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Sensitivity of adipocyte lipolysis to stimulatory and inhibitory agonists in hypothyroidism and starvation

E. David SAGGERSON
Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

1. The responsiveness of lipolysis to the stimulatory agonists noradrenaline, corticotropin and glucagon and to the inhibitory agonists N^6-phenylisopropyladenosine, prostaglandin E_1 and nicotinic acid was investigated with rat white adipocytes incubated with a high concentration of adenosine deaminase (1 unit/ml). 2. The cells were obtained from fed or 48 h-starved euthyroid animals or from fed or starved animals rendered hypothyroid by 4 weeks of treatment with low-iodine diet and propylthiouracil. 3. Hypothyroidism increased sensitivity to and efficacy of all three inhibitory agonists in their opposition of noradrenaline-stimulated lipolysis. Starvation decreased sensitivity to all three inhibitory agonists when opposing basal lipolysis. 4. Hypothyroidism decreased sensitivity to noradrenaline, glucagon and corticotropin by 37-, 4- and 4-fold respectively and decreased the maximum response to these agonists by approx. 50%, 50% and 75% respectively. Starvation reversed decreases in maximum response to these agonists in hypothyroidism. 5. Starvation in the euthyroid state increased sensitivity to glucagon and noradrenaline, but did not alter sensitivity to corticotropin. 6. Cells from hypothyroid rats were relatively insensitive to *Bordetella pertussis* toxin, which substantially increased basal lipolysis in the euthyroid state.

INTRODUCTION

Lipolysis in adipose tissue can be stimulated by the catecholamines, corticotropin and glucagon. Conversely, other agonists, e.g. adenosine, E-series prostaglandins and nicotinic acid, act through specific receptors to inhibit lipolysis. The activity of hormone-sensitive lipase is dependent on protein phosphorylation, which is the resultant of the activities of cyclic AMP-dependent protein kinase and phosphoprotein phosphatase(s). In turn, the cellular content of cyclic AMP is dependent on the relative activities of adenylate cyclase and cyclic nucleotide phosphodiesterase. Receptors for the inhibitory agonists are coupled to adenylate cyclase by a guanine nucleotide-binding protein (N_i) distinct from that (N_s) which couples receptors for stimulatory agonists to the enzyme (Rodbell, 1980; Murayama & Ui, 1983; Olansky *et al.*, 1983; Moreno *et al.*, 1983; Bokoch *et al.*, 1984; Codina *et al.*, 1984). In hypothyroidism adenylate cyclase, the increase in cyclic AMP, and lipolysis all show diminished responsiveness to the stimulatory agonists (Goodman & Bray, 1966; Armstrong *et al.*, 1974; Correze *et al.*, 1974; Malbon *et al.*, 1978; Ohisalo & Stouffer, 1979; Goswami & Rosenberg, 1980). Conversely, the inhibitory effect of adenosine mediated by the A_1 (Van Calker *et al.*, 1979) or R_i (Londos *et al.*, 1980) adenosine receptor is increased in hypothyroidism (Ohisalo & Stouffer, 1979; Malbon & Graziano, 1983; Chohan *et al.*, 1984; Malbon *et al.*, 1985). This enhanced responsiveness is most easily measured in the presence of adenosine deaminase by using the non-metabolized analogue PIA and appears to reflect an increase in the abundance of N_i in the adipocyte plasma membrane (Malbon *et al.*, 1985) without any increase in receptor number (Chohan *et al.*, 1984; Malbon *et al.*, 1985).

The present investigation was undertaken to answer two questions regarding the hypothyroid state. First, does hypothyroidism increase sensitivity to other inhibitory agonists (PGE_1 and nicotinic acid)? Second, in the presence of adenosine deaminase, do cells from hypothyroid rats retain decreased responsiveness to other stimulatory agonists, e.g. corticotropin and glucagon?

Starvation also alters the responsiveness of the adipocyte to adenosine, but in this state sensitivity is decreased (Chohan *et al.*, 1984). Accordingly it was also of interest to see whether sensitivity to PGE_1 and nicotinic acid was decreased in this state.

MATERIALS AND METHODS

Chemicals

Chemicals were obtained and treated as described by Fernandez & Saggerson (1978), Honnor & Saggerson (1980) and Saggerson (1980). In addition, PGE_1 and nicotinic acid were from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey, U.K.), and *Bordetella pertussis* toxin was purchased from the Centre for Applied Microbiology & Research, Porton Down, Wilts., U.K.

Animals

Rats were selected at 115±5 g and subjected to treatments to achieve the euthyroid and hypothyroid states as described by Chohan *et al.* (1984). All animals were subjected to these treatments for 4–6 weeks. Hypothyroid animals had ceased to grow by week 4 of treatment and were significantly lighter than the age-matched euthyroid controls (Table 1). Food was removed for 48 h to induce the starved state.

Abbreviations used: EC_{50}, the concentration of an inhibitory or a stimulatory agonist that causes 50% of its maximum effect; PGE_1, prostaglandin E_1; PIA, N^6-L-phenylisopropyladenosine.

Preparation and incubation of adipocytes

The pooled epididymal fat-pads of two or three rats were disaggregated with collagenase (1 mg/ml) as described by Rodbell (1964). Adipocytes equivalent to one-sixth of a fat-pad were incubated at 37 °C in 25 ml silicone-treated flasks containing 4 ml of Krebs–Henseleit (1932) saline, 4% (w/v) fatty acid-poor albumin, 5 mM-glucose and adenosine deaminase (1 unit/ml). The gas phase was O_2/CO_2 (19:1). After 60 min the flask contents received $HClO_4$ to a final concentration of 6% (w/v) and were then neutralized and treated as described by Fernandez & Saggerson (1978).

Analytical methods

Neutralized extracts from incubations were assayed for glycerol (Garland & Randle, 1962) and DNA was measured (Switzer & Summer, 1971). Adenosine deaminase (EC 3.5.4.4) was centrifuged at 6500 $g_{av.}$ for 3 min to remove $(NH_4)_2SO_4$, diluted in 0.15 M-NaCl to approx. 100 μg/ml and standardized spectrophotometrically at 25 °C by the method of Kalckar (1947). A unit of adenosine deaminase is that needed to deaminate 1 μmol of adenosine/min at 25 °C.

Statistical methods and presentation of data

Throughout, values are shown as means ± S.E.M. Where S.E.M. bars are not shown, these lie within the symbol. Statistical significance was determined by Student's *t* test for unpaired samples. Values of *n* in legends refer to the numbers of separate preparations.

RESULTS AND DISCUSSION

Effect of adenosine deaminase

Adenosine deaminase was added to all incubations at an extremely high concentration (1 unit/ml), which was considered to render adenosine concentrations insignificant. In the fed euthyroid state basal lipolysis in the absence of adenosine deaminase was 0.35 ± 0.03 μmol/h per 100 μg of DNA ($n = 7$; results not shown). Adenosine deaminase increased basal activity to approx. 2 μmol/h per 100 μg of DNA (Table 1). As found by Fernandez & Saggerson (1978), 20–50 munits of adenosine deaminase/ml was sufficient to maximize this response (results not shown). With cells from fed hypothyroid rats, adenosine deaminase up to concentrations as high as 20 units/ml had no effect whatever on basal lipolysis ($n = 3$; results not shown). This finding was unexpected and might suggest that these incubations contained other inhibitory non-adenosine agonists at concentrations sufficient to suppress basal lipolysis. This possibility was discounted because dilution of these cells 10-fold below the concentration generally used did not increase the basal lipolysis per quantity of cellular DNA or increase the sensitivity to stimulation by noradrenaline ($n = 2$; results not shown).

Anti-lipolytic effects of PIA, PGE_1 and nicotinic acid

In the euthyroid state the anti-lipolytic effects of these agents can be compared in two ways. Since basal lipolysis is quite appreciable in the presence of adenosine deaminase, the inhibitory agonists can be tested directly in opposition to this. Alternatively, the inhibitory agonists can be tested against lipolysis in the presence of a submaximal dose of a stimulatory agonist (e.g.

Table 1. EC_{50} values and statistical comparisons

The values in parentheses indicate the numbers of independent measurements. The body wt. data represent a compendium of measurements made throughout the study. Rates of lipolysis were obtained from Figs. 1–4. EC_{50} values for noradrenaline, corticotropin and glucagon were obtained from Figs. 1 and 4. EC_{50} values for PIA, PGE_1 and nicotinic acid were obtained from Fig. 2. For comparison of the hypothyroid and euthyroid states, a, b, c, d indicate $P < 0.05$, < 0.02, < 0.01, < 0.001 respectively. For comparison of the starved and fed states, e, f, g, h indicate $P < 0.05$, < 0.02, < 0.01, < 0.001 respectively.

		Rates of lipolysis (μmol/h per 100 μg of DNA)			EC_{50} values (nM)					
Condition	Body wt.	Basal	Noradrenaline maximum	Glucagon maximum	Noradrenaline	Corticotropin	Glucagon	PIA	PGE_1	Nicotinic acid
Euthyroid, fed	292±6 (33)	1.97±0.12 (13)	7.65±0.26 (9)	7.80±0.60 (4)	4.9±0.8 (4)	0.13±0.02 (4)	2.3±0.5 (4)	5.8±1.4 (4)	87±9 (5)	1900±200 (5)
Euthyroid, starved	255±7 f (10)	3.50±0.30 h (5)	7.20±1.10 (5)	7.30±1.00 (4)	—	0.1±0.01 (4)	0.46±0.04 g (4)	—	—	—
Hypothyroid, fed	210±5 d (33)	0.19±0.04 (13)	4.00±0.30 d (8)	2.10±0.30 d (4)	180±50 b (4)	0.55±0.12 b (4)	9.2±1.7 c (4)	1.2±0.3 b (5)	7.3±1.6 d (5)	280±70 d (5)
Hypothyroid, starved	188±4 df (12)	0.29±0.01 de (4)	6.44±0.74 f (4)	4.10±0.59 ae (4)	54±6 e (4)	0.76±0.02 d (4)	12±2c (4)	—	—	—

noradrenaline). Since basal lipolysis is so low in the hypothyroid state (Table 1), it was necessary to adopt this second approach. However, certain precautions are absolutely necessary. As discussed by Chohan *et al.* (1984), the effectiveness of the anti-lipolytic action of adenosine against noradrenaline-stimulated lipolysis is dependent on the noradrenaline concentration (Stock & Prilop, 1974; Fredholm, 1978). It is therefore important to choose sub-maximal concentrations for the stimulating agent and, when comparing different physiological states, these should be at equivalent points on each of the noradrenaline dose curves. From Fig. 1 it was found that 0.05 µM- and 1 µM-noradrenaline gave 90% of the maximum lipolytic response in the fed euthyroid and hypothyroid states respectively. Accordingly, these concentrations of noradrenaline were used for investigation of the effect of hypothyroidism on the potency of the inhibitory agonists (Fig. 2, Table 1). In the euthyroid state all three inhibitory agonists were only partially effective in inhibiting lipolysis, since the maximum percentage inhibitions were 71 ± 1%, 60 ± 1% and 37 ± 3% with PIA, PGE$_1$ and nicotinic acid respectively. Hypothyroidism changed these effects in two ways. First, all three agonists were now capable of complete inhibition of lipolysis (Fig. 2). Second, there were 5-fold, 12-fold and 7-fold decreases in the EC$_{50}$ values for PIA, PGE$_1$ and nicotinic acid respectively (Table 1).

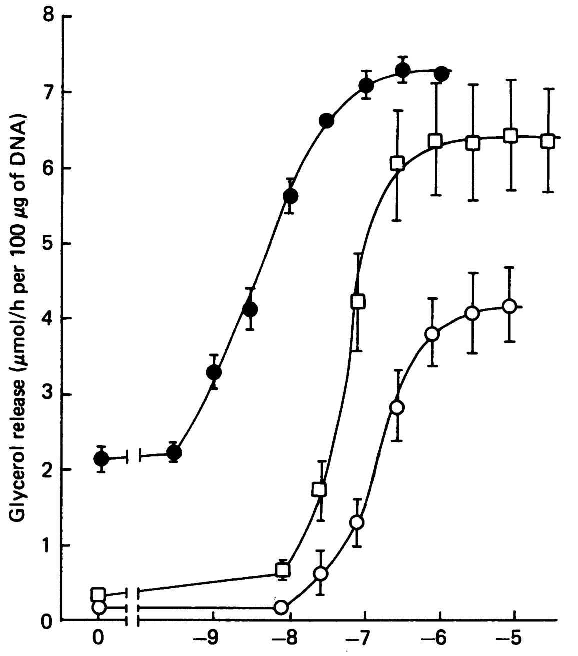

Fig. 1. Noradrenaline dose–response curves in the fed and starved states

●, Euthyroid, fed; ○, hypothyroid, fed; □, hypothyroid, starved ($n = 4$ in each case). Fat-cell DNA was 10.1 ± 1.5, 8.8 ± 1.0 and 8.4 ± 0.9 µg/ml of incubation-flask contents in the fed euthyroid, fed hypothyroid and starved hypothyroid states respectively. All incubations contained adenosine deaminase (1 unit/ml).

To investigate the responsiveness to these inhibitory agonists in the starved state, the simpler approach of opposing only the basal lipolysis (adenosine deaminase present) was adopted (Fig. 3, Table 2). Starvation for 48 h caused 3-fold, 4-fold and 2-fold increases in the EC$_{50}$ for PIA, PGE$_1$ and nicotinic acid respectively (Table 2). In these experiments the EC$_{50}$ for PIA was extremely low, and comparison with receptor-binding studies on fat-cell membranes (Trost & Schwabe, 1981; Chohan *et al.*, 1984; Malbon *et al.*, 1985) suggests that this anti-lipolytic effect must have been achieved by occupation of only a small proportion of the total available adenosine receptors. However, the EC$_{50}$ values for PIA in Table 2 are not inconsistent with the involvement of a set of high-affinity sites for PIA, with $K_D \leqslant 0.24$ nM (see Fig. 4 of Chohan *et al.*, 1984). A surprising observation, shown in Fig. 3, was the effect of a very low concentration of PIA in the fed state; 0.1 pM-PIA had a significantly

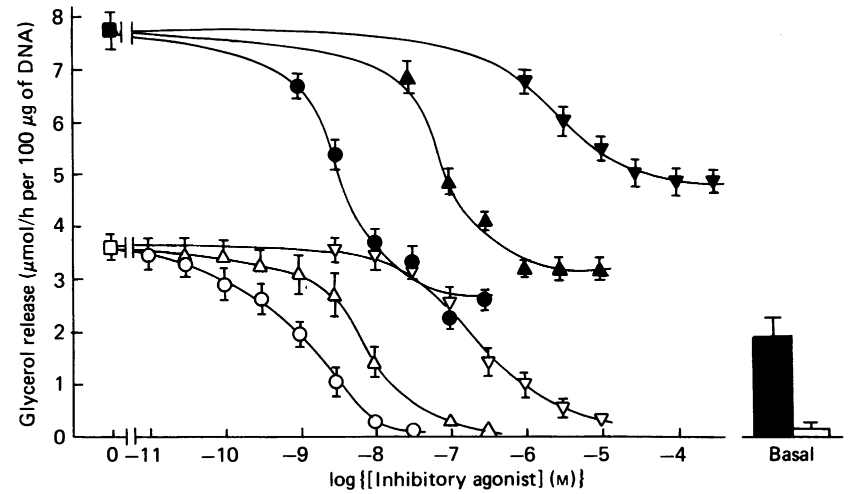

Fig. 2. Dose–response curves for PIA, PGE$_1$ and nicotinic acid opposing noradrenaline-stimulated lipolysis

Black symbols or histogram, fed euthyroid ($n = 4$); white symbols or histogram, fed hypothyroid ($n = 5$). ■, □, With noradrenaline alone (noradrenaline was used at 0.05 µM and 1 µM in the euthyroid and hypothyroid states respectively); ●, ○, with noradrenaline + PIA; ▲, △, with noradrenaline + PGE$_1$; ▼, ▽, with noradrenaline + nicotinic acid; histograms represent basal lipolysis without noradrenaline or inhibitory agonists. Fat-cell DNA was 8.7 ± 1.2 and 8.5 ± 0.8 µg/ml of incubation-flask contents in the euthyroid and hypothyroid states respectively. All incubations contained adenosine deaminase (1 unit/ml).

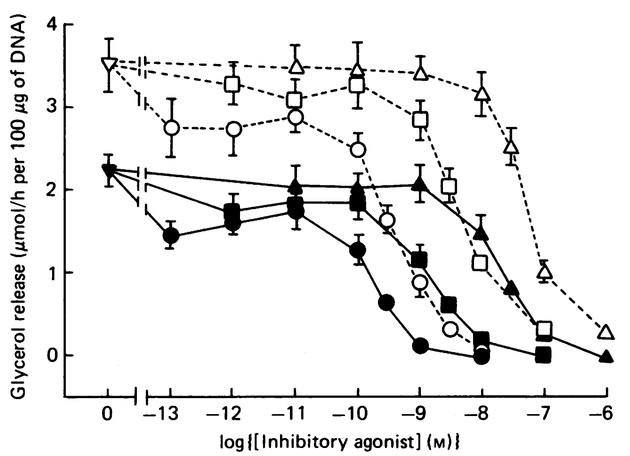

Fig. 3. Dose–response curves for PIA, PGE_1 and nicotinic acid opposing basal lipolysis

Black symbols, fed euthyroid ($n = 6$); white symbols, starved euthyroid ($n = 4$). ▼, ▽, Basal lipolysis with no added agonists; ●, ○, with PIA; ■, □, with PGE_1; ▲, △, with nicotinic acid. Fat-cell DNA was 9.1 ± 0.7 and 8.9 ± 0.9 µg/ml of incubation-flask contents in the fed and starved states respectively. All incubations contained adenosine deaminase (1 unit/ml).

greater anti-lipolytic effect than 1 or 10 pM of this agonist ($P < 0.01$ in both cases on a paired test). This biphasic response was consistently seen in all six separate experiments, but the reason for the phenomenon is unclear.

Lipolytic effects of noradrenaline, corticotropin and glucagon

The effect of physiological state on responsiveness to these agonists is summarized in Figs. 1 and 4 and Table 1. In all instances corticotropin and noradrenaline gave the same maximal response. In the fed or starved euthyroid state glucagon also elicted the same maximal response as noradrenaline, but in the fed hypothyroid state the maximal response to glucagon was only approx. 50% ($P < 0.01$) of those seen with corticotropin or noradrenaline, and these in turn were only approx. 50% of those seen in the euthyroid state. Starvation in the euthyroid state caused no significant change in the maximum response to any of these agonists. However, in the hypothyroid state starvation increased maximum responses to noradrenaline and corticotropin by 60–70%, such that these were now not significantly different from those in euthyroidism. At the same time the maximum response to glucagon was virtually doubled, although it was still significantly less ($P < 0.05$) than those seen with the other agonists.

Changes in sensitivity to stimulatory agonists were seen with some of these alterations in physiological state (Table 1). In the fed state hypothyroidism caused a 4-fold increase in the EC_{50} for both corticotropin and glucagon, but these changes are relatively modest compared with the accompanying 37-fold increase in EC_{50} for noradrenaline. Starvation caused no significant change in the EC_{50} for corticotropin in either the euthyroid or hypothyroid state, whereas the EC_{50} for the other polypeptide agonist, glucagon, was decreased 5-fold by starvation in the euthyroid state but unchanged by food withdrawal in hypothyroidism. With the subsequent realization that starvation decreases sensitivity to adenosine (Table 2; Chohan *et al.*, 1984), it might be questioned whether the change in glucagon sensitivity seen by Honnor & Saggerson (1980), using a submaximal concentration of adenosine deaminase, could be secondary to changes in responsiveness to adenosine. However, the experiments described here using a maximally effective concentration of adenosine deaminase (1 unit/ml) provide reassurance that the change in sensitivity to glucagon in starvation is not secondary and is directly related to the action of the polypeptide. Chohan *et al.* (1984) reported that starvation decreased the EC_{50} for noradrenaline approx. 7-fold in euthyroidism under similar conditions to those used here, and other studies have also demonstrated such an effect (Zapf *et al.*, 1977; Dax *et al.*, 1981). A significant 3-fold decrease in the EC_{50} for noradrenaline was also observed with starvation in the hypothyroid state (Table 1).

Table 2. EC_{50} values for opposition of basal lipolysis by PIA, PGE_1 and nicotinic acid

The values are obtained from Fig. 3. For comparison of the fed and starved states, a and b indicate $P < 0.01$ and $P < 0.001$ respectively. The values in parentheses indicate the numbers of independent measurements.

	EC_{50} values (nM)		
Condition	PIA	PGE_1	Nicotinic acid
Euthyroid, fed	0.13 ± 0.02	1.2 ± 0.2	20 ± 4
	(6)	(6)	(6)
Euthyroid, starved	0.37 ± 0.03 b	4.4 ± 0.3 b	38 ± 1 a
	(4)	(4)	(4)

Interplay between effects of stimulatory and inhibitory agonists

It is apparent from previous studies (Stock & Prilop, 1974) that adenosine decreases the sensitivity of adipocytes to catecholamine hormones without changing the maximum response. However, Fernandez & Saggerson (1978) and Honnor & Saggerson (1980) found that, in the absence of adenosine deaminase, the maximum lipolytic response to glucagon is only 30–40% of that with noradrenaline, whereas these two stimulatory agonists have the same maximum effect in the presence of the enzyme. This suggested that inhibitory agonists such as adenosine might oppose the action of glucagon in a manner which is different from their attenuation of β-adrenoceptor-mediated effects. This phenomenon was investigated more fully (Fig. 5). Fig. 5(*a*) confirms previous work (Stock & Prilop, 1974) and shows a dose-dependent effect of PIA both to decrease basal lipolysis and to shift the noradrenaline dose–response curve to the right without a change in the maximal response (EC_{50} values were 3 nM, 60 nM and 200 nM with zero, 3 nM- and 30 nM-PIA respectively). PGE_1 had a similar effect in that 0.1 µM- and 1 µM-PGE_1 displaced the EC_{50} for noradrenaline from 3 nM to 90 nM and 300 nM respectively (Fig. 5*b*). Quantitatively similar effects were seen when corticotropin was the stimulatory agonist or nicotinic acid was the inhibitory agent (results not shown). Figs. 5(*c*) and 5(*d*) show clearly that these

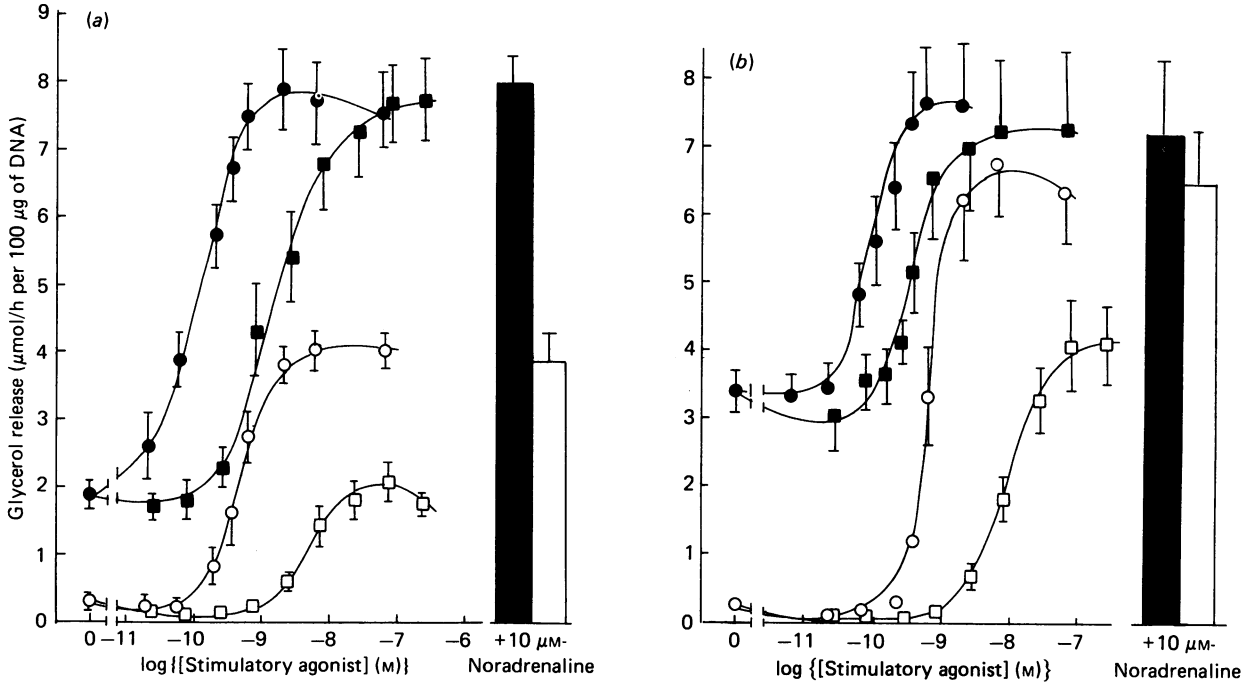

Fig. 4. Corticotropin and glucagon dose–response curves in the fed and starved states

(*a*) Black symbols or histogram, fed euthyroid ($n = 4$); white symbols or histogram, fed hypothyroid ($n = 4$). ●, ○, With corticotropin; ■, □, with glucagon; histograms represent the maximum lipolytic response with 10 μM-noradrenaline. Fat-cell DNA was 10.8 ± 0.7 and 10.3 ± 1.5 μg/ml of incubation-flask contents in the euthyroid and hypothyroid states respectively. (*b*) Black symbols or histogram, starved euthyroid ($n = 4$); white symbols or histogram, starved hypothyroid ($n = 4$); symbols as in (*a*). Fat-cell DNA was 11.1 ± 1.4 and 8.4 ± 0.9 μg/ml of incubation-flask contents in the euthyroid and hypothyroid states respectively. All incubations contained adenosine deaminase (1 unit/ml).

inhibitory agonists opposed glucagon-stimulated lipolysis in a different manner. The most striking difference was that glucagon dose–response curves became biphasic, i.e. higher concentrations of glucagon (1–10 μM) appeared to be inhibitory in the presence of PIA or PGE_1. Because of the complexity of these curves, it was not feasible to estimate EC_{50} values. PIA (3 and 30 nM) decreased the maximum lipolysis with glucagon by 27% and 81% respectively, whereas 0.1 μM- and 1 μM-PGE_1 decreased this value by 57% and 73% respectively. Nicotinic acid had qualitatively similar effects (results not shown). Unlike PIA, PGE_1 and nicotinic acid, the anti-lipolytic effect of insulin does not appear to involve N_i, since it is not blocked by treatment of cells with *Bordetella pertussis* toxin (Kather *et al.*, 1983), and it is therefore noteworthy that insulin differs from the other inhibitory agonists (Fig. 5*d*) in that 1.8 nM-insulin increased the EC_{50} for glucagon 5-fold without altering the maximum response. This effect of insulin is qualitatively similar to its anti-lipolytic action when noradrenaline or corticotropin are the stimulatory agonists, i.e. insulin is effective only against lower concentrations of these agents (Fain *et al.*, 1966; Hepp *et al.*, 1969; Schonhofer *et al.*, 1972). It is suggested that transmembrane signalling via the glucagon receptor is modified when N_i-linked receptors are occupied in an unusual way that is different from alterations in responses initiated through the β-adrenergic or corticotropin receptors.

These experiments are also noteworthy for another reason. In the euthyroid state the maximum response to glucagon without addition of inhibitory agonists is the same as those seen with noradrenaline or corticotropin (Table 1, Figs. 4 and 5). If these incubations contained appreciable amounts of non-adenosine inhibitory agonists of endogenous origin, the maximal response to glucagon would be decreased. It might, however, be argued that the maximum response to glucagon in hypothyroidism is decreased more than that of noradrenaline (Table 1) because of the presence of very small amounts of inhibitory agonists to which the cells have increased sensitivity. This was considered unlikely, because dilution of these cells 5-fold did not restore responsiveness to glucagon ($n = 2$; results not shown).

Effect of *Bordetella pertussis* toxin

This bacterial toxin is reported to catalyse the ADP-ribosylation of the M_r-41000 α-subunit of N_i (Bokoch *et al.*, 1984; Codina *et al.*, 1984) and thereby attenuate the effect of inhibitory agonists that are normally coupled to adenylate cyclase through this protein (Moreno *et al.*, 1983; Olansky *et al.*, 1983). It was expected that, with inhibitory receptors essentially 'empty' under basal conditions (adenosine deaminase present), treatment with pertussis toxin should have no effect on basal lipolysis. Within 30 min of addition of pertussis toxin to euthyroid cells at the relatively high dose of 1 μg/ml, 'basal' lipolysis was increased to a rate which thereafter was not appreciably different from that seen with a maximally effective concentration of noradrenaline (Fig. 6). Pertussis toxin also increased basal lipolysis in hypothyroid cells, but these were relatively resistant to the toxin, since little effect was seen before 90 min and the rate of lipolysis was only one-third of that seen with a maximally effective dose of

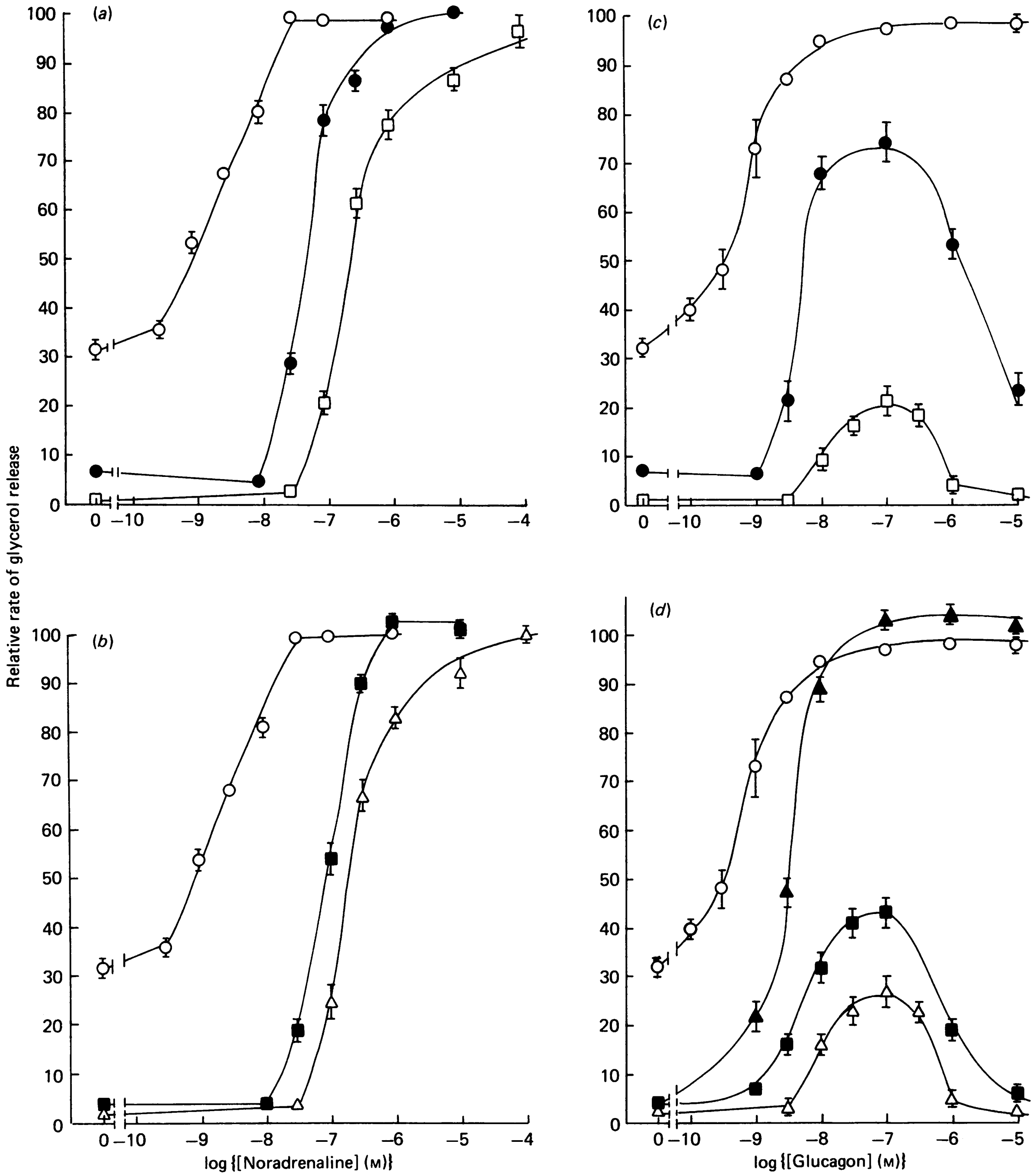

Fig. 5. Dose–response curves for glucagon and noradrenaline in the presence of PIA, PGE_1 or insulin

Cells were obtained from fed euthyroid animals. ○, Without inhibitory agonist ($n = 5$); ●, with 3 nM-PIA ($n = 3$); □, with 30 nM-PIA ($n = 3$); ■, with 0.1 µM-PGE_1 ($n = 3$); △, with 1 µM-PGE_1 ($n = 3$); ▲, with 1.8 nM-insulin ($n = 3$). All rates of lipolysis are relative to the value obtained with 30 nM-noradrenaline, which is set arbitrarily at 100 and was similar to the maximum rate obtained in the fed euthyroid state in Fig. 1 or Fig. 4. Fat-cell DNA contents of incubations were within the range of values observed in Figs. 1–4. All incubations contained adenosine deaminase (1 unit/ml).

noradrenaline (Fig. 6). Malbon *et al.* (1985) also reported that cells from hypothyroid rats are somewhat resistant to the effects of pertussis toxin. In their study the toxin was found to be less effective in the hypothyroid state in abolishing inhibition of forskolin-stimulated cyclic AMP accumulation by PIA. It is presumed that this resistance to the toxin reflects a greater abundance of N_i in hypothyroidism (Malbon *et al.*, 1985). Since arguments have been advanced elsewhere in this paper that inhibitory receptors are essentially 'empty' under basal incubation conditions, these findings possibly suggest that ADP-ribosylation of N_i activates (or deinhibits) stimulatory mechanisms directly in addition to attenuating effects of inhibitory agonists. Additionally it could be argued that increased abundance of N_i by itself could cause some attenuation of

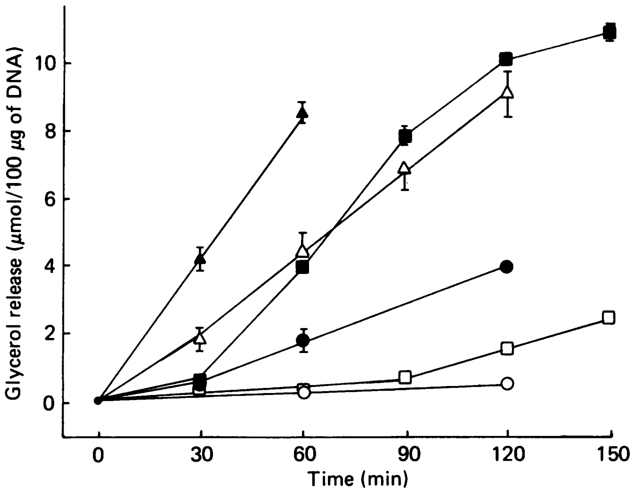

Fig. 6. Time courses of the effect of pertussis toxin on basal lipolysis

Black symbols, fed euthyroid ($n = 3$); white symbols, fed hypothyroid ($n = 3$). ●, ○, No other additions; ■, □, with pertussis toxin (1 μg/ml); ▲, △, with a maximally effective dose of noradrenaline (1 μM and 10 μM in the euthyroid and hypothyroid states respectively). All incubations contained adenosine deaminase (1 unit/ml).

stimulatory mechanisms even in the absence of inhibitory receptor agonists. In accord with this, it is reported by Londos *et al.* (1981) that inhibition of adenylate cyclase by GTP is observed in the absence of any anti-lipolytic compound. It is suggested that increased abundance of N_i in hypothyroidism (Malbon *et al.*, 1985) might contribute to the very low basal lipolysis, the diminished maximum response to glucagon in addition to the increased sensitivity to inhibitory agonists.

General discussion

Changes in sensitivity to and/or efficacy of both the stimulatory and the inhibitory agonists occur in several physiological states. In each case where responsiveness to stimulatory agonists is increased, that to inhibitory agents decreases, and vice versa. Thus hypothyroidism is associated with decreased stimulatory and increased inhibitory input (the present paper; Correze *et al.*, 1974; Malbon *et al.*, 1978; Ohisalo & Stouffer, 1979; Goswami & Rosenberg, 1980; Malbon & Graziano, 1983; Chohan *et al.*, 1984; Malbon *et al.*, 1984, 1985), starvation with increased stimulatory and decreased inhibitory input (the present paper; Zapf *et al.*, 1977; Honnor & Saggerson, 1980; Dax *et al.*, 1981; Chohan & Saggerson, 1982; Chohan *et al.*, 1984), and diabetes with increased responsiveness to stimulatory agonists (Zumstein *et al.*, 1980; Chatzipanteli & Saggerson, 1983) and decreased sensitivity to PIA (K. Chatzipanteli & E. D. Saggerson, unpublished work).

A generalized change in efficacy of, or sensitivity to, stimulatory agonists could be due to alteration in the cellular contents and activities of hormone-sensitive lipase, phosphoprotein phosphatase(s), protein kinase, cyclic AMP phosphodiesterase, adenylate cyclase catalytic unit, or in the number, affinity and coupling of receptors. In hypothyroidism it is established that there is an increase in cyclic AMP phosphodiesterase activity (Armstrong *et al.*, 1974; Correze *et al.*, 1974, 1976; Van Inwegen *et al.*, 1975; Elks & Manganiello, 1985), whereas there is little or no change in adenylate cyclase activity (Malbon & Gill, 1979; Malbon *et al.*, 1978, 1985), in protein kinase activity (Correze *et al.*, 1974; Van Inwegen *et al.*, 1975), or in number and affinity of β-adrenoceptors (Malbon *et al.*, 1978; Goswami & Rosenberg, 1980). Generalized changes in lipolytic responsiveness to stimulatory agonists therefore tell us little beyond describing the overall physiological profile of the process. However, comparisons of changes between specific agonists can be more revealing. Of the three stimulatory agonists, the responsiveness of corticotropin is the most constitutive, showing negligible change in starvation and only a modest 4-fold decrease in sensitivity together with a decreased efficacy in hypothyroidism. Corticotropin therefore provides a baseline, and changes greater than those seen with corticotropin must therefore be agonist-specific, with the implication that these should be at the level of receptors and their coupling. The large (37-fold) decrease in sensitivity to noradrenaline in hypothyroidism is not seen with corticotropin or glucagon, and it appears therefore that a large change in signal transduction betwen the receptor and N_s in this state (Malbon *et al.*, 1984) is confined to the β-adrenoceptor. A decreased efficacy in hypothyroidism is shown similarly by noradrenaline and corticotropin and is therefore not agonist-specific. However, in the fed hypothyroid state the efficacy of glucagon shows a further decrease that appears to be specific to this agonist. As discussed in the preceding section, it is suggested that this change may be related selectively to an increased abundance of N_i. Corticotropin may also be used as a 'reference agonist' in starvation. Both noradrenaline and glucagon show greater increases in sensitivity in this state, and it is suggested again that these changes occur at the level of receptors and/or their coupling. Lastly, responsiveness to the three inhibitory agonists is demonstrated for the first time to be co-ordinately changed. It remains to be established to what extent alterations in abundance of N_i or other adaptations are responsible.

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