Alpha-1 Adrenergic Stimulation of Glucose Uptake in Rat White Adipocytes

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ABSTRACT
We recently demonstrated that adipocyte lactate production depends on alpha-1 adrenergic control and that adipocytes can produce lactate even when insulin-stimulated glucose uptake is markedly impaired. This prompted us to investigate the glucose uptake in response to an alpha-1 adrenergic stimulation. We measured the adrenergic regulation of glucose uptake by adipocytes isolated from epididymal white adipose tissue using agonists (norepinephrine, phenylephrine and isoproterenol) and antagonists (prazosin and propranolol) of alpha-1 and beta adrenoceptor subtypes. Our results show that the maximal glucose uptake obtained in the presence of 10^{-8} M norepinephrine is partially inhibited by prazosin (10^{-6} M, 57%) or propranolol (10^{-6} M, 52%) suggesting that glucose uptake is subjected to both alpha-1 and beta regulation. Indeed, our findings show that glucose uptake is dose-dependently increased by phenylephrine. This stimulation is totally inhibited by prazosin (10^{-6} M). Isoproterenol stimulated glucose uptake. The stimulation of glucose uptake by isoproterenol is totally inhibited in the presence of propranolol (10^{-6} M) in the incubation medium. Our results demonstrate for the first time that alpha-1 adrenergic subtype is involved in the regulation of glucose uptake by white adipocytes.

The adrenergic stimulation of glucose uptake by white adipose cells has been described both in vitro and in vivo in previous studies. Ludvigsen et al. (1980) reported that epinephrine stimulated glucose uptake by isolated white adipocytes. More recently, Liu et al. (1994) showed a stimulated glucose uptake in epididymal adipose tissue in rats perfused with increasing doses of norepinephrine. Nevertheless, these results have drawn little attention to characterization of the adrenergic stimulation of glucose uptake into the alpha or beta effect. Ludvigsen et al. (1980), using the 3-O-methyl glucose transport, suggested that the effect of epinephrine was mediated in large part by beta receptors and that there may be a small alpha component of the stimulation by epinephrine. Furthermore, Kashiwagi and Foley (1982) showed that glucose uptake was under beta adrenergic control in human isolated adipocytes.

Recent studies by our group have brought new insights on the role of alpha-1 adrenoceptor in adipocyte metabolism. Indeed, we recently demonstrated that rat adipocytes can produce lactate independent of lipolysis in insulin-resistant rats (Faintrenie and Géloën, 1996a) and that lactate production depends on alpha-1 adrenergic control (Faintrenie and Géloën, 1996b). These experimental conditions raised the question of the origin of lactate produced by insulin-resistant adipose cells in which insulin hardly stimulates glucose uptake. This prompted us to study glucose uptake in response to alpha-1 adrenergic stimulation.

We studied the adrenergic regulation of glucose uptake in white adipocytes from rat using agonists (norepinephrine, phenylephrine and isoproterenol) and antagonists (prazosin and propranolol) of alpha-1 and beta adrenoceptor subtypes. Our results show that norepinephrine significantly stimulates glucose uptake only at 10^{-8} M. Phenylephrine increases glucose uptake dose-dependently, whereas isoproterenol induces a maximal augmentation at 10^{-10} M; beyond this dose, glucose uptake decreases but remains significantly higher than basal glucose uptake. Maximal glucose uptakes obtained in the presence of phenylephrine (10^{-7} M) and isoproterenol (10^{-10} M) are totally inhibited, respectively, in the presence of prazosin (10^{-6} M) and propranolol (10^{-6} M). These results demonstrate for the first time a significant role of alpha-1 adrenoceptors in the stimulation of glucose uptake by white adipose cells.

Materials and Methods
Male Sprague-Dawley rats (Iffa-Credo, l’Arbresle, France) weighing 300 to 350 g were kept on a 12:12 hr light/dark cycle (lights on

ABBREVIATIONS: KRB buffer, Krebs-Ringer bicarbonate buffer (25 mM HEPES and 6 mM glucose, pH 7.4; BSA, bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
Isolation of adipocytes. Adipocytes were isolated from 2.0 g of epididymal fat by a modification of Rodbell’s original procedure (1964). Adipose tissues were weighed, minced and digested in a 20-ml polyethylene vial containing 8 ml of KRB buffer with 1 mg/ml collagenase (type 2, Clostridium histolyticum), 1% BSA (fraction 5, fatty acid free) and 200 nM adenosine. The vial was shaken at 80 cycles/min at 37°C. The resulting cell suspension was filtered through a nylon mesh (200 μm) and washed three times with 5 ml of fresh KRB buffer and 1% BSA and two times with the same buffer containing 4% BSA. Then, adipocytes were resuspended in KRB buffer and 4% BSA. A sample of the final cellular suspension was counted in a hemocytometer after staining with trypan blue.

Glucose transport assays. The uptake of 2-deoxyglucose by white adipocytes was measured by filtration assay. An aliquot of 300000 cells was preincubated in polyethylene vials containing fresh KRB buffer and 4% BSA with or without different concentrations of adrenergic agonists or antagonists (i.e., norepinephrine, isoproterenol, phenylephrine, prazosin or propranolol). The final volume was 1 ml. Adipocytes were incubated with gentle shaking (50 cycles/min) at 37°C for 30 min. KRB buffer (30 μl) containing 0.2 μCi of 2-[1,2-3H]deoxyglucose (CEA SA; Gif sur Yvette, France, 17 Ci/mmol) and 0.02 μCi of [14C]sucrose (Desitek SA, Orsay, France, 13.4 GBq/mmol) was then rapidly added. After 3 min of incubation, the flux of glucose was stopped by the addition of 5 ml of cold KRB buffer.

The cell suspension was immediately filtered onto a cellulose nitrate filter (8 μm, Sartorius), held by a filtration apparatus (Millipore). The filters were immediately washed with 5 ml and then 2.5 ml of cold KRB solution. The filters were put into scintillation vials containing 4% BSA. Then, adipocytes were resuspended in KRB buffer and 4% BSA. A sample of the final cellular suspension was counted in a hemocytometer after staining with trypan blue.

Statistical analysis. Results are presented as mean ± S.E.M. Statistical significance was tested with a analysis of variance (ANOVA) followed by Fisher’s protected least significant difference test. Differences between mean values were accepted as significant at P < .05.

Results

 Effects of norepinephrine on glucose-uptake by white adipocytes. Norepinephrine significantly increased glucose uptake only at 10⁻⁶ M compared with basal glucose uptake (1.86 ± 0.13 vs. 0.38 ± 0.02 nmol 2DG/300000 cell at 3 min, respectively; P < .05, fig. 1A). Beyond this concentration, glucose uptake returned to basal values.

In another series of experiments, we tested the effects of alpha and beta adrenergic receptor antagonists. Propranolol (10⁻⁶ M), a beta adrenergic antagonist, partially inhibited glucose uptake to 52% of the maximal (i.e., 10⁻⁸ M norepinephrine-stimulated) glucose uptake (0.77 ± 0.02 vs. 1.49 ± 0.10 nmol 2DG/300000 cell at 3 min for norepinephrine + propranolol-stimulated glucose uptake and norepinephrine-stimulated glucose uptake; P < .05). Prazosin, an alpha-1 adrenergic antagonist, inhibited glucose uptake to 57% of norepinephrine-stimulated glucose uptake (0.85 ± 0.04 vs. 1.49 ± 0.10 nmol 2DG/300000 cell at 3 min, respectively; fig. 1B).

Effects of isoproterenol on glucose-uptake by white adipocytes. Isoproterenol, a beta adrenergic agonist, significantly increased glucose uptake compared with basal values. Maximal glucose uptake was obtained in the presence of 10⁻¹⁰ M isoproterenol (1.49 ± 0.12 nmol 2DG/300000 cell at 3 min). Beyond this maximal response, the glucose uptake decreased to ~50% of the maximal response but remained significantly different than basal glucose uptake (0.84 ± 0.04, 0.72 ± 0.04 and 0.69 ± 0.04 nmol 2DG/300000 cell at 3 min for 10⁻⁹, 10⁻⁸ and 10⁻⁷ M isoproterenol vs. 0.43 ± 0.02 for basal glucose uptake; P < .05, fig. 2A).

Prazosin at 10⁻⁶ M had no effect on maximal isoproterenol-stimulated glucose uptake [i.e., in the presence of 10⁻¹⁰ M isoproterenol; 1.37 ± 0.18 vs. 1.44 ± 0.19 nmol 2DG/300000 cell at 3 min for prazosin + isoproterenol (10⁻¹⁰ M) and isoproterenol (10⁻¹⁰ M), P = N.S.]. On the other hand, glucose uptake measured in response to isoproterenol (10⁻¹⁰ M) + prazosin (10⁻⁶ M) was not significantly different from glucose uptake obtained under basal conditions (0.45 ± 0.03 vs. 0.44 ± 0.02 nmol 2DG/300000 cell at 3 min, respectively; P = N.S.). These results show that prazosin totally inhibit the effect of 10⁻¹⁰ M isoproterenol (fig. 2B).

Effects of phenylephrine on glucose uptake by white adipocytes. Glucose uptake increased dose-dependently with phenylephrine concentrations (fig. 3A). The highest dose of phenylephrine, an alpha-1 adrenergic agonist, at a maximal dose (10⁻⁶ M) induced a 2.5-fold increase of basal glucose uptake in epididymal adipocytes (1.09 ± 0.03 vs. 0.44 ± 0.03 nmol 2DG/300000 cell at 3 min, respectively; P < .05).
The effect of the maximal concentration of phenylephrine (10^{-7} M) was totally inhibited by 10^{-6} M prazosin. Indeed, in the presence of prazosin, glucose uptake returned to basal values (0.55 ± 0.03 vs. 0.44 ± 0.02 nmol 2DG/300000 cell at 3 min, respectively; P = N.S.). However, propranolol had no effect on phenylephrine-stimulated glucose uptake [1.11 ± 0.03 vs. 1.01 ± 0.03 nmol 2DG/300000 cell at 3 min for phenylephrine + propranolol- and phenylephrine (10^{-7} M)-stimulated glucose uptake; P = N.S., fig. 3B].

Discussion

The main result of the present study is that phenylephrine dose-dependently stimulates glucose uptake (fig. 3A). The effects of phenylephrine were totally inhibited by 10^{-6} M prazosin (fig. 3B), whereas propranolol had no effect on phenylephrine-induced glucose uptake. These findings show undoubtedly that the specific stimulation of alpha-1 adrenergic receptors results in the stimulation of glucose uptake on epididymal adipocytes.

In the present study, norepinephrine and isoproterenol dose-response curves are biphasic (figs. 1A and 2A), confirming the stimulatory effect of low concentrations of catecholamines (Ludvigsen et al., 1980; Kashiwagi and Foley, 1982) and the inhibitory effect of high concentrations of catecholamines (Taylor et al., 1976) on glucose uptake. Such a biphasic shape has been reported in vitro for the dose-response curves of glucose uptake by brown adipocytes (Marette and Bukowiecki, 1989) and in vivo for glucose uptake by epididymal white adipose tissue (Liu et al., 1994).

It is not easy to explain why the stimulation at the highest concentrations of norepinephrine does not stimulate glucose uptake. One explanation could be the elevation of intracellular cAMP concentration. Indeed, several studies have shown that cAMP inhibits glucose transport in adipocytes (Taylor et al., 1976; Kashiwagi and Foley, 1982). An inverse correlation between rates of glucose transport and intracellular cAMP concentration has been reported (Faintrenie and Géloën, 1996a, 1996b), suggesting that intracellular cAMP could be not significantly increased.

Alpha-1 adrenoceptors are not linked to the adenylate cyclase system and do not increase intracellular cAMP concentration. This may explain why glucose uptake is dose-dependently stimulated in response to phenylephrine. The binding of alpha-1 agonists activates phospholipase C, which releases inositol-1,4,5-phosphate to the cytosol, leaving diacylglycerol...
within the membrane. The primary function of inositol-1,4,5-phosphate is to mobilize calcium from intracellular stores (Berridge, 1987). It has been suggested that inositol-1,4,5-phosphate acts by opening the calcium channel (Smith et al., 1985) through the binding to a specific receptor that may be connected to a calcium channel of the endoplasmic reticulum.

In conclusion, our results show for the first time that a significant part of norepinephrine-glucose uptake is mediated through activation of alpha-1 adrenergic receptors. The study of possible modifications of the expression and activity of alpha-1 adrenergic receptors during the establishment of insulin resistance is needed to determine the role of these receptor subtypes in the onset of obesity and non-insulin-dependent diabetes.

References


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