POSTER SESSION

Thursday 29th October

17.45-19.30

P1

Prion infectivity and neurotoxicity could be prevented by modulation of EndoCannabinoid System

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Interaction of antibodies with toxic prion protein form (PrP scrapie) responsible for neurodegenerative diseases through a not yet understood mechanism, can lead to healing or worsening in animals. Binding of antibody POM1 to prion protein, for instance, increases PrP toxicity in in vitro experiments [1]. Moreover, our collaborators have shown [2] that several compounds, among which Cannabidiol, a low affinity ligand of endocannabinoid receptors 1 and 2, could prevent toxic effects induced by PrP scrapie.

We designed single POM1 antibody mutants that are either non-toxic or, more strikingly, can protect from prion infection even when administered 20 days post PrP scrapie infection. This surprising and novel result has implications for therapy but it also gives as a tool to understand more about PrP toxicity and protection. Comparison of PrP in complex with toxic, non-toxic and protective antibodies might, in fact, reveal differences pinpointing the molecular requirements for PrP toxicity. Furthermore testing the effect of anti-prion antibodies in the presence of agonist or antagonist of endocannabinoid system will allow us to confirm a potential clinical application of these compounds in neurodegenerative diseases.

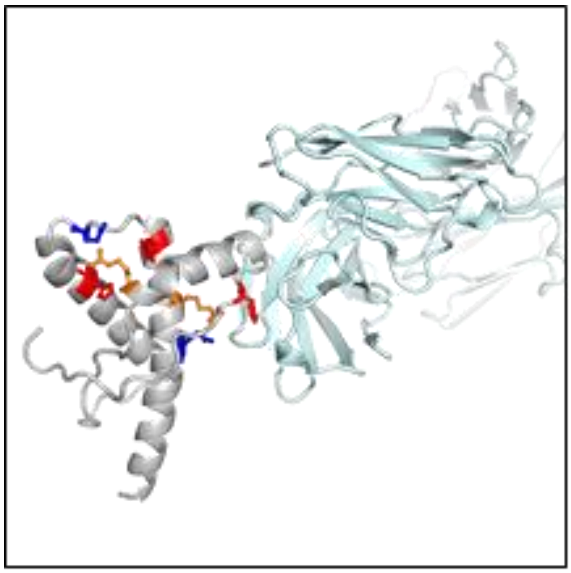

Figure 1. Cartoon representation of human PrP (light blue) in complex with POM1 antibody (light cyan). In orange are highlighted residues that interact differently in bound or free hPrP conformation. Different structural behaviour could be directly correlated to antibody induced neurotoxicity

References

[1] Polymenidou, M., et al., The POM monoclonals: a comprehensive set of antibodies to non-overlapping prion protein epitopes. PLoS One, 2008. 3(12): p. e3872.

[2] Falsig, J., et al., Prion pathogenesis is faithfully reproduced in cerebellar organotypic slice cultures. PLoS Pathog, 2012. 8(11): p. e1002985.

[3] Sonati, T., et al., The toxicity of antiprion antibodies is mediated by the flexible tail of the prion protein. Nature, 2013. 501(7465): p. 102-6.

P2

Investigating the Eicosanoid Pathway in *Caenorhabditis elegans* to Identify Novel Serine Hydrolase Inhibitors

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Caenorhabditis elegans (*C. elegans*) has emerged as a powerful tool for investigating a diverse array of biological mechanisms. Currently, we are investigating the endocannabinoid system (ECS) with a particular focus on arachidonic acid metabolism in *C. elegans*. Our goal is to understand and pharmacologically exploit key eicosanoids within *C. elegans* to modulate the accessibility of arachidonic acid, an essential fatty acid involved in normal cellular processes. Serine hydrolases are a class of enzymes that play a key role in the synthesis of arachidonic acid. Since the components of these serine hydrolases have not been studied extensively in *C. elegans*, we first characterized these enzymes in *C. elegans* with activity-based protein profiling (ABPP) and assessed their role in normal worm phenotypes. We tested the known serine hydrolase inhibitor aldicarb, which is extensively used in research and agriculture to cause paralysis in nematodes via its inhibition of acetylcholinesterase, a serine hydrolase primarily expressed in neurons. Moreover, more than 380 medicinal plant extracts have been screened to identify specific inhibitors of the *C. elegans* arachidonic acid biosynthesis pathways identified in the ABPP as a potentially novel antinematodal strategy

P3

Targeting the Putative Endocannabinoid Transporter: Guineensine and WOBE437

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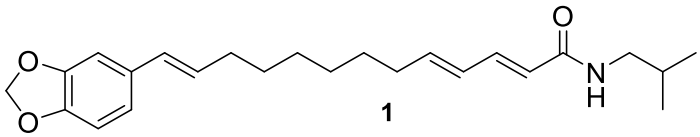
Guineensine (1) is a natural product isolated from Piper nigrum that was recently shown to be a new inhibitor of cellular reuptake of the endocannabinoid anandamide (AEA) in U937 cells (IC_{50} = 290 nM).[1] In order to provide a basis for the exploration of structure-activity relationships (SAR) around 1, we have developed a new total synthesis of this natural product. The synthesis comprises five linear steps and thus is the shortest route to 1 that has been developed to date. Based on this chemistry we have prepared a series of guineensine analogs that were assessed for their ability to inhibit AEA reuptake. The results of these SAR studies reveal that variations of the amide part of 1 can lead to a significant improvement in potency. Furthermore, the importance of the double bonds in 1 for AEA reuptake inhibition was confirmed.



1

WOBE437 is an unsaturated fatty acid amide that is a low nM inhibitor of AEA reuptake and shows exquisite selectivity with respect to inhibition of the anandamide hydrolysing enzyme fatty acid amide hydrolase. We are using WOBE437 as a structural platform for the design of molecular tools that should enable the identification of the putative endocannabinoid membrane transporter (EMT).[2] In this context we have synthesized a highly active (as anandamide reuptake inhibitor) photoaffinity probe (IC_{50} = 16 nM) as well as several fluorescent and biotin-labeled inhibitors, and an alkyne probe which is suitable for immobilization by click-chemistry. The poster will also focus on the latest SAR findings associated to our synthesized AEA uptake inhibitors and tool compounds.

Reference

[1] Nicolussi S. et al., *Pharmacol. Res.* **2014**, 52-65.

[2] Nicolussi, S.; Gertsch, *J. Vitamins and Hormones* **2015**, 98, 441-485.

P4

Novel Urea-based Inhibitors of Anandamide Reuptake with Anti-inflammatory Activity in Keratinocytes in vitro

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We have recently reported *N*-alkylcarbamates as potent inhibitors of cellular reuptake of the endocannabinoid anandamide (AEA).[1] Moreover, a plant derived dodeca-*2E,4E*-diene amide, WOBE437, was described to be a prototype of a novel class of potent and selective endocannabinoid reuptake inhibitors. One goal of this project was to evaluate whether *N*-alkylurea analogs of WOBE440, WOBE701 [1] and WOBE437 would also inhibit cellular AEA uptake. Moreover, we were interested in learning whether such an effect would be mediated by fatty acid amide hydrolase (FAAH) inhibition or by inhibition of the yet to be isolated endocannabinoid membrane transporter (EMT). A series of dodeca-*2E,4E*-diene derived *N*-alkylureas was prepared. Analogs of WOBE440 and
WOBE701 were found to exhibit no or marginal inhibitory activity on cellular AEA reuptake. However, the analog of WOBE437 in which the amide was replaced by a urea showed an EC_{50} of 15 nM. The influence of the alkyl chain, the head group of the urea as well as urea-N methylation was assessed. The most potent compounds were found to inhibit cellular AEA reuptake with single digit nanomolar EC_{50}s.
For a selected compound, WOBE635, FAAH, COX-1 and COX-2 inhibition were determined. WOBE635 inhibited cellular AEA reuptake with an EC_{50} of 10 nM and FAAH with an IC_{50} of 9.2 µM while not inhibiting COX-1 and COX-2. The compound did not bind to CB_1 and CB_2 receptors. Moreover, WOBE635 also exerted anti-inflammatory effects in human epidermal keratinocytes at a non-cytotoxic concentration following TLR-2 activation on gene and protein level.[2]
The results obtained in this study could be the basis for future structure-activity relationship (SAR) studies for this novel class of AEA reuptake inhibitors.

Reference

[1] Nicolussi S. et al. *Biochem. Pharmacol*. 2014, 92: 669-689
[2] methods: Oláh A. et al*. Exp. Dermatol*. under revision

P5

Anti-inflammatory and anti-pruritic properties of a lipophilic *Echinacea purpurea* root extract

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Echinacea purpurea extracts (purple coneflower) are known to have immune-modulatory effects. Several alkamides, the major lipophilic constituents, bind to cannabinoid receptors. Since the endocannabinoid system is of importance in inflammatory skin diseases, anti-inflammatory activity of alkamides was investigated. Therefore the new lipophilic root extract of *Echinacea purpurea* was tested in cultured human keratinocytes. A significant ($p<0.05$) reduction of lipoteichoic acid-induced mRNA expression of IL-1a, IL-1b, and IL-6 was observed. Moreover, the mixture was also able to reduce expression of IL-8 and showed significant anti-inflammatory effects in vitro.

The overall impact of the new developed water-in-oil (W/O) emulsion containing the lipophilic root extract of *Echinacea purpurea* was investigated in different clinical studies.

Long-term efficacy and safety was evaluated in a 3 month half-side trial against comparator (30.2 ± 15.9 years; n = 60). The emulsions were applied at least twice daily on two comparable and contra-lateral located skin areas on the crooks of arms, hollow of the knees, on the trunk, on the wrist or on the shin with slight lesions of atopic dermatitis.

Erythema, pruritus and local SCORAD reduced significantly after 1, 2 and 3 months after application of *Echinacea purpurea* root extract (W/O) emulsion, as well as comparator. Interestingly, *Echinacea purpurea* root extract (W/O) emulsion is superior after prolonged application, indicated by a significant difference to comparator after 2 or at least after 3 months.

To gain further insight in underlying mechanisms, electron microscope analyses of the skin barrier as well as the lipid analysis by HPTLC will be performed and data will be presented.

In summary, application of an *Echinacea purpurea* root extract (W/O) emulsion reduced significantly the local SCORAD, erythema and pruritus without irritation, very likely by improved functions of the epidermal barrier.

Phylogenetic and chemotaxonomic analyses of endocannabinoids and their fatty acid precursors in the plant kingdom

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Endocannabinoids (ECs) are lipid mediators involved in many physiological and pathological conditions by interacting with endocannabinoid receptors (CB_1 and CB_2) and other receptors. The two most studied ligands are 2-arachidonoyl glycerol (2-AG) and arachidonoyl ethanolamide (anandamide, AEA) produced from arachidonic acid. Although the occurrence of endocannabinoids and related lipids in plants has been postulated, there is no systematic data available and the phylogenetic distribution is currently unknown. Therefore, the aim of this study was to systemically analyze arachidonic acid, endocannabinoids, new endocannabinoid-like lipids and their fatty acid precursors in 70 plant species representative of the major plant phylogenic clades by GC-MS and LC-MS/MS analyses. Our study provides new insights into evolution of arachidonic acid and the endocannabinoid system at the level of secondary metabolites found in the plant kingdom prior to the evolution of mammalian cannabinoid receptors.

Beyond Δ^9-Tetrahydrocannabinols: Stereodivergent Dual Catalysis as a Tool for the Divergent Synthesis of Cannabinoid Derivatives and their Pharmacological Analysis

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The abstract is not shown upon authors' request

P8

The CB_1 and CB_2 cannabinoid receptors in bovine fetal pancreas at late gestation: preliminary results.

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The present immunohistochemical investigation studied cannabinoid receptors distribution in the bovine fetal pancreas at late gestation.

The interest in this topic arises from the consideration that data present in the literature report that the bovine pancreas is very similar to the human in endocrine portion development and control, therefore studies on the fetal gland could prove to be very interesting, as an abnormal maternal condition during late pregnancy may be a predisposing trigger for adult metabolic disorders. Moreover, data present in bibliography on the endocannabinoid system expression and distribution in the endocrine pancreas appear scarce and controversial as descriptions are limited to humans and laboratory animals.

Immunohistochemistry showed large islets contained almost only β-cells which co-localized with the CB_1 cannabinoid-receptor. Small islets expressed only the CB_2 cannabinoid- receptor in some cells primarily localized at the edges of islets even if it was possible to observe them also scattered in the center of the cluster. According to the results of the current search, the CB_2 cannabinoid-receptor was localized in neither insulin- nor glucagon-producing cells. Characteristically, also the smooth muscle layers of the smaller arteries, in the interlobular glandular septa, tested positive for the CB_2 endocannabinoid receptor.

Although further studies are needed to better identify the CB_2 positive cells, we hypothesize that the endocannabinoid system is able to play a major role in controlling pancreas functionality in the fetal age, at late gestation, so affecting, in addition to the proper development of the fetal pancreas, also the correct metabolic functioning in adulthood.

P9

Tetracyclic triterpenoids derived from euphol selectively inhibit ABHD12

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Alpha/beta-hydrolase domain-containing 12 (ABHD12) is an integral membrane protein that exhibits monoacyglycerol lipase activity *in vitro* and has been suggested to contribute to the metabolism of the endocannabinoid 2-arachidonoylglycerol (2-AG) in the nervous system [1]. Tetrahydrolipstatin (THL), originally developed as a DAGL inhibitor, is currently the only available compound used for ABHD12 inhibition. However, it has been shown to inhibit several other serine hydrolases, among them the ABHD6 and the ABHD16A (with IC_{50} values of about 100 nM) [2]. A better understanding of the role of ABHD12 in the endocannabinoid system (ECS) is hampered by the lack of selective inhibitors. With the aim of finding potent and selective ABHD12 inhibitors, we established the stable cell line HEK293T-hABHD12 which we used as source of ABHD12 for screening of focused libraries of natural products as well as semisynthetic derivatives. Among the euphol-derivative series, we found two compounds, euphorbone oxide and hydroxyeuphorbone, that exhibit IC_{50} values for ABHD12 inhibition in the sub-micromolar range and with a ≥100 times selectivity over the other endocannabinoids degrading enzymes (ABHD6, MAGL and FAAH). Michaelis Menten analysis of euphorbone oxide, the most selective of them, indicates that this compound inhibits ABHD12 by significantly decreasing its V_{max} at the same that that it increases the K_m. Current work is focused on chemical modifications that would lead to increase the potency of the current hit compounds as well as further studies on the mechanism of inhibition and its impact on whole cell content of 2-AG.

P10

Ginger phenylpropanoids inhibit FAAH activity
Potential benefits of regular ginger intake in preventing GI damage

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Ginger, the rhizome of *Zingiber officinale* L., is one of the most widely consumed spices worldwide. Moreover, it has a vast history as herbal medicine to treat a variety of ailments, in part related to the gastrointestinal (GI) tract, including vomiting, pain, indigestion, and fever [1]. We have previously shown that ginger phenylpropanoids exert potent anti-inflammatory effects via specific inhibition of IL-1b and PLA2, thus modulating free arachidonic acid [2]. Given the involvement of the endocannabinoid system (ECS) in many of these pathophysiological conditions, we studied whether ginger exerts its effects, at least in part, through the modulation of the components of the ECS. Here, we show for the first time that ginger extracts potently inhibit FAAH (IC_{50} ~300 ng/mL). A ginger extract enriched in phenylpropanoids (Flavex) was studied in more detail. While potently inhibiting FAAH it exhibited weak inhibition of ABHD6 and ABHD12 (IC_{50} > 10 µg/mL), no inhibition of MAGL and weak CB receptor binding affinity ($IC_{50}CB_1$ ~10 µg/mL and $IC_{50}CB_2$ ~7 µg/mL). When testing the major active compounds of ginger, we found that shogaols inhibit FAAH with 10-shogaol being particularly potent (IC_{50}~150 nM). As 10-shogaol is at the same time a good COX-2 inhibitor (IC_{50}~500 nM), and it has been reported that FAAH and COX-2 are expressed in abnormally high levels in some inflammatory diseases, e.g. inflammatory bowel disease [3], regularly consuming ginger could be of great benefit for preventing and/or reverting GI tract damage. Here, we show that in the croton-oil mouse model of GI/intestinal inflammation [4], where FAAH expression is significantly increased, the *ex vivo* incubation of Flavex (100 µg/mL) was able to decrease the extent of AEA hydrolysis as assessed in membrane preparations from different organs. Further experiments to assess its efficacy *in vivo* are ongoing.

Reference

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P11

Endocannabinoids in chronic alcoholic liver disease

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Introduction: Cannabinoid receptors CB_1 and CB_2 are implicated in the development of chronic liver diseases. However, the mechanisms by which the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) contribute to ongoing liver damage in alcoholic liver diseases (ALD) are incompletely defined.

Methods: Anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) were measured by gas chromatography and mass spectrometry (GC-MS) in plasma from healthy individuals and ALD patients. Gene expression was assessed by TaqMan PCR. In vivo, liver fibrosis in was induced by combination of ethanol and CCL4 for 5 weeks in C57BL6 mice, which were treated with inhibitors of fatty acid amid hydrolase (FAAH, URB937), monoacyl glycerol lipase (MAGL, JZL184) or vehicle control for 4 weeks. Liver damage was assessed by ALT and AST levels. Collagen content was measured by hydroxyproline determination and Sirius Red stain. Hepatic inflammation and necrosis were semi-quantitatively evaluated from H&E stainings.

Results: AEA and 2AG plasma levels were significantly higher in patients with ALD, whereas FAAH and MAGL mRNA in liver biopsies were 2- and 10-fold downregulated, respectively, compared to healthy controls. Statistical analysis revealed ALT, AST and alcohol levels as predictors of high AEA among alcoholic patients ($p<0.05$). The active metabolite of ethanol - acetaldehyde (AA) slightly inhibited enzymatic activity of MAGL, reflected by a reduced amount of hydrolyzed 2AG. In peripheral blood mononuclear cells AA showed similar effect by reducing MAGL mRNA. In vivo, inhibition of FAAH and MAGL in alcohol-induced liver injury in mice did not affect strongly liver fibrosis, inflammation and necrosis, but modified fibrosis- and inflammation-related gene expression.

Discussion / Preliminary conclusion: Chronic alcohol consumption may induce endocannabinoids AEA and 2AG levels via the blockage of endocannabinoid degradation enzymes activity. Whether this elevation actively contributes to the ongoing alcohol-related liver damage still requires more work for elucidation.

P12

Rimonabant inhibits the heterotrimeric G protein activity in a receptor independent manner

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Rimonabant is the first Cannabinoid type 1 receptor (CB_1) antagonist to be developed [1]. At high micromolar concentrations it behaves as inverse agonist, decreasing [^{35}S]GTPγS binding in rodent and human cerebral cortex and in Chinese hamster ovary (CHO) cells transfected with CB1 receptors [2]. However, in vitro and in vivo studies on brain membranes of CB_1 knockout (KO) and CHO cells not expressing CB_1 receptors suggest that inverse agonist activity of Rimonabant is CB_1 receptor independent [3-4]. The exact mechanism has not been clarified yet. The present study aimed to determine whether the CB_1 receptor-independent effects of Rimonabant are mediated via GPCRs, in particular GABAB and dopamine DR2 receptors, that share the same Gαi/o signaling pathways, or if Rimonabant acts directly on G protein. Using [^{35}S]GTPγS binding assay on native and recombinant system, we discovered that Rimonabant (IC_{50} of 5-10 μM range) decreases basa l and agonist-stimulated [^{35}S]GTPγS binding to cortical membranes of rats, CB_1 and GABAB KO mice, as well as in CHO naive cells and CHO cells stable transfected with GABAB or DR2. In Bioluminescence Resonance Energy Transfer (BRET) experiments Rimonabant increases the Gαβγ heterotrimeric state, induces a rearrangement between DR2 and Gαi subunit, and decreases G protein activity after GABAB agonist application. Moreover Rimonabant, blocking Gαi signaling, leads to an inhibition of Adenylyl Cyclase activity. Finally electrophysiological recordings reveal that Rimonabant blocks the Gβγ mediated K^+ current elicited by Baclofen and Quinpirole in dopamine neurons of CB_1-KO mice and elicited by GTPγS on CHO cells transfected with GIRK1/2.

Reference

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[4] Erdozain et al., *Biochem. Pharmacol.* 83:260–268, 2012

P13

A fluorescently-labelled noladin ether probe to investigate the endocannabinoid bidirectional movement across the plasma membrane and their tissue distribution *in vivo*

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Introduction: The effects of endocannabinoids (ECs) are regulated by cellular biosynthesis, release, reuptake, trafficking and enzymatic metabolism. Unlike the clear knowledge about the biosynthetic and metabolic pathways, the mechanisms of cell membrane trafficking is not yet elucidated. Although the identification of the putative endocannabinoid membrane transporter (EMT) remains still elusive, the best experimentally supported theory relies on a passive membrane transporter-mediated mechanism. One of the main issues in elucidating the uptake process is the tight inter-play between ECs plasma membrane movement and their rapid and almost complete cleavage mainly dependent on FAAH and MAGL activity. Recently we have shown that all ECs compete for the same EMT independently of their intracellular fate (trafficking and enzymatic inactivation). Thus, we have synthesized and characterized a fluorescent-analogue of noladin ether (NBD-2AGE), the only EC hydrolysis-resistant.

Methods: The biological properties of NBD-2AGE were investigated testing CB receptor binding, bidirectional trafficking across plasma membranes and enzymatic cleavage. The stability of the probe was investigated using HPLC and FACS methods. The kinetics of cell uptake and release was investigated in different cell lines and in PBMCs. NBD-2AGE was also incubated with mouse brain slices and the distribution was analyzed by using confocal microscopy and high-resolution laser scanner (Typhoon FLA 9500). The distribution of NBD-2AGE into the brain and peripheral tissues was investigated after i.p. and intracisternal (i.c.) injection in mice. A LC-MS/MS method was established to detect noladin ether in the brain and peripheral tissues.

Results: 1) NBD-2AGE retains all main characteristics of ECs, including binding to CB receptors and trafficking across plasma membranes. 2) NBD-2AGE showed a saturable and inhibitable kinetics of uptake and release in different cell lines and in PBMCs. 3) NBD-2AGE confirmed to be a hydrolytic-resistant EC analogue and to selectively compete with the main ECs for cellular uptake. 4) The fluorescent probe showed a specific distribution in different brain regions which was prevented by the pre-treatment with EMT inhibitors and incubation at low temperature. 5) Upon i.p. and i.c. injection, NBD-2AGE showed a different pattern of distribution in the brain and peripheral tissues. 6) Using a LC-MS/MS method, noladin ether was identified in human and rodent plasma but not in the brain.

Conclusions: Our data suggest that NBD-2AGE is a very useful probe to investigate the kinetics of EC trafficking across plasma membranes with a sensitive and radioactivity-free based method. Unlike the other ECs, NBD-2AGE is resistant to the fast and very efficient FAAH- and MAGL-mediated hydrolysis, which is a well-known confounding factor for studying cellular uptake and trafficking of AEA and 2-AG. Finally, fluorescently-tagged noladin ether would allow monitoring the ECs distribution in different cell types and tissues when applied to complex matrices such as whole blood or brain slices or injected in animals.

P14

2-Oxo-1,2-dihydropyridine-3-carboxamide scaffold as a tool to investigate the molecular pharmacology of the endocannabinoid system

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Cannabinoid receptor (CB1R and CB2R) and their endogenous ligands (endocannabinoids) anandamide and 2-arachidonoylglycerol , together with several enzymes implicated in endocannabinoid biosynthesis (DAGLs, NAPE-PLD) and degradation (FAAH, MAGL, ABDHs) and other proteins (intracellular carrier proteins and the putative endocannabinoid membrane transporter, EMT), constitute the endocannabinoid system (ECS). The ECS is involved in several physiological and pathological processes including cancer, appetite, memory, neuropathic and inflammatory pain, obesity and neurodegenerative diseases.

In a research project aimed at obtaining new CB2R ligands, a series of 6-methyl-2-oxo-1,2-dihydropyridine-3-carboxamide derivatives was developed. Interestingly, it was discovered that the nature of the substituent in position C5 of the pyridine ring is crucially involved in the functional activity of these molecules at CB2R. The present work is aimed at further investigating the structure-activity relationships of this class of compounds, exploiting different approaches: a) insertion of all the different halogens at the C5 position of the pyridine ring b) switch of the methyl group from the C6 to the C4 position; c) insertion of bulky substituents at the C4 or C6 position of the pyridine ring.

The 2-oxo-1,2-dihydropyridine-3-carboxamide derivatives were also tested on all the main targets of the ECS. Some of these compounds, beyond the modulation of CBR activity, are able to inhibit additional targets (FAAH, ABHDs and EMT). Therefore, since the 2-oxo-1,2-dihydropyridine-3-carboxamide derivatives can interact with different targets of the ECS, this scaffold might represent an useful tool to investigate different polypharmacological approaches to modulate the ECS

References

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Cannabinoid system is involved in effort-based and delay-based cost-benefit decision making

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Despite the evidence for disturbed decision making in cannabis abusers, the role of the cannabinoid system during decision making has not been studied. Here, we tested the effects of cannabinoid modulation during cost-benefit decision making in the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC), key brain areas involved in decision making. We trained different groups of rats in a delay-based and effort-based versions of cost-benefit T-maze decision making task. During test days, the rats received local injections of either vehicle or a cannabinoid type-1 receptor (CB1R) agonist, ACEA in the ACC or OFC. Spontaneous locomotor activity following the same treatments were also measured and CB1Rs localization on different neuronal populations within these regions were characterized using immunohistochemistry. We showed that CB1R activation in the ACC impaired decision making such that rats were less willing to invest physical effort to gain high reward. Similar ly, CB1R activation in the OFC induced impulsive pattern of choice such that rats preferred small immediate rewards to large delayed rewards. Control tasks ensured the specificity of the effects for differential cost-benefit tasks. Furthermore, we demonstrated widespread co-localizations of CB1Rs on GABAergic axonal ends but few co-localizations on glutamatergic, dopaminergic and serotonergic neuronal ends. These results provide direct evidence that the cannabinoid system plays a critical role in regulating cost-benefit decision-making in the ACC and OFC and implicate cannabinoid modulation of synaptic ends of predominantly interneurons and to a lesser degree other neuronal populations in these frontal regions. Similar to lesion studies, these results also suggest a double dissociation in the processing of decision costs at the level of cannabinoid modulation.