tissue.

Long chain fatty acids are the major energy providing substrate for most tissues. Their release from adipose tissue and uptake by liver, heart, and skeletal muscle are increased in exercise, fasting, and diabetes. We have recently identified (1) and characterized (2) a transport system for long chain fatty acid in the plasma membrane of isolated adipocytes. The process is freely reversible, does not require metabolic energy, and has other characteristics of facilitated diffusion. It is strongly inhibited by phloretin and DIDS. The possibility that fatty acid transport is regulated by hormones or metabolic factors, as are glucose and amino acid transport, appeared likely and is the subject of this study. Such a regulation in adipocytes could be important for release of fatty acid and could have relevance to clinical problems in which lipid metabolism is disturbed. We report here a strong stimulation by epinephrine of fatty acid influx and efflux in isolated rat adipocytes. Evidence is provided that the hormonal effect is exerted on the membrane transport process.

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1 The abbreviations used are: DIDS, 4,4-diisothiocyanostilbene-2,2'-disulfonate; BSA, bovine serum albumin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
Epinephrine Effect on Fatty Acid Transport

**Fig. 1.** Epinephrine stimulation of \[^{3}H\]oleate influx into adipocytes. Cells prepared as described (see "Methods") were exposed to 1 \(\mu\)g/ml epinephrine (C) for 5 min at 37 °C before testing. Triangular symbols (Δ) show results with cells preincubated with the lipolytic inhibitor RHC 80267 for 15 min at 37 °C before treatment as described above with epinephrine. Filled symbols show parallel controls. The assays of oleate transport were carried out at 23 or at 4 °C (see inset) as outlined under "Methods." In this and all other data shown total fatty acid was 20 \(\mu\)M, BSA, 15 \(\mu\)M, and unbound fatty acid, 0.12 \(\mu\)M. The curves shown are a composite of four experiments which are typical of at least 20 more.

![Epinephrine stimulation of \[^{3}H\]oleate influx into adipocytes](image)

**Fig. 2.** Epinephrine stimulation of \[^{3}H\]oleate efflux from preloaded adipocytes. 30 \(\mu\)l of control or epinephrine-treated cell suspensions (30%, v/v) were incubated for 12 s with 20 \(\mu\)l of medium containing \[^{3}H\]oleate (5 \(\mu\)Ci/ml). Buffer (4 ml) at 23 °C containing fat free BSA (1%) was added to start efflux (0 time) and the cell suspension, diluted about 100-fold, was sampled at the times indicated on the abscissa. Aliquots (1 ml) were added to 5 ml of chilled stop solution and cellular radioactivity determined (see "Methods"). Non-specific fatty acid adsorption to the filters was not subtracted in the data shown and would account for as much as 80% of the radioactivity recovered with the cells after 1 min in the epinephrine-treated cells. The curves shown are a composite of four experiments.

lipolytic inhibitor, RHC 80267, before exposure to the hormone. This compound is a powerful inhibitor of tri- and diglyceride lipases (5). In separate experiments, using the same incubation and assay conditions, it was determined that a 15-min preincubation with RHC 80267 (10 \(\mu\)M) inhibited the stimulatory effect of epinephrine (1 \(\mu\)g/ml) on glycerol production by 85% (data not shown). No effects of the compound itself on fatty acid or glucose transport were observed. As shown in Fig. 1 (triangular symbols) inhibition of lipolysis by RHC 80267 did not alter the epinephrine effect. This suggested that accumulation of intracellular fatty acid after epinephrine treatment could not explain the transport activation.

Of particular importance physiologically was the observa-

![Epinephrine stimulation of \[^{3}H\]oleate efflux from preloaded adipocytes](image)

**Fig. 3.** Dose response of the epinephrine effect on \[^{3}H\]oleate transport. Cells were preincubated with the lipolytic inhibitor RHC 80267 (15 min at 37 °C) before treatment with various concentrations (0.001 to 100 \(\mu\)g/ml) of epinephrine for 5 min at 37 °C. Transport was then assayed at 23°C (see "Methods"). The stimulation by epinephrine is shown as the increase above basal transport which is given the arbitrary value of 1. The bars indicate standard errors \(n = 4-10\).

**Fig. 4.** Effect of DIDS on \[^{3}H\]oleate transport in basal and epinephrine-treated cells. Cells suspended (30%, v/v) in modified Krebs-Ringer medium buffered with Hepes buffer containing 2 mM glucose and 0.2% BSA were incubated without or with DIDS (300 \(\mu\)M) for 45 min at 37 °C in the dark. RHC 80267 was then added (10 \(\mu\)M) and the cells were left at 37 °C for 15 min more. Aliquots from control and DIDS-treated cells were then exposed to epinephrine (1 \(\mu\)g/ml) for 5 min at 37 °C. In some experiments the cells were washed in BSA containing medium following DIDS treatment to remove the unbound compound and were then treated with RHC 80267 and epinephrine as described above. Since this washing did not alter the results, it was subsequently omitted. △ and •, basal cells; ○ and ●, epinephrine treated; △ and ○, DIDS treated. The data is a composite of three experiments.

![Effect of DIDS on \[^{3}H\]oleate transport in basal and epinephrine-treated cells](image)
medium whereas esterified fatty acid would be retained. Further evidence that the epinephrine effect was not due to a stimulation of intracellular disposal of fatty acid was obtained by thin layer chromatography of lipid extracts from basal- and epinephrine-treated cells. In these experiments, the isotope taken up was quantitatively recovered on the silica plate in the free fatty acid band. This result was confirmed by extracting cells in ethanolic NaOH and petroleum ether (see "Methods"). All radioactivity partitioned in the aqueous phase.

**Effect of DIDS**—The permeation of long chain fatty acid in basal adipocytes involves to a limited extent a component with kinetics of simple diffusion as well as the carrier mediated system (1). To determine whether epinephrine might be stimulating the diffusion component the effect of DIDS2 on permeation in epinephrine-treated cells was investigated.

As shown in Fig. 4, DIDS inhibited fatty acid permeation by more than 80%. This effect of DIDS is similar in magnitude to its effect in basal cells (2) and indicates that epinephrine stimulates the carrier mediated component of FA permeation. Phloretin also strongly inhibits epinephrine-activated transport but the inhibition is not complete (data not shown).

**DISCUSSION**

The failure of epinephrine treatment to overcome the inhibitory effect of DIDS (Fig. 4) argues against the stimulation of transport by translocation of carriers to the plasma membrane from an intracellular reservoir as hypothesized for insulin activation of glucose transport (7). Since DIDS does not penetrate into the cell, intracellular carriers, if present, would be protected during exposure to DIDS which, in our studies, preceded epinephrine treatment.

The mechanism of epinephrine action on fatty acid transport could involve the same factors known to mediate its lipolytic effect (8); namely binding of the catecholamine to the receptors leading to increased activation of cAMP production. Our studies, to be reported later, indicate this to be the case.

It is probable, therefore, that transport will be stimulated by other hormones which raise cAMP. We have recent evidence which indicates that insulin (1 nM) can counteract very effectively the epinephrine stimulatory effect. It is well-known that insulin can reduce cAMP levels in epinephrine-activated adipose cells. Thus it appears that changes in the activity of fatty acid transport are physiologically coordinated with changes in lipase activity to bring about the lipolytic and anti-lipolytic effects of epinephrine and insulin in intact cells. Hormonal regulation of fatty acid transport, therefore, could have great significance physiologically for substrate supply and utilization in fasting, feeding, exercise, stress, and diabetes.

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2 This is the well-known inhibitor of band 3 anion transport in human erythrocytes. Its action in intact cell systems, like that of phloretin, is largely or exclusively on the plasma membrane.

3 N. A. Abumrad, P. Perry, and R. R. Whitesell, unpublished observations.