Adrenergic receptor stimulation attenuates insulin-stimulated glucose uptake in 3T3-L1 adipocytes by inhibiting GLUT4 translocation


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Submitted 19 February 2004; accepted in final form 23 May 2005

The sympathetic nervous system plays an important role in the regulation of metabolic processes. For instance, acute activation of the sympathetic nervous system results in a reduced ability of insulin to stimulate tissue glucose uptake (insulin resistance). Orthostatic stress and acute noradrenergic activation produce insulin resistance in forearm skeletal muscle (9, 21), as does mental stress in patients with type 1 diabetes (23). Several conditions, like hypertension, obesity, and aging, are associated with increased sympathetic nervous system activity (22, 24, 31). Interestingly, these conditions are also associated with insulin resistance (2, 4), and it is tempting to link insulin resistance to the chronically increased sympathetic nervous system activity. However, how sympathetic activity influences insulin sensitivity is unknown. Possibly, activation of the adrenergic receptors inhibits insulin-stimulated glucose uptake at the cellular level.

At least nine subtypes of adrenergic receptors have been identified, including three α1-, three α2-, and three β-adrenergic receptors (26, 29). In vitro studies demonstrate that activation of β-adrenergic receptors attenuates insulin-stimulated glucose uptake at the cellular level. The β-adrenergic receptor agonist isoproterenol attenuates insulin-stimulated glucose uptake in isolated rat adipocytes (11, 14, 15, 38), and β-adrenergic stimulation in brown adipose tissue has the same effect (13, 16). The inhibitory effect of catecholamines on insulin-stimulated glucose uptake is not only found in adipose tissue, but is also observed in rat skeletal muscle cells (3, 8, 19).

Several mechanisms have been proposed by which β-adrenergic receptor stimulation may affect insulin-stimulated glucose uptake. Smith et al. (34) found that isoproterenol inhibits insulin-induced GLUT4 translocation to the plasma membrane in rat adipocytes. Others reported that both isoproterenol and norepinephrine attenuate the binding of insulin to its receptor, which probably results in a less stimulatory signal and, as a consequence, in a reduced increase in the rate of glucose uptake (15). However, it is reported that, despite a reduced rate of glucose uptake, the number of glucose transporters at the plasma membrane was not different between insulin-stimulated rat adipocytes incubated with or without isoproterenol (10–12, 38). On the basis of these results, it has been proposed that stimulation of β-adrenergic receptors does not affect insulin-mediated translocation of GLUT4 but modifies the intrinsic activity of glucose transporters (11). In rat skeletal muscle, the inhibitory effect of epinephrine on insulin-stimulated glucose uptake was not observed when the glucose analog 3-O-methylglucose was used (19). Accumulation of 3-O-methylglucose in the cell is, in contrast to accumulation of 2-deoxyglucose (2-DOG), not dependent on phosphorylation by hexokinase. This suggests that epinephrine affects the hexokinase activity rather than lowering insulin-stimulated GLUT4 translocation to the plasma membrane.

As such, various mechanisms have been described to explain the inhibitory effect of adrenergic receptor stimulation on the...
insulin-signaling pathway. However, it is still unknown which adrenergic receptor subtypes are involved. In this study we show that, in 3T3-L1 adipocytes, catecholamines attenuate insulin-stimulated glucose uptake by signaling through different β-adrenergic receptor subtypes. In addition, the reduced insulin-stimulated glucose uptake in catecholine-treated cells can be explained by a reduced translocation of GLUT4.

MATERIALS AND METHODS

Materials. DMEM was purchased from ICN Biomedicals (Aurora, OH), and FCS was purchased from PPA Laboratories (Linz, Austria). All other culture reagents were purchased from GIBCO-BRL (Paisley, Scotland). BSA, IBMX, 2-DOG, goat anti-rabbit IgG peroxidase conjugate, epinephrine bitartrate, norepinephrine bitartrate, isoproterenol bitartrate, adenosine, propanolol, metoprolol, ICI-118551 and SR-59230A were obtained from Sigma (St. Louis, MO). Phenolamine was purchased from Novartis Pharma (Arnhem, The Netherlands). Recombinant human insulin was from Eli Lilly (Nieuwegein, The Netherlands), and [1-3H]-2DOG (spec. act. 481 GBq/mmol) was purchased from Amersham Life Sciences (Amersham, UK). GLUT1 and GLUT4 antibodies were provided by Prof. Dr. A. Schürmann, Dept. of Pharmacology, German Institute of Human Nutrition (Potsdam-Rehbrücke, Germany). All other chemicals were of analytical grade.

Adipogenesis of 3T3-L1 adipocytes. The 3T3-L1 cells (American Type Culture Collection, Manassas, VA) were seeded in T75 flasks and grown to confluence in culture medium composed of DMEM with 10% (vol/vol) FCS, 10 U/ml penicillin, 10 μg/ml streptomycin, and 0.2% (wt/vol) BSA, composed of DMEM with 10% (vol/vol) FCS, 10 U/ml penicillin, 10 μg/ml streptomycin, and 0.2% (wt/vol) BSA, and 0.5 μg/ml amphotericin B for 10 days in a humidified atmosphere of 5% CO2 at 37°C. Subsequently, cells were differentiated into mature adipocytes by incubating them with culture medium supplemented with insulin (1.7 μM), IBMX (1 μM), and dexamethasone (25 μM) for 2 days. After incubation in culture medium with insulin (1.7 μM) for an additional 6 days, the adipocytes were maintained in culture medium and used for experiments within 7–11 days. At the time of the experiments, >90% of the cells had accumulated lipid droplets.

Glucose transport assay. Fully differentiated 3T3-L1 adipocytes, subcultured in 12-well plates, were incubated in DMEM without FCS for 3 h. Subsequently, cells were washed twice with PBS and incubated at 37°C for 45 min in reaction buffer containing 138 mM NaCl, 1.85 mM CaCl2, 1.3 mM MgSO4, 4.8 mM KCl, 0.2% (wt/vol) BSA, IBMX (1 μM), and 1.85 mM CaCl2, 1.3 mM MgSO4, 4.8 mM KCl, 0.2% (wt/vol) BSA, IBMX (1 μM), and 1.5 mM 2-DOG. After 10 min of incubation, 2-DOG uptake was determined by addition of 250 μl of reaction buffer with the indicated different drugs. 27.8 kBq [1-3H]-2DOG and 1.5 mM 2-DOG. After 10 min of incubation, 2-DOG uptake was terminated by washing the cells three times with ice-cold PBS containing 10 mM glucose. Subsequently, cells were lysed in 1% (wt/vol) SDS and 0.2 M NaOH. Incorporation radioactivity was measured by liquid scintillation spectrometry.

Preparation of cell fractions. Differentiated 3T3-L1 adipocytes, grown in T75 flasks, were incubated in DMEM without FCS for 2 h. Subsequently, cells were washed twice with PBS and incubated for 20 min in reaction buffer, as described in the previous section, containing insulin (0.1 μM) with or without epinephrine (1 μM) or norepinephrine (1 μM). Cell fractions were prepared as described previously. After a wash with PBS, adipocytes from a T75 flask were scraped in ice-cold HEPES-EDTA-sucrose (HES) buffer containing 5 mM EDTA, 250 mM sucrose, 1 tablel/15 ml complete miniprotease inhibitor cocktail tablet, and 10 mM HEPES adjusted to pH 7.4. Adipocytes from two flasks were pooled and homogenized at 4°C using a Potter-Braun (Type S) homogenizer (glass-teflon) with 15 strokes (manually). All subsequent procedures were performed at 4°C. The homogenate was centrifuged for 15 min at 8,000 g. The pellet was resuspended (25 strokes in a 1-ml glass-glass potter) in 1 ml of HES buffer and layered on a 1.12 M sucrose cushion. After a 60-min centrifugation at 100,000 g in a swing-out rotor, the top layer was taken off. To this top layer HES buffer was added until the volume was 10 ml, and the mixture was centrifuged for another 60 min at 100,000 g to pellet the plasma membrane (PM) fraction. The supernatant from the first centrifugation (15 min at 8,000 g) step was centrifuged for 20 min at 41,000 g. To pellet the low-density membrane (LDM) fraction, the resulting supernatant was centrifuged for 75 min at 180,000 g. The PM and LDM fractions were resuspended in 200 μl of HES buffer, and total protein content was determined (BCA; Pierce, Rockford, IL). Finally, the fractions were stored at −80°C until further use.

Western blot analysis of GLUT1 and GLUT4. Proteins (20 μg) from the different fractions, yields as described above, were precipitated with 5% trichloroacetic acid, and after 10 min incubation on ice the fractions were centrifuged for 10 min at 16,000 g. The pellets were resuspended in sample buffer to give a final protein concentration of 2 mg/ml. Subsequently, samples were subjected to SDS-PAGE, after which the proteins were transferred to nitrocellulose membranes (Amersham Life Sciences, Amersham, UK) by Western blotting. Membranes were washed twice with PBS and blocked for 2 h at room temperature in TBS (Tris-buffered saline) buffer containing 0.1% (vol/vol) Tween-20, 150 mM NaCl, and 50 mM Tris adjusted to pH 7.4. Subsequently, membranes were incubated for 1 h at room temperature in TBS buffer containing rabbit glucose transporter-isotype-specific antibodies (32), diluted 1:2,000 for the detection of GLUT1, and diluted 1:5,000 for the detection of GLUT4. After being washed three times with TBS buffer, the membranes were incubated for 1 h at room temperature in TBS buffer containing goat anti-rabbit IgG antibodies conjugated to horseradish peroxidase (1:1,000). After being washed three times with TBS buffer, bands were visualized using enhanced chemiluminescence (Amersham). The bands were scanned using the documentation and analysis system Alphalager 1220 (Biozym, Landgraaf, The Netherlands), and the integrated density values were calculated with the computer program AlphaEase v5.1 (Biozym).

Statistical analysis. The results of 2-DOG uptake experiments are presented as means ± SE, and statistical analysis was performed using unpaired Student’s t-tests or univariate analysis of variance (ANOVA) as appropriate. Integrated density values quantifying the amount of glucose transporters (western blot experiments) were presented as median ± interquartile range. Statistical analysis of GLUT1 and GLUT4 translocation experiments was performed using Mann-Whitney tests, as data were not normally distributed. P values < 0.05 were considered significant.

RESULTS

Effect of epinephrine on insulin-stimulated 2-DOG uptake. 3T3-L1 adipocytes were incubated with increasing concentrations of insulin (ranging from 0.001 to 1 μM) in the absence or presence of epinephrine (1 μM). Addition of 0.1 μM insulin stimulated the rate of 2-DOG uptake more than sevenfold from 1.01 ± 0.17 to 7.76 ± 0.39 nmol·10 min−1·well−1. Epinephrine did not affect the rate of glucose uptake in unstimulated cells but significantly decreased the rate of glucose uptake in
insulin-stimulated cells (Fig. 1). In the presence of epinephrine, 0.1 μM insulin augmented the rate of glucose uptake with 4.10 ± 0.60 nmol 2-DOG·10 min⁻¹·well⁻¹, which is a reduction of 39% compared with the rate of glucose uptake in insulin-stimulated cells not treated with epinephrine. Identical results were obtained using 1 μM norepinephrine instead of epinephrine (data not shown). In all subsequent experiments, insulin was used at a concentration of 0.1 μM.

**Effect of adenosine on insulin-stimulated 2-DOG uptake.** 3T3-L1 adipocytes were incubated with insulin (0.01 and 0.1 μM) in the presence or absence of adenosine (1 μM). Addition of 0.01 or 0.1 μM insulin stimulated the rate of 2-DOG uptake more than tenfold. Addition of adenosine had no effect on basal or insulin-stimulated 2-DOG uptake (Fig. 2).

**Effect of α- and β-adrenergic receptor antagonists on catecholamine-induced inhibition of insulin-stimulated 2-DOG uptake.** 3T3-L1 adipocytes were treated with either 10 μM phentolamine (α-adrenergic receptor antagonist) or 0.3 μM propranolol (β-adrenergic receptor antagonist) before addition of 0.1 μM insulin and/or 1.0 μM epinephrine. Neither antagonist had any effect on the basal or the insulin-stimulated rate of glucose uptake of cells not exposed to epinephrine (Fig. 3). Propranolol, but not phentolamine, prevented the inhibitory effect of epinephrine on insulin-stimulated 2-DOG uptake. In experiments with norepinephrine instead of epinephrine, the responses were similar (data not shown).

Next, the basal and the insulin-stimulated rates of glucose uptake were examined in the presence of the β-adrenergic agonist isoproterenol (1 μM). Figure 4 shows that isoproterenol did not affect the basal rate of glucose uptake but significantly reduced insulin-stimulated glucose uptake from 6.39 ± 0.34 to 4.88 ± 0.32 nmol 2-DOG·10 min⁻¹·well⁻¹. Similar to epinephrine and norepinephrine, the inhibitory effect of isoproterenol on insulin-stimulated glucose uptake was abolished by propranolol.

**Effect of selective β-adrenergic receptor subtype antagonists on epinephrine-mediated inhibition of insulin-stimulated 2-DOG uptake.** 3T3-L1 adipocytes were treated with metoprolol (β₁-adrenoceptor antagonist, 1 μM), ICI-118551 (β₂-adrenoceptor antagonist, 1 μM), or SR-59230A (β₃-adrenoceptor antagonist, 1 μM) alone or in combination before addition of insulin and/or epinephrine. Incubation of cells with the above-mentioned antagonists did not affect the basal rate of glucose uptake (data not shown). Incubation with either metoprolol, ICI-118551, or SR-59230A did not counteract the effect of

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**Fig. 1.** Effect of epinephrine on insulin-stimulated 2-deoxyglucose (2-DOG) uptake. 3T3-L1 adipocytes were incubated with different concentrations of insulin (0.01 and 0.1 μM) for 10 min in absence (○) or presence (●) of 1 μM epinephrine (n = 4). Epinephrine significantly decreased insulin-stimulated glucose uptake. *P < 0.05, ANOVA.

**Fig. 2.** Effect of adenosine on insulin-stimulated 2-DOG uptake. 3T3-L1 adipocytes were incubated with insulin (0.01 and 0.1 μM) in the presence or absence of adenosine (1 μM). Addition of 0.01 or 0.1 μM insulin stimulated the rate of 2-DOG uptake more than tenfold. Addition of adenosine had no effect on basal or insulin-stimulated 2-DOG uptake (Fig. 2).

**Fig. 3.** Effect of α- and β-adrenergic receptor antagonists on catecholamine-induced inhibition of insulin-stimulated 2-DOG uptake. 3T3-L1 adipocytes were preincubated without (open bars) or with (closed bars) the α-adrenergic receptor antagonist phentolamine (10 μM; A) or the β-adrenergic receptor antagonist propranolol (0.3 μM; B). After 10 min of preincubation, insulin (ins, 0.1 μM) and/or epinephrine (epi, 1 μM) were added. Epinephrine significantly decreased insulin-stimulated glucose uptake. The inhibitory effect of epinephrine was abolished by propranolol but not by phentolamine. Results were based on ≥5 independent experiments. *P < 0.05, **P < 0.01. NS, not significant.

**Fig. 4.** Effect of selective β-adrenergic receptor subtype antagonists on epinephrine-mediated inhibition of insulin-stimulated 2-DOG uptake. 3T3-L1 adipocytes were treated with metoprolol (β₁-adrenoceptor antagonist, 1 μM), ICI-118551 (β₂-adrenoceptor antagonist, 1 μM), or SR-59230A (β₃-adrenoceptor antagonist, 1 μM) alone or in combination before addition of insulin and/or epinephrine. Incubation of cells with the above-mentioned antagonists did not affect the basal rate of glucose uptake (data not shown). Incubation with either metoprolol, ICI-118551, or SR-59230A did not counteract the effect of
Epinephrine on insulin-stimulated glucose uptake (Table 1). When cells were incubated simultaneously with ICI-118551 and SR-59230A, the inhibitory effect of epinephrine on insulin-stimulated glucose uptake was abolished. Similarly, simultaneous addition of metoprolol, ICI-118551, and SR-59230A prevented epinephrine-induced inhibition of insulin-stimulated glucose uptake.

Effect of epinephrine and norepinephrine on insulin-stimulated GLUT4 and GLUT1 translocation to the PM. The PM fraction was separated from the intracellular vesicles (LDM fraction) of untreated and insulin-treated cells. Insulin (0.1 μM) increased the amount of GLUT4 in the PM fraction and decreased the amount of GLUT4 in the LDM fraction (Fig. 5). Although less profoundly, insulin also increased GLUT1 in the PM fraction and decreased GLUT1 in the LDM fraction. Next, we examined the effect of catecholamines on insulin-stimulated GLUT4 and GLUT1 translocation. The amount of GLUT4 in the PM fraction was profoundly less when the adipocytes were stimulated with insulin in the presence of epinephrine or norepinephrine, and more GLUT4 was retained in the LDM fraction (Fig. 6, left lane). Norepinephrine, and not epinephrine, lowered the amount of GLUT1 in the PM and increased the amount of GLUT1 in the LDM fraction (Fig. 6, right lane).

**DISCUSSION**

In this study, we investigated how adrenergic receptor stimulation affects insulin-mediated glucose disposal in 3T3-L1 adipocytes. We found that the insulin-stimulated glucose uptake could be reduced in the presence of epinephrine, norepinephrine, and isoproterenol. This reduction was established by stimulation of multiple β-adrenergic receptor subtypes. Both epinephrine and norepinephrine reduced the amount of GLUT4 at the plasma membrane of insulin-stimulated adipocytes. Taken together, the results of the present study show that β-adrenergic receptor activation attenuates insulin-stimulated glucose uptake by decreasing translocation of GLUT4.

Insulin-stimulated glucose uptake at concentrations of 0.01, 0.1, and 1 μM. At these concentrations, epinephrine (1 μM) and norepinephrine (1 μM) nearly halved the stimulatory effect of insulin. Treatment of adipocytes with a lower catecholamine concentration (0.1 μM) did not reduce insulin-stimulated glucose uptake (data not shown). The inhibitory effect of epinephrine on insulin-stimulated glucose uptake was abolished by propranolol. Results were based on 9 independent experiments. *P < 0.05, **P < 0.01.

**Table 1. 2-DOG uptake with/without incubation with selective β-adrenergic receptor antagonists**

<table>
<thead>
<tr>
<th></th>
<th>Ins</th>
<th>Ins + Antag</th>
<th>Ins + Epi</th>
<th>Ins + Epi + Antag</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met</td>
<td>6.89±0.88</td>
<td>6.51±1.22</td>
<td>4.73±0.92</td>
<td>4.97±1.12</td>
<td>11.1</td>
</tr>
<tr>
<td>ICI</td>
<td>6.05±0.49</td>
<td>5.36±0.51</td>
<td>3.98±0.44</td>
<td>4.06±0.42</td>
<td>3.9</td>
</tr>
<tr>
<td>SR</td>
<td>6.40±0.47</td>
<td>5.74±0.59</td>
<td>4.42±0.48</td>
<td>5.11±0.37</td>
<td>34.8</td>
</tr>
<tr>
<td>Met + ICI</td>
<td>6.22±0.42</td>
<td>6.19±0.26</td>
<td>3.57±0.35</td>
<td>4.50±0.57</td>
<td>35.1</td>
</tr>
<tr>
<td>Met + SR</td>
<td>6.34±0.76</td>
<td>6.13±0.97</td>
<td>3.39±0.39</td>
<td>5.16±0.78</td>
<td>60.0</td>
</tr>
<tr>
<td>ICI + SR</td>
<td>6.34±0.76</td>
<td>5.49±0.84</td>
<td>3.39±0.39</td>
<td>5.73±0.56*</td>
<td>79.3</td>
</tr>
<tr>
<td>Met + ICI + SR</td>
<td>6.44±0.72</td>
<td>5.69±0.89</td>
<td>3.66±0.49</td>
<td>5.76±0.57*</td>
<td>75.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. 3T3-L1 adipocytes were preincubated with receptor antagonists (Antag) of different β-adrenergic receptor subtypes or with a combination of these antagonists (β1-antagonist: metoprolol (Met, 1 μM); β2-antagonist: ICI-118551 (ICI, 1 μM); β3-antagonist: SR-59230A (SR, 1 μM)). After 10 min of preincubation, insulin (Ins, 0.1 μM) and epinephrine (Epi, 1 μM) were added. Administration of epinephrine significantly reduced insulin-stimulated glucose uptake in all experiments. 2-Deoxyglucose (2-DOG) uptake is expressed as nmol·10 min⁻¹·well⁻¹ (means ± SE). Results are based on 3 independent experiments. In the right column, the difference between Ins + Epi + Antag and Ins + Epi is expressed as %total inhibition by epinephrine (calculated as the difference between Ins and Ins + Epi). *P < 0.05, Ins + Epi + Antag vs. Ins + Epi.
Adenosine stimulates the G₁-coupled receptor and increases glucose uptake, abolishing the inhibitory effect of isoproterenol. The inhibiting effect of isoproterenol on glucose transport can be restored by adding adenosine deaminase (10, 17). It is unknown to what extent adenosine is present in the incubation medium of 3T3-L1 adipocytes cultured in 12-well plates. We expect that the role of adenosine production in our experiments can at best be minor, because the culture medium was replaced two times a week during the adipogenesis and 24 h, 3 h, and 45 min before the experiment to prevent accumulation of metabolites in the medium. In addition, at the start of the experiments, the agents were added together with fresh reaction buffer. As such, we anticipated that the concentration of adenosine in our medium was very low. Because the stimulatory effect of adenosine has been demonstrated only in rat adipocytes, we tested the effect of adenosine in 3T3-L1 adipocytes and found that adenosine (1 μM) had no additional effect on basal or insulin-stimulated 2-DOG uptake in 3T3-L1 adipocytes. Because the experiments were not performed in the presence of adenosine deaminase, we cannot definitely exclude an effect of minor concentrations of adenosine on the inhibitory effect of epinephrine on insulin-stimulated glucose uptake. Nevertheless, we can state that adrenergic receptor stimulation inhibits insulin-stimulated glucose uptake in 3T3-L1 adipocytes. If adenosine indeed was present in our experiments, the inhibition of insulin-stimulated glucose uptake we measured would underestimate the maximum effect of epinephrine. However, we are unable to compare these data in 3T3-L1 adipocytes in detail with the previous data obtained in rat adipocytes.

The inhibitory effect of epinephrine and norepinephrine on insulin-mediated glucose transport in 3T3-L1 adipocytes could be completely abrogated by the β-adrenergic antagonist propranolol. This indicates that catecholamines act via β-adrenergic receptors to attenuate insulin signaling. In agreement with this observation is the inhibitory effect of the β-adrenergic receptor agonist isoproterenol on insulin-stimulated glucose uptake, which was also abolished by propranolol. The observation that catecholamines attenuate insulin-stimulated glucose uptake via the β-adrenergic receptors is observed in other models from either adipogenic (5, 14) or muscular (6, 7, 19, 35) origin. There are no reports that catecholamines attenuate insulin signaling through α-adrenergic receptors, and in agreement with this is our observation that phentolamine did not prevent the reduction in insulin-stimulated glucose uptake by epinephrine and norepinephrine. It should be noted, however, that there are no reports that demonstrate the presence of this type of adrenergic receptors in 3T3-L1 adipocytes.

The presence of all three subtypes of β-adrenergic receptors has been demonstrated in 3T3-L1 adipocytes (20). We used selective β₁-, β₂-, and β₃-antagonists to assess which β-adrenergic receptor subtypes are involved in the inhibitory effect of catecholamines on insulin-stimulated glucose uptake. Incubation insulin-stimulated 3T3-L1 adipocytes with metoprolol, ICI-118551, or SR-59230A (1 μM) did not prevent the inhibitory effect of epinephrine. This indicates that epinephrine can inhibit the stimulatory effect of insulin by more than one β-adrenoceptor subtype. Simultaneous inhibition of β₁- and β₂-adrenoceptors did not prevent the inhibitory effect of epinephrine in insulin-stimulated 3T3-L1 adipocytes, demonstrating the importance of the β₃-adrenoceptor in the inhibitory...
effect of catecholamines. Because a combination of β1- and β3-antagonists could not counteract the inhibitory effect of epinephrine, the β2-adrenoceptor also seems to be involved. Inhibition of both β2- and β3-adrenoceptors at the same time abolished the effect of epinephrine, as did simultaneous inhibition of all three receptor subtypes. Dose-response curves of selective β1-, β2-, and β3-antagonists showed that higher concentrations (>1 μM) partially inhibited the effect of epinephrine, suggesting that at these concentrations selectivity is lost (data not shown). We concluded that epinephrine inhibits insulin-stimulated glucose uptake in 3T3-L1 adipocytes at least by the β2- and the β3-adrenoceptor. The involvement of the β1-adrenoceptor is less likely.

Next, we examined whether a hampered translocation of GLUT4, the main insulin-sensitive glucose transporter, from intracellular vesicles to the plasma membrane could explain the reduced insulin-stimulated glucose uptake upon β-adrenergic receptor stimulation. Insulin profoundly stimulated the translocation of GLUT4, and to a lesser extent GLUT1, from intracellular vesicles (LDM fraction) to the plasma membrane. In the presence of epinephrine, however, the amount of GLUT4 at the plasma membrane in the insulin-stimulated adipocytes was remarkably reduced. It appeared that the GLUT4 transporter was retained in the intracellular vesicles, as its amount was increased in the LDM fraction in cells exposed to catecholamines and insulin. The same results were obtained when norepinephrine was used instead of epinephrine. These results are in line with previous reports showing that isoproterenol inhibits insulin-stimulated glucose uptake in rat adipocytes, at least partially, by inhibiting GLUT4 translocation from the intracellular pool to the plasma membrane (34). In contrast, other investigators reported that, in insulin-treated rat adipocytes, the translocation of GLUT4 was not decreased following treatment with catecholamines (10, 12, 17). It was suggested that phosphorylation of the COOH terminus of GLUT4 by cAMP decreased the intrinsic activity of the glucose transporter (10, 18). However, more recent studies prove that phosphorylation of GLUT4 is not involved in the regulation of the intrinsic activity of the glucose transporter (27, 28). An alternative possibility is that insulin-stimulated GLUT4 can exist in two distinct states within the plasma membrane: one, which is functional and accessible to extracellular substrate, and one that is nonfunctional and unable to bind substrate (38). The discrepancy between our study, in which we report a catecholamine-induced decrease in insulin-stimulated GLUT4 translocation, and the aforementioned studies that did not find an effect of catecholamines on GLUT4 translocation, may be based on the fact that we studied mouse 3T3-L1 adipocytes, whereas the other studies used rat adipocytes. Another possible explanation for the discrepancy between our study and the other studies involves the sequence of addition of the various hormones. In our study and in the previously mentioned study of Smith et al. (34), insulin and catecholamines were added simultaneously, whereas in the studies that did not find an effect of catecholamines on GLUT4 translocation insulin was added before the catecholamines (10, 12, 17).

In our study, the decrease in GLUT4 translocation exceeded the decrease in 2-DG transport capacity induced by epinephrine and norepinephrine. A possible explanation for this discrepancy is that GLUT1, which is not affected by epinephrine and only slightly affected by norepinephrine, significantly contributes to 2-DG transport in these cells. Unfortunately, there is no adequate technique available to determine exactly the relative concentrations of GLUT1 and GLUT4 or their relative contribution to overall glucose transport.

How catecholamines attenuate insulin-stimulated GLUT4 translocation is unknown. Our results suggest that the β-adrenergic receptor subtypes share a pathway that is able to attenuate insulin-stimulated glucose uptake, for example the cAMP-protein kinase A (PKA) pathway. Several reports demonstrated that nonselective cAMP analogs in 3T3-L1 adipocytes and rat adipocytes inhibit insulin-stimulated glucose uptake (14, 37, 39). However, in insulin-stimulated rat adipocytes, glucose transport is inhibited by isoproterenol in a cAMP-PKA-independent manner (11, 17). Moreover, stimulation of PKA with 5-aminoimidazole-4-carboxamide-ribonucleoside stimulates rather than inhibits glucose uptake in 3T3-L1 adipocytes (30). In muscle cells, the inhibition of insulin-stimulated glucose transport by epinephrine was associated with a strong reduction in PI 3-kinase activation (6–8). It is well recognized that PI 3-kinase activity is of crucial importance in the metabolic actions of insulin (33). Possibly, catecholamines, through a yet unidentified pathway, lower the phosphatidylinositol (PI) 3-kinase activity in 3T3-L1 adipocytes (34). In muscle cells, the inhibition of hexokinase activity by accumulation of glucose-6-phosphate released from glycogenolysis presumably is not of concern in adipocytes. This is in agreement with the results of experiments showing that insulin-stimulated 3-O-methylglucose uptake is also inhibited by catecholamines in 3T3-L1 adipocytes (data not shown). Because accumulation of 3-O-methylglucose in the cell is, in contrast to accumulation of 2-DG, independent of phosphorylation by hexokinase, the inhibitory effect of catecholamines on 3-O-methylglucose uptake is not the result of an inhibition of hexokinase activity.

In conclusion, these results show that, in 3T3-L1 adipocytes, β-adrenergic receptor stimulation attenuates insulin-induced glucose uptake by inhibiting GLUT4 translocation to the plasma membrane. We also found that epinephrine can inhibit insulin-stimulated glucose uptake via different β-adrenergic receptor subtypes: at least the β2- and the β3-adrenergic receptors are involved; the role of the β1-adrenoceptor remains unclear. To our knowledge, we are the first to report that catecholamines affect insulin-stimulated glucose uptake in 3T3-L1 adipocytes by more than one β-adrenoceptor subtype by interfering with GLUT4 translocation from intracellular vesicles to the plasma membrane. The question of how β-adrenergic receptor stimulation interferes with the insulin-signaling cascade deserves further investigation.

ACKNOWLEDGMENTS

We thank Helga van Rennes (Dept. of Chemical Endocrinology, Radboud University Nijmegen Medical Centre) for culturing the 3T3-L1 adipocytes, and we thank Sjaak Willemsen (Dept. of Chemical Endocrinology, Radboud University Nijmegen Medical Centre) for preparation of the catecholamine solutions. We gratefully thank Prof. Dr. A. Schirrmann (Dept. of Pharmacology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany) for providing the GLUT1 and GLUT4 antibodies. We owe much gratitude to M. Ouwens (Dept. of Molecular Cell Biology, Leiden University Medical Centre,
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Leiden, the Netherlands) for help and advice and for providing us with the 3T3-L1 adipocytes used in the adenosine experiments.

GRANTS

C. J. Tack is a recipient of a clinical fellowship of the Dutch Diabetes Foundation (no. 2000.01.004).

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