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UCB Pharma research day—25 October 2007 'Glia-neuron interactions and purinergic receptors in neurological disorders'

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UCB Pharma asked Geoffrey Burnstock to propose a programme of 8 international leaders in the glia-neuron interaction and purinergic signalling field to participate in a meeting aimed at introducing the topic to about 30 members of the Company to consider the therapeutic potential of purinergic signalling compounds for the treatment of neurological disorders, including: epilepsy, neurodegenerative diseases, movement disorders, neuroprotection, mood disorders and neuropathic pain.

Geoff Burnstock, acting as the Chairman of this 1-day meeting, held at the Château de Limelette, Belgium, introduced the objectives of the meeting. He stressed the sophisticated biology that was involved in drug development and the advantages of collaborations between basic scientists, clinicians and the drug industry in bringing advances in knowledge from basic science to application in clinical medicine for the treatment of disease.

The morning session was devoted to the mechanisms underlying glia-neuron interactions. **Professor Philip Haydon** from the Department of Neuroscience, University of Pennsylvania, USA, led off by describing '**The tripartite synapse: how astrocytes listen and talk to neurons**', describing the functional as well as structural interactions between three elements: the neuronal presynaptic component, the postsynaptic neuronal component and the perisynaptic astrocyte.

Astrocytes are intimately associated with neurons and, through their extensive contacts with synapses, they are able to regulate synaptic transmission. Since the discovery of the ability of astrocytes to release chemical transmitters, a process that is termed gliotransmission, our understanding of the dynamical regulatory roles for these glial cells has dramatically expanded. Astrocytes are equipped with the necessary machinery to listen and to talk to neurons. Following synaptic activity neurotransmitters acting through glial metabotropic receptors induce glial Ca^{2+} signals. In turn, these Ca^{2+} signals can induce the release of a variety of chemical transmitters, including glutamate, ATP and D-serine.

Using a variety of astrocyte-specific manipulations recent work has identified a variety of physiological and pathological functions for this process of gliotransmission. By releasing ATP, which is converted in the extracellular space to adenosine, astrocytes exert a powerful presynaptic inhibition of synaptic transmission. Glutamate and D-serine released from astrocytes activate neuronal *N*-methyl-D-aspartate (NMDA) receptors. These glial-neuron signalling pathways regulate neuronal excitability and synaptic transmission. When examined in vivo recent studies show that neuronal networks are under the continuous modulatory control of the astrocyte through both purinergic and NMDA receptor-dependent pathways. In addition to providing physiological modulatory actions, astrocytes have the potential to contribute to neurological disorders and psychiatric states. The contribution of gliotransmission to delayed neuronal death was discussed.

He concluded that our understanding of brain function is changing from one in which neuron-based electrical signals

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are the formal code to one in which rapid electrical signals in neuronal networks interact with slow modulatory signals provided by glia. The ultimate function or dysfunction of the nervous system during health and disease is an emergent property of these neuron-glial interactions.

The next lecture was presented by **Professor Andrea Volterra**, from the Department of Cell Biology and Morphology, University of Lausanne, Switzerland. His talk, entitled '**Gliotransmission, synaptic plasticity and synaptic dysfunction**', focused on the process of exocytosis of glutamate and other 'gliotransmitters' released from astrocytes and on modes of rapid communication of glia with neighbouring cells. Using an interdisciplinary approach, he described the mechanisms of gliotransmission in the normal brain physiology and in brain diseases, as well as identifying signalling steps that may contribute to novel drug targets. He discussed how glial activity follows two routes: an intracellular one through gap junctions (connexins) and an extracellular one via release of ATP, glutamate and serine. Astrocytes also contain a synaptic-like microvessel compartment for uptake, storage and release of glutamate. Such gliotransmission plays a role in synapse formation, synaptic function, adult neurogenesis and neurovascular tone.

Astrocytes control excitatory transmission and synaptic strength at hippocampal synapses via purinergic $P2Y_1$ receptor signalling, glutamate and tumour necrosis factor-α (TNFα). Modulatory control in the hippocampal dentate gyrus in response to activation of $P2Y_1$ purinoreceptors ($P2Y_1R$) was shown and immunochemistry demonstrated that $P2Y_1Rs$ are localised preferentially in astrocyte processes directly apposed to asymmetric synapses in the dentate molecular layer. In hippocampal slices, activation of $P2Y_1R$ with the selective agonist, 2-methylthioadenosine-5′-diphosphate (2-MeSADP), triggers glutamate release. $P2Y_1R$ activation with 2-MeSADP causes increased miniature excitatory postsynaptic currents (mEPSC) and spontaneous EPSC (sEPSC) activity in dentate granule cells (CG). $P2Y_1R$ signalling in astrocytes activates presynaptic NMDA receptors via release of glutamate.

Post-embedding immunogold cytochemistry showed gold particles in the extrasynaptic portion of excitatory nerve terminals. Stimulation of astrocyte $P2Y_1R$ induces release of TNFα in addition to glutamate. The two signalling events are correlated, as $P2Y_1R$-evoked glutamate release is dramatically reduced in TNFα knockout mice.

Professor Christian Steinhäuser, Director of the Institute of Cellular Neuroscience, University of Bonn, Germany, gave a talk entitled '**A role for astrocytes in epilepsy**'. He suggested that astrocytes may be active players in the cellular basis of hyperexcitability and synchronization in epilepsy and other neurological diseases. The functional significance of transmitter release from astrocytes involves modulation of the strength of excitatory and inhibitory synaptic transmission by activating receptors and neurons. As each astrocyte can reach thousands of synapses simultaneously, the release of gliotransmitter may lead to synchronization of neuronal firing patterns. Epilepsy is often accompanied by massive glial cell proliferation, even though the exact role of these cells in seizures and epilepsy is still unclear. Not only do glial cells express various ion channels and receptors, but they are probably functionally heterogeneous. Therefore, a better understanding of mechanisms of glial function in brain health and disease may offer the potential for developing novel strategies to treat epilepsy and other brain disorders. The current anticonvulsant drugs and complementary therapies are not sufficient to control seizures in about a third of epileptic patients. Thus, there is an urgent need for treatments that prevent the development of epilepsy and control it better in patients already inflicted with the disease. Despite significant advances in different fields of neuroscience, the pathological mechanisms of epileptic disorders remain poorly understood. An improved understanding of astrocyte biology and the involvement of glial cells in epileptogenesis offers the potential for developing novel strategies to treat epilepsy. Work in his laboratory had recently identified two distinct types of cells with astroglial properties, GluR cells and GluT cells, co-existing in mouse hippocampus. GluR cells express ionotropic glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype, receive synaptic input from hippocampal neurons, and lack functional glutamate transporters and gap junction coupling. GluT cells, in contrast, display glutamate transporter activity, gap junction coupling, touch brain capillaries with their endfeet, but lack ionotropic glutamate receptors. Both types of astroglial cells are also present in the hippocampus of patients presenting with intractable temporal lobe epilepsy (lesion-associated, non-sclerotic epilepsy). However, in patients suffering from Ammon's horn sclerosis, the most common type of neuropathological damage seen in individuals with temporal lobe epilepsy, one subpopulation of these cells (GluT-type) almost completely disappears while the remaining GluR cells undergo molecular and functional alterations of their glutamate receptors. These findings support the hypothesis that glial cells play a key role in the generation and/or spread of seizure activity in human epilepsy. This deleterious effect is brought about by (1) the abundant excitatory neurotransmitter glutamate and (2) impaired gap junction-mediated buffering of K^+ and metabolites, leading to prolonged activation of cells in the hippocampus, a brain region crucially involved in learning, memory and emotional processing. Analyses of more than a hundred brain specimens neurosurgically resected from epilepsy patients were used to substantiate this intriguing

new insight. The findings challenge the common view of epileptogenesis according to which neurons are considered the prime targets affected in this disease.

Professor Francesco Di Virgilio, from the Department of Experimental and Diagnostic Medicine, University of Ferrara, Italy, presented the last talk of the morning session entitled '**Immuno-neural interaction and neuro-inflammatory diseases**'. He discussed the molecular mechanisms underlying neuro-inflammation, in particular, the two-way communication between neurons and microglia, in which purinergic signalling (ATP and the ionotropic ATP receptor subtype $P2X_7$) constitutes a key pathway. Release of ATP from neurons directly modulates microglial function, resulting in secretion of neurotrophic factors, or, if excessive or protracted, in the release of proinflammatory mediators, amongst which interleukin (IL)-1β and IL-18 are the cytokines most tightly controlled by the $P2X_7$ receptor. Conversely, purinergic stimulation of microglia via P2 receptors may elicit microglial ATP release that feeds back onto neurons. $P2X_7$ receptor activation may lead to inflammation by activation of the "inflammasome", a protein complex that activates inflammatory caspases. Unique to the $P2X_7$ receptor is that prolonged stimulation leads to the opening of a large-conductance pore, which corresponds to a gap junction-like hemichannel, the pannexin, panx-1, that participates in the $P2X_7$ receptor-dependent inflammasome activation. This recent view on $P2X_7$ receptor-inflammasome interaction may open unanticipated avenues for the development of novel anti-inflammatory drugs.

Francesco pointed out that we are taught during neurobiology courses that the brain is an immunologically privileged organ; however, this does not mean that the brain is devoid of immune cells and that immuno-mediated reactions cannot occur in the brain. On the contrary, it is becoming increasingly clear that resident immunocompetent cells are crucial in the physiological homeostasis of the central nervous system (CNS). Although it was long thought that the brain was unable to mount an inflammatory response, it is now clear that, with some relevant differences with respect to peripheral tissues, the CNS can undergo all the typical changes of inflammation, can activate endogenous inflammatory cells and generate inflammatory mediators. Neurons are surrounded by a wide population of support cells (oligodendroglia, astroglia and microglia) that establish intimate physical relationships with the neurons and exchange with them a wealth of biochemical information. Among these cells, microglia have a special status as these cells are both supportive and immunocompetent. In this capacity, microglia share all the positive and negative roles of their relatives located in the periphery, i.e. tissue macrophages. In fact, although there are no doubts that microglia have a key protective role in CNS trauma or infections, or even during regeneration, it is equally clear that microglia are a fundamental culprit in CNS dysfunction.

Microglia release several factors that affect neuronal functions: activated oxygen and nitrogen species, cytokines, chemokines and growth factors. In turn, microglia are the target of mediators released from neurons. We have only very recently started to understand the subtleties of this exchange of information and extracellular nucleotides have been identified among the most relevant components of this two-way traffic. Neurons are a rich source of ATP, which has now an established role as a cotransmitter in most major nerve types. Secretory exocytosis seems to be the most common pathway for ATP release. Furthermore, other cells such as astrocytes may also take part in this communication network by releasing nucleotides or neurotransmitters that in turn evoke ATP release from neurons. IL-1β is one of the most important proinflammatory mediators, implicated in a variety of neurodegenerative conditions. We now know that secretion of IL-1β is the end result of a complex chain of tightly controlled intracellular events occurring within a multimolecular structure named the "inflammasome". Dependence of IL-1β release on extracellular ATP makes microglia an obvious target of neuronal activity, especially in the presence of high levels of neurotransmitter release. Under these conditions, if microglia are also primed by exogenous or endogenous proinflammatory factors, this may lead to sustained IL-1β release and neuronal damage. There is scattered evidence that this mechanism may play a role in the pathogenesis of chronic CNS pathologies, notably Alzheimer's disease.

The afternoon session was focused on purinergic receptors in glia-neuron interactions. An overview of '**Purinergic receptors and signalling mechanisms**' was introduced by **Professor Geoffrey Burnstock**, President of the Autonomic Neuroscience Centre, Royal Free and University College Medical School, London, UK. He reviewed how purinergic transmission was proposed in the early 1970s, with evidence that ATP was a neurotransmitter in non-adrenergic, non-cholinergic nerves in the gut and urinary bladder. Later, it was shown to be a cotransmitter with classic neurotransmitters and it is now recognised as a cotransmitter in most, if not all, nerve types in the peripheral and central nervous systems. Implicit in purinergic neurotransmission is the existence of postjunctional receptors for ATP. Released ATP is broken down by ectonucleotidases to ADP, AMP and adenosine, and in 1978 separate receptors for adenosine and AMP (named P1 receptors) and for ATP and ADP (named P2 receptors) were proposed. In the early 1990s, P1 and P2 receptors were cloned and characterised: four P1 receptor subtypes (A_1, A_{2A}, A_{2B} and A_3), seven subtypes of P2X ligand-gated ion channel receptors ($P2X_{1\text{-}7}$) and eight subtypes of P2Y

G protein-coupled receptors ($P2Y_{1,\ 2,\ 4,\ 6,\ 11,\ 12,\ 13,\ 14}$). $P2Y_{2,\ 4,\ 6}$ receptors are activated by the pyrimidines uridine triphosphate (UTP) or uridine diphosphate (UDP) as well as by purine nucleotides. The cation channel is formed by three P2X receptor subunits either forming homomultimers or heteromultimers. $P2X_{1/2}$, $P2X_{2/3}$, $P2X_{1/4}$, $P2X_{1/5}$, $P2X_{2/6}$, $P2X_{4/6}$ and probably $P2X_{4/7}$ heteromultimer receptors are widely expressed in vivo and have different pharmacological properties from homomultimers. Reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemical studies have shown widespread expression of P1 and P2 receptor mRNA and protein in neuronal and non-neuronal tissues. Both short-term purinergic signalling in neurotransmission, secretion and platelet aggregation and long-term (trophic) purinergic signalling in cell proliferation, migration, differentiation and death in development and regeneration have been recognised.

For many years it was assumed that the source of ATP was dying cells, but it is now recognised that many cells release ATP physiologically in response to mechanical stimuli and hypoxia, although the mechanism of ATP transport is still debated. ATP release from nerves and some other cells is vesicular; from others it may involve ABC transporters, connexin or pannexin hemichannels or $P2X_7$ receptor pores. Expression of purinergic cotransmitters and receptors shows plasticity in development and old age, in the nerves that remain after trauma or surgery and in disease.

For many years, it was recognised that adenosine, acting through P1 (usually A_1) presynaptic receptors to inhibit the release of excitatory transmitters, was an important mechanism in the CNS. However, in 1992, evidence was presented for ATP mediating synaptic neurotransmission in the medial habenula and since then there has been an explosion of interest in purinergic neurotransmission and neuromodulation in the different regions of the brain and spinal cord. Multiple purinergic receptors have also been identified on astrocytes, oligodendrocytes and microglia and important mechanisms involving neuron-glial cell interactions have been recognised, as reviewed recently [1]. There is increasing interest in the role of purinergic signalling in the pathophysiology of neurological disorders, including: trauma, stroke and ischaemia; neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's, multiple sclerosis and amyotrophic lateral sclerosis; migraine; neuro-psychiatric disorders such as schizophrenia, anxiety and depression; epileptic seizures; and neuropathic pain. Purinergic therapeutic strategies for the treatment of these conditions are beginning to be explored.

Professor Herbert Zimmermann, from the Institute of Cell Biology and Neuroscience Biocenter, University of Frankfurt, Germany, gave the next talk entitled '**Regulation of purinergic signalling by ectonucleotidases**'. He described how extracellular nucleotides are hydrolyzed by plasma membrane-located enzymes with an extracellulary oriented catalytic site (ectonucleotidases). Ectonucleotidases modulate ligand availability at nucleotide and nucleoside receptors. Apart from that, they serve the recycling of nucleosides via specific cellular transport systems. Substrates of ectonucleotidases include nucleoside triphosphates, diphosphates and monophosphates and dinucleoside polyphosphates. The final hydrolysis products are phosphate or pyrophosphate and the nucleoside whereby several enzyme species can be involved in completing the hydrolysis chain. Ectonucleotidases are molecularly even more diverse than P2 receptors and include several enzyme families. Amongst these are the ectonucleoside triphosphate diphosphohydrolase family (E-NTPDases), the ectonucleotide pyrophosphatase/phosphodiesterase family (E-NPPases), the alkaline phosphatases, ecto-5′-nucleotidase and a variety of enzymes involved in the extracellular interconversion of nucleotides such as ectonucleoside diphosphokinase and ecto-ATP:AMP phosphotransferase (adenylate kinase, myokinase). To date, most of the ectonucleotidases have been identified and characterised in molecular and functional terms. The enzymes are equally abundant as nucleotide receptors and reveal a wide and partially overlapping tissue distribution.

The diversity of the individual family members is considerable and it is still difficult to assign the modulation of purinergic signalling pathways to identified enzymes. In the brain, members of all ectonucleotidase families are expressed. Physiological implications include the modulation of synaptic transmission, the ATP-mediated propagation of glial Ca^{2+} waves, microglial function, adult neurogenesis and the control of vascular tone, haemostasis and thromboregulation. Research on ectonucleotidases now continues in diverging directions, addressing e.g. structure-function relationships, overexpression and knockdown, crystallization and atomic structure analysis and physiological analysis. Yet, considerably more detailed information concerning tissue and cellular localisation of the individual ectonucleotidases is required for understanding their potential interaction with extracellular nucleotide signalling pathways. To date, NTPDase1, NPP1, ecto-5′-nucleotidase and two isoforms of alkaline phosphatase have been deleted in mice. As yet, no inducible knockouts are available. The development of additional knockout models will be of great importance to further define the physiological significance of the individual enzyme isoforms. This needs to be flanked by the development of inhibitors that do not affect nucleotide receptors and target individual enzyme isoforms. Together these studies will provide both an understanding of the molecular structure and of the physiological function of ectonucleotidases at a considerably higher level of resolution.

Professor Peter Illes, Chairman of the Department of Pharmacology, University of Leipzig, Germany, gave the

next talk entitled '**Purinergic receptors in pain, neuroprotection and nerve growth**'. He described how noxious stimuli lead to the outflow of ATP via the damaged cell membrane and subsequently may activate a certain subtype of ionotropic P2X receptor ($P2X_3$), or metabotropic P2Y receptor ($P2Y_1$), both situated at the nociceptive C fibre terminals. These fibres originate from sensory neurons located in nodose or dorsal root ganglia (DRG). $P2X_3$ receptors are algogenic by mediating depolarization, propagated action potentials and, in consequence, glutamate release from the central terminals of small-diameter sensory neurons in the dorsal horn of the spinal cord. In contrast, $P2Y_1$ receptors cause opposite effects through the blockade of voltage-sensitive Ca^{2+} channels, which normally initiate transmitter release. In addition, a negative interaction between P2Y and P2X receptors was described via G protein involvement in the cell bodies of DRG neurons. Moreover, various nucleotides, including ATP, have been suggested to phosphorylate, by means of ecto-protein kinases, the extracellular loop of $P2X_3$ receptors and thereby to increase the conductance of these receptor channels. Eventually, long-lasting contact with subthreshold concentrations of ATP is sufficient to induce a massive desensitization of the $P2X_3$ receptor. Hence, $P2X_3$ and $P2Y_1$ receptors alone or in concert shape the strength and duration of painful stimuli at the level of the peripheral nervous system.

In the CNS, both neuronal damage and mechanical distortion may rapidly increase the extracellular concentration of nucleotides. In particular, the effects of stab wound injury and hypoxia/ischaemia have been extensively investigated. ATP can either aggravate or ameliorate the extent and intensity of the original damage. An aggravation could be due to the activation of $P2X_7$ receptors, which have a low sensitivity to ATP, but may open large holes in the plasma membrane of astrocytes, microglia, or even neurons, initiating apoptotic or necrotic processes. All P2X receptor subtypes may lead, because of their Ca^{2+} permeability, to an overload of cells by Ca^{2+} and thereby to apoptosis. On the other hand, astrocytic $P2Y_1$ receptors may mediate proliferation and the production of glial scars interfering with the re-establishment of normal axonal connections. In the nucleus accumbens, $P2X_1$ and $P2X_7$ receptors were absent on astrocytes of untreated rats, but became expressed after mechanical damage. Similarly, $P2Y_2$ and $P2Y_6$ receptors appeared only after stab wound injury on the accumbal astrocytes. Several P2 receptor subtypes were upregulated by the introduction of an injection cannula into the nucleus accumbens and still more so by the application of the mixed P2X/P2Y receptor agonist 2-methylthio ATP (2-MeSATP) or the $P2Y_{1,\ 12,\ 13}$ selective agonist ADP-β-S. The co-application of selective antagonists [pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), MRS2179] confirmed the preferential involvement of the $P2Y_{1,\ 12,\ 13}$ receptors with a minor contribution of P2X receptor subtypes. The intracerebroventricular application of PPADS alleviated the morphological and functional consequences of ischaemia in an in vivo stroke model (rat medial cerebral artery occlusion) confirming the pathophysiological role of ATP.

Extracellular ATP may promote nerve growth alone or in conjunction with various growth factors. In co-cultures of the entorhinal cortex and the hippocampus, fibre growth was visualized by the anterograde tracer biocytin, which was placed onto the entorhinal part of the co-culture. The entorhinal fibre outgrowth was increased by 2-MeSATP in the absence but not in the presence of PPADS. Hence, some unidentified P2 receptors may exert a trophic influence under these conditions. A similar effect was observed when the ventral tegmental area/substantia nigra complex was co-cultured with the prefrontal cortex. In conclusion, extracellular ATP, through the activation of various P2 receptor subtypes may modulate pain, neuronal damage and nerve growth.

Professor Maria Abbracchio, Department of Pharmacological Science, University of Milan, Italy, gave the final talk, entitled '**Agonists and antagonists of old and new P2 receptors**'. Extracellular purine and pyrimidine nucleotides are ubiquitous signalling molecules that modulate the function of diverse mammalian cell types and tissues under both physiological and pathological conditions. The highly specific actions of these neurotransmitters are mediated by the activation of two distinct families of membrane receptors: the P2X receptor channels and the P2Y receptors belonging to the G protein-coupled seven-transmembrane (7-TM) receptor (GPCRs) superfamily. Seven distinct P2X receptor subunits ($P2X_1$-$P2X_7$) forming multimeric ligand-gated sodium and calcium channels have been identified; conversely, eight subtypes of P2Y receptors ($P2Y_{1,\ 2,\ 4,\ 6,\ 11,\ 12,\ 13,\ 14}$ receptors) are officially recognised by the IUPHAR (International Union of Pharmacology) Subcommittee for P2Y Receptor Nomenclature and Classification. Several non-selective agonists (e.g. hydrolysis-resistant adenine and uracil nucleotide analogs) and antagonists (e.g. suramin, Reactive Blue 2, Brilliant Blue G) have been available during the years, but it has been only recently that ligands displaying selectivity towards specific P2 receptor subtypes have been made available. Besides being important pharmacological tools for the characterization of the pathophysiological roles of P2X and P2Y receptors in native systems, there is agreement that such ligands may represent new therapeutic entities of potential interest in a variety of human diseases, including pain, depression, stroke and chronic neurodegenerative disorders. The design and synthesis of selective P2Y ligands has been greatly aided by the development of three-dimensional structures of these receptors via structure-activity relationships, mutagenesis and homology modelling studies based on the crystallization of

the GPCR rhodopsin. Detailed three-dimensional structures of P2X receptors have not been proposed yet, due to the lack of a suitable protein template. Recent work has identified nucleotide agonists selective for $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors and nucleotide antagonists selective for $P2Y_1$ (MRS2179, MRS2500), $P2Y_{12/13}$ (Congrelor, previously known as AR-C69931MX) and $P2X_1$ receptors. Selective non-nucleotide antagonists have been reported for $P2Y_{1,\ 2,\ 6,\ 12,\ 13}$ and $P2X_{2/3}$/$P2X_3$ and $P2X_7$ receptors. For example, the dinucleotide INS37217 (UP4dC) potently activates the $P2Y_2$ receptor and the non-nucleotide antagonist A-317491 is selective for $P2X_{2/3}$/$P2X_3$ receptors.

Dr. Abbracchio described a recently reported "deorphanization" of a new P2Y receptor, formerly known as the "orphan" GPR17 receptor. Phylogenetically and structurally, GPR17 is closely related to both the $P2Y_{12,\ 13,\ 14}$ subfamily of P2Y receptors and to CysLT1 and CysLT2 receptors. Hence, its ligand specificity could not be predicted simply based on its phylogenetic position. It was shown that this receptor responded dually to both uracil nucleotides and cysteinyl-leukotrienes, a family of proinflammatory arachidonic acid metabolites. Activation of GPR17 by uracil nucleotides can be counteracted by P2Y receptor antagonists such as MRS2179 or cangrelor, whereas its activation by CysLTs can be blocked by already known CysLT antagonists, such as Montelukast and Prankulast. Since both extracellular nucleotides and CysLTs are released in great amounts in ischaemic brain, it was hypothesized that the pathological activation of GPR17 by these endogenous ligands may contribute to ischaemia-associated neuronal death. In line with this hypothesis, the in vivo knockdown of GPR17 by either P2Y/CysLT antagonists or by an anti-sense oligonucleotide strategy markedly prevented ischaemia evolution. GPR17 thus represents the first fully characterised example of a dual GPCR responding to two distinct unrelated classes of non-peptide endogenous ligands. However, the $P2Y_{12}$ receptor has been also reported to respond to the cysteinyl-leukotriene LTE4, suggesting that there may exist several GPCRs characterised by a dual pharmacology, and, in particular, that the $P2Y_{12,\ 13,\ 14}$ subfamily of P2Y receptors may interact with both nucleotides and CysLT with high affinity. The existence of dual receptors opens the possibility of developing dual ligands characterised by previously unexplored therapeutic potency. In particular, it was envisaged that dual GPR17 antagonists may represent a novel class of potent anti-neurodegenerative agents.

The formal presentations were followed by a **discussion session** with lively exchanges of views between the speakers and members of UCB staff. Some promising therapeutic targets were identified. Marc De Ryck thanked the speakers for their authoritative and informative presentations and said that UCB would now consider the way forward.

Reference

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