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Noradrenaline stimulates glucose transport in rat brown adipocytes by activating thermogenesis

Evidence that fatty acid activation of mitochondrial respiration enhances glucose transport

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The mechanisms by which noradrenaline, lipolytic agents and long-chain fatty acids stimulate glucose transport were investigated in rat brown adipocytes. Glucose transport was evaluated with tracer D-[U-^{14}C]glucose and cell respiration was measured polarographically. Noradrenaline increased basal oxygen consumption (8–10-fold) and glucose transport (4–5-fold) in a dose-dependent manner, with a maximal stimulation at 100 nM. The stimulatory effects of noradrenaline on respiration and glucose transport were selectively mimicked by dibutyryl cyclic AMP (DBcAMP), 3-isobutyl-1-methylxanthine, cholera toxin and physiological concentrations of palmitic acid. Cytochalasin B completely blocked the effects of these agents on glucose transport. The β-adrenergic antagonist propranolol inhibited noradrenaline-induced glucose transport, but did not affect the action of DBcAMP, palmitic acid or cholera toxin on this process. The specific inhibitor of mitochondrial carnitine palmitoyltransferase, 2-tetradecylglycidic acid (McN 3802) (50 μM), inhibited the stimulatory effects of noradrenaline (100 nM) and palmitic acid (0.5 mM) on both glucose transport and mitochondrial respiration. Significantly, McN 3802 failed to affect insulin (1 nM) action under identical experimental conditions. These results demonstrate that (*a*) the stimulatory effects of noradrenaline on brown-adipocyte respiration and glucose transport can be dissociated from those induced by insulin, and (*b*) noradrenaline increases glucose transport indirectly, by activating adenylate cyclase via β-adrenergic pathways and by stimulating mitochondrial oxidation of fatty acids.

INTRODUCTION

In homoeotherms, cold exposure increases glucose turnover, improves glucose tolerance, reverses the deleterious effects of long-term high-fat feeding on insulin-sensitivity, and stimulates glucose uptake in peripheral tissues, particularly in brown adipose tissue (BAT) (for a review see Bukowiecki, 1989). Although long-chain fatty acids represent the main fuel for thermogenesis in BAT (Ma & Foster, 1986; Bukowiecki, 1986), that tissue has a high capacity for glycolysis as well as for lipogenesis from glucose (McCormack & Denton, 1977; Cooney & Newsholme, 1982; Saggerson *et al.*, 1988). We have found that cold exposure (5 °C) may increase glucose uptake in rat BAT by more than 100-fold (Vallerand *et al.*, 1987). Although cold exposure amplifies the stimulation of glucose uptake by insulin, the effects of cold exposure do not appear to require the presence of significant levels of circulating insulin. Indeed, cold exposure significantly increases glucose uptake in starved animals, i.e. in animals in which plasma insulin levels are barely detectable by radioimmunoassay (Shibata *et al.*, 1989). Considering that cold exposure activates non-shivering thermogenesis in BAT by stimulating the release of noradrenaline from sympathetic nerve endings, we tested whether the neurohormone would mimic the effects of cold exposure on glucose utilization in isolated brown adipocytes. We found that noradrenaline, in the absence of insulin, stimulates both glucose transport and utilization and that, in addition, it increases insulin-sensitivity (Marette & Bukowiecki, 1989, 1990). It was therefore decided to determine the mechanism by which noradrenaline increases glucose uptake in isolated brown adipocytes incubated in the absence of extracellular insulin. The principal goal of the present studies was to assess whether noradrenaline acted directly on the brown-adipocyte glucose transport system, or whether it stimulated glucose uptake indirectly by activating the adenylate cyclase system, lipolysis and/or thermogenesis. We examined these possibilities by testing the effects of a series of agents affecting different steps of the sequence of metabolic events leading to increased thermogenesis in BAT. In particular, we analysed the effects of agents that (1) activate both lipolysis and thermogenesis [dibutyryl cyclic AMP (DBcAMP), 3-isobutyl-1-methylxanthine (IBMX), cholera toxin], (2) stimulate thermogenesis but retro-inhibit lipolysis (palmitic acid), and (3) inhibit thermogenesis (McN 3802, a specific inhibitor of carnitine palmitoyltransferase). The results indicate that noradrenaline increases glucose transport indirectly by stimulating lipolysis, long-chain fatty acid oxidation and mitochondrial respiration.

MATERIALS AND METHODS

Materials

Type II collagenase (C-6885), DNAase I (D-0876), fatty-acid-free BSA, (−)-noradrenaline bitartrate, (±)-propranolol hydrochloride, palmitic acid, DBcAMP, IBMX, cholera toxin (from *Vibrio cholerae*) and $HgCl_2$ were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. D-[U-^{14}C]Glucose (348 mCi/mmol) was obtained from ICN Biochemicals, Montréal, P.Q., Canada. Insulin (Iletin II) was obtained from Eli Lilly, Indianapolis, IN, U.S.A. McN 3802 (2-tetradecylglycidic acid) was kindly given by Dr. G. F. Tutwiler, McNeil Co., Spring House, PA, U.S.A.

Isolation of brown adipocytes

Male Sprague–Dawley rats weighing 220–300 g were kept at 25 ± 1 °C in metal cages with a 12 h-light/12 h-dark schedule and were fed on laboratory chow *ad libitum*. The rats were killed by decapitation, and brown adipocytes were isolated from interscapular BAT as previously described (Bukowiecki *et al.*, 1980; Marette & Bukowiecki, 1989). The cells were diluted to a

Abbreviations used: BAT, brown adipose tissue; DBcAMP, dibutyryl cyclic AMP; IBMX, 3-isobutyl-1-methylxanthine.
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concentration of (4–8) × 10^5 cells/ml in Krebs–Ringer bicarbonate buffer (KRB) (gassed with O_2/CO_2, 19:1; pH 7.4) containing fatty-acid-free BSA (40 mg/ml) and glucose (2.7 mM).

Oxygen-consumption measurements

Oxygen uptake by brown-adipocyte suspensions [(1–2) × 10^5 cells/ml] was measured polarographically at 37 °C in a water-jacketed Perspex chamber equipped with a Clark-style oxygen electrode in KRB freshly bubbled with O_2/CO_2 (19:1; pH 7.4) containing glucose (2.7 mM) and 40 mg of fatty-acid-free BSA/ml (4 mg/ml for palmitic acid assay), as previously described (Bukowiecki *et al.*, 1980). Because glucose transport was evaluated in the presence of trace concentrations of radiolabelled glucose (see below), we checked in preliminary experiments whether cell respiration was influenced by the absence of extracellular glucose. It was found that glucose did not affect noradrenaline (100 nM)-stimulated respiration (see Fig. 1), even when the adipocytes were incubated in the absence of glucose for 2 h.

Glucose transport determinations

Glucose transport was evaluated by using D-[U-^{14}C]glucose clearance in isolated cells [(4–8) × 10^5 cells/ml] as previously described (Marette & Bukowiecki, 1989). This method, originally developed for white adipocytes by Kashiwagi *et al.* (1983), is based on the premise that glucose uptake provides a measurement of glucose transport when experiments are carried out at very low glucose concentrations (< 5 μM). The cells were first preincubated with hormones or agents for 20 min in KRB containing BSA (40 mg/ml, or 4 mg/ml for palmitic acid assay). Incubations were started by adding 20 μl of trace D-glucose (final concn. 400 nM) containing 0.02 μCi of D-[U-^{14}C]glucose. The cells were incubated for 8 min. The glucose transport rate was expressed as the glucose clearance rate in $fl \cdot s^{-1} \cdot cell^{-1}$, where clearance = volume of medium × (d.p.m. in cells/d.p.m. in medium). This expression has the advantage of being independent of the tracer concentration, since it is known that the uptake of glucose is limited when the glucose concentration is much lower than the K_m for transport (Foley *et al.*, 1980).

Statistics

The data were analysed by analysis of variance. Significant differences between groups were located by the Fisher PLSD *post hoc* test.

RESULTS

Effects of (−)-noradrenaline, DBcAMP, IBMX and palmitic acid on brown-adipocyte respiration and glucose transport

Addition of noradrenaline, DBcAMP, IBMX and palmitic acid to brown-adipocyte suspensions elicited a dose-dependent increase in oxygen consumption (Fig. 1). Noradrenaline increased cellular respiration approx. 8 times above basal values ($V_{max.}$ = 296 ± 20 nmol of O_2/min per 10^6 cells at 100 nM). The respiratory effects of noradrenaline were mimicked by IBMX, palmitic acid and DBcAMP, although the last two compounds were less efficient than noradrenaline or IBMX in increasing adipocyte respiration (4–6-fold increase above basal values). These data obtained with male Sprague–Dawley rats generally agree with our previous observations made with adipocytes isolated from female Wistar rats (Bukowiecki *et al.*, 1981; Bukowiecki & Collet, 1983).

Dose–response relationships for the effects of noradrenaline, DBcAMP, IBMX and palmitic acid on glucose transport are given in Fig. 2. Noradrenaline increased brown-adipocyte glucose transport 4–5-fold above basal values ($V_{max.}$ = 20.2 ± 2.2 fl/s per cell at 100 nM), whereas the other agents enhanced the basal transport rate 2–4-fold. The order of potency ($V_{max.}$), noradrenaline = IBMX > palmitic acid > DBcAMP, and the order of sensitivity (EC_{50}), noradrenaline ⩾ IBMX > palmitic acid > DBcAMP, were similar for the stimulation of respiration (Fig. 1) and glucose transport (Fig. 2). This provided a first indication that stimulation of respiration and glucose transport are functionally linked, at least at low agonist concentrations. Indeed, noradrenaline, palmitic acid and DBcAMP were less effective for stimulating glucose transport when used at high concentrations, resulting in bell-shaped dose–response curves (concentrations of IBMX higher than 0.5 mM were not tested for reasons of solubility) (Fig. 2; Marette & Bukowiecki, 1989). It

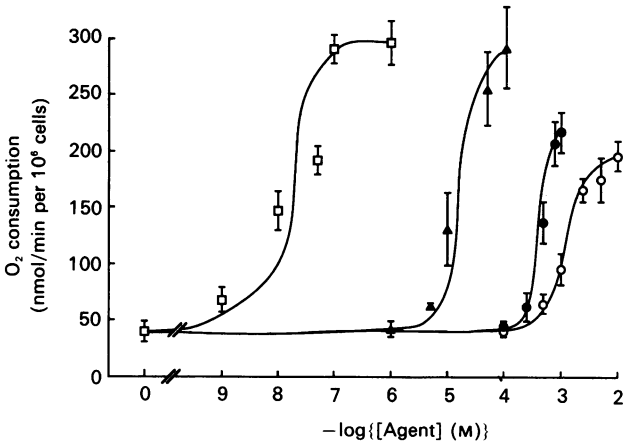

Fig. 1. Dose–response relationships for the effects of noradrenaline (□) DBcAMP (○), IBMX (▲) and palmitic acid (●) on respiration

Adipocytes were isolated from the interscapular BAT and maintained at 37 °C in Krebs–Ringer bicarbonate buffer containing 40 mg of fatty-acid-free BSA/ml (4 mg/ml for palmitic acid assay) and glucose (2.7 mM). Oxygen uptake was measured polarographically by using (1–2) × 10^6 cells/ml exactly as described in the Materials and methods section. Each point represents the mean ± S.E.M. of at least three individual experiments.

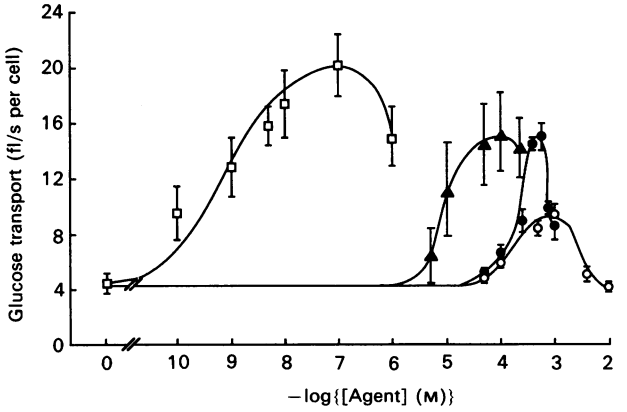

Fig. 2. Dose–response relationships for the effects of noradrenaline (□), DBcAMP (○), IBMX (▲) and palmitic acid (●) on glucose transport

Adipocytes were isolated from interscapular BAT and maintained at 37 °C in Krebs–Ringer bicarbonate buffer containing 40 mg of fatty-acid-free BSA/ml (4 mg/ml for palmitic acid assay) and labelled D-glucose. Glucose transport was determined by using (4–8) × 10^6 cells/ml, exactly as described in the Materials and methods section. Each point represents the mean ± S.E.M. of three to five separate experiments performed in triplicate.

should be emphasized that such bell-shaped curves were not seen for the stimulation of respiration (Fig. 1). This indicates that fatty acids modulate glucose transport via at least two different mechanisms and that the stimulatory mechanism predominates at low concentrations.

We previously found that insulin stimulates glucose uptake in brown adipocytes at the same concentrations (0.1–10 nM) as it inhibits the thermogenic effects of noradrenaline (Marette & Bukowiecki, 1989). In the present studies, we confirmed these observations and found that insulin did not modify the effects of DBcAMP, IBMX or palmitic acid on respiration (dose–response curves were similar to those shown in Fig. 1). This suggests that insulin inhibits the thermogenic effects of noradrenaline at an early step of the stimulus–thermogenesis sequence. It is unlikely that this metabolic step is associated with glucose metabolism itself. Indeed, preliminary experiments showed that (*a*) insulin did not affect basal adipocyte respiration in the presence of extracellular glucose, and (*b*) preincubation of adipocytes in the absence of glucose for 2 h did not modify basal or agonist-stimulated respiration.

Other control experiments (results not shown) revealed that (*a*) oleic acid was as effective as palmitic acid for enhancing respiration and sugar transport, (*b*) supra-maximal doses of noradrenaline (> 1 μM) added alone or in combination with IBMX, palmitic acid or DBcAMP failed to increase further respiration and (*c*) noradrenaline and DBcAMP stimulated sugar uptake in the same range of concentration whether D-[U-^{14}C]glucose or 2-[^{3}H]-deoxyglucose were used (see Marette & Bukowiecki, 1989).

Effect of β-adrenergic blockade on the stimulatory effect of noradrenaline, DBcAMP and palmitic acid on glucose transport

We previously demonstrated that long-chain fatty acids stimulate brown-adipocyte respiration even in the presence of an excess of propranolol, i.e. when endogenous cyclic AMP production is inhibited (Bukowiecki *et al.*, 1981). Addition of propranolol (1 μM) totally inhibited the stimulatory effects of noradrenaline (0.1 μM) on glucose transport without affecting the action of DBcAMP and palmitic acid (result not shown). This strongly suggests that palmitic acid alters glucose transport at post-receptor, post-cyclic AMP, levels.

Effects of cholera toxin on brown-adipocyte respiration and glucose transport

Cholera toxin is known to activate adenylate cyclase by catalysing the ADP-ribosylation of membrane proteins (G_s proteins) (Cassel & Pfeuffer, 1978). Incubation of brown adipocytes with the toxin for 90 min increased basal respiration 5-fold (Fig. 3). Other experiments (results not shown) revealed that cholera toxin had no acute effects on respiration. Addition of noradrenaline (100 nM) to cells treated with cholera toxin further enhanced oxygen consumption to levels observed with noradrenaline alone (Fig. 3). Subsequent addition of propranolol (1 μM) to the respiratory chamber completely blocked the stimulatory effect of noradrenaline (0.1 μM), without affecting cholera-toxin-stimulated respiration. Control experiments revealed that propranolol also failed to inhibit the stimulatory effects of cholera toxin in the absence of noradrenaline.

Cholera toxin, when used under the same incubation conditions as for the respiratory measurements, enhanced brown-adipocyte glucose transport 2-fold [mean ± S.E.M.; 6.42 ± 0.33 versus 11.55 ± 0.90 fl/s per cell ($P < 0.01$) for basal and toxin-stimulated cells respectively]. As expected, propranolol (1 μM) did not block the stimulatory effect of the toxin either on respiration or on glucose transport (results not shown).

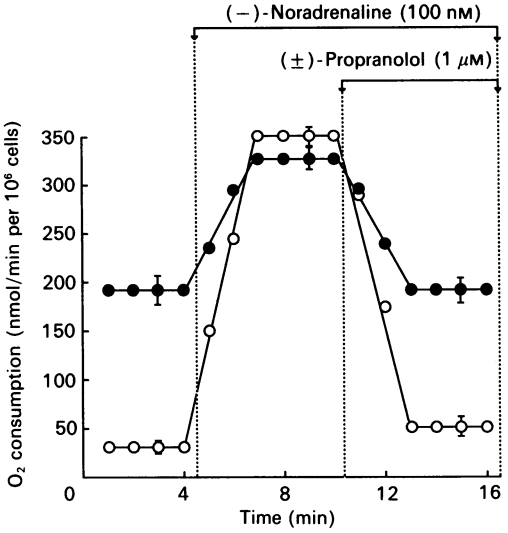

Fig. 3. Effect of cholera toxin on brown-adipocyte respiration

Adipocytes were preincubated either with the toxin (●; 500 ng/ml) or with the buffer (○) for 90 min before being incorporated in the respiratory chamber. Oxygen uptake was measured polarographically exactly as described in the Materials and methods section. Values represent means of two individual experiments performed in duplicate.

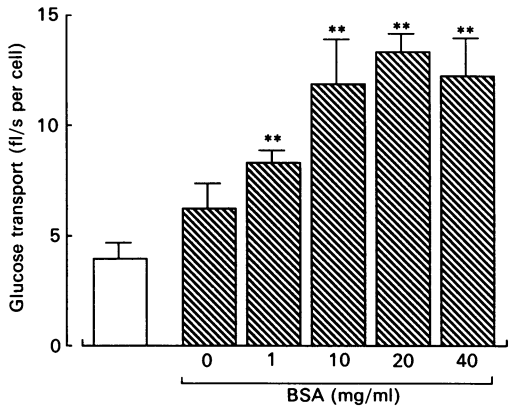

Fig. 4. Effect of BSA concentration on noradrenaline-stimulated glucose transport

Brown adipocytes were maintained in buffer containing 40 mg of BSA/ml before the experiments. The cells were then washed and incubated with noradrenaline and different concentrations of BSA. Glucose transport was measured exactly as described in Fig. 2. Each point represents the mean ± S.E.M. of four to five individual experiments performed in triplicate. Key: □, basal; ▨, (−)-noradrenaline (100 nM).

Modulation of noradrenaline stimulation of glucose transport by BSA

To examine further the critical role of fatty acids on noradrenaline-stimulated glucose transport, the effect of BSA (a potent fatty-acid-binding protein) was investigated over a range of concentrations of 0–4% (Fig. 4). In the absence of BSA, noradrenaline (100 nM) failed to stimulate glucose transport significantly. The addition of only 1 mg of BSA/ml (0.1%) in the incubation medium restored the stimulatory effect of noradrenaline. Maximal stimulation was obtained for BSA levels higher than 10 mg/ml (1%). These results suggest that extracellular fatty acid accumulation retro-inhibits glucose transport. It should be mentioned that (*a*) BSA had no significant effect on basal glucose transport, and (*b*) the levels of fatty acids released from isolated brown adipocytes are relatively low and

Table 1. Effect of McN 3802 on brown-adipocyte glucose transport

Brown adipocytes were preincubated for 20 min in the presence of agents or buffer with or without McN 3802. Glucose transport was measured exactly as described in the Materials and methods section. Values represent means ± S.E.M. for the numbers of individual experiments shown in parentheses. The symbols *($P < 0.01$) and †($P < 0.01$) represent significant differences from control or agent-stimulated values respectively.

Addition	McN 3802 (50 μM)	Glucose transport (fl/s per cell)
No addition	−	4.46 ± 0.29 (6)
Insulin (1 nM)	−	12.32 ± 1.03 (5)*
Insulin (1 nM)	+	10.75 ± 1.35 (5)*
No addition	−	4.46 ± 0.29 (6)
Noradrenaline (0.1 μM)	−	15.20 ± 2.76 (5)*
Noradrenaline (0.1 μM)	+	7.89 ± 1.35 (5)†
No addition	−	5.93 ± 0.80 (6)
Palmitic acid (0.5 mM)	−	17.60 ± 1.70 (6)*
Palmitic acid (0.5 mM)	+	5.30 ± 0.55 (3)†

Table 2. Effect of cytochalasin B on brown-adipocyte glucose transport

Brown adipocytes were preincubated for 20 min in the presence of agents or buffer with or without cytochalasin B. Glucose transport was measured exactly as described in the Materials and methods section. Values represent means ± S.E.M. for the numbers of separate experiments shown in parentheses. The symbols *($P < 0.01$) and †($P < 0.01$) represent significant differences from control or agent-stimulated values respectively.

Addition	Cytochalasin B (10 μM)	Glucose transport (fl/s per cell)
No addition	−	4.46 ± 0.73 (3)
Insulin (10 nM)	−	15.20 ± 1.85 (3)*
Insulin (10 nM)	+	3.74 ± 0.06 (3)†
No addition	−	4.40 ± 0.85 (5)
Noradrenaline (0.1 μM)	−	10.96 ± 1.01 (5)*
Noradrenaline (0.1 μM)	+	1.63 ± 0.56 (4)†
No addition	−	5.93 ± 0.80 (6)
Palmitic acid (0.5 mM)	−	17.60 ± 1.70 (6)*
Palmitic acid (0.5 mM)	+	3.15 ± 1.60 (3)

do not influence noradrenaline-stimulated respiration in the presence of various BSA concentrations (Bukowiecki *et al.*, 1981). Thus the effects of BSA on glucose transport seen in the present experiments cannot be explained by changes in the respiratory response to noradrenaline.

Effect of McN 3802 and cytochalasin B on (−)-noradrenaline-, palmitic acid- and insulin-stimulated glucose transport

To test whether the stimulatory effects of noradrenaline on glucose transport were consequent to mitochondrial stimulation of fatty acid oxidation, we analysed the effects of a specific inhibitor of mitochondrial carnitine palmitoyltransferase, 2-tetradecylglycidic acid (McN 3802) (Tutwiler & Dellevigne, 1979; Bukowiecki *et al.*, 1981). Incubation of brown adipocytes with McN 3802 (50 μM) effectively blocked the stimulatory effects of noradrenaline (0.1 μM) or palmitic acid (0.5 mM) on glucose transport without affecting the action of insulin (1 nM) (Table 1). This observation demonstrates that the stimulatory effects of noradrenaline on brown-adipocyte respiration and glucose transport can be dissociated from those induced by insulin. It also shows that McN 3802 did not antagonize the action of noradrenaline via unspecific deleterious effects to the cell, but rather by inhibiting the activation of respiration by fatty acids released during the activation of lipolysis.

On the other hand, the stimulatory effects of noradrenaline, palmitic acid and insulin on glucose transport were all abolished by addition of cytocholasin B to the incubation medium (Table 2). The fungal metabolite also inhibited the enhancing effects of IBMX and DBcAMP on glucose transport (results not shown). Thus, although the mechanisms by which insulin and noradrenaline affect glucose transport are different, the effects of these (neuro-)hormones are mediated by cytochalasin-B-sensitive glucose transporters. Whether the transporters mobilized by insulin are similar to those activated by noradrenaline (and fatty acids) remains to be determined.

DISCUSSION

Previous studies *in vivo* have demonstrated that glucose uptake is significantly increased in BAT when non-shivering thermogenesis is stimulated in that tissue by cold exposure or by noradrenaline administration at room temperature (Cooney *et al.*, 1985; Ma & Foster, 1986; Smith *et al.*, 1986; Greco-Perotto *et al.*, 1987; Vallerand *et al.*, 1987; Shibata *et al.*, 1989). The present experiments add further evidence obtained *in vitro* for the hypothesis that there is a tight coupling between the triggering of heat production in BAT and the enhancement of glucose transport. Indeed, all agents that enhanced thermogenesis in brown adipocytes (noradrenaline, DBcAMP, IBMX, palmitic acid, cholera toxin) also stimulated glucose transport. Likewise, all agents that inhibited the thermogenic effects of noradrenaline (propranolol and McN 3802) or palmitic acid (McN 3802) also abolished the stimulatory action of noradrenaline and palmitic acid on glucose transport. However, in two situations, there was an apparent dissociation between increased thermogenesis and increased glucose transport. First, at 0.1 nM-noradrenaline, glucose uptake was significantly augmented ($P < 0.05$) (Fig. 2), but thermogenesis was not enhanced (stimulation started at 1 nM) (Fig. 1). This may indicate that noradrenaline stimulates glucose transport via at least two metabolic pathways, dependent and independent of the activation of thermogenesis. Alternatively, it may suggest that the method used for detecting changes in intracellular glucose transport is more sensitive than the polarographic measurements of extracellular oxygen concentration. Secondly, when brown adipocytes are incubated in the presence of noradrenaline (10 nM) and in the absence of BSA, thermogenesis is maximally stimulated (Bukowiecki *et al.*, 1981), but basal glucose transport is not significantly enhanced (Fig. 4). A likely explanation for this observation is that fatty acids released when lipolysis is maximally stimulated by noradrenaline (100 nM) accumulate in the extracellular medium (Bukowiecki *et al.*, 1981) and retro-inhibit glucose transport. BSA (1–4 mg/ml) reverses this inhibition by binding most of the extracellular fatty acids (Fig. 4). This interpretation is also supported by the fact that palmitic acid, added in the presence of BSA (4 mg/ml), is more effective in stimulating glucose transport at low (0.1–0.6 mM) than at high (0.6–1 mM) concentrations (Fig. 2). A similar bell-shaped dose–response curve has been observed not only for palmitic acid, but also for lipolytic agents such as noradrenaline, DBcAMP and IBMX (Fig. 2, and Marette & Bukowiecki, 1989). It therefore appears that fatty acids modulate glucose transport via at least two mechanisms: (1) they stimulate glucose uptake at low concentrations by enhancing mitochondrial respiration (an effect inhibitable by McN 3802), and (2) they inhibit that parameter if allowed to accumulate in the extracellular medium (an effect that may be reversed by BSA).

High concentrations of fatty acids might decrease glucose transport by inhibiting glucose oxidation (Elks, 1990) and/or by uncoupling oxidative phosphorylation, and consequently decreasing ATP levels (glucose transport is an ATP-dependent process). However, the contribution of the latter mechanism to the inhibition of glucose transport is uncertain, since agonist concentrations that inhibited glucose uptake (Fig. 2) did not affect respiration (Fig. 1). Similarly to glucose uptake, respiration is critically dependent on ATP that is required for fatty acid activation. It has been reported that supramaximal concentrations of noradrenaline or fatty acids (concentrations that totally uncouple oxidative phosphorylation) decrease ATP levels by only 30–50% (Williamson, 1970). It therefore appears that substrate-level phosphorylation plays an important role in supplying ATP for energy-requiring processes such as fatty acid activation, glucose transport etc.

The fact that propranolol and McN 3802 failed to affect insulin action on glucose transport (Marette & Bukowiecki, 1989; the present study) demonstrates that the metabolic pathways by which insulin and noradrenaline modulate glucose transport are different, at least in part. Insulin does not stimulate thermogenesis in isolated brown adipocytes; on the contrary, it partly inhibits the thermogenic effects of noradrenaline (Marette & Bukowiecki, 1989). Thus the mechanism by which insulin affects glucose transport appears to be unrelated to the activation of thermogenesis. However, we have previously demonstrated that there is a metabolic interaction between the effects of insulin and noradrenaline on glucose transport, as noradrenaline markedly increases the sensitivity of brown adipocytes to the stimulatory effects of insulin (Marette & Bukowiecki, 1989). Therefore, both mechanisms can operate simultaneously for increasing glucose transport (Marette & Bukowiecki, 1989) and metabolism (Isler *et al.*, 1987; Ebner *et al.*, 1987; Saggerson *et al.*, 1988).

The precise mechanisms responsible for this thermogenesis-dependent increase in glucose transport are still unknown, but they probably involve alterations in the properties of glucose transporters. Indeed, Greco-Perotto *et al.* (1987) have found that cold exposure stimulates glucose utilization in BAT and concurrently increases the translocation of glucose transporters from a microsomal pool to the plasma membrane. In addition, cold exposure appears to increase the affinity of the transporters for glucose. A similar phenomenon has been observed by various groups for the effects of exercise on glucose uptake in skeletal muscle (Hirshman *et al.*, 1988; Fushiki *et al.*, 1989; Douen *et al.*, 1989; Sternlicht *et al.*, 1989). Considering that ATP depletion activates the red-blood-cell glucose transporters (Carruthers, 1986; Hebert & Carruthers, 1986), it is possible that the ATP/ADP ratio, which decreases in noradrenaline-stimulated brown adipocytes (Williamson, 1970), controls the activity of glucose transporters in BAT. However, other metabolites such as glucose 6-phosphate might also control the intrinsic activity of glucose transporters (Foley & Hueckstead, 1984).

The finding that cholera toxin markedly stimulates oxygen consumption and glucose transport in brown adipocytes (Fig. 3, and the Results section) agrees with the observation that the G_s-protein transduction system is present in BAT (Granneman & Bannon, 1989). It has also been reported that chronic treatment of brown preadipocytes in culture with cholera toxin promotes their differentiation and enhances the expression of the genes coding for cytochrome *c* oxidase and lipoprotein lipase (Herron *et al.*, 1989). It therefore appears that the G_s proteins play a major role in the regulation of BAT metabolism.

Joost & Steinfelder (1985) reported that fatty acids enhance glucose transport in white adipose cells by increasing the affinity of hexose transporters for glucose. More recently, Thode *et al.* (1989) observed that palmitic acid increases glucose incorporation in white adipocytes by mechanisms involving intracellular Ca^{2+} translocation. In the present experiments with brown adipocytes, 2-tetradecylglycidic acid significantly inhibited palmitic acid enhancement of glucose transport (Table 1). Considering that acylcarnitine derivatives of long-chain fatty acids cannot be considered as uncoupling messengers (Nicholls & Locke, 1983), it is unlikely that McN 3802 blocked the effects of palmitic acid on glucose transport by inhibiting the formation of these metabolites. Furthermore, McN 3802 did not alter insulin stimulation of glucose transport, as would be expected if the drug displayed non-specific side effects (Table 1). The results strongly indicate that fatty acids need to be oxidized to increase glucose transport. To our knowledge, the effect of McN 3802 on palmitic acid-induced glucose uptake in white adipocytes has not yet been studied.

In summary, the present data demonstrate that: (1) the stimulatory effects of noradrenaline and insulin on glucose transport in BAT can be dissociated, by using propranolol or McN 3802, from those induced by insulin; (2) the effects of noradrenaline are mimicked by agents that increase intracellular cAMP levels and/or thermogenesis (DBcAMP, IBMX, cholera toxin, palmitic acid); (3) stimulation of thermogenesis is usually associated with stimulation of glucose transport; and (4) inhibition of thermogenesis by McN 3802 results in an inhibition of glucose transport. It is therefore concluded that noradrenaline increases glucose transport indirectly by activating adenylate cyclase via β-adrenergic pathways and by stimulating mitochondrial oxidation of fatty acids.

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