The Endocannabinoid System: Directing Eating Behavior and Macronutrient Metabolism

Watkins and Kim 2015

Council of Hypothalamic Metabolites
Agenda

- The Endocannabinoid System (ECS)
  - Intro
  - The ECS in the Brain and Peripheral Organs
  - Endocannabinoid Biosynthesis and Degradation

- Relationship of the ECS and Common Food Intake/Modulation Hormones
  - Control of Food Intake
  - Dietary PUFA and the Endocannabinoids
  - The Muscle, Liver and Adipose

- Conclusion
Intro the ECS and Endocannabinoid Research
Searching for an Answer and Solution

- Food Related Diseases
  - Greater time has been spent looking at the effects that endogenous cannabinoids can have in the homeostasis of our bodies given factors mainly determined by diet of choice
  - Given the effects of Cannabis scientists decided to look at the effects as a probable explanation in over eating in individuals that may have a certain imbalance of an endogenous ligand similar to those provided by cannabis
First Glimpse of the Endocannabinoid System

- **1960’s**
  - The findings of Endocannabinoids and the Endocannabinoid System all began with the discovery for the localization and function of the psychoactive properties of Cannabis in the brain that were merely theorized as early as the late 19th century.

- **1980s and 1990s**
  - Scientists were able to discover both Cannabinoid Receptors and thereafter the Endocannabinoids that our bodies produce.
The Endocannabinoid System

- Responsible for Managing:
  - Energy Intake
  - Metabolism Storage
  - Nutrient Transport

- Two types of GPCRs
  - CB1 Receptors
    - Mainly located in the Brain, digestive track and adipose tissue
  - CB2 Receptors
    - Mainly located in peripheral organs and areas associated with the immune system
Current Dietary Factors on the Synthesis of Endocannabinoids

- PUFA (Polyunsaturated Fatty Acids)
  - Endocannabinoids are products of dietary fatty acids (FA) when they were originally thought to be generated only by demand
    - EC derived from n-6 PUFA Compounds: Linoleic Acid which is synthesized to Arachidonic Acid. AA is the omega-6 fatty acid which helps convert lipid precursors into the endocannabinoids AEA and 2-AG
    - EC derived from n-3 PUFA Compounds: Eicosapentaenoylethanolamide from eicosapentaenoic acid (EPA) and Docosahexaenoylethanolamide from docosahexaenoic acid (DHA)
Overview of the Endocannabinoid System in the Brain and Peripheral Organs
Overview of the Endocannabinoid System in the Brain and Peripheral Organs

- Endocannabinoid system (ECS)
  - Cannabinoid receptors
  - Biosynthesis and degradation enzymes of ECS ligands
  - Signaling pathways

- Cannabis
  - Tetrahydrocannabinol (THC)
  - Cannabinoid receptor 1 (CB1)
Overview Continued

- **Endocannabinoids**
  - Anandamide (AEA)
  - 2-Arachidonoylglycerol (2-AG)
    - Paracrine or autocrine fashion

- **CB1**
  - Liver, muscle, adipose tissue

- **CB2**
  - Immunocompetent cells
  - Bone, muscle, heart, CNS
Overview Continued

- G(i/o) protein coupled receptors
  - Signal transduction pathway
  - Inhibition of adenylyl cyclase, cAMP, protein kinase A
    - Adenylyl cyclase: enzyme that catalyzes cyclization of ATP into cAMP (cyclic adenosine monophosphate)
    - Protein kinase A (PKA): enzyme that phosphorylates other proteins and is regulated by fluctuating levels of cAMPS in cells
      - Responsible for cellular responses due to cAMP second messenger system
  - Therapy?
Overview Continued

- Endocannabinoid System
  - Modify activation of receptors to selectively direct downstream actions
    - Potentially:
      - limit food intake
      - reduce obesity
      - Improve insulin sensitivity
  - n-3 PUFA required for proper cannabinoid signaling
    - moderate amount of AEA and 2-AG in cells
      - Alter ECS signaling → decrease over stimulation of signaling and associated negative health impacts
Endocannabinoid Biosynthesis and Degradation
Endogenous Cannabinoids Biosynthesis and Degradation

- Anandamide (AEA)
  - Main Pathway: NAPE-PLD Pathway
- 2-Arachidonoylelglycerol (2-AG)
  - 2 Pathways in which 2-AG is synthesized
- Degradation
  - Degradation of AEA, and to a lesser degree 2-AG, occurs by FAAH to produce AA and an ethanolamide. The degradation of 2-AG occurs by MAGL to form AA and monoglycerol
Step 1 of NAPE-PLD Synthesis Pathway (Anandamide)

- Begin with the Lipid Precursor: Phosphatidylethanolamine
- Arachidonic Acid (AA) is cleaved as a Phosphatidylethanolamine by N-Acyltransferase (NAT) to form (NAPE)
Step 2 of NAPE-PLD Synthesis Pathway (Anandamide)

- NAPE-PLD is the enzyme that catalyzes the release of N-acylethanolamine (NAE) from N-acyl-phosphatidylethanolamine
  - When AA is the starting substrate, the released NAE is Anandamide (AEA)
Step 1 for Synthesis of (2-AG)

- Begin with the Lipid Precursor: Phosphatidylinositol
  - Phosphatidylinositol can be hydrolyzed by Phospholipase C to form 1,2 Diacylglycerol
  - In combination with AA, Phosphatidylinositol can be hydrolyzed by Phospholipase A1 to form 2-arachidonoyl-lysophospholipid (lysoPI)
Step 2 for Synthesis of (2-AG)

- @ (lysoPl) is then hydrolyzed by lysoPhospholipase C to form 2-AG
- @ (1,2 DAG) is the hydrolyzed in combination with AA by Diacylglycerol Lipase (DAGL) to form 2-AG
Degradation (Anandamide)

- Fatty Acid Amide Hydrolase interaction with Anandamide produces AA and an Ethanolamide
Degradation (2-AG)

- Occurs by Monoacylglycerol Lipase (MAGL) to form AA and monoglycerol
Endocannabinoids and Control of Food Intake

- CNS
- GI tract
Brain Signaling Review

- How EC system and food intake are related
  - Hypothalamus, homeostasis, and eating behavior
  - EC system in hypothalamus
    - High concentration of CB1 found
  - EC system and obesity
  - ECs as retrograde messengers on CB1
    - at presynaptic glutamatergic terminals
    - inhibition of glutamate (excitatory NT)
    - overall suppressive effect on neuroendocrine
Cannabinoid Receptor Signal Transduction
- (Endo)cannabinoids, energy storage, and hyperphagia
  - Δ-9-THC
  - Evolutionary perspective (Piazza et al. 2007)
  - Fasted rats => ↑ AEA
  - Hunger and EC level, EC and hunger drives

- **ECs controls food intake in two ways:** (Di Marzo and Matias, 2005; Monteleone et al., 2005)
  1. Reinforces the motivation to find and consume food with high incentive value
  2. Induces appetite by regulating levels and actions of orexigenic and anorectic mediators

- **ECs induce increase in food intake even in satiated rats**
  - Peripheral injection of AEA
  - Nucleus Accumbens (shell) injection of 2-AG
  - Can be attenuated/prevented by CB1 antagonist treatment/pre-treatment
  - CB2 antagonist had no effect
Brain Signaling Review (cont.)

- CB1 antagonist
  - SR141716 a.k.a rimonabant
  - Rimonabant treatment and increased adiponectin level
    - Adiponectin
  - Side-effect of Rimonabant: anxiety, depression, and etc.

- Leptin lowers EC level in hypothalamus
  - High level of EC found in ob/ob and db/db mice

- CB1 and orexigenic and anorectic mediators
  - CRH (corticotropin releasing hormone, anorectic) in paraventricular nucleus of HT
  - MCH (melanin-concentrating hormone, orexigenic) in lateral HT
  - Pre-pro-orexin (orexigenic) in VM HT
  - CB1 KO mice
    - higher levels of CRH mRNA
    - implications
Gastrointestinal Tract

The GI is a site for EC production

EC signals from the small intestine to the brain regulate energy balance

CB1 is present in the peripheral nervous system connected to the GI

EC mediate the nervous signals sent from orexigenic/anorexigenic hormones
Experimental Overview

- AEA injection in satiated rats = CB1 activation and ↑ appetite
- Injection of ghrelin + CB1 antagonist = normal food intake (no ghrelin effect)
- Injection of leptin or CCK = ↓ 2-AG and AEA
- Inactivation of CB1 = ↓ plasma insulin and leptin
- EC-KO mice = ↑ CRH mRNA /
- Fasting = ↑ AEA levels in small intestine /
- Fasting + consequent eating = ↓ hypoth. 2-AG and vagus/intestinal CB1 activation
- Fatty-sham feeding = also ↓ CB1
- Fasting + EC blocking = ↓ food intake effect
- CB1 activation = ↑ food intake and weight gain
- ob/ob and db/db mice or diet-induced obese mice = ↑ 2-AG and AEA levels /
- CB1 antagonist in obese mice = reverse leptin resistance and ↓ obesity
What we learned from that

Mainly orexigenic

Mediation of energy balance and food intake

EC levels are responsive to nutrient status

High in fasting, low after eating

Response to cephalic signals

Overactivation of the ECS is indicative of obesity

EC levels mediate leptin status

They interact with several other hormones to accomplish their purpose (leptin, ghrelin, insulin, CCK, CRH)
Dietary PUFA and the Endocannabinoids
Dietary PUFA: Effects on Cells

- Plasma membrane PUFA composition
- n-3 PUFA changes phospholipid composition
- Changes in concentrations of long chain n-3 PUFA (EPA and DHA) in vivo and n-6 PUFA (specifically AA)
- Changes to substrates for biosynthesis of EC
- Physiological effects: changes in hunger, satiety, energy consumption and food intake modulation
Changes in EC Levels

- Responsive to substrate PUFA availability
- n-6 PUFA rich diets (higher AA levels) induced greater 2-AG and AEA levels in the brain, liver, and small intestine
- n-3 PUFA rich diets (higher DHA and EPA) has the opposite effect; decreases AA levels and decreases EC in the brain, small intestine, liver, visceral adipose tissue, and plasma
- EC are products of dietary lipids; links how changing our diet can induce changes in our overall EC levels
Differences Between n-3, n-6, and Other Types of PUFA

- Oleic acid -> oleoyl ethanolamide: induces satiety, decreases FA concentration, increases capacity for \(\beta\)-oxidation, and inhibits action of AEA and 2-AG in adipose tissue
- n-6 PUFA: formation of AEA and 2-AG; support excessive energy intake, weight gain
- n-3 PUFA: prevents neuropsychiatric diseases, stops negative effects of non-functional or desensitized CB1 receptors
Case Study: Effects of Controlled Diets

- 80 C57/blk6 mice 21 days old
- Given a modified diet (AIN-93G): contains 11.04% fat (isocaloric and isonitrogenous)
  - Assigned with safflower oil to the control group or
  - Assigned with DHA-enrichments to the experimental group
- Results shown after 62 days and 118 days
  - After 62 days: DHA diet mice had higher levels of 14:0, 16:1n7, 18:1n9, 18:2n6, 20:3n6, 22:5n3, and 22:6n3 (DHA)
    - Ratio of n-6/n-3 PUFA in the brain DHA vs control: 0.71 vs 1.26
    - Ratio of AA to DHA in the diet fed mice: 0.48 vs 0.77
  - After 118 days: DHA diet mice had higher levels of 14:0, 18:1n9, 18:2n6, 20:3n6, 20:5n3 (EPA), 22:5n3, and 22:6n3 (DHA)
    - Ratio of n-6/n-3 PUFA in the brain DHA vs control: 0.71 vs 1.26
    - Ratio of AA to DHA in the diet fed mice: 0.48 vs 0.86
Case Study: Effects of Controlled Diets cont.

- Both intervals (62 and 118 days) produced similar results
- Dietary PUFA alters the fatty acid composition of the brain and the substrates for the biosynthesis of the EC
- n-3 PUFA supplemented diets did increase concentration of EPA and DHA and decrease AA
- But there are more complicated changes based on the other aspects of the body’s biochemistry
Diet, Metabolism, and Peripheral Organs
Metabolism and the ECS

- Macronutrients:
  - Carbohydrates
  - Fats
  - Lipids

- Macronutrient Metabolism
  - Gastrointestinal tract
  - Muscle
  - Liver
  - Adipose
  - Endocrine system
Gastrointestinal Tract

- Gut motility
- Activated cannabinoid receptors: inhibition of peristalsis and gastric secretion, increase in food intake
- Nick DiPatrizio
  - EC signaling in gut drives overconsumption
  - Chronic consumption of high-fat/high-sugar foods → increase in EC
  - Blocking EC from receptors decreased overconsumption
- THC → “munchies”
Mouse Diet and Obesity

- n-3 PUFA consumption may help reduce obesity and diabetes
- Mice fed DHA:
  - Increase in gene expression for cannabinoid receptors, enzymes for EC synthesis/degradation, glucose metabolism
  - Enhance glucose uptake in myoblasts
  - Adiponectin increase
  - Favor fatty acid oxidation and decrease fat accumulation
Mouse Diet and Obesity Continued

- ob/ob mice
  - Recessive mutation
  - Excessive food consumption
  - Leptin deficient
    - Uncontrolled food intake
    - Increased levels of orexin (OX)
    - Overexpression of 2-AG
  - Possible treatment for human obesity
Obesity and Peripheral Organ Actions

- ECS controlling Food Intake
  - Anorexigenic and Orexigenic pathways
    - Ghrelin, leptin, adiponectin, endogenous opioids, and corticotropin-releasing hormones are involved (Viveros et al., 2008).
  - Dysregulates various peripheral organs that are key components of metabolism

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Adipose and Liver

- Adipose Tissue and Liver
  - Increase in Endocannabinoid Release in conjunction with an increase in CB1R expression and the depletion of FAAH
    - This will create a cycle if a diet is mainly built on n-6 PUFA consequently increasing EC produced by Arachidonic Acid
  - Evidence suggests the activation of CB1 receptors in these peripheral tissues promotes lipogenesis, lipid storage, insulin secretion, glucagon secretion and adiponectin modulation
Conclusion
Future Research

- Complex relationships between endogenous ligands, receptors, and signaling
- Modifications to the ECS by endogenous ligands to blunt food intake and improve skeletal muscle response to glucose and insulin
- Downstream action in the signalling pathways and their relative compatibility
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