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BOOK OF
ABSTRACTS

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Department of Medicinal Chemistry
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Department of Pharmacy - Pharmaceutical Sciences
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Department of Pharmacy - Pharmaceutical Sciences
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Maj Institute of Pharmacology Polish Academy of Sciences

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Closing remarks

Prof. Katarzyna Starowicz
Maj Institute of Pharmacology Polish Academy of Sciences

with

Natalia Malek (1), Jakub Mlost (1), Barbara Przewlocka (1), Luigia Cristino (2), Sabatino Maione (3), Enrico Morera (4), Vincenzo Di Marzo (2,5,6)

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BEYOND CB1: UNDERSTANDING THE ACTIONS OF ANADAMIDE IN PAIN AND NOCICEPTION

Anandamide (AEA) and congeners have been shown to play key biological activities both the central nervous system (Di Marzo et al., 2015), and the periphery (Jourdan et al., 2016). This multifaceted ability of AEA to impact on nearly every system of human body depends on a multiplicity of receptor targets that include, besides CB1 and CB2, transient receptor potential vanilloid-1 (TRPV1) channels, G-protein coupled receptors 55 (GPR55) and 119 (GPR119), and peroxisome proliferator activated receptors (PPARs; reviewed e.g., in Di Marzo et al., 2015 and Jourdan et al., 2016). Therefore, a deeper understanding of AEA action for the possible translation of AEA-based drugs into novel therapeutics for human diseases is needed. Despite the wealth of information on TRPV1 and, particularly, CB1 receptor distribution in the brain, much less was known about their potential co-expression thus we determined the neuroanatomical and cellular relationship between the vanilloid and cannabinoid systems in the CNS (Cristino et al, 2008) in order to achieve reduction of nociceptive processing in acute and chronic animal models.

Chronic pain is an unmet clinical problem that urgently requires the development of new treatment. One way this can be achieved is through a better understanding of the functional interaction between endogenous systems involved in the perception and transmission of pain stimuli. The aim of this research was to determine the role of the endocannabinoid (CB1/2) and endovanilloid (TRPV1) receptors systems in the development and treatment of chronic pain. Particular emphasis was placed on the pain associated with nerve damage and osteoarthritis, which constitute the majority of clinical cases of chronic pain. With certain concern on pain and nociception (including brainstem areas involved in the descending antinociceptive pathway, such as the PAG) we examined the effect of TRPV1 activation and antagonism on glutamatergic and GABAergic signaling in the VL-PAG-RVM circuit (Starowicz et al., 2007). We also stressed the importance of spinal AEA concentrations in determining the receptor through which this endocannabinoid/endovanilloid exerts its analgesic effects in chronic pain. Our study supported the hypothesis of an anti-allodynic and anti-hyperalgesic role for spinal TRPV1 and its endogenous ligands in conditions of neuropathic pain, and suggested that indirect modulation of TRPV1 function, as well as strengthening endogenous AEA signaling by inhibiting its enzymatic degradation, both holds promise for the development of novel multi-target pharmacological treatments (Starowicz et al., 2012, 2013; Malek et al. 2015).

Finally, the association between chronic pain, depression and anxiety has gained particular attention due to high rates of comorbidity. Recent data demonstrated that the mesolimbic reward circuitry is involved in the pathology of chronic pain. The endocannabinoid system has emerged as a highly relevant player involved in both pain perception and reward processing (Mlost et al., Pharm Res 2019; Mlost, Bialon et al., submitted). Targeting enzymes involved in the degradation of endocannabinoids seems to be a promising treatment strategy as endocannabioids are produced "on demand" and therefore, inhibition of degradative enzymes (such as FAAH) should be devoid of unwanted side effects as the increase of endocannabinoid levels should be limited specifically to disease-affected tissues. Consequently, we demonstrated (Mlost et al., Front Mol Neurosci 2018) that increasing endocannabinoid tone, counteracts the hypodopaminergic state in mesolimbic and mesocortical brain structures in OA animals without affecting healthy animals.

Acknowledgements

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THE GUT MICROBIOME AND ITS INTERACTIONS WITH THE EXPANDED SYSTEM OF ENDOCANNABINOIDS AND SIMILAR SIGNALING LIPIDS

For several decades, and starting some 25 years from its discovery, the only plant cannabinoid with an established mechanism for its pharmacological actions has been D9-tetrahydrocannabinol (THC). To THC are ascribed the most important euphoric and psychotropic effects of recreational preparations (e.g. marijuana, hashish) obtained from those varieties of Cannabis sativa that are rich in this compound. The discovery of two G-protein-coupled receptors (GPCRs), the cannabinoid CB1 and CB2 receptors, specific for D9-tetrahydrocannabinol (THC) led to the identification of their endogenous agonists, later named endocannabinoids: N-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoyl-glycerol (2-AG). The chemical signaling system composed of CB1 and CB2 receptors, the two endocannabinoids and the anabolic and catabolic enzymes regulating endocannabinoid levels, became known as the endocannabinoid system.

More recently, an expanded endocannabinoid system, including many non-endocannabinoid long chain fatty acid amides and esters, such as: a) congeners of anandamide and 2-AG, b) N-acyl-aminoacids, c) N-acyl-neurotransmitters and d) primary fatty acid amides, has been discovered. These lipid mediators often share with the two endocannabinoids biosynthetic and/or inactivating enzymes, but not necessarily their receptors, which instead include orphan GPCRs, ligand-activated ion channels and peroxisome proliferator-activated nuclear receptors (PPARs). These small molecules, therefore, should not be considered endocannabinoids, but instead as endocannabinoid-like mediators, and this expanded endocannabinoid system is becoming known as the endocannabinoidome (eCBome)(1,2). The eCBome is involved in almost all aspects of mammalian physiology and pathology, and recent work from my and other laboratories have highlighted how this complex signalling system is directly modulated by, and in turn modulates, several other players in physiopathological conditions, including another very complex one: the gut microbiome (3).

Microbiomes are complex ecosystems constituted of different types of microorganisms (bacteria, archea, yeasts and viruses, as well as, in some cases, unicellular prokaryotes, which together form different microbiota), as well as their genes, proteins and metabolites, through which these microorganisms communicate with their typical environments. The microbiota composition in different kingdoms, phyla and taxa depends on the surrounding environment and its changes and, in the case of animal microbiomes, on the genetics of the host. In animals, all exposed parts of the body (and, as suggested by recent evidence, internal organs too) harbor specific microbiomes. The gut microbiome is the best studied of the animal microbiomes, and is defined as the system of trillions of microorganisms belonging to thousands of species that populate the intestine (the gut microbiota), together with their molecular components. The taxonomic composition of the gut microbiome is

regulated by both innate and external factors, in a way that two different individuals will never have the exact same taxonomic profile, especially at the genus or species level. The host microbiomes, and in particular the gut microbiome, is deeply involved in the regulation or, instead, when they are pathologically altered, dysregulation of almost all physiological functions. They do so by producing, often following the processing of different nutrients, small molecule signals, such as short chain fatty acids (SCFAs), tryptophan metabolites and secondary bile acids, among others, which can enter the host circulation or affect its enteric nervous system. Importantly, some commensal bacteria produce a wide range of endocannabinoid-like molecules (the mBendocannabinoidome), thus strengthening the cross-talk between the gut microbiome and the eCBome.

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DUAL PIPERIDINE-BASED HISTAMINE H$_3$ AND SIGMA-1 RECEPTOR LIGANDS IN THE TREATMENT OF NOCICEPTIVE AND NEUROPATHIC PAIN

The treatment of complex, multifactorial diseases by single target-oriented therapies rarely results in good efficacy. For this reason, the approach based on simultaneous modulation of multiple targets' activity captured the interest of the pharmaceutical industry and academia. Importantly, recent studies have shown that some clinically evaluated histamine H3 receptor antagonists (H3R) possess additional affinity at sigma-1 receptors (σ1R), which may play an important role in their pharmacology [1]. Considering the clear relation between H3R and σ1R, great effort should be made to develop such ligands for the treatment of various pain conditions. In our study, we decided to combine chemical, biological and computational methods to reveal molecular properties responsible for histamine H3R and σ1R selective or dual-target binding of the studied compounds [2]. Next, we designed a series of 16 new ligands and performed their pharmacological characterization using in vitro methods. Finally, lead compounds were tested in animal models of nociceptive and neuropathic pain. In a series of novel compounds, we selected three lead structures for further biological evaluation with high affinity at both H3R and σ1R. Compound 12 showed a better safety profile than the other two tested compounds, hence we have selected this ligand for further analysis of its analgesic activity. The high potency of 12 in both, formalin- and capsaicin-induced pain indicated that this compound has the potential to attenuate neurogenic pain, regardless of the mechanism of its induction. Finally, we used two different models of neuropathic pain to test the influence of 12 on pain associated with neuronal tissue damage. The obtained results strongly indicate that compound 12 can alleviate both chemotherapy-induced and sciatic nerve damage-driven neuropathic pain. This confirms its broad spectrum of analgesic activity based on the novel molecular mechanism [2].

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Prof. Emanuele Amata
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with

Maria Dichiara (1), Francesca Alessandra Ambrosio (2), Sang Min Lee (3), Maria Carmen Ruiz-Cantero (4), Jessica Lombino (1), Adriana Coricello (5), Giosuè Costa (5,6), Dhara Shah (3), Giuliana Costanzo (1), Lorella Pasquinucci (1), Kyung No Son (7), Giuseppe Cosentino (1), Rafael González-Cano (4), Agostino Marrazzo (1), Vinay Kumar Aakalu (7), Enrique J. Cobos (4), Stefano Alcaro (5,6)

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DESIGN, SYNTHESIS, MOLECULAR MODELLING AND BIOLOGICAL EVALUATION OF NOVEL SIGMA RECEPTORS LIGANDS WITH POTENT ANTIALLODYNIC ACTIVITY

Over the years, spirocyclic and fused scaffolds have gained increasing interest in the development of bioactive compounds due to their peculiar spatial arrangement affecting crucial parameters of drug candidates, including potency, selectivity, and physicochemical properties.

With the aim of identifying classes of Sigma Receptors (SRs) ligands with high affinity and selectivity, novel SR ligands – based on diazaspiro, diazabiciclo, dihydropyrrolopyrazole, as well as other scaffolds – have been developed. By varying the central cores, and the substituents decorating them, we were able to capture discrepancy among S1R vs S2R affinity and functional profile, as well as the behaviour in a model of in vivo disease.

Our efforts have been aimed at developing new candidates based on affinity and selectivity and on the synthesis of new compounds for additional rounds of drug-target evaluation. Molecular modeling studies were carried out to analyse the binding mode and the interactions established between the ligands and S1R and S2R. The most notable compounds have been subjected to further biological evaluation in *in vitro* and *in vivo* models.

One of the most notable result includes the discovery of AD258, a compound with negligible in vitro cellular toxicity and high binding affinity to both SR (K_{iS1R} = 3.5 nM, K_{iS2R} = 2.6 nM), but not for other pain-related targets, and with high potency in a model of capsaicin-induced allodynia, with maximum antiallodynic effect at very low doses (0.6−1.25 mg/Kg). Functional activity experiments showed that S1R antagonism is needed for the effects of AD258 and that it did not induce motor impairment. In addition, AD258 exhibited a favourable pharmacokinetic profile.

Prof. Manuela Bartolini
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ITALIAN-POLISH CROSSTALK THROUGH BIO-ANALYSIS

This presentation aims to share results achieved within the framework of a joint Italy-Poland mobility project focusing on the production of nanodiscs containing a recombinant form of the β_2-adrenergic receptor (β_2-AR) to be used for in-solution investigations on such receptor.

The isolation of membrane proteins in their functional forms still represents a scientific challenge being the surrounding lipid bilayer an important player in protein stability and function. Hence, different expertise from my research unit at the University of Bologna and the research unit of Prof. Jozwiak at the Medical University of Lublin were combined to achieve the aims of the project.

As embedding system SMALPs (styrene-maleic acid lipid particles) were selected. SMALP technology was recently proposed [1] as solubilization technique for membrane proteins. It exploits the amphiphilic properties of styrene-maleic acid co-polymers (SMA), which can interact directly with cell membranes and isolate membrane proteins embedded in their native environment. This technique was selected since it can be direct applied to crude cell membrane extracts and allow extraction in detergent-free conditions, which allow to preserve protein function to a larger extent.

To facilitate β_2-AR-SMALP isolation, the research group of Prof. Jozwiak purposely designed a recombinant variant of the receptor displaying the cleavable hemagglutinin signal sequence and the FLAG tag on the N-terminal side, as well as the TEV cleavage site and a double His tag (10×His + 12-aminoacid spacer + 6×His) on the C-terminal side, which was then stably transfected into a HEK 293 cell line with the aim of obtaining a sufficiently large amount of β_2-AR-containing cell membranes to be used for SMALPs production.

β_2-AR-SMALPs were prepared using the resulting membrane suspensions and the best performing SMA copolymer, which was selected within a small series of possible candidates; SMALP-embedded β_2-AR were then isolated by an optimized protocol which included the use of immobilized metal affinity chromatography (IMAC).
The presence of FLAG-tagged proteins in the IMAC elution fraction preliminarily confirmed that the SMALP technology can be successfully applied to the preparation of aqueous solutions of human β_2-AR embedded in their native membrane environment.[2]

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FROM SYNTHETIC COMPOUNDS ACTIVE ON THE CENTRAL NERVOUS SYSTEM TO CANNABIS: A JOINT RESEARCH STORY

Once upon a time, there was my research group, always focused on the synthesis of new derivatives active in the central nervous system, particularly on the ionotropic glutamate AMPA receptor. This research led to the development of highly sophisticated techniques for the synthesis and evaluation of the biological activity of synthesized compounds, such as the technique of chiral-achiral bidimensional chromatography coupled with cerebral microdialysis. A fruitful collaboration with Prof. K. Jozwiach's research group allowed us to establish the main interactions with the AMPA receptor and design new derivatives that were increasingly specific and selective. These compounds were also the subject of a joint patent between the Medical University of Lublin and the University of Modena and Reggio Emilia. When in 2016 my research group approached cannabis for the first time, we discovered that both the design and synthesis of highly active derivatives in the central nervous system were carried out by numerous small pharmaceutical industries present on the tiny leaflets of its inflorescence: the glandular trichomes. Here, Darwinian evolution had designed derivatives with remarkable pharmacological activities that humans had always used to treat various conditions, and the secreting cells at the base of the trichome gland produced them abundantly. The initial chemical analyses of these structures led us to focus only on the famous tetrahydrocannabinol (THC) and cannabidiol (CBD). However, just as the triangle in Flatland dreamt of a three-dimensional reality, my research group applied untargeted metabolomic analysis and discovered that there were over 150 phytocannabinoids, many of which were unknown and had never been seen in the two-dimensional reality of the chromatogram. Thus, new phytocannabinoids were discovered, such as tetrahydrocannabiphorol (THCP), at least 30 times more active than THC, no longer synthesized in the laboratory but produced by the tiny glandular trichome of cannabis. The stereoisomerism of the main phytocannabinoids was evaluated using bidimensional chromatographic techniques without the need for complicated stereoselective syntheses in the laboratory; the glandular trichome had taken care of producing them in a single enantiomeric form. In the end, it must be admitted that the glandular trichome of cannabis is much more skilled than our research group at designing and synthesizing compounds active in the central nervous system.

Prof. Krzysztof Jóźwiak
Department of Biopharmacy, Medical University of Lublin

COLLABORATIVE RESEARCH ON MOLECULAR MECHANISMS OF DRUG-RECEPTOR INTERACTIONS

G protein – coupled receptors (GPCR) form the largest group of membrane sensors with very diverse functions. Plenty of recent research lines indicate their tremendously complex signaling behavior; a receptor upon activation may couple to more than one type of G protein triggering a plethora of alternative intracellular pathways while arrestin recruitment may induce additional and G protein independent signaling that affects the cell function. All these concerted cellular actions can be fine-tuned by a ligand molecule bounded to the extracellular binding domain and very important topic of current GPCR pharmacology is biased agonism. I will present current understanding of structural basis and key drug - receptor interactions that are responsible for ligand recognition leading to biased signaling. Structural models and molecular dynamics simulations will be presented to propose key mechanisms and interactions between β2-adrenergic receptor and its biased ligands.

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EXPLORING METABOLIC STABILITY AND LIGAND KINETICS VIA *IN SILICO* APPROACHES

In the current era of information explosion, machine learning methods have become indispensable tools for dealing with large-scale, high-dimensional data. As the predictions made by machine learning methods can often reduce laboratory experiments, their application in drug design campaigns can lower the costs of the search for new potential medicines. Machine learning methods are frequently employed in drug design pipelines, not just for the identification of new active ligands, but also for the optimization of their physicochemical and pharmacokinetic properties [1].

In the study, we used the machine learning methods for achieve two main objectives – prediction and optimization of metabolic stability of selected ligands of serotonin receptor 5-HT7, and assessment of compound kinetics, with a particular focus on residence time. Both of these parameters have crucial impact on the potential ligand efficiency in the organism: low metabolic stability can cause that the compound does not have sufficient time to trigger desired biological response [2]; whereas the duration of compound action is correlated with the endurance of its complex with the protein, following Paul Ehrlich's doctrine Corpora non agunt nisi fixata. Several recent reports stress the importance of residence time for pharmacological action of chemical compounds and indicate that lengthening the duration of the ligand-receptor complex can result in more effective drugs, larger therapeutic window and extended dose intervals [3].

Metabolic stability prediction was addressed using molecular fingerprints and descriptors in the ligand-based approach. Such representation was then analyzed by machine learning algorithms, such as support vector machines, decision trees, Bayesian classifiers, etc. [4] On the other hand, the issue of residence time was tackled from a structure-based perspective, which includes docking and molecular dynamics simulations, followed by the statistical analysis of ligand-protein contacts and examination of compound pose variations in the binding site [5]. The conclusions drawn from this analysis will be beneficial in developing new 5-HT7R ligands with prolonged metabolic stability and residence time.

Acknowledgments

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DEVELOPING OF SEROTONIN 5-HT7 RECEPTOR LIGANDS BEYOND HIGH AFFINITY: THE BINDING KINETIC PROFILE AND METABOLIC STABILITY

The 5-HT7 serotonin receptor is a promising target for innovative therapeutic strategies for neurodevelopmental and neuropsychiatric disorders. The observation that 5-HT7 receptor blockade produced a faster antidepressant-like response than the commonly prescribed antidepressant fluoxetine fueled the search for selective antagonists that culminated with the identification of JNJ-1803868, the first-in-class 5-HT7 receptor antagonist to enter clinical trials. We have contributed to the field by identifying a set of selective 5-HT7 receptor agonists with an arylpiperazine structure, among which LP-211 stood for its selectivity over other relevant central nervous system monoaminergic receptors and for its ability to penetrate the blood-brain barrier [1].

Next, our efforts were dedicated to going deeper into the characterization of our newly designed 5-HT7 receptor agonists from the pharmacokinetic and pharmacodynamic aspects. As for the pharmacokinetic aspect, we have studied novel analogs of LP-211 designed to enhance stability towards microsomal oxidative metabolism through the reduction of the overall lipophilicity, introduction of electron-withdrawing groups, blocking of potentially vulnerable sites of metabolism and found the adopted design strategy does not directly translate into improvements in stability [2]. As for the pharmacodynamic aspect, we focused on the kinetics of drug-target interaction, as this aspect is receiving increasing attention as an important pharmacological parameter in the drug development process. Several studies have suggested that the lipophilicity of a molecule can play an important role. We found that it is not the overall lipophilicity of the molecule that influences drug-target interaction kinetics but rather the position of polar groups within the molecule [3].
The data collected in these studies allowed, from one side, for the construction of a machine learning model that, in a given chemical space, can describe and quantitatively predict the metabolic stability of the compounds and, from the other side, perform a combination of molecular docking studies and molecular dynamics simulations to gain insights into structure-kinetics relationships.

Acknowledgments

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ROLE OF CANNABINOID RECEPTORS IN THE DESCENDING MODULATION OF PAIN

The endogenous antinociceptive descending pathway represents a circuitry of the supraspinal central nervous system whose task is to counteract pain. It includes the periaqueductal grey (PAG)-rostral ventromedial medulla (RVM)-dorsal horn axis, which is the best characterized pain modulation system through which pain is endogenously inhibited. Thus, an alternative rational strategy for silencing pain is the activation of this anatomical substrate. Intra-PAG microinjections of cannabinoid compounds have proved to be analgesic in animal pain models. Like opioids, cannabinoids agonists or Fatty Acid Amide Hydrolase (FAAH) inhibitors produce centrally-mediated analgesia by activating the descending pathway. The consequent outcome is the behavioral analgesia. We also suggested a supraspinal regulation of cannabinoids on release of specific neurotransmitters [g-aminobutyric acid (GABA) and glutamate] which enhance the antinociceptive descending pathway. Finally, we suggested that compounds with a dual target, such as FAAH inhibition and Transient Receptor Potential Vanilloid Type 1 (TRPV1) receptor blockade, may cause analgesic effects that are stronger than they would be if acting on each single target, individually. Taken together these findings would suggest that supraspinal cannabinoid receptors have broad applications, from pain control to closely related central nervous system diseases such as anxiety and depression.

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INHIBITION OF ANANDAMIDE DEGRADATION REVERSES OSTEOARTHRITIS-RELATED HIPPOCAMPAL LTP AND MONOAMINE LEVELS DISRUPTIONS - THE ROLE OF CB1 RECEPTOR

Introduction: The hippocampus plays crucial role in various brain functions such as learning, memory, and emotional processing. Unfortunately, pain research has largely overlooked the hippocampus's involvement in pain perception, likely due to the absence of convincing links to nociceptive signals reaching this region. Nevertheless, Mutso et al. [1] managed to reveal physiological, molecular, and neurogenesis abnormalities within the hippocampus of rodents with neuropathic injuries, thus prompting us to broaden the perspective. We sought to emphasize the significance of understanding chronic pain through the lens of brain learning and memory circuitry. Recent studies on different chronic pain management models strongly support the involvement of the endocannabinoid (EC) system [2]. Our study aimed to investigate alterations in the hippocampus of animals experiencing chronic pain due to osteoarthritis (OA) and the role of EC signaling in hippocampal function in rats enduring persistent pain.

Methods: In this research, male Wistar rats were subjected to intra-articular knee injections of either NaCl (sham) or 1 mg of sodium monoiodoacetate (MIA) to induce lesions resembling osteoarthritis (OA). On the 28th day following MIA injection, the following evaluations were conducted: assessment of mechanical pain threshold using the Pressure Application Measurement (PAM) test, evaluation of tactile allodynia through the von Frey test, induction of long-term potentiation (LTP) in the dentate gyrus (DG) by means of theta-burst stimulations (TBS) originating from the lateral entorhinal cortex (LEC), and the collection of extracellular levels of dopamine and serotonin in the DG via in vivo microdialysis, which were subsequently analyzed using the HPLC method. The experimental groups were divided into Sham or MIA rats that received either the vehicle or URB597 (1 mg/kg, i.p.) alone, or in conjunction with the CB1 inverse agonist, AM251 (1 mg/kg, i.p.), which selectively inhibits the FAAH enzyme.

Results: Behavioral tests revealed that the systemic administration of URB597 effectively reduced knee hyperalgesia in MIA rats, specifically on the 28th day after induction, with the most pronounced impact observed during the 60-120 minutes following drug administration. This beneficial outcome was negated by prior treatment with AM251. Furthermore, this same treatment notably restored the disrupted connectivity between the DG and LEC, as evidenced by increased amplitude and slope of fEPSPs in MIA rats. Additionally, it was observed that MIA-injected animals exhibited lowe r dopamine levels and higher serotonin levels in the hippocampal DG when compared to unaffected

individuals. The administration of URB597 was successful in significantly reversing the abnormal dopamine and serotonin levels in the hippocampal DG of MIA animals.

Conclusions: The latest findings not only demonstrate the pain-relieving effects of URB597 but also highlight its involvement in the establishment of long-term potentiation (LTP) in the LEC-DG pathway and the modulation of neurotransmitter levels in the hippocampal DG. We have identified substantial behavioral, neurochemical, and synaptic alterations in the hippocampus of animals suffering from osteoarthritis (OA). Therefore, addressing these systemic changes in chronic pain could potentially enhance both the quality of life for patients and their actual pain-related behaviors.

Acknowledgements

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STRUCTURE AND FUNCTION OF PROTEIN CORONA AT THE NANOPARTICLES INTERFACE

IIn the current development of nanomedicine, the problem is not the lack of therapeutic substances but an efficient way of delivering them. In contact with body fluids, nanomaterials are immediately coated with proteins. This rapidly forming protein corona determines the physicochemical properties of nanoparticles. Moreover, the interaction with the cell membrane and the cell absorption mechanism is significantly controlled by the proteins adsorbed on the surface of the carrier. Therefore, the protein corona defines the biological identity of nanoparticles, influencing their cytotoxicity, biodistribution in the body, and endocytosis to specific cells. The signature of the protein corona depending on the type of nanocarrier indicates its diversity and complexity and makes it difficult to predict its final action. By defining the role of individual proteins, it is possible to identify specific proteins responsible for the biological pathway of nanocarriers. So far, it has been assumed that predominantly quantitative proteins or proteins with dedicated biological functions have a significant impact on the achievement of the molecular target. Understanding which proteins found in the protein corona define the nanoparticle pathway is critical to the appropriate design of targeted nanocarriers.
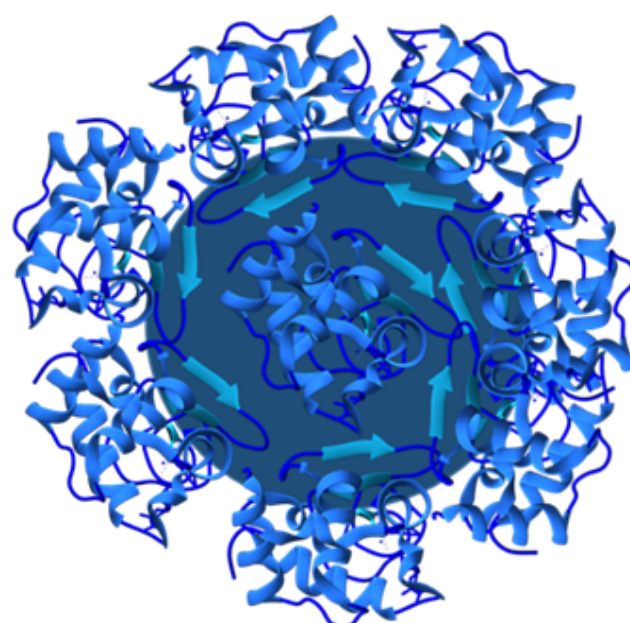
Optimizing the properties of nanocarriers by using the protein corona for specific biomedical applications appears to be a promising tool in personalized medicine.

It should be emphasized that the use of nanomaterials in biomedical systems is currently relatively limited (the number of FDA-approved nanosystems is still less than 30). The presently observed difficulties in the development of nanomedicine are related to the response of the immune system resulting, inter alia, from the action of the protein corona. Therefore, there is a need for systematic research aimed at determining the specific role of the protein corona in biological systems. Advances in this field will allow the development of knowledge in the field of nanotoxicology, but also bring closer the compatibility of in vitro and in vivo models, and thus facilitate the achievement of the expected therapeutic effects at the stage of clinical trials.

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HAS PHYSICAL CHEMISTRY CONTRIBUTED TO THE UNDERSTANDING OF INTERACTIONS IN BIOSYSTEMS? THE CASE OF WEAK AND STRONG ELECTROLYTES

Electrolytes are fundamental constituents of biological fluids where they play the important function to modulate the interactions between biomacromolecules. Weak electrolytes regulate the pH of physiological fluids, whereas strong electrolytes regulate ionic strength and, together, cooperate to set the surface potential of proteins, enzymes, lipids, and nucleic acids. However, although the important role of electrolytes has been recognised since more than 100 years, their specific mechanism of action has only partially been understood. Current theories, mainly based on electrostatics, ignore the role of the ubiquitous dispersion forces that are responsible of electrolytes specificity. The present contribution will overview some recent experiments and the most recent theoretical developments that will show how physical chemistry is on the way to contribute to the understanding of interactions between macromolecules in biological systems.

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BOOSTING THE RESOLUTION OF INFLAMMATION AS AN INNOVATIVE APPROACH IN THE PHARMACOTHERAPY OF BRAIN DISEASES CHARACTERIZED BY NEUROINFLAMMATION (PART 1)

Chronic or unresolved inflammation is a key pathological process in various diseases, including depression, schizophrenia, and neurodegenerative disorders. Successful resolution of inflammation requires the activation of endogenous pathways that can switch from the production of pro-inflammatory to specialized pro-resolving mediators (SPMs). New insights into such pathways are offering novel opportunities to pharmacologically manipulate the resolution of inflammation and, eventually, open new therapeutic approaches for chronic inflammation. The Formyl Peptide Receptor 2 (FPR2), a receptor modulated by several SPMs, such as lipoxin A4 and resolvins, is one of the key players in the resolution of inflammation. At the Department of Pharmacy, University of Bari, a class of non-peptidic FPR2 agonists with a ureidopropanamide scaffold has been identified. Structural manipulation of the ureidopropanamide scaffold led to identifying potent FPR2 agonists endowed with suitable pharmacokinetic properties for in vivo use. In collaboration with the Department of Experimental Neuroendocrinology at Maj Institute of Pharmacology, the effect of the most potent compounds on viability/metabolic activity, necrotic death, and production of pro-and anti-inflammatory mediators in in vitro, ex-vivo, and in vivo models have been studied. The collaboration efforts have led to the identification of FPR2 agonists having therapeutic potential for treating of CNS diseases characterized by neuroinflammation.

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BOOSTING THE RESOLUTION OF INFLAMMATION AS AN INNOVATIVE APPROACH IN THE PHARMACOTHERAPY OF BRAIN DISEASES CHARACTERIZED BY NEUROINFLAMMATION (PART 2)

Alzheimer's disease (AD) is the most prevalent form of dementia globally. The hallmarks of AD include extracellular plaques containing amyloid beta (Aβ) and intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein. A lot of data suggests that chronic neuroinflammation plays an essential role in the onset of AD, whose progression is directly linked to neuroinflammation. Although short-term inflammation is a beneficial phenomenon, and its controlled resolution enables repair and restoration of homeostasis, disturbances in the resolution of inflammation (RoI) lead to chronic inflammation. The long-term inflammatory status is associated with excessive microglia activation, production of toxic factors, pro-inflammatory cytokines, chemokines, and oxidative stress. Several studies have demonstrated that RoI is regulated by endogenous specialized pro-resolving mediators (SPMs), including lipoxin A4 (LXA4). LXA4 affects RoI by activating N-formyl peptide receptor-2 (FPR2) and can stimulate a pro-resolving activation of microglia by reducing both NFκB and NLRP3 activation and increasing anti-inflammatory cytokines release.

So far, the strategy of RoI is greatly hampered by the unfavourable pharmacokinetic properties of endogenous agonists. Hence, new drug-like molecules targeting the FPR2 signalling pathways are urgently needed to repair the endogenous RoI deficits. Recently, our innovative research is concentrated in vivo in a recognized model of Alzheimer's disease using the transgenic animals APPNL-F/NL-F (LOAD model). We combine the age-dependent behavioral studies with various molecular and omics methods to verify the hypothesis that the exogenous treatment with new hybrid agonists of the FPR2 receptor may lead to a booster of the RoI and lead to the limitation of AD-related pathologies.

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