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Selective cyclooxygenase-2 inhibitors stimulate glucose transport in L6 myotubes in a protein kinase Cδ-dependent manner

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ABSTRACT

Selective inhibitors of cyclooxygenase-2 (prostaglandin-endoperoxide synthase-2; COX-2) augment the rate of hexose uptake in myotubes by recruiting glucose transporter-4 (GLUT-4) to the plasma membrane in an insulin- and AMPKα-independent manner [Alpert E, Gruzman A, Lardi-Studler B, Cohen G, Reich R, Sasson S. Cyclooxygenase-2 (PTGS2) inhibitors augment the rate of hexose transport in L6 myotubes in an insulin- and AMPKα-independent manner. Diabetologia 2006;49:562–70]. We aimed at elucidating the molecular interactions that mediate this effect of COX-2 inhibitors in L6 myotubes. The effects of the inhibitors niflumic acid, nimesulide and rofecoxib on activities and phosphorylation state of key proteins in the insulin transduction pathway were determined. These inhibitors did not induce specific tyrosine phosphorylation in IRS-1, could not assemble a functional IRS-PI3K-PKB/Akt complex and did not activate GSK3α/β, JNK1/2, ERK1/2, p38-MAPK or c-Cbl by site-specific phosphorylation(s). Yet, like insulin, they activated mTOR and induced downstream threonine phosphorylation in p70S6K and 4EBP1. However, rapamycin, which inhibits mTOR enzymatic activity, did not interfere with COX-2 inhibitor-induced stimulation of hexose uptake in myotube. Thus, mTOR activation was not required for COX-2 inhibitor-dependent augmentation of hexose transport in myotubes. Because PKCδ has also been shown to activate mTOR, we asked whether COX-2 inhibitors activate mTOR by a prior activation of PKCδ. Indeed, all three inhibitors induced tyrosine phosphorylation in PKCδ and stimulated its kinase activity. Moreover, pharmacological inhibition of PKCδ or the expression of a dominant-negative form of PKCδ in myotubes completely abolished COX-2 inhibitor-dependent stimulation of hexose uptake. This study shows that selective COX-2 inhibitors activate a unique PKCδ-dependent pathway to increase GLUT-4 abundance in the plasma membrane of myotubes and augment the rate of hexose transport.

1. Introduction

Selective cyclooxygenase-2 (COX-2; prostaglandin endoperoxide synthase-2) inhibitors may cause hypoglycemic episodes in man when over-consumed or in a combination therapy with oral antihyperglycemic drugs ([1] and references therein). We have shown recently that some selective COX-2 inhibitors (niflumic acid, nimesulide and rofecoxib) augment

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the rate of hexose transport in L6 myotubes in a dose- and time-dependent manner by increasing the plasma membrane abundance of the insulin-sensitive glucose transporter-4 (GLUT-4) but not of the ubiquitously expressed GLUT-1 [1]. In contrast, COX-1 inhibitors (i.e., acetyl salicylic acid and indomethacin) had no effect on the hexose transport system in L6 myotubes [1]. The translocation of GLUT-4 to the plasma membrane of skeletal muscle usually occurs following the activation of the insulin receptor-dependent transduction pathway or through the 5′-AMP-activated kinase (AMPKα)-dependent pathway in contracting muscles [2]. We have shown that the hexose transport stimulatory effect of these inhibitors is slow (6-h lag period; maximal effect at 12 h), insulin-independent and does not entail AMPKα activation [1]. In the present study, we addressed the hypothesis that COX-2 inhibitors increase the abundance of GLUT-4 in the plasma membrane in myotubes by circumventing the initial step of insulin transduction pathway and activating downstream transducer proteins or by utilizing other unrelated mechanisms.

Insulin-induced translocation of GLUT-4-containing vesicles to the plasma membrane of myotubes occurs upon the binding of the hormone to its cell-surface receptor, induction of tyrosine kinase activity in its β-subunit and activation of a downstream phosphorylation cascade that assembles and activates regulatory complexes [2]. The initial step in this cascade is the phosphorylation of certain tyrosine residues in insulin receptor substrates (IRS) and the formation of insulin receptor-IRS docking complexes for various regulatory proteins with SH2 domain. Both IRS-1 and IRS-2 are expressed in L6 myotube. The former is considered the predominant isoform that mediates GLUT-4 translocation to the plasma membrane in skeletal muscles [2]. Several interactions that enhance the rate of IRS-1 degradation slow or terminate insulin action have been proposed: for instance, glycogen synthase kinase-3 (GSK3α/β)-dependent phosphorylation of target serine residues in IRS-1 directs it to proteasomal degradation. The inactivation of GSK3α/β by serine^{21}- and serine^{9} phosphorylation, respectively, may slow IRS-1 degradation and extend the duration of insulin action [3,4]. The role of IRS-2 in regulating glucose transport in L6 myotubes is ambiguous as silencing of IRS-1 expression, but not of IRS-2, reduced insulin-dependent GLUT-4 translocation to the plasma membrane [5]. The insulin receptor-IRS-1 complex activates PI3-kinase, whose metabolite phosphatidylinositol (PI)-3,4,5-trisposphate then recruits 3-phosphoinositide-dependent protein kinase-1 (PDK1). The latter activates protein kinase B (PKB/Akt) by targeted serine/threonine phosphorylations, allowing it to phosphorylate other protein kinases and regulatory proteins, which mediate a variety of cellular processes, including the translocation of GLUT-4-containing vesicles to the plasma membrane [2].

Insulin-induced targeted tyrosine phosphorylation of c-Cbl has been linked to an enhanced recruitment of GLUT-4-containing vesicles to the plasma membrane in 3T3L1 adipocytes [6,7]. Notwithstanding the dispute whether this pathway functions in insulin-treated adipocytes [8], it might contribute to GLUT-4 translocation in skeletal muscles [9], possibly due to the expression of a novel skeletal muscle-specific c-Cbl associated protein [10]. Members of the MAPK family are also activated by insulin: JNK1/2 facilitate the inactivation of IRS-1 [11]. The intrinsic activity of GLUT-4 might be regulated by p38-MAPK [12]. ERK1/2 have also been linked to insulin signaling in skeletal muscles [13]. The active PI3K-PKB/Akt complex also activates mammalian target of rapamycin (mTOR), which regulates various downstream pathways, such as augmentation of rate of translation of a family of mRNAs that encode components of the protein synthesis machinery or ensues cap-dependent translation initiation by activating p70S6K and 4EBP1, respectively [14,15]. Sampson and colleagues have recently identified a role for PKCδ in insulin action in skeletal muscle cells: myotubes overexpressing a kinase inactive, dominant negative PKCδ became resistant to insulin-induced stimulation of glucose uptake and GLUT-4 translocation, while overexpression of PKCδ increased the rate of glucose uptake, even in the absence of insulin [16].

We used niflumic acid, nimesulide and rofecoxib that represent early and newer generations of selective COX-2 inhibitors [1]. While there is no indication for a COX-2 inhibitor-induced activation of key elements in the classical insulin transduction mechanism, this study shows that these inhibitors stimulate the glucose transport system in myotubes in a unique PKCδ-dependent manner.

2. Materials and methods

2.1. Materials

The sources of materials used in this study were as follows: αMEM and FCS from Biological Industries (Beth-Haemek, Israel); 2-[1,2-^{3}H(N)]-deoxy-D-glucose (2.22 TBq/mmol) from American Radiolabeled Chemicals (St. Louis, MO, USA); 2-deoxy-D-glucose (dGlc), LY294,002, niflumic acid, nimesulide and rottlerin from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Rapamycin from Calbiochem (La Jolla, CA, USA); Protein G- and protein A Sepharose™ beads were from Amersham Pharmacia Biothech (Uppsala, Sweden). Rofecoxib was kindly donated by Merck Research Laboratories (Rahway, NJ, USA). All other chemicals, reagents and solvents were reagent- or molecular biology-grade. The antibodies used were against: IRS-1, IRS-2, phospho-S^{307}-IRS-1, phospho-Y (clone 4G10), PKB/Akt1 (PH domain), phospho-S^{473}-Akt1/PKBα and Cbl (clone 7G10) from Upstate Biotechnology (Lake Placid, NY, USA); phospho-Y^{774}-c-Cbl from Cell Signaling Technology (Beverly, MA, USA); phospho-T^{308}-PKB/Akt1/2/3, PKCδ (C-17), p70S6K, phospho-T^{389}-p70S6K, phospho-T^{69}-4EBP1, phospho-S^{21}-GSK3α and phospho-S^{9}-GSK3β from Santa Cruz Biotechnology (Santa Cruz, CA, USA); phospho-T/Y$^{185/187}$-ERK1/2, phospho-T/Y$^{183/185}$-JNK1/2 and phospho-T/Y$^{180/182}$-p38-MAPK from Biosource International (Camarillo, CA, USA).

2.2. Cell cultures

L6 skeletal myocytes were grown and let to differentiate into multi-nuclear myotubes (85–90% yield) in αMEM supplemented with 2% (v/v) FCS, as described [17].