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EFFECTS OF SULFHYDRYL INHIBITION ON THE REGULATION OF BASAL LIPOLYSIS AND GLUCOSE UPTAKE IN HUMAN ADIPOSE TISSUE

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Abstract—Stimulation of basal lipolysis and inhibition of glucose uptake by *N*-ethylmaleimide (NEM) was demonstrated *in vitro* in human omental adipose tissue. NEM, at concentrations ranging from 4 to 5 × 10^{-4} M, produced a maximal stimulation of basal lipolysis as well as a 50 per cent inhibition of the rate of glucose uptake, whereas concentrations above 1 × 10^{-3}M reduced the basal rate of glycerol release. In contrast, under the conditions in which it stimulated basal lipolysis, NEM strongly inhibited theophylline induced lipolysis. NEM-stimulated lipolysis was not directly related to decreased glucose uptake, to α- or β-adrenergic mechanisms or to inhibition of phosphodiesterase. The lipolytic response of human adipose tissue to NEM was, however, markedly reduced by insulin and nicotinate, suggesting that adenylcyclase activation could be responsible for the lipolytic effect of NEM. The inhibitory effect exerted by NEM on glucose uptake was similarly reversed by the addition of insulin. The rate of basal lipolysis and glucose uptake by fat pads removed from diabetic patients was unaffected by NEM. A possible involvement of insulin in the effects induced by NEM on both basal lipolysis and glucose uptake in normal human adipose tissue is discussed.

Fassina *et al.* [1–3] have shown that, in adipose tissue, stimulated lipolysis is markedly reduced by either inhibitors of oxidative phosphorylation or of glycolysis. These authors, however, have published surprising results concerning the action of these inhibitors on basal lipolysis. They found that dicyclohexylcarbodiimide, antimycin A and piericidin A [3], as well as sodium fluoride [2] have no effect on basal lipolysis in rat adipose tissue, whereas monoiodoacetate stimulates this process and increases simultaneously tissue 3′, 5′-cyclic AMP levels [2].

It is therefore possible that the special activity of monoiodoacetate on basal lipolysis is related to its thiol blocking ability. This led us to study, in a previous investigation [4], the action of another thiol blocking agent, *N*-ethylmaleimide (NEM) [5], on basal lipolysis in rat adipose tissue. We found that NEM, like monoiodoacetate, stimulates basal lipolysis, in contradiction with its inhibitory effect on stimulated lipolysis [4].

Since important differences exist in the factors regulating lipolysis in human adipose tissue compared with the rat, e.g., rate of basal lipolysis [6], insensitivity to ACTH, glucagon and STH [7, 8], we studied, the influence of thiol inhibition induced by NEM on the basal glycerol and free fatty acid (FFA) release in human adipose tissue. Since glucose is an important factor in the regulation of lipolysis and because of the possible involvement of bound insulin in the mechanism by which NEM stimulates lipolysis in rat adipose tissue [4], these investigations were carried out on adipose tissue removed from both normal and diabetic patients.

MATERIAL AND METHODS

Human omental adipose tissue was obtained from 21 adult patients undergoing abdominal surgery. Their ages ranged from 20 to 55 yr. Five of these patients were untreated or uncontrolled diabetics with a mean fasting blood sugar of 204 mg/100 ml (range 135–395). None of the 16 other patients was acutely ill, or had clinical evidence of endocrine disease. The patients were fasted overnight and premedicated before operation with atropine and pethidin. Anesthesia was induced with short acting barbiturates combined with halothane, nitrous oxide, oxygen and succomethonium chloride. Samples of adipose tissue were obtained 10–20 min after the start of the operation and immediately transferred into Krebs–Ringer bicarbonate buffer pH 7·4 [9], containing glucose (5 × 10^{-3}M) and maintained at 37°.

Adipose tissue was divided into sections of approximately 50–60 mg and preincubated in the above medium for 30 min. After preincubation, 150–200 mg of adipose tissue was transferred into stoppered flasks containing 5 ml Krebs–Ringer bicarbonate buffer (pH 7·4) with bovine albumin 4% (w/v) and glucose (5 × 10^{-3}M). Incubations were performed for 3 hr at 37° with gentle shaking and air as the gas phase. Additions of NEM, insulin, nicotinate, propranolol, epinephrine and theophylline to the medium were done at zero time.

At the end of the incubation, lipolysis was determined as described previously [10]. FFA were measured according to Dole and Meinertz [11] and glycerol according to the enzymatic method of Eggstein [12]. FFA and glycerol release respectively are expressed as microequivalents and micromoles liberated in three hours into the medium per g wet weight adipose tissue. Glucose in the medium was assayed by the glucose oxidase method according to Bergmeyer and Bernt [13]; glucose uptake was calculated from the disappearance of glucose from the medium and expressed as micromoles glucose consumed in 3 hr per wet wt adipose tissue.

Results are expressed as the mean ± S.E. and statistical differences between groups were calculated by Student's *t*-test.

Enzymes, coenzymes and substrates were from Boehringer, NEM, theophylline and nicotinate from Merck, propranolol from Sigma, *l*-epinephrine bitartrate and insulin from Calbiochem and bovine albumin (fraction V) from Miles Pentex.

RESULTS

The effects of various concentrations of NEM on lipolysis and glucose uptake by adipose tissue removed from normal human subjects are shown in Fig. 1. As can be seen, NEM stimulates significantly glycerol and FFA release even at the concentration of 3×10^{-4}M. Maximal stimulation occurs at $4{\cdot}5 \times 10^{-4}$M, a concentration at which glycerol and FFA output is approximately 4 times the control level. As observed with rat adipose tissue [4], NEM concentrations equal to, or higher than 1×10^{-3}M produce an inhibition of glycerol release. Glucose uptake by human adipose tissue is also depressed by NEM. Already significant at the concentration of $3{\cdot}5 \times 10^{-4}$M, the rate of inhibition increases rapidly with the increase in concentration of NEM added to the medium; glucose uptake was reduced by 50 per cent by the addition of 5×10^{-4}M NEM, whereas it was almost completely abolished by 5×10^{-3}M NEM.

In contrast, no significant modifications of the rate of basal lipolysis or glucose uptake was observed in adipose tissue removed from diabetic patients after exposure to NEM (Fig. 2).

Lipolysis induced by NEM in human adipose tissue did not seem to be directly related to the inhibitory effect of NEM on the rate of glucose uptake. The results in Table 1 show that the rate of glycerol and FFA release induced by a same concentration of NEM was of the same order of magnitude whether adipose tissue was incubated in the absence or in the presence of glucose (5×10^{-3}M).

In order to gain insight into the mechanism by which NEM stimulates lipolysis *in vitro*, and since lipolysis in human adipose tissue is under the control of both β-and α-adrenergic mechanisms, we studied the influence of a β-blocking agent, propranolol (5×10^{-5}M) [8], as well as the consequence of α stimulation induced by propranolol (5×10^{-5}M) plus epinephrine (5×10^{-5}M) [8], on the lipolytic action of NEM ($4{\cdot}5 \times 10^{-4}$M). Fig. 3 indicates that neither propranolol alone nor propranolol plus epinephrine were able to alter significantly the rate of lipolysis induced by NEM.

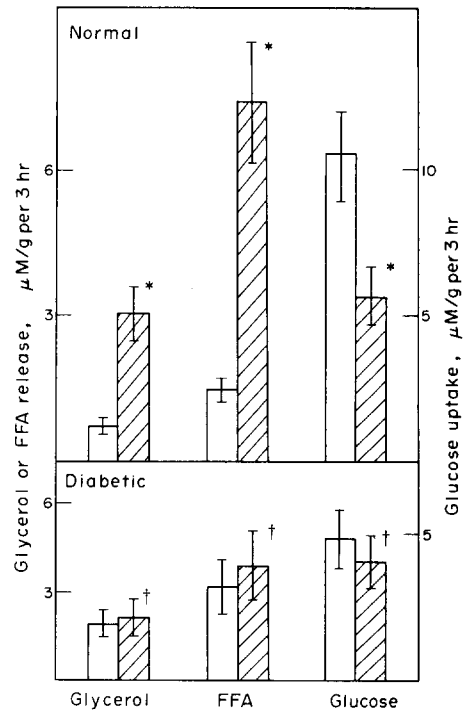

Fig. 2. Effect of *N*-ethylmaleimide ($4{\cdot}5 \times 10^{-4}$M) on lipolysis and glucose uptake by human adipose tissue removed from normal and uncontrolled diabetic patients. Incubation conditions are described in Material and Methods. Shaded bars represent the values observed with *N*-ethylmaleimide. Each vertical bar represents the mean of five determinations. Vertical lines represent 2 S.E. * $P < 0{\cdot}01$ as compared to the control values. † Not significant compared with the control values.

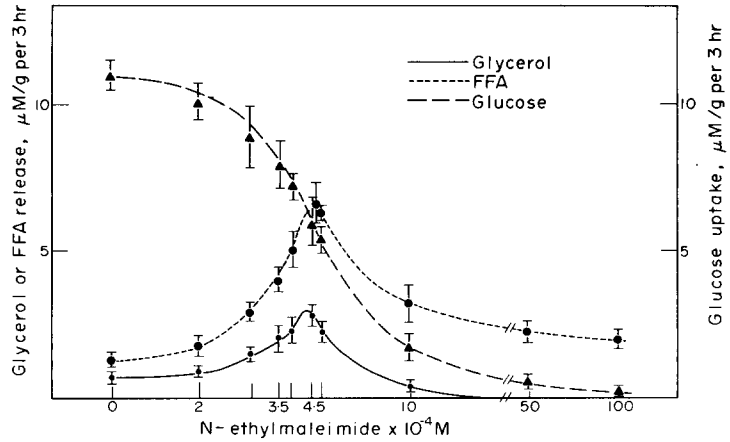

Fig. 1. Effect of various concentrations of *N*-ethylmaleimide on lipolysis and glucose uptake by human omental adipose tissue *in vitro*. Incubation conditions are described in Material and Methods. The vertical lines on the individual points represent 2 S.E. Each point represents the mean of five determinations.

Table 1. Influence of glucose on the lipolytic activity of *N*-ethylmaleimide (NEM) in human omental adipose tissue incubated *in vitro*

	Addition to the medium			
	0	NEM ($4{\cdot}5 \times 10^{-4}$M)	Glucose (5×10^{-3}M)	NEM ($4{\cdot}5 \times 10^{-4}$M) + glucose (5×10^{-3}M)
Glycerol release (μM/g per 3 hr)	0·52 ± 0·06	1·97 ± 0·31*	0·64 ± 0·05	2·41 ± 0·21*†‡
% increase		279 ± 60		276 ± 33
FFA release (μEq/g per 3 hr)	3·14 ± 0·39	8·18 ± 0·24*	2·84 ± 0·54	8·35 ± 0·73*†‡
% increase		160 ± 8		194 ± 26

Results are expressed as the mean ± S.E. of five determinations. NEM added to the medium at zero time. Incubation conditions are described in Material and Methods.

* $P < 0{\cdot}01$ compared with the results observed with no addition to the medium.

† $P < 0{\cdot}01$ compared with the results observed with addition of glucose (5×10^{-3}M) to the medium.

‡ not significant compared with the results observed with addition of NEM ($4{\cdot}5 \times 10^{-4}$M) to the medium.

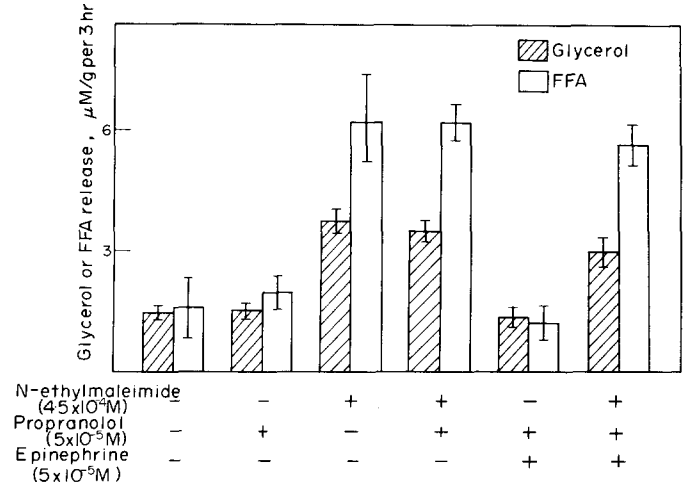

Fig. 3. Influence of propranolol (5×10^{-5}M) and of both propranolol (5×10^{-5}M) and epinephrine (5×10^{-5} M) on the lipolytic effect induced by *N*-ethylmaleimide ($4{\cdot}5 \times 10^{-4}$ M) in human omental adipose tissue *in vitro*. Incubation conditions are described in Material and Methods. Each vertical bar represents the mean of five determinations. Vertical lines represent 2 S.E.

Table 2. Influence of insulin (10 mU/ml) and nicotinate (1×10^{-3}M) on the effects of *N*-ethylmaleimide (NEM) on lipolysis and glucose uptake by human omental adipose tissue incubated *in vitro*

	Addition to the medium					
	0	NEM ($4{\cdot}5 \times 10^{-4}$M)	Insulin (10 mU/ml)	NEM ($4{\cdot}5 \times 10^{-4}$M) + Insulin (10 mU/ml)	Nicotinate (1×10^{-3}M)	NEM ($4{\cdot}5 \times 10^{-4}$M) + Nicotinate (1×10^{-3}M)
Glycerol release (μM/g per 3 hr)	0·89 ± 0·06	2·25 ± 0·13*	1·14 ± 0·14	1·81 ± 0·12†§	1·03 ± 0·15	1·47 ± 0·12‡§
% increase		153 ± 15		59 ± 11		43 ± 12
FFA release (μEq/g per 3 hr)	2·28 ± 0·20	4·89 ± 0·80*	2·15 ± 0·26	2·53 ± 0·45§‖	2·42 ± 0·25	5·04 ± 0·60‡
% increase		114 ± 35		18 ± 21		108 ± 25
Glucose uptake (μM/g per 3 hr)	9·26 ± 0·92	5·90 ± 0·85*	14·62 ± 1·15	8·98 ± 1·23†§‖	—	—
% decrease		36 ± 9		39 ± 8		

Results are expressed as the mean ± S.E. of five determinations. NEM, insulin and nicotinate added to the medium at zero time. Incubation conditions are described in Material and Methods.

* $P < 0{\cdot}01$ compared with the results observed with no addition to the medium.

† $P < 0{\cdot}01$ compared with the results observed with addition of insulin (10 mU/ml) to the medium.

‡ $P < 0{\cdot}01$ compared with the results observed with addition of nicotinate (1×10^{-3}M) to the medium.

§ $P < 0{\cdot}01$ compared with results observed with addition of NEM ($4{\cdot}5 \times 10^{-4}$ M) to the medium.

‖ not significant compared with the results observed without addition to the medium.

Table 3. Effect of *N*-ethylmaleimide (NEM) $4{\cdot}5 \times 10^{-4}$M on theophylline stimulated lipolysis in human omental adipose tissue incubated *in vitro*

Addition to the medium	Glycerol release (μM/g per 3 hr)	FFA release (μEq/g per 3 hr)
0	0·85 ± 0·07	1·90 ± 0·20
NEM ($4{\cdot}5 \times 10^{-4}$M)	2·15 ± 0·14*	7·25 ± 0·80*
Theophylline (5×10^{-3}M)	6·30 ± 0·95*	14·57 ± 1·47*
Theophylline (5×10^{-3}M) + NEM ($4{\cdot}5 \times 10^{-4}$M)	0·70 ± 0·05†‡	1·85 ± 0·19†‡

Results are expressed as the mean ± S.E. of five determinations. NEM and theophylline added to the medium at zero time.

* $P < 0{\cdot}01$ compared with the results obtained with no addition to the medium.

† $P < 0{\cdot}01$ compared with the results obtained with addition of theophylline (5×10^{-3}M) to the medium.

‡ Not significant compared with the results observed without addition to the medium.

Considering the possible interaction of NEM with adenylcyclase, we tested the influence of two inhibitors of this enzyme, insulin [14] and nicotinate [15] on lipolysis induced by NEM (Table 2). Addition of insulin (10 mU/ml) or nicotinate (1×10^{-3}M) produced up to a 60 per cent decrease in the rate of stimulation induced by NEM on glycerol release. Stimulation of FFA release was almost completely abolished by insulin whereas it was unaffected by nicotinate, compared with the value observed with NEM alone.

Results in Table 2 indicate furthermore that the addition of insulin could prevent the inhibition induced by NEM on adipose tissue glucose consumption.

In contrast to the results observed in basal conditions, NEM ($4{\cdot}5 \times 10^{-4}$M) showed a marked inhibitory effect on the rate of lipolysis induced by theophylline (5×10^{-3}M) as indicated in Table 3.

DISCUSSION

Sulfhydryl inhibitors have been shown previously [4, 16–19] to decrease unspecifically the lipolytic response of rat adipose tissue or fat cells to ACTH, TSH, catecholamines or theophylline. The present results show that NEM has the same inhibitory effect on theophylline stimulated lipolysis in human adipose tissue. As postulated for iodoacetate [2], this antilipolytic effect of NEM could be related to inhibition of glycolysis and consequently to a reduction of ATP supply, which is required for stimulated lipolysis both at the steps before and after 3′, 5′-cyclic AMP synthesis [20, 21]. Such a relationship has also been claimed for the inhibition of hormone-induced lipolysis caused by several inhibitors of oxidative phosphorylation [1, 3] or of glycolysis [2], which, contrary to monoiodoacetate and NEM, do not combine with thiol.

The stimulatory activity of NEM on basal lipolysis is interesting, as it is not a common feature of all inhibitors of energy supply. Considering in rat adipose tissue different inhibitors of oxidative phosphorylation and glycolysis, Fassina *et al.* [1, 3] found that monoiodoacetate was the only one which enhanced markedly basal lipolysis as indicated by the glycerol release. It appears thus that the stimulation of basal lipolysis occuring in the presence of either monoiodoacetate or NEM could be related specifically to their thiol blocking activity.

Considering the mechanism of the stimulatory effect of NEM on basal lipolysis, we investigated the possibility that this activity could be mediated either by inhibition of phosphodiesterase and/or stimulation of adenylcyclase.

The marked inhibitory effect of NEM on theophylline-stimulated lipolysis led us to conclude that inhibition of phosphodiesterase is not involved in the mechanism by which NEM stimulates basal lipolysis in human adipose tissue.

Our results indicating a marked reduction of the lipolytic effect of NEM by adenylcyclase inhibitors, such as insulin [14] or nicotinate [15], suggest, on the contrary, that NEM could act in human adipose tissue as in the rat [4], through adenylcyclase stimulation. The mechanism would be the same as that recorded by Fassina *et al.* [2] for monoiodoacetate, another thiol blocking agent which stimulates basal lipolysis and induces an increase in the level of 3′ 5′-cyclic AMP in rat adipose tissue.

Activation of adenylcyclase in human adipose tissue has been found to be partly regulated, at the membrane level, through stimulation of the β-adrenergic receptors, or through inhibition of the α-adrenergic receptors [8]. The present results, showing that inhibition of the β-adrenergic receptors by propranolol [22] as well as activation of the α-receptors by epinephrine plus propranolol [8] fails to reduce the lipolytic effects of NEM, indicate however that lipolysis induced by NEM does not involve either α-or β-adrenergic mechanisms.

The involvement of insulin in the mechanism by which NEM may stimulate adenylcyclase is to be considered, because of the failure of NEM to affect lipolysis in adipose tissue removed from diabetic patients, in whom insulin is either inactive or insufficiently secreted.

Previous reports have shown that pretreatment with NEM of perfused rat heart [23], as well as rat hemidiaphragm and rat adipose tissue [24–27], resulted in an inhibition of the effects of insulin upon glucose metabolism. Because of the important role played in rat tissues by thiol groups at both the active site of glucose entry into the cell [28] and the tissue receptors for insulin [27], it was postulated that the disorders induced in glucose metabolism by sulfhydryl inhibitors could be related to inhibition of either the specific insulin receptors or the active sites of the glucose transport system of the cell [26, 27].

The occurrence of such inhibitions in human adipose tissue exposed to thiol inhibitors is to be considered since it would explain not only the reduction of the basal and the insulin-stimulated glucose uptake, but also the activation of basal lipolysis occurring in these conditions. In fact, inhibition of the insulin receptors would hinder the basal effects exerted by the residual insulin bound to the tissue prior to excision [27]. These effects concern especially a stimulation of the glucose entry and an inhibition of adenylcyclase, and

therefore glucose uptake and basal lipolysis would respectively decrease and increase.

Whereas NEM in a concentration range from 3×10^{-4}M to 5×10^{-3}M stimulates basal lipolysis, higher NEM concentrations are inhibitory. Since NEM has been shown to penetrate cells readily [29], this inhibition probably results from an intra-cellular site of action, which could be localized at the level of some thiol involved in the active site of human adipose tissue triglyceride lipase, as seems to be the case for rat adipose tissue [17]. Such a biphasic effect on lipolysis has also been described for aldehydes [30, 31], compounds which, like NEM, combine with thiol [32].

In conclusion, our results showing opposite effects of thiol inhibitors on basal and stimulated lipolysis suggest the occurrence of two different mechanisms: inhibition of stimulated lipolysis seems to be related to an interference with the energy equilibrium in the adipose cell, whereas enhancement of basal lipolysis could possibly be related to the inhibition of the basal effects exerted on lipolysis by insulin bound to adipose tissue. Further investigations are needed to establish whether such a mechanism occurs and involves a direct inactivation of thiol at the level of human as well as of rat adipose tissue insulin receptors.

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