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Visions & Reflections

The insulysin (insulin degrading enzyme) enigma

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In 1981, Goldstein and Livingston [1] suggested that insulin degradation occurs within the cell. The degradation of insulin was found to be dependent upon binding to the insulin receptor, and thus insulin degradation was believed to occur within an intracellular compartment. The identification of this cellular compartment is still controversial. The enzyme responsible for insulin degradation was identified and named insulin degrading enzyme (IDE) [2–4]. Its IUB name and number are insulysin, EC 3.4.24.56. Insulysin is composed of a polypeptide chain of approximately 110 kDa and contains a molecule of zinc at its active site. It is classified as an inverzincin on the basis of the presence of the zinc-binding motif HXXEH, which is inverted relative to the more common HEXXH zinc-binding motif seen in related metalloproteases. Several lines of evidence support a role for insulysin being involved in insulin catabolism. This includes the observation that injection of an anti-insulysin monoclonal antibody into HepG2 cells increased cellular insulin levels [5]. In addition, insulin internalized into HepG2 cells was able to be cross-linked to intracellular insulysin [6]. The GK rat, which is a widely studied model of type 2 diabetes, exhibits defects in both insulin action and insulin degradation. Although there are a number of loci that map to the defect, a major locus was shown to be the insulysin gene. Two mutations were found, H18R and A890V, which together produced a decrease of about 30% of the wild-type insulysin activity [7]. The amyloid beta peptides 1–40 and 1–42 are also physiological substrates for insulysin. As a consequence of the mutations in the insulysin gene, increased amyloid beta peptide levels are seen in primary neuronal cultures derived from the GK rat, but interestingly no such increase in steady-state levels of amyloid beta peptide in the brain is seen [8]. The latter is postulated to reflect compensatory degradative mechanisms in the brain [8].

In vitro studies showed that insulysin was able to degrade amyloid beta peptides [9, 10]. The enzyme received considerable attention when studies suggested a secreted form of insulysin degraded amyloid beta peptides in cell culture [11, 12]. A definitive role for insulysin in regulating amyloid beta peptide levels in the brain was provided when it was shown that mice deficient in insulysin had elevated levels of both amyloid beta peptide 1–40 and amyloid beta peptide 1–42 [13, 14]. Over expression of insulysin in human amyloid precursor protein transgenic mice led to a decrease in amyloid beta peptide levels [15]. Insulysin activity was found to decline with aging in the hippocampus of a mouse model of Alzheimer's disease (AD) [16], likely due to the susceptibility of insulysin to oxidative inactivation and increased turnover [17]. Furthermore, insulysin is reduced in the hippocampus of AD patients with an apolipoprotein E-4 allele [18]. Together these studies suggest that insulysin contributes to the etiology of AD.

Unlike the other major amyloid beta peptide degrading enzyme neprilysin (NEP), which is localized to the plasma membrane with its active site outside the cell where it can degrade secreted amyloid beta peptides, insulysin is primarily cytosolic [19]. Other than cytosolic insulysin, other cellular localizations have been observed, including a fraction of the enzyme in peroxisomes that is directed to that organelle by a C-terminal peroxisomal targeting signal [19], and a form of the enzyme derived from an al-

ternative translational start site that can localize to mitochondria [20]. However, neither of these compartments is believed to contain either insulin or amyloid beta peptide, and thus neither is a likely site of catabolism. Although a cell surface insulin degrading activity was reported on pancreatic acini [21] as well as on lymphocytes, HepG2 cells, and primary hepatocytes [22], this activity was only indirectly identified as IDE, and, as noted above, more definitive evidence for intracellular insulysin acting on insulin has been obtained. A small fraction of insulysin was found on the plasma membrane of CHO cells [23]. In a recent study [24], it was reported that insulysin exists primarily on the surface of human cerebrovascular endothelial cells, and this cell surface form was the major form of insulysin responsible for degrading amyloid beta peptides.

How insulysin gets to the cell surface is unclear. The enzyme does not contain any obvious signal sequence that would direct it to the secretory pathway, nor is there any obvious amino acid sequence that could serve to anchor the enzyme in the plasma membrane. Sequences for post-translational modification of insulysin that might add a membrane anchoring glycosylphosphatidylinositol (GPI) or myristoyl group are not apparent, and insulysin has not been reported to contain these modifications.

There are many cellular locations proposed for insulysin, yet there is a lack of a definitive study that clearly establishes within which cellular compartment insulin and amyloid beta peptide hydrolysis occurs. Thus, the controversy continues. Does the degradation of insulin and amyloid beta peptides by insulysin occur intracellularly, at the cell surface, or through the action of secreted insulysin? Are insulin and amyloid beta peptides degraded by insulysin in the same cellular compartment? Is the cellular compartment in which degradation of insulin and amyloid beta peptide occurs different in different cell types? A number of different approaches may be needed to answer these questions. These might include the use of specific cell permeable and cell impermeable inhibitors, which could distinguish between cell surface and intracellular IDE or blocking the secretion of IDE or its transport to the cell surface. Clearly mores studies are warranted in this area.

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