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The cGMP-inhibitable phosphodiesterase modulates glucose transport activation by insulin

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Abstract

To assess the role of the cGMP-inhibitable phosphodiesterase (cGI-PDE) in the action of insulin on glucose transport, adipocytes from young, lean rats were preincubated for 20 min at 37°C with and without OPC 3911, a specific inhibitor of cGI-PDE, and 3-*O*-methylglucose uptake was measured. Insulin-stimulated glucose transport was impaired by OPC 3911 (~ 15%) and this impairment became more pronounced in the presence of the degradable cAMP-analogue 8-bromo-cAMP (~ 45%). This analogue alone did not significantly decrease glucose transport. Furthermore, insulin sensitivity was impaired by the combination of OPC 3911 and 8-bromo-cAMP. Maximal insulin-stimulated glucose transport in adipocytes from aging, obese rats was affected similarly by OPC 3911 and 8-bromo-cAMP, suggesting that cGI-PDE activity is not markedly altered in this insulin-resistant state. In conclusion, cGI-PDE exerts a modulating effect on the stimulatory action of insulin on glucose transport. This effect is particularly pronounced when the cellular cAMP levels are elevated.

Key words: Insulin; Insulin receptor; Glucose transport; Phosphodiesterase; cyclic AMP; OPC 3911; Obesity; (Rat adipocyte)

1. Introduction

Insulin stimulates transmembrane glucose transport in different cell types [1]. In adipocytes, cardiac and skeletal muscle this occurs through the translocation of glucose transporters, predominantly the GLUT 4 isoform, from an intracellular pool to the plasma membrane [2–5]. Insulin also seems to stimulate the insertion and/or activation of the glucose transporting proteins in the plasma membrane [6,7]. However, the signalling pathway between the insulin receptor and glucose transporter translocation and activation is incompletely characterized.

The membrane-bound, cGMP-inhibitable low-K_m phosphodiesterase (cGI-PDE), which promotes hydrolysis of 3′-5′-cyclic adenosine monophosphate (cAMP), plays a critical role in insulin's antilipolytic action [8–10]. This enzyme is probably activated by insulin through serine phosphorylation [9,10]. It is also activated by the cAMP-stimulated protein kinase A (PrkA) which, thus, exerts a negative feedback control of the cellular cAMP levels [10].

cAMP is known to counteract several effects of insulin including the stimulation of glucose transport and IGF-II receptor binding [11–13] and the recently reported ability of insulin to unmask its own receptors at the cell surface [14,15]. Thus, normal insulin action is dependent on low cellular cAMP levels as was also suggested long time ago [16,17]. The present study was undertaken to elucidate whether lowering of the cAMP levels through cGI-PDE activation is of importance for the effect of insulin on glucose transport. To assess this mechanism, rat adipocytes were incubated with insulin in the presence or absence of the allegedly specific cGI-PDE inhibitor OPC 3911 [18,19]. Furthermore, two different cAMP analogues were used; 8-bromo-cAMP, which is readily hydrolysed by cGI-PDE and N^6-monobutyryl-cAMP which is resistant to enzymatic degradation [20].

2. Materials and methods

2.1. Materials

Porcine monocomponent insulin and mono [^{125}I]Tyr A_{14}-insulin (spec. act. 200–300 μCi/μg) were pur-

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chased from Novo Nordisk (Copenhagen, Denmark). 3-*O*-[^{14}C]methyl-D-glucose (spec. act. 59 μCi/μmol) was from Amersham (Buckinghamshire, UK). OPC 3911 was generously supplied by Dr. P. Belfrage (University of Lund, Sweden). Collagenase, BSA (fraction V) and the cAMP analogues, 8-bromo cAMP and N^6-monobutyryl-cAMP (N^6-mbcAMP), were from Sigma (St. Louis, MO). Adenosine deaminase (ADA) and N^6-(*R*-phenylisopropyl)adenosine (PIA) were obtained from Boehringer-Mannheim (Mannheim, Germany) and medium 199 from Statens Bakteriologiska Laboratorium (Stockholm, Sweden).

2.2. Cell preparation and incubation conditions

Male Sprague-Dawley rats, fed ad libitum, were stunned and decapitated at a weight of 150–200 g (lean rats used unless otherwise specified) or 350–450 g (obese). The epididymal fat pads were immediately excised and minced. Fat cells were isolated in medium 199 with 1 mg/ml collagenase and 40 mg/ml BSA. The cells were then filtered through a nylon mesh and washed four times with fresh medium. The preincubations were performed in medium 199, lipocrit 5–10%, at 37°C for 20 min in the presence of 1 U/ml ADA (to remove endogenously released adenosine), glucose (2.8 mM for glucose transport and 5.6 mM for insulin binding) and the indicated agents.

2.3. 3-O-[^{14}C]Methyl-D-glucose transport

This assay was performed as previously described (21). Briefly, aliquots of the cell suspension were pulsed with 3-*O*-[^{14}C]methyl-D-glucose (50 μM, 1.0 μCi) and 3-*O*-methylglucose uptake was stopped after 5 or 10 s (for cells preincubated with and without insulin, respectively) through the addition of 0.3 mM ice-cold phloretin. Cell-associated radioactivity was measured and remaining, extracellularly trapped 3-*O*-methylglucose subtracted (i.e., cell-associated radioactivity found when tracer was added after phloretin).

2.4. ^{125}I-Insulin binding to adipocytes

After the preincubations, the cells were energy depleted with 2 mM KCN for 5 min before the binding assays as previously reported [14,15]. To remove insulin and other agents that could interfere with binding, the cells were then washed four times with medium 199 containing 10 mg/ml BSA and 2 mM KCN. This procedure is sufficient to dissociate >90% of the bound insulin [14]. Aliquots of cells and medium were transferred to 16°C and 0.2 ng/ml ^{125}I-insulin was added. After 2 h, when steady state was clearly established, specific ^{125}I-insulin binding was measured as cell-associated radioactivity after the subtraction of non-specific binding (in the presence of 3.5 μg/ml unlabeled insulin).

2.5. Statistics

Statistical significance of differences was tested with Student's two-tailed *t*-test for paired data. Results are means ± S.E. unless otherwise indicated.

3. Results

3.1. Effects of cAMP and cGI-PDE inhibition on glucose transport – lean animals

The combination of 10 μM OPC 3911, a concentration which completely inhibits the antilipolytic effect of insulin (not shown), with 8-bromo-cAMP produced a modest elevation of the 'basal' transport rate in the absence of insulin (Fig. 1). This stimulation was also seen with the non-hydrolysable cAMP analogue N^6-mbcAMP (4 mM) alone (~30% increase, $n = 6$, $P < 0.05$, not shown). The maximally insulin-stimulated glucose transport was slightly reduced (~15%) by OPC 3911 but this inhibition became much more pronounced (~45%) when OPC 3911 was combined with either 8-bromo-cAMP (Fig. 1) or N^6-mbcAMP ($n = 4$, $P < 0.05$). 8-bromo-cAMP alone did not significantly alter maximal glucose transport rate (Fig. 1), whereas

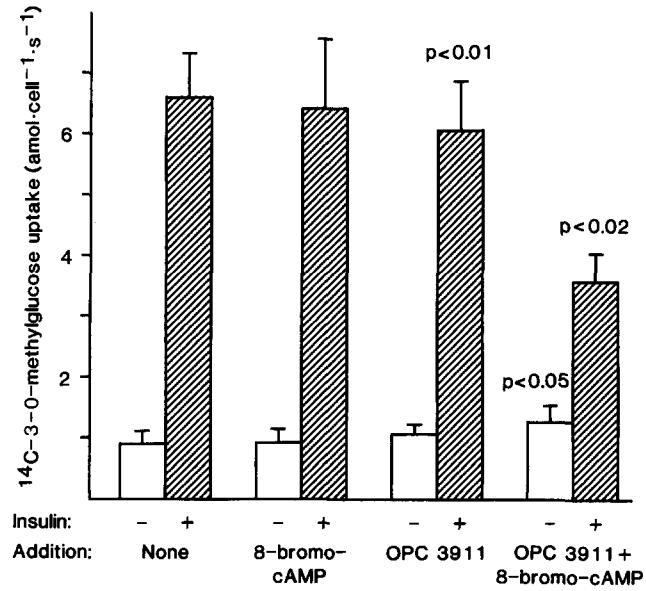

Fig. 1. Effects of insulin, cAMP analogues and OPC 3911 on 3-*O*-[^{14}C]methyl-D-glucose uptake in rat adipocytes. Cells were preincubated at 37°C for 20 min with or without insulin (1000 μU/ml), 8-bromo cAMP (4 mM) and OPC 3911 (10 μM) as indicated. 3-*O*-[^{14}C]methyl-D-glucose uptake was measured as described in Materials and methods. Data are means ± S.E. of 5–7 separate experiments and *P*-values refer to comparisons with the corresponding control group (with or without insulin, respectively).

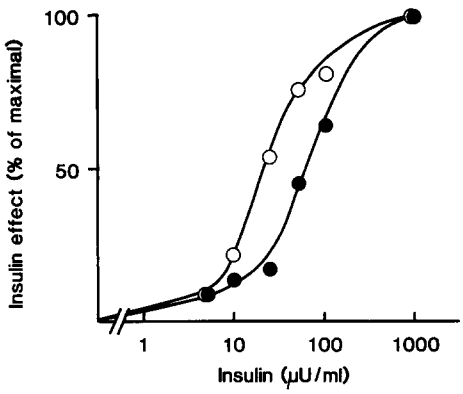

Fig. 2. Dose-response relationship for the effect of insulin on 3-*O*-[^{14}C]methyl-D-glucose uptake in rat adipocytes. Cells were preincubated as in Fig. 1 with the indicated concentrations of insulin alone (○) or in the presence of both 8-bromo-cAMP (4 mM) and OPC 3911 (10 μM; ●) and 3-*O*-[^{14}C]methyl-D-glucose uptake was assessed. Data are from one representative experiment repeated five times and are expressed as percent of maximal insulin effect.

N^6-mbcAMP produced an ~20% decrease ($n = 6$, $P < 0.001$). In some experiments the non-metabolizable adenosine analogue PIA (1 μM) was added to achieve a maximal adenosine effect in order to minimize adenylyl cyclase activity and, hence, cAMP levels. Since ADA was also present, endogenously formed adenosine was degraded and, thus, a defined effect of the analogue could be achieved. Again, the maximally insulin-stimulated glucose transport rate was decreased by 15–20% by OPC 3911 ($n = 4$, $P < 0.01$).

The dose-response relationship for the action of insulin on glucose transport was also assessed (Fig. 2). EC_{50} (concentration exerting half-maximal effect) for insulin was 15–20 μU/ml in control cells. No significant change was produced by adding either 8-bromo-cAMP or OPC 3911 alone ($n = 5$, not shown), whereas these agents in combination impaired insulin sensitivity (2–3-fold increase in EC_{50}, $n = 5$, $P < 0.02$, Fig. 2). N^6-mbcAMP (4 mM) alone also produced an insensitivity to insulin with a 2–3-fold increase in EC_{50} and this was not further affected by the combination with OPC 3911 (not shown).

3.2. ^{125}I-Insulin binding

We recently showed that pretreatment of adipocytes from young insulin-sensitive rats with insulin can lead to the unmasking of a 'pool' of insulin receptors associated with the plasma membrane [14]. OPC 3911 significantly reduced basal ^{125}I-insulin binding by ~20%, whereas it did not consistently alter insulin-stimulated binding (Table 1). However, when OPC 3911 was added together with insulin and 8-bromo-cAMP, a marked decrease in subsequent insulin binding was seen and the effect of insulin to enhance its binding capacity was abolished (Table 1).

Table 1
Effects of preincubation with insulin, 8-bromo-cAMP and OPC 3911 on ^{125}I-insulin binding in rat adipocytes

Addition	^{125}I-insulin bound (pg/10^5 cells)
None	0.36 ± 0.05
OPC 3911	0.28 ± 0.05 [b]
Insulin	0.98 ± 0.11 [c]
Insulin + OPC 3911	0.66 ± 0.12 [a]
Insulin + 8-bromo-cAMP	0.96 ± 0.12 [c]
Insulin + 8-bromo-cAMP + OPC 3911	0.15 ± 0.01 [a,d]

Cells were preincubated at 37°C for 20 min with or without insulin (1000 μU/ml), 8-bromo-cAMP (1 mM) and OPC 3911 (10 μM) as indicated. Following energy depletion with KCN and washing, ^{125}I-insulin binding was assessed as described in 'Materials and methods'. Data are means ± SE of 4–6 separate experiments.
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs no addition; [d] $P < 0.02$ vs insulin alone.

3.3. Effects of cAMP and cGI-PDE inhibition on glucose transport – obese animals

To assess the role of cGI-PDE in insulin resistant states, adipocytes from aging, obese rats (350–450 g) were studied [14]. Data on glucose transport in these cells are shown in Table 2. The maximally insulin-stimulated glucose transport (per cell) was ~40% lower than in control cells from young, lean rats. Similar to what was seen with control cells, OPC 3911 alone significantly decreased insulin-stimulated transport by ~15%. This inhibition was further pronounced by the combination of OPC 3911 and 8-bromo-cAMP (~30%). Again, no consistent decrease in maximally insulin-stimulated transport was seen when 8-bromo-cAMP was added alone.

In adipocytes from obese rats neither OPC nor 8-bromo-cAMP alone or in combination produced any consistent further reduction of insulin sensitivity (not shown). However, cells from the obese rats already displayed an impaired insulin sensitivity in the absence of these agents when compared to cells from lean rats (~2-fold increase in EC_{50}, $n = 6$, $P < 0.01$).

Table 2
3-*O*-[^{14}C]Methyl-D-glucose uptake in adipocytes from obese rats

Addition	3-*O*-Methylglucose uptake (amol/cell per s)	
Insulin:	−	+
None	0.55 ± 0.16	3.81 ± 0.88
8-bromo-cAMP	1.04 ± 0.24 [a]	3.45 ± 0.82
OPC 3911	0.61 ± 0.16	3.32 ± 0.93 [c]
OPC 3911 + 8-bromo-cAMP	1.08 ± 0.30 [a]	2.62 ± 0.78 [b]

Cells from obese rats (350–450 g) were preincubated with or without insulin and the other additions as in Fig. 1 and 3-*O*-methylglucose uptake was measured. Data are means ± SE of 6 separate experiments. [a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$ vs no addition or insulin alone, respectively.

4. Discussion

Several previous studies have shown that elevated cAMP levels markedly impair insulin responsiveness and sensitivity with respect to activation of glucose transport, IGF-II-receptor translocation, the recently described uncovering of cell surface insulin receptors as well as antilipolysis [11–13,15,22]. The ability of insulin to lower the cellular cAMP levels has proven critical for the antilipolytic effect of insulin, which occurs through the activation of cGI-PDE [9,10]. The present study demonstrates that the ability of insulin to activate cGI-PDE is also of importance for insulin action on glucose transport. The specific cGI-PDE inhibitor OPC 3911 clearly impairs the insulin response and this impairment is, as expected, most evident when the cAMP concentration is high.

8-Bromo-cAMP is readily hydrolyzed by cGI-PDE and this can be achieved by a high insulin concentration which essentially overcomes the cAMP effect (Fig. 1). Thus, due to the concomitant cGI-PDE activation, a high concentration of insulin can exert its full effect on glucose transport despite a high cAMP concentration (e.g. producing a maximal stimulation of lipolysis [8]). However, when cGI-PDE was inhibited by OPC 3911, 8-bromo-cAMP at the same concentration exerted a pronounced decrease in insulin responsiveness (Fig. 1). cGI-PDE may also be of importance for the 'basal', non-stimulated cAMP levels, since OPC 3911 alone significantly impaired the insulin response. This was seen in the absence of cAMP analogues, also when endogenous cAMP production was antagonized by the addition of PIA.

Insulin sensitivity assessed as EC_{50} for insulin was also modulated by cGI-PDE, albeit with subtle differences as compared to the cellular responsiveness. Thus, neither 8-bromo-cAMP nor OPC 3911 alone produced any consistent insensitivity to insulin. However, the combination of 8-bromo-cAMP and OPC 3911 significantly decreased the sensitivity by ~ 2-fold. This corresponds to the change in insulin binding, where the effect of insulin to rapidly enhance its cell surface binding capacity [14,15] was abolished by the combination of 8-bromo-cAMP and OPC 3911, whereas each agent alone was without consistent effect. Quantitatively, the inability of insulin to enhance its binding matches the ~ 2-fold increase in EC_{50} for insulin with respect to glucose transport activation [23].

N^6-Monobutyryl cAMP (N^6-mbcAMP) is resistant to hydrolysis by PDE [20]. This probably accounts for its ability to reduce insulin-stimulated glucose transport in the absence of OPC 3911. Similarly, N^6-mbcAMP alone also impairs insulin sensitivity. Surprisingly, the combination of OPC 3911 and 8-bromo-cAMP (or N^6-mbcAMP) exerted a greater inhibition on the insulin response than did N^6-mbcAMP alone. One possible explanation may be that N^6-mbcAMP is not completely resistant to hydrolysis by cGI-PDE [20] and that a pharmacological inhibition of the enzyme by OPC 3911 therefore leads to a small elevation of the cAMP level.

The rise in 'basal' glucose transport rate (in the absence of insulin) following the addition of OPC 3911 and/or cAMP analogues is compatible with other recent results showing a slight cAMP-stimulated translocation of GLUT 4 to the plasma membrane [24]. However, the physiological significance of this is unclear. In human adipocytes, PDE-inhibition per se has also previously been demonstrated to lead to an enhanced 'basal' glucose transport [25].

Cells from obese, aging rats display an impairment of both insulin sensitivity and responsiveness [14]. A possible mechanism for this could be elevated cellular cAMP levels and/or impaired cGI-PDE function in these cells. However, the present data show that inhibition of cGI-PDE with OPC 3911 antagonizes insulin responsiveness to a similar extent as in normal cells from lean rats. Furthermore, the absence of effect of 8-bromo-cAMP alone and the marked effect of the combination of OPC 3911 and 8-bromo-cAMP were analogous to what was seen in the insulin-sensitive fat cells from lean animals. Thus, these data suggest that the insulin resistance in cells from obese rats is not caused by increased cellular cAMP levels nor by a defective activation of cGI-PDE in response to insulin. The lack of consistent effects of 8-bromo-cAMP and OPC 3911 on insulin sensitivity in cells from obese rats may be due to the fact that these cells already are insensitive to insulin, which, in turn, may be accounted for by the inability of insulin to enhance its binding capacity in these cells [14,23].

In conclusion, the present data demonstrate that a normal activation of cGI-PDE by insulin is important for the effect of insulin to increase transmembrane glucose transport. This enzyme is particulary important when the cellular cAMP levels are high, but it also appears to play a role under basal, non-stimulated conditions. The present study also suggests that insulin is able to exert a normal activation of cGI-PDE in insulin-resistant cells from obese rats.

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