



Novel Pain Therapeutics: From Basic Research to Clinical Translation and Rehabilitation

Under the Auspices of

Italian Society of Pharmacology

Japanese Society of Pharmacology

Italian Society of Neurological Rehabilitation

A Collaborative Scientific Initiative of

The University of Calabria, Rende (Cosenza), Italy

The University Magna Graecia, Catanzaro, Italy

The Tohoku Medical and Pharmaceutical University, Sendai, Japan

Daiichi University of Pharmacy, Fukuoka, Japan

Sant'Anna Institute for Neurological Rehabilitation, Kroton, Italy

23rd-25th October, 2019

University Club, University of Calabria,

Rende, (Cosenza), Italy

(Book of abstract, Eds Rombolà L & Scuteri D)

PRELIMINARY PROGRAM

Wednesday 23rd October, 2019

Afternoon 17.00 - 19.00

Opening Ceremony

Meeting rationale, *Giacinto Bagetta* (Cosenza)

Wellcome address, *Gino Mirocle Crisci*, Rector of the University of Calabria (Cosenza)

Wellcome address, *Maria Luisa Panno*, Head of Department of Pharmacy, Health Science and Nutrition, University of Calabria (Cosenza)

Dr Giacomo Giovinazzo, General Manager, Department of Agriculture and Agrifood Resources, Handling Authority for PSR Calabria 2014-2020, Calabria Region (Catanzaro)

Avv. Ezio Pizzi, President of Unionberg & of Consortium for Protection of Bergamot (Reggio Calabria)

Opening Lectures

17.30 - 19.00

Chairpersons: **Shinobu Sakurada** (Sendai) / **Giacinto Bagetta** (Cosenza)

Hiroshi Nagase (Tsukuba) Design and synthesis of orexin receptor selective ligands and their pharmacology

Cristina Tassorelli (Pavia) At the edge of migraine therapy

Welcome Cocktail

Thursday 24th October, 2019

Morning Session 9.30 - 13.00

Basic Mechanisms of Pain

Chairpersons: **Diana Amantea** (Cosenza) / **Makoto Inoue** (Urbana-Champaign)

9.30 - 10.00 *Stefania Ceruti* (Milan) Purinergic mechanisms and glial cells

10.00 - 10.30 *Oliver J Dolly* (Dublin) SNAP-25 cleaving botulinum neurotoxins suppress cytokine-induced elevation of the surface content in DRGs of pain transducers, TRP/V1 and /A1 channels

10.30 - 11.00 *Sabatino Maione* (Naples) Role of glutamate and endocannabinoids in the plastic changes associated with neuropathic pain

Coffee Break

Basic Mechanisms of Pain

Chairpersons: **Luigi Antonio Morrone** (Cosenza) / **Tsukasa Sakurada** (Fukuoka)

11.30 - 12.00 *Shiro Kishioka* (Wakayama) Peripheral administration of $\alpha 4\beta 2$ nAChR agonist ameliorates neuropathic pain elicited by type 2 diabetes mellitus in mice

12.00 - 12.30 *Kengo Hamamura* (Fukuoka) Behavioural effects of continuous subcutaneous administration of bergamot essential oil in mice with partial sciatic nerve ligation

12.30 - 13.00 *Alberto Chiarugi* (Florence) Dexpramipexole blocks $Na_v 1.8$ sodium channels and provides analgesia in multiple nociceptive and neuropathic pain models

13.00 - 15.00 Lunch Break and Poster Discussion

Afternoon Session 15.00 - 18.30

Inflammation and Pain

Chairpersons: **Laura Berliocchi** (Catanzaro) / **Kosuke Aritake** (Fukuoka)

15.00 - 15.30 *Hirokazu Mizoguchi* (Sendai) Management of morphine-resistant inflammatory pain

15.30 - 16.00 *Tomonori Kaifu* (Sendai) A glycosylation enzyme ameliorates experimental autoimmune encephalomyelitis through increasing ligand for an inhibitory C-type lectin receptor

16.00 - 16.30 *Makoto Inoue* (Urbana-Champaign) The role of extra gonad-derived estrogen in neuroinflammation in multiple sclerosis mouse model

Coffee Break

Histamine in Pain and Itch

Chairpersons: **Patrizio Blandina** (Florence) / **Shinobu Sakurada** (Sendai)

17.00 - 17.30 *Tadahiko Nakamura* (Sendai) Behavioral changes related to increased brain histamine

17.30 - 18.00 *Lorenzo di Cesare Mannelli* (Florence) Histamine in pain modulation: a role for opioid tolerance management

18.00 - 18.30 *Mariaconcetta Durante* (Florence) Cross-talk between histamine H4 receptor and A3 adenosine receptor on CD4⁺ T cells in resolution of neuropathic pain states

Friday 25th October, 2019

Morning Session 9.30 - 13.00

Translational Research

Chairpersons: **Alberto Chiarugi** (Florence) / **Hirokazu Mizoguki** (Sendai)

9.30 - 10.00 *Giulia Magni* (Milan) Purple corn extract as adjuvant therapy for the prevention and treatment of trigeminal pain

10.00 - 10.30 *Serena Boccella* (Naples) Role of PEA in the hippocampal plasticity associated with neuropathic pain

10.30 - 11.00 *Giovanni Appendino* (Novara) Botanicals and Pain

Coffee Break

Translational Research

Chairpersons: **Paolo Tonin** (Kroton) / **Hiroshi Nagase** (Tsukuba)

11.30 - 12.00 *Tatsuro Kohno* (Sendai) Acetaminophen metabolite AM404 induces analgesia via TRPV1 receptors expressed on the c-fiber terminals in the spinal dorsal horn

12.00 - 12.30 *Francesca Guida* (Naples) Oral cannabidiol prevents allodynia and neurological dysfunctions in a mouse model of mild traumatic brain injury

12.30 - 13.00 *Roberto Russo* (Naples) TRPM8 modulators in pain perception

13.00 - 15.00 Lunch Break and Poster Discussion

Afternoon Session 15.00 - 18.30

Rehabilitation and Pain

Chairpersons: **Giorgio Sandrini** (Pavia) / **Shiro Kishioka** (Wakayama)

15.00 - 15.30 *Roberto De Icco* (Pavia) Vagus nerve stimulation and pain suppression in migraine

15.30 - 16.00 *Francesco Riganello* (Kroton) Role of the autonomic system in the vegetative status: from pain to consciousness

16.00 - 16.30 *Giorgio Sandrini* (Pavia) Italian consensus conference on pain in neurorehabilitation: evidence and recommendations in dementia

16.30 - 17.00 *Damiana Scuteri* (Cosenza) Neuropsychiatric symptoms of dementia: role of pain and essential oil of bergamot as a novel therapeutic

Coffee Break

Future Perspectives in Pain Research

Chairpersons: **Tatsuro Kohno** (Sendai) / **Oliver J Dolly** (Dublin)

17.30 - 18.00 *Kosuke Aritake* (Fukuoka) Inhibitor for hematopoietic prostaglandin D synthase accelerates wound healing

18.00 - 18.30 *Ernesto Fedele* (Genoa) Modulation of glutamate release by presynaptic NMDA receptors as a functional model to investigate pain mechanisms

18.30 Closing Ceremony

Presidents

Giacinto Bagetta
Maria Tiziana Corasaniti
Shinobu Sakurada
Tsukasa Sakurada
Giorgio Sandrini
Paolo Tonin

International Organizing Committee

Damiana Scuteri
Hirokazu Mizoguchi
Luigi A. Morrone
Chizuko Watanabe

Scientific Committee

Annagrazia Adornetto
Diana Amantea
Laura Berliocchi
Patrizio Blandina
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Stefania Ceruti
Alberto Chiarugi
Marco Cosentino
Oliver J Dolly
Ernesto Fedele
Hiroko Hagiwara
Livio Luongo
Sabatino Maione
Fumito Naganuma
Giovanni Nicotera
Giovanni Pugliese
Laura Rombolà
Rossella Russo
Assunta Tarsitano
Sonia Trombino
Syun Watanabe

Scientific Secretariat

Damiana Scuteri BSc Pharm, PhD
Department of Pharmacy, Health Science and Nutrition
University of Calabria
87036 Rende (CS) Italy
Phone/Fax 39 0984 493462
Email: damiana.scuteri@unical.it

Chizuko Watanabe BSc Pharm, PhD
Tohoku Medical and Pharmaceutical University
4-4-1 Komatsushima, Aobaku
Sendai, Miyagi 981-8558, Japan
Phone +81 222 344181 Fax +81 222 752013
Email: w-chizu@tohoku-mpu.ac.jp

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University of Calabria, Rende (Cosenza)

Department of Pharmacy, Health Science and Nutrition, University of Calabria Rende, (Cosenza)

Calabria Region (Catanzaro)

Bruno Farmaceutici S.p.A. (Rome)

Epitech Group S.p.A. (Padoa)

Meeting Venue

University Club at University of Calabria, Via Pietro Bucci 87036 Arcavacata di Rende, Cosenza. The International Airport of Lametia Terme (Catanzaro) is 60 Km from the University of Calabria. Further travelling information is available on the web (<https://www.unical.it/portale/index.cfm>).

Registration Fee

No fee is to be payed but a registration is needed to attend the meeting and this should be sent to the Scientific Secretariat.

Fellowships and Financial Assistance

A limited number of bursaries will be available upon request for supporting young researchers (PhDs and Post-Docs) attending the meeting. The request should be submitted to the Scientific Secretariat.

<https://pharmacologymeeting.wixsite.com/paintherapeutics>

Meeting Rationale

Chronic pain is a major health care problem that affects some 30%-50% of the world population and almost 20% of the Europeans actually suffers from chronic or intermittent pain; the latter results in suffering and disability for patients and increasing economic loss for society. In Italy, pain prevalence is estimated to be 21,7% and it is calculated that only 1/3 of patients receives pain relief from current analgesics, like nonsteroidal anti-inflammatory drugs (NSAIDs), local anaesthetics, some antidepressants and anticonvulsants, including carbamazepine and gabapentin, opiates. Advancements in the understanding of the mechanisms that produce pain have disclosed new potential therapeutic targets for the development of more effective drugs. For instance, validation of experimental models recapitulating allodynia contributed a great deal of precious information on the long-term plasticity changes occurring in pain transmission and modulation. Thus, changes of long-term potentiation (LTP) of excitatory synaptic transmission have been described to occur at spinal and supra-spinal central nervous system (CNS) regions. Likewise, at cellular level, changes in the activation of postsynaptic signaling pathways and of a host of genes and synthesis of new proteins, including some that can generate permanent structural changes, have also been reported. Release of soluble mediators by non neuronal cells might play an important role in initiating and modulating activity in primary afferent nociceptors; recently, the influence of CNS glial cells (including microglia, astrocytes and oligodendrocytes) on pain processing has also been recognized and investigated. The evidence gathered so far implicates that CNS glia, via modulating fundamental mechanisms of neuronal function and synaptic communication, can contribute to sensitization and pain-related behavior though its translational value to clinic remains to be established. Recent application of functional image analysis technique (fMRI) to the study of pain has proven extremely useful to the study of pharmacologic modulation of pain related brain activity in humans. Thus, it has been reported that, under conditions recapitulating sensitization occurring in neuropathic pain, gabapentin has a measurable antinociceptive effect and a stronger antihyperalgesic effect most evident in the brain areas undergoing deactivation, thus supporting the concept that gabapentin is more effective in modulating nociceptive transmission when central sensitization is present. The latter should also be considered an important example of how basic knowledge is nowadays rapidly translated into valuable therapeutic advancements. Indeed, in spite of the development of specific drugs for neuropathic pain, the treatment of severe pain relies on the use of opioids. Unfortunately, addiction, overdose and death from opioid prescriptions and the development of diversion and illicit activities still characterize opioid use; the latter disorders may stem from inappropriate prescription (see CDC guidelines for treatment of pain unrelated to cancer) and limited pharmacodynamic and safety characteristics of opioids. Among the most disabling chronic pain conditions, one of the hardest challenges is represented by migraine. In this field, botulinum toxin has emerged as noteworthy pharmacological tool for the study of pain and the development of biotechnological drugs (e.g. mAbs) raised against the signalling of calcitonin gene related peptide (CGRP), reaffirming the fundamental role for basic research in drug discovery. Higher prevalence of pain is registered in aging (over 65 patients) since it predisposes to chronic conditions as musculoskeletal disorders, osteoporosis, osteoarthritis, diabetes, herpes zoster, frequent traumas, often accompanied by the development of chronic pain. Moreover, aging is characterized by slower healing and poorer recovery from acute injury. This problem becomes even more burdensome in patients suffering from dementia, due to the impaired communication capabilities which make pain often underdiagnosed and undertreated. Unrelieved pain accounts for the development in 40 to 60% of these patients of neuropsychiatric symptoms of dementia (BPSDs) like agitation; this is managed through off label use of atypical antipsychotics increasing (approx. 2 fold) the risk of mortality, whilst Cochrane meta-analyses of randomized clinical trials support the efficacy of some botanicals. Fundamental is the effort of the Italian consensus conference on pain in neurorehabilitation for the clinical management of chronic pain. Collectively, these and other subjects will form matter of discussion at

the forthcoming international meeting on “Novel Pain Therapeutics: From Basic Research to Clinical Translation and Rehabilitation” to be held at the University Club of the University of Calabria (Rende, CS, Italy). This collaborative meeting is organized in the frame of a scientific and academic agreement signed among the Tohoku Medical and Pharmaceutical University (Sendai, Japan), the Daiichi Pharmaceutical University (Fukuoka, Japan) and the University of Calabria (Rende, Cosenza, Italy); it will host some twenty scientists from several Universities of Japan, including Tokyo, Sendai, Wakayama, Fukuoka, that will join researchers of international caliber. The conference is organized under the aegis of the Italian and Japanese Societies of Pharmacology (SIF & JPS) and the Italian Society of Neurological Rehabilitation (SIRN).

ABSTRACTS

MAIN LECTURES
(L1 – L26)

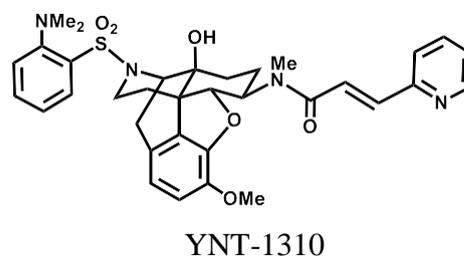
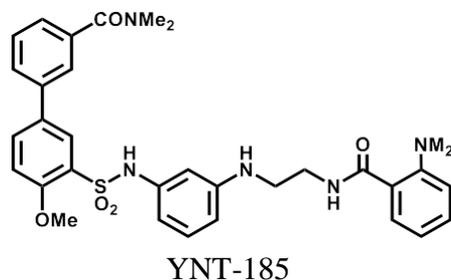
DESIGN AND SYNTHESIS OF OREXIN RECEPTOR SELECTIVE LIGANDS

Nagase H¹, Nagahara T², Yamamoto N¹, Yata M¹, Ohruai S¹, Okada T¹, Saitoh T¹, Kutsumura N¹, Irukayama Y¹, Ogawa Y¹, Goda H³ and Yanagisawa M¹

¹International Institute for Integrative Sleep Medicine (WPI-IIIIS), University of Tsukuba, Tsukuba, Japan; ²School of Pharmacy, Kitasato University, Tokyo, Japan; ³School of Pharmacy, Showa University, Tokyo, Japan

Orexin is a pair of lateral hypothalamic neuropeptides for orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R). An essential role of orexin system is regulation of sleep and wakefulness. Many researchers have attempted to develop non-peptide orexin antagonists to evaluate the role of orexin receptors (OXRs), especially focused on sleep indications. Many selective OX1R and OX2R antagonists have been reported. Quite recently, Merck released suvorexant in Japan and United State for insomnia. However, no orexin agonist has been reported until recently. In 2015, we reported the design and synthesis of the first non-peptide OX2R agonist, YNT-185 which showed potent and selective agonistic activity for OX2R (EC₅₀ = 28 nM, OX1R/OX2R ratio = 100) (1). The icv and ip injection of YNT-185 increased wake time to 53 min and showed improvement of narcoleptic symptom in mouse narcolepsy model. Furthermore, we discovered the antagonistic activity of nalfurafine (k opioid agonist) for OX1R (K_i = 250 nM). The K_i value was improved by the modification of the 3-hydroxy, 6-amide and 17-alkyl groups in nalfurafine to afford YNT-1310 (K_i = 1.36 nM for OX1R, >10,000 nM for OX2R). YNT-1310 attenuated the physical dependence of morphine (2).

We will report the design, synthesis and pharmacological effects of OX2R selective agonist YNT-185 and OX1R selective antagonist YNT-1310.



1. Nagahara T et al, (2015) J Med Chem 58: 7931-7937;
2. Nagase H et al, (2017) J Med Chem 60: 1018-1040.

AT THE EDGE OF MIGRAINE THERAPY

De Icco R, Sances G, Sandrini G and Tassorelli C

Headache Science Centre, IRCCS Mondino Foundation, Pavia, Italy; Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

Migraine is more than a headache, as it represents a complex neurological disorder that affects multiple cortical, subcortical, and brainstem areas that regulate autonomic, affective, cognitive, and sensory functions. The trigeminovascular system plays a pivotal role in migraine pathophysiology, being the final effector of a cascade of events that, originated in the brain, lead to the activation of nociceptors innervating pial, arachnoid, and dural blood vessels. The central process of the trigeminal fibers terminates in the spinal trigeminal nucleus, a pivotal relay station from where nociceptive signals are transmitted to central structures (thalamus and cortex) that ultimately make the patients aware of migraine pain. The nociceptive innervation of intracranial vasculature and the meninges consists of C and A-delta axons containing vasoactive neuropeptides such as substance P and calcitonin gene-related peptide (CGRP) (1,2).

The available pharmacological tools for the prevention of migraine are far from satisfactory, as they have moderate efficacy, poor tolerability and, probably for these reasons, are underused (3).

CGRP has been identified as novel target for migraine prophylaxis and a new class of drug, namely anti-CGRP monoclonal antibodies (mAbs), has now become available (4).

Fremanezumab and Galcanezumab target directly the CGRP ligand, while Erenumab block the CGRP receptor (5).

In a handful of methodologically robust phase 3 studies, the efficacy of these drugs has been confirmed across the entire spectrum of migraine (episodic and chronic forms). Some features are noteworthy: 1) the improvement starts already after the first week of treatment; 2) the clinical response persists for several months (up to 6); 3) a subset of patients experience a 100% reduction in headache frequency (6-8).

Treatment with mAbs seem safe, considering that the reported adverse events are mild to moderate in severity and often limited to injection site reactions (9). Though the potential long-term effects and safety still need to be elucidated, mAbs targeting CGRP appear to be a solid pharmacological class for migraine prevention.

1. Nosedà R, Burstein R. Migraine pathophysiology: Anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain. *Pain*. 2013;154(SUPPL. 1):S44-S53. doi:10.1016/j.pain.2013.07.021;
2. Goadsby PJ, Holland PR, Martins-Oliveira M, Hoffmann J, Schankin C, Akerman S. Pathophysiology of Migraine: A Disorder of Sensory Processing. *Physiol Rev*. 2017;97(2):553-622. doi:10.1152/physrev.00034.2015;
3. Hepp Z, Dodick DW, Varon SF, et al. Persistence and switching patterns of oral migraine prophylactic medications among patients with chronic migraine: A retrospective claims analysis. doi:10.1177/0333102416678382;
4. Edvinsson L, Haanes KA, Warfvinge K, Krause DiN. CGRP as the target of new migraine therapies - Successful translation from bench to clinic. *Nat Rev Neurol*. 2018;14(6):338-350. doi:10.1038/s41582-018-0003-1;
5. Scuteri D, Adornetto A, Rombolà L, et al. New trends in migraine pharmacology: Targeting calcitonin gene-related peptide (CGRP) with monoclonal antibodies. *Front Pharmacol*. 2019;10(APR). doi:10.3389/fphar.2019.00363;
6. Dodick DW, Silberstein SD, Bigal ME, et al. Effect of Fremanezumab compared with placebo for prevention of episodic migraine a randomized clinical trial. *JAMA - J Am Med Assoc*. 2018;319(19):1999-2008. doi:10.1001/jama.2018.4853;
7. Rosen N, Pearlman E, Ruff D, Day K, Abraham J, Nagy J. 00% Response Rate to Galcanezumab in Patients With Episodic Migraine: A Post Hoc Analysis of the Results From Phase 3, Randomized, Double-Blind, Placebo-Controlled EVOLVE-1 and EVOLVE-2 Studies. 2018. doi:10.1111/head.13427;
8. Goadsby PJ, Uwe R, Hallstrom Y, et al. A controlled trial of erenumab for episodic migraine. *N Engl J Med*. 2017;377(22):2123-2132. doi:10.1056/NEJMoa1705848;
9. Lattanzi S, Brigo F, Trinka E, et al. Erenumab for Preventive Treatment of Migraine: A Systematic Review and Meta-Analysis of Efficacy and Safety. *Drugs*. 2019;79(4):417-431. doi:10.1007/s40265-019-01069-1.

PURINERGIC MECHANISMS AND GLIAL CELLS

Ceruti S

Department of Pharmacological and Biomolecular Sciences (DiSFeB), Università degli Studi di Milano, Milan, Italy

In the last decade, the hypothesis that non-neuronal cells, including immune (macrophages and lymphocytes) and glial cells (namely, astrocytes and microglia in the central nervous system and satellite glial cells in sensory ganglia), cooperate with neurons to generate and maintain painful sensations has been clearly demonstrated. Indeed, although several painful conditions are undoubtedly of neuronal origin due to sensitization and increased neuronal firing, glial cells are fundamental to the transition from acute to chronic pain. In fact, the classical “neuron-centric” view of pain has progressively moved to a more integrated approach in which glial cells equal neurons in painful networks (1). This has prompted the search for the signaling pathways that drive glia reactivity to identify innovative targets to be pharmacologically modulated as analgesic strategies. The purinergic system has progressively emerged as one of the most important signaling system controlling both physiological and pathological conditions, including pain. In fact, both neurons and glial cells can modulate pain transmission by releasing pro- or anti-nociceptive mediators, among which a major role is played by ATP and other nucleotides, which bind to either fast ligand-operated P2X channels or to slowly-acting specific G-protein coupled receptors, the P2Y receptor family widely expressed throughout the body (2). Additionally, the rapid ATP breakdown generates high concentrations of adenosine. Far from being a simple “neuromodulator” as long believed, adenosine selectively activates 4 subtypes of P1 G protein-coupled receptors which promote opposite effects with respect to those exerted by ATP and other nucleotides; in most cases in the central nervous system, ATP is excitatory while adenosine is inhibitory on neuronal firing (3). This view has been progressively extended and integrated, with the discovery that adenosine receptors are widely distributed in all tissues and cells of the body (4), where they contribute to normal physiological functions but become even more important in pathologies where the local extracellular concentrations of nucleotides rise several folds, such as tissue damage, hypoxia, but also increased neuronal firing as in epilepsy or during chronic pain. The role of specific nucleotide and adenosine purinergic receptors in controlling neuron and glia functions and communication during acute and chronic pain condition has now been firmly established (e.g., neuronal P2X₃, microglia P2X₄ and P2Y₁₂ and astrocytic A₃ receptor subtypes, as well as several P2Y receptor subtypes expressed by satellite glia in sensory ganglia) (2). Nevertheless, despite several encouraging preclinical and some clinical studies, no purine-based analgesic has reached the market so far. The complexity of both the purinergic system and of the cellular networks controlling pain is one of the key issue which limits the discovery and development of effective purine-based painkillers. Only the synergic efforts of scientists in the field of medicinal chemistry, pharmacology, neuroscience, and system biology will be successful in the exploitation of the purinergic system to address the unmet needs of many pain patients in the future.

1. Ji RR et al., (2013) *Pain* 154 Suppl 1: S10;
2. Magni G, Ceruti S, (2019) *Brain Res Bull* 151: 132;
3. Chen JF et al., (2013) *Nat Rev Drug Discov* 12(4): 265;
4. Borea PA et al., (2018) *Physiol Rev* 98(3): 1591.

SNAP-25 CLEAVING BOTULINUM NEUROTOXINS SUPPRESS CYTOKINE-INDUCED ELEVATION OF THE SURFACE CONTENT IN DRGs OF PAIN TRANSDUCERS, TRP/V1 AND /A1 CHANNELS

Nugent M, Yusef YR, Lawrence G, Zurawski T, Meng J, Wang J and Dolly J O

International Centre for Neurotherapeutics, Dublin City University, Ireland

Transient receptor potential (TRP) vallinoid 1 (TRPV1) and ankyrin 1 (TRPA1) proteins are two transducing channels expressed on peripheral sensory nerves involved in pain sensation. Upregulation of their expression, stimulated by inflammatory cytokines and growth factors in animal pain models, correlate with the induction of nociceptive hyper-sensitivity. Herein, we demonstrate by immuno-cytochemical labelling that TNF α augments the surface content of these channels on cultured dorsal root ganglion (DRG) neurons from early postnatal rats and this, in turn, enhances the electrophysiological and functional responses of the latter to their specific agonists. A molecular basis underlying this TNF α -dependent enhancement was unveiled by pre-treating DRGs with a recently-published chimeric protein, consisting of the protease light chain (LC) of botulinum neurotoxin (BoNT) serotype E fused to full-length BoNT/A (LC/E-BoNT/A). This cleaves synaptosomal-associated protein of Mr 25k (SNAP-25) *in vitro* and was reported previously to exhibit anti-nociceptive activity in a rat model of neuropathic pain (1). Low pM concentrations of this chimera were found to prevent the TNF α -stimulated delivery of TRPV1/A1 to the neuronal plasmalemma and, accordingly, decreased their incremental functional activities relative to those of control DRG cells, an effect accompanied by SNAP-25 cleavage (2). Advantageously, LC/E-BoNT/A did not reduce the basal surface contents of the two channels or their pharmacological responses. Thus, use of multiple complementary methodologies provides evidence that LC/E-BoNT/A abolishes the TNF α -dependent augmented, but not the resting, surface trafficking of TRPV1/A1. As TNF α is known to induce nociceptive hyper-sensitivity *in vivo*, our observed inhibition by LC/E-BoNT/A of its action *in vitro* could contribute to its potential alleviation of pain.

1. Wang, J., Casals, L., Zurawski, T., Meng, J., Moriarty, O., Nealon, J., Edupuganti, O.P. and J Dolly, J.O. (2017) *Neuropharmacology*, 118: 223–232, Doi: 10.1016/j.neuropharm.2017.03.026;
2. Nugent, M., Yusef, Y. R., Meng, M., Wang, J. and Dolly, J. O. (2018) *Neuropharmacology*, 138, 257-266.

ROLE OF GLUTAMATE AND ENDOCANNABINOIDS IN THE PLASTIC CHANGES ASSOCIATED WITH NEUROPATHIC PAIN

Maione S

Department of Experimental Medicine, University of Campania "L. Vanvitelli", Naples, Italy

In the last decade there has been a growing interest in the role of N-acylethanolamines, like anandamide, N-oleoylethanolamide (OEA), N-palmitoylethanolamide (PEA) and N-linoleoylethanolamide (LEA), as lipidic transmitters in the Central Nervous System. PEA, in particular, has attracted much attention because of its capability of inhibiting inflammation and for having some neuroprotective effects through the stimulation of peroxisome proliferator activated receptor- α (PPAR α). In addition, PEA exhibits anti-allodynic and anti-hyperalgesic effects in chronic pain conditions by among other things, modulating the activity of microglia and mast cells. In this study we investigated the effect of a continuous PEA treatment (10 mg/kg, i.p., 15 days) on pain responses in the spared nerve injury (SNI) model in mice. Moreover, pain related-affective and cognitive behaviours have been evaluated. We found that PEA alleviated mechanical allodynia and thermal hyperalgesia and ameliorated cognition impairments and depression-like behaviour in SNI mice 1 or 10 months after injury. Interestingly, pain-related motor deficits or obsessive-compulsive-like behaviour, were not affected by PEA. In sham-operated mice, PEA increased the expression of mGlu1/5 whereas decreased mGlu7 and NR2b receptors. The mTOR pathway was also modified: pAKT and BDNF were increased or decreased, respectively. Accordingly, the PSD-associated proteins (Homers, Shank, etc) were also found modified. In SNI mice, the expression of mGlu1/5 and NR2b receptors were increased, and mGlu7 was decreased. The mTOR pathway was also modified: pAKT and pS6 were increased, BDNF resulted massively decreased. Accordingly, the PSD-associated proteins (Homers, Shank, etc) were also over-expressed in a way that the synapses were strengthened. In SNI mice the continued treatment with PEA normalized the expression level of NR2b and mGlu7, whilst the expression of mGlu1/5 receptors resulted even more over-expressed. Finally, we found the studied mTOR pathway proteins normalized. Our results suggest that N-acylethanolamines, including PEA, have a substantial action on glutamate-related synaptic signaling and provide a new strategy for the treatment of allodynia/hyperalgesia and the associated affective/cognitive impairments. Moreover, this study suggests that PEA physiologically modifies the metabolism of synapse, suggesting a tonic role of N-acylethanolamines and their receptors in controlling synapses.

PERIPHERAL ADMINISTRATION OF $\alpha 4\beta 2$ nAChR AGONIST AMELIORATES NEUROPATHIC PAIN ELICITED BY TYPE 2 DIABETES MELLITUS IN MICE

Kishioka S, Saika F and Kiguchi N

Department of Pharmacology, Wakayama Medical University, Wakayama, Japan

We previously reported that peripheral neuroinflammation, driven by inflammatory macrophages, plays a pivotal role in the pathogenesis of neuropathic pain in partial sciatic nerve ligation (PSL) mouse model. Moreover, the peripheral administration of $\alpha 4\beta 2$, but not $\alpha 7$, nicotinic acetylcholine receptor (nAChR) agonist could suppress inflammatory macrophages, being the amelioration of PSL-induced neuropathic pain (1). On the other hand, type 2 diabetes mellitus (T2DM) is a common metabolic disease, resulting in long-term complications associated with the dysfunction of nervous system, and more than 50% of patients eventually experience neuropathic pain (2). In this study, we determined whether inflammatory macrophage might participate in the T2DM-induced neuropathic pain, and the inhibition of the inflammatory macrophages by peripheral administration of $\alpha 4\beta 2$ nAChR agonist improved the T2DM-induced neuropathic pain in mice.

Neuropathic pain was characterized by mechanical allodynia and thermal hyperalgesia, which were evaluated by von Frey test and Hargreaves test, respectively. For the development of T2DM, C57BL/6J mice were fed by a high fat diet (HFD) or control diet *ad libitum*. In mice fed HFD, body weight rapidly increased, and hyperglycemia was observed on 4 weeks and persisted for at least 24 weeks during HFD feeding. The 50% paw withdrawal threshold, evaluated by von Frey test, was significantly decreased on 16 weeks in HFD feeding mice, indicating that the mechanical allodynia was evoked. The mRNA expression levels of macrophage markers (CD11b, CD68, and F4/80) were significantly upregulated in the sciatic nerve (SCN) on 16 weeks in HFD feeding mice compared to control mice, suggesting infiltration of macrophages in the SCN. Moreover, the mRNA expression levels of pro-inflammatory cytokines (IL-1 β and TNF α) and chemokine (CC-chemokine ligand 3 <CCL3> and CCL4) were also upregulated in the SCN. To eliminate macrophages, saporin-conjugated Mac-1 antibody (Mac-1 Sap) was locally injected around the SCN on 16 weeks of HFD feeding. Upregulated mRNA levels of macrophage and inflammatory mediator (cytokine and chemokine) markers were suppressed by Mac-1 Sap. HFD feeding-induced mechanical allodynia was also significantly improved by Mac-1 Sap. Next, we examined the effects of TC-2559, an $\alpha 4\beta 2$ nAChR agonist, on mechanical allodynia in HFD feeding mice. TC-2559 was injected into the surrounding of SCN on 4 consecutive days from 16 weeks of HFD feeding. The peripheral administration of TC-2559 attenuated the mechanical allodynia. In addition, systemic administration of TC-2559 according to same schedule also improved the mechanical allodynia in HFD feeding mice, indicating that the developed mechanical allodynia associated with T2DM was relieved by TC-2559.

These results suggest that inflammatory macrophages underlie neuropathic pain induced by not only peripheral nerve injury (PSL) but also T2DM, and that activation of $\alpha 4\beta 2$ nAChR located on inflammatory macrophage prevents both types of neuropathic pain. Further investigations, focusing on the other types of neuropathic pain, may be warranted to develop novel pharmacotherapy for neuropathic pain by $\alpha 4\beta 2$ nAChR agonist.

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BEHAVIOURAL EFFECTS OF CONTINUOUSLY ADMINISTERED BERGAMOT ESSENTIAL OIL (BEO) IN MICE WITH PARTIAL SCIATIC NERVE LIGATION

Hamamura K¹, Katsuyama S², Komatsu T³, Aritake K¹, Bagetta G⁴ and Sakurada T⁵

¹Laboratory of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Daiichi University of Pharmacy, Fukuoka, Japan; ²Center for Education of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan; ³Drug analysis laboratory, Faculty of Pharmaceutical Sciences, Daiichi University of Pharmacy, Fukuoka, Japan; ⁴Department of Pharmacy, Health Science and Nutrition, University of Calabria, Cosenza, Italy; ⁵Center for Supporting Pharmaceutical Education, Faculty of Pharmaceutical Sciences, Daiichi University of Pharmacy, Fukuoka, Japan

Neuropathic pain is an intractable chronic pain disease that is mainly caused by allodynia and is resistant to opioid analgesics. We previously reported that intraplantar (i.pl.) administration of bergamot essential oil (BEO) containing an aromatic compound significantly suppresses partial sciatic nerve ligation (PSNL)-induced mechanical allodynia via opioid mu receptors in mice (1). On the other hand, as represented by curry, it has also been reported that inhalation of a dilute aromatic compound has various effects such as appetite promotion (2). Therefore, we aimed to elucidate whether the anti-allodynic action of BEO is mediated by olfactory stimulation by volatile components. In this study, BEO was continuously administered with an osmotic pump immediately after PSNL, and the effects were examined pharmacologically.

PSNL was performed in mice according to the procedure described by our previous report (3). For sham-operation, the sciatic nerve was exposed, but not manipulated. BEO and naloxone, an opioid receptor antagonist, were continuously administered at a dose of 1mg/kg/7day and 5mg/kg/7day, respectively, using the osmotic pump (Alzet[®]: 1 week sustained type 0.5 μ L/hr) placed subcutaneously at the time of PSNL preparation. Animal behavior was analysed by using a double activity monitoring system (Shin Factory Co., Ltd.), which can detect two-dimensional planar motion in the cage with an infrared beam sensor and active motion with a running wheel.

When measured the two-dimensional planar activity, the number of counts in mice with PSNL was increased significantly only in the light phase (8:00 to 20:00) but not in the dark phase (20:00 to 8:00) from the second day after surgery. The increased counts produced by PSNL were abolished by continuously administered BEO. The effect of BEO on the two-dimensional planar counts in mice with PSNL was antagonized by naloxone. In the running wheel activity, the number of rotations was decreased by PSNL in the dark phase from the 8th day after surgery and abolished by BEO. The effect of BEO on the number of rotations was antagonized by naloxone. It is inferred that BEO components exhibit an anti-allodynic action, which is mediated by the opioid mu receptor but not by the olfactory system.

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DEXPRAMIPEXOLE BLOCKS $Na_v1.8$ SODIUM CHANNELS AND PROVIDES ANALGESIA IN MULTIPLE NOCICEPTIVE AND NEUROPATHIC PAIN MODELS

Muzzi M^a, Urru M^a, Coppi E^b, Ranieri G^a, Buonvicino D^a, Camaioni E^c, Coppini R^b, Pugliese AM^b, Tanaka B^d, Estacion M^d, Waxman SG^d, Dib-Hajj SD^d and Chiarugi A[#]

^aDepartment of Health Sciences, Section of Clinical Pharmacology and Oncology, University of Florence, Florence, Italy; ^b Department NEUROFARBA, Division of Pharmacology, University of Florence, Florence, Italy; ^cDepartment of Pharmaceutical Sciences, University of Perugia, Perugia, Italy; ^dDepartment of Neurology, Yale University School of Medicine and Veterans Affairs Medical Center, West Haven, CT, 06516, USA

Selective targeting of sodium channel subtypes has been highly investigated as an approach to development of new pain therapies. Among sodium channels, $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ are preferentially expressed by peripheral nociceptors, thereby representing a unique opportunity to develop analgesics devoid of central side effects. Several compounds that target $Na_v1.7$ and $Na_v1.8$ with different degrees of selectivity have been developed and are currently being tested in clinical trials for multiple pain indications. Among these chemicals, benzothiazole-like compounds emerged as potent sodium channel blockers. Here, we report that dexpramipexole, a benzothiazole-bearing drug with pleiotypic neuroactive properties and a good safety profile in humans, blocks TTX-resistant sodium conductances in cultured rat dorsal root ganglion neurons with an IC_{50} of 294.4 nM, suggesting selectivity towards $Na_v1.8$. In keeping with this, dexpramipexole does not affect sodium currents in DRG neurons from $Nav1.8$ null mice, and acquires binding pose predicted to overlap that of the $Na_v1.8$ channel-selective blocker A-8034637. The drug provides analgesia when parenterally, orally or topically applied in inflammatory and visceral mouse pain models, as well as in mice affected by neuropathic pain induced by oxaliplatin, nerve constriction or diabetes. Pain reduction in mice occurs at doses consistent with those adopted in clinical trials. Overall, the present findings confirm the relevance of selective targeting of peripheral $Na_v1.8$ channels to pain therapy. In light of the excellent tolerability of dexpramipexole in humans, our results support its translational potential for treatment of pain.

MANAGEMENT OF MORPHINE-RESISTANT INFLAMMATORY PAIN

Mizoguchi H, Watanabe C, Yoshizumi M, Aoki Y, Komiyama A and Sakurada S

Department of Physiology and Anatomy, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan

The antinociceptive effects of narcotic analgesics, morphine, fentanyl, oxycodone and methadone, in the inflammatory pain state were described using the complete Freund's adjuvant (CFA)-induced mouse inflammatory pain model. After an i.pl. injection of CFA, mechanical allodynia (decrease of mechanical threshold) in the ipsilateral paw was observed in the von Frey filament test. The antinociceptive effect of morphine injected s.c. and i.t. against mechanical allodynia was reduced bilaterally at 1 day after the CFA pretreatment. Like morphine, the antinociceptive effect of fentanyl and oxycodone injected s.c. against mechanical allodynia in inflammatory pain state was reduced bilaterally at 1 day after CFA pretreatment. However, the antinociceptive effect of methadone injected s.c. against mechanical allodynia in inflammatory pain state was slightly but significantly reduced unilaterally at 1 day after CFA pretreatment. The expression level of mRNA for μ -opioid receptors at 1 day after the CFA pretreatment was reduced bilaterally in the lumbar spinal cord and dorsal root ganglion (DRG). In contrast, the protein level of μ -opioid receptors at 1 day after CFA pretreatment was decreased in the ipsilateral side in the DRG but not in the lumbar spinal cord. Single or repeated i.t. pretreatment with the protein kinase C α (PKC α) inhibitor Ro-32-0432 completely restored the reduced morphine antinociception in the contralateral paw but only partially restored in the ipsilateral paw in the inflammatory pain state. In conclusion, reduced morphine antinociception against mechanical allodynia in inflammatory pain state is mainly mediated via a decrease in μ -opioid receptors in the ipsilateral side and via the desensitization of μ -opioid receptors in the contralateral side by PKC α -induced phosphorylation.

A GLYCOSYLATION ENZYME AMELIORATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS THROUGH INCREASING LIGAND FOR AN INHIBITORY C-TYPE LECTIN RECEPTOR

Kaifu T¹ and Iwakura Y²

¹*Division of Medicine, Tohoku Medicine and Pharmaceutical University, Sendai, Japan;* ²*Center for Animal Disease Models, Research Institution for Biological Sciences, Tokyo University of Science, Noda, Japan*

The C-type lectin receptors are a group of pattern recognition receptors that detect bacteria and fungi. They recognize a broad range of ligands, such as oligosaccharides, lipids, and proteins, and most of them activate a variety of physiological functions that protect a body from pathogen invasion. Dendritic cell immunoreceptor (DCIR) is a transmembrane type of C-type lectin receptors with a canonical signal motif for initiating inhibitory signals in the cytoplasmic region. DCIR is expressed on myeloid cells, like dendritic cells (DCs) and macrophages, and the structural insights were expected to exert inhibitory functions in the immune system. Indeed, *Dcir*^{-/-} mice spontaneously developed autoimmune-like diseases, such as sialadenitis, swelling and redness of joints. *Dcir*^{-/-} mice were high susceptible to experimental autoimmune diseases, like collagen-induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE) (1,2). In addition, *Dcir*^{-/-} mice not only developed aberrant cartilage and bone formation but also increased bone volume, accompanied with the higher number of osteoclasts and osteoblasts (3,4), showing that DCIR is a unique regulator for maintaining the homeostasis of the immune system and bone metabolism. In general, a ligand is important for exerting receptor functions but a functional ligand for DCIR remained unclear. We successfully identified asialo-saccharide as one of functional ligands, which is capable of initiating DCIR-mediated inhibitory signaling. We found that *in vivo* injection of enzyme, which cleaves terminal sugars and increases DCIR ligand, ameliorated clinical conditions of CIA and EAE, which are chronic inflammatory diseases caused by autoreactive T cells. The enzyme reduced T cell responses by regulating the functions of DCs. These data show that the interaction of DCIR with its ligand suppresses immune responses through regulating DC functions, and the disturbance of DCIR-mediated signal leads to a breakdown of homeostasis in the immune system and bone metabolism.

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THE ROLE OF EXTRA GONAD-DERIVED ESTROGEN IN NEUROINFLAMMATION IN MULTIPLE SCLEROSIS MOUSE MODEL

Khaw YM and Inoue M

Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Multiple sclerosis (MS) is a multifactorial autoimmune disease with both genetic and environmental etiologies. MS is characterized by neuroinflammation in the central nervous system (CNS), and an estimated 2.5 million individuals currently have MS worldwide. Patients experience a variety of neurological symptoms including chronic pain that differ in disease type (e.g. relapse-remitting and progressive) and severity (1), and MS is thought to be a disease of diverse subtypes characterized by distinct clinical presentation, immune signature, and reactivity and/or resistance to pharmaceutical treatments (1,2). Several etiological factors, such as exposure to Epstein-Barr virus, low vitamin D levels, stress, and changed balance of steroid hormone play a role in MS susceptibility and disease progression. We developed unique animal models for MS with and without CNS neurodegeneration (3). Using these models, we are investigating how several etiological factors are involved in MS using these mouse models. Here we will present how steroid hormone is involved in MS heterogeneity using experimental autoimmune encephalomyelitis (EAE), an animal model for MS. There is an inverse correlative relationship between gonadal endogenous estrogen levels and disease severity in MS and EAE. We also found that gonad-derived estrogen is involved in the disease *severity*. However it is *not* involved in disease *type* and MS *drug sensitivity*. Estrogen also synthesizes in extra-gonadal sites, such as lymph nodes (LNs), which are critical regions for the activation of immune systems during MS and EAE. We found that LN-derived estrogen is essential to determine the disease *type* and MS *drug sensitivity* as well as CNS *neuronal status* evaluated by newly developed microscopy technique (4). This is a first report on role of extra-gonad-derived estrogen in EAE, which is different from gonad-derived estrogen.

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BEHAVIORAL CHANGES RELATED TO INCREASED BRAIN HISTAMINE

Nakamura T^{1,2}, Naganuma F^{1,2}, Yoshikawa T², Okamura N^{1,2} and Yanai K²

¹*Division of Pharmacology, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai, Japan;* ²*Department of Pharmacology, Tohoku University Graduate School of Medicine, Sendai, Japan*

Histamine neurons are localized in the tuberomammillary nucleus (TMN) of the posterior hypothalamus and project their axons to various brain regions for regulating a wide range of physiological processes, such as sleep-wake cycle, food intake, body temperature, nociception, emotions, learning and memory (1). The dysfunctions of histaminergic nervous system in the CNS are identified in several brain diseases and disorders (2). For instance, histamine levels are decreased in autopsied brains of Alzheimer's disease (3) and in cerebrospinal fluid collected from narcoleptic patients (4). H₁ receptor binding is decreased in major depression (5). In addition, first generation H₁ antagonists induce sedation due to blockade of histamine signalling in the cortices (6). Based on these previous evidences, decreased histamine levels in the CNS need to be corrected to maintain brain functions. Furthermore, brain histamine levels higher than normal range might be protective against brain diseases and disorders. To address whether increasing brain histamine level is beneficial or not, we examined the behavioral changes related to increased brain histamine levels in rodents. We used four approaches to increase the brain histamine levels in rodents: i) oral histidine administration, ii) administration of H₃ antagonists, iii) knockout of histamine-*N*-methyltransferase (HNMT) and iv) specific activation of histamine neurons in the TMN. We found that i) oral administration of histidine, a precursor of histamine, increased brain histamine release and improved memory function (7); ii) JNJ10181457, an H₃ antagonist, ameliorated nociception in a neuropathic pain model; iii) HNMT, a histamine metabolizing enzyme in the CNS, knockout mouse exhibited abnormal sleep-wake cycle and high aggressive behavior related to extremely elevated brain histamine levels (8,9); iv) acute, regional and cellular specific activation of histamine neurons by DREADDs increased wakefulness and induced aggressive behavior. These results indicated extremely high histamine levels could be defined as another dysfunctional status of histaminergic nervous system but normal-high level of histamine in the CNS might have protective or therapeutic effects.

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HISTAMINE IN PAIN MODULATION: A ROLE FOR OPIOID TOLERANCE MANAGEMENT

Di Cesare Mannelli L

Department of Neurosciences, Psychology, Drug Research and Child Health - Neurofarba – Section of Pharmacology and Toxicology, University of Florence, Italy

In spite of the potency and efficacy of morphine, its clinical application for chronic persistent pain is limited by the development of tolerance to the antinociceptive effect. The cellular and molecular mechanisms underlying morphine tolerance are complex and still unclear. Similarly to the condition of chronic pain, tolerance establishment is related to the involvement of the immune system. As a part of the innate immune component, glial cells and the release of glia-derived proinflammatory mediators have been suggested to play a role in the phenomenon. Nevertheless, the interaction of different immune cells should be considered; in particular, the mast cells and their key transmitter histamine are gaining increasingly relevance. Morphine favors mast cell degranulation, histamine possesses proalgesic effects and destabilizing properties on nociceptive neurons.

N-Palmitoylethanolamine (PEA), is an endogenous compound with antinociceptive effects able to reduce mast cells degranulation and glial activation. On this basis, 30 mg kg⁻¹ PEA was subcutaneously daily administered in morphine-treated rats (10 mg kg⁻¹ intraperitoneally, daily). PEA treatment significantly attenuated the development of tolerance doubling the number of days of morphine antinociceptive efficacy in comparison to the vehicle + morphine group. PEA prevented both microglia and astrocyte cell number increase induced by morphine in the dorsal horn, on the contrary the morphine-dependent increase of spinal TNF- α levels was not modified by PEA. Nevertheless, the immunohistochemical analysis revealed significantly higher TNF- α immunoreactivity in astrocytes of PEA-protected rats suggesting a PEA-mediated decrease of cytokine release from astrocyte.

PEA intervenes in the nervous alterations that lead to the lack of morphine antinociceptive effects, a possible application of this endogenous compound in opioid-based therapies is suggested.

CROSS-TALK BETWEEN HISTAMINE H₄ RECEPTOR AND A₃ ADENOSINE RECEPTOR ON CD4⁺ T CELLS IN RESOLUTION OF NEUROPATHIC PAIN STATES

Durante M¹, Lucarini L¹, Ghelardini C¹, Di Cesare Mannelli L¹, Thurmond R² and Masini E¹

¹Department of NEUROFARBA, Section of Pharmacology, University of Florence; ²Janssen Research & Development, San Diego, United States

Neuropathic pain is a severe clinical problem and a chronic debilitating condition very difficult to treat. Deregulation of adenosine signalling at the A₃ adenosine receptor subtype (A₃AR) contributes to the development of neuropathic pain states, suggesting A₃AR agonists as a novel approach for neuropathic pain management (1). In contrast to the limited therapeutic utility of A₁AR and A_{2A}AR agonists due to their cardiovascular side effects, the A₃AR agonists have advanced in clinical trials for non-pain indications and show a good safety profile. Moreover, the central histaminergic system has been implicated in the regulation of pain perception and recent studies demonstrate that H₄R agonists induce relief from painful peripheral neuropathy (2). Both A₃ARs and H₄R are expressed on CD4⁺ T cells and they mitigate chronic inflammation via an IL-10-mediated pathway.

The aim of the study is to investigate a possible synergistic action between A₃AR and H₄R signalling pathways in modulating neuropathic pain conditions.

Male WT and H₄R^{-/-} mice were subjected to Chronic Constriction Injury (CCI), a model of neuropathic pain, and were treated systemically with IB-MECA (1 mg/kg), a selective A₃AR agonist, as well as with a selective H₄R agonist, VUF 8430 (10 mg/kg), during peak of mechanical allodynia.

Our results demonstrate that IB-MECA treatment completely reverses neuropathic pain in WT mice; while, mice lacking of H₄R partially reverse mechanical allodynia in response to the A₃AR agonist's treatment. Adoptive transfer of CD4⁺ T cells isolated from WT donors restores the beneficial effects of A₃AR agonists in H₄R^{-/-} mice. To support the behavioral data, the plasma levels of different cytokines such as IL-6, IL-10 and TNF-α were evaluated by a bead-based multiplex immunoassay.

Our data suggest an involvement of H₄R in the mechanism of action of A₃AR agonists and selective stimulation of neuronal H₄R together with non-toxic doses of A₃AR agonists could have clinical relevance for the treatment of neuropathic pain.

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PURPLE CORN EXTRACT AS ADJUVANT THERAPY FOR THE PREVENTION AND TREATMENT OF TRIGEMINAL PAIN

Magni G¹, Marinelli A², Riccio D³, Lecca D¹, Milani C⁴, Ventura M⁴, Abbracchio MP¹, Tonelli C², Petroni K² and Ceruti S¹

¹Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; ²Department of Biosciences, Università degli Studi di Milano, Milan, Italy; ³Department of Health Science and Technology, Aalborg University, Aalborg (DK); ⁴Department of Chemistry, Life Sciences and Environmental Sustainability, Università degli Studi di Parma, Parma, Italy

Trigeminal pain is a highly debilitating condition whose pharmacological treatment still represents an unmet medical need. In the search of new therapeutic approaches, the pivotal role of a correct diet in promoting health is clearly emerging beyond drugs. Studies performed with different sources of anthocyanins (ACNs) showed that they can protect against several inflammation-related diseases (1), but very few data are available on pain syndromes, with no hints on TG pain. Additionally, accumulating evidence introduces the concept of “gut-brain axis” as a bidirectional signaling between the gut microbiota and the central nervous system (CNS), in both physiology and pathology.

With the present project we tested the role of an ACN-rich dietary supplement in an *in vivo* model of trigeminal sensitization. We utilized isogenic maize model foods: purple corn with increased ACN content, and yellow corn without ACNs as control, both provided as water-soluble granules (2). Dietary supplements were tested on a model of trigeminal sensitization *in vivo* based on the unilateral injection of Complete Freund’s Adjuvant (CFA) in the temporomandibular joint of male rats. Mechanical allodynia was measured by probing the orofacial skin regions with Von Frey filaments (3). The expression of the microglia/macrophages marker Iba1 was evaluated by immunohistochemistry. For the analysis of microbiota composition, the bacterial taxonomic profile was reconstructed from fecal samples by means of 16S rRNA profiling protocol (4).

Animals receiving water and yellow corn developed ipsilateral orofacial allodynia, maintained up to 72 hours, which was instead significantly reduced in rats that received purple corn. The effect was fully comparable to the anti-allodynic action exerted by acetylsalicylic acid (ASA) in animals drinking water and yellow corn. Moreover, purple corn extract was as effective as ASA in inhibiting the TG infiltration of Iba1+ macrophages in CFA-injected rats. Conversely, purple corn alone significantly reduced microglial activation in the brainstem, with no effect exerted by ASA. This latter result was confirmed *in vitro*, since the treatment of LPS-activated microglia with purple corn extract reduced the production of pro-inflammatory mediators and promoted a shift towards an anti-inflammatory phenotype (5). Finally, purple corn administration significantly modified the gut microbiota toward an anti-inflammatory taxonomic profile.

Based on results, we speculate that purple corn extract acts to prevent inflammatory pain through different cellular/molecular mechanisms that could also involve the gut-brain axis. Therefore, we foresee a possible application of ACN-rich dietary supplements as co-adjuvant to pharmacological treatments or as new preventive strategy against TG pain, aimed at reducing drugs dosage and side effects and improving patients’ compliance to therapy.

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ROLE OF PEA IN THE HIPPOCAMPAL PLASTICITY ASSOCIATED WITH NEUROPATHIC PAIN

Boccella S, Luongo L, Guida F, Belardo C, Iannotta M, Mazzitelli M, de Gregorio D, Romano R, Marabese I, Palazzo E, de Novellis V and Maione S

Department of Experimental Medicine, Section of Pharmacology "L. Donatelli", Università degli studi della Campania "Luigi Vanvitelli", via S.M. di Costantinopoli, 16. 80138, Naples, Italy

Patients with chronic pain exhibit increased anxiety, depression, and deficits in learning and memory. Long-term potentiation (LTP) in the hippocampus has received attention as the biological substrate at the base of learning and memory. The activation of cannabinoid receptors, is widely supported by recent studies on neuropathic pain management (1, 2, 3). There is evidence that palmitoylethanolamide (PEA) is able to reduce pain-related behaviors and to restore glutamatergic synapses homeostasis in the medial prefrontal cortex of neuropathic mice (4). In this study, to investigate the impact of chronic pain condition on the hippocampal synaptic plasticity and on the related behavioral responses, electrophysiological, behavioural and biochemical analysis were performed, in a murine model of spared nerve injury (SNI), 30 days post-surgery (5). Moreover, the possible neuroprotective effect of chronic treatment with PEA, was evaluated, in both wild-type and PPAR α -/- SNI mice. Our results showed, in 30 days SNI mice, a reduction of time spent in the target quadrant in Morris Water Maze test (MWM) and of recognition index in the Novel Object Recognition (NOR) test, as compared to the control group (Sham mice). Moreover, both neuropathic wild-type and PPAR α null mice showed either an altered spatial memory retention and an impairment of LTP in dentate gyrus (DG)- lateral entorhinal cortex (LEC) pathway (6). PEA chronic treatment (14 days) was able to improve memory deficits as well as to partially restore the LTP in the dentate gyrus in wild-type SNI mice but not in PPAR α /SNI null mice. Finally, PEA positively modulated the altered glutamatergic signaling together with neurogenesis in hippocampus in SNI mice. These results suggest that neuropathic pain negatively affect the limbic and cognitive functions, which may underlie the deficiency of LTP and memory. Moreover, it opens new perspectives for the possible use of natural compounds such as PEA for the treatment of neuropathic pain and its central behavioural sequelae.

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ABSTRACT NOT ARRIVED

ACETAMINOPHEN METABOLITE AM404 INDUCES ANALGESIA VIA TRPV1 RECEPTORS EXPRESSED ON THE C-FIBER TERMINALS IN THE SPINAL DORSAL HORN

Kohno T¹, Ohashi N² and Uta D³

¹*Department of Anesthesiology, Tohoku Medical and Pharmaceutical University, Sendai, Japan;* ²*Department of Applied Pharmacology, Toyama University, Toyama, Japan;*

³*Division of Anesthesiology, Niigata University, Niigata, Japan*

The widely used analgesic acetaminophen is metabolized to N-acylphenolamine (AM404), which induces analgesia by acting directly on transient receptor potential vanilloid 1 (TRPV1) or cannabinoid 1 (CB1) receptors in the brain. Although these receptors are also abundant in the spinal cord, no previous studies have reported analgesic effects of acetaminophen or AM404 mediated by the spinal cord dorsal horn. We hypothesized that clinical doses of acetaminophen induce analgesia via these spinal mechanisms. We assessed our hypothesis in a rat model using behavioral measures. We also used *in vivo* and *in vitro* whole-cell patch-clamp recordings of dorsal horn neurons to assess excitatory synaptic transmission. Intravenous acetaminophen decreased peripheral pinch-induced excitatory responses in the dorsal horn ($53.1 \pm 20.7\%$ of control), while direct application of acetaminophen to the dorsal horn did not reduce these responses. Direct application of AM404 decreased the amplitudes of monosynaptic EPSCs evoked by C-fibers stimulation ($58.3 \pm 10.3\%$ of control), but not those evoked by stimulation of ATM-fibers ($96.2 \pm 5.7\%$ of control). These phenomena were mediated by TRPV1, but not CB1, receptors. The analgesic effects of acetaminophen and AM404 were stronger in rats experiencing an inflammatory pain model compared to naïve rats. Our results suggest that the acetaminophen metabolite AM404 induces analgesia directly via TRPV1 receptors expressed on central terminals of C-fibers in the spinal dorsal horn and leads to conduction block, shunt currents, and desensitization of these fibers.

ORAL CANNABIDIOL PREVENTS ALLODYNIA AND NEUROLOGICAL DYSFUNCTIONS IN A MOUSE MODEL OF MILD TRAUMATIC BRAIN INJURY

Guida F

Department of Experimental Medicine, University of Campania "L. Vanvitelli", Naples, Italy

Neurological dysfunctions are the most impactful and persistent consequences of traumatic brain injury (TBI). Indeed, previous reports suggest that an association between TBI and chronic pain syndromes, as well as anxious-depressive behaviors, tends to be more common in patients with mild forms of TBI. At present, no effective treatment options are available for these symptoms. In the present study, we used a weight drop mild TBI mouse model to investigate the effect of a commercially available 10% Cannabidiol (CBD) oil on both the sensorial and neuropsychiatric dysfunctions associated with mild TBI through behavioral and biomolecular approaches. TBI mice developed chronic pain associated with anxious and aggressive behavior, followed by a late depressive-like behavior and impaired social interaction. Such behaviors were related with specific changes in neurotransmitters release at cortical levels. CBD oral treatment restored the behavioral alterations and partially normalized the cortical biochemical changes. In conclusion, our data show some of the brain modifications probably responsible for the behavioral phenotype associated with TBI and suggest the CBD as a pharmacological tool to improve neurological dysfunctions caused by the trauma.

TRPM8 MODULATORS IN PAIN PERCEPTION

Russo R

Department of Pharmacy, University of Naples, Federico II, Naples, Italy

Transient Receptor Potential Melastatin-8 (TRPM8) is a non-selective cation channel activated by cold temperature and by cooling agents (1). Several studies have proved that this channel is involved in pain perception (2). Although some evidences indicate that TRPM8 inhibition is necessary to reduce acute and chronic pain, it is also reported that TRPM8 activation produces analgesia (3). These conflicting results could be explained by extracellular Ca^{2+} -dependent desensitization that is induced by an excessive activation. Likely, this effect is due to phosphatidylinositol 4,5-bisphosphate (PIP2) depletion that leads to modification of TRPM8 channel activity shifting voltage dependence towards more positive potentials (4-5). This phenomenon needs further evaluation and confirmation, that would allow us to understand better the role of this channel and to develop new for controlling pain. We have tested several specific TRPM8 compounds, both antagonists and agonists, in acute and chronic animal pain models (male Sprague-Dawley rats and on CD1 male mice), after systemic administration and local application, to better understand the role of this receptor in pain perception.

The wet-dog shake test and the body temperature measurements have highlighted the antagonist activity on TRPM8 channel. Moreover, it has been shown that antagonism on this channel produced an analgesic effect in formalin-induced orofacial pain and in chronic constriction injury-induced neuropathic pain, demonstrating the involvement of TRPM-8 channel in these two pain models. On the other hand, results obtained with agonists suggest and confirm that TPRM8 channel is characterized by dual activity, intensive pain at the lowest doses and analgesic with at the highest doses, probability due to downregulation.

TRPM8 channel is strongly involved in pain: in particular, a selective antagonist of this channel modulates both acute and chronic pain pathway.

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VAGUS NERVE STIMULATION AND PAIN SUPPRESSION IN MIGRAINE

De Icco R, Sances G, Sandrini G and Tassorelli C

Headache Science Centre, IRCCS Mondino Foundation, Pavia, Italy; Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

A possible effect of vagus nerve stimulation in migraine was first suggested from clinical observations. Migraine patients who implanted vagus nerve stimulation for medically refractory epilepsy reported a significant improvement in the frequency and intensity of headache¹.

More recently, Non-invasive Vagus Nerve Stimulation (nVNS) has become practical through a portable device which activates the vagus through transcutaneous electrical stimulation at neck level.

In the PRESTO trial, a large randomized and sham controlled study, nVNS was superior to sham stimulation as acute therapy in migraine patients when pain-free responder rate, pain relief rate and consistency of response were considered². Moreover, the percentage of patients who did not require a rescue medication was significantly lower in nVNS treated patients³.

On the other hand, the PREMIUM trial, designed to test nVNS efficacy as preventive therapy in episodic migraine, proved to be negative for the primary outcome. Anyway, it is noteworthy that when only patients with a high adherence to treatment were considered, the reduction in migraine days was significantly higher in nVNS treated patients respect to sham stimulation⁴.

nVNS proved to be useful also in cluster headache. In particular, nVNS could be used as acute treatment in episodic cluster headache, and as preventive treatment in chronic cluster headache^{5,6}.

The mechanism of action of nVNS is largely elusive and likely multifactorial.

In animal models, nVNS suppressed cortical spreading depression frequency and speed propagation, and clearly elevates its threshold to evoke⁷. Moreover, nVNS reduced spontaneous and dural-evoked responses in the trigeminocervical complex⁸.

These mechanisms seem to be primarily mediated by inhibition of glutamate release in the pivotal relays of the trigeminovascular system⁹.

Finally, a direct effect of nVNS on the trigeminovascular system was demonstrated with fMRI in healthy humans¹⁰.

These effects are likely direct, via the vagal jugular ganglion projections to the trigeminal nucleus, and indirect, via the nodose ganglion bilateral projections to the locus coeruleus, the raphe nuclei, the thalamus and the cortex.

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ROLE OF THE AUTONOMIC SYSTEM IN THE VEGETATIVE STATUS: FROM PAIN TO CONSCIOUSNESS

Riganello F, Cortese D, Arcuri F and Tonin P

Department of Pharmacology, University of Lampedusa, Lampedusa, Italy

With the term Disorders of Consciousness (DOC) (1), we refer to a spectrum of clinical conditions characterized by prolonged impaired awareness following severe brain damage. In this context, patients in a Vegetative State, now also called Unresponsive Wakefulness Syndrome (UWS) (2), show no signs of awareness of self or their environment, hence their motor repertoire is restricted to reflexes. Nowadays, the gold standard to assess the level of consciousness is the clinical assessment, based on patients' behaviour/responsiveness. Because responsiveness represents only an indirect evidence of consciousness (i.e. the lack of responsiveness does not necessarily imply lack of consciousness) reliance on these behavioural markers presents significant challenges and may lead to misdiagnosis. Clinical studies have shown that up to 40% of patients with a diagnosis of UWS may, in fact, retain some level of awareness (3).

Research by advanced positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) techniques in UWS patients has documented stimulus- or condition-related regional brain activation reflecting retained connectivity in isolated networks (4). These findings are thought to be indicative of survived sensory, emotional and "cognitive" modular processing at varying levels of functional complexity also in absence of the integrative processes necessary to consciousness. In this frame, the concept of pain perception is relevant (5). To date, there is no univocal consensus of pain perception in patients with DOC (6). Neuroimaging studies have shown distinctive cerebral responses to noxious stimuli in patients with UWS and in Minimally Conscious State, however, these approaches are not always practical as they require a scanner, are expensive and are not suitable for all patients. Alternative approaches of investigation may provide further information and interpretation of consciousness status in patients with DOC.

The Central Autonomic Network (CAN) is a functional integrated model proposed to describe the Brain-Heart two-way interaction (7). According to this model, the Brain-Heart two-way interaction in DOC can be investigated by the entropy Heart Rate Variability analysis.

HRV represents the fluctuation in the time intervals between adjacent heartbeats and represents a non-invasive and robust method to analyse the Autonomic Nervous System (ANS) sympathovagal balance and, to some extent and indirectly, higher brain functions. Studies show that the HRV entropy reflects functional connectivity changes in the CAN (providing an indirect way to screen and monitor connectivity changes in this neural system), and can be useful to differentiate levels of consciousness and predict the recovery of patients (8,9).

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THE ITALIAN CONSENSUS CONFERENCE ON PAIN IN NEUROREHABILITATION: EVIDENCE AND RECOMMENDATIONS IN DEMENTIA

Sandrini G

Department of Brain and Behavior Sciences, IRCCS C. Mondino Foundation, University of Pavia Italy

Dementia represents one of the most important problem in particular in economically more developed countries, due to its relevance from epidemiological and socioeconomical point of view.

There are many millions of people suffering from dementia worldwide and the number continues to rise related to the progressive increase of the mean age of the population.

It has been evaluate that more than 80% of patients with dementia living in care homes regularly experience pain.

Among the most common conditions inducing pain in dementia are included musculoskeletal, gastrointestinal and cardiac diseases, as well genitourinary infections, pressure ulcers and oral pain. Neuropathic pain is also common in dementia.

Several studies evidenced that the use of pain medication and non pharmacological treatments are often inappropriate in these patients. This fact produces many consequences, including worsening of mental and physical impairment, inducing a significant negative effect on quality of life.

Assessment of pain is essential to ensure effective treatment and ongoing care.

The loss of ability to communicate, particularly in the later stages of the condition, is a crucial problem and to distinguish pain from other behavioral symptoms could be difficult in these patients.

Recently, Italian Society of Neurorehabilitation (SIRN) together other preeminent scientific societies promoted a consensus conference on pain in neurorehabilitation (1).

A specific topic was concerning this field and the experts evaluated the evidence in literature and made recommendations about assessment and treatment of pain in dementia.

Background and conclusions are reported in the presentation.

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NEUROPSYCHIATRIC SYMPTOMS OF DEMENTIA: ROLE OF PAIN AND ESSENTIAL OIL OF BERGAMOT AS A NOVEL THERAPEUTIC

Scuteri D¹, Rombolà L¹, Morrone LA¹, Bagetta G², Sakurada S³, Sakurada T⁴, Tonin P⁵ and Corasaniti MT⁶

¹Preclinical and Translational Pharmacology, Department of Pharmacy, Health Science and Nutrition, University of Calabria, 87036 Rende, Italy; ²Preclinical and Translational Pharmacology, Department of Pharmacy, Health Science and Nutrition, University of Calabria, 87036 Rende, Italy; ³Department of Physiology and Anatomy, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan; ⁴Daiichi College of Pharmaceutical Sciences-First Department of Pharmacology Fukuoka, Fukuoka 815-8511, Japan; ⁵Regional Center for Serious Brain Injuries, S. Anna Institute, 88900 Crotona, Italy; ⁶Department of Health Sciences, University "Magna Graecia" of Catanzaro, 88100 Catanzaro, Italy.

The cluster of behavioral and psychological syndromes that affect demented patients, known as neuropsychiatric symptoms of dementia, remarkably reduce the quality of life and represent the most serious issue aside memory loss. Indeed, some 40-60% patients resident in nursing homes presents these symptoms (1), treated with atypical antipsychotic drugs doubling the risk of death (2). Unfortunately, this attitude to inappropriate treatment of patients suffering from dementia is common in Italy and, also, in our regional real-world context. There is a tight link between the neuropsychiatric symptoms of dementia and unrelieved pain (3). Up to 80% of people with dementia suffers from pain (4) that is likely to arise from age-dependent comorbidities such as osteoarthritis, traumas, tumours, diabetic and post-herpetic neuropathy. Assessment of pain in these patients is very difficult because of impaired communication skills. Hence, diagnostic tools are needed for accurate evaluation of pain and a guideline for assessment of pain in severely demented patients is under evaluation at the Center for Clinical Excellence (Rome). Interestingly, aromatherapy with *Melissa officinalis* provides evidence for control of agitation, though this essential oil is endowed with limited analgesic efficacy and the clinical trial is downgraded by the lack of blindness. Another issue that weakens the use of aromatherapy is the instability of the components of the phytocomplexes hampering reproducibility and titration of the dose. The essential oil of bergamot (BEO) has been extensively studied demonstrating strong analgesic properties both in inflammatory and in neuropathic pain models (5). In order to overcome the above pitfalls of aromatherapy, BEO without furocoumarins has been loaded in a nanotechnological, smell-devoid, delivery system in the pharmaceutical form of a cream, currently under patent consideration. The nanotechnological cream of BEO confirms the analgesic activity of BEO in inflammatory and neuropathic pain models and it allows rigorous double-blind clinical trials.

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INHIBITOR FOR HEMATOPOIETIC PROSTAGLANDIN D SYNTHASE ACCELERATED WOUND HEALING

Aritake K¹, Hamamura K¹, Tanaka A² and Sakurada T¹

¹*Chemical Pharmacology Laboratory, Daiichi University of Pharmacy, Fukuoka, Japan;*

²*RIKEN Center for Life Science Technologies, Yokohama, Japan*

Prostaglandin (PG) D₂ is an allergic and inflammatory mediator produced by mast cells, Th2 cells, Langerhans cells, dendritic cells and microglial cells. PGD₂ activates 2 distinct types of receptor, DP1 and DP2 (CRTH2). PGD₂ causes contraction of airway smooth muscle via DP receptors and mediates the chemotaxis of leukocytes into the skin via DP2 receptors. Thus, PGD₂ coordinately regulates skin reaction such as allergic dermatitis, skin inflammation or wound, via these 2 receptors. PGD₂ is formed from arachidonic acid by successive enzyme reactions mediated by cyclooxygenase (COX) and PGD synthase (PGDS).

There are two distinct types of PGDS: one is lipocalin-type PGDS (L-PGDS); and the other, hematopoietic PGDS (H-PGDS). L-PGDS is localized in the central nervous system, male genital organs, and heart, and is involved in the regulation of sleep. On the other hand, H-PGDS is absolutely requiring glutathione and is localized in mast cells, Th2 cells, microglial cells, langerhans cells, and dendritic cells, and is involved in allergic and inflammatory reactions. Thus, selective inhibitors of H-PGDS are considered to be more useful to suppress allergic and inflammatory reactions without showing adverse effects that COXs inhibitors did.

We have already determined X-ray crystallographic structure of human H-PGDS complexed with co-factor, GSH and also human H-PGDS complex with GSH and a prototype of the H-PGDS inhibitor. Recently, we designed and synthesized a ligand for human HPGDS based on the coordinate of complex structures of human H-PGDS, GSH and prototype H-PGDS inhibitors.

In this present study, we show that newly synthesized compound is a specific inhibitor of human H-PGDS with higher affinity, higher selectivity and good biological availability. Enzyme assay using recombinant HPGDS protein, competitive binding assay using FITC-labeled inhibitor, and complexed structure of human HPGDS, GSH and compound revealed the compound to be a competitive inhibitor against PGH₂ and a non-competitive one against GSH. F-092 highly selectively inhibited the production of PGD₂ catalyzed by H-PGDS without affect the production of other PGs in cell lines. Interestingly, when orally administered, the compound accelerated the wound healing of skin in WT mice. The pharmacological effect of wound healing was also confirmed in H-PGDS gene knockout mice.

We proposed that PGD₂ catalyzed by H-PGDS accelerates wound inflammation and H-PGDS is a new target for the wound healing.

MODULATION OF GLUTAMATE RELEASE BY PRESYNAPTIC NMDA RECEPTORS AS A FUNCTIONAL MODEL TO INVESTIGATE PAIN MECHANISMS

Fedele E

Department of Pharmacy, Center of Excellence for Biomedical Research, Unit of Pharmacology and Toxicology, University of Genova; IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Glutamate is the major excitatory neurotransmitter of the CNS that signals through different membrane receptors. Among ionotropic ones, the NMDA receptor (NMDAR) has attracted the interest of neuroscientists since its discovery in the late 80's, due to its unique activation properties and functions in physiological and pathological conditions. As a matter of fact, this is the only receptor known to date that needs two agonists and membrane depolarization to be activated, making it the ideal coincidence sensor for the expression of synaptic plasticity phenomena which underlay important functions, such as learning and memory. In addition, it is well established that alterations of NMDARs lead to a variety of CNS pathological states, including pain.

All these functions have long been attributed to postsynaptic NMDA receptors. However, in the early 90's, it was shown that NMDA receptors are also localized presynaptically (PreNMDARs) onto nerve terminals where they modulate neurotransmitter release. Since then, using a variety of functional and imaging techniques, a large body of evidence has accumulated confirming the existence of PreNMDARs able to modulate both the spontaneous and the evoked release of different neurotransmitters, and that are increasingly involved in physiological and pathological conditions.

In particular, several pre-clinical studies have shown that PreNMDARs can be involved in opioid induced hyperalgesia and tolerance, as well as in central sensitization and the development of neuropathic pain by enhancing glutamate release in the dorsal horn of the spinal cord.

Synaptosomes (isolated nerve terminals) in superfusion certainly represents a method of choice to study neurotransmitter release and the modulation by presynaptic receptors. Using this technique, in our laboratory PreNMDARs have been confirmed to be present on spinal cord glutamatergic boutons where they enhance the spontaneous release of glutamate in a concentration-dependent manner. The pharmacological characterization revealed that these PreNMDARs are composed of GluN1 subunits lacking the N1 cassette, being very sensitive to acidic pH, GluN2A and GluN2B subunits, since the effect on glutamate release was antagonized by low concentrations of zinc and ifenprodil, respectively. Dimiracetam, a novel compound with potent analgesic properties in different models of neuropathic pain, was able to completely block the effect of PreNMDARs on the release of glutamate. Interestingly, it was found that dimiracetam is more potent in antagonizing PreNMDARs enhancing glutamate release in the spinal cord than those in the hippocampus.

Although more research is needed, there is increasing evidence suggesting that PreNMDARs can be suitable targets for the treatment of neuropathic pain.

POSTERS

THE ROLE OF GLYCOSPHINGOLIPIDS AND GLYCOSYLTRANSFERASES IN THE INFLAMMATORY PAIN

Watanabe S^{1,2}, Morita M^{1,2}, Abe M^{1,2}, Sugama S^{1,2}, Nomura N^{1,2}, Sagawa M^{1,2} and Tanabe M^{1,2}

¹Laboratory of Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan; ²Medicinal Research Laboratories, School of Pharmacy, Kitasato University, Tokyo, Japan

Glycosphingolipids are synthesized via step-by-step glycosylation of ceramides. Their structural heterogeneity is due to the great diversity in the carbohydrate chain moieties synthesized by various glycosyltransferases. In the first glycosylation step, galactose or glucose is attached to ceramide to form galactosylceramide or glucosylceramide. Gangliosides, sialyl glucosylceramide-based glycosphingolipids, are abundant in neural tissue and play important roles in cell-cell adhesion, signal transduction, and cell differentiation. Gangliosides are divided into four groups—sialo-, a-, b-, and c-series gangliosides—based on their biosynthetic pathway. Our previous studies reported that intraplantar injection of b-series but not a-series gangliosides produced mechanical allodynia and hyperalgesia. Ganglioside-induced hyperalgesia involves glutamate accumulation and NMDA receptor activation in skin tissues (1). Furthermore, *Arthrobacter ureafaciens* sialidase that degrades sialyl conjugates such as gangliosides reduced mechanical allodynia during inflammation induced by complete Freund's adjuvant (CFA) (2). Sialidase reduced elongated nerve fibers in epidermis of the inflamed skin tissue and neurite outgrowth of F11 cells (the dorsal root ganglion neuron derived cell line). These results indicated that gangliosides play important roles in hyperalgesia and allodynia; however, the regulation of glycosyltransferases involved in glycosphingolipid biosynthetic pathway during inflammatory pain is unclear.

To address this issue, we examined whether gene expression of six glycosyltransferases in spinal cord are altered during CFA-induced inflammatory pain. Several glycosyltransferase genes in spinal cord were upregulated one day after CFA injection. Next, we investigated whether intrathecal injection of glycosphingolipids synthesized from glycosyltransferases encoded by upregulated genes caused mechanical allodynia. B-series gangliosides and sulfatides (Galactosylceramide-based glycosphingolipids) led to allodynia. The mechanisms underlying sulfatide-induced allodynia are unknown; however, the glial activation inhibitors or TNF-alpha inhibitor decreased the effects of intrathecal injection of sulfatide. On the other hand, gene expressions had different patterns 1 day or 15 days after CFA injection, suggesting that different species of glycosphingolipids in the spinal cord are involved in the molecular mechanisms responsible for early and chronic inflammatory pain.

These results support the view that glycosphingolipids including b-series gangliosides and sulfatides modulate pain signaling in skin and the spinal cord during inflammatory pain.

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POSSIBLE INVOLVEMENT OF HISTAMINE ON NOCICEPTIVE BEHAVIORS INDUCED BY INTRATHECALLY ADMINISTERED CCK-8

Sakurada S¹, Hayashi T², Watanabe C¹, Katsuyama S³, Suzuki T² and Sakurada T⁴

¹Department of Physiology and Anatomy, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan; ²Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan; ³Central for Experiential Pharmacy Practice, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan; ⁴First Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan

The involvement of spinal release of histamine on nociceptive behaviors induced by CCK-8 was examined in mice. Intrathecal (i.t.) administration of CCK-8 produced dose-dependent nociceptive behaviors, consisting of scratching, biting and licking. The nociceptive behaviors induced 10 amol and 10 pmol were markedly suppressed by i.t. pretreatment with antiserum against histamine and were abolished in histidine decarboxylase-deficient mice. In histamine H₁ receptor-deficient mice, the nociceptive behaviors induced by CCK-8 were not abolished after treatment with 10 amol or 10 pmol CCK-8. The i.t. pretreatment with tachykinin NK₁ receptor antagonists eliminated the nociceptive behaviors induced by 10 pmol, but did not affect the nociceptive behaviors induced by 10 amol of CCK-8. On the other hand, the nociceptive behaviors induced by CCK-8 at both 10 amol and 10 pmol were suppressed by i.t. pretreatment with antagonists for NMDA receptor polyamine-binding site. The present results indicate that nociceptive behaviors induced by i.t. administration of CCK-8 are mediated through the spinal release of histamine and are elicited via activation of NMDA receptors.

A POTENTIAL NEW CELL THERAPY USING PERIPHERAL BLOOD MONONUCLEAR CELLS VIA QUALITY AND QUANTITY CONTROLLED CULTURE FOR CHRONIC ISCHEMIC PAIN

Hagiwara H^{1,2}, Tanaka R^{1,2}, Sakurada T³ and Mizuno H^{1,2}

¹Department of Plastic and Reconstructive Surgery, Juntendo University School of Medicine, Tokyo, Japan; ²Center for Genomic and Regenerative Medicine, Juntendo University School of Medicine, Tokyo, Japan; ³Center for Supporting Pharmaceutical Education, Daiichi University of Pharmacy, Fukuoka, Japan

Peripheral arterial disease (PAD), which causes a variety of severe systematic diseases such as cardiovascular disease, is gradually increasing as societies age. Especially, if the blood flow to the peripheral tissues becomes severely restricted in PAD, critical limb ischemia (CLI) can develop. The mortality rate of CLI is the same as that of some types of cancer. The treatment of CLI is a serious challenge. Resting pain of intractable wounds and ulcers are highly debilitating. The only options available are palliative care and amputation, and survival in the year following surgery is <50% (1). The development of more effective limb-conservation treatment and pain relief is thus needed to improve the quality of life of CLI patients. Many studies have investigated cell transplantation therapy using mesenchymal stem cells, CD34-positive cells, and mononuclear cells (MNCs) as means of vascular and tissue regeneration therapy. However, many problems remain unsolved. We recently developed a serum-free, ex vivo cell expansion system called the Mononuclear Cell Quality and Quantity Control Culture System (MNCQQc), using peripheral blood MNCs. The system increases the number and enhances the vasculogenic property of endothelial progenitor cells (EPCs) for enhanced vasculogenesis and tissue regeneration from a small amount of peripheral blood. We conducted a Phase 1 clinical study to investigate the safety of MNCQQc therapy for non-healing wounds, as part of the effort to develop a more practical, simple, minimally invasive, and effective vascular and tissue regeneration therapy in PAD patients, including CLI patients. We also discovered the analgesic effect of MNCQQ cell transplantation in the clinical study. While these and other data suggest that cell transplantation for limb ischemia has analgesic effects in murines and humans, the mechanisms underlying these effects remain unknown (2). In this study, we validated the analgesic effect of MNCQQc transplantation in mouse models of CLI featuring chronic ischemic pain and investigated the mechanisms of this effect. We generated six CLI mouse models because almost all models reported previously recapitulated acute limb ischemia. Two weeks after ischemia, MNCQQ cells or PBS were injected into mouse femur and lower leg. The nociceptive response was determined using the von Frey test and blood flow was measured by laser Doppler flowmetry one day to 2 weeks after cell transplantation. Protein and gene expression levels were quantified by western blotting and real-time PCR, respectively. MNCQQ cells lessened chronic ischemic pain in mice with CLI. The cells secreted various neuroregeneration-related cytokines and growth factors. The collective results indicate that the transplantation of MNCQQ cells may improve severe chronic ischemic resting pain in mice with CLI. We are investigating the mechanisms underlying the nociceptive response following MNCQQ cell transplantation in the mouse model. Further investigations will clarify the effect of MNCQQ cells on pain relief and neuroregeneration.

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NEUROTENSIN IN LATERAL HYPOTHALAMUS

Naganuma F¹, Nakamura T¹, Ramalingam V² and Okamura N¹

¹*Division of Pharmacology, Tohoku Medical and Pharmaceutical University, Sendai, Japan;*

²*Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA*

Neurotensin (Nts) acts as a neuropeptide in the central nervous system and is involved in various physiological events such as pain, locomotion, food intake and thermoregulation. The lateral hypothalamus (LH) comprises a large population of peptidergic neurons neurochemically defined by the presence of Nts. LH has been considered to be an important brain region for sleep-wake regulation. Previous studies reported that Nts neurons in LH (Nts-LH) heavily project to dopaminergic neurons in ventral tegmental area (VTA) and microinjection of Nts into the VTA increases locomotor activity. Thus, we hypothesized that Nts neurons may form the excitatory wake promoting cell group within the LH. In addition, hypothalamus plays a key role in thermoregulation. However, the molecular mechanism of thermoregulation remains unknown. Previous studies reported that ICV injection of Nts altered body temperature, which could be hypothesized that Nts-LH may also contribute to thermoregulation.

To test these hypotheses, we studied the functional roles of Nts-LH neurons in the regulation of sleep-wake cycle, locomotor activity (LMA) and body temperature (Tb), using chemogenetic and optogenetic technologies. First, we found that specific activation of Nts-LH neurons with chemogenetic method produced sustained arousal, higher LMA and hyperthermia. Additionally, optogenetic activation of Nts-LH neurons elicited rapid transition from non-rapid eye movement (NREM) sleep to wakefulness. On the other hand, selective chemogenetic inhibition of Nts-LH neurons attenuates the arousal, LMA and Tb responses to a psychological stress (in a novel environment) and augments responses to a physiological stress (in fasting condition). Finally, neuronal tracing study demonstrated that Nts neurons projected to various brain regions such as preoptic area, VTA, periaqueductal and locus ceruleus. These results indicated that Nts-LH neurons are capable of initiating and sustaining wakefulness and increasing Tb and LMA. Considering the involvement of LH in various physiological functions including sleep-wake, feeding and thermoregulation and close-interrelationship between these functions, our results suggest that Nts-LH neurons could play a crucial role in modulating sleep-wake states, LMA and Tb through the modulation of neuronal activities in the various projected brain regions in response to a variety of physiologic and metabolic demands.

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THE DEVELOPMENT OF BILATERAL PAIN UNDER THE INFLAMMATORY STATE

Watanabe C¹, Yoshizumi M¹, Hayashi T², Sakurada S¹ and Mizoguchi H¹

¹ Department of Physiology and Anatomy, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan; ² Department of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai, Japan

The unilateral peripheral injury or inflammation produces bilateral hypersensitivity to pain in the injured and uninjured sides. The abnormal phenomenon, called mirror image pain, is reported in clinical pain syndromes and in various animal pain models. However, its mechanism is not fully understood. In the present study, we investigated the mechanism of mirror image pain using the complete Freund's adjuvant (CFA)-induced inflammatory pain model and the capsaicin test. Chronic inflammatory pain was induced by injecting CFA into the plantar surface of the left hindpaw and mechanical allodynia was assessed by von Frey filament test. After CFA injection, the paw withdrawal threshold to mechanical stimuli decreased significantly in the ipsilateral paw (CFA injected paw) but not in the contralateral paw (CFA non-injected paw). The decrease in mechanical threshold was observed at 1 day and reached a peak at 3 days after CFA injection. On the other hand, capsaicin is a valuable pharmacological tool for investigating the physiological role of the sensory C-fiber neurons. Capsaicin produced nociceptive behaviors consisting of licking/biting toward the capsaicin-injected paw. Interestingly, in mice pretreated with CFA in the left hindpaw 3 days before, injection of a low-dose, not producing significant pain-related behaviors, of capsaicin in the right hindpaw induced remarkable pain-related behaviors against the left hindpaw (capsaicin non-injected paw). This phenomenon was dose-dependently attenuated by the administration of TRPV₁ receptor antagonist. Moreover, bilateral increase of the mRNA expression level of spinal TRPV₁ receptor was observed at 3 days after CFA pretreatment. These results suggest that the bilateral activation of TRPV₁ receptor under the inflammatory state induced capsaicin related pain in non-injected side.

AGEING AFFECTS NOCICEPTION IN C57BL/6 MICE

Scuteri D¹, Berliocchi L^{2,3}, Morrone LA^{1,3}, Corasaniti MT² and Bagetta G^{1,3}

¹Department of Pharmacy, Health and Nutritional Sciences, Section of Preclinical and Translational Pharmacology, University of Calabria, 87036 Rende, Cosenza, Italy;

²Department of Health Sciences, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; ³Centre of Neuropharmacology of Normal and Pathological Synaptic Plasticity, University Consortium for Adaptive Disorders and Head Pain, 87036 Rende, Cosenza, Italy

The effect of ageing on pain sensitivity and threshold is currently not well understood. The aim of this research was to study the influence of ageing on pain processing. Von Frey's (1), Pin-prick (2), Hargreaves' (3) and Acetone tests (4) have been performed and nociceptive behaviour has been assessed in the Formalin Test (5) in C57BL/6 mice belonging to different age groups and the molecular changes in the spinal cord have been investigated through western blotting. Both mechanical and thermal sensitivity stimuli progressively increased with ageing from 2 to 6 and 12 months of age, reaching a sort of *plateau* from 12 to 18 months. The nocifensive behaviour evoked by intraplantar injection of formalin resulted modified from the classical trend in aged mice with an extra peak and a varied amplitude of the response. A progressive decrease in the expression of Beclin-1, a marker of autophagy, known to be implicated in chronic pain (6), has been highlighted in spinal cord. The level of the L-type voltage-gated Ca²⁺-channel subunit $\alpha_2\delta$ -1 has shown an age-related bell-shaped trend of increase in the spinal cord, that could be responsible for the different nociceptive behaviour observed. These data provide relevant insights on the influence of ageing on nociception and, thus, for the treatment of chronic pain in the fragile population of the elderly.

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PHARMACOEPIDEMOLOGY EVIDENCE IN FAVOUR OF A REDUCED ACCESS TO PAIN TREATMENT IN PATIENTS ASSUMING ACETYLCHOLINESTERASE INHIBITORS AND MEMANTINE IN THE PROVINCIAL HEALTH DISTRICT OF COSENZA

Scuteri D¹, Piro B², Morrone LA¹, Corasaniti MT³, Vulnera M² and Bagetta G¹

¹Preclinical and Translational Pharmacology Section, Department of Pharmacy, Health Science and Nutrition, University of Calabria, Rende (Cosenza), Italy; ²Pharmacovigilance Territorial Service, Pharmaceutical Department, Provincial Health Authority, Cosenza, Italy; ³Department of Health Sciences, University Magna Graecia, Campus of Germaneto, Catanzaro, Italy

It is estimated that in Italy about one million people suffers from dementia, of which more than 50% suffer from Alzheimer's disease. Evidence suggests that up to 80% of people with dementia suffers from pain (1) that is likely to arise from age-dependent comorbidities such as osteoarthritis, trauma, tumors, diabetic and post-herpetic neuropathy. The cognitive impairment of these patients makes the diagnosis of pain extremely difficult, making it misunderstood and likely the basis of the behavioral and psychological disorder frequently treated with atypical antipsychotic drugs that increase the risk of death (2). This pharmacoepidemiological study aims at assessing the treatment of these fragile patients in the local context. The present preliminary investigation was conducted in the territory of competence of the Territorial Pharmacovigilance Service of Cosenza. Data concerned with the prescription of acetylcholinesterase inhibitors, memantine and analgesics in the province of Cosenza for the period 2014-2015 have been collected. The results obtained show a reduced access to pain treatment. In accordance with the data yielded, it is possible to conclude that patients with dementia have limited access to pain therapy in the Province of Cosenza and this, probably, depends on the lack of pain assessment and review of therapy. Therefore, programs for the diagnosis and assessment of pain are needed to improve the prescriptive appropriateness of analgesics for the treatment of moderate-severe chronic pain and to improve quality of life of the patient with dementia.

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INDEX OF AUTHORS

Abbraccio MP	L15
Abe M	P1
Aoki Y	L9
Arcuri F	L22
Aritake K	L7; L25
Bagetta G	P6; P7; L7; L24
Belardo C	L16
Berliocchi L	P6
Boccella S	L16
Buonvicino D	L8
Camaioni E	L8
Ceruti S	L3; L15
Chiarugi A	L8
Coppi E	L8
Coppini R	L8
Corasaniti MT	P6; P7; L24
Cortese D	L22
de Gregorio D	L16
De Icco R	L2; L21
de Novellis V	L16
Di Cesare Mannelli L	L13; L14
Dib-Hajj SD	L8
Dolly J O	L4
Durante M	L14
Estacion M	L8
Fedele E	L26
Ghelardini C	L14
Goda H	L1
Guida F	L16; L19
Hagiwara H	P3
Hamamura K	L7; L25
Hayashi T	P5; P2
Iannotta M	L16
Inoue M	L11
Irukayama Y	L1
Iwakura Y	L10
Kaifu T	L10
Katsuyama S	P2; L7
Khaw YM	L11
Kiguchi N	L6
Kishioka S	L6
Kohno T	L18
Komatsu T	L7
Komiyama A	L9
Kutsumura N	L1
Lawrence G	L4
Lecca D	L15

Lucarini L	L14
Luongo L	L16
Magni G	L15
Maione S	L5; L16
Marabese I	L16
Marinelli A	L15
Masini E	L14
Mazzitelli M	L16
Meng J	L4
Milani C	L15
Mizoguchi H	P5; L9
Mizuno H	P3
Morita M	P1
Morrone LA	P6; P7; L24
Muzzi M	L8
Nagahara T	L1
Naganuma F	P4; L12
Nagase H	L1
Nakamura T	P4; L12
Nomura N	P1
Nugent M	L4
Ogawa Y	L1
Ohashi N	L18
Ohrui S	L1
Okada T	L1
Okamura N	P4; L12
Palazzo E	L16
Petroni K	L15
Piro B	P7
Pugliese AM	L8
Ramalingam V	P4
Ranieri G	L8
Riccio D	L15
Riganello F	L22
Romano R	L16
Rombolà L	L24
Russo R	L20
Sagawa M	P1
Saika F	L6
Saitoh T	L1
Sakurada S	P2; P5; L9; L24
Sakurada T	P2; P3; L7; L25; L24
Sances G	L2; L21
Sandrini G	L2; L21; L23
Scuteri D	P6; P7; L24
Sugama S	P1
Suzuki T	P2

Tanabe M	P1
Tanaka A	L25
Tanaka B	L8
Tanaka R	P3
Tassorelli C	L2; L21
Thurmond R	L14
Tonelli C	L15
Tonin P	L22; L24
Urru M	L8
Uta D	L18
Ventura M	L15
Vulnera M	P7
Wang J	L4
Watanabe C	P2; P5; L9
Watanabe S	P1
Waxman SG	L8
Yamamoto N	L1
Yanagisawa M	L1
Yanai K	L12
Yata M	L1
Yoshikawa T	L12
Yoshizumi M	P5; L9
Yusef YR	L4
Zurawski T	L4

