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MEETING REPORT

TRENDS IN PAIN RESEARCH

THE SOCIETY FOR MEDICINES RESEARCH PAIN MEETING, MARCH 24, 2011, GIRTON COLLEGE, CAMBRIDGE, UK

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SUMMARY

Chronic pain represents a disorder of high unmet medical need. It can be a pain so excruciating that words fail to describe it and doctors cannot explain it, leading some patients to consider committing suicide. It can be described as a malfunction in the central nervous system (CNS), usually following injury to the peripheral nerves or to the CNS. This injury can result from direct damage to the nerves, for instance through amputation, or be triggered by medical conditions such as diabetic neuropathy, AIDS-related neuropathy, degenerative spinal disease and multiple sclerosis. It is a condition that is thought to effect over 25 million people worldwide, with a considerable associated cost to healthcare providers. In the past couple of decades, an important focus of research has been the study of novel pain mechanisms, particularly the biological pathways that lead to the abnormal sensitivity known as spontaneous pain and hyperalgesia. This symposium brings together experts from industry and academia from the U.K., the U.S. and Europe. Speakers covered the latest advances in understanding of new biological pathways, as well as findings from recent drug discovery programs.

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FUNDAMENTAL PAIN TARGETS

Prof. Peter McNaughton (University of Cambridge, U.K.) gave the opening presentation on the research from his group regarding certain fundamental pain targets. Since the discovery of the transient receptor potential channel TRPV1 in the mid-1990s, there has been a lot of work carried out to understand the function and signaling involved with the channel. TRPV1 is a member of the thermo TRP ion channel class which are activated at varying temperatures and all share a common tetramer of 6-transmembrane-spanning domain structure to form the ion channel pore. Prof. McNaughton's research has been involved in identifying at least three different signaling mechanisms that mediate the actions of inflammatory mediators on the TRPV1 channel. Bradykinin (BK) and prostaglandin E_2 (PGE_2) enhance the probability that TRPV1 channels will be activated by a heat stimulus, and they act by promoting serine phosphorylation of TRPV1 (S502 and S800) by protein kinase C (PKCε) and protein kinase A (PKA), respectively. By contrast, nerve growth factor (NGF) increases the expression of TRPV1 channels in the neuronal cell membrane by promoting trafficking from a subcellular vesicle store. Phosphorylation of TRPV1 by PKCε and PKA depends critically on a scaffolding protein, A-kinase anchor protein 5 (AKAP-5, A-kinase anchor protein 79 kDa, AKAP 79), which binds PKA and PKCε into a signaling complex together with TRPV1. Prof. McNaughton's group has identified the key AKAP interaction region in the *C*-terminus of TRPV1 and demonstrated that AKAP-5 mediates the effects of both bradykinin and PGE_2 on the TRPV1 channel. More recent work has shown that transient receptor potential channel TRPM8, which is activated by cool temperatures and menthol, is by contrast inhibited by inflammatory mediators, apparently by a very different mechanism.

Prof. McNaughton also talked about his research into the function of the cyclic nucleotide-regulated channel (HCN) ion channels. The frequency of firing in nociceptive neurons signals the intensity of pain, and proinflammatory mediators such as PGE_2 enhance the sensation of pain by increasing the frequency of action potential firing in response to a given level of painful stimulus. The enhanced firing is abolished by a blocker (ZD-7288) of the I_h inward current, suggesting an involvement of the HCN channel isoform family in modulating the frequency of action potential firing. There are four HCN isoforms responsible for I_h currents (HCN1-4), with HCN2 and HCN4 being cAMP-sensitive. Genetic deletion of HCN1 ablates the fast-activating I_h, which is seen in large neurons and in a small subpopulation of cold-sensitive neurons, but leaves unaffected the slowly-activating I_h seen in small nociceptive neurons. Deletion of HCN2, on the other hand, reduces I_h in small neurons; the remaining current is insensitive to cAMP and probably reflects expression of the cAMP-insensitive HCN3. Importantly it has been shown that deletion of HCN2 abolishes the effect of PGE_2 in accelerating action potential discharge. In subsequent behavioral experiments, it was also shown that genetic deletion of HCN2 abolishes some components of inflammatory pain and has a remarkable effect in abolishing neuropathic pain in an animal model, suggesting that HCN2-selective blockers may have potential in reducing inflammatory and neuropathic pain.

NEUROBIOLOGICAL MECHANISMS UNDERPINNING AFFECTIVE AND COGNITIVE MODULATION OF PAIN

Dr. David Finn (National University of Ireland, Galway) gave a very interesting talk on the research from his studies into how pain perception can be affected by state of mind. Pain shares a bidirectional, reciprocal relationship with affect and cognition, whereby the latter two can both influence and be influenced by the pain experience. In particular, acute stress or fear tends to suppress pain through the phenomenon of stress/fear-induced analgesia, while chronic anxiety or depression is often associated with enhanced pain perception or hyperalgesia. By employing a rat model that combines the formalin test of tonic, persistent nociception with classical contextual fear conditioning, Dr. Finn's group investigated the role of the endocannabinoid, opioid and GABAergic systems in fear-induced analgesia. The results demonstrated that pharmacological blockade of the cannabinoid CB_1 receptor with rimonabant prevents, whereas pharmacological inhibition of endocannabinoid degradation (fatty-acid amide hydrolase inhibition with URB-597) enhances, fear-induced analgesia in rats. Some evidence for endocannabinoid–opioid interactions during fear-induced analgesia was also generated, as the fatty-acid amide hydrolase inhibitor (URB-597)-induced enhancement of fear-induced analgesia was attenuated by μ opioid receptor blockade with naloxone. The brain regions involved in endocannabinoid-mediated fear-induced analgesia were also investigated. Data suggested a key role for the endocannabinoid system in the dorsolateral periaqueductal grey and ventral hippocampus in mediating fear-conditioned analgesia. In vivo microdialysis and site-specific intracerebral microinjections also demonstrated a role for GABA in the basolateral amygdala and dorsal periaqueductal grey during fear and pain responding and fear-induced analgesia.

Dr. Finn also presented work on attentional modulation of pain, which can be achieved through the phenomenon of distraction-induced analgesia, whereby exposure to non-stressful distracting stimuli can reduce pain perception. A rat model of distraction-induced analgesia was established and experiments demonstrated the likely involvement of the monoaminergic and endocannabinoid systems in mediating attentional modulation of pain. He also outlined some newer research into stress effects on analgesia, where it has been demonstrated that hyperalgesia in the stress-hypersensitive Wistar–Kyoto rat strain is associated with alterations in levels of endocannabinoids and monoamines in key brain regions regulating pain and affect. Dr. Finn concluded that the use of animal models may enhance our understanding of the neurobiological mechanisms underpinning affective and cognitive modulation of pain, which, in turn, may aid in the identification of novel pharmacological targets for the treatment of pain, mood disorders and their comorbidity.

DOES CHROMATIN OR EPIGENETIC MODIFICATION OFFER AN ALTERNATIVE APPROACH TO THE TREATMENT OF CHRONIC PAIN STATES?

Prof. Chas Bountra (Structural Genomics Consortium, University of Oxford, U.K.) proposed a new approach to identify potential treatments for chronic pain utilizing epigenetic modulation. Chemical modification of either DNA (e.g., methylation) or histone tails (e.g., methylation, acetylation and phosphorylation) can affect gene expression in a cell- or tissue-specific manner, leading to gene activation or repression. Prof. Bountra highlighted recent studies where peripheral nerve injury (PNI) had led to an increase in RE1-silencing transcription factor (neural-restrictive silencer factor; *NRSF*) in dorsal root ganglia cells (DRGs); RE1-silencing transcription factor then binds to neuron-restrictive silencer element (NRSE), which in turn leads to decreased histone acetylation on histone H3 and/or H4 and decreased translation of pain implicated proteins, the μ opioid receptor, the voltage-gated sodium channel $Na_V1.8$ and the voltage-gated potassium channel $K_V4.3$. Subsequent *NRSF* knockdown studies were able to block the PNI-induced decrease in the μ opioid receptor, the $Na_V1.8$ channel and the $K_V4.3$ channel, indicating that modulation of this epigenetic pathway could provide a potential route to new pain therapeutics with multiple effects from a single point of intervention. Other studies have also shown that the epigenetic modulation of histone acetylation by histone deacetylase inhibitors leads to antihyperalgesic effects. Histone deacetylase inhibitors increase acetylation of p65RelA, which in turn leads to increased expression of the metabotropic glutamate $mGlu_2$ receptor and decreased primary neurotransmitter release from primary afferent neurons, culminating in an antihyperalgesic effect. This is readily demonstrated in vivo, where the histone deacetylase inhibitor MS-275 (entinostat) has antihyperalgesic effects in the second phase of the formalin test after 5 days of s.c. administration, which is blocked by preadministration of the mixed $mGlu_{2/3}$ antagonist LY-341495. In vivo studies have also demonstrated that the histone deacetylase inhibitor SAHA can increase acetylation of p65RelA and expression of $mGlu_2$. Prof. Bountra closed by proposing that intervention within epigenetic pathways could be a better way to treat chronic diseases, as many relevant genes can be modulated in a specific fashion by a single point of intervention, which might be more useful than trying to treat the disease by modulating a highly specific downstream single gene product. The work being carried out at the Structural Genomics Consortium in Oxford is generating protein structures and

small-molecule chemical probes for tractable epigenetic targets, which aim to help delineate these disease pathways.

DISCOVERY OF SELECTIVE $NA_V1.8$ MODULATORS FOR THE TREATMENT OF CHRONIC MIXED PAIN

Dr. Sharan Bagal (Pfizer, Sandwich, U.K.) presented efforts from Pfizer to discover novel, clinically selective voltage-gated sodium channel $Na_V1.8$ ligands as potential treatments for chronic pain. She highlighted the clear association and history of sodium channel blockers in this therapeutic area and, in particular, stressed the need for the development of subtype-selective agents which could achieve an improved efficacy versus tolerability window. Pfizer took the decision to target $Na_V1.8$ specifically due to its localization in C and Aδ pain-sensing fibers, the enhanced channel activity caused by inflammatory mediators, its increased expression near painful injury sites and the antisense oligonucleotide knockdown data showing reduced nociceptive responses in animal models.

The lead molecule for this approach was A-803467 (Abbott/Icagen), with high selectivity for $Na_V1.8$ but poor pharmacokinetics (1). The Pfizer lead was generated from a weakly potent, nonselective 3-phenylpyridyl hit by incorporation of features of the marketed pan-voltage-gated sodium channel blocker lamotrigine (Fig. 1). The metabolic instability of the *N*-acetyl lead was improved by reversing the amide and led to the first clinical candidate. This molecule was a potent, selective $Na_V1.8$ blocker with good pharmacokinetics and animal model efficacy; however, 1-month toxicological evaluation identified retinal atrophy in the rat, which terminated its progression into development.

Despite this setback, a second clinical candidate was identified following exploration of the amide group, which led to a 3-methylisoxazole (Fig. 2). As before, this molecule was a potent (0.26 μM) and selective $Na_V1.8$ channel blocker with good predicted human pharmacokinetics and efficacy in the rat carrageenan (thermal hyperalgesia) model. Unfortunately, however, as before, this molecule failed to make it to the clinic due to a lack of safety margin over CNS effects in rats during toxicity studies (although no retinal atrophy was observed). Dr. Bagal ended on a more positive note with the disclosure that both of these toxicology liabilities had been overcome in subsequent compounds.

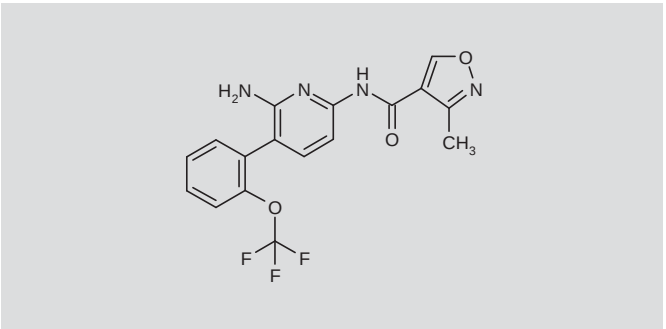


Figure 2. 3-Methylisoxazole.

SEPARATING EFFICACY FROM HYPERTHERMIC EFFECTS IN TRPV1

Dr. Phil Kym (Abbott, U.S.) gave a presentation from the considerable body of work at Abbott to identify blockers of the TRPV1 channel as new treatments for chronic pain. He emphasized that there remains a significant unmet medical need in both neuropathic and inflammatory pain indications and outlined the strong supporting target validation behind the TRPV1 target, as well as the wealth of literature published on both preclinical and clinical antagonists.

The first Abbott candidate, ABT-102 (Fig. 3), is a potent, selective TRPV1 blocker capable of inhibiting channel activation by multiple stimuli, including capsaicin, lipids, acidic pH and heat (2). It is active

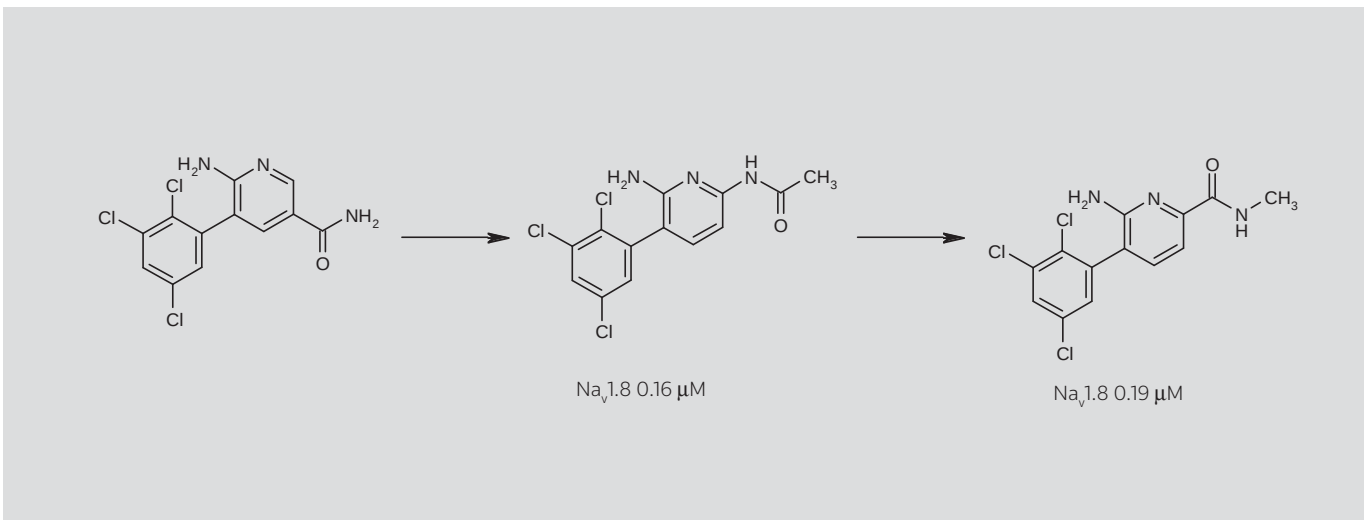
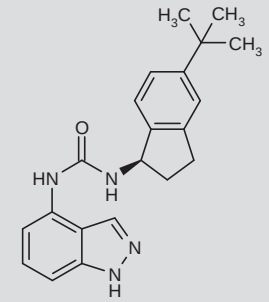
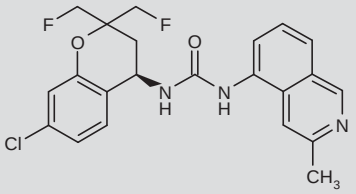


Figure 1. Initial Pfizer lead evolution (electrophysiology data).



ABT-102
IC_{50} = 4 nM: brain:plasma = 0.3



A-1165442
IC_{50} = 7 nM

Figure 3. TRPV1 antagonists.

in a wide range of preclinical pain models: rat acute capsaicin, complete Freund's adjuvant (CFA) model of inflammatory pain, model of osteoarthritis and evoked bone cancer pain. Importantly, in the chronic dosing osteoarthritis model, Dr. Kym highlighted the increase in potency observed over time without any increase in plasma concentrations. This finding is not replicated for other mechanisms of other drugs such as celecoxib or morphine, and was hypothesized to be either attributable to a decreased sensitization state of the TRPV1 channel over time or an increased occupancy of the channel.

Dr. Kym addressed the key issue of marked hyperthermia observed clinically for the lead Amgen molecule AMG-517, which persisted in a phase Ib study with no dose-dependency and considerable variability. For ABT-102, a modest, dose-limiting temperature elevation was observed which was attenuated on repeat dosing. Interestingly, Abbott was able to contextualize these data by also running a human experimental ultraviolet burn pain model, which showed potent efficacy for ABT-102, which has now successfully completed phase I evaluation.

Follow-on efforts were then directed to identification of a further differentiated blocker with improved clinical tolerability. A screening cascade was devised to assess thermoregulation liability at the point of initial in vivo characterization and this approach identified a new series of molecules which were optimized to A-1165442. This molecule had good developability properties, and interestingly, completely blocked activation of TRPV1 by capsaicin, NADA and heat, but not by acidic pH. Furthermore, in vivo, A-1165442 was effective, but did not cause significant elevation in core body temperature in rats. From analysis of all data generated by Abbott, Dr. Kym argued that the profile of efficacy without effects on body temperature was directly associated with the selective pattern of channel block depending on the mode of activation. Additionally, this molecule also caused the same increase in efficacy on chronic dosing and represents a promising lead for future development.

$P2X_3$ RECEPTOR ANTAGONISTS AND THEIR POTENTIAL TO TREAT PAIN AND URINARY DYSFUNCTION

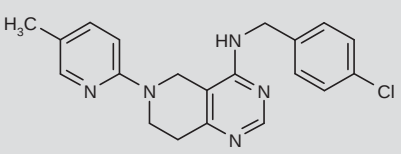
Dr. David Hallett (Evotec, Oxford, U.K.) described their efforts towards new pain drugs through the antagonism of the $P2X_3$ receptor. This receptor is predominantly restricted to the sensory neurons, suggesting a limited risk of on-target CNS side effects. The industry efforts around this target suggest that finding high-quality chemistry leads can be problematic. Evotec overcame this by screening a proprietary ion channel-based library of 2,500 compounds against a chimeric human $P2X_{2-3}$ receptor transfected into an astrocytoma cell line. This yielded an initial tetrahydropyrido[4,3-*d*]pyrimidine hit (Fig. 4).

This starting point was more potent against $P2X_{2-3}$ than $P2X_3$ and had a number of drug metabolism/pharmacokinetic (DMPK) issues; however, it was felt that it was in a good physicochemical space for further elaboration and optimization. Using e-phys assays alongside fluorescent imaging plate reader (FLIPR) assays, optimization was achieved, improving its DMPK profile and reversing the selectivity to give a 20-fold window for $P2X_3$ and increasing the selectivity window for $P2X_3$ over $P2X_{2-3}$ (structure not disclosed; Fig. 5). This compound was profiled further in pain models, showing efficacy at 1 mg/kg p.o. in the rat CFA inflammatory pain model, as well as efficacy in a model of diabetic neuropathy.

In summary, a novel, selective and potent series of $P2X_3$ antagonists have been discovered through directed screening and lead optimization. The compounds behaved like allosteric modulators (noncompetitive with ATP). Key compounds from the series have been profiled further through animal models, showing good efficacy at oral doses.

TARGETED SECRETION INHIBITORS: A NOVEL APPROACH TO THERAPY?

Dr. John Chaddock (Syntaxin, U.K.) outlined the proprietary technology developed by Syntaxin (a spin-out company from the Health Protection Agency, formed in 2005) over the past 15 years. The



$hP2X_{2-3}$ IC_{50} ~ 400 nM
$hP2X_3$ IC_{50} ~ 1000 nM
$hP2X_{2-3}$ IC_{50} ~ 400 nM
$hP2X_{2/3}$ IC_{50} ~ 440 nM
MWt = 366
PSA = 54

cLogP = 4.4
Rat hepatocytes: $t_{1/2}$ = 29 minutes

Solubility (pH 7.4) < 5 μmol
Pan CYP inhibition (5-10 μmol)
hERG: 68% @ 10 μmol
Bioavailability < 1% (rats)

Figure 4. Initial Evotec hit.

$hP2X_3$ FLIPR IC_{50} = 10 nM
$hP2X_3$ EPhys IC_{50} = 20 nM

$hP2X_{2/3}$ FLIPR IC_{50} = 260 nM
$hP2X_{2/3}$ EPhys IC_{50} = 260 nM

Solubility (pH 7.4) 335 μmol
PSA = 80
cLogP = 2.1

Hepatocytes: $t_{1/2}$/minute
Rat/dog/cyano/human > 180/111/18/> 180
PPB (% free) rat/human 20/10
Pan CYP inhibition > 50 μmol
hERG: 15% @ 10 μmol
AMES (± S9) negative
Cerep Screen Clean
Bioavailability (%) rat/dog 77/45

Figure 5. Optimized profile.

research is based on bacterial endopeptidase manipulation to produce novel biopharmaceuticals that inhibit cell secretion (see technology overview at www.syntaxin.com). Botulinum neurotoxins are made up of three regions –a heavy-chain binding domain which targets neuromuscular cell vesicles, an Hn transport domain which mediates translocation from the endosome vesicle to the cytosol and the LC enzyme, which is a zinc-dependent metalloproteinase that cleaves a specific bond in a soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE). Cleavage of these SNARE proteins blocks vesicular secretion of neurotransmitters from nerve cells. By replacing the heavy-chain binding domain of botulinum with a protein or antibody specific for a different cell type, novel targeted secretion inhibitors (TSIs) can be generated. TSIs are generated recombinantly in *Escherichia coli* and have been shown to tolerate the incorporation of a heavy-chain ligand as large as 50 kDa. TSIs have a long duration of action after a single dose, with efficacy in animal models lasting for at least a month. In the case of the pain endpoint, biological data showed efficacy with subcutaneously dosed TSI in the mouse hot plate model and a model of diabetic neuropathy, with pain reversal comparable to a systemically administered gold standard lasting 3-4 months. This technology platform has led to the nomination of a novel TSI (AGN-214868) in collaboration with Allergan, which is currently progressing through phase II clinical trials for the treatment of postherpetic neuralgia and urinary incontinence by local administration.

DISCLOSURES

R. Davenport and R. Armour are employees of Takeda and Lectus Therapeutics, respectively. S. Ward states no conflicts of interest.

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