New trends in pain research: from basic research to clinical translation

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The small town of Parghelia on the Tyrrhenian coast (Calabria, Italy) recently provided an attractive setting for a two-year-awaited international meeting on "NEW TRENDS IN PAIN RESEARCH: From Basic Research to Clinical Translation" (13-15 September, 2012).
The event, attended by around sixty participants, brought together young researchers, PhD students, international scientists and academics interested in sharing the latest acquisitions in the field of pain research.

Translational research on itching

The meeting opened with an invited lecture on translational research on itching given by Prof. Hiroshi Nagase (Kitasato University, Japan). Prof. Nagase's iconography underlined the urgent need for knowledge in this hitherto little explored field. He recounted the "almost accidental" discovery of the antipruritic effect of the K-receptor agonist nalfurafine hydrochloride, a discovery that subsequently led to its launch on the market (in 2009) for the treatment of kidney dialysis patients suffering from intractable itching.
Nalfurafine, originally designed to challenge morphine-induced addiction, displayed a powerful analgesic action without addictive properties. It underwent a clinical trial as a treatment for post-operative pain but this trial was interrupted because the compound displayed a sedative effect. Attracted by Kuraishi's itchy mouse model presented at the annual meeting of the Japanese Pharmacological Society, Prof. Nagase and his colleagues decided to test nalfurafine in this model. This led to the chance discovery of the antipruritic effect that in turn prompted the development of a clinical trial in kidney dialysis patients with intractable pruritus. During this trial, no serious side effects such as addiction and aversion emerged.

The rest of the meeting was structured in four sessions concerned with different aspects of pain research: cellular and molecular aspects of pain, mechanisms of pain transmission, the rational basis for pain treatment, and advanced pain therapy. Moreover, an entire session was devoted to the research work of young PhD students, and part of the meeting to clinical treatment of chronic pain.

Cellular and molecular aspects of pain

The first presentation of the morning, after the opening lecture, was given by prominent academic Prof. Stephen Hunt (University College London, UK). Mechanisms underlying ongoing neuropathic pain involve pathological modifications occurring after injury, manifesting themselves in territories progressing from the brainstem to the spinal cord and, further, to the peripheral nerves. It is possible that targets for pain therapy such as the trkA receptor could be located on C-fibers along these structures; more importantly, mTOR and its translational machinery on A-fibers, which can be negatively modulated by mTOR inhibitors (of which rapamycin is the main compound) and, as recently shown, by metformin, seem to be druggable for chronic pain control (Obara et al., 2012).
The focus then shifted to autophagy in neuropathic pain, with the presentation, by Dr Laura Berliocchi (University "Magna Graecia" of Catanzaro, Italy), of research that underlined the importance of basic cellular and molecular research for opening up new avenues of study in drug discovery. She demonstrated that 7 days after injury the autophagy machinery is impaired in the spinal cord of spinal nerve ligated mice. In fact, levels of the conjugated form of LC3, i.e. LC3-II, a marker of increased autophagosome formation, were higher in the L4-L5 portion of the spinal cord ipsilateral to the ligation and this increase was paralleled by accumulation of p62. These LC3-II levels appeared to correlate with overexpression of the $\alpha 2\delta$-1 subunit of the voltage-dependent calcium channel, typically overexpressed under neuropathic conditions but not found in mice that underwent a sham procedure. This compelling evidence supports Dr Berliocchi's original hypothesis that impaired autophagy plays an important role in neuropathic pain and offers novel targets for its control (Berliocchi et al., 2011; Klionsky et al., 2012).
From the cell membrane perspective, Dr Santina Chiechio (University of Catania, Italy) highlighted the value of the metabotropic glutamate receptors as potential new targets for chronic pain treatment. In fact, the original data presented demonstrated that activation of the mGluR2 subtype is responsible for the analgesic action

produced by group II mGluR agonists. The experimental use of L-acetylcarnitine and "epigenetic drugs", such as the histone deacetylase inhibitors SAHA and MS-275, was instrumental in up-regulating mGluR2 in the dorsal root ganglia and in the dorsal horns of the spinal cord to produce analgesia in chronic pain conditions. The latter data are strengthened by mGluR2 down-regulation obtained by administering curcumin, a histone acetyltransferase inhibitor (Chiechio and Nicoletti, 2012).

Mechanisms of pain transmission

Prof. Sabatino Maione (Second University of Naples, Italy) focused on the large family of transient receptor potential cation channels as possible new targets for therapeutic strategies. In his opinion, modulation of TRPV1, and the TRPA1 congener, expressed in the descending pain pathway, in particular in the periaqueductal gray and in the rostral ventromedial medulla, may offer interesting perspectives (Palazzo et al., 2012).
Prof. Shinobu Sakurada (Tohoku Pharmaceutical University, Sendai, Japan) underlined that nociceptin plays an important role in the nociceptive spinal transmission system. Nociceptin/orphanin is a 17-amino-acid peptide identified as the endogenous ligand of the opioid receptor like-1 (ORL-1) receptor, also called the nociceptin/orphanin FQ peptide receptor. Intrathecal administration of nociceptin activates the ORL-1 receptor, and this activation may produce disinhibition of histaminergic neurons. H1 receptors located on SP-containing neurons are activated by histamine causing the spinal cord-mediated nociceptive response. Nociceptin undergoes cleavage induced by aminopeptidase N and endopeptidase 24.15 to yield two N-terminal fragments, of which nociceptin (13-17) shows an interesting pharmacological profile and binds to spinal H1 receptors (Mizoguchi et al., 2012).

Rational basis for pain treatment

Dr Roberta Lattanzi (Sapienza University of Rome, Italy), focusing on pro-nociceptive systems, looked at pain killers from the opposite perspective. She highlighted the importance, in neuropathic pain development, of prokineticin 2 (Bv8/PK2), an inflammatory cytokine-like molecule expressed by macrophages and neutrophils. Bv8/PK2, whose levels are increased in the spinal cord after chronic constriction injury, induces mononcytes to express TNF, IL-1 and the chemokines CCL4, CXCL1, CXCL8, and might modulate the neuroimmune interactions occurring during neuropathic pain states (Negri and Lattanzi, 2012).
The Epitech-sponsored lecture by Prof. Salvatore Cuzzocrea (University of Messina, Italy) also focused on immune cells and, in particular, on the role of mast cells in pain pathophysiology.
The puzzling neuro-immune communication involved in the onset and maintenance of neuropathic pain has been a hot topic in pain research for many years. Dr Marzia Malcangio (King's College London, UK) highlighted the marked involvement of microglia and astrocytes in central sensitization. In a collagen-induced arthritis animal model, inhibitors of cathepsin S, a lysosomal cysteine protease also implicated in joint degradation, and centrally active CX3CR1 antagonists, have been shown to be useful in the therapy of pain (Old and Malcangio, 2012).
The role of other chemokines, e.g. macrophage inflammatory proteins (MIPs), in pain processing was discussed by Prof. Shiro Kishioka (Wakayama Medical University, Japan). These molecules, in particular MIP-1α, MIP-1β and MIP-2, binding respectively to CCR1, CCR5 and CXCR2 receptors located on Schwann cells, are responsible for microglia activation and recruitment of neutrophils and macrophages and found to be up-regulated after partial sciatic nerve ligation (PSL). MIP-1α could be considered a central sensitization mediator, as supported by the finding that perineural injection of antibodies against MIPs, and in particular MIP-1α, or of antagonists of their receptors was able to prevent PSL-induced mechanical allodynia and thermal hyperalgesia (Kiguchi et al., 2012).
This meeting also brought participants up to date with the latest findings relating to key questions in migraine care. Dr Stefania Ceruti (University of Milan, Italy) focused on the cross-talk between trigeminal neurons and "satellite" glial cells (SGCs), which could be involved in migraine pain processing. ATP and UTP purinergic receptors, and, in particular, some P2Y-receptor subtypes present on SGCs (ADP-responsive P2Y1 and UTP-sensitive P2Y2), can be up-regulated by calcitonin gene-related peptide (CGRP) induced by bradykinin that mediates this interaction (Magni and Ceruti, 2013).
Prof. Cristina Tassorelli (C. Mondino Foundation, IRCCS, Pavia, Italy) described the increased enzymatic degradation of endocannabinoids and the up-regulation of their receptors found in the brains of animals subjected to "nitroglycerin-induced hyperalgesia", findings which suggest that a dysfunction of the endocannabinoid system is probably implicated in migraine (Perrotta et al., 2012).

Advanced pain therapy

The workshop then moved on to more technologically advanced approaches to the diagnosis and control of chronic pain states, focusing particularly on those states that will never derive benefit from intervention at peripheral level.
Dr Gianfranco Spalletta (IRCCS Santa Lucia Foundation, Rome, Italy) drew attention to the Janus face of pain and depression. He described a study analyzing, with the aid of MRI, the brain structures of a cohort of 34 old patients with mild cognitive deterioration, some affected by Alzheimer's disease and some by mild cognitive impairment. In patients affected by pain states, the nucleus accumbens gray matter was shown to have a significantly ($p<0.005$) increased volume. This might be at the root of a deregulation of mood tone probably responsible, in turn, for increased perception of pain.
Neural stem cells (NSCs) exert an anti-allodynic and an anti-hyperalgesic effect and reduce pro-inflammatory cytokines in neuropathic mice. Human NSC technology is not yet sufficiently advanced to be of clinical use in the treatment of chronic pain. On the basis of this reasoning, Prof. Paola Sacerdote (University of Milan, Italy) highlighted the possibility of using human adipose tissue

derived stem cells (hASCs), given intravenously, to reduce allodynia after nerve injury. It was demonstrated, using hASCs, that these cells exert all the actions of NSCs and could therefore be useful for reversing neuropathic pain symptoms (Sacerdote et al., 2013).

Prof. Tsukasa Sakurada (Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan) spoke about the therapeutic possibilities offered by the plant world, which provides a wealth of natural products for pain control. Prof. Sakurada looked, in particular, at bergamot essential oil (BEO), linalool, a volatile component of BEO, and β-caryophyllene (BCP), which is a common constituent of the essential oils of several plants including cannabis. Intraplantar injection (i.pl.) of BEO or linalool into the ipsilateral hindpaw of mice submitted to PSL reduced mechanical allodynia and, as assessed by Western blot experiments, inhibited PSL-induced spinal ERK activation (Kuwahata et al., 2013). Administration (i.pl.) of BCP exerted a dose-dependent antinociceptive action in the capsaicin test and this appeared to be CB2 receptor-dependent since it was minimized by AM630 (s.c. and i.pl.), a selective CB2-antagonist, but not by AM251, a selective CB1-antagonist (Katsuyama, 2012).

International regulatory agencies have recently approved the use of botulinum neurotoxin in the treatment of chronic, migraine pain resistant to conventional drugs. This provides further confirmation of the importance of funding basic research focusing on fundamental cellular processes (exocytosis in this case). Thirty years ago, nobody would have predicted the myriad (some one thousand) clinical applications in which this toxin is now used. The meeting closed with a presentation by Prof. Oliver Dolly (Dublin City University, Ireland), a speaker of considerable academic stature whose research efforts have produced a vast amount of knowledge over the past three decades. He spoke of the therapeutic perspectives that may be opened up in the near future through the engineering of botulinum toxin chains from different serotypes. He explained, for instance, that BoTIMA, a BoNT/A (botulinum neurotoxin A) inactive mutant, is effective in migraine care since it cleaves SNARE proteins in trigeminal neurons, and in particular SNAP-25E, and blocks CGRP exocytosis [more effectively the light chain (LC)E-conjugated form of the toxin]. The latter pharmacological approach also alleviates mechanical and cold hypersensitivity in spared nerve injury rats, opening a new avenue of research for these technologies (Dolly and O'Connell, 2012).

At the end of the meeting, in the stimulating atmosphere generated by the above report, young researchers and PhD students had the opportunity to meet scientists of international renown to discuss, with them, their own projects and broaden their research horizons.

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