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Therapeutic targeting of insulin-regulated aminopeptidase: Heads and tails?

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Abstract

Insulin-regulated aminopeptidase, IRAP, is an abundant protein that was initially cloned from a rat epididymal fat pad cDNA library as a marker protein for specialized vesicles containing the insulin-responsive glucose transporter GLUT4, wherein it is thought to participate in the tethering and trafficking of GLUT4 vesicles. The same protein was independently cloned from human placental cDNA library as oxytocinase and is proposed to have a primary role in the regulation of circulating oxytocin (OXY) during the later stages of pregnancy. More recently, IRAP was identified as the specific binding site for angiotensin IV, and we propose that it mediates the memory-enhancing effects of the peptide. This protein appears to have multiple physiological roles that are tissue- and domain-specific; thus the protein can be specifically targeted for treating different clinical conditions.

Keywords: Insulin-regulated aminopeptidase; GLUT4; Memory; Diabetes

Abbreviations: ACD, acyl coenzyme A dehydrogenase; APA, aminopeptidase A; APB, aminopeptidase B; APN, aminopeptidase N; Ang II, angiotensin II; Ang IV, angiotensin IV; AS160, Akt substrate of 160 kDa; AVP, arginine vasopressin; CBD, chitin-binding domain; ER, endoplasmic reticulum; FHOS, forming homologue overexpressed in spleen; GAP, GTPase-activating protein; GST, glutathione *S*-transferase; GSV, GLUT4 storage vesicle; HC, hexanoyl CoA; HDM, high-density microsomes; i.c.v., intracerebroventricular; IRAP, insulin-regulated aminopeptidase; LDM, low-density microsomes; LTA4H, leukotriene A4 hydrolase; LVV-H7, LVV-hemorphin 7; MPA, 3-mercaptoproprionic acid; OXY, oxytocin; PM, plasma membrane.

Contents

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1. Introduction

Insulin-regulated aminopeptidase — IRAP; LNPEPEC; cystinyl aminopeptidase, oxytocinase, placental leucine aminopeptidase (P-LAP); EC 3.4.11.3.

The diverse functions of IRAP are reflected in its identification based on different physiological or biochemical properties by 3 different groups. In 1995, IRAP was purified and cloned by Keller et al. as a marker protein of GLUT4 vesicles (Keller et al., 1995). In the following year, it was cloned under the moniker oxytocinase as an aminopeptidase produced by the placenta and postulated to function as a degradative enzyme for oxytocin (OXY) as the name implies (Rogi et al., 1996). In 2001, IRAP was also identified as the angiotensin AT_4 receptor, a binding site for the peptide angiotensin IV (Ang IV), characterized in a range of tissues, including the brain.

IRAP is a type II transmembrane protein of 1025 amino acid residues with 3 domains (Rogi et al., 1996; Laustsen et al., 1997; Rasmussen et al., 2000). The hydrophilic N-terminal intracellular domain is 110 amino acids in length. It contains motifs involved in endocytosis and trafficking (Keller et al., 1995) and interacts with a number of proteins. The hydrophobic transmembrane segment is 22 residues (amino acids 111–131) in length and is followed by an extracellular domain of 893 residues (amino acids 132–1025). The extracellular domain contains the catalytic site consisting of the GAMEN motif (amino acids 428–432) and the $HEXXH(X)_{18}E$ Zn^{2+}-binding motif (amino acids 464–487), which identifies IRAP as an M1 aminopeptidase (Keller et al., 1995; Fig. 1).

Regulation of IRAP is of therapeutic potential based on the 3 known properties of the protein. This review will focus on the 2 functional domains of IRAP: (i) HEAD, which contains the catalytic site of IRAP — inhibition of the aminopeptidase activity in this domain has the potential to (a) facilitate memory and (b) induce labour; (ii) TAIL, the intracellular domain of IRAP — modulation of proteins that interact with this domain have the potential to enhance glucose uptake in insulin responsive cells providing a treatment for type 2 diabetes.

1.1. Tissue distribution of IRAP

IRAP colocalizes with the insulin-responsive glucose transporter GLUT4 in insulin-responsive tissues, including skeletal muscle and adipose. In addition, IRAP is found in a number of other tissues, many of which do not express GLUT4, such as the adrenal gland, spleen, kidney medulla and placenta (reviewed in Chai et al., 2004). This broad tissue distribution supports the concept of a range of physiological roles for IRAP.

IRAP was originally identified as a marker protein of the specialized vesicles containing GLUT4; recycling with the glucose transporter within intracellular compartments in the basal state and accompanying the protein to the plasma membrane (PM) in response to insulin (reviewed in Bryant et al., 2002; Fig. 2). Mobilization of GLUT4 to the cell membrane by insulin facilitates a large influx of glucose into fat cells. The role of IRAP in insulin-responsive tissues is not fully defined, but it is proposed to play a role in the tethering of GLUT4 vesicles within the cell under basal conditions (see Section 3).

IRAP is also produced in the placenta during pregnancy, wherein it is present mainly in the apical membrane of syncytiotrophoblasts. The extracellular domain is proteolytically cleaved at the PM and is secreted into maternal serum as a soluble form (Yamahara et al., 2000). In contrast to the widespread tissue distribution of membrane-bound IRAP, the soluble form of the enzyme is only detected in serum during pregnancy. Serum levels of IRAP increase with advancing gestation until just before the onset of labor, before diminishing postpartum (Mizutani et al., 1996; Yamahara et al., 2000).

In the brain, IRAP is present in high concentrations in neurones in the CA1–CA3 regions of the hippocampus, frontal, prefrontal, insular and entorhinal cortices and basolateral amygdala — areas associated with cognition. However, the distribution of IRAP is not restricted to these sites in the brain as it is also found in all motor neurones and motor-associated regions, some hypothalamic, thalamic and sensory nuclei (Fernando et al., 2005). In neurones, IRAP is found predominantly in intracellular compartments, in vesicular-like structures. In some brain regions, IRAP exhibits a high degree of colocalisation with GLUT4, reminiscent of that observed in fat cells (Fernando et al., 2005; Fig. 3). However, unlike insulin-responsive cells, it remains unclear as to whether IRAP and/or GLUT4 translocation to the PM is responsive to a particular stimuli or simply constitutive.

1.2. IRAP knockout mice

Limited insights into the physiological importance of IRAP have been provided by a study on the IRAP knockout mouse (Keller et al., 2002). Although the IRAP null mice exhibited impaired insulin-stimulated glucose uptake in muscle and adipose cells, possibly due to the concurrent 40–85% decrease in GLUT4 levels in the different muscles and adipocytes, they maintained normal glucose homeostasis — fed and fasted blood glucose and insulin levels were indistinguishable from wild-type controls (Keller et al., 2002). The weights of adipose tissue and skeletal muscles were not significantly different from wild-type controls; however, their hearts were on average 20% larger — cardiomegaly has also been observed in the GLUT4 null mice. These mice displayed decreased GLUT4 levels of between 40%