

NEW TRENDS IN PAIN RESEARCH
From Basic Research to Clinical Translation

Panta Rei Hotel (www.hotelpantarei.com)
Parghelia (VV), Calabria, Italy
13th-15th September, 2012

WITH THE SUPPORT OF
THE PRESIDENT OF THE ITALIAN REPUBLIC

UNDER THE AUSPICES



FINAL PROGRAMME AND ABSTRACT BOOK
(Eds. Laura Berliocchi, Maria Maiarù, Damiana Scuteri and Luigi Antonio Morrone)

A COLLABORATIVE SCIENTIFIC INITIATIVE OF



The Tohoku Pharmaceutical University
Department of Physiology and Anatomy



The University of Calabria
Department of Pharmacobiology



The University Magna Graecia of Catanzaro
Department of Health Sciences



The University Consortium for Adaptive
Disorders and Headache (UCADH)



Headache Science Center, The IRCCS
Mondino Foundation PAVIA



The IRCCS Santa Lucia Foundation/CERC
Rome

PRESIDENTS

Giacinto Bagetta (Cosenza)
Maria Tiziana Corasaniti (Catanzaro)
Shinobu Sakurada (Sendai)
Cristina Tassorelli (Pavia)

INTERNATIONAL ORGANIZING COMMITTEE

Giacinto Bagetta (Italy)
Carlo Caltagirone (Italy)
Maria Tiziana Corasaniti (Italy)
Oliver J. Dolly (Ireland)
Stephen P Hunt (UK)
Shinobu Sakurada (Japan)
Giorgio Sandrini (Italy)

INTERNATIONAL SCIENTIFIC BOARD

Maria Pia Abbracchio (Milan)
Bruno Amantea (Catanzaro)
Francesco Amato (Cosenza)
Sebastiano Andò (Cosenza)
Lucio Annunziato (Naples)
Fabio Blandini (Pavia)
Norman G. Bowery (Birmingham)
Gianfranco Di Renzo (Naples)
Stephen P. Hunt (London)
Rosario Iannacchero (Catanzaro)
Shiro Kishioka (Wakayama)
Marcello Maggiolini (Cosenza)
Sabatino Maione (Naples)
Giuseppe Micieli (Pavia)
Hirokazu Mizoguchi (Sendai)
Luigi A. Morrone (Cosenza)
Iroshi Nagase (Tokyo)
Giuseppe Nappi (Pavia)
Lucia Negri (Rome)
Giovanni Nicotera (Cosenza)
Pierluigi Nicotera (Bonn)
Domenicantonio Rotiroti (Catanzaro)
Paola Sacerdote (Milan)
Tsukasa Sakurada (Fukuoka)
Diego Sisci (Cosenza)
Gianfranco Spalletta (Rome)
Chizuko Watanabe (Sendai)
John N. Wood (London)
Akihiko Yonezawa (Sendai)

LOCAL SCIENTIFIC COMMITTEE

Annagrazia Adornetto (Cosenza)
Diana Amantea (Cosenza)
Laura Berliocchi (Catanzaro)
Maria Cristina Caroleo (Cosenza)

Laura Rombolà (Cosenza)
Rossella Russo (Cosenza)

EXECUTIVE COMMITTEE

Michelangelo Certo (Cosenza)
Federica Cavaliere (Cosenza)
Antonella Ciociaro (Catanzaro)
Maria Maiarù (Catanzaro)
Francesco Petrelli (Cosenza)
Damiana Scuteri (Cosenza)
Giuseppe Varano (Catanzaro)

SCIENTIFIC SECRETARIAT

Laura Berliocchi
Department of Health Sciences,
University Campus “Salvatore Venuta”
University Magna Graecia of Catanzaro
88100 Catanzaro
Tel 39 0961 369 4109
Email: Berliocchi@unicz.it

EXECUTIVE SECRETARIAT

Mr. Nicola Fico
Department of Pharmaceutical Sciences, University of Calabria, Via Pietro Bucci, 87036 Rende
(Cosenza) Italy Tel 39 0984 493248; Fax 39 0984 493462 Email: fico@unical.it

SPONSORS

Calabria Region
University of Calabria, Rende (Cosenza)
University Magna Graecia, Catanzaro
Department of Pharmacobiology, Rende, (Cosenza)
Pharmacists Association

MEETING VENUE

Panta Rei Hotel (tel. 39 0963 600000) is located at Parghelia (Vibo Valentia), in one of the loveliest spots of the Tirrenian Coast facing the still active Stromboli volcano. The international airport of Lamezia Terme (Catanzaro) is 30 Km from the Hotel. Further travelling information are available on the web at www.pantarei.com. A reduced price has been agreed for the full board accommodation of participants to the workshop.

LANGUAGE

The official language of the conference is English.

ABSTRACTS SUBMISSION

Participants are welcome to submit abstracts for presentation at the meeting in the form of poster communications to be held in the afternoon session of the 14th september. The abstract should be written according to the instructions given below. Deadline for submission: 15th July 2012. Abstracts must be submitted by e-mail to the secretariat (fico@unical.it), indicating “Pain meeting” as subject of the correspondence. Authors will be notified the acceptance of the abstract by August, 10th, 2012. Each abstract, written in times new roman (12 character), should contain a sentence stating the study objective; a brief statement of methods, if pertinent; a summary of the results and a

statement of the conclusions. It is not satisfactory to say “the results will be discussed”. Use a short, specific title. Capitalize initial letters of trade names. Use standard abbreviations for units of measure. Other abbreviations should be spelled out in full at first mention, followed by the abbreviation in parentheses. Include the source of research support on the bottom line of the abstract. The presenting author assures the merit of the presentation and that all authors listed have had a significant role in the research being reported. The size of the poster will be 70 cm width and 100 cm height. Posters should be mounted on the numbered boards at the corresponding number as listed in the book of abstract (e.g. P1 to P10) and should be attended by one of the Authors during the whole duration of the poster session; the relative information will be communicated by the scientific secretariat via the letter of acceptance. Posters should be dismantled at the end of the poster session.

A selected number of presentations will be incorporated in the proceedings of the meeting and these will appear in an indexed Journal linked to Pubmed, ensuring that articles can be cited on Medline. Instructions to Authors will be available at <http://gbagetta.jimdo.com> as soon as possible.

REGISTRATION FEE

A registration fee of 350 € must be paid at the time of abstract submission. The fee includes access to the meeting room, congress kit, cocktail at the opening ceremony, coffee breaks. Payment should be made to “New Trends in Pain Research”, Department of Pharmacobiology, University of Calabria, Via P. Bucci, 87036 Rende (CS) Italy. Bank details: MONTE DEI PASCHI DI SIENA FILIALE 8473, Via Ponte Pietro Bucci, 87036 Arcavacata di Rende (Cosenza). Cin W, ABI 01030, CAB 80880, Bank Account 000000010947, IBAN IT 52W 01030 80880 000000010947; BIC PASCITMMXXX.

Further administrative information can be obtained from Dr Daniela Marsili (tel. 39 0984 493219; email: dmarsili@unical.it).

A number of bursaries for young scientists are made available by the Italian Society of Pharmacology and by The Italian Society of Neuroscience. These will be attributed on a competitive basis; to this end, the participants should follow the instructions available upon request to the Societies or at <http://gbagetta.jimdo.com>.

SCIENTIFIC PROGRAMME

Thursday, 13th September

18.00 Welcome address

18.15 Invited **Opening Lecture**

Chairperson: Giacinto Bagetta (Cosenza)

Invited Speaker: **Hiroshi Nagase** (Tokyo), Translational Research in Itching: Research and Development of nalfurafine hydrochloride and its application to kidney dialysis patients with intractable pruritus

Friday, 14th September

Morning session

9.00-10.30 **MECHANISMS OF PAIN TRANSMISSION**

Chairpersons: Shinobu Sakurada (Sendai), Cristina Tassorelli (Pavia)

9.00-09.30 **Stephen P Hunt** (London), Pain mechanisms and new targets

9.30-10.00 **Sabatino Maione** (Naples), Transient receptors in pain transmission

10.00-10.30 **Marzia Malcangio** (London) Neuron-glia interactions in chronic pain

10.30-11.00 Coffee Break

11.00-13.30 **CELLULAR AND MOLECULAR ASPECTS OF PAIN**

Chairpersons:, Oliver J Dolly (Dublin), Giorgio Sandrini (Pavia)

11.00-11.30 **Laura Berliocchi** (Catanzaro), Autophagy in neuropathic pain

11.30-12.00 **Shiroh Kishioka** (Wakayama), Novel mediators of peripheral sensitization

12.00-12.30 **Lucia Negri** (Rome), New trends in prokinetins research

12.30-13.00 **Paola Sacerdote** (Milan) Stem cells as advanced therapy of chronic pain

13.00-13.30 General Discussion

Lunch Break

Afternoon session

16.30 Epitech Sponsored Lecture

Salvatore Cuzzocrea (Messina), "Mast cell modulation and pain: a challenging perspective on PNS and CNS"

17.15 Free poster communications

Chairpersons: Laura Berliocchi (Catanzaro), Cristina Tassorelli (Pavia)

Saturday, 15th September

Morning session

9.00-10.30 **RATIONAL BASIS FOR PAIN TREATMENT**

Chairpersons: Carlo Caltagirone (Rome), Pierluigi Nicotera (Bonn),

9.00-9.30 **Stefania Ceruti** (Milan), Nucleotide receptors in trigeminal satellite glial cells: new targets for the pharmacological control of migraine pain

9.30-10.00 **Santina Chiechio** (Catania), Metabotropic glutamate receptors as target for chronic pain control

10.00-10.30 **Tsukasa Sakurada** (Fukuoka), Natural products for pain control

10.30-11.00 Coffee Break

11.00-13.00 **ADVANCED PAIN THERAPY**

Chairpersons: Lucia Negri (Italy), Shiroh Kishioka (Japan),

11.00-11.30 **Gianfranco Spalletta** (Rome) Pain and depression

11.30-12.00 **Shinobu Sakurada** (Sendai), Involvement of H1 receptor in pain related behaviors induced by nociceptin and its metabolites in the mouse spinal cord

12.00-12.30 **Cristina Tassorelli** (Pavia), Endocannabinoids and migraineous pain

12.30-13.00 **Oliver J Dolly** (Dublin), Targeting SNARE for migraine control

13.00-13.30 General Discussion

Lunch Break

Social programme

Thursday, 13th September
Friday, 14th September

19.00 Welcome cocktail
22.30 Folk music concert

ABSTRACTS ORAL COMMUNICATIONS

(L1-L15)

TRANSLATIONAL RESEARCH IN ITCHING: RESEARCH AND DEVELOPMENT OF NALFURAFINE HYDROCHLORIDE AND ITS APPLICATION TO KIDNEY DIALYSIS PATIENTS WITH INTRACTABLE PRURITUS

Hiroshi Nagase

School of Pharmacy, Kitasato University

Morphine was known to show potent analgesic effect but also severe addiction. In 19th century, since the structure of morphine was determined, many researchers have been trying to eliminate the addiction of morphine and designed an ideal analgesics.

In 1980th, three types of opioid receptor (μ , δ , κ) were proposed and then, the receptor types were isolated by biological methods. Portoghese synthesized irreversible μ antagonist, β -FNA which showed that addiction would derive from μ receptor type. Since then, the fierce competition for development of κ agonists which were expected to be strong analgesic drugs without the addiction. In 1982, Upjohn' researcher reported the first κ selective agonist, U-50488H. Then most of the researches in the world imitated the structure. Although the synthesized Upjohn type κ agonists showed no addiction liability, these agonists showed the aversive effect like psychotomimetic effects and were dropped out in early clinical trials.

We designed a novel κ agonist (containing Tyr-Gly partial structure of endogenous opioid peptides) with the quite different structure from U-50488H on the basis of message-address concept and the accessory site theory from κ antagonist, nor-BNI . Our agonist, nalfurafine showed potent analgesic effect ($ED_{50} = 0.003$ mg/kg s.c., in mouse acetic acid writhing test) with neither addiction nor aversion and applied to the clinical trial for post-operative pain. Although nalfurafine showed potent analgesic effect, the trial was ceased because of sedative effect. When we examined another indication of nalfurafine, we happened to find the presentation for the specific itchy mice model by Kuraishi on the annual meeting of Japanese Pharmacological Society. Soon we examined the antipruritic effect of our agonist using Kuraishi' s model and found the potent effect ($ED_{50} = 0.007$ mg/kg, p.o.). Next we started the clinical trial applied for kidney dialysis patients with intractable pruritus. Fortunately, we obtained the strong antipruritic effect without any serious side effects (no addiction and aversion) in the trial.

In 2009, our κ agonist, nalfurafine hydrochloride (commercial name: Remitch capsule ®) was launched as an antipruritic agent for kidney dialysis patients with the intractable pruritus.

In post marketing surveillance study, any serious problem has not been reported and the satisfactory degree of patients has been extremely high. We will report design and synthesis of nalfurafine, and also detail clinical data.

PAIN MECHANISMS: NEW TARGETS FOR THE CONTROL OF CHRONIC PAIN STATES

Stephen P Hunt

Cell and Developmental Biology, University College London, London, WC1E6BT, UK

Pain has been divided into three categories, nociceptive pain that is brief with minimal consequences, inflammatory pain that is associated with injury and generally resolves as tissue repairs and neuropathic pain that results from damage or disease of the somatosensory nervous system. Chronic pain includes neuropathic pain and long-term inflammatory conditions such as osteoarthritis and can be difficult to control with current drug therapy.

The neurobiology of pain and nociception is complex and the relative importance of peripheral and central components of pain pathways in generating particular pain states still generates considerable debate. For example, research has suggested that pathological changes in damaged peripheral nerves underlie neuropathic pain primarily because on-going pain, one of the major characteristics of neuropathic pain, can be alleviated by administration of a local anaesthetic to the peripheral area of injury. However, there is also good evidence that the maintenance of chronic pain is the result of changes in neural networks within the spinal cord and brainstem that support persistent central sensitization of dorsal horn neurons. To resolve these different points of view will require a detailed molecular neurobiological understanding of nociceptive pathways and the changes that occur following injury.

Primary afferents fall into two broad categories: myelinated A- fibers that signal noxious or innocuous stimuli and unmyelinated C-fibers that in rat and mouse are largely nociceptors. A-nociceptors mediate 'first' pain perceived as rapid and sharp and C-fibers signal 'second' pain, delayed, diffuse and dull. Inflammation or injury provokes the release of a variety of cytokines and growth factors that increase the sensitivity of some nociceptors to noxious thermal stimulation. Nociceptors have sensory end organs in the skin, muscle, joints and viscera that selectively respond to noxious or potentially tissue-damaging stimuli. Most small fiber nociceptors sensitize as demonstrated by a reduction in their threshold and an increase in the magnitude of the response to noxious stimulation. In addition, previously ineffective stimuli may become effective and spontaneous activity may also develop. Inflammatory pain is signaled primarily through C-fibers while neuropathic pain also has an A-fiber component.

Targeting C-fibers. A critical role in this process of primary sensitization is played by the capsaicin-sensitive TRPV1 receptor that acts as a focus for many signaling pathways within the axon terminal of the majority of C-fibres. While all C-fibers (but few A-fibers) express TRPV1 receptors and release the neurotransmitter glutamate, approximately 50% of fibers also express the trkA receptor (which binds nerve growth factor, NGF) and synthesize and release neuropeptides such as calcitonin gene related peptide (CGRP) and substance P. NGF is released by inflamed and damaged tissue and blocking NGF activity at the trkA receptor is proving to be one of the most effective ways of blocking inflammatory pain in humans. A major point of controversy is whether labeled lines for particular submodalities exist within the peripheral nerve. There is good evidence for a dedicated 'itch' afferents containing gastrin releasing peptide but the evidence in favor of mechanical or thermally distinct pathways is not yet compelling.

Targeting A-fibers. Recent research suggests that targeting peripheral A-nociceptors may be effective in controlling some symptoms of neuropathic pain. The mechanical sensitivity (secondary hyperalgesia and allodynia) that originates from around the site of injury is generated by central amplification of signals generated in A-fiber nociceptors and is similar in many respects to the increased mechanical sensitivity seen in some neuropathic pain patients. More importantly, experimentally induced secondary hyperalgesia and allodynia can be replicate and studied both in animals and in healthy patients as a model for human neuropathic pain. A-nociceptors rather than C-fibres therefore represent an interesting target for therapeutic intervention and one that we have exploited. mTOR (mammalian target for rapamycin) and the related machinery for mRNA translation has been localized to the axons of this subpopulation of A-nociceptive primary afferent sensory fibers and neuropathic pain is reduced by treatment with mTOR inhibitor rapamycin. Ongoing local translation of mRNA maintains the sensitivity of a subset of A-nociceptors and offers a therapeutic target for the control of pain states. Recently, translational possibilities of this approach for control of human pain states has been brought a step nearer by the demonstration that metformin a drug widely used for the control of type II diabetes has been shown to inhibit mTOR and also to be effective in rodent models of chronic pain and itch.

Conclusions. There is evidence that a new range of drug treatments for the control of chronic pain preferentially target the peripheral nervous system. However, it should be remembered that the majority of currently available drugs effective in the control of neuropathic pain target both peripheral and central nervous system and it is unlikely that relief from all chronic pain states will ever be achieved by the peripheral route alone. Pain is a symptom of complex and variable changes in nociceptive networks with each pain condition defined by its own underlying molecular signature.

TRANSIENT RECEPTORS IN PAIN TRANSMISSION

Sabatino Maione

Department of Experimental Medicine, Division of Pharmacology L. Donatelli, Faculty of Medicine and Surgery, The Second University of Naples, Via Costantinopoli 16, 80138 Naples, Italy

Transient receptor potential vanilloid type 1 (TRPV1), a ligand-gated cation channel, is a polymodal nociceptor widely expressed within pain transmitting/modulating areas of the peripheral and central nervous system. TRPV1 is both activated and sensitized by inflammatory endogenous mediators during inflammatory pain conditions and appears to be up regulated in neuropathic pain conditions. Due to its role as pain integrator, its widespread expression in pain neuraxis and its involvement in pain development TRPV1 offers an exciting opportunity for therapeutic interventions in pain management. In particular, its supraspinal expression within the antinociceptive descending pathway, which includes periaqueductal grey (PAG) and rostral ventromedial medulla (RVM), represents an endogenous switch for extinguishing pain in pathological conditions. Moreover, together with TRPV1, TRPA1 is also expressed in neurons of the PAG matter and cooperate with several other receptors or TRP channels for modulating of descending antinociceptive/pronociceptive pathways. Endogenous lipids such as unsaturated fatty acids and their cyclooxygenase, lipoxygenase or epoxygenase related metabolites can modulate TRPV1 channel activity by direct binding or by modulating its activity throughout morphofunctional changes. Knowledge on lipidergic mediators which affect TRPV1 channel activity will provide more opportunities to exploit this channel for novel therapeutic strategies.

NEURON-GLIA INTERACTIONS IN CHRONIC PAIN

Marzia Malcangio

Chronic pain represents a major problem in clinical medicine because it causes debilitating suffering and is largely resistant to currently available analgesics. A characteristic of chronic pain is abnormal response to somatic sensory stimulation. Thus, patients suffering peripheral neuropathies may experience pain by simple touching of the skin or by changes in temperature, both stimuli which are normally non-painful (allodynia). As pain, touch and temperature activate specific sets of peripheral receptors and recruit discrete CNS pathways, the mechanism for allodynia has been the subject of intense pre-clinical and clinical studies. Under pathological conditions, the occurrence of allodynia means that innocuous stimuli can access the nociceptive system.

Convincing evidence suggests that allodynia is the result of pain remaining centralized (central sensitization) (Woolf, 2011). Thus, following peripheral nerve/tissue damage, the increased activity and/or ectopic activity in primary afferent fibres drives the pain towards the CNS. Here the gain of neurons in the pain pathways is increased and neurons begin to be activated by innocuous inputs. In recent years, it has become appreciated that in addition to neuronal plasticity, changes in microglia and astrocytes contribute to the maintenance of central sensitization, which is represented behaviourally by pain hypersensitivity including allodynia (McMahon & Malcangio, 2009). Indeed, specific and selective control of neuron-glia interactions results in attenuation of allodynia in inflammatory and neuropathic pain models. Specifically, the activation of chemokine receptor CX3CR1 receptor which is exclusively localized in microglia in the CNS results in the release of mediators which contribute to central sensitization and CX3CR1 antagonism results in anti-allodynic effect in neuropathic and inflammatory pain (Clark *et al.*, 2011; Clark *et al.*, 2012). CX3CR1 is exclusively activated by the chemokine fractalkine (FKN) which is mainly expressed by spinal cord neurons and the central terminals of primary afferent fibres. As FKN is a transmembrane protein, in order to activate the CX3CR1 receptor on microglia, the extracellular, chemokine domain of the chemokine needs to be cleaved off by a protease. In the spinal cord soluble fractalkine is liberated by the cysteine protease cathepsin S (CatS) which is expressed and released by microglial cells.

We propose that CX3CR1 receptor and cathepsin S represent microglial targets for chronic pain therapy.

References

- Clark AK, Grist J, Al-Kashi A, Perretti M, Malcangio M. Spinal cathepsin S and fractalkine contribute to chronic pain in collagen induced arthritis. *Arthritis Rheum.* 64: 2038-47, 2012
- Clark AK, Malcangio M. Microglial signalling mechanisms: Cathepsin S and Fractalkine. *Exp Neurol.* 234: 283-292, 2012
- McMahon S.B., Malcangio M. Current challenges in glia-pain biology, *Neuron*, 64: 46-54, 2009.
- Woolf, C.J. (2011). Central sensitization: Implications for the diagnosis and treatment of pain. *Pain*, 152, S2-S15.

AUTOPHAGY IN NEUROPATHIC PAIN

Laura Berliocchi^a, Rossella Russo^b, Maria Maiarù^{a,b}, Cristina Tassorelli^c, Giuseppe Nappi^c, Giacinto Bagetta^b and Maria Tiziana Corasaniti^a

^aDepartment of Health Sciences, University “Magna Graecia” of Catanzaro, 88100 Catanzaro, Italy; ^bDepartment of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy; ^cIRCCS National Neurological Institute “C. Mondino” Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia, Pavia, Italy

Autophagy, a major degradative pathway for proteins and organelles, is essential for cellular remodelling and survival. An imbalance in this process can impact basal functions leading to cellular dysfunction and this has recently been implicated in several human diseases, including neurodegeneration and cancer.

Recently, we have shown that autophagy is impaired in the spinal cord following peripheral nerve injury and suggested a potential role for this degradative pathway in neuropathic pain (Berliocchi et al., 2012). Here, we have further studied the role of autophagy in the spinal dorsal horn following peripheral nerve injury and investigated the consequences of autophagy impairment on pain behaviour.

Spinal nerve ligation (SNL) was performed on male C57BL/6 mice (20-22g). Mechanical sensitivity was assessed by the Von Frey’s behavioural test and the expression of the main autophagic markers LC3, beclin 1 and p62, was investigated by western blot analysis together with immunofluorescence and confocal microscopy in the dorsal horn of mice that underwent either SNL or sham surgery.

A slight increase in LC3-I and beclin 1 expression was observed 7 days following SNL in the L4-L5 portion of the spinal cord ipsilateral to the ligation. At the same time point, SNL promoted the appearance of LC3-II, the phosphatidylethanolamine-conjugated LC3 form indicative of increased autophagosomes formation. Also, LC3-II accumulation was paralleled by a drastic elevation of p62 levels, strongly supporting the hypothesis of a blockade in the autophagic flux. The increased expression of these autophagic markers was restricted to the spinal cord side ipsilateral to the ligation in SNL mice, correlated with the up-regulation of the calcium channel subunit $\alpha_2\delta-1$, and was not present in mice that underwent sham surgery. Analogous changes were observed also in the formalin test, a model of acute inflammatory pain characterised by a peripheral and a central component. In this model, autophagy seemed to be impaired only transiently, thus suggesting a role for these alterations in the case of persistent pain states. Also, immunofluorescence studies suggested that the changes in the expression of the autophagic markers occurred within specific cell populations in the spinal dorsal horn. Seven days after surgery, increased p62 immunoreactivity was detectable in the most superficial laminae of the spinal dorsal horn on the side of injury in SNL mice and was less evident in mice that underwent sham surgery, thus confirming previous western blot data. P62 was markedly expressed in several cell somata, but also in the neuropil. Double immunostainings with the main cellular markers, indicated the presence of p62 mainly in NeuN-positive cell bodies, occasionally in GFAP-positive processes, but not in Iba1-positive cells, thus suggesting a predominant expression in the neuronal compartment.

Our data indicate that autophagy is impaired in the spinal cord following spinal nerve ligation and suggest that this event may be playing a relevant role in pain processing.

The experimental protocols were in accordance to the guidelines of the Italian Ministry of Health for animal care (D.M. 116/1992). This work is supported by IRCCS “C. Mondino” (RC2012).

NOVEL MEDIATORS OF PERIPHERAL SENSITIZATION

Shiroh Kishioka, Norikazu Kiguchi, Yuka Kobayashi, Naoki Wakida

Department of Pharmacology, Wakayama Medical University

Neuropathic pain, which is characterized by allodynia and hyperalgesia, is caused by peripheral and central sensitization. There is growing evidence indicating that peripheral neuroinflammation associated with cytokine-chemokine network involves in the pathogenesis of neuropathic pain. We investigated the role of chemokine, such as macrophage inflammatory proteins (MIPs), in neuropathic pain.

Neuropathic pain was produced by partial sciatic nerve ligation (PSL) in mice. Symptoms of neuropathic pain, such as tactile allodynia and thermal hyperalgesia were evaluated by von Frey test and Hargreaves test, respectively. Western blotting, RT-PCR and immunohistochemical analyses were performed according to conventional methods.

The mRNA levels of MIPs (MIP-1 α , MIP-1 β , and MIP-2) and these receptors (CCR1, CCR5 and CXCR2) were up-regulated in the injured sciatic nerve (SCN). These molecules are localized on Schwann cells and recruited leukocytes (neutrophils and macrophages). Perineural injection of neutralizing antibodies for MIPs or antagonists for those receptors prevented PSL-induced tactile allodynia and thermal hyperalgesia, and suppressed the PSL-induced up-regulation of inflammatory cytokines (IL-1 β and TNF α) in the injured SCN. Moreover, perineural injection of recombinant MIPs elicited tactile allodynia and thermal hyperalgesia, dose-dependently. In regard to signal transduction related to chemokine, the activation of JAK2-STAT3 pathway by PSL produced MIP-1 α in macrophage. The MIP-1 α acted on CCR1 and CCR5, and activated MEK-ERK pathway in the injured SCN, leading to neuropathic pain. The expressions of MIP-2 and its receptor CXCR2 were regulated by epigenetic augmentation, i.e. hyper-acetylation of histone H3 on their promoter region.

PSL elicited the activation of microglia located in spinal dorsal horn, and perineural injection of neutralizing antibody for MIP-1 α after PSL inhibited the microglial activation. Intrathecal injection of neutralizing antibody for MIP-1 α prevented PSL-induced tactile allodynia and thermal hyperalgesia. Intrathecal injection of recombinant MIP-1 α elicited tactile allodynia and thermal hyperalgesia, dose-dependently.

These results suggest that MIPs derived from Schwann cells and recruited leukocytes in the injured peripheral nerves elicit peripheral sensitization, leading to neuropathic pain. Moreover, one of these key molecules (such as MIP-1 α) might also be a novel mediator of central sensitization.

NEW TRENDS IN PROKINETICIN RESEARCH

Lucia Negri

Department of Physiology and Pharmacology, Sapienza University of Rome

Bv8 (Prokineticin 2, PK2) is an inflammatory cytokine-like molecule expressed predominantly by macrophages and neutrophils infiltrating sites of tissue damage and behaves as a main pain player. It belongs to a family of small proteins identified in several species from reptiles to mammals characterized by a conserved N-terminal sequence and by the presence of five disulfide bridges. In mammals they bind two closely related (85% sequence identity) family A G-protein coupled receptors (prokineticin receptor 1 and 2, PKR1 and PKR2) localized in the brain, dorsal root ganglia (DRG) neurons, granulocytes, macrophages and endothelial cells. Most sequence variation between the PKR subtypes is concentrated in the extracellular N terminal region as well as in the second intracellular loop (ICL2) and in the C terminal tail. The receptors' endogenous ligands are relatively large proteins, which most likely bind the extracellular surface of the receptors. In different tissues, specific signalling outcomes following receptor activation is achieved by coupling to several different G proteins. Computational analysis suggest an identical TM-bundle binding site for hPKR1 and hPKR2 so that small-molecule antagonists are not likely to easily differentiate between the subtypes. Our group synthesized a triazine-guanidine derivative (named PC1) that preferentially binds PKR1 and appears very promising in controlling acute and persistent pain.

Intensive research of the prokineticin system over the past decade has revealed a dazzling array of biological activities, including angiogenesis, hematopoiesis, immune processes, inflammation and nociceptive transmission. Bv8/PK2 is overexpressed in inflamed tissues and has a crucial role in neutrophil-dependent inflammatory hypernociception. In vitro Bv8 activates macrophages to migrate and to produce proinflammatory cytokines IL-1 and IL-12. Many reports indicate the Bv8/PK as main inflammatory mediators also in humans. PKs induce human monocytes to express TNF, IL-1 and the chemokines CCL4, CXCL1, CXCL8. There are now various reports of prokineticin system dysregulation in inflammatory diseases, for example, in type II collagen-induced arthritis in mice, a model of the human rheumatoid arthritis. The Pk2 gene expression is up-regulated in biopsy samples from ulcerative colitis patients, and similar elevations were observed in rodent models of inflammatory colitis.

Considering that prokineticins and their receptors are expressed in neurons, glia cells and immune cells and that this system is involved both in nociception and in immunoregulation, Bv8/PK2 may acts as pivotal mediator of the neuroimmune interactions in neuropathic pain. In a mouse model of neuropathic pain, the chronic constriction injury of the sciatic nerve, a clear activation of the Bv8/PK system takes place in peripheral and central nervous system together with development of mechanical allodynia and thermal hyperalgesia. In CCI sciatic nerve and spinal cord Bv8/PK2 levels appear significantly increased. PKR2 levels increased both in the peripheral and the central nervous system while high PKR1 levels were only observed in CCI sciatic nerve. In CCI animals sciatic nerve and spinal cord IL-10 concentrations were decreased, while IL-1 levels were significantly increased. One-week therapeutic treatment with the Bv8-antagonist PC1 significantly reduced allodynia and thermal hyperalgesia, and restored physiological levels of the cytokines, since the antagonist was able to reduce IL-1 both in the peripheral and the central nervous system, and to increase the production of the anti-inflammatory cytokine IL-10 in the sciatic nerve. Our data demonstrate that reducing Bv8 levels in damaged tissues or antagonizing PKRs might be an innovative strategy to control persistent, invalidating pain.

STEM CELLS AS ADVANCED THERAPY OF CHRONIC PAIN

Paola Sacerdote, Alberto E. Panerai.

Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy

Neuropathic pain affects 1% of the population, it can occur secondarily to injury of the central nervous system, but most commonly peripheral nervous system. These injuries can be iatrogenic (amputation cholecystectomy etc), traumatic, due to tumors compressing peripheral nerves, drugs, metabolic (diabetes) or viral (Herpes Zoster, HIV) diseases, ischemia. Whatever the cause, neuropathic pain is unresponsive to classic analgesics and antidepressant or anticonvulsant drugs, Transcutaneous Electrical Nerve Stimulation and psychological/cognitive help are used. All these treatments relief a limited percentage of patients (30%, e.g. comparable to placebo), before pain inevitably reappears. The diversity of therapeutic approaches sharing an equal percentage of failure suggests that each of them targets only a few out of the multiple pathological changes observed during the development of the disease. In order to understand neuropathic pain, most of the attention has been directed to the central anatomical and biochemical modifications that occur in the Central Nervous System (spinal cord, CNS) following peripheral nerve lesion. We and others showed changes in several neurotransmitters and neuropeptides in the CNS but also that a pathological interaction between the neuron and non neural cells in the peripheral nerve and in the spinal cord is crucial for the development and maintenance of neuropathic pain. Following a peripheral lesion, activated Schwann cells, resident and infiltrating macrophages, activated glia cells in the DRG and glia and microglia in the spinal cord, secrete pro/anti-inflammatory cytokines in the peripheral and central nervous system and start a neuroinflammatory cascade of events that progressing toward the CNS leads to neuronal sensitization and the development and maintenance of neuropathic pain. A well-accepted hypothesis for the development of neuropathic pain is that overall the above-mentioned interactions among the various cell types lead to a non-physiological repair of the lesioned nerve, resulting in the formation of neurinomas, deranged nerve conduction and generation of spontaneous firing. Thus, stem cells appeared as the ideal tool in order to obtain a physiological repair and a new approach to therapy to restore normal physiological conditions in the damaged tissues and to limit the activation of pathological biochemical and molecular pathways. On these premises, we successfully treated peripheral nerve lesion-derived neuropathic pain, showing that the intravenous administration of murine Neural Stem Cells (NSC) exerted a curative effect on active pathology in the mouse. Moreover, NSC effects increase in entity and duration by increasing cell number and can be restored by a new dose when it decreases in time. In parallel to the anti-allodynic and anti-hyperalgesic effect, we observed a reduction of pro-inflammatory cytokines that are stimulated

following the lesion. Behavioral and biochemical effects were evident by day 3 after NSC administration, while nerve repair initiated only over 4 weeks later. Moreover, we observed that NSC massively reached the lesion site shortly after administration and disappeared in the following days, although the effects on biochemical parameters and neuropathic pain were maintained. Since we want to repair or modulate a damage of the nervous system, the use of neural stem cells should give a plus in the model of neuropathic pain. Neural stem cells should be more committed to regeneration of nervous system and/or normalize abnormal neuronal activation. On the other hand, it is evident that for human therapy, species-specific neural stem cells appear quite difficult or impossible to obtain. On the contrary mesenchymal stem cells (MSC) are easier to be obtained and isolated. In particular, the availability of MSCs from adipose tissue is high and does not need invasive procedures. Moreover the MSC anti-inflammatory properties to induce a general immunosuppression are well demonstrated and accepted. These MSC properties can be particularly useful in neuropathic pain where the immuno/inflammatory component plays an important role. We replicated NSC effects on neuropathic pain symptoms (allodynia and hyperalgesia) also using human MSC derived from adipose tissue (hASC). hASCs were able to completely reverse hyperalgesia and reduce allodynia starting 24 hours after injection. The effect began to fade 21 days after administration, but it could be restored by a new cell injection (1×10^6). In parallel we observed a recovery of cytokines balance both for pro- and anti-inflammatory ones. In particular the levels of IL-1 β and IL-6, that were significantly enhanced in CCI mice, were restored when mice are treated with cells. As far as anti-inflammatory cytokines are concerned, hASCs treatment induced a significant increase in IL-10 level respect to both sham-operated and CCI animals. In conclusion peripheral administration of stem cells of different origin and species (murine NSC and hASC) therapeutically reversed neuropathic pain symptoms in the CCI mouse model. For this reason we believe that a bidirectional interaction between stem cells and the lesioned-inflamed nerve is at the basis of the positive modulation of pain and inflammation.

NUCLEOTIDE RECEPTORS IN TRIGEMINAL SATELLITE GLIAL CELLS: NEW TARGETS FOR THE PHARMACOLOGICAL CONTROL OF MIGRAINE PAIN

Stefania Ceruti

Laboratory of Molecular and Cellular Pharmacology of Purinergic Transmission – Department of Pharmacological and Biomolecular Sciences – Università degli Studi di Milano, Milan, Italy.

According to the WHO, migraine is a rather common disorder affecting 15% of adults in the Western World. It has been included in the list of the 20 more disabling pathologies, due to the dramatic reduction of the patient's quality of life, and also to massive personal and social costs, in terms of both medical expenses and lost workdays. Despite the recent introduction to the market of new, potent and effective anti-migraine drugs (e.g., triptans), still a significant number of migraineurs is insensitive to the currently available pharmacological approaches, suggesting that there are yet-to-be identified molecular and cellular players at the basis of the disease, which are not targeted by currently utilized drugs. For example, it is now believed that the trigeminal (TG)-brainstem sensory system plays a central role in the development and maintenance of migraine pain. Currently available pharmacological approaches to migraine mostly modulate neuronal activity, but it is now clear that TG neurons act in strict synergy with non-neuronal cells, in particular "satellite" glial cells (SGCs) that envelop neuronal bodies within the ganglion, to promote and maintain migraine-associated pain. Therefore, others and we have become interested in verifying whether SGCs might represent innovative pharmacological targets to migraine pain, and we have focused our research on the purinergic system, based on the knowledge that the crosstalk between neurons and SGCs is at least partly mediated by the activation of purinergic receptors responding to endogenously released nucleotides, like ATP and UTP. We are also interested in studying the cross-talk between the purinergic system and other known pro-algogenic signals, in order to test whether the modulation of purinergic receptors is involved in the mechanism of action of known pain-killers currently utilized by patients.

To test our hypotheses, we utilized both mixed neuron-glia and purified glial cultures from mouse TG. We previously showed that the algogenic mediator bradykinin (BK) potentiates G protein-coupled purinergic P2Y-receptors on SGCs in primary trigeminal cultures (Ceruti *et al.*, *Cell Calcium* 43:576-90, 2008), through the neuronal release of the pro-algogenic mediator calcitonin gene-related peptide (CGRP; Ceruti *et al.*, *J Neurosci* 31:3638-49, 2011). Interestingly, the anti-migraine drug sumatriptan fully inhibited both CGRP release and glial P2Y-receptor potentiation, therefore suggesting a possible role for receptors activated by adenine and uracil nucleotides in the mechanism of action of this drug. Indeed, exposure to BK led to an increased production of PGE₂, which was fully inhibited by the predominant COX-1 inhibitor acetyl salicylic acid (ASA), sometimes utilized to abort migraine attacks. The latter also blocked neuronal CGRP release, thus highlighting the existence of a complex cross-talk between arachidonic acid metabolites, the CGRP system and purinergic receptors, which modulate glial and neuronal cell functions in the TG. By a pharmacological approach employing selective antagonists, we have identified the P2Y receptor subtypes that are up-regulated in TG glial cells by BK and CGRP exposure, namely the ADP-responsive P2Y₁ and the UTP-sensitive P2Y₂ subtypes. Our data also suggest that their increased activity is both due to an increased receptor protein expression, but also, mostly for the P2Y₁ subtype, to the modulation of their subcellular localization to membrane lipid rafts. Studies on the possible pro- or anti-algogenic role of these receptor subtypes are currently in progress. Interestingly, this complex molecular cross-talk between the purinergic system and known pro-algogenic substances was further potentiated in cell cultures from Ca_v2.1 α 1 R192Q mutant knock-in (KI) mice expressing a human mutation causing familial hemiplegic migraine type 1, further suggesting the possible involvement of glial purinergic receptors in the onset and maintenance of migraine-associated pain. Overall, our findings suggest, for the first time, that P2Y receptors on glial cells act might represent new targets for the development of innovative therapeutic agents against migraine pain.

The financial contribution of the Italian Comitato Telethon (project #GGP10082A) and of the Fondazione Cariplo (project #2011-0505) is gratefully acknowledged.

METABOTROPIC GLUTAMATE RECEPTORS AS TARGET FOR CHRONIC PAIN CONTROL

Santina Chiechio

Department of Drug Sciences, University of Catania, Italy

Metabotropic glutamate receptors (mGluRs) belong to the superfamily of G-protein coupled receptors. Eight subtypes of mGluRs have been identified and divided into three groups that act through different intracellular pathways. Group I (mGluR1 and mGluR5) are coupled to $G_{\alpha q}$ proteins and activate phospholipase C (PKC), whereas group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) are coupled to $G_{\alpha i}$ proteins and inhibit adenylate cyclase. In addition, mGluRs can also alter ion channel currents via $G_{\beta\gamma}$ subunits. Moreover, mGluRs have been shown to form homodimers and heterodimers at the plasma membrane. With the exception of mGluR6 that is selectively expressed in the retina, the presence of all mGluR subtypes has been demonstrated in the pain neuraxis, including peripheral nerve endings, dorsal root ganglia (DRG), dorsal horns (DH) of the spinal cord, and supraspinal sites. Activation of mGluRs can either increase or decrease cell excitability depending on the specific receptor subtype activated and the anatomical and cellular localization, differently modulating pain transmission. In particular activation of group II mGluRs have been demonstrated to induce analgesic effects in inflammatory and neuropathic pain conditions. Studies from our lab indicate that the analgesic effects mediated by the activation of group II mGluRs are most likely related to the activation of the mGluR2 subtype. Moreover, pharmacological interventions that up-regulates the expression of mGluR2 in DRG and DH, with the use L-acetylcarnitine or the so called epigenetic drugs such as the histone deacetylase (HDAC) inhibitors, SAHA and MS-275, are able to induce analgesia in chronic pain models. On the contrary, preliminary data from our lab indicate that repeated administration with the histone acetyltransferase (HAT) inhibitor, curcumin, induces a significant down-regulation of the mGlu2 receptors in the spinal cord together with a marked decrease of histone 4 (H4) acetylation level.

NATURAL PRODUCTS FOR PAIN CONTROL

Tsukasa Sakurada, Takaaki Komatsu

Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8511, Japan

Bergamot essential oil (BEO) is one of the most common essential oil containing linalool and linalyl acetate as major volatile components. This study investigated the effect of intraplantar (i.pl.) BEO or linalool on neuropathic hypersensitivity induced by partial sciatic nerve ligation (PSNL) in mice. The i.pl. injection of BEO or linalool into the ipsilateral hindpaw to PSNL reduced PSNL-induced mechanical allodynia. Peripheral (i.pl.) injection of BEO or linalool into the contralateral hindpaw did not yield anti-allodynic effects, suggesting a local anti-mechanical allodynic effect of BEO or linalool in PSNL mice. We also examined the possible involvement of spinal extracellular signal-regulated protein kinase (ERK) in BEO or linalool-induced anti-allodynia. In western blotting analysis, i.pl. injection of BEO or linalool resulted in a significant blockade of spinal ERK activation induced by PSNL. These results suggest that i.pl. injection of BEO or linalool may reduce PSNL-induced mechanical allodynia followed by decreasing spinal ERK activation. β -Caryophyllene (BCP) is also a common constituent of the essential oils of numerous spice, food plants and major component in *Cannabis*. We investigated the contribution of peripheral cannabinoid (CB) and opioid systems in the antinociception produced by i.pl. injection of BCP in mice. The i.pl. injection of BCP produced a dose-dependent antinociceptive effect in the capsaicin test. BCP-induced antinociception was prevented by subcutaneous (s.c.) and i.pl. pretreatment with AM630, a selective CB₂ receptor antagonist, but not by AM251, a selective CB₁ receptor antagonist. Pretreatment with naloxone hydrochloride, an opioid receptor antagonist, and β -funaltrexamine, a selective μ -opioid receptor antagonist, reversed the antinociceptive effect of BCP in a dose-dependent manner. Pretreatment with naloxone methiodide (s.c.), a peripherally acting antagonist for opioid receptors and i.pl. pretreatment with β -endorphin antisera, an endogenous opioid peptide, resulted in a significant antagonizing effect on BCP-induced antinociception. Our results provide evidence for the involvement of peripheral CB₂ and opioid receptors in the peripheral, local nature of the antinociception induced by i.pl. BCP. In conclusion, the present results have shown that BEO, linalool and BCP are effective after local treatment, and that their peripheral use may be of therapeutic interest in acute and chronic pain.

PAIN AND DEPRESSION

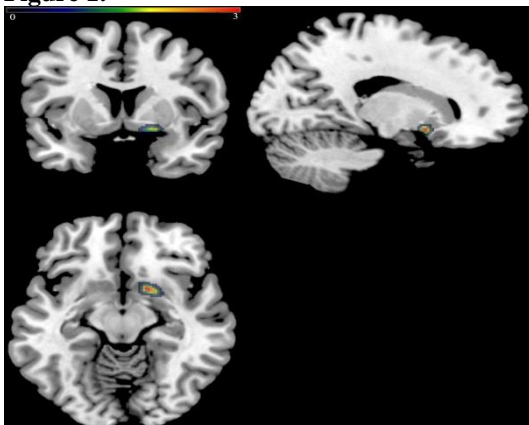
Gianfranco Spalletta

Department of Clinical and Behavioral Neurology, Neuropsychiatry Laboratory, IRCCS Santa Lucia Foundation, Via Ardeatina, 306 – 00179 Rome, Italy.

Tel: 06-51501575. Email: g.spalletta@hsantalucia.it

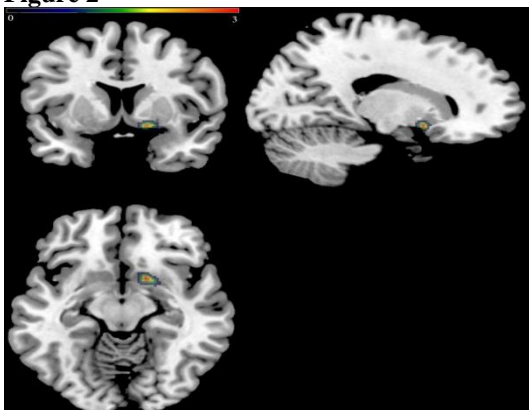
Patients with pain often suffer from depression, and the two disorders are frequently associated in patients with physical/geriatric illnesses. Depressive mood reduces pain threshold and increases pain perception. On the other hand, pain, especially chronic pain, may induce firstly demoralization and eventually a full depressive disorder. A longitudinal study in elderly subjects has recently confirmed the strict relationships between pain and depression. Indeed, in this population pain was an independent risk factor for developing depression two years later (OR=1.54; 95% CI=1.27-1.88), and vice-versa (OR=1.45; 95% CI=1.18-1.77). Here, we focused on some aspect of the affective pain in which depression is an emotional pain state caused by a dysregulation of several sub-systems modulating body or/and emotional homeostasis of the human organism. In particular, we analyzed, using the SPM8 toolbox (see Fig 1), 3D high-resolution (1x1x1 mm³) T1 weighted images acquired with a 3T Siemens Allegra MRI machinery of a sample of 34 old age patients with mild cognitive deterioration (13 with mild Alzheimer Disease and 21 with Mild Cognitive Impairment). We found that patients suffering from pain (n=19) have a statistically significant ($p < 0.005$) increased gray matter volume in the right nucleus accumbens in comparison with patients without pain (n=15).

Figure 1.



Using a Visual Analogue Scale as a continue measure of pain we also confirmed (see Figure 2) that there is a statistically significant relationship ($p < 0.005$) between pain severity and gray matter volume of the right nucleus accumbens

Figure 2



These data suggest that the macrostructure of the right nucleus accumbens may be implicated in the affective pain mechanisms in old age people with mild cognitive deterioration. A dysregulation of the hedonic tone, reward and mood, all behaviors well linked with the medial forebrain bundle pathophysiology, may be caused by an increased volume of the nucleus accumbens, thus impairing the motivated approach to pleasure and impeding to avoid pain.

INVOLVEMENT OF H₁ RECEPTOR IN PAIN RELATED BEHAVIORS INDICED BY NOCICEPTIN AND ITS METABOLITES IN THE MOUSE SPINAL CORD

Sakurada S.

Department of Physiology and Anatomy, Tohoku Pharmaceutical University, Sendai, Japan

Nociceptin/orphanin FQ (N/OFQ) is a newly discovered 17-amino acid neuropeptide encoded by the proN/OFQ gene described in mice, rats and humans. Nociceptin is metabolized by aminopeptidase N and endopeptidase 24.15. into two major *N*-terminal fragments, nociceptin(13-17) and nociceptin(14-17). Intrathecal (i.t.) injection of nociceptin or its *N*-terminal fragments at extremely low-doses elicited a pain-related behavior mainly consisting caudally directed biting and licking in mice. Nociceptin- and nociceptin(14-17)-induce responses were attenuated by i.t. co-administration of [Nphe¹]nociceptin(1-13)NH₂, a N/OFQ peptide (NOP) receptor antagonist, whereas nociceptin(13-17)-induced response was not affected by the NOP antagonist. H₁ receptor knockout (H₁R-KO) mice did not show the nociceptive response induced by nociceptin and two *N*-terminal fragments, which was observed in wild-type mice. Histidine decarboxylase knockout (HDC-KO) mice did not show the nociceptin- or nociceptin(14-17)-induced nociceptive response, whereas the nociceptin(13-17)-induced response was enhanced in HDC-KO mice. Pretreatment with a histamine antiserum or a histidine decarboxylase inhibitor resulted in a significant reduction of the response to nociceptin or nociceptin(14-17) but not nociceptin(13-17). Nociception induced by nociceptin and two *N*-terminal fragments was inhibited by co-administration of H₁ receptor antagonists. These results suggest that i.t. injected nociceptin(13-17) may act directly on spinal H₁ receptors, whereas the activation of NOP receptors by i.t. injection of nociceptin or nociceptin(14-17) may induce the disinhibition of histaminergic neurons, which subsequently enhances the release of histamine and acts on the H₁ receptor to produce the spinal cord mediated nociceptive behavior.

THE ENDOCANNBINOID SYSTEM AND MIGRAINE

*C. Tassorelli^{1,2}, R. Greco¹, A. S. Mangione¹, D. Piomelli³, G. Sandrini^{1,2}, G. Nappi^{1,2}

¹Headache Science Centre, IRCCS National Neurological Institute "C. Mondino" Foundation, Pavia, Italy;

²University of Pavia, Italy; ³Dept. of Pharmacology and Biological Chemistry, University of California, Irvine, CA 92617-4625, USA and Unit of Drug Discovery and Development, Italian Institute of Technology, Genova 16163, Italy

In collaboration with the Consortium for the Study of Adaptive Disorder and Head pain (UCADH), Pavia, Italy

The endocannabinoid system (ECS), which includes endocannabinoids and the proteins that metabolize and bind them, is involved centrally and peripherally in the processing of pain signals. Endocannabinoids inhibit, through a cannabinoid type-1 receptor (CB1R)-dependent retrograde mechanism, the release of neurotransmitters controlling nociceptive inputs and that the levels of these lipids are high in those regions (such as sensory terminals, skin, dorsal root ganglia) known to be involved in transmission and modulation of pain signals. Migraine is a neurovascular disorder that occurs with episodic headaches or, in the most severe forms, with chronic pain. Migraine has been associated to an abnormal processing of sensory information due to peripheral and/or central sensitization. Although the exact ECS-dependent mechanisms underlying migraine are not fully understood, clinical and, even more so, experimental data strongly suggest that a dysfunction of the ECS is likely involved in migraine.

In an animal model of migraine and hyperalgesia, our group has shown an increased activity of the enzymatic pathways that degrade the main endocannabinoids (anandamide and 2-arachidonoylglycerol). These changes are associated to an increase in the density of cannabinoid receptors in brain areas. It is noteworthy that purely peripheral inhibition of anandamide degradation proved capable of counteracting the hyperalgesic condition associated with our animal model of migraine, to suggest that endocannabinoids exert their effect probably at the meningeal level.

The importance of the ECS in migraine is also supported by sparse, but reliable data in humans, to suggest that modulation of this system might represent a promising therapeutical tool for the preventive treatment of migraine attacks.

Oliver J Dolly

ABSTRACT NOT ARRIVED

EFFECTS OF SELECTIVE PERIPHERAL FAAH INHIBITOR ON AN ANIMAL MODEL OF MIGRAINE PAIN: THE ROLE OF PERIPHERAL COMPONENTS

Mangione AS (1), Greco R (1), Bandiera T (3), Siani F (4), Pucci E (1,2), Nappi G (1,2), Piomelli D (3), Tassorelli C (1,2)

(1)Headache Science Centre, IRCCS National Neurological Institute “C. Mondino” Foundation , Pavia - (2)University Consortium for the Study of Adaptive Disorder and Head pain (UCADH), Pavia - (3)Medicinal Chemistry Drug Discovery and Development, Italian Institute of Technology, Genova - (4) Laboratory of Functional Neurochemistry, IRCCS National Institute of Neurology “C Mondino”

Anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) endocannabinoids, hydrolysed by fatty-acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) respectively, are promising pain modulators in migraine. Systemic administration of nitroglycerin (NTG) has been used as an animal model of migraine pain since it induces a condition of hyperalgesia in the rat through the activation of spinal and brain structures involved in nociception. We already demonstrated that the central inhibitors of FAAH and MAGL (URB597 and URB602 respectively) reduce hyperalgesia at the Tail flick and Formalin tests, suggesting that the theoretical consequent increase in spinal content of AEA and 2-AG, may modulate pain perception in a specific animal model of migraine. In this study we evaluated whether URB937, a FAAH inhibitor that does not penetrate the blood-brain barrier, may alter nociceptive responses in this model. The data show that URB937 did inhibit hyperalgesia at the Formalin test with only a minimal influence on the hyperalgesia at the Tail flick. The results suggest that availability of anandamide, probably at the meningeal level, is associated with a decrease of *migraine pain*. In this frame, URB937 could be effective in preventing pain in a specific animal model of migraine with fewer side effects.

DOES BV8/PK-SYSTEM CONTRIBUTE TO G-CSF INDUCED PAIN?

L. A. Giancotti, D. Maftai, V. Marconi, L. Negri and R. Lattanzi.

Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Italy.

Granulocyte-colony stimulating factor (G-CSF) is a current therapy to increase neutrophil counts in peripheral blood of patients that underwent chemotherapy or radiotherapy for cancer treatment. The G-CSF therapy is well tolerated, but some side effects such as abdominal pain, bone pain and muscle-skeletal pain limit its applicability. G-CSF is the major inducer of Bv8/Prokineticin2 (PK2) expression in BMMC and in circulating and tissue-infiltrating granulocytes (1). PK2 or mBv8 is a new chemokine which acting on two G-protein coupled receptors [prokineticin receptors 1 (PKR1) and prokineticin receptors 2 (PKR2)], on peripheral nociceptors and on circulating neutrophils produce pro-algesic and pro-inflammatory effects (2). We brought evidence that Bv8/PK2 is a link between polymorphonuclear cells (PMN) infiltrating the inflamed tissue and the development of inflammatory pain (3). It was already demonstrated that receptors and signaling mediators of G-CSF are functionally expressed on sensory nerves: nearly all isolectin-B4-binding and about 30% of CGRP positive peptidergic neurons in the DRG express G-CSF receptors (G-CSFR). G-CSF exposure induces significant up-regulation of the heat activated channel TRPV1 in cultured DRG cells (4).

Aim: Aim of our research is to verify whether G-CSF-induced pain is mediated by Bv8/PK2 system.

Methods and results: In 8 female patients bearing breast cancer, mastectomized, subjected to intravenous chemotherapy according to the scheme FEC 100 (5-Fluorouracil, Epirubicin, Cyclophosphamide), treated with G-CSF (Pegfilgrastim, 100 µg/Kg, s.c.) we measured PK2-mRNA levels in circulating granulocytes and PK2 serum levels. G-CSF treatment induced a significant increase in granulocyte PK2-mRNA levels (RT-PCR analysis) and a significant increase in PK2 protein levels in serum (ELISA assay). Unfortunately, the low number of patients impaired us to obtain a significant temporal correlation between Bv8/PK2 serum levels and painful state.

We evaluated G-CSF-induced pain behaviour in groups of mice pre-treated with saline or with the PKR1 antagonist, PC1, and correlated the behavioural data with granulocytes mobilization and PK2 expression levels.

In CD1 mice G-CSF (1, 5, 10 µg, s.c.) induced dose-dependent granulocytes mobilization and dose-dependent tactile allodynia (Von Frey filaments) on fore and hind paws. Allodynia was already evident one hour after G-CSF injection, peaked at five hours and recovered to baseline values eight-ten hours after injection. The subcutaneous (s.c.) injection of the PKR1 preferring antagonist, PC1 (150 µg/kg, s.c.), ten minutes before G-CSF treatment inhibited the development of tactile allodynia for 3h; when injected in correspondence of the peak abrogated tactile allodynia for 3h. Repeated administrations of G-CSF (10 µg s.c.) for 6 days also induced a significant decrease in base-line allodynic threshold since day 3 after treatment. Such a decrease in base-line pain threshold temporally correlates with the increase in Bv8/PK2 serum levels. Chronic treatment with PC1 (150 µg/kg, s.c., twice day for 6 days) abolished systemic allodynia.

In WT mice intraplantar (i.pl.) injection of G-CSF (300 ng) decreased the nociceptive threshold to thermal stimuli (plantar test) in the injected paw, but left thermal threshold of contra-lateral paw unchanged. Thermal hyperalgesia induced by G-CSF was already evident 2h after G-CSF injection, peaked at 4h, and recovered to baseline levels after 8h. PKR2-KO mice displayed similar sensitivity to G-CSF hyperalgesic effect as WT mice, whereas PKR1-KO mice were significantly less sensitive than WT mice (> 1 mcg in PKR1-KO vs 300 ng in WT mice). The PKR1 antagonist, PC1 (50 ng), administered 3h after G-CSF, antagonized G-CSF-induced thermal hyperalgesia for 4h in WT and in PKR2-KO mice, whereas it was inefficacious (up to 500 ng) in PKR1-KO mice. The TRPV1 antagonist, NF1-56 HCl (50 ng), administered 3h after G-CSF, antagonized the thermal hyperalgesia in all the three genotypes for 2 h.

Conclusions: Our data correlate G-CSF induced allodynia with activation of the Bv8/PK system, and demonstrated a positive cooperation of PKR1 in G-CSF inducing hyperalgesia to agree with literature data demonstrating that G-CSF induces thermal hyperalgesia via TRPV1 sensitization (5). We have already demonstrated that PKR1, mainly localized on peptidergic fibers, cooperates with TRPV1 (6) hence we suppose a crosstalk between G-CSFR and PKR1 via TRPV1 sensitization.

References:

- Shojaei et al., (2007). *Nature*, 450:825-831.
- Negri et al., (2007). *Life Sciences*, 81:1103-1116.
- Giannini et al., (2009). *PNAS*, 106:14646-14651.
- Schweizerhof et al., (2009). *Nature Med*, 15(7):802-7.
- Stösser et al., (2011). *J Mol Med*, 89:321-329.
- Negri et al., (2006). *J Neuroscience*, 26(25):6716-27.

PHARMACOLOGICAL CHARACTERIZATION OF NOVEL BV8-RECEPTOR ANTAGONISTS

V. Marconi¹, R. Lattanzi¹, D. Maffei¹, L.A. Giancotti¹, T. Costa³, G. Balboni², C. Ambrosio³ and L. Negri¹
¹*Department of Physiology and Pharmacology "Vittorio Ersamer", Sapienza University of Rome, Italy;* ²*Department of Toxicology, University of Cagliari, Italy;* ³*Department of Pharmacology, Istituto Superiore di Sanità, Rome, Italy.*

The small protein Bv8, isolated from amphibian skin, and the mammalian Prokineticin 1 (PK1 or EG-VEGF) and Prokineticin 2 (PK2 or mammalian-Bv8) make up a new family of chemokines characterized by the presence of five disulfide bridges and an identical amino terminal sequence, AVITGA, which is critical for the biological activity such as: circadian rhythms, neurogenesis, angiogenesis, haematopoiesis and pain sensitization (Negri *et al.*, 2007). These chemokines activate two G-protein linked PKR receptors [prokineticin receptors 1 (PKR1) and prokineticin receptors 2 (PKR2)] (Negri *et al.*, 2002) distributed in mammalian tissues: PKR1 is more widely distributed in the periphery and PKR2 is highly expressed throughout the central nervous system. In rodents, exogenous administration of Bv8/PK2 reduces the nociceptive threshold to thermal and mechanical stimuli acting on PKRs in primary sensitive nerves and in spinal cord (Negri *et al.*, 2002) and PK2 released from inflammatory granulocytes is the main mediator of inflammatory pain (Giannini *et al.*, 2009). These data demonstrate that PKRs may represent a therapeutic target for the development of novel antinociceptive drugs. A triazine derivative compound, named PC1, recently designed and synthesized demonstrated to be a PKRs antagonist. *In vitro*, binding experiments indicated that PC1 is a PKR1 preferring ligand with an affinity 30 times higher for PKR1 than for PKR2 (Balboni *et al.*, 2008). *In vivo*, PC1 selectively antagonizes Bv8-induced nociceptive sensitization. Modifications in PC1 structure, like the insertion of halogen groups, leads to more potent antihyperalgesic compounds: PC7, PC25 and PC27.

Structure-activity relationship (SAR) study have been performed *in vitro* and *in vivo*:

1) *In vitro*: we evaluated the effect of PKR antagonists on Bv8-induced activation of G-protein coupled PKRs using BRET assay (bioluminescence resonance energy transfer).

2) *In vivo*: we analyzed the ability of the new compounds to antagonize Bv8-induced thermal hyperalgesia (Paw immersion test 48°C) in PKR1-KO and PKR2-KO mice. Bv8 was injected by intraplantar (i.pl.) route at a dose of 630 fmol, a dose which decreases the nociceptive threshold to thermal stimuli only in the injected paw. The antagonists were injected by i.pl. route 5' minutes before Bv8. After drugs administration the animals were observed for three hours at established time intervals.

Results: In BRET assay PC1 displayed 20 times higher affinity for PKR1 than for PKR2, confirming the binding results. PC7 was 70 time more selective for PKR1.

PC25 displayed the higher affinity for PKR1 (~17 times higher than PC1) and the higher selectivity for PKR1 (~300 times).

PC27 showed the lower affinity for both receptors: 40 and 8 folds lower than that of PC1 for PKR1 and PKR2, respectively.

In vivo, PC1 antagonized Bv8-induced thermal hyperalgesia at a dose of 150 pmol in PKR1-KO mice and at a dose of 15 pmol in PKR2-KO mice, accordingly with its preferential affinity for PKR1, the receptor still present in PKR2- KO mice.

PC7 and PC25 antagonized Bv8-induced thermal hyperalgesia in PKR1-KO mice at doses of 15 pmol and 14 pmol, while in PKR2-KO mice at doses of 0.15 pmol and 0.04 pmol, respectively, confirming a selectivity of 100 and 300 times for PKR1.

In spite of *in vitro* low affinity for both receptors, PC27 antagonized Bv8-induced thermal hyperalgesia at a dose of 1.4 pmol in PKR1-KO mice and at a dose of 0.14 pmol in PKR2-KO mice showing an efficacy comparable with PC25. This data let us to suppose that PC27 compound might act as a pro-drug.

Conclusions: Our data demonstrate that the antihyperalgesic efficacy of PKR-antagonists correlates with the PKR1 affinity confirming the main role of PKR1 in peripheral pain perception.

References:

- Negri *et al.* (2002). *Br. J. Pharmacol.*, 137: 1147-1154.
 Negri *et al.* (2007). *Life Sciences*, 81: 1103-1116.
 Balboni *et al.* (2008). *J. Med. Chem.*, 51: 7635-7639.
 Giannini *et al.* (2009). *PNAS*, 106: 14646-14651.

ACTIVATION OF GLIAL CELLS IN THE CERVICAL SPINAL CORD AND IN THE TRIGEMINAL GANGLION FOLLOWING INDUCTION OF INFLAMMATORY PAIN

G. Magni^{1,2}, Peter T. Ohara⁴, Luc Jasmin^{3,4}, Maria P. Abbracchio^{2*}, Stefania Ceruti^{2*}.

*equally contributing

¹Dept. Drug Discovery and Development – Italian Institute of Technology (IIT) - Genova, Italy; ²Dept. Pharmacological and Biomolecular Sciences – Università degli Studi di Milano – Milano, Italy; ³Los Angeles Neurosurgical Institute, Los Angeles, CA 90211, USA;

⁴Dept. of Anatomy, University of California, San Francisco, CA 94143, USA.

It is now well known that glial cells in the central and peripheral nervous system do not merely provide nutrients and trophic support to pain-transducing neurons, but rather directly participate to the genesis and maintenance of chronic pain, through their functional cross-talk with neuronal cells.

Thus, the overall aim of the present work was to study the contribution of glial cells in the spinal-trigeminal system to the development and maintenance of inflammatory pain, and to verify whether the purinergic system might be a part of the complex and still largely unknown molecular network at the basis of the neuron-to-glia communication.

We therefore set up a sub-chronic inflammatory model in vivo, characterized by inflammatory pain and trigeminal hypersensitivity, by injecting complete Freund adjuvant (CFA) into the temporomandibular joint (TMJ) of rats. Glial cell (i.e., astrocytes and microglia in the spinal cord and satellite glial cells in the trigeminal ganglion) activation was then evaluated in the spinal-trigeminal system by immunohistochemistry.

CFA-injected animals showed ipsilateral mechanical allodynia and TMJ edema. In the ipsilateral trigeminal ganglion, a highly significant increase in the number of reactive satellite glial cells encircling neurons was also observed, paralleled by activation of resident macrophages. Seventy-two hours after CFA injection, activated microglial cells were evident in the ipsilateral trigeminal subnucleus caudalis and in the cervical dorsal horn, with a significant up-regulation of Iba1 immunoreactivity. Conversely, no signs of reactive astrogliosis were detected, indicating no role for spinal astrocytes in pain transmission, at least at these early time points. Since the purinergic system has been implicated in the activation of microglial cells during neuropathic pain, we have also evaluated the expression of the microglial-specific P2Y₁₂ receptor subtype. No upregulation was detected following induction of TMJ inflammation, suggesting that any possible role of P2Y₁₂ receptor in this specific model of inflammatory pain does not involve changes in its expression. We are now evaluating the pro- or anti-algogenic role of other P2Y receptors through their selective inhibition in vivo. Our data suggest that specific glial cell populations might represent innovative targets for controlling pain during trigeminal nerve sensitization, such as during migraine attacks.

SPINAL EXPRESSION OF AUTOPHAGIC MARKERS FOLLOWING INTRAPLANTAR FORMALIN INJECTION

Federica Cavaliere^a, Maria Maiarù^a, Damiana Scuteri^a, Rossella Russo^a, Laura Berliocchi^b, Luigi A. Morrone^a, Giacinto Bagetta^a and Maria Tiziana Corasaniti^b

^aDepartment of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy ^bDepartment of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy

Autophagy is a physiological intracellular mechanism contributing to protein and organelles degradation, cellular remodelling and survival. An imbalance on the fine-tuning of this process can impact basal functions leading to cellular dysfunction and this has recently been implicated in several human diseases, including neurodegeneration and cancer.

Recent data from our group have shown autophagy impairment in the spinal cord following spinal nerve ligation and suggested a potential role for this degradative pathway in this experimental model of neuropathic pain (Berliocchi et al, 2012).

Aim of this study was to investigate whether spinal expression of autophagic markers can be altered also in models of inflammatory pain.

Inflammatory pain was induced by a single intraplantar injection of 5% buffered formalin solution (20ul) in the left hindpaw of male C57BL/6 mice (22-25g). Pain-related behaviour was assessed by scoring licking/biting of the injected paw in 5min bins, for 50 minutes.

The expression of the main autophagic markers beclin-1, LC3 and p62 was investigated in spinal cord lysates by western blot analysis.

Formalin injection into the hind paw induced a biphasic pain response characterised by a first phase of 10 minutes in which licking/biting of the paw was high, followed by a transient decline in these behaviours and a subsequent second phase of pain response lasting about 30 minutes.

A decrease in beclin-1 and LC3 expression was observed 4 days following formalin injection in the L4-L5 portion of the spinal cord ipsilateral to the injured paw in comparison to the contralateral side.

The two distinct phases of the test may be used to address different aspects of nociception since the first phase seems to be due to direct activation of primary afferent sensory neurons, whereas the second phase is dependent on peripheral inflammation and changes in central processing. The analysis of the autophagic markers beclin-1 and LC3 revealed time dependent changes at early time points, and a decreased spinal expression 4 days after formalin injection. Our data indicate that autophagy is modulated in the spinal cord following intraplantar injection of formalin and prompt further studies for the characterization and the understating of these changes.

The experimental protocols were in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992). This work was supported by University of Calabria (ex quota 60%).

CHARACTERISATION OF AUTOPHAGIC MARKERS EXPRESSION IN THE SPINAL DORSAL HORN FOLLOWING PERIPHERAL NERVE INJURY

Maria Maiarù^a, Laura Berliocchi^b, Rossella Russo^a, Cristina Tassorelli^c, Giacinto Bagetta^a, Maria Tiziana Corasaniti^b

^aDepartment of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy; ^bDepartment of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; ^cIRCCS "National Neurological Institute C. Mondino" Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia, Pavia, Italy

Neuropathic pain is a form of chronic pain that occurs after injuries to the central or peripheral nervous system and is characterized by spontaneous pain, excessive responses to painful stimuli (hyperalgesia), and pain response to innocuous stimuli (allodynia).

Changes occurring in spinal processing of sensory information as a consequence to a peripheral lesion are important for chronic pain conditions. Recently, we have shown that autophagy is impaired in the spinal cord following spinal nerve ligation and suggested a potential role for this degradative pathway in this experimental model of neuropathic pain (Berliocchi et al., 2012).

Here, we have further characterized the expression of the main autophagic and lysosomal markers in the spinal dorsal horn in the same experimental model.

Spinal nerve ligation (SNL) was performed on male C57BL/6 mice (22-25g). The localization of the autophagic marker p62 was investigated by immunofluorescence and confocal microscopy in the dorsal horn of mice that underwent either SNL or Sham surgery. Seven days after surgery, increased p62 expression was detectable in the most superficial laminae (I-II) of the spinal dorsal horn on the side of injury in SNL mice and was less evident in mice that underwent sham surgery, thus confirming previous western blot data. P62 was markedly expressed in several cell soma, but also in the neuropile. Double immunostainings with the main cellular markers, showed the presence of p62 mainly in NeuN-positive cell bodies, occasionally in GFAP-positive processes, but not in Iba1-positive cells, thus suggesting a predominant expression in the neuronal compartment.

These data support previous data by western blot analysis, provide new information about the distribution of p62 and other autophagic markers in the dorsal horn of the spinal cord and indicate that, following peripheral nerve injury, p62 expression is increased mainly in neurons (NeuN-positive), occasionally in astrocytes (GFAP-positive), but not microglia (Iba1-positive).

Further studies are addressing the meaning of these cell-specific changes and their role in central pain processing.

The experimental protocols were in accordance to the guidelines of the Italian Ministry of Health for animal care (D.M. 116/1992). This work is supported by IRCCS "C. Mondino" (RC2012).

A BV8-RECEPTOR ANTAGONIST CONTROLS DEVELOPMENT AND RESOLUTION OF NEUROPATHIC PAIN

D. Maftai¹, R. Lattanzi¹, L.A. Giancotti¹, V. Marconi¹, E. Borsani², L. Luongo³, F. Florenzano⁴, L. Negri¹
¹ Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Rome, Italy; ² Department of Biomedical Sciences and Biotechnologies, Division of Human Anatomy, University of Brescia, Brescia, Italy; ³ Department of Experimental Medicine, Second University of Naples, Naples, Italy; ⁴ Confocal Microscopy Unit, EBRI and S. Lucia Foundation, Rome, Italy

Mammalian Bv8 (also called prokineticin-2, PK2) belongs to a new family of small proteins identified in several species from reptiles to mammals, which can lower pain threshold and modulate immune responses. It activates two closely related G-protein coupled receptors (GPCRs): prokineticin receptor 1 (PKR1) and 2 (PKR2) localized in the brain, dorsal root ganglia (DRG), neurons, granulocytes, macrophages and endothelial cells as well as in several other organs [1].

In an animal model of CFA-induced paw inflammation, we brought evidence that the granulocyte-derived Bv8/PK2 is a major determinant in triggering inflammatory pain and blocking PKRs is a winning strategy to abrogate hypernociception [2].

We synthesized a non-peptide molecule (a triazine derivative) named PC1, which acts as a PKR1 preferring antagonist selectively antagonizes Bv8-induced hyperalgesia and is highly effective in inflammatory pain treatment [3].

Although inflammatory and neuropathic pain are often considered distinct syndromes, emerging evidences suggest that proinflammatory cytokines released in the injured nerve are a necessary prelude to pain development. Since PK2 and PKRs are constitutively expressed in neurons, microglia, astrocytes and immune cells and the chronic constriction injury (CCI) model of neuropathy combines nerve compression with an epineurial inflammatory lesion, Bv8/PK2 may act as a pivotal mediator of the neuroimmune interactions in neuropathic pain.

Aim: Here we evaluated the antihyperalgesic and anti-inflammatory effects of PC1 administration in CCI model of neuropathic pain.

Methods: CCI of the sciatic nerve was induced in mice [4]. Thermal hyperalgesia (Plantar Test) and tactile allodynia (von Frey filaments) were assessed up to the 42nd day. PC1 (150 µg/kg) or saline were administered two times a day by subcutaneous route to different groups of mice: group 1 received PC1/saline from day 3 (when thermal hyperalgesia peaked) to day 6; group 2 from day 17 (when allodynia peaked) to day 20 after CCI; group 3 was sacrificed nine days after surgery and immunohistochemical studies on spinal cord, sciatic nerve and plantar skin were done.

Results: In our hands all mice developed thermal hyperalgesia from day 3 and tactile allodynia from day 17 after surgery, on the lesion side, while sham-operated mice did not. Acute administration of PC1 abolished CCI-induced thermal hyperalgesia and tactile allodynia for ≈ 2h. In group 1 repeated administrations of PC1 significantly reduced the development of thermal hyperalgesia and prevented development of tactile allodynia. In group 2, PC1 repeated administrations significantly reduced both thermal hyperalgesia and tactile allodynia. Thermal hyperalgesia and mechanical allodynia persisted in saline-treated group of CCI mice for 42 days. Immunohistochemistry showed lower PK2 expression in neutrophils infiltrating sciatic nerve and lower microglia activation in spinal cord in PC1 treated mice compared to saline treated mice. CCI of the sciatic nerve caused partial denervation and significant epidermal thinning in the injured paw. In PC1 treated animals the epidermal thinning was significantly reduced compared to saline treated mice.

Conclusion: In conclusion our data demonstrate that blocking Bv8/PK system might be a successful strategy in preventing / reducing neuropathic pain.

References:

- Negri et al. (2007). *Life Sciences*, 81:1103–1116.
- Giannini et al., (2009). *PNAS*, 106:14646-14651.
- Balboni et al. (2008). *J Med Chem.* 51, 7635-7639.
- Bennett et al., (1988). *Pain*, 33:87.

CHARACTERIZATION OF ALPHA2-DELTA1 EXPRESSION AND RESPONSE TO CHRONIC TREATMENT WITH GABAPENTIN IN A MOUSE MODEL OF NEUROPATHIC PAIN

Damiana Scuteri^{a#}, Michelangelo Certo^{a#}, Laura Berliocchi^b, Luigi A. Morrone^a, Cristina Tassorelli^c, Giacinto Bagetta^a, Maria Tiziana Corasaniti^b

^aDepartment of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy; ^bDepartment of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; ^cIRCCS "National Neurological Institute C. Mondino" Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia, Pavia, Italy

Neuropathic pain is a form of chronic pain consequent to an injury delivered to single or multiple nerves in the Peripheral Nervous System (PNS) or Central Nervous System (CNS). Almost 20% of the European population is affected by chronic or intermittent pain and this causes a reduction of the patient's life quality. Gabapentin is a widely used drug in the treatment of neuropathic pain likely acting via the voltage-dependent calcium channel subunit alpha2-delta1. It has been shown that the anti-allodynic effect of gabapentin depends on the expression of this target molecule and that parameters like circadian oscillation can affect $\alpha 2\delta$ -1 expression and consequently gabapentin's efficacy. Aim of this study was to verify the anti-allodynic effect of a chronic treatment with gabapentin and to correlate it with $\alpha 2\delta$ -1 expression in an experimental model of neuropathic pain. Furthermore, $\alpha 2\delta$ -1 expression was characterized in mice of different age.

The study was conducted using male mice (22-25g) that underwent Spinal Nerve Ligation (SNL), according to the model described by Kim & Chung (1992). Both sham (n=2) and SNL animals (n=8) were subjected to behavioural tests to assess their mechanical (Von Frey's test) and thermal (Hargreaves' test) sensitivity. Western blot experiments were conducted on sham and SNL animals to assess the expression of alpha2-delta1 subunit of voltage gated Ca^{2+} channels in C57Bl/6 mice of different age (2-6-11 months).

In SNL, but not sham animals, severe mechanical allodynia is fully developed at day 1-3 after ligation and peaks at day seven after surgery, lasting for up to thirty days. Western blotting results demonstrate that behavioural effects are paralleled by $\alpha 2\delta$ -1 overexpression in the spinal cord sections ipsilateral to the nerve injury and this is detectable on day 3 and 7 after surgery; the latter observation is conserved in SNL animals of 6 and 11 months of age. No sensitization to thermal sensitivity develops in SNL animals. Under these experimental conditions, administration of gabapentin (100 mg/Kg given i.p. once daily 1 h before behavioural tests), starting from day seven after surgery, minimizes allodynia assessed for up to two weeks. By contrast, this treatment schedule does not affect allodynia measured in later phases of neuropathic pain.

In conclusion, our data provide further evidence to the rational use of gabapentin in the early treatment of neuropathic pain in view of its action on $\alpha 2\delta$ -1 subunit, which is overexpressed in the initial stages of neuropathic state development. Also, these results open new horizons to further investigations on the role of $\alpha 2\delta$ -1 subunit in the mechanisms underlying neuropathic pain development in aged animals.

The experimental protocols were in accordance to the guidelines of the Italian Ministry of Health for animal care (D.M. 116/1992). This work was supported by the Italian Ministry of Health (Ricerca Finalizzata 2005, ex 56/05/15).

GENDER DIFFERENCES IN PAIN RESPONSE TO PERIPHERAL INJURY IN MICE.

Giuseppe Varano^a, Maria Maiarù^b, Damiana Scuteri^b, Laura Berliocchi^a, Luigi A. Morrone^b, Cristina Tassorelli^c, Giorgio Sandrini^c, Maria Tiziana Corasaniti^a

^aDepartment of Health Sciences, University “Magna Græcia” of Catanzaro, 88100 Catanzaro, Italy; ^bDepartment of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy; ^cIRCCS “National Neurological Institute C. Mondino” Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia, Pavia, Italy.

Gender differences exist in both pain thresholds and response to pain and pain medications. Also, chronic pain conditions are more frequent in women than in men, thus suggesting that might be a role for sex hormones in pain perception.

In this study, we have assessed mechanical and thermal sensitivity in male and female C57BL/6 mice (22-25g) by Von Frey’s and Haregreaves’ tests, respectively. Nociceptive responses were measured in males and females at baseline and following spinal nerve ligation (SNL) according to Kim and Chung, a widely used experimental model of neuropathic pain.

No significant differences in pain thresholds to mechanical and thermal stimulation were observed between sexes. Spinal nerve ligation induced a robust and long-lasting mechanical hypersensitivity starting from the third day post surgery. The extent and time course of development of increased mechanosensitivity was identical between males and females. However, the recovery phase that in males started around 28 days after surgery was delayed to about 52 days in females. The longer lasting pain response after peripheral injury observed in females seems to confirm the participation of sex-specific mechanisms to pain processing. No significant differences in thermal sensitivity were observed between males and females. Pain behaviour to both mechanical and thermal stimulation was monitored for up to 112 days.

This study characterise a valuable experimental model in which cellular and molecular mechanisms underlying gender differences in pain processing and perception, and in response to pharmacological treatments, can be investigated.

The experimental protocols were in accordance to the guidelines of the Italian Ministry of Health for animal care (D.M. 116/1992). This work is supported by IRCCS “C. Mondino” (RC2012).

ANTINOCICEPTIVE PROPERTIES OF BEO IN EXPERIMENTAL MODELS OF PAIN.

Antonella Ciociaro^{a,b}, Rossella Russo^{a,b}, Annagrazia Adornetto^{a,b}, Diana Amantea^{a,b}, Laura Berliocchi^c, Rocco Gentile^a, Luigi Antonio Morrone^{a,b}, Laura Rombolà^{a,b}, Maria Tiziana Corasaniti^c, Giacinto Bagetta^{a,b}

^aDepartment of Pharmacobiology and ^bUniversity Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, 87036 Arcavacata di Rende, Italy; ^cDepartment of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy.

Several essential oils and natural substances have important anti-inflammatory and analgesic properties. Among these, the essential oil of Bergamot (BEO, from *Citrus Bergamia*, Risso) has been shown from our previous works to interfere with synaptic mechanisms such as neurotransmitter release [1] and to be neuroprotective both *in vitro* [2] and *in vivo* [3].

Aim of this study was to investigate the effects of BEO on nociceptive behaviour in experimental models of pain.

We used the spinal nerve ligation (SNL) model (Kim & Chung, 1992) and the formalin test as models of neuropathic and inflammatory pain, respectively. Male C57BL/6 mice, that had undergone SNL, were administered BEO (1ml/Kg; s.c.) in a single daily injection, 1 hour before surgery and then once daily for 14 days. The Von Frey's and Haregreaves' test were then used to assess mechanical and thermal sensitivity up to 28 days after SNL.

In the formalin test, mice received a subcutaneous BEO injection 15 minutes before the subcutaneous administration of formalin (s.c., 5%, 20 µl) either in the hind paw or on the scruff of the neck. Licking/biting behaviour was then monitored at intervals of 5 min for the following 60min.

In the formalin test, BEO modified either one or both phases of the licking/biting behaviour test depending on the dose and on the way of administration used. In particular, BEO administered intraplantarly significantly reduced the first phase of licking/biting behaviour with no effect on the second phase. Instead, the same dose of BEO administered subcutaneously in the scruff of the neck reduced both the first and the second phase of this inflammatory pain model. The subcutaneous administration of a lower dose in the scruff of the neck showed anti-nociceptive effect on the second but not on the first phase of the test.

Following SNL, a robust mechanical allodynia developed and lasted over weeks. A daily dose of BEO (1 ml/kg s.c.) administered daily for 7 days attenuated mechanical allodynia compared to SNL vehicle-treated animals. Altogether, our data suggest that BEO is able to interfere with pain sensitivity possibly acting via two different mechanisms (peripheral and central) and may be a useful adjuvant drug for pain treatment [4]. However, BEO toxic profile on cell survival and proliferation suggest a cautionary approach to the use of inappropriate dilutions of the oil [5].

The experimental protocols were in accordance to the guidelines of the Italian Ministry of Health for animal care (D.M. 116/1992). Financial support from the University of Calabria (Ex quota 60%) is gratefully acknowledged.

References

1. Morrone, L.A. et al *Pharmacol Res* 2007, 55, 255-262
2. Corasaniti, M.T. et al *Brit J Pharmacol* 2007, 151, 518-529
3. Amantea, D. et al *Int Rev Neurobiol* 2009, 85, 389-405
4. Bagetta, G. et al *Fitoterapia* 2010, 81, 453-461
5. Berliocchi, L. et al *Food Chem Toxicol* 2011, 49, 2780-2792

INDEX OF AUTHORS

Abbracchio P. Maria	P4
Adornetto Annagrazia	P10
Amantea Diana	P10
Ambrosio C.	P3
Bagetta Giacinto	L5, P5, P6, P8, P10
Balboni G.	P3
Bandiera T.	P1
Berliocchi Laura	L5, P5, P6, P8, P9, P10
Borsani E.	P7
Cavaliere Federica	L5, P5
Certo Michelangelo	P8
Ceruti Stefania	L9, P4
Chiechio Santina	L10
Ciociaro Antonella	P10
Corasaniti Maria Tiziana	L5, P5, P6, P8, P9, P10
Costa T.	P3
Dolly J. Oliver	L15
Florenzano F.	P7
Gentile Rocco	P10
Giancotti L. A.	P2, P3, P7
Greco R.	L14, P1
Hunt P Stephen	L2
Jasmin Luc	P4
Kiguchi Norikazu	L6
Kishioka Shiroh	L6
Kobayashi Yuka	L6
Komatsu Takaaki	L11
Lattanzi R.	P2, P3, P7
Luongo L.	P7
Maftei D.	P2, P3, P7
Magni G.	P4
Maiarù Maria	L5, P5, P6, P9
Maione Sabatino	L3
Malcangio Marzia	L4
Mangione A. S.	L14, P1
Marconi V.	P2, P3, P7
Morrone Antonio Luigi	L5, P5, P8, P9, P10
Nagase Hiroshi	L1
Nappi G.	L14, P1
Negri Lucia	P2, P3, P7
Ohara T. Peter	P4
Panerai E. Alberto	L8
Piomelli D.	L14, P1
Pucci E.	P1
Rombolà Laura	P10
Russo Rossella	L5, P5, P6, P10
Sacerdote Paola	L8
Sakurada S.	L13
Sakurada Tsukasa	L11
Sandrini G.	L14, P9
Scuteri Damiana	L5, P5, P8, P9
Siani F.	P1
Spalletta Gianfranco	L12
Tassorelli Cristina	L14, P1, P6, P8, P9
Varano Giuseppe	P9
Wakida Naoki	L6

