Increased sensitivity of adipocyte adenylate cyclase to glucagon in the fasted state

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1. INTRODUCTION

It has been known since 1959 that glucagon can stimulate adipocyte lipolysis in vitro. With rat adipocytes glucagon has been shown to bind specifically to whole cells [1], to plasma membranes [2], to stimulate adenylate cyclase in ghosts [3] and to raise the intracellular content of cyclic AMP [4]. A cell-surface hormone receptor coupled to adenylate cyclase is therefore proposed and it is generally assumed that the resultant cyclic AMP promotes phosphorylation and activation of the hormone-sensitive lipase by protein kinase action. However, glucagon is not generally assigned a significant lipolytic role in mammals in vivo [5]. In part, this is because in vitro, high, unphysiological concentrations of glucagon are necessary to elicit a lipolytic response which, even at its fullest, is considerably smaller than that seen with maximally effective concentrations of other lipolytic agents such as adrenaline, noradrenaline, corticotropin or theophylline. As discussed below, it is possible that this relative ineffectiveness of glucagon in mammalian adipose tissue could result from the use of inappropriate conditions for incubation of cells.

It is now recognised that low concentrations of adenosine, acting through 'R-site' adenosine receptors [6–10], can decrease adipocyte adenylate cyclase activity and inhibit hormone-stimulated lipolysis. In adipocyte incubations, effects of endogenously-derived adenosine may be controlled and minimised by addition of adenosine deaminase [11–13]. Work from this laboratory [13,14] has suggested that the lipolytic action of glucagon is particularly sensitive to opposition by adenosine. This was seen in [14] where lipolysis in adipocytes from 24 h-fasted rats was found to be more sensitive to glucagon (over 10^{-10}–10^{-8} M) than in cells from fed animals. However, this change was only apparent if low concentrations of adenosine deaminase were present in the cell incubations [14]. It was therefore concluded that onset of the fasted state might result in a sensitization of adipocytes to glucagon, that this change is only seen when attempts are made to control the endogenous adenosine level and that there might be a physiological role for glucagon in adipose tissue in the fasted state [14]. These findings might be explained by fasting-induced changes at one or more sites in the pathway of activation of lipolysis by glucagon. Alternatively, or additionally, fasting-induced changes in the effect of adenosine might be involved. As a start to investigating these possibilities we have examined the response to glucagon of adenylate cyclase in plasma membranes from fed and fasted rats. It is shown that the response to glucagon is enhanced in the fasted state with high sensitivity to the hormone being observed.

2. MATERIALS AND METHODS

Male Sprague–Dawley rats (160–180 g body wt) were bred in the animal colony at University College London. Animals were sacrificed at about 11:00 h. Chemicals were supplied as in [14,15]. In addition, Percoll was from Pharmacia, papaverine from Sigma and cyclic AMP binding protein was prepared from bovine muscle [16].

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Adipocytes were isolated after disaggregation of epididymal adipose tissues with collagenase [17]. For measurement of lipolysis, adipocytes (equivalent to ⅙ of those obtained from one rat, ~25 μg DNA) were incubated in Krebs–Ringer bicarbonate buffer containing defatted albumin (40 mg/ml), 5 mM glucose and adenosine deaminase (4 munits/ml), as in [14]. After 1 h the incubations were terminated by deproteinisation with $HClO_4$ and glycerol measured [18].

Plasma membranes were prepared from the epididymal adipocytes of 4 rats. The cells were broken by agitation on a vortex mixer in 20 ml 0.25 M sucrose, 10 mM Tris–HCl, 2 mM EGTA, pH 7.4 at 0–4°C and rapidly fractionated by the method in [19] which employs density-gradient centrifugation in Percoll. Finally, the plasma membrane preparation was suspended in 1.0 ml 20 mM Tris–HCl buffer (pH 7.4) (~0.85 mg protein/ml).

Adenylate cyclase (EC 4.6.1.1) was assayed by a modification of the method in [20]. This was performed at 25°C in 0.2 ml total vol. containing 30 mM Tris–HCl (pH 7.5), 4 mM $MgCl_2$, defatted albumin (1 mg/ml), 1 mM ATP, 2 mM creatine phosphate, creatine kinase (25 units/ml), adenosine deaminase (0.4 units/ml), 10 μM papaverine, 10 μM guanylyl-imidodiphosphate (Gpp(NH)p) and the indicated concentration of glucagon. The reaction was initiated by addition of 20 μl plasma membranes (10–20 μg protein) and terminated after 20 min by boiling for 3 min followed by cooling in ice. After brief centrifugation to remove precipitated protein, cyclic AMP was measured by a binding protein assay [21]. Fresh, non-frozen preparations of membranes were always used for this assay.

5′-Nucleotidase (EC 3.1.3.5), monoamine oxidase (EC 1.4.3.4), NADP–cytochrome *c* reductase (EC 1.6.2.4) and succinate–cytochrome *c* reductase (EC 1.3.99.1) were assayed as described by [22–25], respectively. Measurements of adipocyte DNA of protein were as in [26] and [27], respectively. In all cases, preparations of adipocytes from fed and fasted rats were made in parallel on the same day.

3. RESULTS AND DISCUSSION

Plasma membranes were rapidly prepared using Percoll as in [19]. Using adipocytes from fed rats this method gave an 8-fold enrichment of 5′-nucleotidase specific activity over that found in the whole homogenate; 13 ± 2% of the homogenate 5′-nucleotidase with spec. act. 34 ± 5 nmol . min^{-1} . mg $protein^{-1}$ (mean ± SEM of 10 preparations) was found in the preparation. The plasma membrane fraction contained only 2 ± 1% and 5 ± 2%, respectively, of the total homogenate succinate–cytochrome *c* reductase and NADP–cytochrome *c* reductase activities and was therefore judged to be reasonably free of inner mitochondrial and microsomal membranes. The distribution of monoamine oxidase in this fractionation procedure was not reported in [19]. Surprisingly, before the final washing, the plasma membrane layer obtained from the Percoll gradient contained 45% of the total monoamine (tyramine) oxidase activity and the final

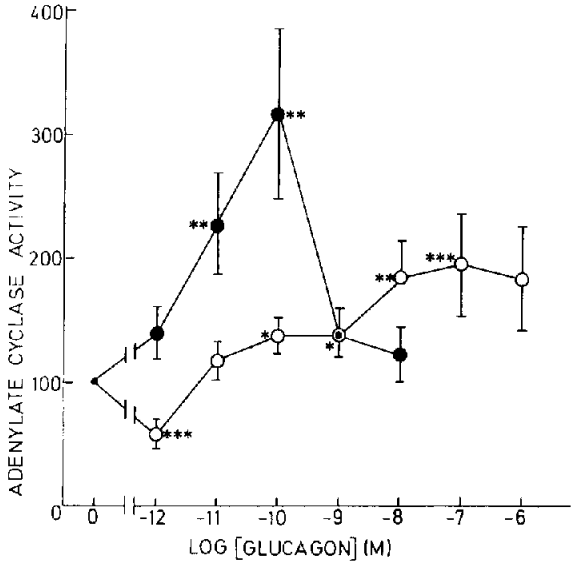

Fig.1. Effect of glucagon on adenylate cyclase. Plasma membranes were prepared from adipocytes of fed (○) and 24 h-fasted rats (●) and assayed for adenylate cyclase as in section 2. The values are means ± SEM of 9 and 8 expt for the fed and fasted states, respectively, and are expressed as percentages of the basal activity (with 10 μM Gpp(NH)p). These basal activities were: fed, 67 ± 10 and fasted, 88 ± 27 pmol . min^{-1} . mg $protein^{-1}$. *, **, *** indicate $P < 0.05$, < 0.02, < 0.01, respectively, for effects of glucagon *vs* the basal state. The percentage response differed significantly between the fed and fasted states at 10^{-12} M ($P < 0.01$), 10^{-11} M and 10^{-10} M (both $P < 0.05$) glucagon.

washed plasma membranes still contained 11 ± 3% of this activity. These results suggest either that there is contamination of the plasma membrane fraction by mitochondrial outer membrane or that adipocyte plasma membrane contains monoamine oxidase activity. Perhaps this might not be unreasonable in adipose tissue with its rich adrenergic innervation.

As in [20] the assay of adenylate cyclase contained a relatively large amount of adenosine deaminase (400 munits/ml) to minimise inhibitory effects of adenosine. Papaverine, which does not have adenosine agonist or antagonist actions in adipocyte membranes [28] was added to prevent cyclic AMP destruction. Under these conditions adenylate cyclase assays were linear with time and cyclic AMP formation was increased by Gpp(NH)p and glucagon (fig.1) and also (not shown) by isoproterenol, noradrenaline, corticotropin and fluoride. Fig.1 shows that, in the presence of Gpp(NH)p, membranes from fed animals exhibited a maximum response of adenylate cyclase with 10^{-7} M glucagon. This represented an approximate doubling of activity over the basal and may be compared with [3] in which the enzyme in membranes from fed animals was stimulated ~2.5-fold by 5×10^{-6} M glucagon (10 μM Gpp(NH)p was present). In the fed state 10^{-12} M glucagon consistently decreased the cyclase activity by ~50% (fig.1). The basis of this latter effect is unexplained. By contrast, in membranes from fasted rats, cyclase activity was trebled by 10^{-10} M glucagon and even 10^{-11} M hormone was sufficient to give more than a doubling of activity. In the presence of 10 μM Gpp(NH)p there was no significant difference between the basal cyclase activities in the fed and fasted states. It was noteworthy that 10 μM Gpp(NH)p alone caused a larger percentage increase in cyclase activity in the fed state (from 13 ± 6–67 ± 10 pmol · min^{-1} · mg protein^{-1}, $P < 0.001$) compared with the fasted state (from 48 ± 18–88 ± 27 pmol · min^{-1} · mg protein^{-1}).

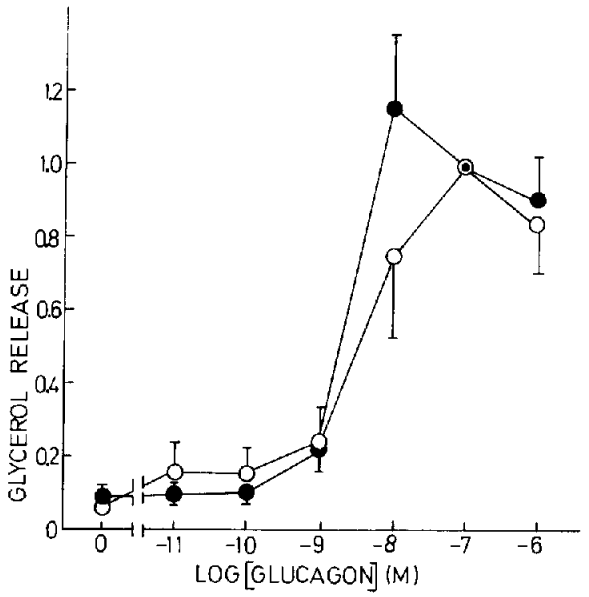

Fig.2. Effect of glucagon on lipolysis. Adipocytes from fed (○) and 24 h-fasted (●) rats were incubated for 60 min and glycerol assayed as in section 2. The values are means ± SEM of 5 expt and are expressed relative to the value at 10^{-7} M glucagon. Basal rates of lipolysis in the absence of glucagon were: fed, 0.6 ± 0.3 and fasted, 1.0 ± 0.3 μmol · h^{-1} · mg DNA^{-1}. The cells were taken from the same preparations that were used for assay of adenylate cyclase (fig.1).

Plasma glucagon levels in the rat have been reported over 80–2000 pg/ml; i.e., ~2×10^{-11}–5×10^{-10} M [29–31]. Under the conditions used here these concentrations are sufficient to activate adenylate cyclase in the fasted state, but are unlikely to have any appreciable stimulatory effect in the fed state.

In some experiments aliquots of adipocytes were also tested for their lipolytic response to glucagon (fig.2). Whilst there was reasonable correlation between the concentrations of glucagon effective on cyclase (fig.1) and lipolysis (fig.2) in the fed state, there was considerable disparity between the ranges of hormone concentration acting on these processes in the fasted state.

In conclusion, activation of adipocyte adenylate cyclase by physiological concentrations of glucagon is substantially enhanced in the fasted state. It is unclear whether this reflects altered properties of the glucagon receptor and/or its coupling via a guanyl nucleotide-binding protein to the catalytic entity of the cyclase. The effect of the hormone on intact cells from fasted rats appears to be attenuated. At present it is unclear whether in vivo the adenylate cyclase (and hence lipolysis) is more responsive to glucagon after fasting, in which case the attenuation of response presumably is an artefact of the cell incubation system. Alternatively,

attenuation of the glucagon response might occur in vivo, in which case sensitization of the cyclase by fasting makes little physiological sense.

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