S 10-1

ISLET CELL DEATH AND CALCIUM

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We have reported that IFN γ/TNF α synergism is the most important death effector in type 1 diabetes. We studied the intracellular events associated with pancreatic β-cell apoptosis by IFN γ/TNF α. IFN γ/TNF α treatment of MIN6N8 insulinoma cells increased the amplitude of high voltage-activated Ca^{2+} currents. $[Ca^{2+}]_c$ was also increased by IFN γ/TNF α. Blockade of L-type Ca^{2+} channel abrogated death of insulinoma cells by IFN γ/TNF α. Diazoxide that attenuates voltage-activated Ca^{2+} currents inhibited MIN6N8 cell death by IFN γ/TNF α, while glibenclamide that accentuates voltage-activated Ca^{2+} currents augmented insulinoma cell death. A PKC inhibitor attenuated MIN6N8 cell death and the increase in $[Ca^{2+}]$c by IFN γ/TNF α. Following the increase in $[Ca^{2+}]_c$, calpain was activated, and calpain inhibitors decreased insulinoma cell death by IFN γ/TNF α. As a downstream of calpain, calcineurin was activated and the inhibition of calcineurin activation by FK506 diminished insulinoma cell death by IFN γ/TNF α. BAD phosphorylation was decreased by IFN γ/TNF α because of the increased calcineurin activity, which was reversed by FK506. IFN γ/TNF α induced cytochrome c translocation from mitochondria to cytoplasm and activation of caspases. These results indicate that IFN γ/TNF α synergism induces pancreatic β-cell apoptosis by Ca^{2+} channel activation followed by downstream intracellular events such as mitochondrial events, and also suggest therapeutic potential of Ca^{2+} modulation in type 1 diabetes. Because mitochondria are the center player in islet cell function and death, we studied functional role of peripheral benzodiazepine receptor (PBR) on mitochodrial membrane in apoptosis and insulin secretion. A PBR agonist PK11195 induced insulinoma cell apoptosis. Death of insulinoma cells by PK11195 was inhibited by CsA. PK11195 induced dissipation of mitochondrial potential and cytochrome *c* translocation. PK11195 induced an increase in cytoplasmic $[Ca^{2+}]$, which was reversed by CsA. Rhod-2 staining showed decreased mitochondrial $[Ca^{2+}]$ by PK11195. PK11195 potentiated glucose-induced insulin secretion due to the increased cytoplasmic $[Ca^{2+}]$. Calpain was activated following Ca^{2+} release, and calpain inhibitors attenuated insulinoma cell death by PK11195. These results suggest that PBR agonists induce mitochondrial potential loss, cytochrome c translocation in conjunction with an increase in cytoplasmic $[Ca^{2+}]$ and calpain activation, which collectively leads to apoptosis of insulinoma cells.

S 10-2

THE ROLE OF CALCIUM AND MYOSIN LIGHT CHAIN KINASE IN THE ACTION OF INSULIN ON GLUCOSE TRANSPORT

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In adipocytes, insulin stimulates glucose transport principally by promoting translocation of glucose transporter GLUT4 from an intracellular compartment to the plasma membrane. Requirements for Ca^{2+}/calmodulin during insulin-stimulated GLUT4 translocation have been demonstrated; however, the mechanism of action of Ca^{2+} in this process is unknown. Recently, myosin II, whose function in non-muscle cells is primarily regulated by phosphorylation of its regulatory light chain (RLC) by the Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK), was implicated in insulin-stimulated GLUT4 translocation. We have investigated, using 3T3-L1 and 3T3-F442A adipocytes, the possibility that MLCK may be involved in the insulin-stimulated translocation of GLUT4. Insulin significantly increases phosphorylation of the myosin II RLC in a Ca^{2+}-dependent manner. ML-7, a selective inhibitor of MLCK, as well as inhibitors of myosin II, such as blebbistatin and 2,3-butanedione monoxime, block insulin-stimulated GLUT4 translocation and subsequent glucose transport. In addition, suppression of MLCK expression via stably expressing antisense-MLCK decreases insulin-stimulated glucose transport. Our studies strongly suggest that MLCK may be a regulatory target of Ca^{2+}/calmodulin and may play an important role in insulin-stimulated GLUT4 translocation in adipocytes.