Congenital Hyperinsulinism in Infancy: The Profiles of Insulin Secretary Granules Are Markedly Different in Focal- and Diffuse β-Cells

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**Background & Objectives**

Congenital Hyperinsulinism of Infancy (CHI) is a potentially lethal condition of profound hypoglycaemia caused by unregulated insulin release in the neonatal period and early infancy. CHI mainly arises due to mutations in ATP-sensitive K-channel genes (ABCC8 and KCNJ11) which can manifest in all islets cells – diffuse-CHI, or can be localised to a focal lesion, focal-CHI. The mechanisms responsible for inappropriate insulin release have largely focused upon defects in KATP channels. As little is known about insulin biogenesis in CHI, our objectives were to assess the profiles of insulin-containing granules in β-cells from patients with diffuse- and focal disease.

1. Insulin-Containing Granules

β-cell and insulin secretory granules were identified using immuno-gold labeling (Fig. A) and routine TEM labeling (Fig. B). Three different stages of insulin granules were characterised: mature, dense-core / crystalline granules (labelled as C); immature secretory granules (labelled as I) and secretory granules that were depleted of insulin (labelled as D). Scale bars = 1μm.

2. The Profile of Insulin-Containing Granules

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<th>Focal Lesion (n=4)</th>
<th>Diffuse (n=4)</th>
<th>Control (n=4)</th>
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<tr>
<td>Depleted Granules</td>
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<td>Cryotome Granite</td>
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<td>Immature Granite</td>
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Panel A illustrates that there are marked differences in the profiles of insulin biogenesis in focal CHI compared to control and diffuse CHI tissue. Panel B indicates that the percentage of depleted granules is significantly higher in focal-CHI lesion while immature granules are significantly lower than control and diffuse CHI tissue. Mature insulin in the form of crystalline granules was similar between focal- and diffuse-CHI but did reach significance; \( P=0.045 \). ‘\( P<0.01 \); ‘\( P<0.001 \); ‘\( P<0.0001 \). Panels C and D show representative TEM images for diffuse-CHI and focal-CHI, respectively. Note that there are far fewer depleted granules in islets from diffuse CHI tissue. Scale bars = 0.5μm.

**Methods**

Tissue was obtained following surgery from patients with diffuse-CHI (n=4 patients with ABCC8 gene defects), lesion from focal-CHI (n=4 patients with ABCC8 gene defects) and control samples (n=4 with no known genetic mutations related to CHI disease). Immunohistochemistry stains (IHC) with SNAP25 were performed on histological sections (5 μm). Ultrathin sections (70 nm) and cryo-sections (70nm) were cut for routine Transmission Electron Microscopy (TEM) and immuno-gold labelling, respectively. Insulin-containing granules were identified and quantified in images J and data were analysed for significance using One-way ANOVA followed by Tukey’s post hoc test.

3. The Profile of Multi-vesicular Structures

Panel A shows the incidence of multi-vesicular insulin-containing granules in the different forms of CHI compared with controls. Note that insulin-containing secretory granules in focal-CHI are mainly found in multi-vesicular structures compared with diffuse and control β-cells. No significant differences were detected between the diffuse-CHI and control. ‘\( P<0.0001 \). Panel B shows a representative TEM image of the multi-vesicular structure (red arrow) in focal-CHI lesion. Scale bar = 0.5μm.

4. Expression of Crucial Exocytosis Related Protein

Snap25 is a core component of exocytosis β-cells and amongst the top 0.2% of genes that are upregulated in focal-diffuse lesions (\( P=2.1 \times 10^{-7} \), False Discovery Rate =1.1 \( \times 10^{-4} \), unpublished). Using immunohistochemistry we found that the localisation of SNAP25 was markedly different in focal-CHI β-cells (A) compared to diffuse-CHI (B) and control tissue (C). Note how in focal CHI, SNAP25 has a far more marked association with the plasma membrane compared to diffuse disease. Scale bars = 50μm.

**Conclusions**

Our data imply that β-cells in focal-CHI have a greater secretory capacity (increased number multi-vesicular secretory granules, depleted granules, altered localisation of SNAP25) than in diffuse disease, despite the fact that both conditions associate with ABCC8 gene defects.