Differential effects of noradrenaline and glucagon on lipolysis and fatty-acid utilization in brown adipose tissue

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The relative effects of noradrenaline (300 pM–3 μM) and glucagon (30 pM–300 nM) upon lipolysis and fatty acid utilization rates in brown adipose tissue from warm- (WA) and cold-acclimated (CA) rats, were: (i) lipolytic sensitivity and responsiveness to the agonists were reduced in CA tissue; (ii) in CA tissue, at 300 pM, glucagon promoted fatty acid utilization more than noradrenaline; (iii) glucagon at 300 pM increased fatty acid utilization in WA tissue. The data suggest that glucagon has a physiological role in brown adipose tissue, modulating events subsequent to NA- and glucagon-induced lipolysis, promoting fatty acid utilization.

Brown fat *Lipolysis* *Fatty acid* *Glucagon* *Noradrenaline* *Cold acclimation*

1. INTRODUCTION

The activation of brown adipose tissue (BAT), a principal site of thermogenic metabolism [1,2], appears to be via noradrenaline- (NA) induced lipolysis resulting from increased sympathetic activity [3]. While NA is undoubtedly the principal agonist regulating BAT, other humoral factors, such as the thyronines have permissive effects upon adrenergic activation [4]. Similarly, glucagon may have a role. An age-related stimulation of glycerol release by glucagon is seen in rat BAT [5], glucagon infusion into rats results in fatty acid release from BAT [6], and isolated rat brown adipocytes exhibit a thermogenic response to glucagon [7]. While it has been suggested [8] that the concentrations of glucagon required to elicit these effects are supraphysiological, a recent report from this laboratory [9] showed substantial increases in arginine-stimulated glucagon secretion by the isolated perfused rat pancreas during cold acclimation. Glucagon-induced lipolysis has been demonstrated in brown adipocytes [10] and it has been suggested this is responsible for its thermogenic effects (e.g. [11]). Chronic glucagon administration causes mitochondriogenesis in BAT [12], and it may be that glucagon exerts effects upon BAT distinct from those upon lipolysis. To assess this possibility, we have made simultaneous measurements of fatty acid and glycerol production by BAT in the presence of NA or glucagon, to gain indications of agonist-related changes in lipolytic and post-lipolytic events. The data suggest a role for glucagon in BAT thermogenesis distinct from its lipolytic actions, and from the actions of the catecholamines.

2. EXPERIMENTAL

Male Wistar albino rats (~200 g body wt) were kept 3/cage, receiving food and water ad libitum and a 12 h photoperiod (08:00–20:00). Control (warm-acclimated) animals were held at 21 ± 1.0°C, and experimental (cold-acclimated) animals

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were held at 4 ± 1.0°C, for 6 weeks in each case. Interscapular BAT (IBAT) from these animals was incubated in Krebs-Ringer bicarbonate buffered medium (KRB) containing NaCl (118.5 mM), KCl (3.0 mM), $CaCl_2$ (2.0 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (1.2 mM) and $NaHCO_3$ (25 mM), with D-(+)-glucose at 2.7 mM [13]. The medium was gassed with 95%:5% O_2/CO_2 at 37°C for 1 h; defatted bovine serum albumin (Cohn fraction V) was added to 4.5% (w/v), and the pH adjusted to 7.4 with 0.1 M NaOH.

Animals were killed by cervical dislocation and IBAT dissected free of white adipose and muscle tissue, and held in KRB without glucose at room temperature. IBAT was divided into fragments which were blotted dry and weighed. 7–9 fragments of total weight 100–150 mg were placed in 5 ml KRB with albumin and glucose. After 30 min preincubation, saline, NA (as the bitartrate; Levophed®, Winthrop Laboratories, England) or glucagon (Sigma) were added in 10 μl volumes to give concentrations of NA from 300 pM to 3 μM, and of glucagon from 30 pM to 300 nM. Incubation was at 37°C in a Dubnoff bath at 80 strokes/min, continuously flushing with 95% O_2/5% CO_2, for 1 h. Metabolic activity was halted by the addition of 2.0 ml of 2.5 M H_2SO_4 [14], followed by centrifugation (1000 × g at 4°C; 15 min). Supernatants were kept at −20°C for later analysis.

Glycerol was determined by a NAD-linked microenzymatic method [15], after deproteinisation with 0.67 M perchloric acid; free fatty acids (FFAs) were estimated using a commercial kit (NEFA-C Test, Wako Pure Chemicals, Japan). Lipolysis rates were expressed as (glycerol release × 3) and fatty-acid utilization rates as (rate of lipolysis − FFA release). Data, as nmol·min^{-1}·g wet wt of tissue^{-1}, were assessed a priori by analysis of variance, followed by Student's t-test (unpaired samples) for point to point comparisons. Statistical significance was assumed at $p \leqslant 0.05$.

3. RESULTS AND DISCUSSION

The derivations of lipolysis and fatty acid utilization rates assume complete triacylglycerol hydrolysis with glycerol release in toto. Some resynthesis of triacylglycerols may occur in BAT [16] causing small under-estimates of lipolysis rates when determined from measurements of glycerol release, as here.

3.1. *Lipolysis*

Analysis of variance of the data shown in fig.1 indicated significant effects of both acclimation states and agonists ($p < 0.001$ and $p < 0.01$, respectively) with significant interaction ($p < 0.01$), indicating reduced responsiveness to both NA- and glucagon-stimulation in CA IBAT. In the case of NA, elevations above basal levels, in both WA and CA IBAT, were observed at 30 nM (WA $p < 0.0005$; CA $p < 0.02$), while for glucagon the threshold of response of WA IBAT was 300 pM ($p < 0.03$) and of CA IBAT at 30 nM, demonstrating reduced glucagon sensitivity in the latter tissue. For NA at and above concentrations of 30 nM, and glucagon at and above concentrations of 300 pM responsiveness was greater in WA than CA IBAT ($p < 0.002$ and $p < 0.04$, respectively).

3.2. *Fatty acid utilization*

Analysis of variance of the data presented in fig.2 indicated a significant effect of acclimation state on the response to glucagon ($p < 0.01$), while acclimation state did not affect the response to NA. A significant effect of concentration ($p < 0.02$) indicated that the response to glucagon was dose dependent. At 300 pM, the effects of NA and glucagon upon fatty acid utilization were indistinguishable in WA IBAT, while in CA IBAT the response to glucagon was significantly greater than that to NA ($p < 0.05$). At agonist concentrations of and in excess of 3 nM, in both WA and CA IBAT, glucagon had consistently greater effects upon fatty acid utilization than NA (p always <0.002). At glucagon concentrations of 300 pM and 3 nM, WA IBAT showed greater responsiveness than CA IBAT ($p < 0.04$ and $p < 0.01$, respectively) whereas at concentrations of and above 30 nM, CA IBAT was more responsive than WA IBAT ($p < 0.02$). The data thus indicate reduced sensitivity with increased responsiveness to glucagon in CA IBAT.

The data show that CA IBAT fragments are less responsive than WA fragments to lipolytic agonists, consistent with earlier reports (e.g. [17]). Glucagon-induced, relative to NA-induced, lipolysis was greater at higher dose levels (3 nM in WA IBAT, 30 nM in CA IBAT). In the isolated

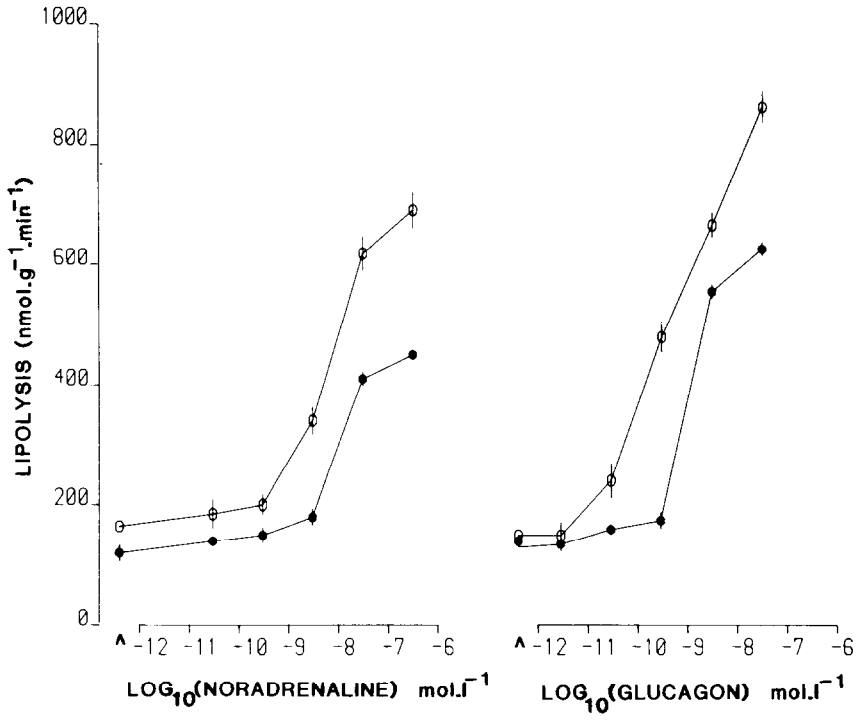

Fig.1. Lipolytic effects of glucagon and noradrenaline upon IBAT fragments. Data, as functions of $\log_{10}$ [agonist concentration], represent mean ± SE, for $n = 6$ in all cases. (○) IBAT from warm-acclimated animals; (●) IBAT from cold-acclimated animals.

brown adipocyte preparation, the sensitivity of lipolysis to glucagon is greater than to NA [18], consistent with the present data, but the same report indicated lower responsiveness of respiration to glucagon. As respiration and lipolysis are closely coupled in BAT [19], lower responsiveness to glucagon would be expected, at variance with the data in fig.1. The present finding of a greater lipolytic response to glucagon than to NA may relate to the adventitious presence of NA in IBAT fragments containing disrupted adrenergic fibres. Support for this view may be adduced from a recent report [20] that in vivo exogenous glucagon has no effect upon V_{O_2} at thermoneutrality, while in acute cold exposure the same agonist increases V_{O_2}, suggesting interaction between NA and glucagon in thermoregulation.

Fatty acid utilization rates indicated relatively low activity of NA and high activity of glucagon, particularly at concentrations greater than 30 pM. At the highest glucagon concentrations, substantial increases in fatty acid utilization rates are seen. Glucagon appears to promote fatty acid utilization in IBAT, subsequent to lipolytic events induced by itself or by NA. Chronic administration of glucagon to rats at thermoneutrality results in increased BAT mitochondriogenesis, the enlarged mitochondria showing well developed cristae [12] consonant with enhanced β-oxidative capacity, and markedly improved cold tolerance [21]. Mitochondriogenesis is a longer-term event and it is evident from the present data that glucagon promotes fatty acid utilization in the short-term, as seen in WA IBAT. There are no relevant reports of metabolic regulation, apart from lipolysis, by glucagon in IBAT. In liver, it has been suggested that a glucagon-induced reduction of α-glycerophosphate concentration thereby reduces triacylglycerol synthesis, promoting fatty acid oxidation [22]. Similarly, glucagon reduces hepatic concentrations of malonyl coenzyme A, an inhibitor of carnitine acyltransferase [23], permitting reciprocal regulation of fatty acid oxidation and esterification [24]. The present data do not permit

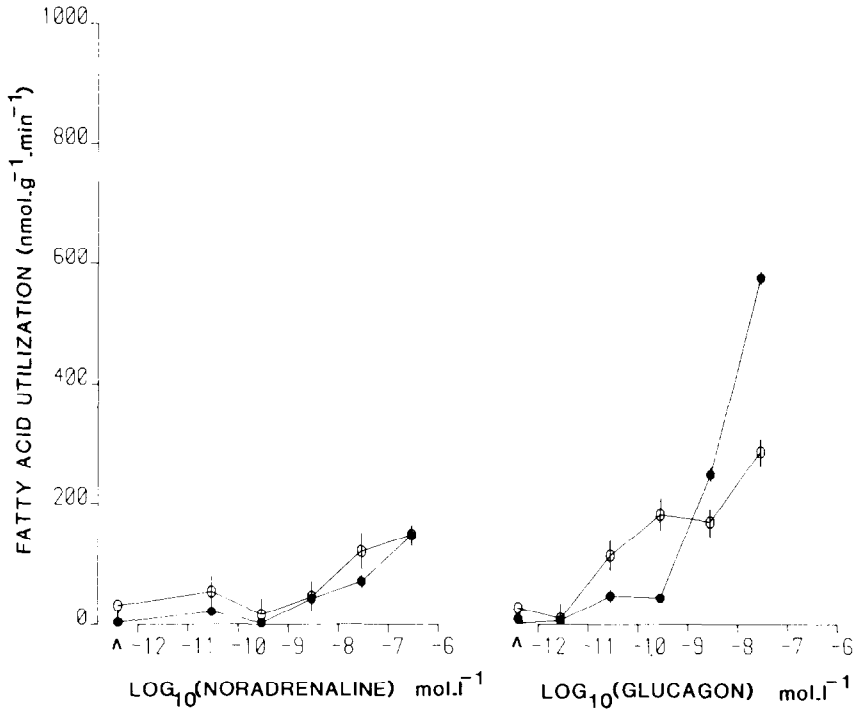

Fig.2. Effects upon fatty acid utilization by IBAT of glucagon and noradrenaline. Data and symbols as in fig.1.

conclusions as to the fate of the additional fatty acid retained in the presence of glucagon. However, for significant levels of re-esterification of liberated FFA to occur, insulin is required [25]. In the present case, tissues from fed animals were incubated with 2.7 mM glucose in the absence of exogenous insulin; it is thus reasonable to assume adequate oxalacetate to support β-oxidation of FFA, and to speculate that this process is enhanced by glucagon at concentrations in excess of 30 pM in WA IBAT, and 3 nM in CA IBAT.

A function of glucagon in IBAT activation may thus be to modulate post-lipolytic events, but the concentrations required in vitro are high. However, in cold exposed rats (60 min at $-5°C$), glucagon in venous plasma rose from 60 to 360 pM [26], at which concentration fatty acid utilization is increased in WA IBAT (fig.2). Also, arginine-stimulated glucagon outputs from the perfused pancreas of the rat, after 2 weeks at 4°C, increase to 3.5 nM during phase I secretion [9]. The present data therefore suggest that glucagon may have a physiological role in BAT, distinct from its lipolytic activity, during cold exposure.

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