

Insulin Action on Adipocytes

EVIDENCE THAT THE ANTI-LIPOLYTIC AND LIPOGENIC EFFECTS OF INSULIN ARE MEDIATED BY THE SAME RECEPTOR

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1. The dose–response relationships of insulin stimulation of lipogenesis and inhibition of lipolysis were studied simultaneously by using rat adipocytes to determine whether these different effects of insulin are mediated through the same or different sets of receptors. 2. The sensitivity (defined as the concentration of insulin required to produce a half-maximal effect) of the stimulated lipogenic response to insulin was not significantly different from the sensitivity of the anti-lipolytic response to insulin. The addition of different adrenaline and glucose concentrations did not alter the half-maximal concentration of insulin required to inhibit lipolysis. 3. The specificities of the lipogenic and anti-lipolytic responses were studied by using insulin analogues. The sensitivities of the lipogenic and anti-lipolytic responses were the same for five chemically modified insulins and hagfish insulin, which have potencies compared with bovine insulin of between 3 and 90%. 4. Starving rats for 48h significantly increased the sensitivities of both the anti-lipolytic and lipogenic responses to insulin, but the changes in the sensitivities of the two effects were not significantly different. After re-feeding for 24h the sensitivities of both lipogenesis and anti-lipolysis returned to that of fed rats. 5. We conclude that insulin stimulates lipogenesis and inhibits lipolysis over the same concentration range. These observations provide powerful evidence that the different effects of insulin are mediated through the same set of receptors.

In adipose tissue insulin increases the cellular uptake and metabolism of glucose (Rodbell, 1964; Vinten *et al.*, 1976) and inhibits lipolysis stimulated by adrenaline (Jungas & Ball, 1963), but the mechanisms whereby insulin exerts these two different effects are not fully understood. It was reported that insulin inhibits lipolysis in adipocytes over a significantly lower concentration range than that required to stimulate glucose metabolism (Fain *et al.*, 1966; Hepp *et al.*, 1967), and Kono (1969) has proposed that separate sets of insulin receptors may be involved in mediating the two effects of insulin.

We have investigated the dose–response relationships for the stimulation of lipogenesis and inhibition of lipolysis by insulin, using the same preparations of isolated rat adipocytes, in order to determine whether these two effects of insulin are mediated through the same or different receptors. The specificities of the two responses to insulin were investigated by using a number of chemically modified insulins with widely varying potencies and binding affinities compared with bovine insulin. In a further attempt to differentiate between two different sets of insulin receptors, the lipogenic and anti-lipolytic responses to insulin were studied in adipocytes prepared from

starved and starved–re-fed rats. The results indicate that these two biological responses are mediated through the same set of receptors. A short report has appeared (Thomas *et al.*, 1978).

Experimental

Materials

Collagenase was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A. [^3H]-Glucose was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Adrenaline and bovine serum albumin were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Other biochemicals and enzymes were obtained from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K. Hagfish (*Myxine glutinosa*) insulin was a gift from Dr. S. Emdin, Umea School of Medicine, Sweden. [N^{α} -Acetoacetyl-Phe $^{\text{B1}}$]insulin and [N^{α} -acetoacetyl-Gly $^{\text{A1}}$]insulin were gifts from Dr. D. G. Lindsay, University of Sussex, Brighton, Sussex, U.K.

Methods

Isolated adipocytes were prepared from the epididymal fat-pads of 100–120g male Wistar rats

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(Cumming–Sprague–Europe strain) by the method of Rodbell (1964) as modified by Gliemann (1967). The rats were allowed access to food, starved for 48 h, or starved for 48 h and re-fed for 24 h before being killed.

Since the sensitivity of adipocytes to insulin varies between different cell preparations, lipogenesis and lipolysis were measured at the same time in the same cell preparation, although incubations for the two bioassays were separate. Lipogenesis was measured by the method of Moody *et al.* (1974) as the incorporation of [^3H]glucose into toluene-extractable lipid after incubation of adipocytes (0.2×10^5 – 0.4×10^5 cells) for 90 min at 37°C in Krebs–Ringer bicarbonate buffer (Cohen, 1957) containing 30 mg of bovine serum albumin and 0.55 mM-glucose. The incubation mixture (1 ml total volume) also contained $0.01 \mu\text{Ci}$ of [^3H]glucose and various concentrations of bovine insulin or chemically modified insulins (see Figure legends). Lipolysis was measured as the release of glycerol from 1×10^5 – 2×10^5 cells incubated for 30 min at 37°C in 2 ml of the above buffer containing albumin and glucose. Lipolysis was stimulated by the concentrations of adrenaline shown in the Figure legends. The incubation was stopped by cooling in an ice bath and the cells were allowed to float to the surface. A 1.5 ml sample of the infranatant was taken and added to 1.5 ml of 10% (w/v) HClO_4 . The precipitate was removed by centrifugation (100g for 10 min) and the supernatants were neutralized with 20% (w/v) KOH. Glycerol concentration was measured enzymically by the method of Eggstein & Kreutz (1966).

Biological potencies were calculated by using the combined results for each analogue plotted as the log dose against the percentage response (where the basal response was set at zero and maximal response at 100%). Data in the linear portion of the log (dose)–percentage-response curves were analysed by using parallel-line bioassay techniques (Finney, 1964; McArthur *et al.*, 1966). The potency of an analogue relative to bovine insulin was derived from the horizontal distance between fitted linear-regression lines of percentage response against the log dose for insulin and the analogue. The log (dose)–percentage-response curves were tested for linearity, non-parallelism and heterogeneity of variance. From the variance of the data, 95% fiducial limits were derived for each calculated potency relative to bovine insulin.

Results

Effect of adrenaline and glucose on the anti-lipolytic action of insulin

The effects of different concentrations of adrenaline and glucose on the inhibition of lipolysis by insulin

were studied to determine whether the sensitivity of the response to insulin varied with the concentration of adrenaline or glucose added. The sensitivity of both the lipogenic and anti-lipolytic effect of insulin was defined as the concentration of insulin required to produce a half-maximal effect. Fig. 1 shows the effect of different concentrations of insulin up to 1 nM on basal (no adrenaline added) lipolysis and lipolysis stimulated by four different adrenaline concentrations. The amount of glycerol released in the uninhibited (no insulin added) and maximally inhibited (1 nM-insulin added) conditions was increased by concentrations of adrenaline from 0.5 to 50 μM . However, the concentration of insulin required for half-maximal inhibition of adrenaline-stimulated and basal lipolysis was not significantly different. In the following experiments lipolysis was stimulated by 10 μM -adrenaline.

The sensitivity of the anti-lipolytic effect of insulin without added glucose and in the presence of 0.55 mM- and 5.0 mM-glucose was not different (Fig. 2). In subsequent experiments 0.55 mM-glucose was present in the incubation buffer.

Effect of insulin on lipogenesis and lipolysis

The lipogenic and anti-lipolytic responses to increasing concentrations of bovine insulin were measured by using adipocytes from the same cell preparation. Fig. 3 shows the mean responses \pm S.E.M.

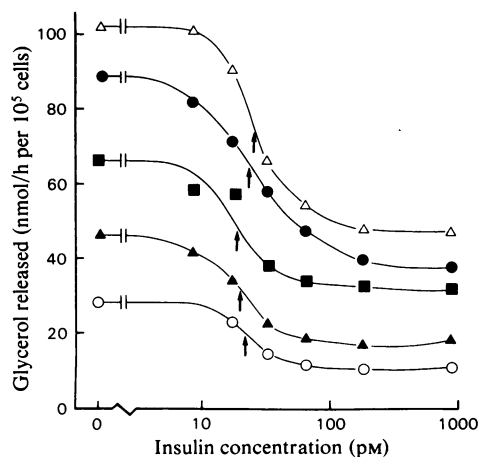


Fig. 1. *Effect of increasing concentrations of insulin on lipolysis stimulated by different concentrations of adrenaline* Lipolysis was stimulated by (\blacktriangle) 0.5 μM -, (\blacksquare) 2 μM -, (\bullet) 10 μM - and (\triangle) 50 μM -adrenaline. The effect of insulin on basal (no adrenaline added) lipolysis is also shown (\circ). The arrows indicate the concentrations of insulin required for a half-maximal inhibition of lipolysis. The points represent means of three separate experiments.

for 11 separate experiments. Lipogenesis was maximally stimulated 6-fold above basal (no insulin added) by 872 pM-bovine insulin. The maximal inhibition of lipolysis was 3.5-fold by concentrations of bovine insulin between 87.2 and 872 pM. When higher insulin concentrations were used (800–8000 pM) a paradoxical reversal of the anti-lipolytic

effect was observed (results not shown), as has been previously observed (Jungas & Ball, 1963; Clouverakis, 1967; Lavis & Williams, 1975).

The concentrations of insulin required to produce a half-maximal stimulation of lipogenesis or a half-maximal inhibition of lipolysis were not significantly different [28.1 ± 1.7 pM ($n = 11$); 28.2 ± 1.2 pM ($n = 11$) respectively]. The similarity between the sensitivities of the lipogenic and anti-lipolytic effects of insulin was emphasized when the insulin responses were plotted as the percentage of the maximal response (results not shown). The slope and position of the log (dose)–percentage–response curves for the two effects of insulin were not significantly different.

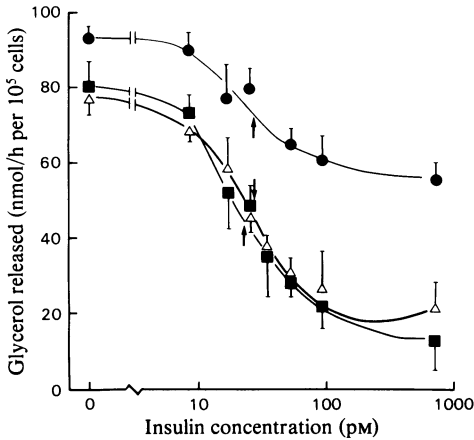


Fig. 2. Effect of glucose on the anti-lipolytic response of insulin

The effect of increasing concentrations of insulin on 10 μM-adrenaline-stimulated lipolysis was measured in medium containing (■) 0, (Δ) 0.55 mM- and (●) 5.0 mM-glucose. The arrows indicate the concentration of insulin required for half-maximal inhibition of lipolysis. The points represent means and the range of two separate experiments.

Effect of chemically modified insulins on lipogenesis and lipolysis

Five chemically modified insulins and hagfish insulin were used to stimulate lipogenesis and inhibit lipolysis in isolated adipocytes. The chemically modified insulins were either modified at the available amino groups ([N^α-acetoacetyl-Gly^{A1}]insulin, [N^α-acetoacetyl-Phe^{B1}]insulin and [N^ε-acetyl-Lys^{B29}]insulin) or were insulin dimers linked by a suberoyl chain ([N^α-Phe^{B1}-suberoyl-N^ε-Lys^{B29}]insulin dimer and [N^ε-Lys^{B29}-suberoyl-N^ε-Lys^{B29}]insulin dimer). The potencies compared with bovine insulin of these analogues and hagfish insulin ranged from 4 to 90% (Table 1) and were similar for insulin-stimulated lipogenesis or insulin inhibition of lipolysis. Moreover the concentrations of each of the chemically modified insulins required to produce a half-maximal stimulation of lipogenesis or inhibition of lipolysis were not statistically different by a paired *t* test (Table 1).

Effect of starvation and re-feeding on the lipogenic and anti-lipolytic effects of insulin

Starving rats for 48 h decreased the maximum response of adipocytes to insulin and the maximum rate of lipolysis stimulated by adrenaline (Fig. 4). Adipocytes from 48 h-starved rats were more sensitive to insulin than were adipocytes from fed rats. The concentration of insulin required to produce a half-maximal effect on both lipogenesis and anti-lipolysis was significantly decreased ($P < 0.05$) compared with adipocytes from fed rats. However, the changes in the half-maximally effective concentrations of insulin for both lipogenesis and anti-lipolysis were not significantly different (Table 2).

After a 24 h re-feeding of 48 h-starved rats the sensitivity of both the lipogenic and anti-lipolytic responses to insulin was not significantly different from that of fed rats (Fig. 4; Table 2). The maximum effect of insulin on lipogenesis and adrenaline on lipolysis in adipocytes from 24 h-re-fed rats was similar to or greater than that of fed rats.

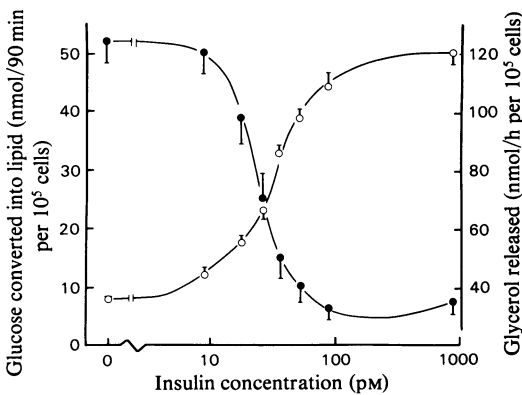


Fig. 3. Effect of increasing concentrations of bovine insulin in stimulating lipogenesis and inhibiting lipolysis

Lipogenesis (○) and 10 μM-adrenaline-stimulated lipolysis (●) were measured in media containing 0.55 mM-glucose. The points represent means ± S.E.M. for 11 separate experiments.

Table 1. *Biological potencies of analogues relative to insulin in stimulating lipogenesis and inhibiting lipolysis*

Biological potencies relative to bovine insulin were calculated as described in the Experimental section. The biological potencies for the two insulin dimers relative to bovine insulin were calculated by using equal weights of dimers and bovine insulin. The concentration of bovine insulin or insulin analogue required to produce a half-maximal stimulation of lipogenesis or inhibition of lipolysis was derived from the individual log (dose)-response curves, and the combined results are expressed as means \pm S.E.M. for the numbers of experiments shown. Results for potency are means with 95% fiducial limits in parentheses.

Analogue	No. of experiments	Potency relative to insulin (%)		Concentration for half-maximal response (pM)	
		Lipogenesis	Anti-lipolysis	Lipogenesis	Anti-lipolysis
Bovine insulin	11	100	100	28.1 \pm 1.2	28.2 \pm 1.2
[N ^ε -Acetyl-Lys ^{B29}]insulin	4	89 (78-103)	92 (71-120)	34.0 \pm 5.0	30.2 \pm 4.1
[N ^ε -Acetoacetyl-Phe ^{B1}]insulin	4	89 (73-109)	95 (79-114)	36.5 \pm 5.5	30.0 \pm 4.2
[N ^ε -Acetoacetyl-Gly ^{A1}]insulin	6	25 (17-36)	31 (19-59)	81.3 \pm 5.4	82.0 \pm 8.3
[N ^ε -Phe ^{B1} -suberoyl-N ^ε -Lys ^{B29}]insulin dimer	5	41 (36-46)	46 (37-58)	82.0 \pm 5.8	75.0 \pm 5.4
[N ^ε -Lys ^{B29} -suberoyl-N ^ε -Lys ^{B29}]insulin dimer	4	5.0 (4.0-6.3)	4.5 (3.6-5.5)	661.0 \pm 106.0	644.0 \pm 131.0
Hagfish insulin	4	3.8 (3.1-4.7)	4.4 (3.6-5.6)	762.0 \pm 60.0	746.0 \pm 69.0

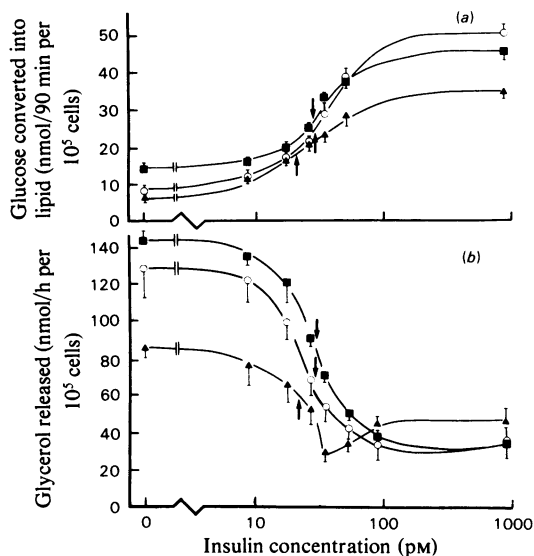


Fig. 4. *Effect of increasing concentrations of insulin on adipocytes prepared from starved, starved-re-fed and fed rats*

(a) Stimulation of lipogenesis; (b) inhibition of lipolysis. The points represent means \pm S.E.M. for fed rats (\circ , 11 separate cell preparations), 48 h-starved rats (\blacktriangle , six separate cell preparations) and 48 h-starved-24 h-re-fed rats (\blacksquare , four separate cell preparations). The arrows indicate the concentrations of insulin required for a half-maximal effect.

Discussion

In this paper we have compared the sensitivities to insulin of adrenaline-stimulated lipolysis and glucose metabolism using isolated adipocytes from

the same cell preparations and incubated under the same conditions. Figs. 1 and 2 show that stimulation of lipolysis with different concentrations of adrenaline and in the presence of different concentrations of glucose did not alter the concentration of insulin required for half-maximal inhibition of lipolysis.

We were unable to show any significant difference between the sensitivities to bovine insulin of lipogenesis and adrenaline-stimulated lipolysis (Fig. 3). This contrasts with previous reports that the inhibition of corticotropin- and catecholamine-stimulated lipolysis by insulin was 5 times more sensitive than insulin stimulation of glucose metabolism (Hepp *et al.*, 1967; Jacobsson *et al.*, 1976). Fain *et al.* (1966) have reported that lipolysis stimulated by somatotropin and dexamethasone was inhibited by a 1000-fold smaller concentration of insulin than that required to stimulate glucose metabolism, whereas corticotropin-stimulated lipolysis and glucose metabolism were affected by similar insulin concentrations. Stimulation of lipolysis by somatotropin and dexamethasone was measurable only after a 2 h incubation, whereas adrenaline-stimulated lipolysis was an immediate effect.

Stimulation of lipogenesis and inhibition of lipolysis by insulin represent multi-step metabolic processes. When the two responses to insulin were plotted with basal response as zero and the maximum response as 100% (results not shown), the log (insulin dose)-percentage-response curves for the two effects were almost identical, with similar position and slope, indicating that the rate-limiting step for insulin stimulation of lipogenesis and inhibition of lipolysis is common to both processes. Haring *et al.* (1978) have suggested that there is a common rate-limiting process between receptor binding and effects on membrane function, such as glucose transport or the cyclic AMP system.

Table 2. *Effect of starving and re-feeding on the sensitivities of lipogenesis and anti-lipolysis to bovine insulin*
 Groups were treated as described in the Experimental section. The concentration of bovine insulin required to produce a half-maximal stimulation of lipogenesis or inhibition of lipolysis was derived from the individual log (dose)-response curves, and the combined results are expressed as means \pm S.E.M. for the numbers of experiments shown.

Treatment	No. of experiments	Concentration of insulin for half-maximal response	
		Lipogenesis	Anti-lipolysis
Unchanged	11	28.1 \pm 1.7	28.2 \pm 1.2
48 h-starved	6	20.6 \pm 2.1	20.6 \pm 2.2
48 h-starved, 24 h-re-fed	4	29.5 \pm 2.1	28.3 \pm 1.9

The characteristics of the two effects of insulin were studied by using chemically modified insulins of widely different potencies. It has been shown that the potencies, compared with bovine insulin, of most insulin analogues as measured by lipogenesis in adipocytes is similar to their binding affinity compared with bovine insulin (Gliemann & Gammeltoft, 1974; Freychet *et al.*, 1974). However, this relationship may be different for the [N^{α} -Phe^{B1}-suberoyl- N^{ϵ} -Lys^{B29'}]insulin dimer, [N^{ϵ} -Lys^{B29}-suberoyl- N^{ϵ} -Lys^{B29'}]insulin dimer (Willey *et al.*, 1978) and hagfish insulin (Emdin *et al.*, 1977). If the lipogenic and anti-lipolytic responses to insulin were mediated through two different sets of receptors, this could be reflected in a difference between the potencies of the insulin analogues in inhibiting lipolysis and stimulating lipogenesis. The results presented show that it was not possible to identify different receptors mediating the two effects of insulin, since the lipogenic and anti-lipolytic activities of all the insulin analogues were similar whether expressed as potency compared with bovine insulin or dose required to produce a half-maximal response. In a preliminary investigation, Ellis *et al.* (1978) also showed that the biological potencies relative to insulin of [N^{α} -acetyl-Gly^{A1}, N^{ϵ} -acetyl-Lys^{B29}]insulin, cross-linked insulin analogues [N^{α} -Gly^{A1}-suberoyl- N^{ϵ} -Lys^{B29}]insulin and [N^{α} -Gly^{A1}-dodecyl- N^{ϵ} -Lys^{B29}]insulin and hagfish insulin were similar whether measured as a lipogenic or an anti-lipolytic response. These results contrast with the observations of Rudman *et al.* (1968), who observed, using des-Ala^{B30}-insulin and des-Ala^{B30}-des-Asn^{A21}-insulin, that the anti-lipolytic response to these two insulin analogues was more sensitive than the stimulation of glucose oxidation. However, in their experiments glucose oxidation was measured in rat adipocytes, whereas anti-lipolysis was measured in hamster fat-cells.

In a further attempt to distinguish between sets of receptors responsible for mediating the lipogenic and anti-lipolytic responses of insulin, rats were starved for 48 h. Previous reports have shown that starvation produces an increased specific binding

capacity (Bar *et al.*, 1976) or an increased receptor affinity (Olefsky, 1976). Fig. 4 and Table 2 show that after starvation for 48 h the sensitivity (as measured as the half-maximally effective dose) of the lipogenic and anti-lipolytic response to insulin was significantly increased. However, the sensitivities of the two responses to insulin were the same after 48 h starvation, again suggesting that the receptors mediating a stimulation of lipogenesis and an inhibition of lipolysis are the same. The changes in sensitivities of the two effects of insulin after starvation and re-feeding could relate to changes in serum insulin concentrations. Increased insulin concentrations have been shown to cause a decrease in insulin receptor number (Gavin *et al.*, 1974).

We have been unable to distinguish any differences in the insulin dose-response relationships for stimulation of lipogenesis and inhibition of adrenaline-stimulated lipolysis either by changing the nutritional state of the rats or by using chemically modified insulins with different binding affinities from that of bovine insulin. We suggest that insulin exerts its effects on lipogenesis and lipolysis through the same set of receptors.

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