Diabetes & The Endocannabinoid System: Prospects For Therapeutic Control

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Quick Outline

- This will be a very detailed discussion, so lets put it in perspective
- First we'll discuss causes of diabetes
- Then move on to insulin receptor signaling and defects in this mechanism
- Next we will focus on the PPARã and cannabinoid CB1 & CB2 receptors
- Finally, it will all be tied together; how cannabinoid therapy treats the symptoms of Type 1 & Type 2 Diabetes

Diabetes Background

- Over 28 million Americans have diabetes (Type 1 or 2)
- 80% of cases are diagnosed as Type 2
- The leading cause of blindness and amputations
- Diagnosed cases are rising exponentially-directly related to diet
- For every kg bodyweight over healthy BMI, a 7% increase in getting Type 2 is found

What is Diabetes?

- Type 1 (Diabetes Mellitus)
  - An autoimmune disorder characterized by islet â-cell destruction
  - Plasma glucagon levels may be increased
  - No detectable plasma insulin

What Is Diabetes?

- Type 2 (Diabetes Insipidus)
  - Often environmentally induced in predisposed individuals
  - Characterized by:
    - Obesity

- Impaired IRS phosphorylation
- Impaired PI3K activity
- Impaired GLUT-4 translocation
- Increased FFA

Common Attributes To Both

- Both Type 1 and 2 patients have;
  - Hypo/hyperglycemia
  - Dyslipidemia
  - Decreased immune function
  - Poor wound healing
  - Microangiopathies
    - Neuropathy, retinopathy, nephropathy
  - Depression & weight gain
    - Both attributable to inflamm. TNFá, IL-2, and IL-6

Causes of Diabetes

- Type 1:
  - Only 30% identical twins will both have it
  - MHC genes on chromosome 6
    - Of 21 known DR alleles, DR3 & DR4 found in 95%
  - â-cell autoantibodies
    - Directed against GAD (glutamic acid decarboxylase), unique to â-cells

Causes of Diabetes

- Type 2
  - A variety of theories, we'll focus on PPAR based
  - Interruption of lipid homeostasis
    - Leads to increased FFA
    - FFAs normally decreased by PPAR activation
  2. Activation of inflammatory cytokines normally suppressed by PPAR

Insulin Receptor Signaling

1. Insulin binds to the heterotetrameric IR (Insulin Receptor)
   - Causes autophosphorylation of tyrosine residues

2. Tyrosine autophosphorylation causes dissociation of IRS-1 (Insulin Receptor Substrate-1)
   - 4 IRS proteins;
     * IRS-1 – immediate activation of PI3K
     * IRS-2 – prolonged activation of PI3k
     * IRS-3 & -4 – inhibit PI3K activation

Insulin Receptor Signaling

3. Activation of PI3K

- Responsible for:
  * Activ. of Akt/PKB (serine phosphorylation)
  * GLUT-4 translocation

4. Activation of Ras/Raf
   - Both PKB mediated or directly IRS activated
   -Activates the MEK- ERK1/2 pathway
5. MEK & ERK1/2 Pathway
   - Responsible for glycolysis & protein synthesis
   - Activation of PPARã

Insulin Desensitization

- Besides tyrosine autophosphorylation, the IR has;

  - Both serine & threonine residues capable of autophosphorylation

  - Upon excess agonist activity, serine/threonine autophosp. causes a dissociation of IRS-1 without activation

  - Results in loss of function IR, or only activation of IRS-2
    * This is why we see ª IRS-2 activity in both Types

Insulin Desensitization

- Increased Fatty Acids
  - Elevated FFAs lead to accumulation of
    * DAG *fatty acyl-CoA
    * ceramide
  - These compounds are known to activate membrane bound PKCè
  - PKCè causes serine phosphorylation of IRS-1 in lieu of IR mediated IRS-1 tyrosine phosphorylation
    * Serine phosphorylation causes a dissociation between IRS-1 & PI3K

Insulin Resistance

3. TNFá and inflammatory adipokines
   - Chronic exposure to TNFá to 3T3-L1 adipocytes resulted in 90% « in GLUT-4 mRNA
   - TNFá has been found to:
     * Repress expression of IRS-1 & GLUT-4
     * Induce serine phosphorylation of IRS-1
     * Increase FFA plasma levels
   - TNFá levels >2.5x higher in both Type 1 & 2 than in healthy patients

PPARã

- Peroxisome-proliferator activated gamma (PPARã)
- A nuclear receptor when activated dimerizes with retinoic X receptor

- A downstream mediator of IR – MEK- ERK1/2 pathway
- Both PPARã & retinoic X receptor activation shown to enhance insulin sensitivity
- Ligands include mono- & poly-unsaturated fatty acids, PGs, the most commonly prescribed Type 2 diabetes medications thiazodolines (TZDs), and some NSAIDs (possible breakdown to AM404)

Functions of the PPARã

- Originally discovered to inhibit lipid peroxidation
- Agonist activity found to down regulate TNFá gene
- Stimulates adipocyte differentiation & apoptosis
  - Beneficial mostly for Type 2
- Represses gene expression of chemokines involved in insulin resistance:
  - Leptin * Plasminogen activator-inhibitor-1
  - Resistin * IL-6 & IL-11
- Induces gene expression of insulin sensitizing factors:
  - Adiponectin * Fatty acid transport protein
  - IRS-2

The Endocannabinoid System

- The CB1 & CB2 receptors are the most abundant G-protein coupled receptors in the human body
- Besides CB1 & CB2 endo- & phyto- cannabinoids also bind to the PPARã and TRPV1 vanilloid receptor
  - The vanilloid receptor is expressed both in the islet â-cells and smooth muscle cells
  - Vanilloid receptor activation found to enhance insulin secretion and sensitivity
- Anandamide (arachidonylethanolamide) & 2-AG (arachidonylglycerol) are endocannabinoids
  - These are under negative control of leptin

Endocann. Continued

- Leptin is a hormone secreted by adipose tissue and exerts its effects in the hypothalamus
- As previously mentioned, leptin increases insulin resistance
- Endocannabinoids are down-regulated by leptin
  - Leptin causes an inhibition in the MAPK stimulated glycogen synthase activity of the CB1 receptor

The Cannabinoid Receptors

- The CB1 & CB2 receptors
  - Both GPCR with $G_{\acute{a}i/o}$ coupling
  - CB1 also has $G_{\acute{a}s}$ coupling ability under certain conditions
  - Both coupled to activation of the PI3k-Akt/PKB pathway
  - Both receptors shown to activate MAPKs via the Ras/Raf pathway
    - P38 & p42/p44 MAPKs activated
    - Shown to increase glycogen storage, glucose metabolism, c-fos expression

CB Receptors Continued

- Both receptors found to activate PLC

    - PLC cleaves IP3
    - IP3 releases Ca2+ from intracellular storage vesicles
  - CB1 receptor also shown to inhibit K+ outflow & Ca2+ efflux
  - CB2 not coupled to ion channels

CB & IR Interactions
CB Agonists

- Thus CB1 activation beneficial to insulin sensitivity and glucose metabolism
- CB2 is found predominantly in immune cells & adipocytes
- CB2 activation in B-cells, macrophages, T-cells, and monocytes is found to:
  - Reduce TNFá, IL-2, IL-6, and IL-11; all elevated in diabetics and correlated to insulin resistance
  - Balance Th1/Th2 inflammatory cell profile
    - Autoimmune Type 1 diabetes has ª activation of T_H1/T_H2
    - IFN-ã, IL-12, and TNFá associated with ª T_H1, treatment with THC showed a marked decrease in mRNA levels of all

CB Receptors & â-Cells

- Insulin secretion by â-cells follows an oscillatory pattern
  - Stimulated by ª & « pattern of intracellular Ca2+
- Receptor localization:
  - CB1 found mostly on á-cells
  - CB2 found on both á- & â-cells
  - TRPV1 also found on â-cells
- Cannabinoids found to/may:
  - Reduce insulin secretion (metabolic syndrome X)
  - CB1 may reduce cAMP dependent release of glucagon
  - Enhance effects of insulin signaling

CB Receptors & â-Cells

- The Evidence:
  - Anandamide & 2-AG concentration in â-cells ª under hyperglycemic conditions and decreases under hypoglycemic conditions
  - Administration of insulin « endocannabinoid levels
  - Chronic activation of CB1 leads to up-regulation of PPARã (in adipocytes)
  - Personal data:
    - Smoking + insulin = ~18%> reduction in BGL
    - Smoking alone = ~8% reduction
    - No reduction when large quantities cannabis used + food
    - Dangerous enhancement between exercise + cannabis + insulin combination can reduce insulin by 1/5

Non-CB Mediated Effects

- Both endo- & phyto- cannabinoids bind to the PPARã receptor
- Diabetics have a marked reduction in immune function & O_2 transport
  - IgA glycosylation 4x ª in both types of diabetics w/o complications, 33% more in Type 1
  - IgM glycosylation ªeven in healthy diabetics, 8% more in Type1
  - Healthy individuals have 1-3% hemoglobin glycosylation, uncontrolled diabetics 20%

(diagnostic tool HbA1c)
- Poor O_2 transport by Hb leads to microangiopathies
- Other long lived proteins also get glycosylated; collagen, albumin, myelin

Non-CBR Mediated Effects

– Since protein glycosylation is an oxidative process, antioxidants have proven useful
  • Preventative effects of Cannabis derived antioxidants on Hb glcosylation at [.5], [5], and [10]ìg
    – Quercitan (flavanoid) 3%, 37%, 52%
    – Kaempferol (terpenoid) 10%, 12%, 15%
    – 20 other flavanoids, also THC, CBD, CBC, and CBG all have antioxidant properties
– Hb glycosylation a Fenton Reaction
  • NIH published paper on cyclic voltammetry & rat focal ischemia model: THC 20X potent the antioxidant than ascorbate

3. Cannabinoids (CBD) protect against myelin degradation, and excessive glutamatergic firing, a cause of one type of diabetic neuropathy (sensory)
   - NMDA receptor induced intracellular Ca2+ accumulations cause neurotoxicity

Diabetic Retinopathy

• 2 Phases:

- Nonproliferative
  • Neovascularization – resp. for dev. of new blood vessels in many tissues, especially the retina
  • Growth mediated by VEGF

- Proliferative phase
  • Advanced stages of retinopathy
  • Neovasc. Causes optic nerve damage & macular edema
  • Leading cause of blindness
  • ¾ all diabetics after 15 yrs

Retinopathy

• The VEGF Pathway
  – Also actiavtes the PI3K-AKT/PKB pathway (like the CB receptors)
  – Also activates the Ras/Raf dep. MAPK pathway just like the CB receptors
  – Yet again, also activates the PLCã-PKC pathway, and IP3 mediated intracellular Ca2+ release, like the CB receptors
  – How then, can cannabinoids be beneficial?

Retinopathy & The CB Receptors

How Cannabinoids Benefit Retinopathy:

• Remember, 20 flavanoids + cannabinoid are antioxidants
  - The eye is rich with FFAs which are subject to oxidation (COX-2), typically elevated in diabetics
  - Cannabinoids prevent superoxide anion formation, and increase fatty acid metabolism
- VEGF
  - While VEGFR2 & CB receptors share nearly identical transduction mechanisms, cannabinoids inhibit VEGF gene transcription via other receptors, may not share similar phosphorylation patterns
  - TNFá increases VEGF mRNA, as does the Ils that are inhibited by CB activation
- PEDF
  - Pigment epithelial derived factor, a potent inhibitor of neovascukaarization via VEGF
  - PEDF is inhibited by oxidative stress & TNFá

Conclusions

• Diabetes is a simple disorder with complex pathways regulating insulin resistance/sensitivity and secondary pathology
• Nearly all complications to diabetes are the result of hyperglycemia

- After reviewing the IR, PPARã, CB1, CB2, and VEGF, we find that cannabinoid therapy for diabetes can:
  - Reduce BGLs 2. Reduce HbA1c
  - ª insulin sensitivity 4. ª glucose & lipid metabolism
  - Prevent retinopathy 6. Inhibit inflammatory chemokines
  - Neuroprotection 8. Improve O_2 transport

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