UNIVERSITY OF CALABRIA

UNIVERSITY OF CATANZARO

ITALIAN SOCIETY OF PHARMACOLOGY

Monothematic Meeting

Sponsored by the Italian Society of Pharmacology on

CELLULAR AND MOLECULAR ASPECTS OF PHARMACOLOGIC CONTROL OF PAIN

Hotel Porto Pirgos, Parghelia (Vibo Valentia)

September 23rd, 2010

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Thursday, 23nd September

Invited Lecture

Chairperson: Giacinto Bagetta (Cosenza)

15.45-16.30 Silvana Gaetani (Rome)

Endocannabinoids, synaptic plasticity and pain control

Oral Communications

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

16.30-17.30 Communications (C 1 – C 5)

17.30-17.45 General discussion

17.45-18.00 Coffee Break

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

18.00-19.00 Communications (C 6 – C10)

19.00-19.15 General discussion

19.15-20.30 **Poster communications** (P 1 – P 13)

Oral Communications

C1) P Ambrosino, M V Soldovieri, D Viggiano, G Cacciola & M Taglialatela (Campobasso) Molecular mechanisms underlying intracellular calcium increases in peripheral sensory neurons (F11 cells) by the analgesic compound palmitoylethanolamide (PEA)

C2) S Watanabe, K Tan-No, T Tadano & H Higashi
(Sendai) Intraplantar injection of gangliosides produces nociceptive behavior and hyperalgesia

C3) R Lattanzi, D Maftei, G Balboni, S Salvadori & L Negri
(Rome) New non-peptide antagonists of the prokineticin receptors

C4) L Luongo, F Guida, C Giordano, D Siniscalco, L Cappellacci, E Palazzo, M Grifantini, V de Novellis, F Rossi, & S Maione (Naples) 5'-Chloro-Deoxy-(±)-ENBA, a novel A1 adenosine receptor agonist, alleviates neuropathic pain through functional microglial changes in mice

C5) H Watanabe, S Sakurada & G Bakalkin (Uppsala) Mu2-opioid receptors involve in the antinociceptive activity induced by Tyr-W-MIF-1 in the mouse spinal site

C6) M Maiarù, R Russo, A Levato, L Rombolà, H Mizoguchi, G Bagetta, MT Corasaniti & L Berliocchi (Catanzaro) Autophagy and neuropathic pain

C7) C Nozaki, C Gaveriaux-Ruff, D Reiss, A Matifas & B L. Kieffer (Illkirch) Conditional knock-out of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia

C8) T Hayashi, S Katsuyama, H Nakamura, T Suzuki & S Sakurada (Sendai) Antinociceptive mechanism of epibatidine analogue ABT-594 in mice

C9) F Pavone, S Marinelli, S Cobianchi, V Vacca & S Luvisetto (Rome) Botulinum toxins in animal models: innovative therapeutic approaches against pain

C10) S Franchi, A Rossi, E Borsani, L Rodella, D Ferrari, C Zaffa, A Vescovi, P Sacerdote, M Colleoni & A E Panerai (Milan) Mouse adult neural stem cells endovenous administration exerts a long lasting relief of neuropathic pain and re-establish a correct balance between pro- and antiinflammatory cytokines in the chronic constriction injury mouse model.

Poster communications

P1) Y Kobayashi, N Kiguchi, T Maeda & S Kishioka (Wakayama) Functional role of spinal ceramide in the development of neuropathic pain

P2) M Canestrelli, R Lattanzi & L Negri (Rome) The Bv8 mutant, [Ala^{24}]Bv8, is endowed with antihyperalgesic effect

P3) Y Tokunaga, H Mizoguchi, C Watanabe, A Yonezawa & S Sakurada (Sendai) Spermine-induced allodynia in mice hind-paw skin

P4) M Cipriano, D De Filippis, L Luongo, E Palazzo, M P Cinelli, S Maione & T Iuvone (Naples) Palmitoylethanolamide: control of hyperalgesia during chronic inflammation in vivo

P5) R Greco, A S Mangione, M Maccarrone, M Bolla, G Sandrini, G Nappi & C Tassorelli (Pavia) The relationship between nitroglycerin-induced hyperalgesia and levels of endocannabinoids – A therapeutic role for inhibitory hydrolases?

P6) Y Aoki, H Mizoguchi, C Watanabe, A Yonezawa & S Sakurada (Sendai) Changes in spinal m-opioid receptor on the inflammatory pain state

P7) C Parenti, G Aricò, G Ronsisvalle, O Prezzavento, L Pasquinucci & G M Scoto (Catania) The blockade of NOP receptor in the vlPAG reduces the development of tolerance to opioid-induced antinociception in rats

P8) S Marinelli, V Vacca, C Eleuteri, S Cobianchi, F Florenzano, S Luvisetto & F Pavone (Rome) Botulinum neurotoxin serotype A and morphine combination: a synergistic action on inflammatory and neuropathic pain

P9) Y Banya, H Mizoguchi, C Watanabe, A Yonezawa & S Sakurada (Sendai) Lack of the rewarding effect and locomotor-enhancing effect of m-opioid receptor agonist amidino-TAPA

P10) D Siniscalco, C Giordano, L Luongo, V de Novellis & S Maione (Naples) Human mesenchymal stem cells as novel neuropathic pain therapeutics

P11) K Okuyama, A Yonezawa, S Ogawa, M Hagiwara, T Morikawa, T Sakurada & S Sakurada (Sendai) A tetrapeptide of dermorphin analogue produces an extremely potent antinociceptive effect in mice

P12) L Pasquinucci, R Turnaturi, C Parenti, G Aricò, G M Scoto, Z Georgoussi, D-D Fourla & G Ronsisvalle (Catania) New benzomorphan-based LP1 ligand as suitable mixed MOP/DOP receptors agonist for chronic pain treatment

P13) A Levato, D Amantea, V Regio, H Mizoguchi, T Sakurada, D Rotiroti, G Bagetta, M.T. Corasaniti & L. Berliocchi (Cosenza) Essential oil of bergamot reduces pain behavior induced by formalin in mice

ABSTRACTS ORAL COMMUNICATIONS

(C1-C10)

C 1

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

P. Ambrosino[1], M. V. Soldovieri[1], D. Viggiano[1], G. Cacciola[1] and M. Taglialatela[1,2]

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

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

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

The possible involvement of PPARα activation in PEA-induced Ca^{2+} response was assessed using biochemical measurements of PPARα phosphorylation, indicative of an enhanced protein activation, and pharmacological tools such as PPARα activators (clofibric acid) and blockers (GW-6471). Immunofluorescence

experiments using a phospho-specific anti-PPARα antibody revealed that PEA induced an early (5-10 min) increase in PPARα phosphorylation. 30-sec exposure to clofibric acid (0.1-10 μM) induced a marked increase in $[Ca^{2+}]_i$, an effect largely abolished (>80%) by the PPARα selective antagonist GW-6471 (10 μM); more importantly, GW-6471 (10 μM) reduced by almost 70% PEA-induced $[Ca^{2+}]_i$ increase, suggesting that PPARα activation plays a major role in PEA-induced enhancement of $[Ca^{2+}]_i$.

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

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C 2

Intraplantar injection of gangliosides produces nociceptive behavior and hyperalgesia

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

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

New non-peptide antagonists of the prokineticins receptors

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Bv8, prokineticin1 (PK1) and prokineticin 2 (PK2) make up a new family of chemokines that lowers pain threshold and modulates immune responses. They activate two G-protein linked receptors (PKR1 and PKR2) in the central nervous system, dorsal root ganglia (DRG) and in cells participating to immune and inflammatory responses. In the animal model of complete Freund's adjuvant (CFA)-induced paw inflammation we brought evidence that the granulocyte-derived Bv8/PK2 is a major determinant in triggering inflammatory pain and we demonstrated that the non-peptide Bv8-antagonist, PC1 (a triazine derivative), selectively antagonizes Bv8-induced nociceptor sensitization and abolishes the inflammation-induced hyperalgesia (1). These promising results suggest that blocking Bv8/PK system might be a winning strategy to treat pain in other inflammatory pathologies which usually develop from early neutrophil infiltration and flow into a chronic pain perception such as neurophatic pain. In the mouse model of neurophaty, the sciatic nerve chronic constriction injury (CCI), the development of neurophatic pain involves not only neurons but also immune cells, Schwann cells, satellite cells in the DRG, spinal microglia and astrocytes which express prokineticins and their receptors.
Aim of this work is to evaluate, in this CCI model in mice, the antihyperalgesic effect of PC1 chronically administered in preventive and therapeutic schedule.
Sciatic ligation induced thermal hyperalgesia (Plantar test), that appeared in 3 days, and mechanical allodynia (Von frey filaments) that appeared 17 days after surgery. PC1 was administered subcutaneously 150 µg/kg s.c., twice/day for four days starting on day 3, in a group of mice, and on day 17 in another group. In all animals heat hypersensitivity and mechanical allodynia was evaluated in comparison with another group of neurophatic mice treated with saline. In mice (n=5), repeated systemic administration of PC1 from day 3 to 6 after surgery completely reverted thermal hyperalgesia bringing the nociceptive threshold of injuried paw (9.8 ± 0.9 sec.) toward that of contralateral non-injuried paw (10.2 ± 1.2 sec.). At the end of PC1 treatment, from day 5 to 30, thermal nociceptive hyperalgesia slowly reappeared after withdrawal of PC1 treatment but at levels significantly lower (7.5 ± 0.3 sec) that that of saline-treated mice (4.5 ± 0.3 sec). Interestingly, mice chronically injected with PC1 from day 3 to 6 after surgery,

never developed mechanical allodynia. Repeated systemic administration of PC1 from day 17 to 20, significantly reduced both thermal and mechanical hyperalgesia that reappeared after PC1 treatment withdrawal. In animal model of inflammation (CFA in the paw) chronic administration of PC1 150 µg/kg, s.c., twice/day from day 1 to day 4, completely abolished, in mice, the inflammation-induced thermal (Plantar test) and mechanical (Von frey filaments) hypernociception and reduced paw edema (Plethismometer) accelerating the recovery to normal pain value after the insult.

New synthesized non-peptide PKRs-antagonists (PC2-PC36) preinjected (-10 min) into the right paw of mice, antagonized the hyperalgesic effect (paw-immersion test, 48°C) of exogenous Bv8 (0.5 ng/paw). PC27 acted at doses 100 fold lower than the lead compound.

These data demonstrate that PKRs may represent a therapeutic target for the development of novel peripherally acting antinociceptive drugs.

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

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5'-chloro-deoxy-(±)-ENBA, a novel a1 adenosine receptor agonist, alleviates neuropathic pain through functional microglial changes in mice

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

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

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

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

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

C 5

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

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

C 6

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

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The contribution of spinal neuronal cell death to neuropathic pain has been investigated in several animal models, but it remains still controversial. Moreover, previous studies have exclusively focussed on mechanisms of apoptosis. Aim of this study was to investigate the occurrence of novel cell death mechanisms, such as autophagic processes, in the dorsal horn of the adult mouse spinal cord following nerve injury.
Spinal nerve ligation was performed on C57/BL6 mice (20-22g) according to Kim and Chung (Kim S.H. & Chung J.M., *Pain* 1992). Mechanical and thermal sensitivity were assessed by the Von Frey's and Haregreaves' behavioural tests, respectively. The expression and modulation of the two main autophagic markers, LC-3 and beclin-1, were investigated by western blot analysis in the dorsal horn of mice undergone either SNL or sham surgery.
No changes in beclin-1 and LC3 expression were detected 3 days after surgery. However, an increase in beclin-1 expression was observed 7 days following SNL in the L4-L5 portion of the spinal cord ipsilateral to the ligation. At the same time point, SNL promoted the appearance of LC3-II, the phosphatidylethanolamine-conjugated LC3 form indicative of increased autophagosomes formation. The increased expression of the markers was restricted to the spinal cord side ipsilateral to the ligation in SNL mice and was not present in mice undergone sham surgery. The increased expression of beclin 1 and LC3-II appeared to correlate with the upregulation of the calcium channel subunit $\alpha 2\delta$-1.
The present study indicates that degenerative mechanisms other than apoptosis may be activated and participate to the development of neuropathic pain.
The experimental protocols were in accordance to the guidelines of the Ministry of Health for animal care (D.M. 116/1992).

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Modulation of capsaicin-induced pain behavior by opioid receptors

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

Antinociceptive mechanism of epibatidine analogue ABT-594 in mice

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

C 9

Botulinum toxins in animal models: innovative therapeutic approaches against pain

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

C 10

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

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

present. The nerve IL-1 and IL-6 overexpression in NSC treated animals appear significantly reduced, while a slight increase of IL-10 production is observed.
Conclusion: The peripheral administration of NSC therapeutically reverses neuropathic pain in the CCI mouse model. We believe that a bidirectional interaction between NSCs and the lesioned-inflammed nerve is at the basis of the positive modulation of pain and inflammation.

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ABSTRACTS POSTER COMMUNICATIONS
(P 1- P 13)

P 1

Functional role of spinal ceramide in the development of neuropathic pain

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

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

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

P 2

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

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

administration of Bv8. I.t. preinjection (-5 min) of [Ala^{24}]Bv8 at 10 ng dose abolished the biphasic hyperalgesia induced by i.t. Bv8 (0.5 ng/rat).
In mice, systemic administration of [Ala^{24}]Bv8 (20 µg/kg, s.c.) abolished the thermal hyperalgesia and significantly reduced the tactile allodynia induced by ip.l injection of Bv8.
-In CFA-induced paw inflammation
In rats, systemic, s.c. and i.v., injection of [Ala^{24}]Bv8, dose-dependently abolished the CFA-induced mechanical hyperalgesia (paw-pressure test): the antihyperalgesic effect lasted for 8 h, 4 h and 3 h after the s.c. injection of 20, 5, and 2 µg/kg respectively. I.v. administration of [Ala^{24}]Bv8 (0.5 µg/kg) abolished hyperalgesia for 2 h.
In mice, [Ala^{24}]Bv8 at 20 µg/kg abolished the thermal hyperalgesia (paw-immersion test, 48°C) for more than 6 h.

Spermine-induced mechanical allodynia in the mouse hind-paw

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

P 4

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

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Introduction: Palmitoylethanolamide (PEA) belongs to the family of ALIAmides (*Autacoid Local Injury Antagonism Amide*), that exhibit local effects mainly through the control mast cell (MC) activation. MCs are immune-competent cells mainly localized in sites directly interfacing with external environment, where they orchestrate the inflammatory reaction by the release of pro-inflammatory mediators. PEA has been recently shown to reduce the progression of chronic inflammation, in a model of granuloma sustained primarily by MC activation in rats. Moreover, recent evidences indicate a bidirectional cross-talk between MCs and sensory nerves (SNs) suggesting that MCs and SNs may be functionally and anatomically assembled within certain tissues as the skin, where MCs are frequently co-localized near nerve fibers. Starting from the assumption that MC granules contain pro-inflammatory and pro-algogenic mediators, primarily Nerve Growth Factor (NGF), the present study addresses its attention to investigate whether PEA is able also to control granuloma-associated hyperalgesia in rat.
Materials and Methods: Granuloma, a typical chronic inflammation, was induced by subcutaneous implantation of two λ-carrageenin (1%)-soaked sponges on the back of male Wistar rats. PEA was injected into each sponge at the concentration of 200, 400, 800 μg/mL. The mechanical allodynia was evaluated by using the Von Frey filaments with calibrated bending forces, that were used to deliver punctuate mechanical stimuli of varyious intensity, in the middle of and around the granulomatous tissue; the frequency of withdrawals induced by consecutive applications of the same filament was evaluated, too. After 96 hours to the implantation, rats were sacrificed and the new nerves formation was evaluated in the granulomatous tissue by histological analysis. Western blot analysis for NGF and Protein Gene Product 9.5 (PGP 9.5) was conducted. In parallel, rat Dorsal Root Ganglia (DRG) were excised and transverse sections treated to perform immunohistochemical analysis, to evaluate citotypes involved in the granuloma-induced DRG sensitization. Obtained slides were incubated with primary antibody

solutions for the pro-inflammatory markers TNF-α, NGF and COX-2, co-labeled with TRPV1 and satellite cells marker.
Results: Our results show the analgesic properties of PEA, evaluated by its efficacy in reducing mechanical allodynia, by using the Von Frey filaments. Moreover, the histological image of granulomatous tissues evidenced the massive presence of degranulated MCs in tight contact with nerve fibres that were both significantly reduced by PEA. These data were confirmed by the reduction of the pro-inflammatory/pro-algogen mediators expression from MCs, in granuloma. Finally, we found that granuloma-induced mechanical allodynia was associated with an increased expression of COX-2, iNOS, TNF-α and NGF in rat dorsal ganglia (DRG), that were significantly reduced by PEA treatment.
Conclusions: The present data corroborate the evidence of an analgesic role played by PEA in several model of pain, recognizing in MCs the leading cell-type affected by PEA action in the model of carrageenin–induced granuloma in rats. Thus, according to our results it is conceivable to hypothesize the use of PEA and its congeners in the treatment of several painful conditions since by controlling MC degranulation and by modulating NGF release, PEA shows to act as a promising analgesic drug in chronic inflammation model.

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P 5

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

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Keywords: endocannabinoids, migraine, hydrolases, hyperalgesia

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

P 6

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

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

P 7

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

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

References

1) Scoto GM, Aricò G, Ronsisvalle S, Parenti C. Blockade of the nociceptin/orphanin FQ/NOP receptor system in the rat ventrolateral periaqueductal gray potentiates DAMGO analgesia. Peptides. 2007;28:1441-6.
2) Ueda H, Inoue M, Mizuno K. New approaches to study the development of morphine tolerance and dependence. Life Sci. 2003; 74:313-20.
3) Scoto GM, Aricò G, Iemolo A, Ronsisvalle G, Parenti C. Selective inhibition of the NOP receptor in the ventrolateral periaqueductal gray attenuates the development and the expression of tolerance to morphine-induced antinociception in rats. Peptides. 2010 Apr; 31:696-700.

P 8

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

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

P 9

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

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

P 10

Human mesenchymal stem cells as novel neuropathic pain therapeutics

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

P 11

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

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

P 12

New benzomorphan-based lp1 ligand as suitable mixed mop/dop receptors agonist for chronic pain treatment

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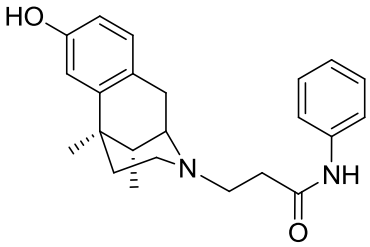
Successfull management of pain with opioid analgesics is based on use of a drug at its minimal effective dose with lower adverse effects incidence.[1] Powerful analgesics relieve pain primarily through agonism on mu-opioid peptide (MOP) receptor. Unfortunately, the clinical utility in chronic treatment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

the mixed MOP/DOP receptors agonist LP1 seems to be an useful analgesic agent for chronic pain treatment.



LP1

[1] H. L. Pan et al, *Pharmacol. Ther.* **2008**, *117*, 141. [2] S. S. Negus et al, *Eur. J. Pharmacol.* **2009**, *602*, 92. [3] L. Pasquinucci et al, *Bioorg. Med. Chem.* **2010**, *18*, 4975.

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

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

The subcutaneous administration of a lower dose in the scruff of the neck showed anti-nociceptive effect on the second but not on the first phase of the test. Our data suggest that BEO can modulate pain sensitivity possibly acting *via* two different mechanisms (peripheral and central). This natural substance could, therefore, be a useful adjuvant drug for pain treatment.

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

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