(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2006 (28.12.2006)

PCT

(10) International Publication Number
WO 2006/137106 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/IT2006/000488

(22) International Filing Date: 23 June 2006 (23.06.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
RM2005A000332 24 June 2005 (24.06.2005) IT

(71) Applicant *(for all designated States except US)*: **LAY LINE GENOMICS S.p.A** [IT/IT]; Via di Castel Romano, 100, I-00128 Roma (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PAVONE, Flaminia** [IT/IT]; c/o CNR Istituto di Neuroscienza, Via del Fosso di Fiorano 65, I-00143 Roma (IT). **MARINELLI, Sara** [IT/IT]; c/o Lay Line Genomics S.p.A., Via di Castel Romano, 100, I-00128 Roma (IT). **CATTANEO, Antonio** [IT/IT]; c/o Lay Line Genomics S.p.A., Via di Castel Romano, 100, I-00128 Roma (IT). **UGOLINI. Gabriele** [IT/IT]; c/o Lay Line Genomics S.p.A., Via di Castel Romano, 100, I-00128 Roma (IT).

(74) Agents: **CAPASSO, Olga** et al.; De Simone & Partners S.p.A., Via Vincenzo Bellini, 20, I-00198 Roma (IT).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2006/137106 A2

(54) Title: METHOD FOR THE POTENTIATION OF OPIOID ANALGESICS EFFECTS ON PAIN

(57) Abstract: According to the invention there is provided use of an anti-TrkA antibody capable of inhibiting the binding between NGF and TrkA combined with at least one opioid analgesic for the preparation of a medicament for treating and/or preventing pain.

Method for the potentiation of opioid analgesics effects on pain

BACKGROUND TO THE INVENTION

The present invention relates to molecules that are able to block TrkA activity for t potentiation of opioid analgesics effects on pain. In particular, the molecules are anti-Trk antibodies showing a potentiation of the analgesic effect of opioids such as morphine.

STATE OF THE ART

The nociceptive signals afferent to the spinal cord are carried by the fibres Aδ and C, t cell bodies of which (primary sensitive neurons) are located in the spinal dorsal gang (DRG). The primary sensitive neurons release glutamate together with ATP as excitatory neurotransmitter, and various other substances such as substance P and CGI (calcitonin-gene-related-peptide), (Hunt and Mantyh, 2001). The release of these excitat neurotransmitters is controlled by various classes of receptors present on the affer terminals including those sensitive to capsaicin (vanilloid receptors, VR1), those activa by GABA, those activated by ATP itself and those activated by cannabinoids (CI (Sivilotti and Nistri, 1991; Hunt and Mantyh, 2001; Khakh, 2001; Morisset et al., 200 One of the physiopathological whereby chronic pain occurs is allodynia, i.e. transformation of stimuli that are not normally painful into painful sensations. T phenomenon involves various ionic currents and therefore different channels of "ligand-gated" type, including the receptor for the capsaicin, VR1, and the ionotro receptors for ATP (Khakh, 2001). The simultaneous activation of the receptors for V and of those for ATP on spinal nociceptive interneurons generates a considera accumulation of the excitatory synaptic signals with reinforcement of the painful stimu transmission (Nakatsuka et al., 2002). From these observations it is therefore clear that ATP receptors (especially those belonging to the P2X3 class) play a fundamental role the pain pathways (Burnstock, 2001). These receptors are present on the peripheral ne terminals activated by algogenic stimuli, on the cell bodies of the neurons in the DRGs on the presynaptic terminals thereof, as well as on postsynaptic terminals in the spinal c (Khakh, 2001). There is considerable evidence showing an involvement of the ne growth factor (NGF) and its high-affinity receptor TrkA (Levi-Montalcini, 1987; L Montalcini et al., 1996; Frade and Barde, 1998; Kaplan, 1998) in the molecular proces underlying the main kinds of "persistent" pain, indicating a major therapeutic area (tha

pain, with particular reference to the "tonic" forms), for the antibodies which block NGF/TrkA system (Levine, 1998). The development of sensitive nociceptive neur depends greatly on NGF, and the responses of the adult nociceptors are modulated by same factor (Julius and Basbaum, 2001). In particular, NGF exerts acute sensitisation to capsaicin algogenic stimulus (Shu and Mendell, 1999). From a functional standpo nociceptive neurons, following chronic inflammation, develop alterations in the freque and duration of their action potential. These phenomena regress by blocking endogen NGF, leading to a significant attenuation of the hyperexcitability typical of states chronic pain (Djouhri et al., 2001). NGF action in defining the pain threshold in ac nociceptors is mediated by the TrkA receptor, also through modulation of the respo mediated by the VR1 receptor present on the nociceptive terminals. The TrkA depend potentiation of the VR1 response is thought to occur through the intracellular transduct pathway of the phospholipase C gamma (PLCgamma, Chuang et al., 2001). This ra TrkA mediated potentiation of nociceptor signalling and function could have an algoge effect in acute and chronic pain settings. The peripheral NGF levels are increased inflammatory processes, while the administration of exogenous NGF has a hyperalge effect on rats and produces muscular pain in humans. Furthermore, NGF produ hypersensitisation to heat stimulation in humans and mammals in general. NGF is releas by mast cells, fibroblasts and other cell types in the peripheral sites where inflammat processes occur. In particular, mast cells appear to play a fundamental role (Woolf et a 1996). As they produce NGF and at the same time express functional TrkA receptors their surface (Nilsson et al., 1997), they are able to respond to NGF itself, in the preser of lysophosphatidylserine (Horigome et al., 1993; Kawamoto et al., 2002). As a result, t NGF/TrkA system appears to mediate mastocyte activation through an autocrine positi feedback mechanism which allows local amplification of the algogenic inflammatc signal.

High levels of NGF are also found in neurons, where this neurotrophin is apparen responsible for the modifications of the nerve fibres, associated with pain (Harpf et a 2002). In certain forms of cancer, the excess of NGF facilitates the growth and infiltrati of nerve fibres with induction of oncological pain (Zhu et al., 1999). Recent experimen studies demonstrate how, by blocking NGF, it is possible to significantly reduce t formation of neuromas, responsible for neuropathic pain, without damaging the c bodies of the lesioned neurons (Kryger et al., 2001). These results generated significa

interest in therapeutic approaches based on the reduction of NGF effects for the treatn of acute and persistent pain (Saragovi and Gehring, 2000). In recent years, the involvem of the NGF/TrkA system in the molecular processes of pain transduction was a genetically demonstrated. In particular, mutations of the TrkA gene (localised on chromosome 1q21-q22) are responsible for a hereditary recessive autosomic syndrc known as CIPA ("congenital insensitivity to pain with anhydrosis"), characterised recurrent episodic fever, anhydrosis, absence of reaction to nociceptive stimuli, me retardation and a tendency to self-mutilation (Indo et al., 1996; Saragovi and Gehri 2000; Indo, 2001; Indo et al., 2001). Further confirmation of the involvement of NGF the nociceptive response was recently obtained by the inventors with the characterisatior anti-NGF transgenic mice phenotype (AD11). In these animals, the ectopic expressior the anti-NGF antibody αD11 produces a functional block of NGF in adult age. Such bl consistently translates into an increase in the latency time of the response to harmful h stimuli (Capsoni et al., 2000; Ruberti et al., 2000). Numerous evidence indicates the syst constituted by the nerve growth factor (NGF) and its high-affinity receptor TrkA a possible target for pain therapy. For this reason, antibodies capable of neutralising biological activity of the NGF/TrkA system by blocking the TrkA receptor may repres an important resource for pain therapy.

The authors of the present invention make use of antibodies (directed against the TrkA receptor) which are able to block the biological effects of NGF mediated by TrkA. The reagent MNAC13 is of particular interest.

The MNAC13 antibody is a mouse monoclonal antibody directed against the human Trl receptor (Cattaneo et al., 1999; Pesavento et al., 2000), particularly in the inhibition TrkA activation by NGF and the downstream biological functions, both *in vitro* and *in vi* (Cattaneo et al., 1999; Pesavento et al., 2000). Anti-TrkA antibodies, including t MNAC13 antibody, having an antagonist activity preventing the functional activation TrkA by NGF" are disclosed in EP 1.181.318. Derivatives of such antibody are al disclosed in WO2005/061540. However, the potentiation of the analgesic effect of opioi by such molecules is not disclosed.

The antibodies were characterised in detail from the point of view of the structı (Covaceuszach et al., 2001) and as for the molecular interaction with the TrkA recep (Covaceuszach et al., 2005). On the basis of such in-depth structural knowledge, by mea of an innovative method a humanised version of MNAC13 was generated (Hu-MNAC1

with the same antigen binding features as the parental antibody (patent applicat WO2005/061540).

The action of the MNAC13 antibody, as well as of the humanized MNAC13 antibc version (huMNAC13), was investigated in a classical model of persistent inflammat pain, i.e. the mouse formalin test (Porro and Cavazzuti, 1993). When animals undergo t test, two behavioral phases can be distinguished, in terms of pain response. The two pha are separated by an interval of a few minutes during which the response to the pain stimulus is mild or absent. Phase 1 is determined by direct stimulation of nocicepti terminals (by formalin), whilst phase 2 is due to the subsequent inflammation. The acti of the MNAC13 antibodies was compared to that of morphine, a traditional stro analgesic belonging to the opioid class, largely used to treat mild to severe pa (Przewlocki and Przewlocka, 2001), and the anti-NGF antibody αD11. Opioid analgesi include all active principles, natural or synthetic, whose action is similar to that morphine. Synthetic or semi-synthetic principles are derivatives of five chemical class (phenanthrenes, phenylethylamine, phenylpiperidines, morphinans, and benzomorphinan From the pharmacological point of view, they have different activities: they may be stro opioid receptor agonists (like morphine), moderate or weak agonists of the same receptc (like codeine), compounds with mixed antagonist/agonist activity (like nalbuphine), partial agonists (like nalorphine). Of all these compounds, morphine remains the mc largely used. However, despite its therapeutic properties, it has several side effec (sedative effect, nausea, vomiting, etc.). Moreover, morphine and the compoun belonging to this analgesic class show a fundamental disadvantageous characteristic that the development of tolerance and physical addiction. There is therefore an urgent need develop treatments using doses of this class of analgesics as low as possible, reducing th incidence of side effects and the probability to develop tolerance and or/addiction.

For this purpose potentiation of the analgesic effect of opioids was investigated, wi antibodies blocking the NGF/TrkA system.

The patent application WO2004/096122 describes a method for the treatment or th prevention of pain including the administration of an NGF antagonist and an opioi analgesic. The application refers, in particular, to an anti-NGF antibody and not to a antagonist of its receptor, TrkA.

SUMMARY OF THE INVENTION

The authors of the present invention have found that small amounts (below their effic threshold) of molecules able to block TrkA biological activity can potentiate the analge effect of opioids. Such combined therapy allows using a reduced opioid amount to obt the same level of pain relief that would be achieved with a higher opioid dose administered alone.

The object of the present invention is the use of an anti-TrkA antibody that is able inhibit the binding between NGF and TrkA combined with at least one opioid analgesic the preparation of a medicament for the treatment and/or the prevention of pain. Suital the antibody blocks the biological activity of TrkA i.e. is an antagonistic antibody.

A molecule that blocks the biological activity of TrkA refers to a molecule that acts as antagonist in terms of the NGF binding to the TrkA receptor, and which can be defined a synthetic molecule or a monoclonal antibody or a biological/synthetic derivative ther which:

i) binds to TrkA; and

ii) inhibits the binding of NGF to the "native" TrkA receptor expressed on surface of living cells; and

iii) blocks the biological activity deriving from NGF binding to the same Tr receptor.

The term "blocking the biological activity" does not simply mean blocking activation the receptor, defined as blocking the conversion process of the receptor itself into "active" state, but also the functional neutralisation of biological consequen downstream of the activation process: second messengers, new gene expressi phenotypic and functional modifications both at cell and system level. The molecule of invention is not only able to block TrkA in a classic *in vitro* test (test of neuritic growth PC12 cells), but also *in vivo* (functional block of the cholinergic neurons of the ba forebrain and block of the nociception in a classic "hot plate" test).

As noted above, antagonistic TrkA antibodies are disclosed in EP 1181318 and WO2005/061540.

In an aspect of the invention the variable region of the antibody light chain comprises least one of the complementarity determining regions (CDRs) having the sequence selec from aa. 24 to aa. 33 of SEQ ID No.1; from aa. 49 to aa. 55 of SEQ ID No. 1; from aa. to aa. 96 of SEQ ID No. 1, more preferably two of the above CDRs, most preferably th

of the above CDRs. The variable region of the antibody light chain may, for examp comprise essentially the sequence of SEQ ID No.1.

CDR L1 CDR L2

DIVLTQSPAIMSASLGEEVTLTCSASSSVSYMHWYQQKSGTSPKLLIYTTSNLASGVPSRFS

CDR L3

GSGSGTFYSLTISSVEAEDAADYYCHQWSSYPWTFGGGTKLEIK **(SEQ ID No 1).**

In an aspect of the invention the variable region of the antibody heavy chain comprises at least one of the complementarity determining regions (CDRs) having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 66 of SEQ ID No. 2; from aa. 99 to aa. 112 of SEQ ID No. 2, more preferably two of the above CDRs, most preferably three of the above CDRs. The variable region of the antibody light chain may, for example, comprise essentially the sequence of SEQ ID No.2.

CDR H1 CDR H2

EVKLVESGGGLVQPGGSLKLSCAASGFTFSTYTMSWARQTPEKRLEWVAYISKGGGSTYYPDTVKGRFTI

CDR H3

SRDNAKNTLYLQMSSLKSEDTALYYCARGAMFGNDFFFPMDRWGQGTSVTVSS **(SEQ ID No 2).**

The antibody may be in single chain form and comprises a light chain variable region an heavy chain variable region joined by a linker.

Alternatively the antibody may comprise two light chains and two heavy chains.

Alternatively the antibody may be a Fab (monovalent fragment antigen binding) fragme Fab fragments may be prepared by recombinant means *ab initio* or may be prepared enzymatic (eg papain)_proteolysis of a whole antibody.

Alternatively the antibody may be a Fab2 fragment. Fab2 fragments may be prepared recombinant means *ab initio* or may be prepared by enzymatic (eg pepsin)_proteolysis o whole antibody.

In a preferred aspect of the invention the anti-TrkA antibody is a human or humanis antibody. The skilled in the art shall select the proper humanisation method to design antibody, a preferred method is the method as disclosed in W0 2005/061540. Exempl humanised antibodies comprise a light chain variable region which is a humanis derivative of SEQ ID No 1 (a mouse origin sequence). Exemplary humanised antibod

comprise a heavy chain variable region which is a humanised derivative of SEQ ID No 2 mouse origin sequence).

In a preferred aspect of the invention the variable region of the humanised antibody li chain comprises essentially the sequence from aa. 1 to aa. 106 of SEQ ID No. 3.

In a more preferred aspect the humanised antibody light chain has essentially the sequer of SEQ ID No. 3.

DIVLTQSPSSLSASVGDRVTITCSASSSVSYMHWYQQKPGQAPKLLIYTTSNLASGVPSRFSGSGSGTDYT
*ISSLQPEDVATYYCHQWSSYPWTFGGGTKVEIK*RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
hMNAC13 Vk hCk (**SEQ ID No. 3**).

In a preferred aspect of the invention the variable region of the humanised antibody hea chain comprises essentially the sequence from aa. 1 to aa. 123 of SEQ ID No. 4 .

In a more preferred aspect the humanised antibody heavy chain has essentially a sequer selected from SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6.

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTIS
*NSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPSSKSTSGG
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
HNHYTQKSLSLSPGK hMNAC13 VH hIgG1 (**SEQ ID No. 4**).

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTIS
*NSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPSSKSTSGG
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV
RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
HNHYTQKSLSLSPGK hMNAC13 VH hIgG1 (N297A) (**SEQ ID No. 5**).

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYTMSWARQAPGKGLEWVAYISKGGGSTYYPDTVKGRFTIS
*NSKNTLYLQMNSLRAEDSAVYYCARGAMFGNDFFFPMDRWGQGTLVTVSS*ASTKGPSVFPLAPCSRSTSES
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV

RVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH
YTQKSLSLSLGK hMNAC13 VH hIgG4 (**SEQ ID No. 6**).

Italics: variable regions, Bold: mutations in the mouse sequence in the humanizati process, Underlined: CDRs.

A still further object of the present invention is the use of an anti-TrkA antibody that able to inhibit the binding between NGF and TrkA combined with at least one opi analgesic to prepare a remedy for treatment of pain of any etiology, including but limited to acute and chronic pain, any pain with an inflammatory component, and any p in which an opioid analgesic is usually prescribed. More preferably the pain is caused pancreatitis, kidney stones, headaches, dysmenorrhoea, musculoskeletal pain, sprai visceral pain, ovarian cysts, prostatitis, cystitis, interstitial cystitis, inflammatory bov disease post-operative pain (including dental pain), post-surgical pain, migraine, trigemi neuralgia, pain from burns and/or wounds, pain associated with trauma (includi traumatic head injury), neuropathic pain, post-herpetic neuralgia, pain associated w musculoskeletal diseases, rheumatoid arthritis, osteoarthritis, ankylosing spondili periarticular pathologies, oncological pain (including "break-through pain" and p associated with terminal cancer), pain from bone metastases, pain from HIV, pain fr myocardial infarction .

According to International Association for the Study of Pain (IASP, www.iasp-pain.c <http://www.iasp-pain.org/>), pain is generally defined as "An unpleasant sensory a emotional experience associated with actual or potential tissue damage, or described terms of such damage or both". The essential element in all forms of pain is the activati of specialized high-threshold receptors and nerve fibers to warn the organism of potent tissue damage. The involvement of inflammatory cells and processes is a common elem in many pain states. The term "acute pain" means immediate, generally high thresho pain brought about by injury such as a cut, crush, burn, or by chemical stimulation. T term "chronic pain," as used herein, means pain other than acute pain. It is understood t chronic pain often is of relatively long duration, for example, months or years and can continuous or intermittent.

In one particular embodiment of the invention the pain to be treated is a persistent form pain, e.g. oncological, neuropathic or rheumatic pain.

The anti-TrkA antibody of the invention is suitably administered systemically. Syster administration can be performed by injection, e.g. continous intravenous infusion, bo intravenous infusion, subcutaneous or intramuscular injection. Alternatively other forms administration (e.g. oral, mucosal, via inhalation, sublingually, etc.) may also be us Local delivery of the antibody can be performed by local administration eg intra-articu injection or subcutaneous, intramuscular injection in the affected tissue area.

The anti-TrkA antibody will suitably be formulated in a pharmaceutical compositi appropriate for the intended route of administration. Solutions for injection will suital contain the antibody dissolved or dispersed in an aqueous medium (eg water for injecti as appropriate containing appropriate buffers and molarity modifiers eg phosphate, s and/or dextrose.

Treatment regimen i.e. dose, timing and repetition, can be represented by single repeated administrations (eg injections) of the product by the chosen administration rou The interval of dose administration can be subject to modifications depending on the ext and duration of the clinical response, as well as the particular individual and the individ clinical history.

Combined administration includes both simultaneous administration and/or administrati at different times. The TrkA antagonist and the opioid analgesic may be administered w different frequencies and doses, i.e. a TrkA antibody may be administered 1 to 3 time week or maybe 1 to 3 times a month in single doses in the 0,01-500 mg/kg range (eg 0.1- mg/kg), whilst the opioid analgesic may be administered with greater frequency, witl lower or equal dose to that normally used for this category of analgesics. For exampl suitable dose of morphine (as morphine hydrochloride) would be in the 0,05-5mg/kg ran For example a suitable dose of fentanyl (as fentanyl citrate) would be in the 0,0005-0. range. TrkA antibody and the opioid analgesic may be administered in different ways a the doses may vary during the administration according to patient's response to t treatment. Adequate administration pathways possible (for one and/or the other compon of the combination) are the following: oral, intravenous, sublingual, subcutaneo intraarterial, intramuscular, rectal, intraspinal, intrathorax, intraperitoneal, intraventricul transdermic, by inhalation. The administration pathway may be systemic (as in the case

intravenous administration) or localized. A preventive administration, before the onset pain, may also be considered.

Suitably the anti-TrkA antibody has a long duration of action. In particular the clinic effect of the antibody extends following administration may be as long as 21days determined from animal studies. Furthermore preliminary data implies that anti-Trk antibodies may manifest clinical benefit for a longer period than that in which its presen can be detected in a relevant biological matrix such as serum or plasma following administration. .

In a preferred aspect, the opioid analgesic is a compound or a combination of one or mc compounds selected from the following list: morphine, codeine, dihydrocodeii diacetylmorphine, hydrocodone, hydomorphone, levorphanol, oxymorphone, alfentar buprenorphine, butorphanol, fentanyl, sufentanyl, meperidine, methadone, nabulfi propoxyphene, pentazocine; and their pharmaceutically acceptable salt derivatives, In o embodiment the opioid analgesic is morphine or a pharmaceutically acceptable salt there In another embodiment the opioid analgesic is fentanyl or a pharmaceutically acceptal salt thereof.

Suitably the quantity of anti-TrkA antibody is such that the opioid dose is reduced by least 5% eg at least 20% for example at least 50% of that necessary to produce the sa analgesic effect by itself.

Suitably the quantity of opioid analgesic is such that the TrkA antibody dose is reduced at least 5% eg at least 20% for example at least 50% of that necessary to produce the sa analgesic effect by itself.

Another object of the invention is a pharmaceutical formulation comprising pharmaceutically acceptable and effective doses, at least one anti- TrkA antibody and least one analgesic opioid. Preferably, the formulation is contained in a sin pharmaceutical composition. More preferably, the formulation is constituted by t pharmaceutical compositions, the first one comprising the molecule able to block biological activity of TrkA receptor, and the second one including the analgesic opioid.

A further object of the invention is an anti-TrkA antibody of the invention for use combination with an analgesic opioid for the treatment of pain.

Another object is a method of treatment and/or prevention of pain in a subject compris administering to the subject an effective amount of an anti-TrkA antibody and an effect amount of an analgesic opioid to treat and/or prevent pain in said subject.

There is also provided a kit comprising a composition containing an anti-TrkA antibo and an analgesic opioid together with instructions directing administration of s composition to a subject in need of treatment and/or prevention of pain thereby to tr and/or prevent pain in said subject.

This invention will now be described providing non limiting examples thereof w particular reference to the following figures:

FIGURE 1: Effect of morpine **(A)** 1 mg/kg, **(B)** 2.5 mg/kg, **(C)** 5 mg/kg on mo formalin test (n=10 for each experimental group). Morphine was injected intraperitonea 15 minutes prior to starting the test and the total "Licking" time (response to pain) w measured in seconds. Saline solution (Sal) was injected to negative controls. Time interv of 5 minutes were considered. Each time interval was analyzed by means of one fac variance analysis test (ANOVA). The first 8 intervals (=total time 40 min) correspond the duration of the test. The animals were also observed during the 20 minutes immediat after the test. **(A)** 1 mg/kg of morphine is completely ineffective; **(B)** 2.5 mg/kg morphine have a significant analgesic effect; **(C)** 5 mg/kg of morphine block the respor to formalin consistently.

FIGURE 2: Formalin test in mouse: Effect of the anti-NGF αD11 and anti-Trl MNAC13 monoclonal antibodies on the pain response (Licking) when antibodies w injected 1 hour prior to the test, as indicated in the method section. **(A)** anti-NGF αD monoclonal antibody (Mab format: 12.5 µg/20 µl) and anti-TrkA MNAC13 monoclo antibody (Mab format: 60 µg/20 µl); **(B)** Fab (monovalent fragment antigen binding) of t αD11 antibody (12,5 µg/20 µl) and Fab of the MNAC13 antibody (60 µg/20 µ Irrelevant mouse immunoglobulins (IgG, 60 µg/20 µl) and murine irrelevant Fab (Fab, µg/20 µl) were used as controls. The total licking time is divided into phase 1 (ea phase= 0-15 min) and phase 2 (late phase= 15-40 min).

Experimental groups: **(A)** IgG (n=11), αD11 (n=10), MNAC13 (n=10); **(B)** Fab (n= αD11 Fab (n=10), MNAC13 Fab (n=10). ** $p<0.01$ vs. control group (Fisher's PL Test). Fabs of the 2 antibodies show analgesic properties (limited to phase 2).

FIGURE 3: Formalin test in mouse: Effect of the anti-NGF αD11 and anti-Trl MNAC13 monoclonal antibodies on pain response (Licking) when the antibodies w injected 18 hours prior to the test, as indicated in the method section. **(A)** anti-NGF αD monoclonal antibody (Mab format: 12,5 µg in a total injection volume of 20 µl) and an

TrkA MNAC13 monoclonal antibody (format Mab: 60 μg, in a total injection volume 20 μl); **(B)** Fab (monovalent fragment antigen binding) of the αD11 antibody (12.5 μg i total injection volume of 20 μl) and Fab of the MNAC13 antibody (60 μg, in a tc injection volume of 20 μl). Irrelevant mouse immunoglobulins (IgG, 60 μg, in a tc injection volume of /20. μl) and irrelevant murine Fab (Fab, 60 μg in a total injecti volume/20 μl) were used as control. The total licking time is divided into phase 1 (ea phase= 0-15 min) and phase 2 (late phase= 15-40 min).

Experimental groups: **(A)** IgG (n=10), αD11 (n=8), MNAC13 (n=9); **(B)** Fab (n=10), αD Fab (n=8), MNAC13 Fab (n=8). ** $p<0.01$, vs. control group (Fisher's PLSD Te: MNAC13 (Mab) produces a significant analgesic effect (limited to phase 2).

FIGURE 4: Dose-response study of the anti-TrkA MNAC13 antibody in the formalin t (mouse), both for the early phase (phase 1= 0-15 min), and for the late one (phase 2= 15- min). The antibody was administered, 18 hours prior to the test as indicated in the meth section. Experimental groups for single dose of MNAC13: 0.9 μg: n=8; 1.875 μg: n= 3.75 μg: n=10; 7.5 μg: n=10; 15 μg: n=10; 30 μg: n=10; 60 μg: n=9. Mou Immunoglobulins (IgG, 60 μg n = 12) were injected as control.

* $p<0.05$, ** $p<0.01$,*** $p<0.001$ vs. control IgG group (Fisher's PLSD Test). Only t lowest dose (0.9 μg, equal to approximately 25 μg/kg) did not show any analge property. The intermediate dose of 15 μg (equal to approximately 0.4 mg/kg) is alrea able to produce the maximal effect observed (reduction by approximately 52 % of t licking time in phase 2) and is the only dose displaying a statistically significant effect phase 1 of the test.

FIGURE 5: Effect of anti-TrkA MNAC13 monoclonal antibody and its humaniz counterpart (Hu-MNAC13) in the formalin test (mouse). **(A)** 15 μg of MNAC13 w given either sc (subcutaneously) or ip (intraperitoneally) to mice, 18 h before testi Irrelevant mouse immunoglobulins (IgG) were used as controls. Experimental groups: I (ip, n= 10); IgG (sc, n=9); MNAC13 (ip, n=9); MNAC13 (sc, n=8). Statistical analy (two way ANOVA) of both early and late phase results showed a significant effect treatment ($F_{13,66}$- P0.0008 for early phase; $F_{21,657}$-$P<0.0001$ for late phase), but significance for the administration route and interaction (treatment*administration rout Post-hoc comparisons (Tukey/Kramer) revealed a significant difference (** $p<0.($ between MNAC13 and IgG treatment groups for both early and late phase. MNAC induces analgesia in the formalin test, independently of the administration route. **(B)** A

μg dose of Hu-MNAC13 (IgG4) was given ip to mice, 18 h before testing. Irrelev human immunoglobulins (IgG) were used as controls. Experimental groups: Human I (n=6); Hu-MNAC13 (n=9). T-Student test * $p<0.05$, ** $p<0.005$. The results w comparable to those obtained with the mouse MNAC13 antobody (panel A).

FIGURE 6: Potentiation effect of MNAC13 (anti-TrkA) on opioid-induced analge (formalin test in mouse: **A**, morphine; **B**, fentanyl). Each mouse was randomly assigned one of the different experimental groups and subcutaneously (sc) injected 18 hours bef the test with anti-TrkA (mouse MNAC13, 0.9 μg per animal, approximately equivalent 25 μg/kg, ineffective dose per se) antibody or irrelevant mouse IgGs (antibody dose: μg/kg per animal) as indicated in the method section. **(A)** Morphine hydrochloride v injected i.p.15 min before testing. **(B)** Fentanyl was injected sc 20 min before testi Experimental groups: Saline(Sal) and IgG (n=10); IgGs and morphine 1 mg/kg (M (n=9); IgGs and morphine 2.5 mg/kg (Mo2.5) (n=9); MNAC13 (mouse) and saline (n=1 MNAC13 (mouse) and morphine 1 mg/kg (Mo1) (n=11); IgGs and fentanyl 0.005 mg/ (FEN 0.005) (n=8); IgGs and fentanyl 0.01 mg/kg (FEN 0.01) (n=7); MNAC13 a fentanyl (0.01 mg/kg (FEN 0.01) (n=8). One-way ANOVA followed by post-h comparisons (Tukey/Kramer). * $p<0.05$, ** $p<0.005$ vs. control group.

FIGURE 7: Potentiation effect of humanized MNAC13 (Hu-MNAC13) on morphi induced analgesia (formalin test in mouse).

Each mouse was randomly assigned to one of the different experimental groups a subcutaneously (sc) injected 18 hours before the test with anti-TrkA antibody (H MNAC13; antibody dose: 25 μg/kg, per se ineffective) or irrelevant human Ig (antibody dose: 25 μg/Kg), as indicated in the method section. Morphine hydrochloride mg/kg = Mo-1, dose per se ineffective) was injected i.p. 15 min before testing. Morphi negative controls were injected with saline (Sal). Experimental groups: Sal and I (n=8); IgG and Mo-1 (n=6); Sal and Hu-MNAC13 (n=8); Hu-MNAC13 and Mo-1 (n= One-way ANOVA followed by post-hoc comparison (Tukey/Kramer): ** $p<0.001$ control group.

METHODS

Monoclonal antibodies production

The monoclonal antibodies MNAC13 and αD11 were produced from a hybrido supernatant, according to standard methods, disclosed above (Galfre and Milstein, 19

Cattaneo et al.,.1988; Cattaneo et al., 1999). The supernatant containing each antibody v subjected to precipitation (29% ammonium sulphate), followed by dialysis against PBS (Spectra-Por 12/14K membrane, Spectrum) and affinity chromatography on sephar protein G column (4-Fast Flow, Amersham Biosciences). Elution was performed by me of a low pH (HCl 5 mM) solution that was neutralised upon collection. The final eluate v concentrated (Amicon Ultra-15, 50K, Millipore) to obtain preparations of purified antibc with concentrations between 1 and 5mg/ml.

The Fab (Monovalent Fragment Antigen binding) version of the αD11 and MNAC antibodies were produced as previously described (patent application WO 05/0615 Covaceuszach et al., 2004).

Briefly, Fab fragments were obtained from the correspondent whole monoclonal antibodies (IgG format) by papain proteolysis, followed by a ion exchange chromatography purification step and concentration of the Fab fragments collected in the flow-through. In order to separate the Fab fragments from the quite low amount of uncleaved IgG that was still present, size exclusion chromatography on a Superdex G75 column (Pharmacia) was performed using an FPLC system (Pharmacia), followed by a final concentration step.

As far as the humanised versions of the 2 antibodies (Hu-αD11 and Hu-MNAC13) concerned, they were also purified as disclosed above, starting from the supernatants stably transfected cell lines, which were stable cotransfectants for the heavy ch (pVH/CMVexpress) and the light chain (pVL/CMVexpress) of each antibody. The vect used were previously disclosed (patent application WO 05/061540). The stable transfected clones were obtained through double selection with G418 and mycophenc acid. In order to produce the IgG4 variant of Hu-αD11, since the pVH/CMVexpress vec comprises the constant part of human IgG1, this was replaced by the corresponding region of IgG4 (cloned by RT-PCR from human lymphocyte RNA). The IgG1* vari (=IgG1 with the N297A mutation described by Bolt et al., 1993) was generated by s directed mutagenesis.

Animals

Male CD1 mouse (Charles River Labs, Como, Italia) weighing approximately 35g w used. On their arrival to the laboratory (at least 2 weeks before the experiment), they w housed in standard transparent plastic cages (4 per cage), at a constant temperat (22±1°C) and relative humidity (60%), with a light/dark cycle rule (light 07:00-19:00) a

with no water or food limitation. The experiments took place between 09:00 and 14: The animals were treated and handled in compliance with the IASP Ethical Commit ("International Association for the Study of Pain") guidelines and with the Italian natio law (DL116/92, application of the European directive 86/609/EEC) on the use of anim for research purposes. All the necessary efforts were made to minimize animal suffer and to use the lowest possible number of animals, sufficient to produce reliable scient data.

Formalin test

The mice nociceptive response to formalin was tested. Each experimental group inclu 10 animals (unless stated otherwise). Mice were randomly assigned to experimer groups. 30 minutes prior to the test, the animal to be tested was placed in a transpar plastic cage (30 x 12 x 13 cm), where it was free to move in all directions. A mirror pla underneath the cage and a video camera placed opposite allowed a complete view of hind legs during the observation period. Each mouse was treated with antibodies block NGF (αD11) or TrkA (MNAC13 or huMNAC13), e.g. with irrelevant antibodies (mo IgG) or saline, through subcutaneous injection (s.c) in the dorsal part of the right hind p performed 1 to 18 hours prior to the injection (s.c) of formalin (5% in saline solution) the same paw. A Hamilton micro syringe with a 26 diameter needle was used for injections (injection volume equal to 20 µl). In some experiments, antibody (MNAC1 Hu-MNAC13) injection was performed intraperitoneally (ip) 18 h before testing.

Morphine (morphine hydrochloride, Guieu) was injected intraperitoneally (i.p) at a fi concentration between 1 mg/kg and 5 mg/kg (in saline solution), 15 min prior to formalin test.

Fentanyl citrate (Hameln pharmaceuticals GmbH) , doses employed : 0.01 or 0.005 mg/k was administered subcutaneously (dorsal injection) to animals 20 min before testing. Th choice of fentanyl doses to be tested was based on published protocols (Seguin et al., 19 Meert and Vermeirsch, 2005).

The "Licking" activity, that is the total time spent by the animal licking and/or biting injected paw, was recorded continuously for 40 minutes (divided into observation ti intervals of 5 min). During each session of the test, as well as the "Licking", gene activities and "self-grooming" activities (intended as grooming of the face) were a recorded. General activity is conventionally intended as the time the animal spends

horizontal locomotory activities and in exploring activities, including moments when the animal is in a vertical position or leans out.

With regards to the antibodies administration, the tested doses are summarized in the following table:

Antibody	Format	Used doses (μg/ 20 μl)
MNAC13	Whole monoclonal (Mab)	60 μg; 30 μg; 15 μg; 7.5 μg; 3.75 μg; 1.875 μg; 0.9 μg
MNAC13	Monovalent fragment (Fab)	60 μg
MNAC13	Humanized antibody (huMNAC13) in Mab format	15 μg; 7.5 μg; 3.75 μg; 1.875 μg; 0.9 μg
αD11	Whole monoclonal (Mab)	12.5 μg
αD11	Monovalent fragment (Fab)	12.5 μg
Mouse IgG	Whole monoclonal (Mab)	60 μg
Mouse IgG	Monovalent fragment (Fab)	60 μg

In some experiments, MNAC13 (15 μg, roughly equivalent to 0.4 mg/Kg), Hu-MNAC1 (15 μg, roughly equivalent to 0.4 mg/Kg) and mouse/human control Immunoglobulins (1 μg, roughly equivalent to 0.4 mg/Kg) were also given through the ip administration route.

Statistical analysis

The formalin test was performed in a double-blinded manner. Formalin pain respons were separated into an early phase (from 0 to 15 min) and a late phase (from 15 to 40 mi The 2 phases were analyzed separately using one factor variance analysis test (ANOV except for experiments summarized in Fig. 5A, were a two way ANOVA (treatment a administration route factors) was performed. The same type of statistical analysis w performed on the general and "self-grooming" activities. When appropriate, "post-hc comparisons were drawn using either Fisher PLSD or Tukey/Kramer tests, as indicated.

A simple Student T-test was employed to analyse results of Fig. 5B (effect of a single d of Hu-MNAC13 vs human IgG, given ip).

RESULTS

Formalin produced the typical two-phase response, with a first phase of "Licking" activ and a second phase of intense response to pain, starting 15 minutes after formalin injecti Subcutaneous administration of irrelevant mouse immunoglobulins (IgG) or of th

monovalent version (Fab) had no effect on the response to pain. The response of animals treated with IgG matched with that of the animals treated with Saline. In additi general activity and "grooming" were indistinguishable amongst both the treated group a the control group, proving the specific feature of the pharmacological effect of treatment.

Single dose studies

The first series of experiments evaluated the analgesic effect of 3 different doses morphine (1 mg/kg; 2.5 mg/kg; 5 mg/kg) on the response to pain induced by forma injection. The graphics (Fig. 1) relate to a period of observation of 60 minutes (divided i 5 min intervals), including the time of the actual test (first 40 minutes) and the follow 20 minutes.

Considering the first 40 min, while the highest morphine dose was able to strongly redu the pain response (Fig. 1C) and the intermediate dose substantially reduced by 50% licking time (Fig. 1B), the lowest dose (1 mg/kg) proved to be totally ineffective, both the first (0-15 min) and in the second phase (15-40 min, Fig. 1A).

When anti-NGF and anti-TrkA antibodies were used, the pain response (measured "Licking" time), was differentially affected, depending on the format of the antibc (Whole immunoglobulin = Mab; monovalent fragment antigen binding = Fab).

In the first single dose study, the MNAC13 and αD11 antibodies were administered c hour prior to the test in Mab format (Fig. 2A) or in Fab format (Fig. 2B). The variar analysis did not highlight any significant analgesic effect for the antibodies in Mab form both in the early phase ($F_{2.28}$: 1.217; p = 0.3114), and the late phase ($F_{2.28}$: 1.3 p=0.2650). On the contrary, the administration of the antibodies in Fab format (αD Fab: 12.5µg/20µl; MNAC13 Fab: 60µg/20µl) revealed a significant reduction of response to pain (analgesic effect) in the late phase of the test ($F_{2.26}$: 8.340 p<0.01), wh no statistically significant changes were observed in the early phase ($F_{2.26}$: 1.6 p=0.2195).

When the antibodies were administered (same single doses), 18 hours prior to the forma test (Fig. 3A and 3B), the anti-NGF (αD11) and anti-TrkA (MNAC13) antibodies revea an effect on the licking response that was different from that observed when administere hour prior to the test. When compared to irrelevant mouse immunoglobulins (IgG), neitl of the antibodies showed a significant effect on the early phase ($F_{2.24}$: 1.226; p=0.311 however, as for the late phase, whilst MNAC13 produced a significant reduction in t

response to pain ($F_{2,24}$: 5.129; $p<0.01$), the effect of αD11 was not statistically signific
(Fig. 3A). This result showed the non-equivalence of the two molecular targets represent
by the NGF ligand and the TrkA receptor. In a well known model of persist
inflammatory pain (formalin test), the functional block of the receptor (with MNAC
blocking antibody) produced an analgesic effect, that was not detectable (at least with t
same administration protocol), by blocking the ligand (with the αD11 blocking antibody)
As shown on Fig. 3B, neither antibody, if administered 18 hours prior to the test in F
format, was able to produce a significant analgesic effect both in the early phase (F_2
0.468; p=0.6318) and in the late phase ($F_{2,23}$: 0.074; p=0.9293).

Dose-response study for MNAC13

A dose-response study (formalin test in mouse) was performed with the MNAC13 an
TrkA antibody, based on the significant results obtained in the single dose stud
(administration 18 hours prior to the test). The analysis of the response to pain ("Lickin
time) showed (Fig. 4) a significant analgesic effect for all tested doses for the late phase
the test (F7,71: 4,134; $p<0.001$), with the exception of the lowest (0.9 µg/20µl volume
injection, equal to approx. 25 µg/kg). It should be noted that three of the MNAC
effective doses (1.875 µg; 3.75 µg; 7.5 µg) were smaller than the αD11 dose (12.5 µ
used in the single dose studies. This confirms a fundamental difference in analge
efficacy between anti-NGF and anti-TrkA antibodies in the formalin test using tl
administration protocol.

MNAC13 doses higher than 15 µg (equal to approx. 0.4 mg/kg) did not show any increa
in the average analgesic efficacy in the late phase of the test: The maximum effect
reduction of pain response (about 52% of the control) could be already obtained with
µg. This is also the only dose for which a small but statistically significant effect w
observed in the early phase of the formalin test (Fig. 4).

Fig. 5A shows that the analgesic properties of the 15 µg dose are independent of t
administration route. Indeed, the same effect on licking behaviour was observed when t
antibody was injected s.c. or i.p.

When the Hu-MNAC13 was administered i.p. in the same kind of experiment at the dc
of 15 µg, a similar analgesic effect, as compared to the corresponding dose of paren
MNAC13, was observed (Fig. 5A).

Synergistic effect of anti-TrkA antibody MNAC13 and opioids

By employing the formalin test, it was also possible to show that MNAC13 (and humanized counterpart) could act synergistically with opioids. Using the previou described administration protocol (18 hours prior to the test), animals were treated with ineffective dose (25 μg/kg) of MNAC13 after an i.p ineffective dose (1mg/kg) morphine, or fentanyl (0.005 mg/kg). The results of the combined treatment (compared all related cross check controls in which MNAC13 was replaced by irrelev immunoglobulins, while opioids analgesics were replaced by saline solution) showe surprising as well as significant potentiation effect (Fig. 6). The joint administration ineffective doses of MNAC13 and morphine produced a significant reduction of p response ("Licking time"), which was more evident in the late phase of the test.

As for the combined administration of morphine and MNAC13 (Fig. 6A), in phase 2, licking time was reduced by 40% compared to the respective IgG+Sal control group. I both phases of the formalin test, the combined administration of two sub-threshold do of MNAC13 (25 μg/kg) and morphine (1 mg/kg) produced an analgesic effect that v comparable to the (per se effective) 2.5 mg/kg dose of morphine.

Similar results were obtained when another opioid (fentanyl) was given in combinat with MNAC13 (Fig. 6B), indicating that the potentiation of opioid-induced analgesia co be obtained independently of the opioid used. For both phases of the formalin test, combined administration of two sub-threshold doses of MNAC13 (25 μg/kg) and fenta (0.005 mg/kg) produced an analgesic effect that was comparable to the (per se effecti 0.01 mg/kg dose of fentanyl (Fig. 6B).

Similar results were obtained when MNAC13 was replaced by its humanized variant (H MNAC13) as shown in Fig. 7.

The potentiation of opioid-induced analgesia by antibodies blocking the TrkA recep presents remarkable consequences from a therapeutic point of view. The jo administration of MNAC13 (or of its humanised version, as well as of any other derivat that maintains the blocking properties of the parental antibody) allows the use of lov doses of morphine (or other opioids), normally ineffective, consequently reducing incidence of the side effects and the probability of developing drug tolerance or addictio

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CLAIMS

1. Use of an anti-TrkA antibody capable of inhibiting the binding between NGF and Tr combined with at least one opioid analgesic for the preparation of a medicament treating and/or preventing pain.

2. Use according to claim 1 wherein the antibody is capable of blocking the biologi activity of TrkA.

3. Use according to claim 2 wherein the variable region of the antibody light ch comprises at least one of the complementarity determining regions (CDRs) having sequence selected from aa. 24 to aa. 33 of SEQ ID No.1; from aa. 49 to aa. 55 of SEQ No. 1; from aa. 88 to aa. 96 of SEQ ID No. 1.

4. Use according to claim 1 or 2 wherein the variable region of the antibody light cha comprises at least two of the complementarity determining regions (CDRs) having t sequence selected from aa. 24 to aa. 33 of SEQ ID No.1; from aa. 49 to aa. 55 of SEQ No. 1; from aa. 88 to aa. 96 of SEQ ID No. 1.

5. Use according to claim 1 or 2 wherein the variable region of the antibody light cha comprises all three complementarity determining regions (CDRs) having the sequen selected from aa. 24 to aa. 33 of SEQ ID No.1; from aa. 49 to aa. 55 of SEQ ID No. from aa. 88 to aa. 96 of SEQ ID No. 1.

6. Use according to claim 1 or 2 wherein the variable region of the antibody light cha comprises essentially the sequence of SEQ ID No.1.

7. Use according to any one of claims 1 to 6 wherein the variable region of the antibo heavy chain comprises at least one of the complementarity determining regions (CDR having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 6 of SEQ ID No. 2; from aa. 99 to aa. 112 of SEQ ID No. 2.

8. Use according to any one of claims 1 to 6 wherein the variable region of the antibo heavy chain comprises at least two of the complementarity determining regions (CDR having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 6 of SEQ ID No. 2; from aa. 99 to aa. 112 of SEQ ID No. 2.

9. Use according to any one of claims 1 to 6 wherein the variable region of the antibod heavy chain comprises all three of the complementarity determining regions (CDR having the sequence selected from aa. 26 to aa. 35 of SEQ ID No. 2; from aa. 50 to aa. 6 of SEQ ID No. 2; from aa. 99 to aa. 112 of SEQ ID No. 2.

10. Use according to any one of claims 1 to 6 wherein the variable region of the antibc heavy chain comprises essentially the sequence of SEQ ID No. 2.

11.Use according to any one of claims 1 to 10 wherein the antibody is in single chain fo and comprises a light chain variable region and a heavy chain variable region joined b linker.

12. Use according to any one of claims 1 to 10 wherein the antibody comprises two li chains and two heavy chains.

13. Use according to any one of claims 1 to 10 wherein the antibody is a Fab (monoval fragment antigen binding) fragment.

14. Use according to any one of claims 1 to 13 wherein the anti-TrkA antibody is a hun or humanised antibody.

15. Use according to claim 14 wherein the variable region of the humanised antibody li chain comprises essentially the sequence from aa. 1 to aa. 106 of SEQ ID No. 3.

16. Use according to claim 14 or claim 15 wherein the the variable region of t humanised antibody heavy chain comprises essentially the sequence from aa. 1 to aa. 1 of SEQ ID No. 4.

17. Use according to any one of claims 14 to 16 wherein the humanised antibody li chain has essentially the sequence of SEQ ID No. 3.

18. Use according to any one of claims 14 to 17 wherein wherein the humanised antibo heavy chain has essentially a sequence selected from SEQ ID No. 4, SEQ ID No. 5, SI ID No. 6.

19. Use according to any of previous claims wherein the opioid analgesic is a compound a combination of one or more compounds selected from the following list: morphi codeine, diyhydrocodeine, diacetylmorphine, hydrocodone, hydomorphone, levorphan oxymorphone, alfentanil, buprenorphine, butorphanol, fentanyl, sufentanyl, meperidi methadone, nabulfina, propoxyphene, pentazocine; and their pharmaceutically acceptal salt derivatives.

20. Use according to claim 19 wherein the opioid analgesic is morphine or pharmaceutically acceptable salt thereof.

21. Use according to claim 20 wherein the opioid analgesic is fentanyl or pharmaceutically acceptable salt thereof.

22. Use according to one of the previous claims in which the pain is chronic pain.

23. Use according to one of the previous claims 1 to 21 in which the pain is acute pain.

24. Use according to claims 22 or 23 wherein the pain is caused by pancreatitis, kid stones, headaches, dysmenorrhoea, musculoskeletal pain, sprains, visceral pain, ova cysts, prostatitis, cystitis, interstitial cystitis, inflammatory bowel disease post-opera pain (including dental pain), post-surgical pain, migraine, trigeminal neuralgia, pain fi burns and/or wounds, pain associated with trauma (including traumatic head inju neuropathic pain, post-herpetic neuralgia, pain associated with musculoskeletal disea rheumatoid arthritis, osteoarthritis, ankylosing spondilitis, periarticular patholog oncological pain (including "break-through pain" and pain associated with term cancer), pain from bone metastases, pain from HIV.

25. Use according to one of the previous claims in which the amount of anti-TrkA antib is such that the opioid dose is reduced by at least 5% of that necessary to produce the sa analgesic effect by itself.

26. Analgesic pharmaceutical formulation comprising in pharmaceutically acceptable effective doses, at least one anti-TrkA antibody as disclosed in any one of claims 1 to and at least one analgesic opioid.

27. Formulation according to claim 26 wherein the formulation is contained in a sin pharmaceutical composition.

28. Formulation according to claim 27 wherein the formulation is constituted by t pharmaceutical compositions, the first one comprising the anti-TrkA antibody, and second one including the analgesic opioid.

29. Formulation according to any one of claims 26 to 28 wherein the opioid analgesic i compound or a combination of one or more compounds selected from the following l morphine, codeine, diyhydrocodeine, diacetylmorphine, hydrocodone, hydomorpho levorphanol, oxymorphone, alfentanil, buprenorphine, butorphanol, fentanyl, sufentan meperidine, methadone, nabulfina, propoxyphene, pentazocine; and their pharmaceutica acceptable salt derivatives.

30. Formulation according to claim 29 wherein the opioid analgesic is morphine o pharmaceutically acceptable salt thereof.

31. Formulation according to claim 30 wherein the opioid analgesic is fentanyl o pharmaceutically acceptable salt thereof.

32. An anti-TrkA antibody as disclosed in any of claims 1-18 for use in combination w an analgesic opioid for the treatment of pain.

33. The anti TrkA antibody according to claim 32 for use in combination with an analge opioid for the treatment of pain wherein the the opioid analgesic is a compound o combination of one or more compounds selected from the following list: morphi codeine, diyhydrocodeine, diacetylmorphine, hydrocodone, hydomorphone, levorphar oxymorphone, alfentanil, buprenorphine, butorphanol, fentanyl, sufentanyl, meperidi methadone, nabulfina, propoxyphene, pentazocine; and their pharmaceutically accepta salt derivatives.

34. Method of treatment and/or prevention of pain in a subject comprising administering the subject an effective amount of an anti-TrkA antibody as disclosed in any of claims 1- and an effective amount of an analgesic opioid to treat and/or prevent pain in said subjec

35. Method according to claim 34 wherein the opioid analgesic is a compound o combination of one or more compounds selected from the following list: morphi codeine, diyhydrocodeine, diacetylmorphine, hydrocodone, hydomorphone, levorphan oxymorphone, alfentanil, buprenorphine, butorphanol, fentanyl, sufentanyl, meperidi methadone, nabulfina, propoxyphene, pentazocine; and their pharmaceutically acceptal salt derivatives.

36. Method according to claim 35 wherein the opioid analgesic is morphine or pharmaceutically acceptable salt thereof.

37. Method according to claim 36 wherein the opioid analgesic is fentanyl or pharmaceutically acceptable salt thereof.

38. A kit comprising a composition containing an anti-TrkA antibody as disclosed in a one of claims 1 to 18 and an analgesic opioid together with instructions directi administration of said composition to a subject in need of treatment and/or prevention pain thereby to treat and/or prevent pain in said subject.

39. Kit according to claim 38 wherein the opioid analgesic is a compound or combination of one or more compounds selected from the following list: morphir codeine, diyhydrocodeine, diacetylmorphine, hydrocodone, hydomorphone, levorphan oxymorphone, alfentanil, buprenorphine, butorphanol, fentanyl, sufentanyl, meperidir methadone, nabulfina, propoxyphene, pentazocine; and their pharmaceutically acceptat salt derivatives.

40. Kit according to claim 39 wherein the opioid analgesic is morphine or pharmaceutically acceptable salt thereof.

41. Kit according to claim 40 wherein the opioid analgesic is fentanyl or pharmaceutically acceptable salt thereof.

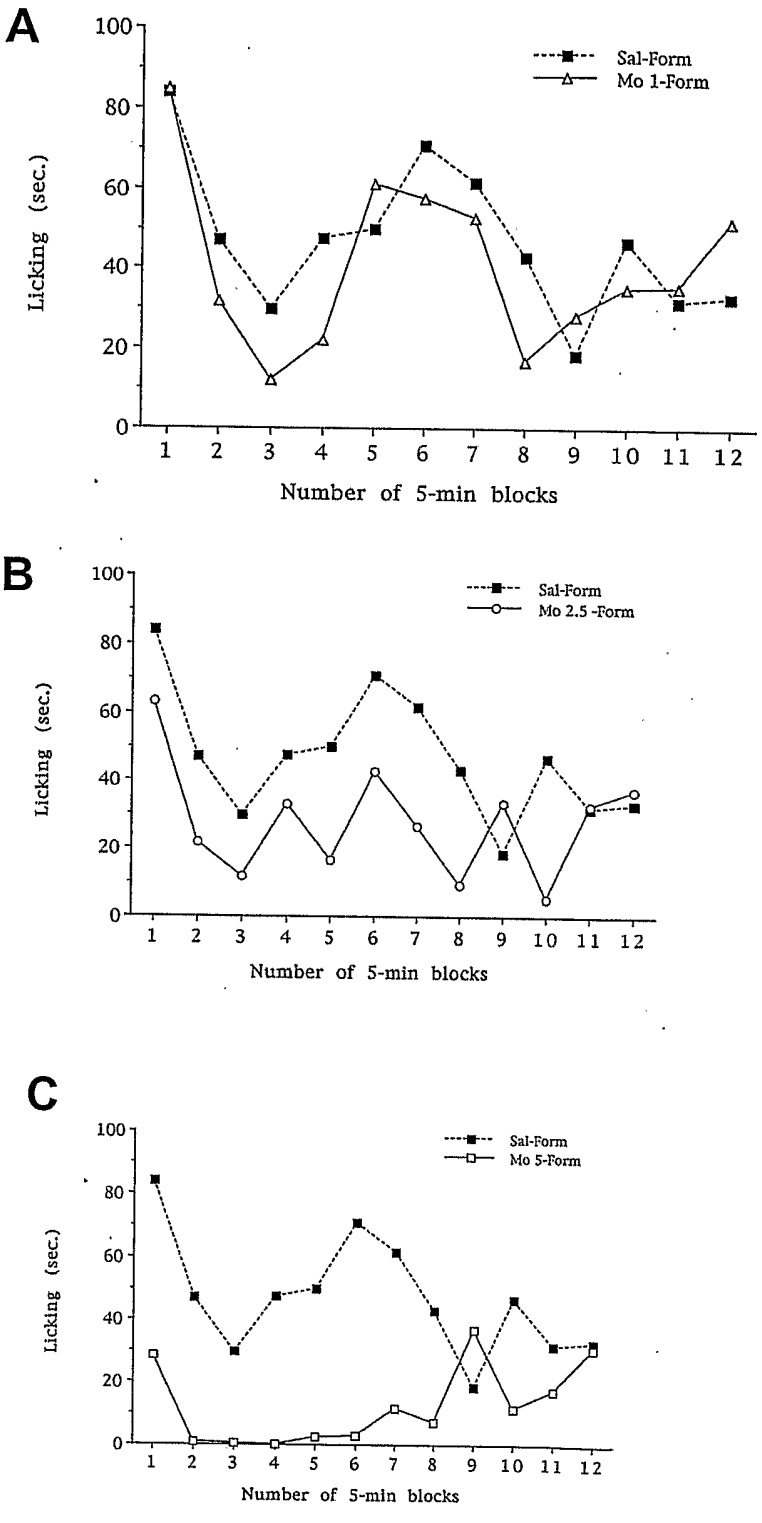
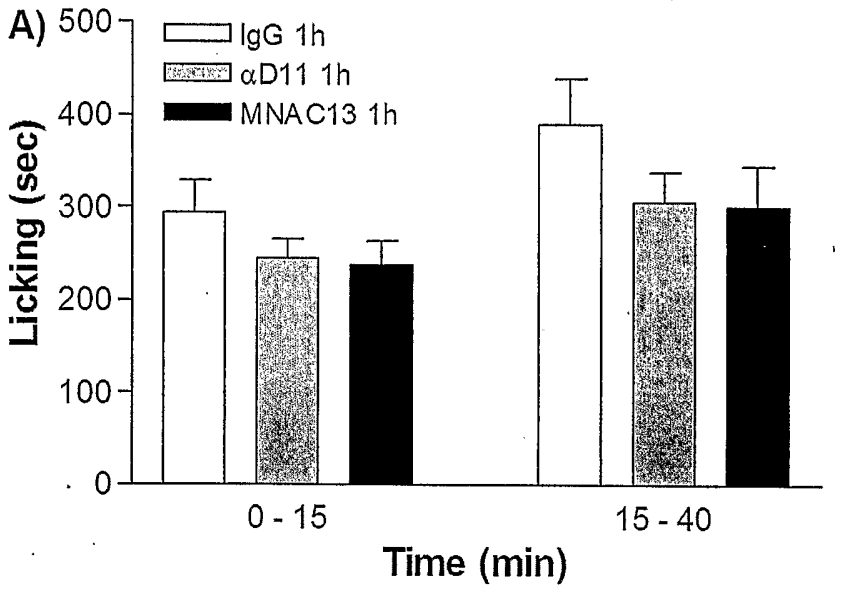

A
Licking (sec.)
Number of 5-min blocks
Sal-Form
Mo 1-Form
B
Licking (sec.)
Number of 5-min blocks
Sal-Form
Mo 2.5 -Form
C
Licking (sec.)
Number of 5-min blocks
Sal-Form
Mo 5-Form

Fig. 1


A)
500
400
300
200
100
0
IgG 1h
αD11 1h
MNAC13 1h
Licking (sec)
0 - 15
15 - 40
Time (min)

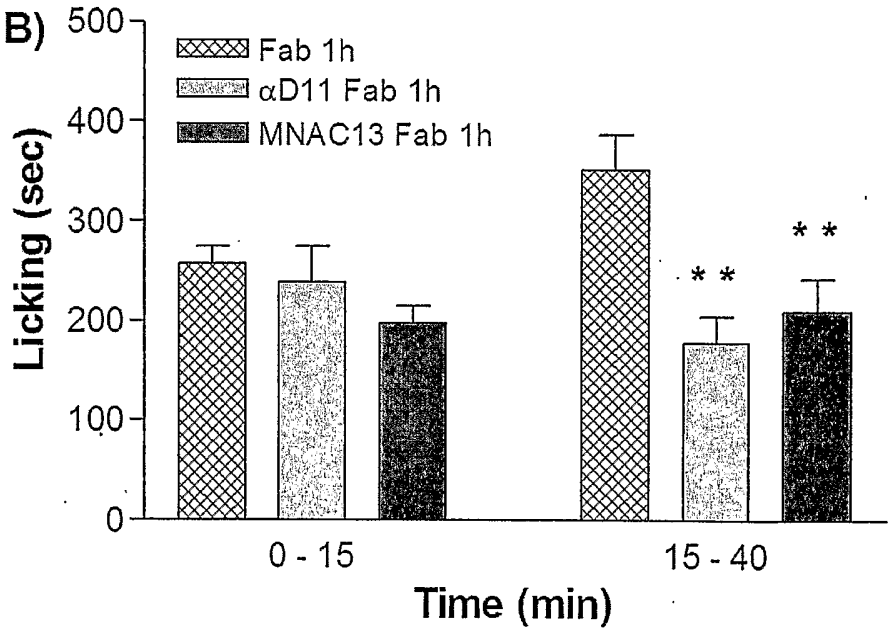

B)
500
400
300
200
100
0
Fab 1h
αD11 Fab 1h
MNAC13 Fab 1h
Licking (sec)
* *
* *
0 - 15
15 - 40
Time (min)

Fig. 2

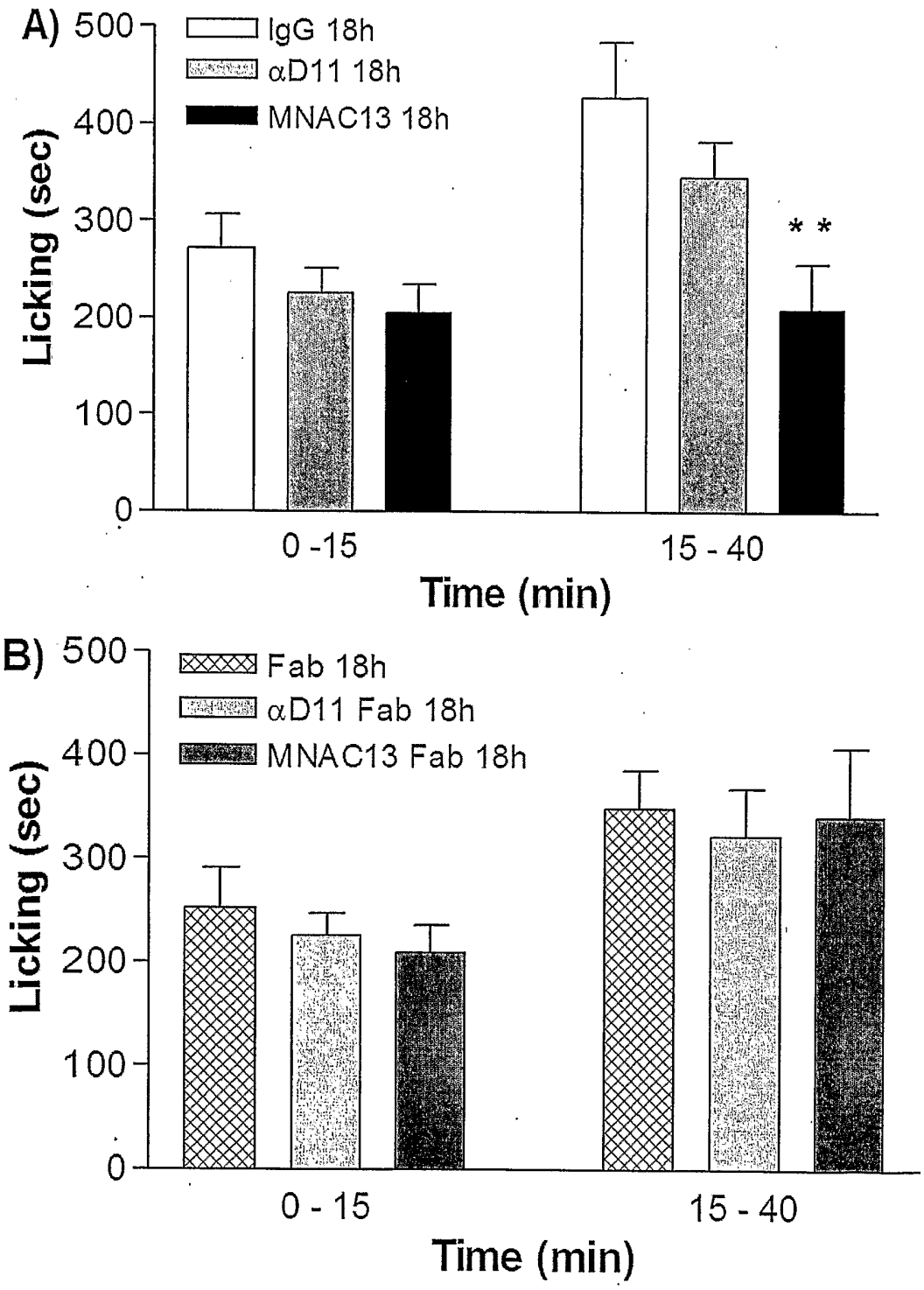

A)
500
400
300
200
100
0
IgG 18h
αD11 18h
MNAC13 18h
Licking (sec)
* *
0 -15
15 - 40
Time (min)
B)
500
400
300
200
100
0
Fab 18h
αD11 Fab 18h
MNAC13 Fab 18h
Licking (sec)
0 - 15
15 - 40
Time (min)

Fig. 3

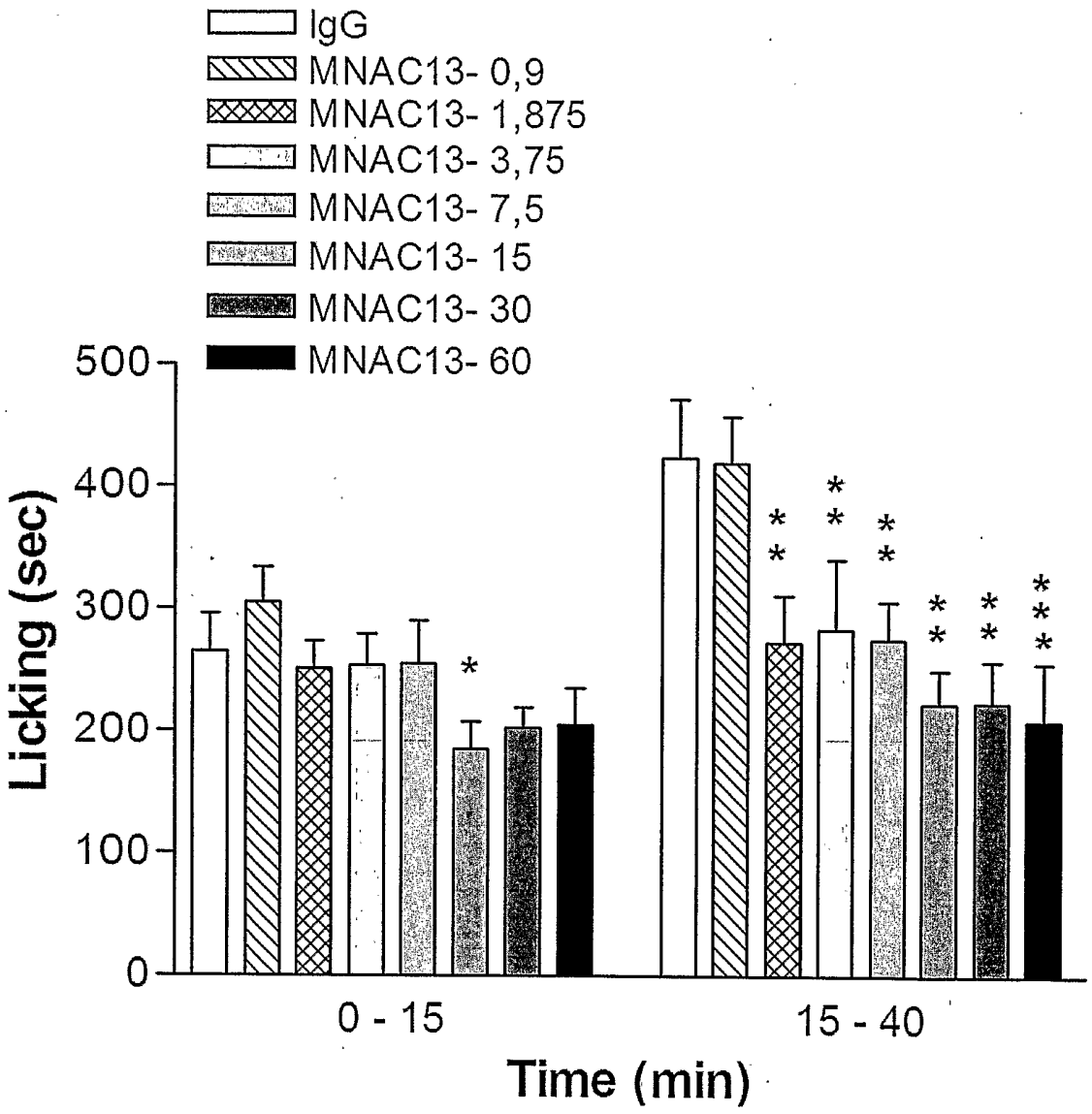

IgG
MNAC13- 0,9
MNAC13- 1,875
MNAC13- 3,75
MNAC13- 7,5
MNAC13- 15
MNAC13- 30
MNAC13- 60
Licking (sec)
500
400
300
200
100
0
0 - 15
15 - 40
Time (min)

Fig. 4

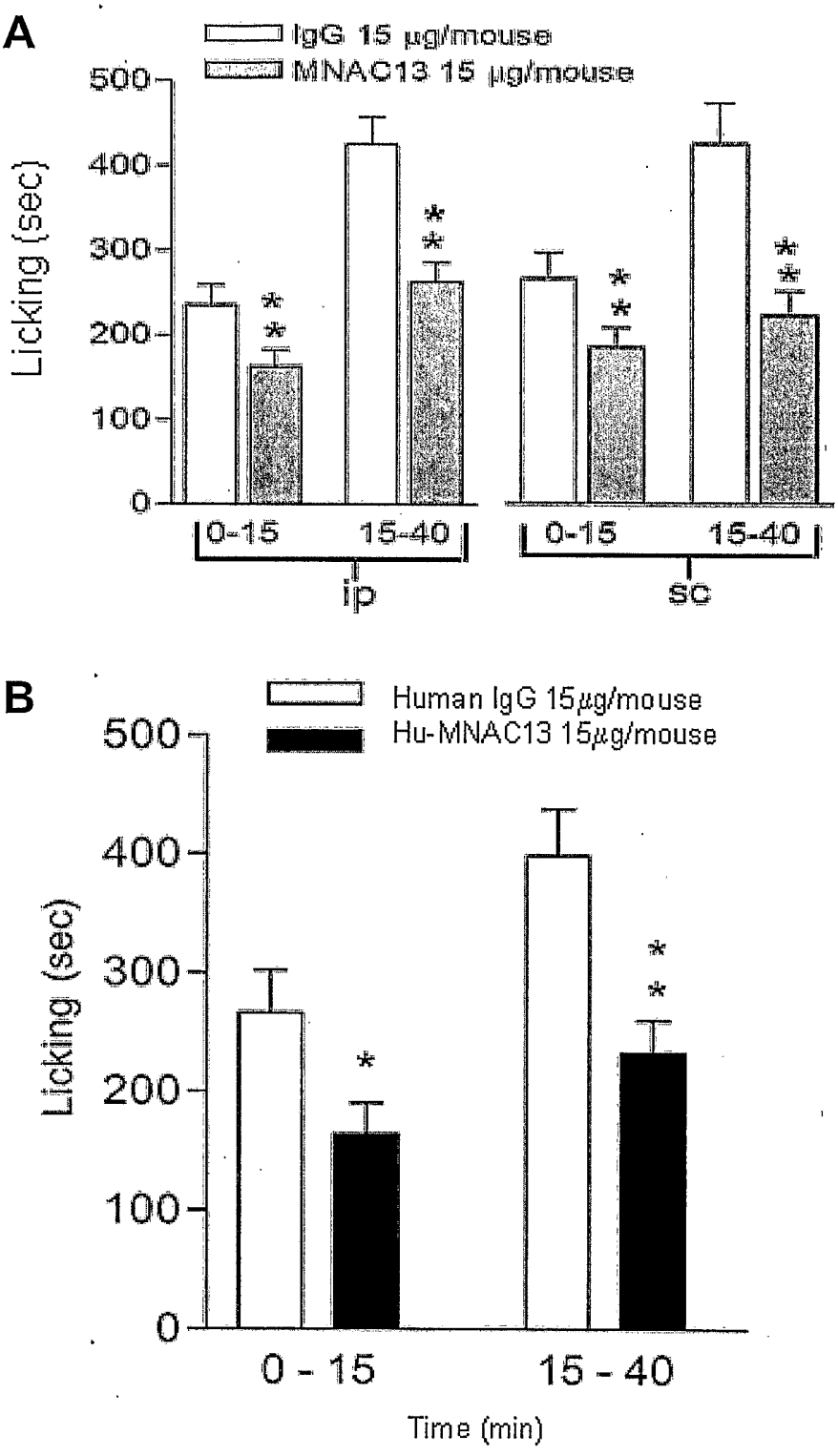

A
IgG 15 µg/mouse
MNAC13 15 µg/mouse
Licking (sec)
500
400
300
200
100
0
0-15
15-40
ip
sc
B
Human IgG 15µg/mouse
Hu-MNAC13 15µg/mouse
Licking (sec)
0 - 15
15 - 40
Time (min)

Fig. 5

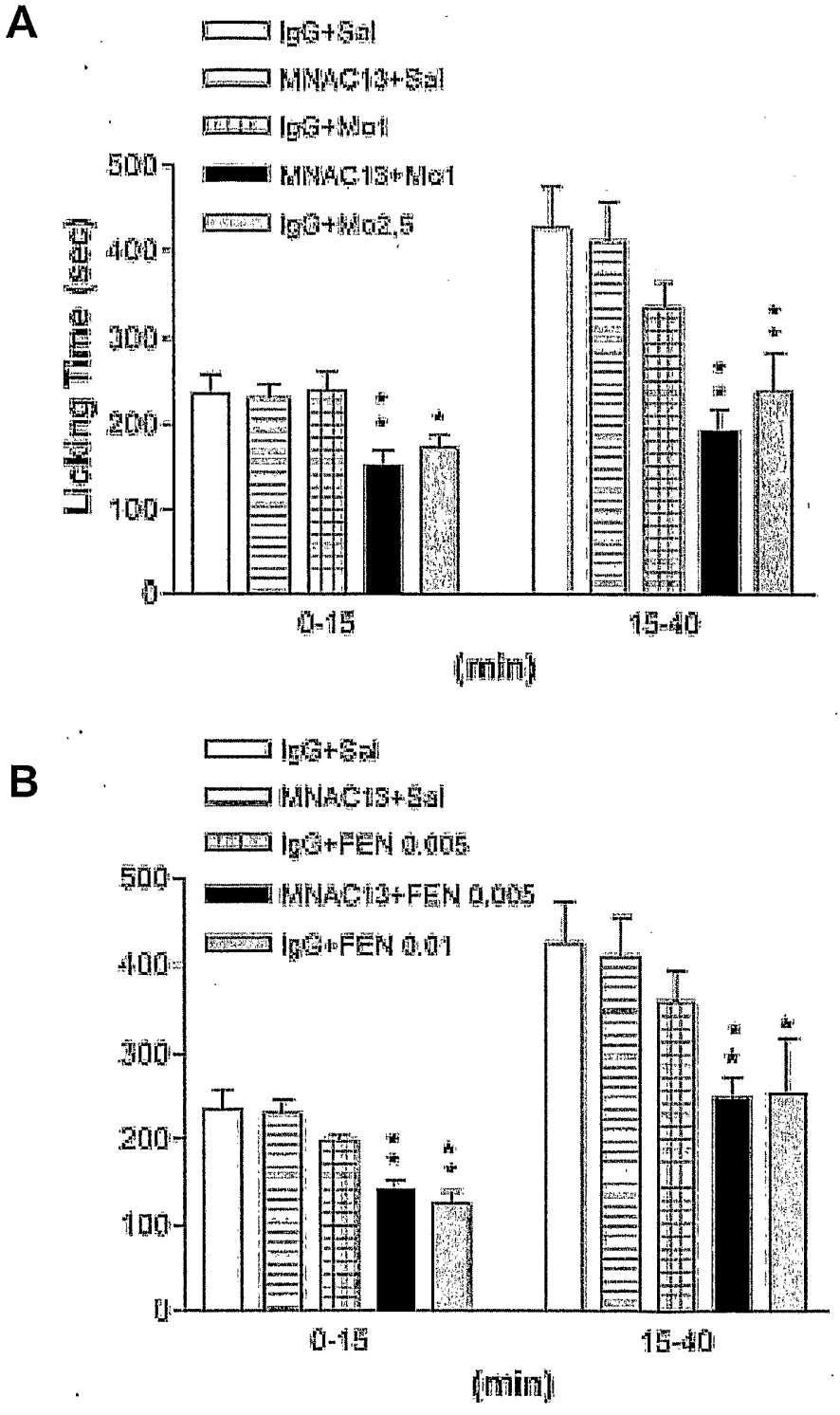

A
IgG+Sal
MNAC13+Sal
IgG+Mo1
MNAC13+Mo1
IgG+Mo2,5
Licking Time (sec)
500
400
300
200
100
0
0-15
15-40
(min)
B
IgG+Sal
MNAC13+Sal
IgG+FEN 0.005
MNAC13+FEN 0.005
IgG+FEN 0.01
500
400
300
200
100
0
0-15
15-40
(min)

Fig. 6

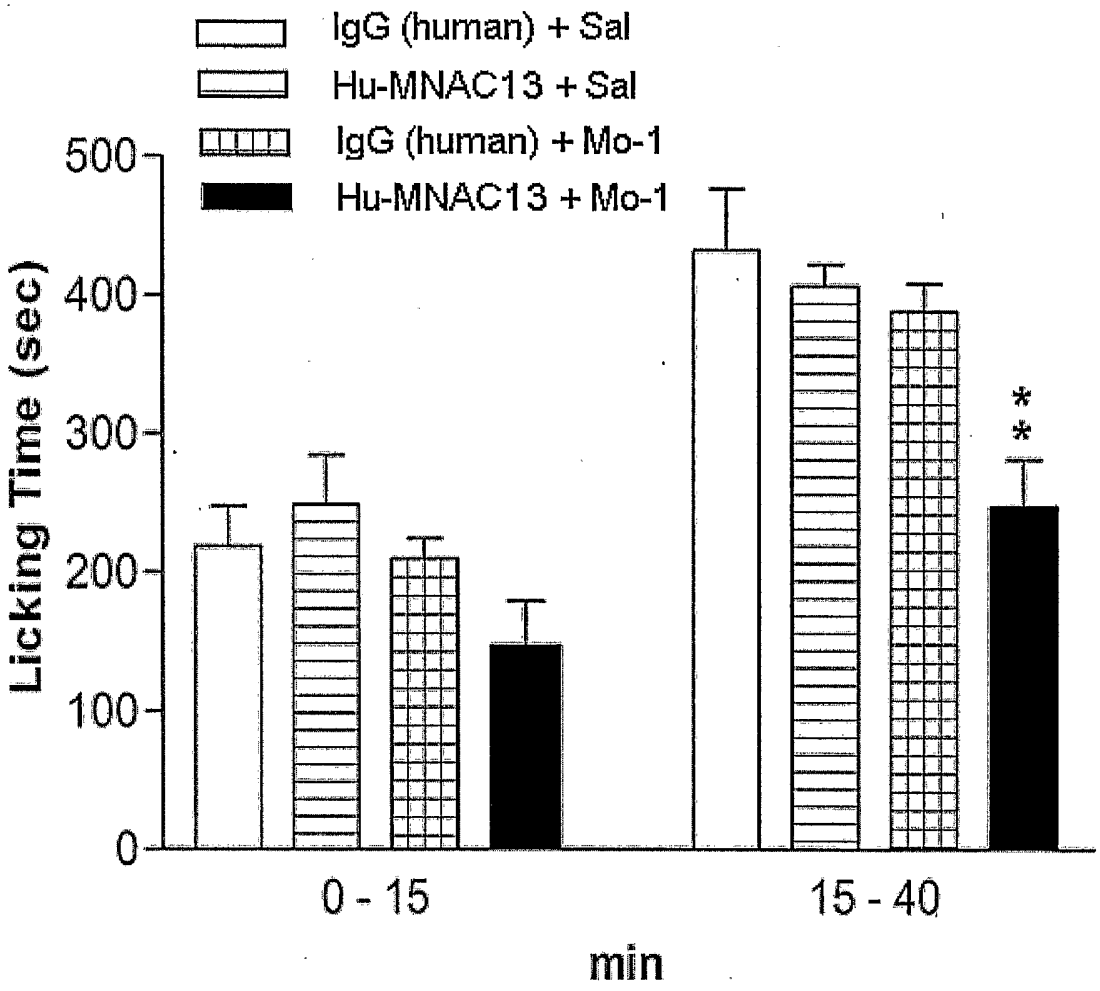

IgG (human) + Sal
Hu-MNAC13 + Sal
IgG (human) + Mo-1
Hu-MNAC13 + Mo-1
Licking Time (sec)
500
400
300
200
100
0
**
0 - 15
15 - 40
min

Fig. 7