MOLECULAR TARGETS FOR NOVEL PAIN THERAPEUTICS
From basic research to clinical translation

Porto Pirgos Hotel (www.portopirgos.com)
Parghelia (VV), Calabria, Italy
22nd-24th September, 2010

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FINAL PROGRAMME AND ABSTRACT BOOK
(Eds. Laura Berliocchi, Hirocazu Mizoguchi and Luigi Antonio Morrone)

A COLLABORATIVE SCIENTIFIC INITIATIVE OF

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MEETING VENUE

Porto Pirgos Hotel (tel. 39 0963 600 351) 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.portopirgos.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 oral or poster communications to be held in the afternoon session of the 23rd. All abstracts will be for oral or poster presentation and should be written according to the instructions given below. Deadline for submission: 15th July 2010. 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, 2010. 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 dismounted 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 345 US$ must be payed 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 "MOLECULAR TARGETS FOR NOVEL PAIN THERAPEUTICS", 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).

FELLOWSHIPS AND FINANCIAL ASSISTANCE
In this final programme and abstract book the asterisk indicates the PhDs and Post-Docs whose participation in the meeting has been partially supported by a grant from the International Society of Neurochemistry.

SCIENTIFIC PROGRAMME

Wednesday, 22nd September

18.00 Welcome address

18.15 Invited Opening Lecture

Chairperson: ***Oliver J. Dolly*** (Dublin)

Invited Speaker: *Shinobu Sakurada (Sendai)*

Nociceptin-mediated nociceptive spinal transmission system

Thursday, 23rd September

Morning session

9.00-11.00 **MECHANISMS OF PAIN TRANSMISSION**
Chairpersons: ***Flaminia Pavone*** *(Rome) &* ***Shinobu Sakurada*** *(Sendai)*

9.00-09.30 *John N. Wood (London)*
From transduction to sensation – genes, cells and circuits

9.30-10.00 *Takaaki Komatsu (Fukuoka)*
Intrathecal morphine-3-glucuronide induces nociception through δ_2-opioid receptors in the spinal cord

10.00-10.30 *Lucia Negri (Rome)*
Bv8/Prokineticins and thei receptors: A new pronociceptive system

10.30-11.00 *Kazuhiko Yanai (Sendai)*
Roles of histamine in pain perception: Studies using histamine-related genes knockout mice

11.00-11.30 ***Coffee Break***

11.30-14.00 **PAIN CONTROL AND SENSITIZATION PROCESS**
Chairpersons: ***Pierluigi Nicotera*** *(Bonn) &* ***AkihikoYonezawa*** *(Sendai)*

11.30-12.00 *Steve P. Hunt (London)*
Descending control and chronic pain

12.00-12.30 *Sabatino Maione (Naples)*
Forebrain TRPV1 receptor and pain mechanisms: role of glutamate and endovanilloids/endocannabinoids in microglial caspase signalling and neural activity in the prefrontal cortex of neuropathic mice

12.30-13.00 *Laura Berliocchi (Catanzaro)*
Differential phenotype features of neuropathic pain in mice bearing a dystonic genotype

13.00-13.30 *Giandomenico Iannetti (London)*
Functional neuroimaging studies of central sensitization

13.30 **General Discussion**

Lunch Break

Afternoon session

Free oral and poster communications

Thursday, 23rd September

Monothematic Meeting
Sponsored by the Italian Society of Pharmacology

CELLULAR AND MOLECULAR ASPECTS OF PHARMACOLOGIC CONTROL OF PAIN

Invited Opening Lecture

Chairperson: Giacinto Bagetta (Cosenza)

15.45-16.30 Silvana Gaetani (Rome)

Endocannabinoids, synaptic plasticity and pain control

Chairpersons: Laura Berliocchi (Catanzaro) & Takehiko Maeda (Wakayama)

16.30-17.30 Free Oral Communications (O1-O5)

17.30-17.45 General discussion

17.45-18.00 Coffee Break

Chairpersons: Hirokazu Mizoguchi (Sendai) & Luigi A. Morrone (Cosenza)

18.00-19.00 Free Oral Communications (O6-O10)

19.00-19.15 General discussion

19.15-20.30 Poster communications (P1-P13)

Friday, 24th September

Morning session

8.30-14.00 **INFLAMMATORY MECHANISMS AND PAIN**
Chairpersons: ***Tatsuro Kohno*** *(Niigata) &* ***Flavio Moroni*** *(Florence)*

8.30-9.00 *Marzia Malcangio (London*)
Glial cells: new functions in chronic pain

9.00-9.30 *Tatsuro Kohno (Niigata)*
Role of bradykinin and antagonistic action of NSAID zaltoprofen on bradykinin-mediated enhancement of AMPA receptor activity in spinal dorsal horn neurons

9.30-10.00 *Shiroh Kishioka (Wakayama)*
Leptin and neuropathic pain

10.00-10.30 *Cristina Tassorelli (Pavia)*
The endocannabinoid system and migraine

10.30-11.00 *Norikazu Kiguchi (Wakayama)*
Role of macrophage inflammatory protein-1 in the pathogenesis of neuropathic pain

11.00-11.30 ***Coffee Break***

11.30-14.00 **NOVEL THERAPEUTIC APPROACHES**
Chairpersons: ***Norman G. Bowery*** *(Birmingham) &* ***Mitsuo Tanabe*** *(Tokyo)*

11.30-12.00 *Paola Sacerdote (Milan)*
Recent advances in the molecular pharmacology of opioids are driving to a better pain therapy

12.00-12.30 *Mitsuo Tanabe (Tokyo)*
Analgesic effects of gabapentin in neuropathic states and the descending noradrenergic system

12.30-13.00 *Hirokazu Mizoguchi (Sendai)*
A peptidic analgesic for treatment to morphine-resistant intractable pain

13.00-13.30 *Hiroshi Nagase (Tokyo)*
Design and synthesis of novel delta opioid agonists and their pharmacologies

13.30-14.00 *Oliver J Dolly (Dublin)*
Novel SNARE-inactivating biotherapeutics with anti-nociceptive potential

14.00 **General Discussion**

Lunch Break

Friday, 24th September

Afternoon session

17.30-20.00 **UCADH ROUND TABLE ON PAIN: FROM BASIC RESEARCH TO REHABILITATION**

Chairpersons: ***Giuseppe Nappi*** (*Pavia*) & ***Giacinto Bagetta*** (*Cosenza*)

17.30-17.45 ***Introduction by*** *Giuseppe Nappi (Pavia)*

17.45-18.00 *Laura Berliocchi (Catanzaro)*
Experimental models of neuropathic pain

18.00-18.15 *Cristina Tassorelli (Pavia)*
Experimental models of headache

18.15-18.30 *Armando Genazzani (Novara)*
Pharmacogenetics of migraine

18.30-18.45 *Armando Perrotta (Pozzilli, IS)*
Neurophysiology of headache

18.45-19.00 *Giovanni Nicotera (Cosenza)*
Pain clinic and networking in Calabria

19.00-19.15 *Giorgio Sandrini (Pavia)*
Pain and rehabilitation

19.15-19.30 *Maria Gabriella Buzzi (Rome)*
Aging brain and pain

19.30-20.00 ***General Discussion Chaired by Fabio Antonaci (Pavia)***

Saturday, 25th September

Closing remarks and Departure

Social programme

Wednesday, 22th September	19.00 Welcome cocktail
Thursday, 23th September	22.00 Folk music concert
Friday, 24th September	20.30 Farewell Dinner

ABSTRACTS
MAIN LECTURES
(L1-L19)

Nociceptin-mediated nociceptive spinal transmission system

S. Sakurada

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

Nociceptin/orphan a 17-amino-acid peptide has been identified as the endogenous ligand for opioid receptor like-1 (ORL-1) receptor. Intrathecal (i.t.) injection of nociceptin elicited a behavioral response mainly consisting of biting and licking, which were eliminated by the i.t. co-administration of opioid receptor-like-1 (ORL-1) receptor antagonists. The behavioral response induced by nociceptin was characteristically similar to that by i.t.-administered histamine, and was attenuated by i.t. co-administration of the H_1 receptor antagonists, but not by the H_2 receptor antagonists, whereas the H_3 receptor antagonist promoted the nociceptin-induced behavior. H_1 receptor knockout (H_1R-KO) mice did not show the nociceptin-induced nociceptive behavior, which was observed in wild-type mice. Pretreatment with a histamine antiserum or a histidine decarboxylase inhibitor resulted in a significant reduction of the response to nociceptin. The previous studies showed that NK_1 receptor antagonists and a novel substance P (SP)-specific antagonist given i.t. could reduce the behavioral response to nociceptin and histamine. On the other hand, the nociceptive response induced by nociceptin, but not histamine, was completely attenuated by the i.t. co-administration of agonists for $GABA_A$ and $GABA_B$ receptors. In contrast, the antagonists for $GABA_A$ and $GABA_B$ receptors injected i.t. showed same nociceptive response with nociceptin and histamine, and their nociceptive responses were significantly blocked by the i.t. co-administration of the H_1 receptor antagonists, but not H_2 receptor antagonists or ORL-1 receptor antagonists. The present results suggest that the activation of the ORL-1 receptor by nociceptin may induce the disinhibition of histaminergic neuron and enhance the release of histamine, which subsequently acts on the H_1 receptor located on the SP-containing neurons to produce the spinal cord-mediated nociceptive response.

From transduction to sensation - genes, cells and circuits

J. N. Wood FRS,

University College London, Gower Street London WC1 E 6BT, UK; J.wood@ucl.ac.uk

Whilst the central representation of pain studied through functional imaging remains uncertain, the essential role of peripheral sensory neurons in initiating pain is well established. The pivotal studies of Ed Perl's lab established the existence of modality-specific sets of sensory neurons that transduce noxious stimuli and signal to neurons in the dorsal horn of the spinal cord. How particular pain sensations arise from peripheral input into the central nervous system is now amenable to study using mouse genetics coupled with behavioural assays. Tissue-specific promoters driving Cre recombinase allow the manipulation of gene expression in sets of sensory neurons. A number of approaches can then be used to examine the functions of these cells in pain pathways. Cells can be deleted through inducing the expression of diphtheria toxin or by deleting growth factor receptors (e.g. c-ret). In addition, cells can be rendered silent by the deletion of glutamate uptake transporters or compromised in their function by deleting voltage-gated channels. Remarkably, there is a close association between cells that express a voltage-gated sodium channel Nav1.8, and the response to intense noxious mechanical pressure, cold pain and the establishment of inflammatory pain. Nav1.7 expressed in Nav1.8 negative neurons seems to play a critical role in acute pain based on studies using Nav1.7 selective inhibitors and Nav1.7 gene deletion in subsets of DRG neurons. 'Early' ret neurons seem to play an important role in mechanosensation and other sets of functionally distinct cells have been described by a number of groups. In this presentation we will discuss aspects of peripheral wiring and approaches to treating chronic pain through manipulating sodium channel expression and function.

Abrahamsen B, Zhao J, Asante CO, Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN.The cell and molecular basis of mechanical, cold, and inflammatory pain. Science. 2008 Aug 1;321(5889):702-5.

Cavanaugh DJ, Lee H, Lo L, Shields SD, Zylka MJ, Basbaum AI, Anderson DJ. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli Proc Natl Acad Sci U S A. 2009 Jul 7;106(27):11424.

Golden JP, Hoshi M, Nassar MA, Enomoto H, Wood JN, Milbrandt J, Gereau RW 4th, Johnson EM Jr, Jain S. RET signaling is required for survival and normal function of nonpeptidergic nociceptors. J Neurosci. 2010 Mar 17;30(11):3983-94.

Luo W, Enomoto H, Rice FL, Milbrandt J, Ginty DD. Molecular identification of rapidly adapting mechanoreceptors and their developmental dependence on ret signaling. Neuron. 2009 Dec 24;64(6):841-56.

L 3

Intrathecal morphine-3-glucuronide induces nociception through δ_2-opioid receptors in the spinal cord

T. Komatsu[1], S. Sakurada[2] and T. Sakurada[1]

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Morphine-3-glucuronide (M3G), a main metabolite of morphine, has been proposed as a responsible factor when patients are presented with the neuroexciatory side effects (nociceptive behavioral responses such as hyperalgesia and allodynia) observed following systemic administration of large doses of morphine. Indeed, both M3G (3nmol/5μl) and high-dose morphine (60nmol/5μl) elicit a serve hindlimb scratching followed by biting and licking when administered intrathecally (i.t.) into mice.
Our previous research has demonstrated that both M3G and high-dose morphine may stimulate a simultaneous release of substance P and glutamate from primary afferent terminals, which induces secondary activation of the spinal extracellular signal-regulated kinase (ERK) signaling through the nitric oxide (NO)- cGMP-PKG pathway.
Elevation of spinal dynorphin content has been observed during the continuous spinal infusion of morphine. Although dynorphin has a relatively high affinity for the κ-opioid receptor and may act as an endogenous antinociceptive peptide, high doses of i.t. dynorphin have previously been reported to produce nociceptive behaviors. Here, we investigated the possible involvement of spinal dynorphin in M3G-induced behavioral response consisting of scratching, biting and licking (SBL). Pretreatment with antisera against dynorphin inhibited i.t. M3G-induced behavioral response and ERK activation. Dynorphin is rapidly degraded into [Leu^5]enkephalin by dynorphin-converting enzymes (cystein protease). The behavioral response and ERK activation evoked by i.t. M3G were significantly suppressed by i.t. injection of *p*-hydroxymecuribenzoate, an inhibitor of cystein proteases. Furthermore, M3G-induced behavior and ERK activation was inhibited dose-dependently by i.t. co-administration of the non-selective δ–opioid receptor antagonist naltridole or the selective δ_2-opioid receptor antagonist naltriben, while the selective δ_1-opioid receptor antagonist BNTX was without effect. We further showed that [Leu^5]enkephalin co-administered with [Leu^5]enkephalin-converting enzyme inhibitors, phosphoramidon, (an endopeptidase 24.11 inhibitor) and bestatin, (a general aminopeptidase inhibitor), produced both a series of SBL behavior and ERK activation. [Leu^5]enkephalin-induced behavior and ERK activation were blocked by NMDA receptor antagonists, nitric oxide synthase inhibitors, the non-selective δ–opioid receptor antagonist and the selective δ_2-opioid receptor antagonist. These results suggest that M3G–induced SBL behaviors may be triggered through the δ_2-opioid receptor activated by [Leu^5]enkephalin which is formed from dynorphin in the spinal cord.

Bv8/Prokineticins and their receptors: A new pronociceptive system

L. Negri and R. Lattanzi

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The accumulation of neutrophils in damaged tissues is commonly associated with hyperalgesia resulting from the neutrophil release of proinflammatory pronociceptive factors including products of arachidonic acid metabolism and several cytokines. Bv8/Prokineticin 2 (PK2) is a new chemokine, characterized by a unique structural motif comprising five disulfide bonds, and expressed in lymphoid organs, myeloid cells and in inflamed tissues. Homologs of the mammalian chemokine Bv8/PK2 have been previously identified in the skin secretion of the frog *Bombina ariegate* (Bv8) and in the venom of the snake black mamba (MIT-1). Bv8/PK2 activates two G protein-linked receptors (PKR1 and PKR2) localized in regions of the nervous system associated with pain, but also present on neutrophils and macrophages and on endothelial cells. In rodents, administration of the amphibian homologue, Bv8, lowers pain threshold to a broad spectrum of physical and chemical stimuli. Primary sensitive neurons expressing PKRs also express the vanilloid receptor TRPV1, providing an anatomical basis for PKR1/TRPV1 co-operative interaction in nociceptor sensitization. Activation of PKRs on neutrophils and macrophages promotes chemiotaxis and cytokine release and activation of PKRs on capillary endothelial cells stimulates angiogenesis. In animal models of inflammation, development and duration of pain temporally correlate with the expression levels of Bv8/PK2 in the inflamed paw skin. Such an increase in PK2 mRNA depends on a marked up-regulation of PK2, in neutrophils and macrophages infiltrating the tissue. Inflammation-induced PK2 over-expression is significantly reduced in RelB-KO mice. Mice lacking the PKR1 develop significantly lower inflammation-induced hyperalgesia and PK2 up-regulation demonstrating a role of PKR1 in settling PK2 levels during inflammation. Blocking PKRs with a non-peptide PKR antagonist (named PC1) dose-dependently reduces or abolishes the inflammation-induced hyperalgesia and accelerates recovery of normal paw volume after the injury. In animal models of neuropathic pain (CCI), acute subcutaneous administration of PC1 (20 mcg/kg, s.c.) abolishes thermal hyperalgesia and tactile allodynia. Repeated s.c. administration of PC1 from day 3 to day 7 after CCI prevents development of allodynia. The above data demonstrate that the prokineticin system is a new pharmacological target to control pain.

This work was supported by grants from the Italian Ministry of University and Scientific Research (PRIN 2004057339, PRIN 2004037781) and from the University "Sapienza" of Rome.

L 5

Roles of histamine in pain perception: Studies using histamine-related genes knockout mice

K. Yanai and S. Sakurada

Department of Pharmacology, Tohoku Graduate University School of Medicine and Department of Physiology and Anatomy, Tohoku Pharmaceutical University, Sendai, Japan

Histamine is known to be involved both in peripheral and central mechanisms. When tissues are injured, stimulated mast cells release histamine resulting in local vasodilation, plasma exudation, as well as depolarization of sensory nerve endings. These peripheral neurological events trigger subsequent processes of pain or itch including release of various neuropeptides from primary afferent fibers, post-synaptic excitation of secondary neurons in the spinal dorsal horn, and signal transduction into the central nervous system. Participation of histamine as a neurotransmitter in central seonsory modulation is also proposed. Histamine releasing neurons are found exclusively in the tuberomammillary nucleus of the posterior hypothalamus. Efferent fibers raised from that region reach diverse areas from the olfactory bulb to the spinal cord that is thought to be important in various neuronal functions.

To study the participation of histamine in pain perception, H1, H2 receptor and histidine decarboxylase (HDC) knockout (KO) mice were examined for pain threshold by means of three kinds of nociceptive tasks. These included assays for thermal, mechanical, and chemical nociception. H1KO mice showed significantly fewer nociceptive responses to the hot-plate, tail-flick, tail-pressure, paw-withdrawal, formalin, capsaicin, and abdominal constriction tests. Sensitivity to noxious stimuli in H1KO mice was significantly decreased when compared to wild-type mice. The antinociceptive phenotypes of H2KO and HDC-KO were relatively less prominent when compared to H1KO mice. We also examined the antinociceptive effects of intrathecally-, intracerebroventricularly-, and subcutaneously-administered morphine in H1KO and H2KO mice. In these nociceptive assays, the antinociceptive effects produced by morphine were more enhanced in both H1KO and H2KO mice.

These results suggest that histamine plays a stimulatory role in nociception and an inhibitory role on morphine-induced antinociception in the spinal and supra-spinal levels. Although the role of the histamine system in pain perception is not simple, the histamine-related genes knockout mice will enable greater understanding of these issues.

Descending control and chronic pain

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Injury results in a rapid sequence of events in the dorsal horn of the spinal cord that support the general increase in excitability referred to as 'central sensitization'. These include, for example, activation of signaling pathways leading to insertion of AMPA glutamate receptor subunits into the postsynaptic membrane, induction of long-term potentiation and transcriptional and translational events that result in increased levels of certain proteins such as the products of the immediate-early genes c-fos and zif268. Injury also initiates a series of epigenetic changes in neurons that result in increased access of transcription factors to DNA. These include the phosphorylation of DNA binding proteins such as MeCP2 leading to decreased acetylation of histone protein. Yet within hours following injury many of these molecular and physiological events begin to subside and the responsibility for maintaining increased excitability of dorsal horn neurons passes to the brainstem which projects through well defined pathways to the superficial layers of the dorsal horn. This coincides with the appearance of increased mechanical sensitivity around the site of injury referred to as secondary hyperalgesia and allodynia and reflects the importance of central processing in monitoring the progress of repair to damaged tissue. The most well defined brainstem pathways are those that express noradrenalin and inhibit dorsal horn neurons and generate analgesia when stimulated: other pathways originate from the rostroventral medulla (RVM). Subsets of RVM neurons are termed 'ON' cells and express the mu opiate receptor. Morphine injected at this site generates a powerful analgesia probably by inhibiting descending excitatory projections to the dorsal horn. However other RVM neurons contain serotonin (5HT) and are also important for setting the level of excitability of dorsal horn neurons. These brainstem pathways are controlled by areas of the forebrain that include the anterior cingulate cortex and amygdala setting the gain of nociceptive signalling as dictated by behavioral priorities in which pain input 'is prioritized relative to other competing behavioral needs and homeostatic demands'. Descending controls are also activated by a specific group of dorsal horn projection neurons situated within lamina I and characterized by their nocispecific receptive fields, expression of the substance P preferring NK1 receptor and ability to support long-term potentiation following strong peripheral stimulation. The control of neuropathic pain following nerve damage has proved extremely challenging clinically and has generated experimental animals models that mirror some of the sensory sequelae seen in humans. Investigation of these models has in turn lead to the idea that bidirectional communication between the dorsal horn and the brain may be disturbed in neuropathic pain states. Thus ablation of descending controls or of the ascending pathway from the superficial dorsal horn substantially reduces the increased pain sensitivity seen after peripheral nerve damage in rodents. Nevertheless, while in humans nerve damage or disease may well predispose a small proportion of patients to long-term pain conditions, the development of pain pathology is unpredictable. This suggests that animal models of neuropathic pain may not be reliable indicators of drug efficacy in humans. In other words, the neural networks and the molecular switch that converts uneventful recovery into uncontrolled pain state remain elusive.

L 7

Forebrain TRPV1 receptor and pain mechanisms: role of glutamate and endovanilloids/endocannabinoids in microglial caspase signalling and neural activity in the prefrontal cortex of neuropathic mice

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N-arachidonoyl-serotonin (AA-5-HT) is a fatty acid amide hydrolase (FAAH) inhibitor and a transient receptor potential vanilloid-1 (TRPV1) antagonist. The effect of a chronic treatment with AA-5-HT on the development of thermal hyperalgesia and mechanical allodynia, the expression of pro-apoptotic and pro-inflammation genes, the endocannabinoid and related enzyme levels in the pre-limbic and infra-limbic (PL-IL) cortex has been tested in mice with spared nerve injury (SNI) of the sciatic nerve. In vivo single-unit extracellular recordings and microdialysis have been also carried out for monitoring electrical activity and extracellular glutamate in the PL-IL cortex. 2-arachidonoyl glycerol (2-AG) level, caspase-1, -8, -9 and -12 genes and caspase-3 protein proved to be increased in SNI mice. Neuronal activity and glutamate release appeared also increased in SNI mice. AA-5-HT alleviated thermal hyperalgesia and mechanical allodynia and normalized the 2-AG and glutamate level and reverted neuronal hyperactivity of PL-IL cortex of SNI mice. AA-5-HT decreased the mRNA levels of the caspase-1, -8, -9 and -12 genes and the protein levels of the caspase-3. This study provides evidence that the simultaneous blockade of TRPV1 receptor and FAAH enzyme by AA-5-HT, might represent a useful tool in the therapy of neuropathic pain symptoms. Chronic treatment with AA-5-HT is also able to revert all the central sequelae such as caspase activation, glutamatergic hyper-tone, increased 2-AG level, and the neuronal hyperactivity associated to nerve injury in the PL-IL cortex.

L 8

Differential phenotype features of neuropathic pain in mice bearing a dystonic genotype

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Early-onset primary dystonia is a syndrome of involuntary muscle contractions characterized by twisting of the limbs, repetitive movements and abnormal postures. Symptoms usually appear in childhood. Many cases of early-onset primary dystonia are associated with a GAG deletion in the TOR1A gene, which results in a single absent glutamic acid residue near the C-terminus of the encoded protein torsinA. Although the pathophysiology of dystonia has been related to basal ganglia dysfunctions, general loss of inhibition and abnormal sensory processing have also been reported and could contribute to the abnormal plasticity documented in the CNS of dystonic patients. Purpose of this study was to establish whether pain processing could also be affected by the altered plasticity and sensory abnormalities observed in dystonic patients. To this aim, spinal nerve ligation according to Kim and Chung (Kim S.H. & Chung J.M., *Pain* 1992) was performed on a genetic DYT1 mouse model of dystonia. Behavioural tests were performed on mice overexpressing the mutant torsinA (hMT), wild-type littermates overexpressing normal torsinA (hWT) and non-transgenic littermates (NT) before and after peripheral nerve injury. Mechanical and thermal sensitivity were assessed by the Von Frey's and Haregreaves' tests, respectively. Pain thresholds to punctate mechanical stimulation were not different between the groups and among genders. In contrast, thermal pain thresholds showed differences between the three genotypes and among genders. Females overexpressing mutant torsinA showed lower latency in comparison to NT females. The same trend was present in hWT in comparison to NT females although it was not statistically significant. On the contrary, hWT males showed response latency comparable to NT males, whereas thermal pain thresholds of hMT males were lower than hWT males. Moreover, females showed lower thermal pain thresholds than males among mice overexpressing normal torsinA. Spinal nerve ligation induced a robust mechanical allodynia starting form the third day post surgery. The extent and time course of development of increased mechanosensitivity was identical between the three genotypes and among genders. However, the recovery phase that started 30 days after surgery in NT males was significantly delayed in hWT and hMT males up to 72 days post-surgery. No significant differences in mechanical sensitivity were observed between females of the three groups, though in general females showed a prolonged hypersensitivity in comparison to males.

The present data point toward a possible role of torsinA in the mechanisms of thermal sensitivity and pain processing.

The experimental protocols were in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992).

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Functional neuroimaging studies of central sensitization

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In this talk I will illustrate some new concepts on the functional significance of the functional neuroimaging responses elicited by nociceptive stimuli in humans (i.e. the so-called "pain matrix"), and on the issues and confounding factors that have to be taken into account when using these responses to investigate the action of centrally-acting pharmacological agents. Indeed, it has been recently shown that the "pain matrix" is only minimally related to the activity of nociceptive-specific neurons in the brain. Starting from these new concepts, I will show how functional neuroimaging can be used to explore the sensitization of the central nervous system (central sensitization) and its pharmacological modulation. Central sensitization is the neural mechanism underlying the behavioural phenomenon of secondary hyperalgesia, i.e. the increased sensitivity to mechanical stimulation occurring in a large, uninjured skin area surrounding following a skin injury. Experimental hyperalgesia is a valid human surrogate model for the study of neurogenic hyperalgesia in patients, and support the view that central sensitization is an important feature of neuropathic pain states.

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Glial cells: new functions in chronic pain

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Mounting preclinical evidence suggests that the generation of chronic neuropathic pain requires neuroimmune activation and neuroinflammatory input for chronicity. Following peripheral nerve damage an important contribution to increased nociception is played by microglial cells and astrocytes which become activated in the dorsal horn of the spinal cord. Recently the signal(s) that mediate spread of nociceptive signalling between neurones and glial cells in the dorsal horn are being actively investigated. We have discovered that the lysosomal cysteine protease *cathepsin S (CatS)* expressed and released from microglia in the spinal cord contributes to the maintenance of chronic pain via liberation of neuronal fractalkine (FNK), a chemokine which is emerging as a novel neuron/microglia signal (Clark et al., 2007). Specifically, CatS is released from microglia by high concentrations of ATP following activation of P2X7 receptor and intracellular phospholipase C and phospholipase A_2 (Clark et al., 2010) whilst the liberation of the chemokine domain of fractalkine (FKN) is critically dependent on microglia-derived CatS cleavage in the dorsal horn of the spinal cord (Clark et al., 2009). FKN further activates microglial mechanisms via activation of CX3CR1 receptor and intracellular p38 MAPK, thereby establishing a *positive feedback* which contributes to nociceptive signaling. Indeed, we have recently shown that mice lacking the CX3CR1 receptor do not develop pain in models of inflammatory and neuropathic pain (Staniland et al., 2010). Furthermore, intrathecal administration of CatS inhibitors reverses neuropathic allodynia and hyperalgesia (Clark et al., 2007). Thus, centrally active CX3CR1 antagonists and CatS inhibitors constitute new therapeutic targets for neuropathic pain.

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L 11

Role of bradykinin and antagonistic action of NSAID zaltoprofen on bradykinin-mediated enhancement of AMPA receptor activity in spinal dorsal horn neurons

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Bradykinin (BK), a peripherally acting inflammatory mediator that has a major role in producing peripheral sensitization. BK also plays a role in pain transmission in CNS. We previously demonstrated that B_2 receptor is expressed by dorsal root ganglion and dorsal horn neurons. We found, moreover, that BK potentiates synaptic glutamate release and action in the spinal cord via B_2 receptors, contributing thereby to pain hypersensitivity. We further examined the signaling pathways that are responsible for the action of BK on glutamatergic transmission in dorsal horn neurons. B_2 receptors are co-expressed in dorsal horn neurons with PKA and PKC, and we find that the augmentation by BK of AMPA receptor-mediated currents requires co-activation of both PKA and PKC. We conclude that BK, by activating multiple kinases, potentiates glutamatergic synaptic transmission to produce pain hypersensitivity. These results suggest that preventing BK production or action will provide a useful means of controlling acute pain hypersensitivity. However, unfortunately these drugs are not clinically available to us. Therefore, we focused on zaltoprofen, a propionic acid derivative of NSAIDs.

Zaltoprofen is a preferential Cox-2 inhibitor as a kind of NSAIDs. In addition, zaltoprofen has a unique action in inhibiting BK-induced nociceptive responses. Accordingly, we examined whether zaltoprofen has an antagonistic action towards BK in AMPA currents in dorsal horn neurons. AMPA currents were significantly potentiated by pre-application of BK. However, zaltoprofen and ibuprofen, blocked the BK-mediated potentiating effect. The inhibitory effect of ibuprofen, but not zaltoprofen, was removed by adding PGE_2. Although pre-incubation with BK, zaltoprofen and PKC activator augmented AMPA currents, the potentiation of AMPA currents was eliminated when PLC activator was substituted for PKC activator. These results suggest that zaltoprofen blocks the augmenting effect of BK on AMPA currents through inhibition of PKC activation, without affecting Cox. We thus conclude that zaltoprofen may be a promising potent analgesic for clinical treatment of pain by an antagonistic action towards BK in dorsal horn neurons.

Leptin and neuropathic pain

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Nerve injury may result in neuropathic pain, characterized by allodynia and hyperalgesia. Accumulating evidence suggests a molecular substrate for neuropathic pain produced by neurons, glia and immune cells. On the other hand, adipokines are hormones derived from the adipocytes, which regulate lipid and glucose metabolism, and have roles in pathological life style disease. Here, we show that leptin, an adipokine exclusively produced by adipocytes, plays a critical role for the development of neuropathic pain through macrophage activation in mice with PSL.

In mice with partial sciatic nerve ligation (PSL), tactile allodynia and thermal hyperalgesia were elicited, as revealed by von Frey test and Hargreaves test, respectively. PSL increased leptin expression in adipocytes distributed at the epineurium of the injured sciatic nerve (SCN). Intraperitoneal leptin aggravates PSL-induced tactile allodynia. Leptin-deficient animals, *ob/ob* mice, showed an absence of PSL-induced allodynia, which was reversed by the administration of leptin to the injured SCN. Perineural injection of a neutralizing antibody against leptin reproduced this attenuation of PSL-induced allodynia. Macrophages recruited to the perineurium of the SCN expressed not only the leptin receptor but also phosphorylated Janus kinase (Jak) and phosphorylated signal transducer and activator of transcription 3 (STAT3), a transcription factor downstream of leptin. PSL also up-regulated the accepted mediators of neuropathic pain – namely, cyclooxygenase-2, inducible nitric oxide synthase and matrix metalloprotease-9 – in the injured SCN, with transcriptional activation of their gene promoters by Jak-STAT3 pathway. This up-regulation was partly reproduced in a macrophage cell line treated with leptin. Administration of peritoneal macrophages treated with leptin to the injured SCN reversed the failure of *ob/ob* mice to develop PSL-induced tactile allodynia. We also found that adipokines took part in development of peripheral neuropathy including neuropathic pain in obesity and prediabetics.

These results suggest that leptin induces recruited macrophages to produce pronociceptive mediators for the development of tactile allodynia. We propose that adipocytes associated with primary afferent neurons may be involved in the development of neuropathic pain through adipokine secretion.

The endocannabinoid system and migraine

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Migraine is a neurovascular disorder characterized by recurrent episodic headaches and caused by abnormal processing of sensory information due to peripheral and/or central sensitization. The pathophysiology of migraine is not fully understood, although it is now widely accepted that the trigeminovascular system, which densely innervates dural vessels, represents one of the main actors in casuing and maintaining migraine pain.
Experimental animal models exploring the effects of nociceptive activation of the trigeminovascular system and aimed to understand the pathophysiology of migraine have suggested the existence of several interactions between the endocannabinoid system and pain mediation in migraine. The endocannabinoid system is centrally and peripherally involved in the processing of pain signals and cannabinoid 1 (CB1) receptors are widely distributed throughout the central nervous system, including the dorsal root ganglia and spinal dorsal horn. A relation between the CB1 gene and headaches was reported, thus suggesting a possible effect of CB1 gene on migraine headaches related to the alteration of peripheral trigeminovascular activation. Furthermore, recent clinical studies have suggested the existence of an endocannabinoid deficiency in migraineurs.
Besides its pathophysiological role in migraine, the endocannabinoid system seems to represent a potential targets for new therapeutic pathways.

L 14

Role of macrophage inflammatory protein-1 in the pathogenesis of neuropathic pain

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Growing evidence indicates that activated glial cells and inflammatory cells constitute complex cytokine-chemokine network leading to neuroinflammation and play an important role in the pathogenesis of neuropathic pain. Two types of macrophage inflammatory protein-1 (MIP-1alpha and MIP-1beta) are major inflammatory chemokines and key regulators on not only inflammatory process but also pain process. Therefore, we investigated the role of MIP-1 in the pathogenesis of neuropathic pain elicited by partial sciatic nerve ligation (PSL) in mice.

In PSL-operated mice, long-lasting tactile allodynia and thermal hyperalgesia, which were evaluated by von Frey test and Hargreaves test, respectively, were observed on the ipsilateral but not contralateral paws. MIP-1alpha and MIP-1beta mRNA were up-regulated in the injured sciatic nerve (SCN) after PSL, and were localized on recruited macrophages and activated Schwann cells. PSL-induced neuropathic pain was prevented by the perineural injection of neutralizing antibodies for MIP-1alpha or MIP-1beta (anti-MIP-1). Perineural injection of recombinant MIP-1alpha or MIP-1beta elicited long-lasting pain behavior. MIP-1 receptors (CCR1 and CCR5) mRNA and their proteins were also up-regulated in the injured SCN after PSL, and they were localized on macrophages and Schwann cells. PSL-induced neuropathic pain was attenuated by perineural injection of siRNAs against CCR1 and CCR5. It is well known that inflammatory cytokines, including interleukin-1beta (IL-1beta), are critical mediators of neuropathic pain. IL-1beta mRNA and its precursor protein were up-regulated in the injured SCN after PSL, and IL-1beta was also localized on macrophages and Schwann cells. PSL-induced neuropathic pain was prevented by the perineural injection of neutralizing antibody for IL-1beta. Up-regulation of IL-1beta after PSL was suppressed by the perineural injection of anti-MIP-1alpha or anti-MIP-1beta.

Taken together, we propose that MIP-1alpha and MIP-1beta derived from macrophages and Schwann cells in the injured nerve are novel key molecules and appear to play a crucial role in the pathogenesis of nerve injury-induced neuropathic pain.

L 15

Recent advances in the molecular pharmacology of opioids are driving to a better pain therapy

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Opiates are among the most effective analgesics known and elective in the treatment of severe pain. However clinical use of opiates is limited because they also produce nausea, vomiting, constipation, respiratory depression and their chronic administration is associated with tolerance, physical dependence and addiction. As a class opiates share a common profile of unwanted effects but there are also significant differences in ligand liability for producing these actions.These drugs produce their effects by acting on GPCRs denominated MOR, DOR and KOR. These receptors when activated, decrease adenylate cyclase activity, increase K currents, inhibits Ca channels. Novel strategies for the development of new and better analgesics are those that take advantage of allosteric properties of GPCRs and their ability to adopt active conformations that differ in their pharmacologically, signalling and regulatory properties. MORs are capable of interacting with and activating numerous Gα subtypes. Recent observations indicate that the interaction of MOR with different Gα subunits is dictated by the type of ligand bound to the receptors. A full agonist, like DAMGO, might activate all Gα proteins expressed in a cell, but a partial agonist may only be able to stimulate some Gα coupled signalling. Therefore after binding identical receptors each agonist determines the classes of GTP – binding regulatory transducer proteins to be activated, suggesting that it is possible to dissociate analgesic actions from unwanted side effects. Following activation, MOR undergoes regulation by a cascade of events that promote receptor desensitization and internalization, and is thereafter recycled into plasma membrane in active state. However not all agonist ligands at the MOR promote the same degree of receptor desensitization and internalization. A correct balance between desensitization and internalization is important for controlling tolerance development to the analgesic effects of the opiates. For example the endogenous peptide ligands at the MOR induce rapid desensitization, internalization and recycling. By contrast, morphine induces partial desensitization and little endocytosis, while methadone is an efficient drug in promoting functional MOR recycling. This important molecular mechanism could partially explain the fact that in clinical setting the switch from one opiate drug to another can restore the loss of analgesic responses. Significant differences among opioid drugs are present also when considering their ability to modulate the immune function. Morphine in fact, induces a general immunosuppression that could be particularly contraindicated in pain treatment in special populations. However, not all opiate drugs exert the same immunosuppressive properties. From several experimental studies it is emerging that opioid drugs such as tramadol and buprenorphine do not exert a negative impact on the immune system. In an experimental model of surgery –induced immunosuppression, morphine, fentanyl, buprenorphine and tramadol were compared at equi-analgesic doses for their ability to prevent surgery pain and to counteract surgery stress–induced immunosuppression. Although all the opioids were able to alleviate pain, only in tramadol and buprenorphine treated animals a complete prevention of immune alterations related to surgery was observed.
In conclusion, the new acquisitions on the molecular mechanisms linked to opioid receptors and ligands will hopefully lead to the development of new analgesics or to a better drug choice for each patient.

L 16

Analgesic effects of gabapentin in neuropathic states and the descending noradrenergic system

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The antiepileptic drugs gabapentin and pregabalin have been used successfully in the treatment of patients with several neuropathic pain conditions, including diabetic neuropathy and postherpetic neuralgia. Based on the importance of spinal plasticity and sensitization following peripheral nerve injury, attention has been focused on the spinal cord as the primary site of analgesic action of gabapentin and pregabalin. We have been focusing on the supraspinal structure as a possible site for their action, and have demonstrated the supraspinally mediated analgesic action of gabapentin and pregabalin, together with their spinal action, in mice developing thermal and mechanical hypersensitivity after partial ligation of the sciatic nerve. This novel supraspinally mediated analgesic effect was markedly suppressed by either depletion of central noradrenaline (NA) or blockade of spinal alpha2-adrenergic receptors. Moreover, intracerebroventricular (i.c.v.) injection of gabapentin and pregabalin increased spinal NA turnover. Importantly, these effects were observed only under hyperalgesic conditions, indicating that gabapentin (and also pregabalin) binding sites or proteins in supraspinal loci appear to be up-regulated after nerve injury. To clarify how gabapentin supraspinally activates the descending noradrenergic pathway, whole-cell recordings were made from locus coeruleus (LC) neurons in brainstem slices prepared from mice after either sciatic nerve ligation or a sham operation, and the effects of gabapentin on GABAergic synaptic transmission were evaluated. Gabapentin concentration-dependently reduced the $GABA_A$-receptor-mediated inhibitory postsynaptic currents (IPSCs). By contrast, glutamate-mediated excitatory synaptic transmission was hardly affected. Moreover, gabapentin did not reduce IPSCs in slices taken from mice given a sham operation. Since gabapentin reduced IPSCs together with an increase in the paired-pulse ratio, it appears that gabapentin acts on the presynaptic GABAergic nerve terminals in the LC. Together, the data suggest that gabapentin presynaptically reduces GABAergic synaptic transmission, thereby removing the inhibitory influence on LC neurons only in neuropathic pain states, leading to activation of the descending noradrenergic pain inhibitory system.

L 17

A peptidic analgesic for treatment to morphine-resistant intractable pain

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Amidino-TAPA is a N-terminal tetrapeptide derivative of dermorphin. Amidino-TAPA shows very high affinity and selectivity for m-opioid receptors and produces potent and longer lasting antinociception than morphine after s.c. and p.o. injections. Interestingly, its antinociceptive profile is quite distinct from morphine. The antinociception induced by i.t.-administered amidino-TAPA is mediated by the spinal release of the endogenous k-opioid peptides dynorphin A, dynorphin B and a-neo-endorphin via activation of spinal m-opioid receptor. These distinct antinociceptive profiles of amidino-TAPA are mediated by the activation of MOR-1J, MOR-1K and MOR-1L, which are amidino-TAPA-sensitive but morphine-insensitive m-opioid receptor splice variants. Unlike morphine, amidino-TAPA dose not show a rewarding effect after s.c. injection. The activation of k-opioid receptor is well established to suppress the development of psychological dependence to m-opioid receptor agonists. Since amidino-TAPA causes the release of endogenous k-opioid peptides via activation of m-opioid receptors, amidino-TAPA may lack the rewarding effect. Most notable character of amidino-TAPA as analgesics is its effectiveness against morphine-resistant intractable pain, especially neuropathic pain. As reported well, the spinal antinociception of morphine is markedly suppressed in ipsilateral paw in compare with that in contralateral paw in the neuropathic pain model (Seltzer model). In contrast, amidino-TAPA shows same magnitude of spinal antinociception in both paws. After the sciatic nerve ligation, mRNA expression of exon-1-containing splice variants, but not MOR-1K or MOR-1L, are markedly reduced in DRG of ipsilateral side in compare with that in DRG of contralateral side. The reduction of spinal antinociception of morphine after sciatic nerve ligation may be caused by the reduction of the morphine-sensitive splice variants in DRG. On the contrary, the spinal antinociception of amidino-TAPA is maintained after the sciatic nerve ligation, since amidino-TAPA-sensitive splice variants in DRG are maintained. In conclusion, amidino-TAPA, that lacks the rewarding effect and is effective against neuropathic pain, is a new analgesic, which has more clinical benefits than morphine.

Design and synthesis of novel delta opioid agonists and their pharmacologies

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Three types of opioid receptors (mu, delta, kappa) are now well established not only by pharmacological studies but by molecular biological studies. Recently we developed a novel kappa selective agonist, TRK-820 (nalfurafine) which produced neither preferential nor aversive effects and it was launched in Japan as an antipruritic for hemodialysis patients. Next our target is design and synthesis of a novel delta agonist. We already reported a highly selective delta agonist, TAN-67. However, it was not enough potent as well as many SNC-80 derivatives to examine the real pharmacological character of delta receptor.

Quite recently, we reported a novel delta selective agonist, SN-28 which was 15 times and 334 times more potent in vitro assay than TAN-67 and SNC-80, respectively and almost equivalent delta selectivity as TAN-67. However, the agonist did not show analgesic effect over 30 mg/kg in acetic acid writhing test by s.c. administration. However, the i.t. injection of SN-28 showed very potent analgesic activity (ED_{50}=0.095 nM) by the same test. We postulated that SN-28 cannot penetrate enough through BBB and designed a new delta agonist KNT-127 which showed potent analgesic effect (1 mg/kg) by s.c. injection.

We will report the rational drug design of KNT-127 and its derivatives, furthermore, their pharmacologies.

Novel SNARE-inactivating biotherapeutics with anti-nociceptive potential

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Owing to problems with efficacy, tolerability, adverse effects and short duration of action of current analgesics, there is an unmet need for long-term and effective pain therapeutics. Thus, an enzymically-inactive mutant ($BoTIM_A$) of the persistently-acting botulinum neurotoxin A ($BoNT_A$) was exploited to deliver into sensory neurons the light chain of type E (LC_E), and to stabilize this protease ─ the most potent inhibitor of TRPV1-mediated CGRP release. Ligating the LC_E gene to that encoding $BoTIM_A$, followed by expression in *E. coli* and purification, yielded a novel LC_E-$BoTIM_A$ protein. Not only does this target neurons, its inhibitory LC_E enters the cytosol and deletes 26 C-terminal residues from SNAP-25 to disable exocytosis. Importantly, these endowed activities transformed the transient effect of LC_E (< 5 days for $BoNT_E$) into a prolonged neuromuscular paralytic action (~ 1 month in mice) and persistent cleavage of SNAP-25, due to stabilisation of the protease by $BoTIM_A$. Mutagenesis identified two leucines in the C-terminal of LC_A that are essential for this therapeutically-advantageous, extended life-time. Moreover, LC_E-$BoTIM_A$ potently inhibits release from sensory neurons of CGRP elicited by capsaicin, and attenuates its evoked nociceptive behaviour in rats over 18 days when administered intra-plantar. Thus, by combining an efficacious blocker of neuro-exocytosis with the acceptor binding, delivery and stabilisation moieties of $BoTIM_A$, a synergistically-acting biotherapeutic was obtained that has enormous potential for long-term pain relief, in addition to its inhibition of cholinergic transmission.

Abstracts Oral Communications
(O 1 - O10)

O 1

Molecular mechanisms underlying intracellular calcium increases in peripheral sensory neurons (F11 cells) by the analgesic compound palmitoylethanolamide (PEA)

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Palmytoilethanolamide (PEA) is an endocannabinoid lipid neuromodulator endowed with anti-inflammatory and analgesic properties; despite intense investigation, the molecular mechanism(s) responsible for these pharmacological actions remain mostly unclear. To investigate the molecular basis for the analgesic actions prompted by PEA in peripheral sensory neurons, Ca^{2+}-imaging and immunocitochemical experiments were performed in differentiated mouse neuroblastoma/rat dorsal root ganglionic hybrid neurons (F11 cells).

Exposure of F11 cells to PEA (0.5-30 μM) for 30 sec elicited a dose-dependent transient increase in intracellular calcium concentration ($[Ca^{2+}]_i$), with an E_{max} of about 400% above basal $[Ca^{2+}]_i$, and an EC_{50} of 3 μM. PEA (10 μM)-induced $[Ca^{2+}]_i$ increase did not involve the activation of cannabinoid (CB) receptors, since it was not affected by either CB_1- (SR-141716A, 10 μM) or CB_2- (SR-144528, 10 μM) -selective receptor antagonists; moreover, ERK phosphorylation, a major downstream event following CB1 receptor activation, was unaffected by PEA.

The removal of extracellular Ca^{2+} or the blockade of L- (nimodipine, 10 μM), N- (ω-conotoxin, 1 μM) and P-Q (ω-agatoxin, 150 nM) type of voltage-gated Ca^{2+} channels (VGCC) completely abolished PEA-induced Ca^{2+} increase, suggesting that PEA depolarized the neuronal plasmamembrane to a level sufficient to trigger extracellular Ca^{2+} influx through VGCC. In addition, vanilloid receptors of the TRPV1 type, which are expressed in F11 cells and whose pharmacological activation by capsaicin (50 μM) also led to a marked enhancement of $[Ca^{2+}]_i$, also appear to contribute to PEA-induced plasmamembre depolarization; in fact, capsazepine, when used at a concentration (1 μM) which selectively blocks TRPV1 receptors but does not directly affect voltage-gated Ca^{2+} channels, caused a significant reduction of PEA-induced $[Ca^{2+}]_i$ increase.

The possible involvement of PPARα activation in PEA-induced Ca^{2+} response was assessed using biochemical measurements of PPARα phosphorylation, indicative of an enhanced protein activation, and pharmacological tools such as PPARα activators (clofibric acid) and blockers (GW-6471). Immunofluorescence experiments using a phospho-specific anti-PPARα antibody revealed that PEA induced an early (5-10 min) increase in PPARα phosphorylation. 30-sec exposure to clofibric acid (0.1-10 μM) induced a marked increase in $[Ca^{2+}]_i$, an effect largely abolished (>80%) by the PPARα selective antagonist GW-6471 (10 μM); more importantly, GW-6471 (10 μM) reduced by almost 70% PEA-induced $[Ca^{2+}]_i$ increase, suggesting that PPARα activation plays a major role in PEA-induced enhancement of $[Ca^{2+}]_i$.

Finally, in order to investigate the potential interaction between PEA and inflammatory/algogenic mediators, the effects of PEA on bradykinin (BK)-induced

changes in $[Ca^{2+}]_i$ in F11 cells were also studied. BK (250 nM) induced a significant $[Ca^{2+}]_i$ increase which appear to depend mostly on extracellular Ca^{2+} influx. BK-induced $[Ca^{2+}]_i$ increase was significantly reduced (by about 40%) upon co-application of PEA. This effect was evident not only at concentrations of PEA (10 μM) effective in increasing $[Ca^{2+}]_i$, but also when concentrations of PEA unable to interfere with $[Ca^{2+}]_i$ (0.5 μM) and closer to those possibly obtained in vivo were used. These results strongly suggest a cross-interaction between PEA- and bradykinin-associated molecular pathways, possibly explaining the PEA-associated analgesic effects observed in animal models of inflammatory pain.

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O 2

Intraplantar injection of gangliosides produces nociceptive behavior and hyperalgesia

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Gangliosides are abundant glycolipids in neural tissue and play important roles in cell-cell adhesion, signal transduction, and cell differentiation. Gangliosides are divided into several groups (asialo-, a-, b-, and c-series gangliosides) based on their biosynthetic pathway. *St8sia1* knockout mice, which lack b- and c-series gangliosides, exhibit altered nociceptive responses (1). The mechanism underlying this defect, however, remains unclear. To address this issue, we first investigated whether gangliosides in peripheral tissues are involved in nociception. Intraplantar injection of the b-series ganglioside GT1b, but not a-series gangliosides such as GM1, produced nociceptive responses and enhanced low-dose formalin-induced nociception. Glutamate receptor antagonists inhibited GT1b-induced hyperalgesia. Furthermore, microdialysis analysis revealed elevated glutamate content in the subdermal tissues by the intraplantar injection of GT1b. These findings suggested that GT1b induced extracellular glutamate to accumulate in subdermal tissues, thereafter activating glutamate receptors, which in turn resulted in hyperalgesia and nociception. On the other hand, intraplantar injection of sialidase, which cleaves sialyl residues from glycoconjugates such as gangliosides, attenuated the late phase of 2% formalin-induced nociception. Thus, the antinociceptive effects of sialidase and the nociceptive effects of GT1b indicated that endogenous gangliosides are involved in nociceptive responses. These results suggest that gangliosides play important roles in nociceptive responses originating in peripheral tissues and that ganglioside-manipulating agents such as sialidase are useful for pain relief.

(1) Handa Y et al., (2005) *Pain*, 117(3): 271-279.

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New non-peptide antagonists of the prokineticins receptors

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Bv8, prokineticin1 (PK1) and prokineticin 2 (PK2) make up a new family of chemokines that lowers pain threshold and modulates immune responses. They activate two G-protein linked receptors (PKR1 and PKR2) in the central nervous system, dorsal root ganglia (DRG) and in cells participating to immuno and inflammatory responses. In the animal model of complete Freund's adjuvant (CFA)-induced paw inflammation we brought evidence that the granulocyte-derived Bv8/PK2 is a major determinant in triggering inflammatory pain and we demonstrated that the non-peptide Bv8-antagonist, PC1 (a triazine derivative), selectively antagonizes Bv8-induced nociceptor sensitization and abolishes the inflammation-induced hyperalgesia (1). These promising results suggest that blocking Bv8/PK system might be a winning strategy to treat pain in other inflammatory pathologies which usually develop from early neutrophil infiltration and flow into a chronic pain perception such as neurophatic pain. In the mouse model of neurophaty, the sciatic nerve chronic constriction injury (CCI), the development of neurophatic pain involves not only neurons but also immune cells, Schwann cells, satellite cells in the DRG, spinal microglia and astrocytes which express prokineticins and their receptors.
Aim of this work is to evaluate, in this CCI model in mice, the antihyperalgesic effect of PC1 chronically administered in preventive and therapeutic schedule.
Sciatic ligation induced thermal hyperalgesia (Plantar test), that appeared in 3 days, and mechanical allodynia (Von frey filaments) that appeared 17 days after surgery. PC1 was administered subcutaneously 150 µg/kg s.c., twice/day for four days starting on day 3, in a group of mice, and on day 17 in another group. In all animals heat hypersensitivity and mechanical allodynia was evaluated in comparison with another group of neurophatic mice treated with saline. In mice (n=5), repeated systemic administration of PC1 from day 3 to 6 after surgery completely reverted thermal hyperalgesia bringing the nociceptive threshold of injuried paw (9.8 ± 0.9 sec.) toward that of contralateral non-injuried paw (10.2 ± 1.2 sec.). At the end of PC1 treatment, from day 5 to 30, thermal nociceptive hyperalgesia slowly reappeared after withdrawal of PC1 treatment but at levels significantly lower (7.5 ± 0.3 sec) that that of saline-treated mice (4.5 ± 0.3 sec). Interestingly, mice chronically injected with PC1 from day 3 to 6 after surgery, never developed mechanical allodynia. Repeated systemic administration of PC1 from day 17 to 20, significantly reduced both thermal and mechanical hyperalgesia that reappeared after PC1 treatment withdrawal. In animal model of inflammation (CFA in the paw) chronic administration of PC1 150 µg/kg, s.c., twice/day from day 1 to day 4, completely abolished, in mice, the inflammation-induced thermal (Plantar test) and mechanical (Von frey filaments) hypernociception and reduced paw edema (Plethismometer) accelerating the recovery to normal pain value after the insult.
New synthesized non-peptide PKRs-antagonists (PC2-PC36) preinjected (-10 min) into the right paw of mice, antagonized the hyperalgesic effect (paw-immersion test, 48°C)

of exogenous Bv8 (0.5 ng/paw). PC27 acted at doses 100 fold lower than the lead compound.
These data demonstrate that PKRs may represent a therapeutic target for the development of novel peripherally acting antinociceptive drugs.

1) Giannini E., Lattanzi R., Nicotra A., Campese A.F., Grazioli P., Screpanti P., Balboni G., Salvadori S., Sacerdote P. and Negri L. (2009). PNAS, 106, 14646-14651.

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O 4

5'-chloro-deoxy-(±)-ENBA, a novel A1 adenosine receptor agonist, alleviates neuropathic pain through functional microglial changes in mice

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Study objective: A1 adenosine-mediated effect in microglia activation occurring in neuropathic pain

Introduction: Neuropathic pain is devastating disease which strongly affects the quality of life although the mechanisms leading to development and maintenance are still poorly understood. Proliferation and activation of spinal microglia in the pathogenesis of neuropathic pain has recently been shown. Therefore, microglia represent an hopeful candidate to investigate neuropathic syndromes, as well as a potential pharmacological target. Purinergic system regulates microglial phenotypical changes. Here we have investigted the role of A1 adenosine receptor (A1R) in microglial morphological changes in the spinal cord of neuropathic mice

Methods: We used spared nerve injury (SNI) mouse model of neuropathic pain to assess the possible use of A1R agonist as a antiallodynic-hyperalgesic drug. Bio-molecular, immunocytochemical and immunohistochemical analysis were carried out in order to verify the A1R modulation on microglial morphological changes *in vivo* and *in vitro*.

Results: Chronic systemic administrations of 5′-chloro-5′-deoxy-(±)-ENBA (0.5 mg/kg, i.p) reduced both thermal hyperalgesia and mechanical allodynia 3 and 7 days post-SNI, in a way prevented by DPCPX (3 mg/kg, i.p.), a selective A1 receptor antagonist. SNI induced spinal changes in microglial activation ipsilaterally to the nerve injury. Chronic treatment with 5′-chloro-5′-deoxy-(±)-ENBA (0.5 mg/kg, i.p) prevented the microglial activation in the spinal cord dorsal horn ipsilaterally to the nerve injury. We performed in vitro experiment, on microglial cell cultures in order to evaluate the expression of A1AR in microglia and the A1AR agonist effectiveness on microglial phenotypical and morphological changes. We found that A1AR mRNA and protein were expressed on microglia and underwent to up-down-regulation depending of the activation challenge (LPS, ATP, LPS+ATP). The incubation with 5′-chloro-5′-deoxy-(±)-ENBA reduced the number of morphological activated cells in the LPS, ATP, LPS+ATP treated cells as compared to the same challenges-activated cells treated with vehicle.

Conclusions: Our result demonstrated an involvement of A1 receptor in the amplified nociceptive thresholds and in spinal microglial changes changes occurred in neuropathic pain.

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O 5

Mu2-opioid receptors involve in the antinociceptive activity induced by Tyr-W-MIF-1 in the mouse spinal site

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Tyr-Pro-Trp-Gly-NH2 (Tyr-W-MIF-1), which is named for its structural similarity to the melanocyte-stimulating hormon-reloease inhibiting factor-1 (MIF-1) family peptides, has been isolated from human cerebral cortex and bovine hypothalamus. Tyr-W-MIF-1 has high affinity to mu-opioid receptor, and has prolonged antinociceptive activity after intrathecal (i.t.) administration. The antinociception induced by Tyr-W-MIF-1 can be diminished by the i.t. pretreatment with a selective mu-opioid receptor antagonist, beta-funaltrexamine. These results clearly suggest that Tyr-W-MIF-1 is a potent agonist for mu-opioid receptor in spinal site. The mu-opioid receptor had been divided into mu1- and mu2-opioid receptors, and these subtypes can be discriminated by the selective mu1-opioid receptor antagonist naloxonazine. Moreover, it has been already reported that D-Pro2-endomorphin-1 and D-Pro2-endomorphin-2, which were endomorphin analogues containing D-Pro2, may be useful tool to discriminate the antinociceptive effects mediated by mu2- and mu1-opioid receptor, respectively. This study has been designed to identify the involvement of mu-opioid receptor subtype on the antinociceptive effect induced by i.t. administered Tyr-W-MIF-1 in mice. The i.t. treatment of Tyr-W-MIF-1 produced a dose-dependent antinociceptive effect, and it was completely diminished by beta-funaltrexamine, but not by a delta-opioid receptor antagonist, naltrindole, nor a kappa-opioid receptor antagonist, nor-binaltorphimine. Furthermore, the antinociceptive effect induced by Tyr-W-MIF-1 was significantly attenuated by the i.t. co-administration with a selective mu2-opioid receptor antagonist, D-Pro2-endomorphin-1. However, the i.t. co-administration of selective mu1-opioid receptor antagonists, naloxonazine and D-Pro2-endomorphin-2, failed to affect the antinociceptive effect of Tyr-W-MIF-1. These results suggest that the antinociceptive effect of Tyr-W-MIF-1 is mediated through the mu2-opioid receptor. This study provides the pharmacological evidence to prove that Tyr-W-MIF-1 acts as the selective mu2-opioid receptor agonist at the spinal site.

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O 6

Neuropathic pain promotes the expression of the autophagic markers LC3 and beclin in the mouse spinal cord

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The contribution of spinal neuronal cell death to neuropathic pain has been investigated in several animal models, but it remains still controversial. Moreover, previous studies have exclusively focussed on mechanisms of apoptosis. Aim of this study was to investigate the occurrence of novel cell death mechanisms, such as autophagic processes, in the dorsal horn of the adult mouse spinal cord following nerve injury.
Spinal nerve ligation was performed on C57/BL6 mice (20-22g) according to Kim and Chung (Kim S.H. & Chung J.M., *Pain* 1992). Mechanical and thermal sensitivity were assessed by the Von Frey's and Haregreaves' behavioural tests, respectively. The expression and modulation of the two main autophagic markers, LC-3 and beclin-1, were investigated by western blot analysis in the dorsal horn of mice undergone either SNL or sham surgery.
No changes in beclin-1 and LC3 expression were detected 3 days after surgery. However, an increase in 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. The increased expression of the markers was restricted to the spinal cord side ipsilateral to the ligation in SNL mice and was not present in mice undergone sham surgery. The increased expression of beclin 1 and LC3-II appeared to correlate with the upregulation of the calcium channel subunit $\alpha 2\delta$-1.
The present study indicates that degenerative mechanisms other than apoptosis may be activated and participate to the development of neuropathic pain.
The experimental protocols were in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992).

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O 7

Modulation of capsaicin-induced pain behavior by opioid receptors

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Capsaicin, the pungent main ingredient of hot chilli peppers, elicits a sensation of burning pain by selectively activating nociceptive sensory neurons. Injection of capsaicin into the hindpaw has been employed as a model of chemogenic-heat nociception in mice. Here we examined involvement of opioid receptors in capsaicin-induced pain behavior by comparing behavioral response in opioid receptors knockouts (KO) and their controls (WT).
First, we evaluated capsaicin-induced pain in mice lacking delta or mu opioid receptors. Intraplantar capsaicin evoked similar pain-related behavior in mu KO and WT mice. However, capsaicin response was significantly reduced in delta KO mice. This reduction was observed in delta KO mice of both 100% B6 and 50%129/50%B6 genetic backgrounds.
Acute morphine treatment almost completely inhibited the capsaicin-induced pain behavior in WT and delta KO mice but not in mu KO mice. On the other hand, acute treatment with delta agonist SNC80 produced significant antinociception in WT mice, and this effect was completely abolished in delta KO mice.
Furthermore, we compared the mRNA expression levels of 4 genes related to capsaicin-induced pain (TRPV1, ENK, SP, CGRP) in DRGs from both WT and delta KO mice by quantitative RT-PCR. Although mRNA expression of TRPV1, ENK and SP did not differ between WT and KO mice, expression level of CGRP mRNA was significantly lower in KO mice than in WT mice, suggesting that reduction of CGRP expression in delta opioid receptor KO mice may be partially involved in the decreased capsaicin-induced behavior.
Altogether, capsaicin-induced pain behavior was drastically reduced by delta opioid receptor deletion. This is the first evidence that suggests that delta opioid receptor is involved in pain transmission initiated by activation of TRPV1. These new findings together with recently reported effects of delta receptor activation on chronic pain strongly suggest the possibility to use delta opioid receptor as new potential therapeutic target for pain.

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O 8

Antinociceptive mechanism of epibatidine analogue ABT-594 in mice

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Epibatidine isolated from the South American frogs, *Epipedobates tricolor*, was discovered as a potent nicotinic agent with antinociceptive and toxic effects. ABT-594, the analogue of epibatidine, was shown to be potency antinociceptive effects without toxicity. It was reported that ABT-594 has higher affinity for the alfa-4-beta-2 nicotinic acetylcholine receptor subunit in the central nervous system. The aim of this study, we were determined the antinociceptive mechanism of ABT-594 by the formalin test, tail-flick test, tail-pressure test and hot-plate test in ddY-strain mice (weight 20-24 g). ABT-594 was dose-dependently shown to be analgesic effects in the formalin test (0.05-0.20 micro-mol/kg, s.c.), hot-plate test (0.1-0.4 micro-mol/kg, s.c.) and tail-pressure test (0.8-1.6 micro-mol/kg, s.c.), respectively. In contrast, the antinociceptive effect of ABT-594 was not shown in the tail-flick assay (dose>1.6 micro-mol/kg, s.c.). Pretreatment with mecamylamine (1 mg/kg i.p.), the nicotine acetylcholine receptor antagonist, was blocked the effect of ABT-594 in the formalin test, tail-pressure test and hot-plate test, respectively. Moreover, pretreatment with naloxone (5 mg/kg, i.p.) was only partially blocked the effects of ABT-594 in the formalin test (first phase: 2% formalin into the right hind paw after the treatment of ABT-594) and the tail-pressure test. The results from the present study demonstrate that ABT-594 produced the antinociception in persistent chemical (formalin), acute thermal (hot-plate, but not tail-flick) and mechanical (tail-pressure) pain states. Then, it was found ABT-594 has produced the antinociception induced the nicotine acetylcholine receptor within the respective noxious stimuli, and included with opioid-related mechanism.

O 9

Botulinum toxins in animal models: innovative therapeutic approaches against pain

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Although the understanding of pain mechanisms has significantly improved in the recent years, much more is yet to be discovered. Broadening of our knowledge is needed to improve basic and clinical research in this field in order to find innovative treatments for fighting pain. In the last years both experimental and clinical studies brought to encouraging results on bacterial toxins as pain relievers. Our research group has investigated the effects of *Botulinum* neurotoxin serotype A (BoNT/A) on inflammatory and neuropathic pain in animal models. BoNT/A exerts its action targeting the SNARE complex responsible of the fusion of neurotransmitter vescicle with presynaptic membrane and blocking neuroexocytosis, including glutamate release, whose role in pain transmission is well known. Following BoNT/A administration, behavioural responses to chemical, mechanical and thermal stimuli and functional recovery were assessed in male CD1 mice. Moreover, investigations on regenerative processes of nerve injured after BoNT/A were carried out. BoNT/A is able to induce analgesic effects on both inflammatory and neuropathic pain and to speed up the functional recovery; in addition it contributes to peripheral processes of nerve regeneration enhancing the expression of Cdc2, S100β and GFAP, proteins associated with nerve injury and repair. Taken altogether, our findings provide new insights in the comprehension of neurobiological mechanisms involved in pain modulation and indicate the way for the development of new pharmacotherapeutic approaches, in particular against neuropathic pain, which represents a severe chronic pathology extremely difficult in treating.

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O 10

Mouse adult neural stem cells endovenous administration exerts a long lasting relief of neuropathic pain and re-establish a correct balance between pro- and antiinflammatory cytokines in the chronic constriction injury mouse model

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Aim: In our study we investigate the biochemical effects of adult neural stem cells systemic administration in a well-established model of peripheral nerve lesion, and correlate them with neuropathic pain development and symptoms. We believe that Neural Stem Cells (NSCs) may condition the local milieu at the lesion site, thus preventing or attenuating the cascade of events that leads to the development of pathological (neuropathic) pain.
Method: We use the chronic constriction injury model in the mouse (CCI) because it produces a robust Wallerian degeneration with additional inflammation. Moreover, since some nerve fibers survive the injury, behavioural testing can also be used to assess pain. NSCs are isolated from the subventricular region (SVZ) of the lateral ventricles of C57BL/6 mice and infected with lentiviral vector expressing the GFP protein, in order to trace NCS after transplant. NSCs are injected in the tail vein at the doses of 1 or 2 millions/200 μl, at day 7 after CCI, when both thermal hyperalgesia, measured by plantar test, and mechanical allodynia, by Dynamic Plantar Aesthesiomether, are maximal. The localization of NSCs in the lesioned nerve is achieved by appropriate immunocytochemistry against the GFP. At 7 days after NSC treatment, sciatic nerves are collected and the expression of the proinflammatory cytokines IL-1 and IL-6 and of the anti-inflammatory cytokine IL-10 measured by real-time RT-PCR.
Results: Twenty four hours after NSC administration, cells are selectively localized in the injured sciatic nerves where they remain up to 7 days. Intravenous NSC administration dose-dependently decreases painful behaviour. The antihyperalgesic and antiallodynic effect starts to be evident 3 days after NSC, and is maximal 7 days later. A significant reduction of hypernociception is always observed: thermal hyperalgesia is completely reversed by the highest NSC treatment, while mechanical allodynia is only partially abolished. The effect is still significant 14 days after NSC administration. As expected, in CCI sciatic nerves a significant increase of both pro and anti inflammatory cytokine expression is present. The nerve IL-1 and IL-6 overexpression in NSC treated animals appear significantly reduced, while a slight increase of IL-10 production is observed.
Conclusion: The peripheral administration of NSC therapeutically reverses neuropathic pain in the CCI mouse model. We believe that a bidirectional interaction between NSCs and the lesioned-inflammed nerve is at the basis of the positive modulation of pain and inflammation.

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Abstracts Poster Communications
(P 1- P 13)

P 1

Functional role of spinal ceramide in the development of neuropathic pain

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Peripheral nerve injury induces neuroinflammation due to activation of glial cells, which is implicated in neuropathic pain. Ceramide is one of bioactive lipid mediators and belongs to sphingolipid family. Ceramide is generated by sphingomyelinases through the breakdown of sphingomyelin or is synthesized *de novo* by ceramide synthase. Although growing evidence indicates that ceramide involves in neuroinflammation, it is unclear whether ceramide contributes to the development of neuropathic pain. In this study, we investigate the involvement of spinal ceramide in partial sciatic nerve ligation (PSL)-induced tactile allodynia which is one of the symptoms of neuropathic pain.

Tactile allodynia was evaluated by von Frey test. Intrathecal (i.t.) injection of Fumonisin B1 (FB1: 1-10 nmol), ceramide synthetase inhibitor, at 3 hrs and 3 days after PSL significantly attenuated the PSL-induced tactile allodynia. FB1 single injection at 3hrs but not 7 days after PSL transiently attenuated tactile allodynia. Similarly, i.t. injection of GW4869 (0.1-1 nmol), neutral sphingomyelinase inhibitor, at 3 hrs and 3 days after PSL attenuated tactile allodynia. By RT-PCR, we observed the increment of neutral sphingomyelinase mRNA expression as well as inflammatory cytokines (IL-1beta, TNF-alpha) and microglial specific molecule (Iba-1, CD11b, CD14) in the spinal cord after PSL. By immunohistochemistry, PSL-induced increase in Iba-1 positive cells in the spinal dorsal horn was prevented by the i.t. injection of FB1 and GW4869. Exogenous ceramide (3 nmol, i.t.) elicited tactile allodynia, accompanied by microglial activation in the spinal cord. By RT-PCR, the up-regulation of inflammatory mediators mRNA, such as IL-1beta, TNF-alpha, MCP-1, and CD14, were observed in the spinal cord after ceramide i.t. injection.

These results suggest that ceramide may play a crucial role in PSL-induced neuropathic pain through microglial activation and inflammatory cytokines release in the spinal cord.

The Bv8 mutant, [Ala^{24}]Bv8, is endowed with antihyperalgesic effect

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Bv8 is a small protein of 8 kDa, isolated from the amphibian skin *Bombina variegata*, that induces hyperalgesia in rodents. Bv8 belongs to AVIT protein family, whose members are highly conserved from non-mammals (such as Mamba Intestinal Toxin 1 or MIT-1) to humans. All these proteins share a conserved N-terminal amino acid sequence AVITGA, which is responsible for the interaction to 2 metabotropic receptors, prokineticin receptor 1 (PKR1) and prokineticin receptor 2 (PKR2) and a C-terminal domain that contributes to hyperalgesic effect.
Analyzing the evolutionary conservation grade of each residue of Bv8 homologous proteins onto the modelled structure of Bv8 suggests that modification in any position from 6 to 40 of the primary structure of Bv8 will produce molecules possibly endowed with altered affinity and/or efficacy for the PKRs. We have suggested that members of the AVIT family could interact with PKRs receptors by orienting the protein region that comprises the AVIT sequence and Trp24. To verify if Trp24 could play a fundamental role in the interaction with PKRs, we generated Bv8 mutants in which the tryptophan in position 24 is substituted by alanine.
Here we describe the pharmacological activity of a variant of Bv8, named [Ala^{24}]Bv8, in which tryptophan in position 24 was substituted by alanine, *in vitro* and *in vivo animal pain models*.
Receptor Affinity
Substitution of Ala for Trp24 reduced the Bv8 affinity for PKR1 of 30 times (Bv8, IC_{50}= 1 nM and [Ala^{24}]Bv8 IC_{50}=30.5 nM) and for PKR2 of only 8 times (Bv8, IC_{50}= 1.07 nM, and [Ala^{24}]Bv8 IC_{50} = 8.60 nM).
Animal pain models
-In Bv8-induced hyperalgesia models
In rodents, s.c injection (200 ng/kg) and i.t. injection (0.5 ng/rat) of Bv8 produces a characteristic biphasic hyperalgesia to thermal (Planter test, Ugo Basile), mechanical (paw-pressure test) and tactile stimuli (Von Frey), and local ip.l injection of Bv8 (0.5ng) induces hyperalgesia with a monophasic time-course. The [Ala^{24}]Bv8 compound, at doses 100 times higher than Bv8 hyperalgesic doses, produces a biphasic hyperalgesia. However at doses that are uneffective, when previously (5-15 min) administred, it was able to antagonize Bv8-induced hyperalgesia, in rats and mice. In rats, s.c. injection (-15 min) of [Ala^{24}]Bv8 (0.1 µg/kg – 20 µg/kg) abolished the hyperalgesia induced by s.c., i.t. and ip.l administration of Bv8. I.t. preinjection (-5 min) of [Ala^{24}]Bv8 at 10 ng dose abolished the biphasic hyperalgesia induced by i.t. Bv8 (0.5 ng/rat).
In mice, systemic administration of [Ala^{24}]Bv8 (20 µg/kg, s.c.) abolished the thermal hyperalgesia and significantly reduced the tactile allodynia induced by ip.l injection of Bv8.
-In CFA-induced paw inflammation
In rats, systemic, s.c. and i.v., injection of [Ala^{24}]Bv8, dose-dependently abolished the CFA-induced mechanical hyperalgesia (paw-pressure test): the antihyperalgesic effect lasted for 8 h, 4 h and 3 h after the s.c. injection of 20, 5, and 2 µg/kg respectively. I.v. administration of [Ala^{24}]Bv8 (0.5 µg/kg) abolished hyperalgesia for 2 h.
In mice, [Ala^{24}]Bv8 at 20 µg/kg abolished the thermal hyperalgesia (paw-immersion test, 48°C) for more than 6 h.

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P 3

Spermine-induced mechanical allodynia in the mouse hind-paw

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The spermine-mediated mechanical allodynia was described in the mouse hind-paw. The intraplantar (i.pl.) injection of spermine induced a transient mechanical allodynia, which peaked at 2 min and disappeared by 30 min after the injection. The spermine-induced mechanical allodynia was dose-dependently inhibited by i.pl. co-administration of D-APV, a competitive antagonist for NMDA receptor, and arcaine, an antagonist for the polyamine recognition site of NMDA receptor. Spermine antiserum also inhibited spermine-induced mechanical allodynia. These results suggest that spermine-induced mechanical allodynia is mediated through the polyamine recognition site of NMDA receptor in the plantar surface of the mouse hind-paw. On the other hand, repeated i.pl. injection of spermine induced a persistent mechanical allodynia in the mouse. The spermine-induced persistent mechanical allodynia was inhibited by i.pl. co-administration of arcaine. The i.pl. injection of complete Freund's adjuvant（CFA） induced the inflammation, persistent thermal hyperalgesia and persistent mechanical allodynia. The repeated i.pl. injection of spermine antiserum also suppressed CFA-induced mechanical allodynia. The present results suggest that acute and consistent increase of spermine in the plantar surface causes the transient and persistent mechanical allodynia, respectively. The consistent increase of spermine in the plantar surface may also contribute to the development of CFA-induced mechanical allodynia.

Palmitoylethanolamide: control of hyperalgesia during chronic inflammation *in vivo*

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Introduction: Palmitoylethanolamide (PEA) belongs to the family of ALIAmides (*Autacoid Local Injury Antagonism Amide*), that exhibit local effects mainly through the control mast cell (MC) activation. MCs are immune-competent cells mainly localized in sites directly interfacing with external environment, where they orchestrate the inflammatory reaction by the release of pro-inflammatory mediators. PEA has been recently shown to reduce the progression of chronic inflammation, in a model of granuloma sustained primarily by MC activation in rats. Moreover, recent evidences indicate a bidirectional cross-talk between MCs and sensory nerves (SNs) suggesting that MCs and SNs may be functionally and anatomically assembled within certain tissues as the skin, where MCs are frequently co-localized near nerve fibers. Starting from the assumption that MC granules contain pro-inflammatory and pro-algogenic mediators, primarily Nerve Growth Factor (NGF), the present study addresses its attention to investigate whether PEA is able also to control granuloma-associated hyperalgesia in rat.

Materials and Methods: Granuloma, a typical chronic inflammation, was induced by subcutaneous implantation of two λ-carrageenin (1%)-soaked sponges on the back of male Wistar rats. PEA was injected into each sponge at the concentration of 200, 400, 800 μg/mL. The mechanical allodynia was evaluated by using the Von Frey filaments with calibrated bending forces, that were used to deliver punctuate mechanical stimuli of varyious intensity, in the middle of and around the granulomatous tissue; the frequency of withdrawals induced by consecutive applications of the same filament was evaluated, too. After 96 hours to the implantation, rats were sacrificed and the new nerves formation was evaluated in the granulomatous tissue by histological analysis. Western blot analysis for NGF and Protein Gene Product 9.5 (PGP 9.5) was conducted. In parallel, rat Dorsal Root Ganglia (DRG) were excised and transverse sections treated to perform immunohistochemical analysis, to evaluate citotypes involved in the granuloma-induced DRG sensitization. Obtained slides were incubated with primary antibody solutions for the pro-inflammatory markers TNF-α, NGF and COX-2, co-labeled with TRPV1 and satellite cells marker.

Results: Our results show the analgesic properties of PEA, evaluated by its efficacy in reducing mechanical allodynia, by using the Von Frey filaments. Moreover, the histological image of granulomatous tissues evidenced the massive presence of degranulated MCs in tight contact with nerve fibres that were both significantly reduced by PEA. These data were confirmed by the reduction of the pro-inflammatory/pro-algogen mediators expression from MCs, in granuloma. Finally, we found that

granuloma-induced mechanical allodynia was associated with an increased expression of COX-2, iNOS, TNF-α and NGF in rat dorsal ganglia (DRG), that were significantly reduced by PEA treatment.
Conclusions: The present data corroborate the evidence of an analgesic role played by PEA in several model of pain, recognizing in MCs the leading cell-type affected by PEA action in the model of carrageenin–induced granuloma in rats. Thus, according to our results it is conceivable to hypothesize the use of PEA and its congeners in the treatment of several painful conditions since by controlling MC degranulation and by modulating NGF release, PEA shows to act as a promising analgesic drug in chronic inflammation model.

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P 5

The relationship between nitroglycerin-induced hyperalgesia and levels of endocannabinoids – A therapeutic role for inhibitory hydrolases?

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Keywords: endocannabinoids, migraine, hydrolases, hyperalgesia

Endocannabinoids are present in most tissues and, in some pain states, their levels are elevated at key sites involved in pain processing. Endocannabinoids are hydrolysed by specific enzymes: fatty-acid amide hydrolase (FAAH) is an intracellular hydrolase that catalyzes the cleavage of bioactive of several endogenous fatty acid amides, such as anandamide (AEA), while the hydrolysis of 2-arachidonoylglycerol (2-AG), another important endocannabinoid, is mainly catalysed by the monoacylglycerol lipase (MAGL). Recent studies have reported that inhibition of FAAH produces analgesia and reduces inflammation in models of acute-inflammatory pain. The development of MAGL inhibitors could offer an opportunity to study the anti-inflammatory and anti-nociceptive role of 2-AG, which have not yet been elucidated. In this study we evaluated whether systemic inhibition of FAAH and MAGL may alter nociceptive responses in a well-known animal model of migraine based on the hyperalgesia induced by nitroglycerin administration at the tail flick and formalin tests. The tail-flick test reflects a spinal reflex, whereas nociceptive responses after formalin injection require higher brain functions. The tests were performed in male Sprague-Dawley rats that were pre-treated with nitroglycerin (10mg/kg, i.p.) or saline (4 hours before) and treated with URB597 (a FAAH inhibitor, 2mg/Kg, i.p.) or URB602 (a MAGL inhibitor, 2mg/Kg, i.p.) 60 minutes before the experimental tests. URB597 induced significant analgesia already in baseline condition and it abolished nitroglycerin-induced hyperalgesia at the tail flick test as well as in phase II of formalin test. URB602 did not show any analgesic effect per se at the tail flick test, while it inhibited nitroglycerin-induced hyperalgesia. In the formalin test, URB602 in baseline condition inhibited nociceptive behavioral only in phase I, while it significantly reduced nitroglycerin-induced hyperalgesia in phase II. These findings demonstrate that theoretical elevations in content of AEA and 2-AG, at the spinal and, possibly, supraspinal level, through inhibition of FAAH and MAGL activities, may modulate pain perception in a specific animal model of migraine, therefore prompting new targets for the development of symptomatic/prophylactic drugs for migraine.

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P 6

Changes in mu-opioid receptor on the mouse spinal cord in inflammatory pain state

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An inflammatory pain is a chronic pain induced by allergic or inflammatory substances such as bradykinin, ATP, cytokine and prostaglandin. The predominant symptoms of inflammatory pain are edema, thermal hyperalgesia and mechanical allodynia around the inflammation sites.
It is well established that morphine is effective against the thermal hyperalgesia during inflammatory pain state. However, we found that morphine is ineffective for mechanical allodynia under the condition of inflammatory pain.
In the present study, the changes in the spinal mu-opioid receptors, which is involved in the morphine analgesia, is investigated in the inflammatory pain state.
To develop the inflammatory pain, complete Freund's adjuvant (CFA) was injected i.pl to the hind-paw of ddY mice. mRNA levels for mu-opioid receptors were quantified by reverse transcription polymerase chain reaction.
The significant mechanical allodynia was observed 1day after the CFA injection on ipsilateral side. mRNA level of exon-1-containing splice variants of mu-opioid receptors were significantly decreased in the DRG on ipsilateral side 1day after the CFA injection. However, any changes in mRNA level of exon-1-containing splice variants were not observed in the lumber spinal cord.
In conclusion, the down-regulation of exon-1-containing splice variants in DRG may be responsible for the reduced efficacy of morphine against inflammatory pain.

P 7

The blockade of NOP receptor in the vlPAG reduces the development of tolerance to opioid-induced antinociception in rats

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The ventrolateral periaqueductal gray (vlPAG) is a main output pathway involved in the descending pain-control system (1) and it plays a major role in the development of opioid tolerance. In addition to mechanisms at cellular level, the development of opioid tolerance can be discussed in terms of plasticity through neuronal networks and an increased activity of "antiopid systems", as N/OFQ/NOP receptor (2), have been proposed as a possible mechanism for this opioid side effect. The present experiments tested whether NOP receptor blockade, in the vlPAG, affected the tolerance to the antinociceptive action of morphine and [D-Ala^2-NMe-Phe^4-Gly-ol^5]-enkephalin (DAMGO), a selective MOP opioid agonist. The analgesic effect of morphine (10mg/kg s.c.) and DAMGO (1µg/1µl intra vlPAG), estimated by tail flick and hot plate test, gradually decreased during repeated opioid treatment (twice a day) up to 3 days when rats became tolerant. Intra vlPAG administration of (±)–J 113397(4 µg/1µl), a non-peptidic NOP receptor antagonist, or UFP-101 (19µg/1µl), a peptidic NOP receptor antagonist, on day 4, restored the analgesic effect of both morphine (3) and DAMGO. Moreover the daily pre-treatment with NOP antagonists prevented the development of opioid tolerance that reappeared if the NOP antagonists were suspended.
These results indicate a role for N/OFQ/NOP receptor in the vlPAG on the development and expression of tolerance to opioid analgesic effect confirming that this system would act as a functional antiopioid antagonist.

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P 8

Botulinum neurotoxin serotype A and morphine combination: a synergistic action on inflammatory and neuropathic pain

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The use of morphine and other opiates, analgesics for several types of severe persistent pain, is limited by significant side effects, including the development of tolerance phenomena. The combined administration of subthreshold doses of morphine with other analgesics may open the possibility of significantly lowering the effective dose of morphine.
In recent years a growing interest in the utilization of Botulinum neurotoxins (BoNTs) for treating pain developed, both in humans and in animal models. In particular, analgesic effects of the serotype A of BoNTs (BoNT/A) were shown.
The aim of the present research was to verify a possibile pharmacological interaction of morphine with BoNT/A.
A number of experiments were carried out using formalin test, as inflammatory pain model, and Chronic Constriction Injury (CCI) as neuropathic pain model, in CD1 male mice. Synergistic analgesic effects of BoNT/A with morphine were examined. Moreover, the effects of BoNT/A on the tolerance-induced by chronic administration of morphine, in inflammatory and neuropathic pain were tested. The behavioural effects of BoNT/A on morphine-induced tolerance were also correlated with immunofluorescence staining of inflammatory markers at the spinal cord level.
The results showed that BoNT/A and morphine exert a synergistic analgesic action and that morphine-induced tolerance is inhibited by previous injection of BoNT/A, both in inflammatory and neuropathic pain models. Moreover, BoNT/A modulates the expression of astroglial cells in the spinal cord.
The possibility of using BoNT/A for lowering the effective dose of morphine and preventing the development of opioid tolerance would have a tremendous impact in terms of clinical application.

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P 9

Lack of the rewarding effect and locomotor-enhancing effect of mu-opioid receptor agonist amidino-TAPA

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We recently developed new mu-opioid receptor agonist amidino-TAPA, which has a distinct antinociceptive profile from morphine that is the release of endogenous kappa-opioid peptides. The activation of kappa-opioid receptor has been suggested to suppress the development of psychological dependence of mu-opioid receptor agonists. In the present study, the psychological dependence liability and its related locomotor-enhancing effect of amidino-TAPA were evaluated. Amidino-TAPA injected s.c. produced extremely potent and long-lasting antinociception than morphine in ddY mice. Unlike morphine, amidino-TAPA injected s.c. did not induce remarkable locomotor-enhancing effect and rewarding effect at antinociceptive dose even at more high doses in ddY mice. However, amidino-TAPA produced potent locomotor-enhancing effect and rewarding effect at antinociceptive dose in prodynorphin-knockout mice. The present results suggest that amidino-TAPA is a potent analgesics lacking psychological dependence liability by releasing the endogenous kappa-opioid peptides.

P 10

Human mesenchymal stem cells as novel neuropathic pain therapeutics

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Study objective: human mesenchymal stem cell transplantation as cell-based therapy for neuropathic pain.
Introduction Neuropathic pain is a very complex disease, involving several molecular pathways. Current available drugs have a generalized nature and act only on the temporal pain symptoms rather than being targeted towards the several mechanisms underlying the generation and propagation of pain. Nowadays, pain research is directing towards new molecular and cellular methods, such as stem cell therapy. Stem cells have been used in a variety of nervous system injury models. As neurodegenerative disease, also neuropathic pain could undergo to stem cell therapy.
Methods We used spared nerve injury (SNI) mouse model of neuropathic pain to assess the possible use of human mesenchymal stem cells (hMSCs) as anti-neuropathic tool. Bio-molecular, immunocytochemical and immunohistochemical analysis were carried out in order to verify stem cell-mediated changes in molecular mechanisms underlying pain development and maintenance.
Results Human MSCs were transplanted in the mouse lateral cerebral ventricle. Stem cells injection was performed 4 days after sciatic nerve surgery. Neuropathic mice were monitored 7, 10, 14, 17, and 21 days after surgery. Human MSCs were able to reduce pain like behaviours, such as mechanical allodynia and thermal hyperalgesia, once transplanted in cerebral ventricle. Anti-nociceptive effect was detectable from day 10 after surgery (6 days post cell injection). Human MSCs reduced the mRNA levels of the pro-inflammatory interleukin IL-1ß mouse gene. Transplanted hMSCs were able to reduce astrocytic and microglial cell activation. Human mesenchymal stem cells were able to reduce premature senescence-associated neuronal suffering. Indeed, hMSCs were able to decrease the ß-galactosidase over-activation positive profiles in the cortex of SNI/hMSC-treated mice compared to SNI/vehicle mice.
Conclusions Despite over fifty years of research there are no valid treatments over time and neuropathic pain can be classified as an incurable disease without treatment. Mesenchymal stem cell therapy represents the new promising potential treatment for neuropathic pain relief.

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P 11

A tetrapeptide of dermorphin analogue produces an extremely potent antinociceptive effect in mice

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FYK-1258 is a newly synthetized analogue of dermorphin *N*-terminal tetrapeptide. We investigated the antinociceptive effect of FYK-1258 in the paw-withdrawal test in mice. Peripheral administration (s.c. or p.o.) of FYK-1258 produced potent antinociception with extraordinary durability. Antinociception induced by intracerebroventricularly (i.c.v.) administered FYK-1258 was about 1000-fold more potent than that of morphine. The antinociceptive effect of i.c.v. FYK-1258 was blocked by pretreatment with beta-funaltrexamine (40 mg/kg, s.c.) or naloxonazine (35 mg/kg, s.c.). Pretreatment with nor-binaltorphimine (4 nmol, i.c.v.) inhibited FYK-1258-induced antinociception, whereas morphine-induced antinociception was unaffected by the kappa-opioid receptor antagonist. In addition, pretreatment with antisera against alpha-neoendorphin markedly attenuated FYK-1258-induced antinociception. These results suggest that FYK-1258 may stimulate the distinct subtypes of mu-opioid receptors through the release of dynorphins. Especially, alpha-neoendorphin may be involved in the antinociceptive mechanism of FYK-1258.

New benzomorphan-based LP1 ligand as suitable mixed mop/dop receptors agonist for chronic pain treatment

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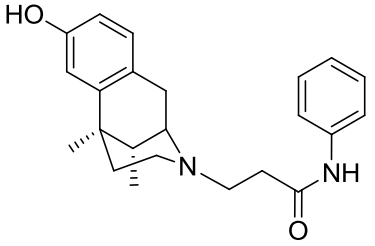
Successfull management of pain with opioid analgesics is based on use of a drug at its minimal effective dose with lower adverse effects incidence.[1] Powerful analgesics relieve pain primarily through agonism on mu-opioid peptide (MOP) receptor. Unfortunately, the clinical utility in chronic treatment of MOP receptor agonists, such as morphine, is limited by the development of tolerance and physical dependence. Recently, it has been observed that simultaneous MOP and DOP receptors activation produce analgesia with reduced tolerance.[2] So, the aim of this work was to investigate the tolerance-inducting capability of our new benzomorphan-based ligand LP1 which showed a mixed MOP/DOP receptors agonist profile in vivo and in vitro assays. In fact, in previous studies LP1 displayed a nanomolar affinity for MOP and delta-opioid peptide (DOP) receptors (K_i = 0.83 nM, K_i = 29 nM, respectively). Moreover, LP1 acted as a mixed MOP/DOP receptors agonist in adenylyl cyclase assay (IC_{50} = 4.8 nM, IC_{50} = 12.0 nM, respectively), and exhibited an analgesic potency similar to morphine in the tail flick test (ED_{50} 2.03 and 2.7 mg/kg for LP1 and morphine, respectively).[3] Here we evaluated the pharmacological effect of LP1 administered at the dose of 4mg/kg s.c. (100% antinociception in acute administration after 20min) twice per day for 9 consecutive days to Male Sprague-Dawley rats. Data obtained by the radiant heat tail flick test showed that LP1 maintained its analgesic profile until the ninth day, while the same experimental protocol with morphine (10mg/kg s.c. twice a day) triggered a strong tolerance effect by day 3. In conclusion, LP1 together with an analgesic potency similar to morphine in acute administration, displayed low incidence on the development of tolerance. Due to these findings, the mixed MOP/DOP receptors agonist LP1 seems to be an useful analgesic agent for chronic pain treatment.



LP1

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Essential oil of bergamot reduces pain behavior induced by formalin in mice

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The essential oil (EO) of bergamot (*Citrus Bergamia*, Risso) (Bergamot Consortium, Reggio Calabria) has been previously shown to interfere with mechanisms of synaptic plasticity and to be neuroprotective *in vitro* and *in vivo*. Also, several essential oils and natural substances are known to attenuate both inflammatory and neuropathic pain. However, no studies have investigated the anti-nociceptive properties of bergamot EO (BEO) in experimental models of pain. Aim of the present study was to investigate the effects of BEO on nociceptive behaviour in models of pain. To this end, we used the spinal nerve ligation (SNL) model and the formalin test as models of neuropathic and inflammatory pain, respectively. In the SNL model, the phytocomplex was administered (1ml/kg; s.c.) in a single daily injection, 1 hour before surgery and then once daily for 14 days in C57BL6 mice. Mechanical and thermal sensitivity were then assessed by the Von Frey's and Haregreaves' tests, respectively, up to 28 days after SNL. In the formalin test, C57BL6 mice received either an intraplantar or a subcutaneous EO injection (20µl/mouse) 15min before the intraplantar administration of formalin (5%, s.c., 20µl). Licking/biting behaviour was then monitored in 5min bins for the following 60min. Chronic BEO treatment did not reduce mechanical allodynia induced by SNL significantly, though a trend to reduction was evident at day 7. Previous work from our group has shown that chronic treatment with (-)-linalool, a major volatile component of BEO significantly attenuated mechanical allodynia at the same time point. This suggests that linalool may be the EO component responsible for this trend to reduction and other components of the phytocomplex may mask its anti-nociceptive properties. In the formalin test, BEO modified either one or both phases of the licking/biting behaviour test depending on the dose and route of administration used. In particular, for intraplantar administration BEO significantly reduced the first phase, with no effects on the second phase. Instead, the same dose of bergamot EO administered subcutaneously in the scruff of the neck reduced both the first and the second phase of the licking/biking behavior. 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. Our data suggest that BEO can modulate pain sensitivity possibly acting *via* two different mechanisms (peripheral and central). This natural substance could, therefore, be a useful adjuvant drug for pain treatment.
The experimental protocols were in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992).

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Abstracts UCADH Round Table (R1-R7)

Experimental models of neuropathic pain

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Chronic pain is a debilitating condition that reduces life quality of the afflicted individuals and has a dramatic economic impact on society. Our ability to treat chronic pain states, particularly neuropathic pain, is currently limited. Up to one-third of neuropathic patients do not benefit from currently available medications, and those who do usually achieve only partial pain relief.

Our understanding of the mechanisms underlying neuropathic pain remains limited. However, the development and characterization of a number of animal models that mimic some aspects of the pain reported by patients has contributed to the recent progress in understanding some of these basic mechanisms. Most of these models are based on the induction of injuries to peripheral nerves by either physical trauma or chemicals and are associated with typical pain behaviours. Behavioural tests offer useful endpoints by which pain and potential pain-relieving drugs can be assessed and characterized. Also, animal models are in general responsive to drugs shown to be active in human neuropathic pain and do not respond to drugs lacking of clinical efficacy. Recently, the search for novel analgesic drugs has been stimulated by the new progress in basic pain research driven by innovative molecular and cellular biology techniques. These techniques, together with the availability of a wide range of transgenic mice, allow the identification of genes potentially important in pain processing and the analysis of molecular mechanisms *in vitro* and *in vivo*. Despite some shortcomings and the ability to model only some features of the clinical condition, pain models represent a valuable tool in predicting the potential clinical efficacy of novel therapeutic strategies and in planning for clinical trials.

Finally, pain share similarities with other neurobiological processes, i.e. learning and memory, and it is an important model system for many still open questions in neurobiology. The integration of clinical observations and pharmacological methods with cellular and molecular techniques is instrumental to a better understanding and more successful management of pain.

Experimental models of headache

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Migraine is characterized by intense and throbbing unilateral or bilateral cranial pain associated with anorexia, nausea, vomiting, photophobia and phonophobia. Initially, the experimental models were based on the evaluation of the vascular and/or neurovascular components of migraine, while in recent years, molecular and genetic models have been also developed. All these models cannot replicate the multifaceted nature of this clinically heterogeneous disorder, nevertheless, *in vivo* and *in vitro* animal models of migraine have contributed, at several levels, to a better understanding of migraine pathophysiology as well as to develop novel antimigraine drugs. This is true for the simple models based on the study of the vasoconstrictive effect upon cranial vessels, for those focused on the study of presynaptic action on sensory nerve endings that inhibit the "neurogenic" inflammation", and for those based on the genetic engineering of mouse mutants expressing human migraine mutations. These latter have been developed to provide an understanding of familial hemiplegic migraine and possibly, by extrapolation, of more common migraine subtypes. In our experience, the most reliable and complete model of migraine is represented by the study of biochemical, neurophysiological and behavioural changes induced by nitric oxide (NO) or its precursors (NO donors). NO indeed has been proposed to play a crucial rule in the activation of the trigeminovascular system by activating perivascular sensory afferent nerve fibres in the meninges. An outstanding advantage of the NO model is that it is available also in the "human" version, which allows the comparison/integration of findings obtained in the pre-clinical and clinical settings.

Pharmacogenetics of migraine

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Serotonin 5-$HT_{1B/1D}$ ***agonists*** **(*triptans)*** are recommended as first-line therapy for migraine patients with moderate-to-severe attacks and in those patients with mild-to-moderate attacks that are not adequately controlled by other agents. In spite of the well-established ***efficacy*** and safety of ***triptans***, up to 40% of migraine patients do not respond to triptan treatment. Failure to have consistent responses after triptan administration is likely to be due to a variety of factors, including individual genetic differences. However, little information is currently available on the genetic basis for the variability in the therapeutic effects of triptans. In this respect, among the most interesting findings are associations with the polymorphic variants in the genes encoding the β-3 subunit of G protein (GNB3) and DRD2. The GNB3 C825T polymorphism has been associated with a splice variant with increased biological activity that ultimately enhances G protein signaling. Although this polymorphism has been recently associated to triptan response in cluster headache, it is not known whether it is also involved in the clinical response of migraine patients to triptan treatment. Furthermore, allele frequency and genotype distribution of the DRD2 NcoI polymorphism have been found significantly different in responders and non-responders to rizatriptan, yet given ***the small number of migraine patients examined***, ***these findings need to be replicated*** in larger studies. In an exploratory, naturalistic study we assessed the value of polymorphic variants in the GNB3 and DRD2 genes, chosen on the basis of previous positive association studies, as predictive factors for consistency in headache response to triptans in migraine patients. Furthermore, we also evaluated the predictive value of polymorphisms in serotonin transporter gene (SLC6A4), which had not been previously investigated. Results obtained do not support GNB3 C825T and DRD2 NcoI polymorphisms polymorphisms as genetic determinants of consistent response to triptans in migraine patients. In contrast, STin2 VNTR polymorphism of SLC6A4 and two other SNPs in strong linkage disequilibrium with STin2 VNTR have been found associated to consistently responses to triptans, either as single markers or in haplotypic combination. Our results provide a useful proof of pharmacogenetics in the field of headache research and suggests the importance of the serotonin transporter. However, larger prospective studies are needed to confirm our results and additional studies are required to elucidate the mechanism by which SLC6A4 gene affects clinical response to triptan treatment. In addition, polymorphic variants in genes encoding drug metabolizing enzymes and drug transporters could also be involved in the individual variability of triptan response and should be evaluated in future studies.

Neurophysiology of headache

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In patients with primary headache, the differential diagnosis is based on the history of the disease and, in particular, on the clinical characteristics of pain.
Although many neurophysiological examinations are of little or no value in the clinical setting, most of the tools have a vast potential for further exploring the pathophysiology of headaches and the effects of pharmacological treatment. Indeed, many aspects of headache pathophysiology have been made clear by neurophysiological studies.
In headaches, clinical neurophysiology studies evaluated the pathophysiology of the neural processing of a large series of stimuli, including visual, acoustic, somatosensorial and nociceptive, by which have been explored the functional state of cortical and sub-cortical neural structures involved in pain processing.
Several central dysfunctions, involving in particular cortex and brainstem structures, were documented by neurophysiological investigation in headache, especially in migraine, including the deficit of habituation to non-painful and painful stimuli processed at cortical and brainstem level during the interictal phase as well as the sensitization of the pain pathways at brainstem and spinal level during the pain phase.
More recently, in chronic form of primary headaches, neurophysiological studies permitted to identify a series of abnormalities in pain processing, including structural and functional abnormalities, that could be helpful to clarify the pathophysiology at the basis of the headache chronification.
In conclusion, neurophysiological testing has become a valuable tool for investigating brain excitability and nociceptive systems in headache disorders.

R 5

Pain clinic and networking in Calabria

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Pain is a devastating and widespread problem in Europe.

- Chronic pain strikes one in five (19%) adults across Europe
    - Prevalence is highest in Norway, Poland and Italy, where just over one in four adults report suffering from chronic pain
    - Prevalence is lowest in Spain, even so more than one in ten (11%) suffers from chronic pain
- Over one third of European households have at least one pain sufferer (chronic or otherwise).

Real Pain

- Two-thirds of chronic pain sufferers experience moderate pain, while one-third experience severe pain (as rated on a 1-10 scale).

A Long Term Problem

- Europeans with chronic pain have been suffering on average for 7 years, some for even longer than 20 (21%).
- A third of people are suffering chronic pain at all times – 24 hours a day, 365 days a year.
- A third of sufferers were so weighed down with pain that they didn't feel they could tolerate any worse.

Work and Social Life Ruined

Untreated chronic pain can leave sufferers' lives in ruins – impacting on their work and families and often causing depression.

- One in five chronic pain sufferers have lost a job as a result of their pain.
- Those employed were forced to take more than 15 days off work every year because of the pain.
- One in five chronic pain sufferers have been diagnosed with depression as a result of their pain
    - Spain has the highest rate of depression (29%) as a result of pain, followed by Norway (28%). The lowest rate is in Denmark (11%).
- Over 40% of sufferers report feelings of helplessness or inability to think or function normally.
- The problem of social isolation seems most acute in France where the greatest number of sufferers (39%) felt unable to discuss their pain with other people.

Pain Killer

- Over one in six European chronic pain sufferers feel their pain is sometimes so bad they want to die.

Doctor / Patient relationships

Despite advances in the management of chronic pain, many patients still suffer unnecessarily due to inadequate evaluation, assessment and monitoring.

- 62% of European patients are satisfied with the doctor who treats their pain. Satisfaction with doctors was highest in Belgium (78%) and lowest in Poland, where only 20% or people were "extremely" or "very satisfied".

- And yet almost two-thirds report that their medication is inadequate at times and only one in ten have been evaluated on a pain scale.
- Only 23% have ever been to a pain management specialist.
- 43% of chronic pain sufferers believe that their physicians are more focused on their illness then their pain.

Treatment of Pain

Chronic pain sufferers report that there is considerable room for improvement in the effectiveness of pain treatments.

- On average NSAIDs are by far the most frequently used non-prescription pain medication (55%), together with paracetamol (43%) and weak opioids (13%).
- Strong opioids (the strongest of all painkillers) are hardly used at all in Italy and Spain, whereas in Ireland, the UK and Denmark they are used more frequently (24%).
- Finland made the greatest use of alternative treatments with 91% of chronic pain sufferers using one or more treatment, while Spain had the lowest with just 56% making use of such treatments.

Lack of Information

- Magazines/newspapers and television are two leading sources of information on new pain treatments, with the internet not a widely used source. At 16%, pain sufferers in Denmark are the most likely to report using the Internet to learn about new treatments. (From: pain in europe)

In Italy, one in four people suffer from persistent pain / chronic.

This means that in Calabria about 250,000 people suffer from pain often misdiagnosed and consequently treated.

Male: 974.680 Female:1.023.372 Total: 1.998.052

Is taken into account the current situation of Medicine/Pain in Calabria. Given that in most hospitals there are Doctors, mainly mining anesthesia, which have felt the need to address this issue, quite far from being addressed adequately the requirements.

Currently there are different forms of approach to pain by the Calabrian public and private health facilities.

An overview made on the internet, shows how varied and diverse both supply.

The first Unit Complex (UOC) of Pain Medicine with inpatient admissions for the ordinary scheme and beds for day hospital was founded in 1999 at the ISTITUTO NAZIONALE DI RIPOSO E CURA PER ANZIANI (INRCA) of Cosenza, which is a Public Institute for Hospitalization and Care Scientific (IRCCS), affiliated and accredited with the NHS, recently reconfirmed by the Quality Management System certification.

Currently has two beds for ordinary wards and two beds for performance in Day Hospital. Here were made at the first neurostimulation systems for rear Spinal Cord (Spinal Cord Stimulation or SCS) neurolesions radiofrequency trigeminal nerve and a wide range of invasive methods of pain therapy. L 'UOC has a tool, also first in Calabria, to study thermal and vibratory sensory thresholds (Quantitative Sensory Testing or QST). Ambulatory activity is conducted through a single point of booking and after challenging the General Practitioner. We work in addition to writing two other executives in Anesthesiology and Intensive Care Specialists and three nurses, all dedicated full time to the management of pain. Recently he was appointed Research Center and Pain Management of the Elderly.

Another Simple Unit is allocated at the Hospital of Cosenza, specifically at the headquarters of "Mariano Santo". And 'directed by Dr Francesco Amato and relies on the cooperation of three professional nurses. We are running plants programmable pump for the pain and systems for the SA.
In the province of Catanzaro, the following are true.
University Magna Graecia: at the Department of Anesthesiology and Intensive Care directed by Professor Bruno Amantea, known national leader in pain medicine, and at the Department of Neurosurgery by Prof. Angelo Lavano, where they also performed invasive procedure.
Hospital Pugliese-Ciaccio Catanzaro: central pain therapy directed by Dr. Rosario Russo.
Nursing Home Villa del Sole, with units of anesthesia and pain therapy conducted by Dr. Gianfranco Rocca, former director of the Pain Therapy Unit of the Hospital Pugliese-Ciaccio.
The territory of the region is also different hospital clinics, whose activities are limited, in most cases, visits and therapies to infiltration and regional block and / or cord.

R 6

Pain and rehabilitation

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The complex relationship that bind pain, headaches and rehabilitation can be simplified in two main issues:

1) the role of rehabilitation in pain and headache treatment
2) the impact of pain and headache on rehabilitation treatment outcome in the different neurological diseases

1) There are defined evidence on efficacy of non-pharmacological treatment and physiotherapy in pain and headache, and in particular in Tension Type Headache (TTH).
Recently the European Federation of Neurological Society Task-Force defined the guidelines of non-pharmacological treatment in TTH. The available evidences suggest the efficacy of counseling in pain and tension type headache treatment, whereas there aren't defined evidences on behavioural and EMG biofeedback technologies. The physical therapy – postural treatment, mass-therapy, spinal manipulation, physical exercise, ultrasound therapy and TENS – reveal a more clear evidence of efficacy.
Some review drafts suggest that physiotherapy may be more efficacious than acupuncture in primary headaches, where the evidence is still contrasting in headache efficacy treatment.

2) The greater part of neurological diseases in the Rehabilitation Unit can present pain and headache conditions. In particular, the main diseases correlated to pain and headache are Stroke, mainly in post-acute phase, and Multiple Sclerosis. A multidisciplinary approach in rehabilitation team is necessary to face this complex problem, also considering the important impact of this condition on rehabilitation and pharmacological treatment and patient assistance approach.

Aging brain and pain

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By definition, aging represents the process of maturation and change over time within physical, social and psychological contexts. A variety of molecular, cellular, structural and functional changes occur in the brain during aging. Neural cells have the opportunity to respond to these changes adaptively through multiple mechanisms in order to maintain the integrity of nerve cell circuits and to facilitate responses to environmental demands. Otherwise, neuronal cells may succumb to neurodegenerative cascades underlying disorders such as Alzheimer's (AD) and Parkinson's (PD) diseases or others. A relevant role in this process is played by neurotrophic factors, which are essential for many aspects of nervous system function since they regulate the development, maintenance and survival of neurons and neuron-supporting cells such as glia and oligodendrocytes. Many evidence indicates that alterations in levels of neurotrophic factors or in their receptors can lead to neuronal death and contribute to aging as well as to the pathogenesis of diseases due to abnormal trophic support (such as neurodegenerative diseases and depression) and diseases of abnormal excitability (such as epilepsy and central pain sensitization). In age-related pain studies, it has been shown that the nociceptive response of the sympathetic system appears to be especially vulnerable to age and to cognitive decline and age and cognitive status seem to affect the pain response system independently from each other. Demented patients have been found to not only differ in their autonomic responses to noxious stimulation but also in their motor, facial, subjective as well as in their cerebral responses evaluated by fMRI. The neurotrophin family of neurotrophic factors are well-known for their effects on neuronal survival and growth. Over the past decade, considerable evidence has accumulated from both humans and animals that one neurotrophin, nerve growth factor (NGF), is a peripheral pain mediator, particularly in inflammatory pain states. and NGF-neutralizing molecules are effective analgesic agents in many models of persistent pain. NGF regulates the expression of a second neurotrophin, brain-derived neurotrophic factor (BDNF), in nociceptors. BDNF is released when nociceptors are activated, and it acts as a central modulator of pain.
The decline of neurotrophins in the central nervous system may be relevant for dictating the event of neurodegeneration and therefore changes in pain processing, whereas, an altered neurotrophin activity in the periphery may interfere with reduced responses to acute pain in the elderly as well as with central modulation of pain.

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