

LECTURES

THE ENDOCANNABINOID SYSTEM: QUO VADIMUS?

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The endocannabinoid system was unknown 15 years ago. Now we are aware of the existence of 2 well defined cannabinoid receptors, with a few more in various stages of discovery. Two endogenous cannabinoids, anandamide and 2-AG, have been found in the brain and the periphery, and several more are being looked into. The enzymes associated with their biosynthesis and degradation have been described. Hundreds of publications on the actions of the endocannabinoid system have appeared.

Do they have a common denominator?

I would like to propose that the endocannabinoid system is part of the general protective system of the mammalian body. We are all aware that the immune system is our guardian against protein attacks, such as those caused by microorganisms. But certainly not all potential damages are due to proteins. Neurodegenerative diseases, cancers, physical traumas and many others are examples of non-protein attacks. The immune system is certainly involved in the reactions of the body to the physiological changes that take place. But the mammalian body has numerous other strategies to avoid or reduce damage. The endocannabinoid system is known to react swiftly in cases of brain trauma, to affect cancer cell proliferation, to be involved in damage control in numerous neurological diseases – multiple sclerosis, Parkinson's disease, Huntington's disease, Alzheimer's disease, ALS. Tourette's disease, epilepsy. Recent data indicates that it affects osteoporosis. And it is certainly involved in the reaction of the body to cancer. Research on many of these protective mechanisms has only started.

Like the immune system, the endocannabinoids may cause damage if they come to dinner uninvited. In some cases enhancement of neurological damage has been noted.

An enormous amount of work has to be done before we can establish conclusively that the central task of the endocannabinoid system is a protective one. Theories serve as catalysts for novel ideas. And sometimes they are even correct.

WHAT'S NEW IN CANNABINOID RECEPTOR PHARMACOLOGY?

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There have been a number of recent advances in the area of cannabinoid CB1 and CB2 receptor pharmacology and some of the more important of these are summarized below.

* There is now evidence that for clinical objectives such as pain relief, the psychotropic effects of CB1 receptor agonists could be avoided/minimized by targeting CB1 receptors outside the CNS either by administering these agonists transdermally [e.g. 1] or by giving patients CB1 receptor agonists that do not readily cross the blood-brain barrier.

* It has recently been shown that cannabinoid receptor expression levels increase in models of neuropathic pain [2, 3]. This raises the possibility that a partial cannabinoid receptor agonist such as delta-9-THC may be more suitable for managing pain in the clinic than a full agonist. Thus, since the maximal effect of a partial agonist often increases in response to an elevation in the expression level (or coupling efficiency) of its receptors, any tendency for an increase in cannabinoid receptor expression levels to be limited to pain pathways might well produce a selective increase in the analgesic efficacy of this type of agonist.

* Evidence has emerged that CB2 receptor agonists are effective not only against inflammatory but also against neuropathic pain [4, 5].

* Evidence now exists for an allosteric site on the CB1 receptor [6]. This opens up the possibility of developing allosteric CB1 enhancers, e.g. as analgesics, and allosteric CB1 antagonists, e.g. as anti-obesity agents.

* Schering Plough recently obtained evidence that CB2 antagonists/inverse agonists with higher inverse efficacy than SR144528 have therapeutic potential as inhibitors of leucocyte migration and hence as anti-inflammatory agents [7, 8]. This is an interesting development, not least because CB2 receptor agonists are also thought to have anti-inflammatory properties.

* Finally, several ligands that behave as neutral competitive antagonists at CB1 receptors have been developed [see 9]. Two of these, NESS 0327 and VCHSR, are analogues of SR141716A. Two others are analogues of delta-8-THC (O-2050) or cannabidiol (O-2654). Since neutral antagonists are not expected to reduce the constitutive activity of cannabinoid receptors, they should serve as useful tools for establishing whether or not any particular increase in tonic activity of the endocannabinoid system is due to an increase in endocannabinoid-induced cannabinoid receptor activation. Whether neutral CB1 antagonists have advantages in the clinic over CB1 antagonists/inverse agonists remains to be established. Another ligand that behaves as a neutral CB1 receptor antagonist is delta-9-tetrahydrocannabivarin [10]. Interestingly, although this plant cannabinoid is a CB1 receptor antagonist, it appears to have a second target at which it can antagonize R-(+)-WIN55212 and anandamide in a highly potent manner.

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REGULATION OF ENDOCANNABINOID LEVELS: AN UPDATE

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Of the several endocannabinoids identified so far, *N*-arachidonylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) are undoubtedly the best studied. These two compounds are produced “on demand” following the enhancement of intracellular Ca^{2+} concentrations. By activating presynaptic cannabinoid CB_1 receptors, endocannabinoids reduce neurotransmitter release and alter both short- and long-term synaptic plasticity by acting as retrograde synaptic messengers in the central nervous system. Furthermore, emerging data indicate that endocannabinoids are also produced in the brain during neuroinflammatory conditions to activate up-regulated CB_2 receptors in brain-resident immune-competent cells. In fact, both cannabinoid receptors and endocannabinoids are subject to regulation during physiological and pathological conditions. For example, dramatic changes in anandamide and 2-AG rat brain concentrations occur when passing from the light to the dark phase of the day. Disorders such as Parkinson’s and Alzheimer’s disease as well as eating disorders are accompanied by alterations in endocannabinoid levels. It has been proposed that these mediators are over-produced in the attempt to minimize neurochemical unbalances and inflammatory reactions occurring at the onset of pathological states, and that permanently altered endocannabinoid levels may instead account for some of the symptoms of chronic neurological and eating disorders. Enzymes catalyzing the biosynthesis of anandamide and 2-AG (i.e. the *N*-arachidonoylphosphatidyl ethanolamine-selective phospholipase D and the *sn*-1 selective diacylglycerol lipases, respectively), or their breakdown (the fatty acid amide hydrolase and the monoacylglycerol lipase, respectively) have now been cloned and characterized. Their properties, distribution and regulation account for the observed changes in endocannabinoid levels and function during physiological and pathological conditions. How the pharmacological manipulation of these enzymes can be used in basic and clinical research will be briefly discussed.

PHARMACOLOGICAL PROPERTIES OF MONOACYLGLYCEROL LIPASE

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The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) have a short duration of action due to effective degradative pathways. In the case of AEA, the key enzyme involved is fatty acid amide hydrolase (FAAH), and both selective inhibition and genetic deletion of this enzyme produce potentially beneficial effects in models of inflammatory pain. AEA can also be metabolised by cyclooxygenase-2 and by lipoxygenases in vitro, and evidence is emerging that the cyclooxygenase-2 pathway may have physiological importance in vivo. The metabolism of 2-AG is more complicated: it is a substrate for both FAAH and cyclooxygenase-2, but in the brain the principal metabolic enzyme is monoacylglycerol lipase (MAGL), a 33 kDa serine hydrolase that catalyses the hydrolysis of 1- and 2-monoglycerides to form glycerol and fatty acids¹. In the brain, the enzyme has a more restricted distribution than FAAH, high levels of mRNA coding for MAGL being found in the hippocampus, cerebellum, cortex and in part of the thalamus¹. In the hippocampus and cerebellum, MAGL shows a presynaptic localisation, whereas FAAH is postsynaptically located².

In contrast to the situation for FAAH, selective inhibitors of MAGL are not available. Compounds like arachidonoyltrifluoromethylketone, methyl - arachidonoyl - fluorophosphonate and hexadecylsulfonyl fluoride can inhibit MAGL, but with lower potencies than for inhibition of FAAH¹. The FAAH substrates palmitoylethanolamide and arachidonoylglycine do not inhibit MAGL-catalysed hydrolysis of 2-oleoylglycerol, whereas AEA and lineoylethanolamide do inhibit the enzyme, albeit with a much lower potency than seen for interaction with FAAH³. 2-AG, 1-AG and α -methyl-1-AG inhibited cytosolic MAGL activity towards 2-oleoylglycerol with IC₅₀ values of 13, 17 and 11 μ M, respectively, whereas the amide and ether analogues of 2-AG (arachidonoyl serinol and noladin ether) were less potent (IC₅₀ values of 73 and 36 μ M, respectively). Cyclo-oxygenation of the arachidonoyl side chain of either arachidonic acid, 1-AG or arachidonoyl serinol to produce the prostaglandin equivalents resulted in an almost complete loss of inhibitory activity of the compounds towards soluble MAGL⁴.

One issue that is unclear is as to whether MAGL is a single enzyme, or whether enzyme heterogeneity exists. 2-oleoylglycerol metabolising activity is found both in cytosolic and membrane-bound fractions¹, and initial data from our laboratory suggests that the membrane-bound enzyme has slightly different pharmacological properties compared to the cytosolic enzyme. Hopefully, continued structure-activity relationship studies will shed light on this issue, and generate selective compounds which can be used to probe the function of 2-AG in the brain.

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ENDOCANNABINOIDS ARE RETROGRADE MODULATORS OF SYNAPTIC TRANSMISSION

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The CB₁ cannabinoid receptor is widely distributed in the central nervous system. The most frequently observed effect of exogenous cannabinoids is inhibition of neurotransmission. Thus, activation of CB₁ receptors leads to presynaptic inhibition of glutamatergic, GABAergic, noradrenergic and serotonergic neurotransmission in many brain regions, for example, in the cortex, cerebellum, hippocampus, caudate-putamen and amygdala.

Recently it has been shown that presynaptic neurotransmitter release-inhibiting CB₁ receptors are activated not only by exogenous cannabinoids, but also by endogenous cannabinoids (endocannabinoids). Endocannabinoids are synthesised by postsynaptic neurons. After release from the postsynaptic neuron they diffuse retrogradely to presynaptic axon terminals, where they activate release-inhibiting presynaptic CB₁ receptors. Two signals can trigger the synthesis of endocannabinoids in postsynaptic neurons. One signal is depolarisation. The depolarisation activates voltage-dependent calcium channels, and the resulting increase in intracellular calcium concentration triggers endocannabinoid synthesis. If the depolarisation leads to retrograde inhibition of inhibitory neurotransmission, then the phenomenon is called depolarisation-induced suppression of inhibition (DSI). If the depolarisation leads to retrograde inhibition of excitatory neurotransmission, then the phenomenon is called depolarisation-induced suppression of excitation (DSE). The other signal triggering endocannabinoid synthesis is activation of G_q protein-coupled receptors; activation of such receptors leads to calcium release from intracellular stores.

We studied DSI in brain slices with patch-clamp techniques. Cerebellar cortical Purkinje cells receive a prominent GABAergic input from neighbouring basket cells. Depolarisation of Purkinje cells by 9 pulses at 1 Hz (each lasting for 100 ms), led to inhibition of GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) recorded in Purkinje cells. The maximal inhibition was a 30-40 % inhibition of cumulative sIPSC amplitude (i.e., DSI was 30-40 %). DSI lasted for about 60 s. DSI was prevented by the CB₁ receptor antagonist rimonabant, verifying involvement of endocannabinoids and CB₁ receptors. Two series of experiments have been carried out in order to determine the endocannabinoid which was released by the depolarisation. In the first series of experiments, diacylglycerol lipase (DAGL), the enzyme thought to be important for the synthesis of 2-arachidonoylglycerol, was inhibited by orlistat or RHC-80267. Orlistat as well as RHC-80267 significantly inhibited DSI, pointing to the involvement of 2-arachidonoylglycerol in DSI. In the second series of experiments, fatty acid amide hydrolase (FAAH), the enzyme thought to be important for the degradation of anandamide, was inhibited by AA-5-HT and URB597. These two drugs did not change the extent and duration of DSI. This observation argues against the role of anandamide in DSI.

Our results show that depolarisation-induced suppression of inhibition (DSI) is operating at

the basket cell – Purkinje cell synapse in the cerebellar cortex. Since inhibitors of 2-arachidonoylglycerol synthesis inhibited DSI, but inhibitors of anandamide degradation did not change it, it is likely that short-term retrograde signaling is mediated by 2-arachidonoylglycerol.

PROTEIN KINASES IN CANNABINOID RECEPTOR SIGNALLING

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Cannabinoid receptors belong to the superfamily of G-protein-coupled receptors and by activating pertussis toxin-sensitive G proteins (Gi/Go proteins) they affect several signal transduction systems.

In the last few years much work has been done to delineate the cannabinoid agonist-induced downstream signalling events. We now know that CB1 receptor stimulation can cause the closure of Ca⁺⁺ channels, opening of K⁺ channels, inhibition of adenylyl cyclase and protein kinase A activity and activation of other protein kinases such as extracellular signal-regulated kinases (ERK), focal adhesion kinase (FAK) and phosphatidylinositol-3-kinase (PI3K).

More interestingly, recent papers have addressed the physiological relevance of kinases involvement in cannabinoid signalling. For example, PI3K/Akt signalling seems to be the main pathway involved in cannabinoid-mediated protection of neurons from excitotoxicity. In cancer cells of different nature (breast and prostate cancer, PC12 pheochromocytoma and malignant gliomas) cannabinoid-induced cell death appears to involve the family of mitogen-activated protein kinases (MAPKs). Anandamide's inhibition of epidermal (keratinocyte) differentiation occurs through a CB1-dependent mechanism involving PKC modulation. Another phenomenon where protein kinases are largely involved is neural plasticity. Cellular responses that elicit synaptic plasticity might involve CB1 receptors and their link to PKA, MAPKs and FAK. Like any endogenous system, the cannabinoid one can adapt to maintain homeostasis, showing a high degree of plasticity. There is ample evidence that tolerance to cannabinoid receptor agonists arises after prolonged exposure and that in animals this tolerance is associated with downregulation of CB1 receptors and desensitization of CB1 receptor-mediated G protein activation. Recent works provided strong evidence that besides PKA, the MAPK cascade is involved in plasticity associated with cannabinoid tolerance. The role of the Ras/ERK pathway in the development of tolerance to delta-9-tetrahydrocannabinol (THC)-induced reduction in spontaneous locomotor activity was demonstrated by a genetic (Ras-specific guanine nucleotide exchange factor (Ras-GRF1) knock-out mice) and pharmacological approach (pretreatment of wild-type mice with SL327 a specific inhibitor of mitogen-activated protein kinase kinase (MEK), the upstream kinase of ERK). Moreover, the impact of the inhibition of ERK activation on the biological processes involved in cannabinoid tolerance (receptor downregulation and desensitization) was investigated by autoradiographic cannabinoid CB1 receptor and cannabinoid-stimulated [³⁵S]GTPγS binding studies in THC chronically treated mice. In the caudate putamen and cerebellum of Ras-GRF1 knock-out mice and SL327 pretreated wild-type mice, CB1 receptor downregulation and desensitization did not occur, suggesting that ERK activation might account for CB1 receptor plasticity involved in the development of tolerance to THC hypolocomotor effect. In contrast, the hippocampus and prefrontal cortex showed CB1 receptor adaptations regardless of the genetic or pharmacological inhibition of the ERK pathway, suggesting regional variability in the cellular events underlying the altered CB1 receptor function.

These data provide strong evidence that modulation of kinase activity represent a key event in cannabinoid physiological/pharmacological responses.

PAST AND PRESENT OF CANNABINOID RECEPTOR ANTAGONISTS

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Rimonabant (SR 141716) is a cannabinoid CB₁ receptor antagonist showing high selectivity for the central CB₁ receptor compared to the peripheral cannabinoid receptor CB₂ in rat tissues and in CHO cells expressing human CB₁ and CB₂ receptors.

It is now generally accepted that endocannabinoid systems are involved with brain reward function. Consistent with the expression of CB₁ receptors in limbic areas, Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and synthetic CB₁ agonists (like WIN 55212-2) activate the mesolimbic dopaminergic system, which is a recognised pathway in mediating the addictive properties of drugs. Δ⁹-THC enhances, and SR141716 reduces, the rewarding effects of electrical stimulation of brain reward circuits.

A great of interest has been centred on the role of CB₁ receptors and eating behavior as they appear to be largely distributed in brain areas involved in the control of feeding behavior (i.e. lateral hypothalamus, limbic system) and additionally seem to be implicated in food intake control.

Recent results show that endocannabinoids in the hypothalamus may tonically activate CB₁ receptors to maintain food intake, and may increase the incentive value of food. Further evidence shows that the CB₁ receptors may be involved in the motivational aspects of eating by enhancing the satisfaction derived from eating through activation of the meso-limbic dopaminergic system. All this evidence would seem to indicate that specific CB₁ antagonists like rimonabant should have some effect in body weight control and this has been confirmed in numerous pharmacological studies in different species.

Nicotine itself and environmental cues associated with nicotine delivery are critically important for sustaining smoking in humans and nicotine self-administration in animals. Recent studies have shown that pre-treatment with SR 141716 decreases nicotine self-administration and decreases compulsive behavior maintained by conditioned stimuli in rats. SR 141716 has also been shown to reduce ethanol intake and ethanol-induced dopamine release in rodents.

These results suggest that the activity of drugs of abuse, like nicotine and ethanol, to facilitate the meso-limbic dopaminergic transmission also involves activation of CB₁ receptors.

Initial clinical studies with rimonabant have shown that it reduces hunger, caloric intake and body weight in obese patients. In smoking cessation studies in man, the compound produced an increased abstinence in patients as well as preventing the secondary weight gain often seen in this situation. In addition, the compound has shown a very good safety profile. Results from the Phase III clinical studies have confirmed these promising results in that overweight/obese patients with untreated dyslipidemia (high triglycerides and/or high total cholesterol/HDL cholesterol ratio) lost weight in one year while improving their lipid and glucose profiles, and that smokers, who had previously unsuccessfully tried to stop smoking, were able to stop in 10 weeks without post cessation weight gain.

The dual role of the compound against risk factors, essentially cardiovascular, associated with obesity and smoking, is likely to make rimonabant a cornerstone therapy in the future management of patients with cardiovascular risks.

CANNABINOID MECHANISM IN DRUG-SEEKING BEHAVIOR

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Although cannabis derivatives have been used and abused by humans for ten thousand years, cannabinoids were long considered “anomalous” as drugs of abuse, in comparison with other more “classical” drugs such as cocaine, heroin, alcohol, nicotine etc. However in more recent years, due to the impressive progress reached in this scientific field, unequivocal evidence demonstrates that cannabinoids act on brain reward processes in a very similar fashion to “classical” drugs of abuse. Such evidence has been obtained by means of electrophysiological, biochemical and behavioural studies in different animal species. Thus, a selective cannabinoid-induced increase in Dopamine (DA) firing in mesolimbic neurones has been shown by different authors. Other laboratories have demonstrated enhancement of extracellular DA overflow in reward-related forebrain DA terminal loci following cannabinoid administration. Although a number of conflicting results, have been reported, it is now clear that cannabinoids act as a positive reinforcer in all animal models of reward and drug-seeking behaviour. One of the first evidence of the rewarding properties of Δ^9 -THC in rats was given by Gardner and coll. by brain electrical stimulation reward model in rats. Δ^9 -THC and synthetic cannabinoid agonists were proved to induce conditioned place preference in mice and rats by different labs. Despite negative results reported in the older literature, cannabinoid self-administration has been described in mice, rats and monkeys. More recently, very suggestive results from independent labs have indicated that the cannabinoid system might be implicated in the mechanisms of relapse to drug-seeking behaviour, opening the possibility of a potential therapeutic application of cannabinoid antagonists in the treatment of addiction. Taken together all this evidence demonstrates that cannabinoids enhance brain reward processes in a similar fashion to other addictive drugs, and therefore the old concept of cannabinoids as “anomalous” drugs of abuse is no longer acceptable.

CANNABINOIDS AND OTHER DRUGS OF ABUSE: OPIOIDS

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After more than 20 years of behavioural electrophysiological and biochemical evidences, cannabinoids and opioids appear to possess several similar pharmacological properties including the stimulation of brain reward circuit that are believed to underlie drug addiction and reward. A lot of papers has provided behavioural evidences of the presence of interaction between the cannabinoid and opioid systems in the different phases of addiction (dependence, tolerance, sensitization) and the cross talk appears to be specific and often reciprocal. Several hypothesis have been formulated to explain the cross modulation including the release of opioid peptides by cannabinoids or endocannabinoids by opioids and interaction at the level of receptor and/or their signal transduction mechanisms. The presence of reciprocal alteration in receptor density and efficiency as well as the modification in opioid cannabinoid endogenous system often do not reflect the behavioural results.

The individuation of the precise anatomical substrates and molecular mechanisms of such interaction needs further studies since a better knowledge of the opioid cannabinoid interaction may lead to exciting therapeutic possibilities.

NEUROFUNCTIONAL CONSEQUENCES OF DEVELOPMENTAL EXPOSURE TO CANNABINOIDS

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Even though marijuana is the most widely used illegal drug among women at reproductive age, reports dealing with the effects of prenatal exposure to this substance of abuse on the length of gestation, fetal growth, and offspring behavior are still controversial. Confounding factors, such as possible impurities in the drug and concomitant tobacco smoking, may be responsible for inconsistencies in the results reported in studies to date. It is likely that many of these conflicting results are due to methodological problems such as the measurement of neonatal outcomes and the context in which the research is conducted. More complex and less understood is the scenario concerning the possible long-term consequences of in utero exposure to cannabis derivatives on cognitive functions. In fact, data on this issue are sparse, and the identification of alterations in brain development and adult expression of cognitive and behavioral functions is far from definitive. These inconclusive results may depend on ethical, practical, and interpretative difficulties surrounding research with human subjects. In this regard, animal models provide a useful tool for examining the possible developmental and long-term effects of prenatal exposure to cannabinoids.

In animal studies a number of behavioral, hormonal and neurochemical effects of developmental cannabinoid exposure have been reported, since the 1960s. The majority of these reports have shown subtle somatic and functional impairments, such as those found in dopamine-dependent motor functions, in the response of the hypothalamic-pituitary-adrenal axis to stress, in the susceptibility to morphine self-administration and in cognitive functions.

Our recent studies (1-3), showed that prenatal exposure to the cannabinoid WIN55,212-2 (WIN) caused long lasting neurochemical and behavioral alterations in the offspring. In particular WIN-exposed pups revealed a poorer performance in homing behavior and a decrease in the rate of separation-induced ultrasonic emission. Behavioral deficits resulted long-lasting, since prenatal WIN exposure caused a disruption of memory retention in young and adult offspring subjected either to a passive or an active avoidance task. Such impairment was associated to electrophysiological and neurochemical alterations. In fact, hippocampal long term potentiation was affected by the prenatal treatment with WIN and resulted consistent with an alteration of hippocampal glutamate extracellular levels, which resulted decreased, both in basal and stimulated conditions. The hippocampal formation was not the only brain area affected by WIN. Both in vitro and in vivo microdialysis experiments showed, indeed, that glutamate release was also decreased in the prefrontal cortex. Finally, morphological experiments demonstrated that neurochemical alterations in both areas are accompanied by significant effects of prenatal treatment on neuronal architecture.

Interestingly, all these alterations were observed after the developmental exposure to a low dose of WIN devoid of overt signs of toxicity and that did not affect gestational and reproduction parameters, such as pregnancy length, dam weight gain, litter size at birth, pup postnatal mortality and pup weight gain. This observation has quite a clinical

relevance, because the dose of WIN considered in these studies may be comparable to that of delta9-tetrahydrocannabinol absorbed during a moderate or even low exposure to cannabis in humans.

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CANNABINOIDS AND MOTOR DISORDERS

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Pharmacological, biochemical and anatomical evidence strengthen the idea of a prominent role for the endocannabinoid signaling system in the control of movement. The abundance of the cannabinoid CB₁ receptor subtype, as well as of endocannabinoids, in the basal ganglia and the cerebellum in comparison with other brain structures, but also the presence in these regions of CB₂ and vanilloid TRPV1 receptors, support this prominent role (1). This is also supported by the occurrence of marked motor anomalies in the two models of CB₁ receptor-deficient mice developed so far (2,3). The location of endocannabinoids and their receptors in the vicinity of classic neurotransmitters acting at the basal ganglia is suggestive that the role of the endocannabinoid transmission in the control of movement might be exerted through regulating the function of these classic neurotransmitters (1,4,5). Thus, CB₁ receptors are located on striatal projection GABAergic neurons (6,7) and on subthalamonigral glutamatergic neurons (7), making possible a stimulatory action of these receptors on GABA transmission and an inhibition of glutamate release. Nigrostriatal dopaminergic neurons do not contain CB₁ receptors, so that the effects of cannabinoids on this transmitter seem indirect, although they express TRPV1 receptors (8) thus allowing certain endocannabinoids and their analogs to inhibit dopamine release through the stimulation of these receptors (9). Therefore, as a result of this regulatory action on different basal ganglia neurotransmitters, cannabinoid agonists or antagonists are able to produce important motor effects. This has been largely demonstrated in a series of pharmacological studies addressed to elucidate in laboratory animals the motor effects of compounds targeting the different proteins (receptors, transporter, degradative enzymes) that form the endocannabinoid system (1,4,5). In general, these studies demonstrated that direct cannabinoid agonists, as well as the inhibitors of the endocannabinoid inactivation, so-called indirect agonists, produce powerful inhibitory effects on motor activity, with the only exception of stimulatory effects at very low doses (1,4,5). There are differences in the magnitude and duration of motor effects of the different cannabinoid agonists assayed, but they can be related to their differences in receptor affinity, potency and/or metabolic stability. By contrast, cannabinoid receptor antagonists attenuated the effects of agonists and produced hyperlocomotion by themselves (1,4,5).

The above evidence also supports that the endocannabinoid signaling system in the basal ganglia might serve as a novel target for the development of specific pharmacotherapies in the treatment of motor-related disorders. Several studies have recently examined whether CB₁ receptors or other key elements of this regulatory system are altered in the basal ganglia of humans affected by several neurological diseases directly (Parkinson's disease, Huntington's chorea, dystonia, dyskinesias) or indirectly (multiple sclerosis) related to motor function (1,5,10). These observations have been corroborated in different animal models of these motor disorders (1,5,10). Thus, the endocannabinoid transmission becomes hypoactive in the basal ganglia in Huntington's disease (11,12), which is compatible with the hyperkinetic phenotype of this disorder, whereas this becomes overactive in Parkinson's disease (13-15), in concordance with the hypokinetic profile of this disease. In any case, these changes have provided support to the idea that cannabinoid-based

compounds, that act at key steps of the endocannabinoid transmission (receptors, transporter, FAAH, MGL), might have interest for their potential use to alleviate motor symptoms, as well as to provide neuroprotection, in pathologies affecting the basal ganglia. In this respect, endocannabinoid uptake inhibitors having an additional capability to activate TRPV1 receptors might serve as antihyperkinetic compounds in Huntington's disease where they acutely correct GABA deficits (11,16). Direct or indirect agonists of CB₁ receptors might serve to reduce tics in Tourette's syndrome (17), to alleviate spasticity in multiple sclerosis (18) or to decrease dystonia (10). By contrast, CB₁ receptor antagonists might be used to reduce bradykinesia (19) or to delay L-DOPA-induced dyskinesia (20) in Parkinson's disease patients, although agonists might be better to attenuate parkinsonian tremor due to their capability to reduce glutamate release and, then, to attenuate the overactivity of subthalamic nucleus (4). Lastly, cannabinoid agonists, by acting through mechanisms that mainly involve antiexcitotoxic, antioxidant and/or antiinflammatory actions, might provide protection against neuronal injury that chronically develops in these diseases (21).

The present lecture will address all these previous pharmacological, biochemical, anatomical and pathological evidence, trying to establish the bases that support the therapeutic potential of the endocannabinoid system in motor-related disorders.

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ROLE OF THE ENDOCANNABINOID SYSTEM IN NEURODEGENERATIVE DISEASE

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One of the most intriguing aspects of the endocannabinoid system (ECS) is its possible role in pathological conditions. Numerous studies have addressed the physiological functions of this system in lower mammals. Partially based on the anatomical distribution of the diverse elements of this system, we now know that the ECS participates in critical aspects of motor control, memory processing, neuroendocrine function, etc. In most of these processes, endocannabinoids act as retrograde messengers and control the synaptic homeostasis of several neurotransmitters. In addition, dramatic changes in the presence of cannabinoid receptors and of endocannabinoids take place when animals are subjected to experimental paradigms of brain damage. Several animal models have provided valuable information and allow us to postulate neuroprotective properties for the ECS. It must be emphasized, however, that we still lack knowledge on the pathophysiological relevance of the ECS in higher mammals. Autoradiographic, *in situ* hybridization and immunohistochemical data have shed light on the distribution of CB₁ receptors and FAAH in the normal brain of macaques and humans. These data point to a mainly neuronal nature of the ECS thus suggesting a prominent role in synaptic control. Importantly, other elements of the ECS (CB₂ receptors) have been classically thought to be absent of the CNS and limited to the immune system in the periphery.

Specially relevant for human pathology are animal models of multiple sclerosis. In these models, CB₁ receptor activation has been proven to be a useful strategy in crucial events such as remyelination as well as in symptomatic relief of spastic episodes. Clinical trials seem to confirm some of these beneficial effects in humans.

Recent data suggest that, under pathologic conditions, the ECS becomes overactivated in glial cells. Specifically, *neuroinflammatory* processes seem to be accompanied by an increase in the expression level of several elements of the ECS and by significant changes in their distribution patterns. A recent definition of “neuroinflammation” describes this process as composed of “chronic, sustained cycles of injury and response, in which the cumulative ill effects of immunological microglial and astrocytic activation contribute to and expand the initial neurodestructive effects, thus maintaining and worsening the disease process through their actions” (Streit et al., *Journal of Neuroinflammation* 1:14, 2004). Other hypothesis suggest that glial response may be partially beneficial, but long-term effects will make it harmful. In any case, there is a general consensus on the pivotal role of glial activation in neurodegenerative processes.

Studies performed in macaque and human brains reveal that the ECS may participate in these harmful processes within the CNS through several pathways. First, the induction of CB₂ expression in microglial cells seems particularly intense in brain regions subjected to proinflammatory insult, such as beta-amyloid plaques in Alzheimer’s disease or

perivascular infiltrates in viral encephalitis. Second, astrocytic FAAH may become a major source of arachidonic acid and, subsequently, of proinflammatory derivatives in the vicinity of focal areas of inflammation. And third, neuronal CB₁ receptors may be the target for massive amounts of endocannabinoids known to be released under conditions of acute brain damage, providing a certain degree of protection and favouring neuronal survival. Taken together, these data suggest a consistent pattern of adaptation of the ECS under neuroinflammatory conditions and open new therapeutic perspectives for its modulation.

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CANNABINOIDS AND CONTROL OF NEURONAL EXCITABILITY

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Numerous reports have shown that both exogenous and endogenous cannabinoids are able to convey neuroprotection. However, the beneficial effects of cannabinoids depend on the experimental paradigms used. In quite a few cases, the activation of cannabinoid receptor type 1 (CB1) can also cause a worsening of the status. In further understanding the mechanisms underlying the involvement of the endocannabinoid system in neuroprotection, we focused on the model of kainic acid (KA)-induced seizures. Here we show that forebrain cortical glutamatergic pyramidal neurons are a major direct target of endocannabinoid action. In conditional mutant mice, lacking expression of the cannabinoid receptor type 1 (CB1) exclusively in the majority of cortical glutamatergic neurons, including hippocampal pyramidal neurons, kainic acid induces more severe seizures than in wild-type littermates. Conversely, there is no difference in the severity of KA-induced seizure between wild-type mice and those lacking CB1 expression in GABAergic neurons. This strongly indicates that CB1 is expressed on glutamatergic principal neurons. Indeed, CB1 mRNA and protein are present in neurons expressing a specific marker of glutamatergic neurons, namely vesicular glutamate transporter 1 (VGluT-1). Analysis of CB1 protein distribution revealed an intense staining of the inner third of the molecular layer of the dentate gyrus, where terminals of mossy cells of the dentate gyrus are located. This staining is conserved in mice lacking CB1 expression in GABAergic neurons, indicating that control of neuronal excitability in mossy cells of dentate gyrus likely represent a primary site of action of the endocannabinoid system in protection against seizures induced by KA. These data provide strong evidence that direct endocannabinoid-mediated control of glutamatergic neurotransmission exists in the hippocampus.

ENDOCANNABINOID AND ENDOVANILLOID MECHANISMS IN NEUROPROTECTION

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Over the last decade cannabinoid and endovanilloid systems have received increasing attention for their neuroprotective effects in the brain.

Exogenous and endogenous cannabinoids exert neuroprotection either *in vitro* or *in vivo* under different experimental conditions. Thus, cannabinoid receptor activation protects hippocampal or granule cerebellar neurons from excitotoxicity (Skaper et al., 1996; Shen and Thayer, 1998; Hampson and Grimaldi, 2001), from hypoxia and glucose deprivation (Nagayama et al., 1999), from acute brain trauma (Panikashvili et al., 2001), ouabain-induced neurotoxicity (van der Stelt et al., 2001) and oxidative cell death (Kim et al., 2005). The size of cerebral infarcts after middle cerebral artery occlusion is increased in CB1-knockout mice (Parmentier-Batteur et al., 2002). The protective effect has been ascribed to inhibition of glutamate transmission, reduction of Ca⁺⁺ influx and subsequent inhibition of noxious cascades, such as tumor necrosis factor- α (TNF- α) generation and oxidative stress (van der Stelt et al., 2002). More recently, exogenous 2-arachidonoylglycerol (2-AG) has been found to reduce brain edema, infarct volume and hippocampal death after closed head injury (Panikashvili et al., 2005), in part via CB1 receptor-mediated mechanism. However, cannabinoid CB1 receptor activation does not prevent the toxicity of glutamate towards embryonic chick telencephalon primary cultures (Nilsson et al., 2003). Increased production of endocannabinoid-related compounds modulate the inflammatory response to ischemia (Franklin et al., 2003). Therefore, endogenous cannabinoid signaling mechanisms may represent a key component of protection and repair programs mobilized in the injured brain.

On the other hand, in addition to the CB1 receptor, the endocannabinoid N-arachidonylethanolamine (AEA) or its metabolites may convey neuroprotection via other molecular targets (Grotenhermen, 2004). AEA is also a full agonist at the transient receptor potential channel vanilloid subfamily member 1 (VR1), recently reported to be involved in neurodegeneration. It is present in regions highly susceptible to neurodegenerative insults and it is influenced by temperature and pH changes (Mezey et al., 2000; Marinelli et al., 2002). In addition, during brain injury, AEA and lipoxygenase products accumulate in the brain (Marinelli et al., 2000, Muthian et al., 2004). Capsazepine, the selective VR1 vanilloid antagonist, has been shown to protect against neuronal injury caused by oxygen glucose deprivation by inhibiting I (h) (Veldhuis et al., 2003). A role of vanilloid receptors and lipoxygenases in neuroprotection by AEA and arvanil against *in vivo* excitotoxicity in the rat, has been reported (Ray et al., 2003) and a protective role of cannabinoid receptors against apoptosis induced by AEA via vanilloid receptors (Maccarrone et al., 2000).

Here we report the results recently obtained after acute post-ischemic treatment of different exogenous cannabinoids agonists (CP 55,940, Cannabidiol, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), CB1 cannabinoid receptor antagonists (SR 141716), VR1 vanilloid receptor agonists (Capsaicin) or antagonists (Capsazepine) in a model of transient global cerebral ischemia in Mongolian gerbils. The neuroprotection was quantified in terms of complete recovery of EEG total and relative spectral power, spontaneous motor activity, memory function and hippocampal CA1 neuronal density, starting from 1 to 7 days. All the compounds protected against ischemia-induced EEG flattening, hyperlocomotion and memory impairment with a dose-dependent bell-shaped curve. In addition, a survival of hippocampal neurons was obtained.

From the present lecture it can be concluded that there is a considerable experimental evidence for a neuroprotective role of both CB1 and VR1 cannabinoid and vanilloid receptors. However, the activation of both receptors does not completely explain the neuroprotective effect observed during brain injury. The data are critically reviewed and possible explanations are given.

CANNABINOIDS IN THE NEUROPATHIC PAIN

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The cannabinoid system is one of the endogenous systems that modulate pain perception. In fact, i) cannabinoid receptors are localized in central and peripheral nervous structures involved in pain processing, ii) the endogenous cannabinoids suppress pain, iii) the CB1 agonists exhibit antinociceptive effects in acute and inflammatory pains, iv) electrophysiological studies provided evidence that CB1 receptor agonists modulate both spinal and supraspinal neural circuits that transmit nociceptive signals. Collectively these pharmacological, anatomical and electrophysiological evidence suggest that among the various therapeutic potentials of cannabinoids is their application against chronic pain. Among the most debilitating types of chronic pain is the neuropathic one, which typically initiates by a primary lesion or dysfunction in the nervous system and results from a variety of etiologies, including trauma, infections, diabetes, immunodeficiencies, ischaemic disorders. Neuropathy affects millions of people worldwide and it can be intense, unremitting and often resistant to all currently available therapies; consequently this type of pain remains an area of unmet therapeutic need and cannabinoids may offer new opportunities of treatment.

The effectiveness of synthetic cannabinoids has been examined in several animal models of neuropathic pain. The first data was obtained by Herzberg et al. in 1997 (*Neurosci. Lett.* 221, 157) showing that WIN55,212-2 attenuated the thermal hyperalgesia and cold allodynia after a single administration in rats with chronic constriction injury of the sciatic nerve (CCI) and that this effect was CB1-mediated. The WIN55,212-2 effect has been confirmed also in the model of spinal nerve ligation (SNL) (Bridges et al., *Br. J. Pharmacol.* 133, 586, 2001) and in diabetic rats (Ulugol et al., *Neurosci. Lett.* 371, 167, 2004). The antinociceptive efficacy of THC in CCI model has been reported by Mao et al. (*Neurosci. Lett.* 280, 13, 2000). Fox et al. (*Pain* 92, 91, 2001) demonstrated that other synthetic cannabinoids, CP,55,940 and HU-210, evoked pronounced inhibition of hyperalgesia and allodynia in neuropathic rats via CB1 receptors, at doses per se active in the behavioural “tetrad” of cannabinoids. However, data recently obtained in our laboratory highlighted the antihyperalgesic efficacy of WIN55,212-2 in CCI rats following repeated administration of a dose per se ineffective and devoid of psychoactive effects (Costa et al., *Br. J. Pharmacol.* 141, 4, 2004). The recent findings demonstrating that CB1 receptors are upregulated in the contralateral thalamic region after a unilateral nerve injury (Siegling et al., *Eur. J. Pharmacol.* 415, R5, 2001) and within the ipsilateral superficial spinal cord dorsal horn in CCI rats (Lim et al., *Pain* 105, 275, 2003) could account for the findings obtained by us, leading to the hypothesis of an enhanced sensitivity to cannabinoids in pathological condition. The main problem linked to the clinical employment of selective or non selective CB1 agonists is the difficult in separating their analgesic properties from a lot of CNS side effects, including sedation, hypomotility, cognitive defects and potential abuse. To bypass this problem, different approaches have been proposed: the development of CB1 agonists that selectively target peripheral (non-CNS) receptors, the use of CB2 selective agonists, the upregulation of endocannabinoid tone through FAAH inhibitors or AMT blockers. The first evidence demonstrating the

effectiveness of CB2 agonists in neuropathic pain was obtained by Ibrahim et al. in 2003 (*Proc. Natl. Acad. Sci. USA*, 100, 10529), who highlighted that systemic AM1241 reversed nerve injury-induced tactile and thermal hypersensitivity in rats and that this effect was reversed by the selective CB2 antagonist, AM630. In the same work authors reported that AM1241 retained its efficacy in CB1 receptor knockout mice, so showing that the activation of CB2 receptors reversed the sensory hypersensitivity produced by peripheral nerve injury. More recently Elmes et al. (*Eur. J. Neurosci.* 20, 2311, 2004) have shown that intraplantar injection of the CB2 receptor agonist JWH-133 inhibited mechanical evoked responses of WDR dorsal horn neurons in the SNL model. These findings suggest that the activation of CB2 receptor may promote relief of pain, without the side effects due to the stimulation of CB1 receptor. The anatomical localization of CB2 receptors (essentially in immune cells) leads to the intriguing question regarding the relationship between CB2 receptors and nociception. One possibility is that CB2 receptors act on the peripheral terminals of primary sensory neurons, even if evidences concerning the expression of CB2 receptors on these neurons are conflicting. Alternatively, CB2 receptor agonists could inhibit pain responses by an indirect mechanism, which involves the activation of the receptors on immune cells thereby decreasing the release of molecules that sensitise primary afferent neurons (e.g. prostanoids, cytokines, ATP, histamine, 5-hydroxytryptamine). A recent in situ hybridization study (Zhang et al., *Eur. J. Neurosci.*, 17, 2750, 2003), has shown that peripheral nerve injury induced the CB2 expression in specific cell types within the lumbar spinal cord that appear to correspond to the activated microglia. This finding suggests that CB2 agonists may be effective against neuropathic pain via CB2 receptor-expressing microglia which modulates the spinal processing of nerve injury-induced pain signals. Recent researches performed in our laboratory aimed to target components of endocannabinoid system to obtain relief of pain without adverse psychotropic effects. Particularly, we have demonstrated that daily administration of AM404, an inhibitor of anandamide (AEA) uptake, to CCI rats resulted in inhibition of both hyperalgesia and allodynia and that this effect reached the maximum after 7 days of treatment. The dose employed did not affect the pain response of contralateral paw and it was ineffective when given acutely to CCI rats. Furthermore, the repeated administration of FAAH inhibitor, URB597, led to a dose-dependent antihyperalgesic effect in CCI rats only after 14 days of treatment. All together our findings suggest that the increase in the endocannabinoid tone, by modulating AEA uptake and metabolism, can relieve pain and highlight these processes as drug targets for compounds useful in the treatment of chronic pain states, such as neuropathic one. The pain relief induced by the enhancement of endogenous level of AEA may be also due to the ability of this endocannabinoid to bind TRPV1 receptor which acts as molecular integrator of noxious physical and thermal stimuli and whose sensitivity and expression is increased in pathological condition, such as neuropathy. Another compound able to bind TRPV1 receptor and to inhibit, at least in vitro, AEA reuptake and metabolism is cannabidiol (CBD), a major component of *Cannabis sativa* that lacks the psychotropic effects, since it is a very weak ligand for both cannabinoid receptor subtypes. We have shown that repeated oral administration of CBD to CCI rats, starting following the onset of painful symptoms, evoked a dose-related inhibition of thermal hyperalgesia and mechanical allodynia. Furthermore, the CBD-induced antihyperalgesic effect was completely reversed by pre-treatment with the selective TRPV1 receptor antagonist and unaffected by the pre-treatment with CB1 or CB2 specific antagonists, highlighting the vanilloid system as the molecular target for CBD

effect. Finally, despite the substantial evidence of the cannabinoids efficacy against neuropathic pain in animals, there are currently very few prospective trials of their efficacy in humans. Attal et al. (*Eur. J. Pain* 8, 173, 2004) showed no benefit of THC in pain and quality of life in patients with refractory neuropathic pain. On the contrary, GW Pharma is undertaking a major research programme in the UK to develop and market distinct cannabis-based prescription medicines (THC:CBD, High THC, High CBD). Recently Berman et al. (*Pain* 112, 299, 2004) have shown the efficacy of two whole plant extracts of *Cannabis sativa*: GW-1000-02 (Sativex), containing THC:CBD in a 1:1 ratio and GW-2000-02, containing primarily THC, for the relief of pain from brachial plexus avulsion, an human model of central neuropathic pain.

SATIVEX[®]: NEW DATA AND PERSPECTIVES

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Sativex[®] is a cannabis-based medicine, manufactured from highly standardised extracts, Tetranabinex[®] (delta-9-tetrahydrocannabinol (THC)-rich Botanical Drug Substance (BDS)) and Nabidiolex[®] (Cannabidiol (CBD)-rich BDS), presented as an oromucosal spray. It is indicated as adjunctive treatment for the symptomatic relief of neuropathic pain in patients with multiple sclerosis. Patients self-titrate Sativex[®]: each 100µl actuation delivers 2.7mg delta-9-tetrahydrocannabinol (THC) and 2.5mg of cannabidiol (CBD), allowing patients to optimise the therapeutic window for symptom relief.

There is potential for interaction and synergy between the principal cannabinoids, minor cannabinoids and non-cannabinoid components present in cannabis, and this may play a role in the therapeutic potential of cannabis as a medicine as they have their own pharmacology. This may explain why a cannabis-based medicine using extracts containing multiple cannabinoids, in defined ratios, and other non-cannabinoid fractions, may provide better therapeutic success and be better tolerated than the single synthetic cannabinoid medicines currently available.

The efficacy and tolerability of Sativex[®] has been investigated in a series of randomised, double-blind, placebo-controlled clinical studies, in symptomatic multiple sclerosis (MS) and chronic pain patients. Outcome was assessed using an 11-point Numerical Rating Scale (0-10). Study medication was added to patients' existing therapies. The benefit observed was incremental to any benefit they had previously gained on other medications. Long-term open-label studies have been undertaken to evaluate the long-term safety and durability of effect. Primary efficacy data from a range of clinical studies is presented in Table 1.

Table 1 – Primary Outcome measures in Sativex[®] Studies

	CNP in MS ¹ (n=66)	CNP in BPA ² (n=48)	PNP ³ (n=125)	Spasticity in MS ⁴ (n=189)	Cancer Pain ⁵ (n=177)	RA ⁶ (n=58)
Baseline NRS Score	6.5	6.7	7.2	5.4	5.7	6.7
Treatment Difference (ITT) [§]	-1.25	-0.58	-0.96	-0.52	-0.67	-0.95
p-value	0.005	0.005	0.004	0.048	0.014	0.044 [#]
Week 52 NRS Score	2.8	4.2	5.1	3.7	NA	NA

NRS = Numerical rating Scale, CNP = Central Neuropathic Pain, BPA = Brachial Plexus Avulsion, PNP = Peripheral Neuropathic Pain;

RA = Rheumatoid Arthritis, [§] Treatment Difference between Sativex[®] & placebo, [#] = Pain on movement

The beneficial effects of Sativex[®] are maintained over time, with no evidence of tolerance. It is well tolerated, with low levels of intoxication-type reactions (Visual Analogue Scale (VAS) scores <5 out of 100) in long-term use.

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CANNABINOIDS AND CANCER

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Cannabinoid receptors represent a new endogenous signaling system that can be targeted pharmacologically for the inhibition of cancer growth. The endocannabinoid system regulates cell proliferation in several cancer cell types. We have previously observed that anandamide inhibits the proliferation of human breast cancer cells by blocking the G₀/G₁-S phase transition of the cell cycle through interference with CB₁ receptor-coupled signal transducing events. We have also shown that a metabolically stable anandamide analog (Met-F-AEA) reduces the growth rate of K-ras-dependent thyroid tumors, induced and/or already established, *in vivo*: these effects are CB₁ receptor mediated. In order to understand the molecular mechanism involved in these processes and to better clarify the role of the endocannabinoid system in tumor progression, we have analysed the anti-proliferative effect of anandamide on estrogen receptor-negative human breast cancer cell line MDA-MB-231. Our data suggest that the decrease in cyclin A and cyclin E expression and increase in p21^{waf} expression lead to the suppression of phosphorylation of pRb, that contributes to the cell cycle arrest. We also hypothesize that anandamide and CB₁ biochemical pathways are strongly influenced by their intracellular trafficking within the cells and that anandamide itself might trigger functional event into rafts. We believe that alteration of CB₁ trafficking and its activation after AEA binding have a role in the inhibition of breast cancer cell proliferation. Moreover, endocannabinoids are now emerging as suppressors of tumor invasion and angiogenesis. We evaluated the potential role of CB₁ receptor in metastatic processes using a model of *in vivo* metastatic infiltration, the murine breast cancer cell line (TSA-E1) in syngenic mice. Met-F-AEA significantly reduced number and dimension of metastatic nodes in a way antagonized by the selective CB₁ receptor antagonist SR141716A. Furthermore, *in vitro* adhesion and migration assays on type IV collagen showed that Met-F-AEA inhibited MDA-MB-231 and TSA-E1 cell adhesion and migration; these effects were antagonized by SR141716A. Our data suggest that MET-F-AEA, by modulating FAK tyrosine phosphorylation and integrin expression, can inhibit tumor cell invasion and metastasis. Furthermore our recent data show that Met-F-AEA significantly inhibits, in tumors as well as in transformed cells, the expression of the vascular endothelial growth factor (VEGF) as well as of one of its receptors, flt-1/VEGFR-1. All these effects were antagonized by SR141716A. VEGF released from cancer cells can also exert a role as a paracrine factor able not only to stimulate the proliferation of endothelial cells and the formation of new vessels, but also, and possibly more importantly, the migration and spreading of cancer cells. Therefore, the down-regulation of both VEGF and Flt-1 induced *in vivo* by AEA may also lead to a direct effect on growth, metastasis and neoangiogenesis. We also reported an antiangiogenic effect of anandamide in porcine aortic endothelial cells (PAE), by a two-dimensional and a three-dimensional model of angiogenesis.

Our findings strongly suggest that CB₁ receptor agonists may be promising anti-tumoral agents as they inhibit growth, angiogenesis and metastatic spreading.

CANNABINOIDS AND REPRODUCTION

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Anandamide (*N*-arachidonylethanolamine, AEA) is known to impair mouse pregnancy and embryo development and to induce apoptosis in blastocysts. In humans, high levels of AEA in maternal blood coincide with pregnancy failure, attributable to dysregulation of the lymphocyte-mediated signalling at the feto-maternal interface. Here, I shall review the roles of AEA, of the AEA-binding cannabinoid (CB) receptors, of the selective AEA membrane transporter (AMT), of the AEA-synthesizing enzyme NAPE-specific phospholipase D (NAPE-PLD), and of the AEA-hydrolyzing enzyme fatty acid amide hydrolase (FAAH) in fertility. In particular, I shall discuss the role of AEA degradation in human reproduction, showing that FAAH in maternal lymphocytes has a critical role for successful pregnancy, and that key-modulators of fertility like follicle-stimulating hormone, progesterone and leptin regulate FAAH activity and/or *FAAH* gene expression at specific sites of the promoter region. I shall also discuss new findings on the role of the endocannabinoid system in regulating sperm functions critical for male reproduction. Taken together, available evidence points towards a key-role for endocannabinoids in the hormone-cytokine networks that regulate human reproduction, at both female and male sides.

CANNABINOIDS AND THE GUT

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The digestive tract contains endogenous cannabinoids (i.e. the endocannabinoids anandamide and 2-arachidonylglycerol) and mechanisms for endocannabinoid inactivation (i.e. endocannabinoids uptake and enzymatic degradation). CB₁ receptors have been detected on enteric nerves and pharmacological effects of their activation include gastroprotection, relaxation of the lower oesophageal sphincter, reduction of gastrointestinal motility and secretion. The endogenous cannabinoid system has been found to be involved in the physiological control of intestinal motility and in some pathophysiological states, including paralytic ileus, intestinal inflammation and cholera toxin-induced diarrhoea. Moreover, cannabinoids inhibits the proliferation of colorectal carcinoma cells. A pharmacological modulation of the endogenous cannabinoid system could potentially provide new therapeutics for the treatment of a number of gut diseases, including irritable bowel syndrome, ulcerative colitis, secretory diarrhoea, paralytic ileus, gastroesophageal reflux disease and colon cancer.

CANNABINOIDS AND THE CARDIOVASCULAR SYSTEM

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The cardiovascular actions of cannabinoids are complex (Randall *et al.*, 2004). In general they cause vasorelaxation in isolated blood vessels, whilst in anaesthetised animals they cause characteristic multiphasic responses which involve an early bradycardia, a transient pressor action and long-lasting hypotension. However, in conscious animals the picture is one of bradycardia followed by pressor responses. Clearly, the responses to cannabinoids are dependent on the experimental conditions.

In terms of mechanisms involved in the vascular responses to cannabinoids the following have been implicated: (a) the involvement of 'classical' cannabinoid receptors, (b) the involvement of a novel endothelial cannabinoid receptor which is coupled to the release of endothelium-derived hyperpolarising factor (EDHF), (c) the release of nitric oxide, (d) the activation of TRP vanilloid receptors leading to CGRP-mediated vasodilatation, (e) metabolism of endocannabinoids to vasoactive molecules, (f) both peripheral inhibition and central excitation of the sympathetic nervous system. In addition, it is also emerging that synthetic cannabinoids and endocannabinoids may act via different mechanisms of vasorelaxation.

In addition to our advances in understanding of the physiology and pharmacology of the cannabinoid system, alterations in the endocannabinoid system have also been reported in hypotensive shock, ischaemia and hypertension.

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