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THE BRAIN ENDOGENOUS CANNABINOID SYSTEM: A ROLE IN REWARD/CRAVING OF ADDICTION?

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ABSTRACT. CANNABINOID SIGNALING in the central nervous system and periphery of mammals is controlled through a mechanism known as the endogenous cannabinoid system (ECS) that consists of the cannabinoid receptors 1 and 2, a growing family of endogenous neuromodulatory fatty acid amide ligands, the "endocannabinoids", and their principal inactivating enzyme, fatty acid amide hydrolase (FAAH). The ECS represents a product of vertebrate nervous system evolution that is now recognized as an important retrograde signaling pathway for continuous modulation of the release of many classical neurotransmitters while also having the potential to influence diverse and at times profoundly abnormal neurobehavioral consequences under pathological conditions. The purpose of this review is to summarize some of the recent advances in our knowledge of the ECS and to propose a hypothesis for a role of the ECS as a risk factor for reward and craving behaviors in some addictions. Evidence is presented that the ECS influences addictive behavior in both animal models and human disorders of reward and craving. These findings support the concept that brain endogenous cannabinoid signaling may be a useful future target for novel therapeutic strategies in selected addiction disorders.

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1. INTRODUCTION

HERBAL CANNABIS has been used and abused by humans for thousands of years due to its medicinal and psychoactive properties but only in slightly more than one decade have the molecular mechanisms of the brain endogenous cannabinoid system (ECS) been revealed as a basis for understanding cannabinoid neurobiological effects (see ref. [1] for historical review). With the cloning and sequencing of the rat [2] and human [3] cannabinoid 1 receptor (CB1), it became evident that the ECS is a phylogenetically ancient signaling pathway that was only discovered recently and that has the potential to be a unique modulator of nervous system physiology and behavior. Both exogenous cannabinoids like delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis, and the neuromodulatory endogenous cannabinoids bind the CB1 receptor located in the presynaptic membrane of many synaptic terminals to result in a retrograde signaling mechanism that acts backwards to inhibit release of neurotransmitters in many neuronal circuits in the brain [4,5]. In the reward and craving behaviors of drug abuse, the most relevant neuronal circuits are located within the ventral striatum in the mesolimbic addiction and reward pathway where CB1 receptors are located on inhibitory interneuron terminals on dopaminergic synaptic projections to the nucleus accumbens septi [6-8]. In this pathway, cannabinoids, binding to CB1 receptors may downregulate gamma amino butyric acid (GABA) inhibitory transmission by depolarization induced suppression of inhibition [4,9,10] and through GABA disinhibition may, in turn, upregulate dopamine release in the nucleus accumbens (FIG. 1) that has been associated with addiction/reward behaviors [11]. In animal gene deletion experiments, mice with CB1 receptor deletion lack the addiction-reward response for morphine [12] and alcohol [13]. Because endogenous cannabinoids bind to the same CB1 G-protein-coupled receptors in the brain as THC and since cannabinoid signaling is involved in many human reward behaviors, the hypothesis advanced here is that ECS malfunction with increased endocannabinoid tone in critical brain circuits could result in the facilitation of reward and craving behavior in some vulnerable individuals potentially increasing susceptibility to some drugs of abuse or other addictive disorders.

2. THE ENDOGENOUS CANNABINOID SYSTEM (ECS)

Cannabinoid signaling in the mammalian central nervous system (CNS) and periphery is mediated by a growing family of endogenous neuromodulatory fatty acid amide lipids known as the "endocannabinoids" [14,15]. The molecular binding targets of the endocannabinoids are the two known cannabinoid receptors, CB1 and CB2, which also bind the main psychoactive compound of cannabis, THC [1,16]. CB1 receptors are expressed at high levels in specific CNS regions and selected tissues throughout the body, mostly in peripheral nervous system endings and innervated organs, including the gut, heart, lung, and reproductive organs [1]. CB2 is present mainly in immunocytes, particularly lymphocytes, macrophages and natural killer cells [17,18]. This system, now known as the endogenous cannabinoid system [19], is a retrograde signaling pathway with the capacity to modulate the ongoing release of chemical signals and transmitters [10,20-22]. In the nervous system, endocannabinoids act to inhibit the release of most of the known excitatory, inhibitory and specialized neurotransmitters by activation of the presynaptic G-protein coupled receptor, CB1 in the CNS or elsewhere in the body [14,19]. It would be expected that if endocannabinoids serve an important signaling role in the nervous system or in the body, these amidated lipids would be closely linked to a rapid inactivating mechanism (catabolic enzyme) capable of locally modulating endocannabinoid levels at multiple sites of action [15].

Recently, an integral cell membrane enzyme in CNS synapses and liver cells was found to inactivate expeditiously a variety of the endogenous neuromodulatory fatty acid amides that are ligands for CB1 and this enzyme has been designated the fatty acid amide hydrolase (FAAH) [15,23]. FAAH was isolated, cloned and sequenced in humans and lower mammals where comparative analysis demonstrates phylogenetic conservation with greater than 80% amino acid sequence identity [24,25]. Several studies now show that FAAH is capable of inactivating many endogenous fatty acid primary amides and N-acyl-ethanolamides with similar

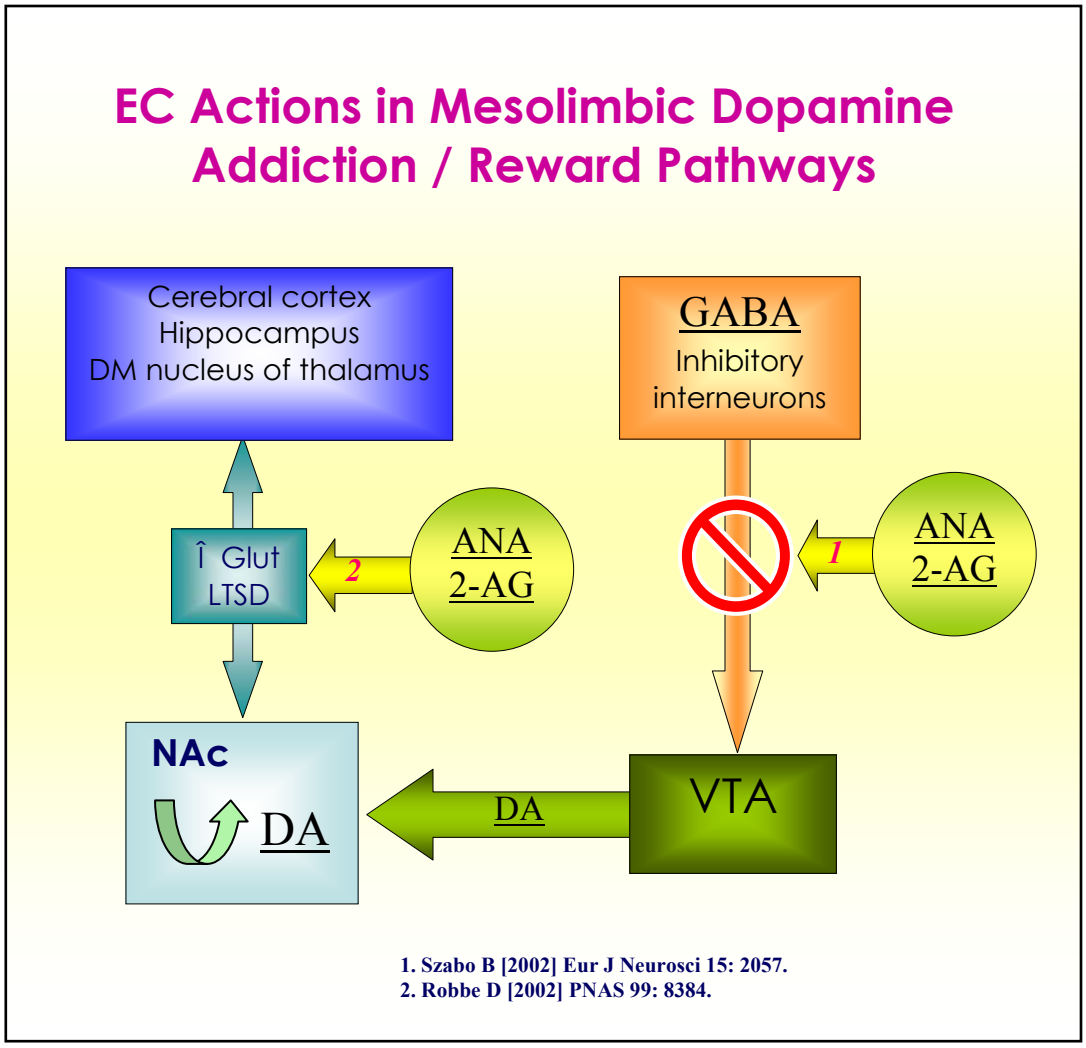

FIGURE 1. SCHEMATIC DIAGRAM OF ENDOCANNABINOID ACTIONS IN THE VENTRAL STRIATUM. Elevated ANA (anandamide) and 2-AG (2-arachidonylglycerol) due to impaired FAAH function could inhibit GABA inhibition in the VTA and increase long term depression in nucleus accumbens (NAc) excitatory glutamatergic synapses by translating post synaptic glutamatergic signaling to presynaptic CB1 signaling. Both mechanisms may increase dopamine levels and induce long-term synaptic plasticity in the mesolimbic dopamine addiction/reward circuits.

hydrolytic efficiencies [26]. FAAH was found to hydrolyze rapidly anandamide and several other bioactive fatty acid amides including 2-arachidonyl glycerol, N-palmitoyl ethanolamide and the sleep-inducing lipid, oleamide [19,26]. Most of these bioactive lipids are endogenous ligands for receptors of the endocannabinoid system, and anandamide, both a CB1 and CB2 agonist, is the most well studied of these [14,27]. Thus, one of the most biologically relevant properties of FAAH is that this enzyme appears to be the principal inactivating enzyme for a broad range of fatty acid amides that are being increasingly recognized as serving a wide variety of neuromodulatory functions in the CNS with behavioral consequences together with functions in other peripheral organs. Genetically engineered mice with functional deletion of FAAH showed markedly elevated levels of brain anandamide and supersensitivity to the biological effects of injected anandamide suggesting that FAAH controls both the amplitude and duration of the endocannabinoid response in vivo and that

abnormal FAAH function may have neurobehavioral sequelae [28]. Moreover, the pharmacological activity of different endocannabinoids appears to be regulated by FAAH activity in vivo [29].

3. ENDOCANNABINOID SIGNALING IN MAMMALIAN BRAIN

Recent reviews of the ECS have demonstrated the widespread distribution of CB1 receptors, FAAH and endogenous ligands in specific brain regions known to be important in memory, motor control and drug abuse and reward behaviors [1,30]. Iverson [31] has summarized the current literature on cannabinoids and the brain, including the endocannabinoids and their distribution in the mammalian brain. Using specific high affinity cannabinoid receptor ligands or antibodies, the neuroanatomical distribution of CB1 receptors and FAAH has been mapped in the brain of mammals [1,32,33]. These studies reveal that CB1 receptors are mainly present on synaptic terminals and axons but are mostly absent from neuron cell bodies and dendrites. The highest densities of CB1 receptors in animals and man are in the cerebral cortex, especially the frontal regions, the hippocampus and anterior cingulate cortex, the basal ganglia including the ventral striatum (mesolimbic addiction/reward pathway including the medial forebrain bundle and nucleus accumbens), and the cerebellum. High levels of FAAH protein expression were found to be correlated with brain regions enriched in CB1 receptors [30] and this supports the concept that FAAH is positioned for prompt regulation of endocannabinoid levels locally at the synaptic level in critical brain circuits [29].

The current model of the brain ECS is one of an important retrograde signaling mechanism [4] in which endocannabinoids are released by the post-synaptic cell in response to synaptic activation by many types of neurotransmitters, then travel backward across the synaptic cleft to bind the CB1 receptor and produce inhibition of pre-synaptic transmitter release by activating G-protein coupled signaling cascades and subsequently are inactivated by FAAH situated in the postsynaptic membrane. The crystal structure of FAAH has recently been elucidated and confirms the membrane bound hydrolytic characteristics of this enzyme that controls and terminates brain endocannabinoid signaling at synapses [34]. Thus, endocannabinoids are critically positioned with abundant synaptic expression in brain regions associated with the reward and craving aspects of drug abuse. Considering that elevated dopamine levels in the striatum and mesolimbic addiction/reward pathway are thought to be of central importance in the rewarding aspects of several drugs of abuse [35], endocannabinoid activity has been linked to the inhibition GABA release which in turn can result in increased dopamine levels in the nucleus accumbens of the addiction/reward pathway [9]. Therefore, pathophysiological alterations of FAAH function or other components of the ECS may be in a position to alter behavior that could increase the risk of reward or craving behaviors in susceptible individuals.

4. NEUROBIOLOGY OF ENDOCANNABINOIDS IN THE BRAIN

Several remarkable studies demonstrating the critical neurophysiological role of endocannabinoids have recently been published by different research groups. In the brain, the phenomenon of depolarization induced suppression of inhibition (DSI), a fast feedback system from post-synaptic neurons to pre-synaptic inhibitory terminals was shown by Wilson and Nichol [4] to be due to endocannabinoids acting as retrograde transmitters to suppress release of an inhibitory transmitter. Independent experimental work by Ohno-Shosaku et al. [22] reached the same conclusion about DSI and identified the inhibition of GABA release as the neurotransmitter altered by cannabinoid effects. Additional support came from Varma et al. [36] who reported that DSI was entirely absent in hippocampal slice preparations from mice with CB1 receptor gene deletion. Kreitzer and Regehr [37] determined that retrograde signaling is not limited to inhibitory synapses by demonstrating an endocannabinoid mechanism in depolarization-induced suppression of excitation (DSE). The CB1 receptor and endocannabinoids are present in brain memory regions [10,38] and a recent study found extinction of aversive memories by selective cannabinoid inhibitory effects on GABA inhibitory networks in the amygdala [39]. Recent studies have

shown that long-term depression (LTD) in the striatum and nucleus accumbens is mediated by endocannabinoids acting as retrograde messengers [8,40] in these brain circuits associated with drug reward and craving. Collectively, these studies confirm that endocannabinoids are involved in both rapid, short-term and long-term modulation of several types of neurotransmitters in multiple neuronal networks or circuits and thus, may play an important role in modulation of neural activity and the associated psychomotor behaviors, for example reward/craving, controlled by these circuits [41].

5. PRECLINICAL EVIDENCE OF THE ECS IN DRUG REWARD AND CRAVING

Recently studied animal models of drug abuse have provided accumulating evidence of endocannabinoid modulation of synaptic transmission in brain regions associated with drug reward or craving. As noted above, activation of CB1 receptors in the ventral tegmental area inhibits GABA inhibitory transmission that in turn increases dopamine release in the nucleus accumbens of the addiction/reward pathway [9]. In addition, a CB1 receptor agonist reduced psychostimulant drug self-administration in rats while a CB1 receptor antagonist significantly increased it, suggesting that the ECS could play different roles depending on the particular drug of abuse under study [42]. It has been further suggested that psychodepressant drugs such as morphine and heroin are likely to be under the tonic control of the ECS [35]. Tanda and Goldberg [43] have reviewed the recent preclinical data on the neurochemical mechanisms of the cannabinoids underlying drug reward and dependence. This report summarizes the major functional interactions between the endogenous cannabinoid, opioid and dopamine neurotransmitter systems in drug reward, reinforcement and tolerance. Animal models of cannabinoid addiction have also been the subject of a recent review and the evidence provides support for interaction with opioid and dopamine transmission in brain reward circuits [44]. Morphine dependent rats were recently found to have region-dependent changes in CB1 receptor binding sensitivity in regions implicated in drug dependence [45].

Behavioral studies have demonstrated that the selective CB1 receptor antagonist, SR141716A, attenuates relapse to cocaine in a drug reinstatement paradigm while a synthetic CB1 receptor agonist, HU210, provokes a relapse to cocaine seeking after prolonged withdrawal in laboratory rats [46]. The endocannabinoid, 2-arachidonyl-glycerol (2-AG) was recently shown to attenuate naloxone-precipitated withdrawal signs in morphine dependent mice suggesting a role for cannabinoid signaling mechanisms in morphine dependence [47]. Because there is functional cross-talk between cannabinoid and opioid systems, a study designed to evaluate the reward and motivational effects of heroin revealed that these effects are mediated at least in part by CB1 receptors in the ECS [48] and this work suggested a rationale for the use of cannabinoid antagonists in the treatment of opioid addiction. Inhibition of methamphetamine self-administration in rats was also shown by the use of the cannabinoid receptor antagonist, AM251, again suggesting a role for synthetic antagonists in the treatment of stimulant abuse [49]. These and other preclinical studies add to the mounting evidence that endocannabinoids and their receptors [50] are involved in a constant process of modulation and adaptation within the brain addiction/reward pathways that may influence the reward and craving aspects of drug seeking behavior and that could contribute to reward or reinforcement in chronic drug abuse (reviewed in Gerdeman et al. [41]). In addition to the direct addictive potential of cannabinoids [51], there is evidence that cannabis may influence the sensitivity or vulnerability to other drugs of abuse [51-53]. Thus, in both animal and human studies, there is evidence from independent studies that repeated use of cannabinoids might facilitate progression to the consumption of other illicit drugs in vulnerable individuals [51,54-56].

6. POTENTIAL ROLE OF THE ECS IN HUMAN DISORDERS

6.1. DRUG ABUSE/ADDICTION AND ALCOHOL

A number of studies have investigated possible connections of the ECS with human disorders but most have focused on the possible role of the CB1

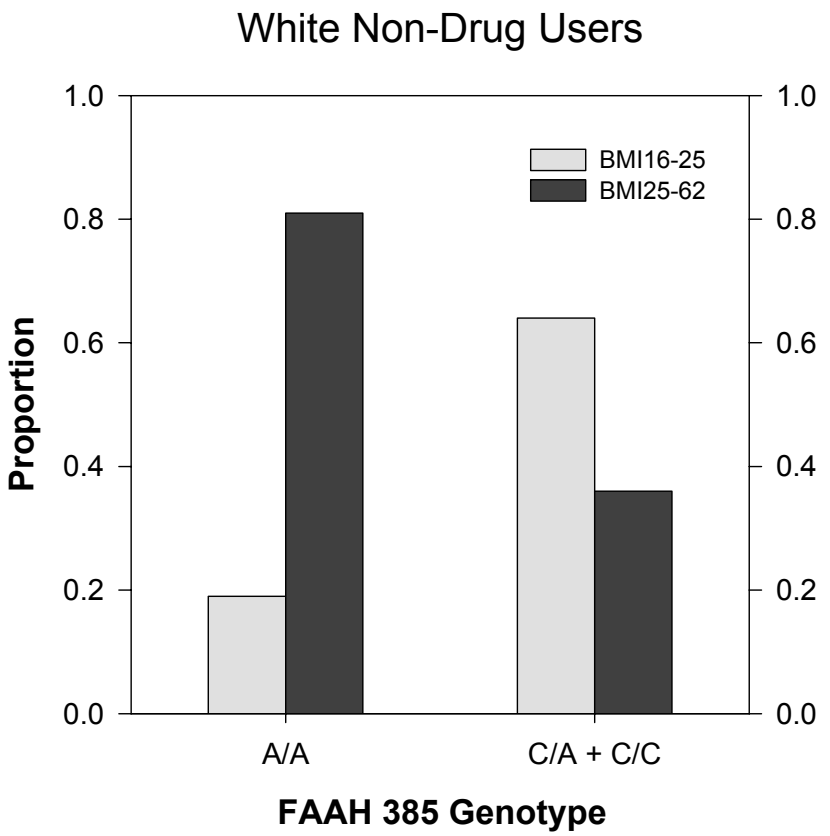

FIGURE 2. BAR GRAPH OF ALL WHITE SUBJECTS WHO DENIED ANY STREET DRUG USE (N = 1688) AND WHO WERE SELECTED RANDOMLY AND GENOTYPED FOR THE FAAH 385 LOCUS. Proportion of genotypes are shown for normal weight subjects (BMI = 16-25) and combined overweight and obese subjects (BMI = 25-62). The homozygous 385 A/A mutation genotype is over-represented in the overweight/obese group (Fisher exact test; P = 0.0069, odds ratio = 0.43).

receptor in neurobehavioral disorders or drug addiction. Typically, investigations have not shown an association of CB1 gene mutations with psychiatric disorders or addiction except in the case of isolated reports of a triplet repeat polymorphism in the CB1 promoter associated with mood disorders [57] and intravenous drug use [58]. Cannabinoid transmission in the reward/reinforcement circuits has been implicated in the addictive potential of cannabinoids in vulnerable individuals [52,59] and suggests a potential mechanism for activation of reward-relevant pathways by endogenous cannabinoids. Self-administration or craving behavior has recently been demonstrated in a primate model maintained by the psychoactive ingredient of marijuana [60]. Another study has shown blockade of the effects of smoked marijuana in humans by the selective CB1 antagonist, SR141716A [61]. These and other studies [43] have suggested a potential role for the ECS in human disorders characterized by abnormal reward or craving responses.

In an evaluation of potential ECS genetic risk factors for reward/reinforcement, Sipe et al. recently published a population study of the gene encoding fatty acid amide hydrolase (FAAH), the principal inactivating enzyme for most endocannabinoids and identified a naturally occurring missense mutation in FAAH (cDNA 385 C→A) that results in a conserved amino acid substitution, Proline 129→Threonine (P129T) and was associated with increased likelihood of street drug use and drug or alcohol abuse [62]. Biochemical studies of the P129T variant revealed an increased sensitivity of this mutant FAAH protein to proteolysis [62].

Subsequent FAAH enzyme activity studies in isolated human lymphocytes have shown a statistically significant >2-fold deficiency of FAAH enzymatic activity in homozygous FAAH 385 A/A mutant subjects compared to wild type 385 C/C matched control subjects (Sipe et al., unpublished data).

Alcohol abuse may be under different complex controls than some of the other drugs of abuse but there are suggestions that endocannabinoids may also play a neuromodulatory role in the development of alcoholism [63]. One recent report observed an association of a CB1 receptor gene polymorphism with severe alcohol dependence and increased risk of delirium [64]. Endocannabinoid signaling is involved in ethanol preference in mice since CB1 inhibitors and CB1 gene deletion results in significantly reduced ethanol and sucrose intake [65,66]. However, in our population study of the FAAH 385 mutation associated with street drug abuse there was no observable association of this missense mutation with alcohol overuse [62].

6.2. NATURAL REWARDS: OVERWEIGHT AND OBESITY

Natural rewards such as food and sweets are likely to be controlled by the same or similar neural reward and craving circuits modulated by the ECS. Available evidence indicates that endocannabinoids regulate both feeding behavior through reward/craving pathways [67] and body fat metabolism via hypothalamic peptidergic circuits [68,69]. Down-regulation (activation) of CB1 receptors associated with dietary obesity was found in the nucleus accumbens and hippocampus of rats suggesting an endocannabinoid role in the hedonic aspects of eating [70]. The evidence for a role of cannabinoid signaling in both the homeostatic and hedonic control of eating has recently been reviewed by Harrold and Williams [71] and leptin regulated tonic endocannabinoid activity appears to be involved in maintaining food intake [72]. To assess the possible role of the ECS in dietary overweight and obesity, Sipe et al. recently completed a preliminary study of the frequency of the FAAH 385 mutation in nearly 1700 white non-drug using subjects stratified by body mass index (BMI) while attending a health maintenance clinic for annual examinations (FIG. 2). The frequency of the FAAH 385 homozygous A/A mutation was found to be approximately 2% in normal BMI subjects (N=594) but nearly 5% (>2-fold increase) in overweight and obese subjects (N=1094) (Fisher exact test, P=0.0069) (Sipe et al., unpublished data). This epidemiological study suggests that the FAAH 385 A/A (P129T) mutation may be one risk factor in Whites for susceptibility to reward behaviors, including street drug abuse and natural food rewards associated with overeating and obesity. Collectively, these findings support the hypothesis that abnormal brain cannabinoid signaling may constitute important risk factors for both natural and drug-induced disorders of reward and craving and suggest a potentially expanded view of reward or craving behavioral consequences associated with the homozygous FAAH 385 A/A naturally occurring mutation that may range from drug abuse to natural rewards in susceptible individuals.

7. FUTURE CONSIDERATIONS

In a current review of mechanisms of drug addiction, Cami and Farre [73] emphasize the multiple and complex factors influencing drug abuse and dependence. These include the properties of individual drugs of abuse, concurrent neurobehavioral or psychiatric disorders, genetic risk factors and social or environmental factors among others. Understanding the complex picture of drug abuse and addictive disorders will require a detailed evaluation of the factors associated with specific drugs of abuse to determine the exact role and degree of contribution of each factor. In the future more data will be available to test the hypothesis of the ECS as a risk factor for vulnerability to disorders associated with reward and craving behaviors. Current work includes evaluating the frequency of the FAAH 385 mutation in subjects with abuse of the major classes of drugs of abuse and in different overweight/obese ethnic groups to more precisely correlate this risk factor with specific drug or natural reward disorders.

The ECS is now an attractive candidate in humans and other mammals for the study of addiction and reward behavior since it has been linked to modulation of critical brain functions and behavior under normal physiological circumstances

and has been implicated as a potential pathological mechanism during endocannabinoid malfunction. Genetic or protein abnormalities could produce functional dysregulation of the ECS with potential clinical consequences but the specific risk factors for at least the initial reward or craving aspects of drugs, food or other addictions remain to be elucidated. Nonetheless, the ECS appears to be gaining importance as a possible clinical therapeutic target in reward and craving disorders through the application of novel synthetic chemical, genome or proteome based technologies.

8. ACKNOWLEDGEMENT

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9. REFERENCES

[1] ELPHICK MR AND EGERTOVA M [2001] The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc Lond B Biol Sci 356: 381-408.

[2] MATSUDA LA, LOLAIT SJ, BROWNSTEIN MJ, YOUNG AC AND BONNER TI [1990] Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346: 508.

[3] GERARD CM, MOLLEREAU C, VASSART G AND PARMENTIER M [1991] Molecular cloning of a human cannabinoid receptor which is also expressed in testis. Biochem J 279: 129-134.

[4] WILSON RI AND NICOLL RA [2002] Endocannabinoid signaling in the brain. Science 296: 678-682.

[5] CHRISTIE MJ AND VAUGHAN CW [2001] Neurobiology: Cannabinoids act backwards. Nature 410: 527-530.

[6] HOFFMAN AF AND LUPICA CR [2001] Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. J Neurophysiol 85: 72-83.

[7] ROBBE D, ALONSO G, DUCHAMP F, BOCKAERT J AND MANZONI OJ [2001] Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. J Neurosci 21: 109-116.

[8] ROBBE D, KOPF M, REMAURY A, BOCKAERT J AND MANZONI OJ [2002] Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. Proc Natl Acad Sci USA 99: 8384-8388.

[9] SZABO B, SIEMES S AND WALLMICHRATH I [2002] Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. Eur J Neurosci 15: 2057-2061.

[10] WILSON RI AND NICOLL RA [2001] Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. Nature 410: 588-592.

[11] AMERI A [1999] The effects of cannabinoids on the brain. Prog Neurobiol 58: 315-348.

[12] COSSU G, LEDENT C, FATTORE L, IMPERATO A, BOHME GA, PARMENTIER M AND FRATTA W [2001] Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav Brain Res 118: 61-65.

[13] RACZ I, BILKEI-GORZO A, TOTH ZE, MICHEL K, PALKOVITS M AND ZIMMER A [2003] A critical role for the cannabinoid CB1 receptors in alcohol dependence and stress-stimulated ethanol drinking. J Neurosci 23: 2453-2458.

[14] DI MARZO V [1998] 'Endocannabinoids' and other fatty acid derivatives with cannabimimetic properties: Biochemistry and possible physiopathological relevance. Biochim Biophys Acta 1392: 153-175.

[15] CRAVATT BF, GIANG DK, MAYFIELD SP, BOGER DL, LERNER RA AND GILULA NB [1996] Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384: 83-87.

[16] DI MARZO V AND DEUTSCH DG [1998] Biochemistry of the endogenous ligands of cannabinoid receptors. Neurobiol Dis 5: 386-404.

[17] KLEIN TW, LANE B, NEWTON CA AND FRIEDMAN H [2000] The cannabinoid system and cytokine network. Proc Soc Exp Biol Med 225: 1-8.

[18] KLEIN TW, NEWTON C AND FRIEDMAN H [1998] Cannabinoid receptors and immunity. Immunol Today 19: 373-381.

[19] PIOMELLI D, GIUFFRIDA A, CALIGNANO A AND RODRIGUEZ DF [2000] The endocannabinoid system as a target for therapeutic drugs. Trends Pharmacol Sci 21: 218-224.

[20] MAEJIMA T, OHNO-SHOSAKU T AND KANO M [2001] Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. Neurosci Res 40: 205-210.

[21] KREITZER AC AND REGEHR WG [2001] Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. Neuron 29: 717-727.

[22] OHNO-SHOSAKU T, MAEJIMA T AND KANO M [2001] Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29: 729-738.

[23] UEDA N, PUFFENBARGER RA, YAMAMOTO S AND DEUTSCH DG [2000] The fatty acid amide hydrolase (FAAH). Chem Phys Lipids 108: 107-121.

[24] WAN M, CRAVATT BF, RING HZ, ZHANG X AND FRANCKE U [1998] Conserved chromosomal location and genomic structure of human and mouse fatty-acid amide hydrolase

genes and evaluation of clasper as a candidate neurological mutation. Genomics 54: 408-414.

[25] GIANG DK AND CRAVATT BF [1997] Molecular characterization of human and mouse fatty acid amide hydrolases. Proc Natl Acad Sci U S A 94: 2238-2242.

[26] PATRICELLI MP AND CRAVATT BF [2001] Proteins regulating the biosynthesis and inactivation of neuromodulatory fatty acid amides. Vitam Horm 62: 95-131.

[27] SELF DW [1999] Anandamide: A candidate neurotransmitter heads for the big leagues. Nat Neurosci 2: 303-304.

[28] CRAVATT BF, DEMAREST K, PATRICELLI MP, BRACEY MH, GIANG DK, MARTIN BR AND LICHTMAN AH [2001] Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. Proc Natl Acad Sci USA 98: 9371-9376.

[29] LICHTMAN AH, HAWKINS EG, GRIFFIN G AND CRAVATT BF [2002] Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. Pharmacol Exp Ther 302: 73-79.

[30] EGERTOVA M, CRAVATT BF AND ELPHICK MR [2003] Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: Evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119: 481-496.

[31] IVERSEN L [2003] Cannabis and the brain. Brain 126: 1252-1270.

[32] Egertova M and Elphick MR [2000] Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB. J Comp Neurol 422: 159-171.

[33] EGERTOVA M, GIANG DK, CRAVATT BF AND ELPHICK MR [1998] A new perspective on cannabinoid signalling: Complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. Proc R Soc Lond B Biol Sci 265: 2081-2085.

[34] BRACEY MH, HANSON MA, MASUDA K, STEVENS RC AND CRAVATT BF [2002] Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. Science 298: 1793-1796.

[35] PAROLARO D AND RUBINO T [2002] Is cannabinoid transmission involved in rewarding properties of drugs of abuse? Br J Pharmacol 136: 1083-1084.

[36] VARMA N, CARLSON GC, LEDENT C AND ALGER BE [2001] Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. J Neurosci 21: RC188.

[37] KREITZER AC AND REGEHR WG [2001] Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. J Neurosci 21: RC174.

[38] WILSON RI, KUNOS G AND NICOLL RA [2001] Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31: 453-462.

[39] MARSICANO G, WOTJAK CT, AZAD SC, BISOGNO T, RAMMES G, CASCIO MG, HERMANN H, TANG J, HOFMANN C, ZIEGLGANSBERGER W, DI MARZO V AND LUTZ B [2002] The endogenous cannabinoid system controls extinction of aversive memories. Nature 418: 530-534.

[40] GERDEMAN G AND LOVINGER DM [2001] CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. J Neurophysiol 85: 468-471.

[41] GERDEMAN GL, PARTRIDGE JG, LUPICA CR AND LOVINGER DM [2003] It could be habit forming: Drugs of abuse and striatal synaptic plasticity. Trends Neurosci 26: 184-192.

[42] BRAIDA D AND SALA M [2002] Role of the endocannabinoid system in MDMA intracerebral self-administration in rats. Br J Pharmacol 136: 1089-1092.

[43] TANDA G AND GOLDBERG SR [2003] Cannabinoids: reward, dependence, and underlying neurochemical mechanisms — a review of recent preclinical data. Psychopharmacology (Berl) 169: 115-134.

[44] MALDONADO R AND RODRIGUEZ DF [2002] Cannabinoid addiction: Behavioral models and neural correlates. J Neurosci 22: 3326-3331.

[45] GONZALEZ S, SCHMID PC, FERNANDEZ-RUIZ J, KREBSBACH R, SCHMID HH AND RAMON JA [2003] Region-dependent changes in endocannabinoid transmission in the brain of morphine-dependent rats. Addict Biol 8: 159-166.

[46] DE VRIES TJ, SHAHAM Y, HOMBERG JR, CROMBAG H, SCHUURMAN K, DIEBEN J, VANDERSCHUREN LJ AND SCHOFFELMEER AN [2001] A cannabinoid mechanism in relapse to cocaine seeking. Nat Med 7: 1151-1154.

[47] YAMAGUCHI T, HAGIWARA Y, TANAKA H, SUGIURA T, WAKU K, SHOYAMA Y, WATANABE S AND YAMAMOTO T [2001] Endogenous cannabinoid, 2-arachidonoylglycerol, attenuates naloxone-precipitated withdrawal signs in morphine-dependent mice. Brain Res 909: 121-126.

[48] DE VRIES TJ, HOMBERG JR, BINNEKADE R, RAASO H AND SCHOFFELMEER AN [2003] Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. Psychopharmacology (Berl) 168: 164-169.

[49] VINKLEROVA J, NOVAKOVA J AND SULCOVA A [2002] Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM-251. J Psychopharmacol 16: 139-143.

[50] YAMAMOTO T AND TAKADA K [2000] Role of cannabinoid receptor in the brain as it relates to drug reward. Japanese J Pharmacol 84: 229-236.

[51] GARDNER EL [2002] Addictive potential of cannabinoids: The underlying neurobiology. Chem Phys Lipids 121: 267-290.

[52] GARDNER EL [2000] What we have learned about addiction from animal models of drug self-administration. Am J Addict 9: 285-313.

[53] KUHAR MJ, JOYCE A AND DOMINGUEZ G [2001] Genes in drug abuse. Drug Alcohol Depend 62: 157-162.

[54] LAMARQUE S, TAGHZOUTI K AND SIMON H [2001] Chronic treatment with delta(9)-tetrahydrocannabinol enhances the locomotor response to amphetamine and heroin. Implications for vulnerability to drug addiction. Neuropharmacology 41: 118-129.

[55] MUSCHAMP J AND SIVIY S [2002] Behavioral sensitization to amphetamine follows chronic administration of the CB(1) agonist WIN 55,212-2 in Lewis rats. Pharmacol Biochem Behav 73: 835.

[56] MAS-NIETO M, POMMIER B, TZAVARA ET, CANEPARO A, DA NASCIMENTO S, LE FUR G, ROQUES BP AND NOBLE F [2001] Reduction of opioid dependence by the CB(1) antagonist SR141716A in mice: Evaluation of the interest in pharmacotherapy of opioid addiction. Br J Pharmacol 132: 1809-1816.

[57] TSAI SJ, WANG YC AND HONG CJ [2001] Association study between cannabinoid receptor gene (CNR1) and pathogenesis and psychotic symptoms of mood disorders. Am J Med Genet 105: 219-221.

[58] COMINGS DE, MUHLEMAN D, GADE R, JOHNSON P, VERDE R, SAUCIER G AND MACMURRAY J [2003] Cannabinoid receptor gene (CNR1): Association with i.v. drug use. Mol Psychiatry 2: 161-168.

[59] GARDNER EL AND VOREL SR [1998] Cannabinoid transmission and reward-related events. Neurobiol Dis 5: 502-533.

[60] TANDA G, MUNZAR P AND GOLDBERG SR [2000] Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci 3: 1073-1074.

[61] HUESTIS MA, GORELICK DA, HEISHMAN SJ, PRESTON KL, NELSON RA, MOOLCHAN ET AND FRANK RA [2001] Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 58: 322-328.

[62] SIPE JC, CHIANG K, GERBER AL, BEUTLER E AND CRAVATT BF [2002] A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci USA 99: 8394-8399.

[63] BASAVARAJAPPA BS AND HUNGUND BL [2002] Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. Prostaglandins Leukot Essent Fatty Acids 66: 287-299.

[64] SCHMIDT LG, SAMOCHOWIEC J, FINCKH U, FISZER-PIOSIK E, HORODNICKI J, WENDEL B, ROMMELSPACHER H AND HOEHE MR [2002] Association of a CB1 cannabinoid receptor gene (CNR1) polymorphism with severe alcohol dependence. Drug Alcohol Depend 65: 221- 224.

[65] WANG L, LIU J, HARVEY-WHITE J, ZIMMER A AND KUNOS G [2003] Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. Proc Natl Acad Sci USA 100: 1393-1394.

[66] PONCELET M, MARUANI J, CALASSI R AND SOUBRIE P [2003] Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. Neurosci Lett 343: 216-218.

[67] COTA D, MARSICANO G, LUTZ B, VICENNATI V, STALLA GK, PASQUALI R AND PAGOTTO U [2003] Endogenous cannabinoid system as a modulator of food intake. Int J Obes Relat Metab Disord 27: 289-301.

[68] HORVATH TL [2003] Endocannabinoids and the regulation of body fat: The smoke is clearing. J Clin Invest 112: 323-326.

[69] COTA D, MARSICANO G, TSCHOP M, GRUBLER Y, FLACHSKAMM C, SCHUBERT M, AUER D, YASSOURIDIS A, THONE-REINEKE C, ORTMANN S, TOMASSONI F, CERVINO C, NISOLI E, LINTHORST AC, PASQUALI R, LUTZ B, STALLA GK AND PAGOTTO U [2003] The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112: 423-431.

[70] HARROLD JA, ELLIOTT JC, KING PJ, WIDDOWSON PS AND WILLIAMS G [2002] Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: A role for endogenous cannabinoids in driving appetite for palatable food? Brain Res 952: 232-238.

[71] HARROLD JA AND WILLIAMS G [2003] The cannabinoid system: A role in both the homeostatic and hedonic control of eating? Br J Nutr 90: 729-734.

[72] DI MARZO V, GOPARAJU SK, WANG L, LIU J, BATKAI S, JARAI Z, FEZZA F, MIURA GI, PALMITER RD, SUGIURA T AND KUNOS G [2001] Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410: 822-825.

[73] CAMI J AND FARRE M [2003] Mechanisms of disease: Drug addiction. N Engl J Med 349: 975-986.

ABBREVIATIONS USED

ECS, endogenous cannabinoid system; FAAH, fatty acid amide hydrolase; CB1 (or CB2), cannabinoid 1 (or 2) receptor; THC, delta-9-tetrahydrocannabinol; GABA, gamma amino butyric acid; CNS, central nervous system; DSI, depolarization-induced suppression of inhibition; LTD, long-term depression.

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