Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 2009–2017
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Inhibition of insulin-like growth factor I receptor tyrosine kinase by ethanol

Raphael Rubin[a,*], Rob Harrison[b], Xian-Feng Chen[b], Joseph Corzitotto[a], Jan B. Hoek[a], Hazem Hallak[a]

[a]*Department of Pathology, Anatomy and Cell Biology, Jefferson Medical College, 226 Alumni Hall, Philadelphia, PA 19107, USA*
[b]*Departments of Computer Science and Biology, Georgia State University, Atlanta, GA 30303, USA*

Received 3 May 2004; accepted 28 June 2004

Abstract

Ethanol inhibits insulin and insulin-like growth factor-I (IGF-I) signaling in a variety of cell types leading to reduced mitogenesis and impaired survival. This effect is associated with inhibition of insulin receptor (IR) and insulin-like growth factor-I receptor (IGF-IR) autophosphorylation, which implicates these receptors as direct targets for ethanol. It was demonstrated previously that ethanol inhibits the autophosphorylation and kinase activity of the purified cytoplasmic tyrosine kinase domain of the IR. We performed computer modeling of the ethanol interaction with the IR and IGF-IR kinases (IRK and IGF-IRK). The analysis predicted binding of alcohols within the hydrophobic pocket of the kinase activation cleft, with stabilization at specific polar residues. Using IGF-IRK purified from baculovirus-infected insect cells, ethanol inhibited peptide substrate phosphorylation by non-phosphorylated IGF-IRK, but had no effect on the autophosphorylated enzyme. In common with the IRK, ethanol inhibited IGF-IRK autophosphorylation. In cerebellar granule neurons, ethanol inhibited autophosphorylation of the apo-IGF-IR, but did not reverse IGF-IR phosphorylation after IGF-I stimulation. In summary, the findings demonstrate direct inhibition of IGF-IR tyrosine kinase by ethanol. The data are consistent with a model wherein ethanol prevents the initial phase of IRK and IGF-IRK activation, by inhibiting the engagement of the kinase activation loop.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Ethanol; Insulin receptor; Insulin-like growth factor-I receptor; Tyrosine kinase; Cerebellar granule neurons

The interaction of ethanol with intracellular signal transduction pathways has been linked to disturbances in cell proliferation and survival [1]. Such perturbations may play a role in the pathogenesis of certain disorders of tissue and organ function associated with acute and chronic alcohol exposures, including neuronal degeneration, cirrhosis, myopathy, and cardiomyopathy. Maternal alcohol consumption during gestation causes profound disorders of fetal brain development (fetal alcohol syndrome) [2].

IGF-I and insulin induce proliferation and survival in most cell types [3,4]. Pharmacologically relevant concentrations of ethanol inhibit intracellular signaling responses to IGF-I and insulin, leading to impaired cell growth and survival [5–12]. As a common feature, ethanol inhibits the autophosphorylation of the IGF-I receptor (IGF-IR) and the insulin receptor (IR) [5,6,11–14], which is the initial step in receptor activation following ligand binding. Inhibition of tyrosine autophosphorylation of the platelet-derived growth factor-bb receptor [15] and the FGF receptor [16] have also been observed in certain cell types. By contrast, the EGF receptor and other members of the PDGF receptor family are largely insensitive to these acute actions of ethanol.

The IR and IGF-IR are closely homologous [17], and the repertoire of signaling molecules that are activated by their ligands are remarkably similar [3]. The receptors are composed of α2–β2 heterodimers, the individual chains linked by α–α and α–β disulfide bridges. The extracellular ligand binding domain is constituted on the α chain, and the tyrosine kinase domain is on the intracellular β domain.

Abbreviations: IGF-I, insulin-like growth factor-I; IGF-IR, IGF-I receptor; IGF-IRK, IGF-IR kinase; IR, insulin receptor; IRK, IR kinase; FGF, fibroblast growth factor; IRS-1, insulin-related substrate-1; EGF, epidermal growth factor; PDGF, platelet-derived growth factor.

* Corresponding author. Tel.: +1 215 503 4517; fax: +1 215 955 8703.
E-mail address: raphael.rubin@jefferson.edu (R. Rubin).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.06.032

Sequence homology is greatest within the tyrosine kinase domain (>80%), and diverges within the carboxyterminal domains, which may account for some of the different functions of the IR and IGF-IR. Notably, IGF-I and insulin principally exert mitogenic and metabolic actions, respectively [18]. Upon ligand binding, the receptors undergo rapid autophosphorylation within the tyrosine kinase domain (Y1131, Y1135 and Y1136 in the IGF-IR and Y1158, Y1162 and Y1163 in the IR). Other tyrosines that are targets for phosphorylation are found within the juxtamembrane and carboxyterminus domains.

The mechanism by which ethanol inhibits the IGF-IR and IR has only been partially characterized. Ethanol inhibits the tyrosine autophosphorylation of IGF-IR within immunoprecipitants [13]. This result suggests that that ethanol directly interacts with the IGF-IR, although a role for ethanol interaction with other proteins contained within the IGF-IR immunoprecipitant complex could not be excluded. To address this issue, we tested the effect of ethanol on the activity of the minimally-defined IR kinase (IRK) [19]. Ethanol inhibited the tyrosine kinase activity of soluble GST-IR, both in terms of the phosphorylation of peptide substrate and autophosphorylation. Kinetic analysis of peptide phosphorylation indicated a non-competitive inhibition by ethanol, with a reduction in the V_{max} of 21% at 150 mM ethanol. These data suggested that the inhibitory effect of ethanol on this class of receptors is localized to the cytoplasmic kinase domain.

The crystal structures of the IR and the IGF-IR kinases (IRK and IGF-IRK) have been solved [20–24], which has provided insights into their activation dynamics. The IR and IGF-IR cytoplasmic kinase domains are highly homologous with 84% overall identity, and 100% identity within the ATP binding cleft [17]. Indeed, by X-ray crystallography, the root-mean square deviation for the Cα atoms of the IR and IGF-IR is 2.5 Å [21]. The kinases are comprised of an N-terminal lobe comprising a five beta-stranded β sheet and one α helix, and a larger carboxyterminal lobe that is mainly α-helical. The activation loop (A-loop) is located in the larger carboxyterminal lobe. ATP binds within the cleft between the two lobes, and tyrosine-containing substrates bind to the carboxyterminal lobe.

The conversion of inactive IRK/IGF-IRK to their active forms involves conformational changes in the protein with a swing-out of the A-loop, thereby allowing access of substrates to the catalytic site [25]. A key regulatory feature of both the IRK and IGF-IRK is that tyrosines within the A-loop (Y1162 for IR and Y1131 for IGF-IR) are bound within the active site, hydrogen-bonded to conserved polar residues within the catalytic loop [20,22]. In the unphosphorylated (0P) state, internal stabilizations prevent phosphorylation of substrates ('gate-closed' configuration) resulting in an autoinhibited state. However, autoinhibition is sufficiently weak to allow for a transient 'gate-open' configuration thereby allowing for transphosphorylation after ligand binding. Thus, autophosphorylation of the IR and the IGF-IR kinases increases their catalytic efficiencies. Based upon these considerations, it was demonstrated that intermolecular transphosphorylation is required to relieve this steric constraint [21,23,26].

In the current study, we generated a model for the interaction of ethanol with the IR and IGF-IR kinases, which predicts binding of ethanol and higher chain n-alkanols within the kinase activation clefts. To validate this model, we tested the effect of ethanol on the IGF-IRK tyrosine kinase domain. The data demonstrate inhibitory action of ethanol on the IGF-IRK, and are consistent with a mechanism whereby ethanol interferes with the initial phase of kinase activation involving the mobilization of the conserved kinase activation loop.

1. Materials and methods

1.1. Materials

Synthetic IRS-1 peptide KKEEEEYMMMMG was synthesized by Biosource (Camarillo, CA). [γ-^{32}P]ATP was from DuPont NEN (Boston, MA). Human recombinant IGF-I was from Calbiochem (San Diego, CA). Anti-IGF-IR β-subunit rabbit polyclonal antibodies and anti-phosphotyrosine (PY99) mouse monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-MAP kinase antibodies, anti-phosphotyrosine and phosphothreonine MAP kinase (E10) antibodies, horseradish peroxidase-conjugated goat anti-rabbit IgG, horse anti-mouse IgG antibodies and signal-enhanced chemiluminescence reagents were obtained from New England Biolabs (Beverly, MA). All other chemicals and biochemicals were of the highest purity commercially available.

1.2. Expression and purification of IGF-IR kinases

The BAC-To-BAC Baculovirus expression system (Invitrogen; Carlsbad, CA) was used to express the IGF-IR cytoplasmic kinase domain fused to a 6-His tag. cDNA corresponding to IGF-IRK residues 956–1256 subcloned into pFB vector was obtained from Dr. Todd Miller, Stoneybrook, NY. IGF-IR was released with *Eco*RI and *Hin*dIII, and ligated into pFastBac HTc plasmids. Generation of recombinant bacmid, infection of *Spodoptera frugiperda* (Sf9) cells, and purification over NiNTA resin columns were performed according to the manufacturers specifications. Protein were concentrated using Microcon YM-10 centrifugal filter devise (Millipore; Bellerica, MA), resuspended in 50 mM Hepes, pH 7.5, and stored at 4 °C. Protein concentration was determined using the Bradford method (Bio-Rad; Hercules, CA).

Download English Version:

https://daneshyari.com/en/article/2515980

Download Persian Version:

https://daneshyari.com/article/2515980

Daneshyari.com