

NOVEL PEPTIDES WHICH HAVE ANALGESIC EFFECTS AND WHICH INHIBIT ASIC CHANNELS

DESCRIPTION

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Technical field

The present invention relates to novel peptides which induce analgesia and which inhibit ASIC channels (Acid Sensing Ion Channels), more particularly homomeric ASIC1a channels, homomeric ASIC1b channels and/or heteromeric channels containing at least one subunit chosen from ASIC1a and ASIC1b, to the polynucleotides encoding said peptides, and also to the pharmaceutical compositions, host cells and vectors contained therein. In particular, said peptides exhibit a minimum of 56% identity with the amino acid sequence SEQ ID No: 1 in the appended sequence listing. They are in particular the two 57-amino acid peptides ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) isolated from the venom of the snake *Dendroaspis polylepis* (black mamba), of sequences SEQ ID No: 2 and SEQ ID No: 3, respectively.

The present invention also relates to the use thereof for obtaining a diagnostic tool or a medicament, in particular an analgesic, and also for identifying analgesic molecules or molecules which inhibit ASIC channels.

The present invention has in particular an application in the prevention or treatment of pain, in particular pain associated with the activation of ASIC channels (for example inflammatory, neuropathic, cancer-related, post-operative, musculoskeletal, visceral, etc., pain), of central neurological diseases (for example, post-traumatic stress, depression, anxiety, strokes, epilepsy, multiple sclerosis, cerebral inflammations, neurodegenerative diseases, etc.), and of pathological

conditions in which the involvement of ASIC channels has been proposed (for example, inflammations, cancers, fibromyalgia, irritable bowel syndrome, etc.).

- 5 In the description below, the reference between square brackets ([]) refer to the list of references presented at the end of the text.

State of the art

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The taking into account and treatment of pain are essential aspects of improving the quality of life of patients. Pain affects a considerable number of individuals, about 60 million in Europe each year, which represents an annual cost of 1 billion dollars in analgesic medicaments for treating said pain. The amounts spent annually throughout the world on analgesic medicaments can be evaluated at approximately 25 billion dollars, and should reach 42 billion in 2010. Pain is divided up into two categories: acute pain and chronic pain. Acute pain corresponds to rapid and brief pain which is limited over time. Conversely, chronic pain is a persistent pain which can be linked, for example, to hyperalgesia, and which constitutes an enormous disease burden, affecting approximately 20% of adults and 50% of the elderly population.

The treatment of pain is based essentially on the prescription of anti-inflammatories, whether they are nonsteroidal (NSAIDs) or steroidal (corticoids), and of weak or strong opiates. NSAIDs form the therapeutic class most widely prescribed throughout the world, owing to their great efficacy both on inflammation and on pain itself. They are used in all types of inflammatory pain, whether acute or chronic [Bertin and Vergne-Salle, 2007] [1]. When NSAIDs and/or corticoids are not sufficient to relieve inflammatory pain, the prescribing physician combines a non-anti-inflammatory

analgesic, for example paracetamol, weak opioids (codeine, tramadol), and, if the pain continues to be resistant to the treatment, strong opioids (morphine, oxycodone, fentanyl) [Gutstein & Akil, 2006] [2].

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While NSAIDs are very effective, they nevertheless remain great purveyors of adverse side effects. Among the most standard adverse side effects, digestive effects are very frequent and limit the use of NSAIDs
10 in many clinical situations. There are also renal, cutaneous, mucosal, allergic and respiratory, hematological, hepatic and, finally, neurosensory and psychological adverse side effects [Bertin and Vergne-Salle, 2007, mentioned above] [1]. In addition, NSAIDs
15 are not effective in all types of pain. Opioids also play a major role in combating pain, but can cause hallucinatory phenomena and cardiorespiratory depression. Analgesics can also be a source of dependence, for instance morphine, methadone, etc.,
20 dependence. There are also cases of habituation to analgesics, that is to say the dose necessary to obtain a constant effect must be increased. This habituation increases over time and therefore leads to the need to increase the doses and can lead to ineffectiveness of
25 the medicament. Indeed, the dose necessary to relieve pain can become greater than the toxic dose of said medicament. Finally, treatment with opioids can also be associated with adverse effects such as severe constipation or hyperalgesia when the treatment is
30 stopped (post-operative pain, for example) [Gutstein & Akil, 2006, mentioned above; Bannister & Dickenson, 2010] [2, 3].

Despite the diversity of the existing therapeutic
35 arsenal, many types of pain remain relatively insensitive to the known analgesics, such as neuropathic pain following damage to the nervous system (50% of patients experience no relief), chronic

visceral pain such as irritable bowel syndrome or chronic inflammatory bowel disease, fibromyalgia, pain associated with cancers and bone metastases, etc. [Yennurajalingam et al., 2004; Mizoguchi et al., 2009]
5 [4, 5].

In this context, the discovery of novel analgesics compensating for these deficiencies and drawbacks and/or novel analgesic targets should therefore
10 represent real progress.

Research in the pharmaceutical industry on pain has over the past few years resulted only in a few limited developments. Mention may, for example, be made of
15 triptans for migraine and certain novel medicaments of which the use still remains limited, such as the combination of tetrahydrocannabinol and cannabidiol for cancer-related and neuropathic pain. In fact, the progress made over the last two decades comes
20 essentially from a better use and adjustments of the dosage of the available analgesics. None of the major families of these analgesics has a benefit/risk ratio which is optimal, because of a limited efficacy and/or considerable side effects.

25 Among the molecular targets identified over the past few years, ion channels hold a particularly important place because they are directly involved in the detection and transmission of pain signals by sensory
30 and central neurons. ASIC channels (Acid Sensing Ion Channels) are cationic channels activated by acidification of the extracellular medium (extracellular acidosis) [Waldmann & Lazdunski, 1998; Wemmie et al., 2006; Lingueglia et al., 2007] [6, 7,
35 8]. Thus far, four genes encoding at least seven subunits (ASIC1a, ASIC1b, ASIC1b2, ASIC2a, ASIC2b, ASIC3 and ASIC4) have been identified in mammals. Functional ASIC channels result from the association of

various ASIC subunits as trimers [Jasti et al. 2007] [9] resulting in homomeric or heteromeric channels [Lingueglia et al., 1997; Benson et al., 2002; Hesselager et al., 2004] [10, 11, 12]. ASIC channels
5 are essentially expressed in nociceptive sensory neurons of the peripheral nervous system and in central nervous system neurons [Waldmann et al., 1997a; Lingueglia et al., 2007, mentioned above; Noel et al., 2010] [13, 8, 14]. Although the ASIC1a and ASIC2
10 isoforms are present in both the central nervous system and the peripheral nervous system, the expression of the ASIC1b and ASIC3 isoforms is restricted to sensory neurons [Waldmann et al., 1997b; Bassler et al., 2001; Chen et al., 1998] [15, 16, 17].

15 It has been postulated that the ASIC channels expressed by sensory neurons, and in particular the ASIC3 channel, are capable of detecting extracellular acidifications that may develop during ischemia, inflammation, a hematoma, a fracture, a lesion, a
20 surgical procedure (post-operative pain), or the development of certain tumors [Reeh and Steen, 1996] [18]. As it happens, it has been known for several years now that extracellular acidosis causes pain
25 [Steen et al., 1995a; Issberner et al., 1996] [19, 20], and experiments carried out on healthy human volunteers [Ugawa et al., 2002; Jones et al., 2004] [21, 22] have shown the involvement of ASIC channels in acidic skin pain by means of amiloride and certain NSAIDs, which
30 are nonspecific inhibitors of ASIC channels [Waldmann et al., 1997a, mentioned above; Voilley et al., 2001] [13, 23]. The important role of certain ASIC channels expressed in central nervous system neurons in neuronal activity (synaptic plasticity of the hippocampus, of
35 the amygdala) and the neuromodulation of the transmission of pain information (ASIC1a channels) by spinal cord neurons has also been demonstrated [Noel et al., 2010] [14].

Until recently, the repertoire of active ligands capable of inhibiting ASIC channels was mainly limited to amiloride, to certain NSAIDs and to the compound
5 A-317567 [Dubé et al., 2005] [24]. However, none of these molecules is absolutely specific for ASIC channels or for a particular ASIC subunit. With the aim of identifying effectors specific for ASIC channels, a very large number of scorpion, bee, spider, snake or
10 sea anemone venoms have been screened. Recently, two animal peptide toxins, PcTx1 and APETx2, have been identified which inhibit homomeric ASIC1a channels and the channels containing the ASIC3 subunit, respectively [Escoubas et al., 2000; Diochot et al., 2004] [25, 26].
15 The peripheral (subcutaneous) injection of APETx2 induces an analgesic effect on inflammatory and acidic pain in rats [Deval et al., 2008] [27] and on post-operative pain in rats after intra-operative application of APETx2 [Deval et al., J. Neurosci.,
20 31(16): 6059-6066, 2011] [36], while the central injection of PcTx1 induces a powerful analgesic effect in mice [Mazzuca et al., 2007] [28]. The analgesic effects of these two toxins have made it possible to demonstrate the involvement in ASIC channels in the
25 perception and transmission of pain information.

There is therefore a real need to identify other effectors specific for ASIC channels or for a particular ASIC subunit, capable of exhibiting an
30 analgesic effect while at the same time compensating for the deficiencies, drawbacks and obstacles of the prior art analgesics.

Description of the invention

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The inventors have now discovered and identified, from the venom of the snake *Dendroaspis polylepis* (black mamba), novel peptides which induce analgesia and which

inhibit ASIC channels (Acid Sensing Ion Channels), more specifically homomeric ASIC1a channels, homomeric ASIC1b channels, heteromeric ASIC1a+2a channels and heteromeric ASIC1a+1b channels.

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They are in particular the peptides ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) which, after central (intrathecal and intracerebroventricular) injection *in vivo* in mice, exhibit a powerful analgesic effect on different pain modes (chemical, thermal, inflammatory), which is comparable to that of morphine but largely independent of opiate receptor activation. In addition, these peptides do not exhibit neurotoxic effects when they are injected centrally. When administered by subcutaneous peripheral injection, these peptides also exert an analgesic effect with reversion of inflammatory hyperalgesia. These two peptides are the first peptides extracted from the venom of *Dendroaspis polylepis* (black mamba) to exhibit an analgesic effect.

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Without being limited by this explanation, the most probable mechanism of action of these peptides appears to result from the inhibition of ASIC ion channels which play an important role in the perception, transmission and modulation of pain information. Indeed, these peptides efficaciously inhibit homomeric ASIC1a channels, homomeric ASIC1b channels and/or heteromeric channels containing an ASIC1a and/or ASIC1b subunit of rats and humans. These two peptides are in particular the first known inhibitors of homomeric ASIC1b and ASIC1a channels and heteromeric ASIC1a+ASIC1b and ASIC1a+ASIC2a channels.

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The ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides also inhibit the native ASIC currents present in sensory neurons and in central neurons. They have no effect on the TRPV1 current activated by capsaicin and by heat and which is itself also involved in pain

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perception. They do not modify the electrical properties of neurons in the basal state, but reduce neuronal excitability in response to an extracellular acidification capable of activating ASIC channels.

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The ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides therefore have, in rodents, powerful analgesic properties comparable to those of morphine, which continue to be observed when the opiate receptors are blocked, and which can be explained by their capacity to specifically inhibit certain rodent and human ASIC channels. The central injection of said peptides induces no toxicity (neurotoxicity, convulsions, etc.), nor any effect on motor activity (accelerating rotarod test).

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The ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides can therefore be envisioned as novel molecules having a therapeutic potential against pain (for example, cancer-related pain, neuropathic pain, post-operative pain, etc.) in humans.

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A subject of the present invention is therefore a peptide comprising:

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(i) the amino acid sequence

LKCX⁴QH GKVV TCH RDMKFCYHNTGMPFRNLKLILQGCSSSCSETENNKCCSTDR
CNK (SEQ ID No: 1)

wherein X⁴ represents any amino acid;

or

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(ii) a natural or synthetic sequence exhibiting an identity of at least 56% with the sequence SEQ ID No: 1, preferably of at least 70%, preferentially of at least 80%, most preferentially of at least 98%, and retaining the biological properties of the peptides comprising the sequence SEQ ID No: 1 as described above, namely which induce analgesia and which inhibit at least one ASIC channel containing at least one subunit chosen from the group consisting of the ASIC1a

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and ASIC1b subunits.

Said peptides according to the invention operate more particularly as a blocker of homomeric ASIC1a channels, homomeric ASIC1b channels and/or heteromeric ASIC channels containing at least one subunit chosen from the group consisting of the ASIC1a and ASIC1b subunits, in particular heteromeric ASIC1a+ASIC1b channels and/or heteromeric ASIC1a+ASIC2a channels.

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For the purpose of the present invention, the term "blocker" is intended to mean a peptide capable of inhibiting in a concentration-dependent manner the current produced by the abovementioned channels. For example, it is a peptide which, like PcTx1 [Escoubas et al., 2000, mentioned above] [25], is capable of inhibiting, at a concentration of 1 nM, 50% of the current produced by the homomeric ASIC1a channels, or which, like APETx2 [Diochot et al., 2004, mentioned above] [26], is capable of inhibiting, at a concentration of 2 μ M, 50% of the current produced by the rat heteromeric ASIC1a+ASIC3 channels.

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Preferably, said peptides according to the invention are extracted from the venom of the snake *Dendroaspis polylepis*. For example, they are isolated from the venom by sequential fractionation by means of a reverse-phase-polarity high performance liquid chromatograph (RP-HPLC). Said peptides can also be prepared by DNA recombination methods or by chemical synthesis.

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Preferably, said peptides according to the invention comprise the ASICalgin-1 (π -Dp1) or ASICalgin-2 (π -Dp2) peptide having the sequence SEQ ID No: 1 wherein X⁴ represents Y or F, respectively (SEQ ID No: 2 and 3, respectively).

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A subject of the present invention is also a polynucleotide comprising a nucleotide sequence encoding a peptide according to the invention.

5 Preferably, said polynucleotide according to the invention comprises a nucleotide sequence:

atgaaaactctgctgctgaccttgctggtggtgacaatcgtgtgcctagacttag
gatactccctgaaatgt⁷³txx⁷⁵caacatggtaaagttgtgacttgtcatcgaga
tatgaagttttgctatcataacactggcatgccttttcgaaatctcaagctcatc
10 ctacagggatgttcttcttcgtgcagtgaaacagaaaacaataagtgttgctcaa
cagacagatgcaacaaatag (SEQ ID No: 4);

or

ctgaaatgt¹⁰txx¹²caacatggtaaagttgtgacttgtcatcgagatatgaagt
tttgctatcataacactggcatgccttttcgaaatctcaagctcatcctacaggg
15 atgttcttcttcgtgcagtgaaacagaaaacaataagtgttgctcaacagacaga
tgcaacaaatag (SEQ ID No: 22);
wherein ⁷³txx⁷⁵ and ¹⁰txx¹² represent tac, tat, ttt or
ttc.

20 Preferably, said polynucleotide according to the invention comprises a nucleotide sequence such that it can hybridize under stringent conditions with the nucleotide sequence SEQ ID No: 4 or SEQ ID No: 22, or a sequence complementary thereto. For example, it is a
25 polynucleotide which comprises a natural or synthetic sequence exhibiting an identity of at least 76% with the sequence SEQ ID No: 4 or the sequence SEQ ID No: 22, preferably of at least 80%, most preferentially of at least 98%.

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A subject of the present invention is also a vector comprising a polynucleotide according to the invention.

35 Preferably, said vector according to the invention is an expression vector.

A subject of the present invention is also a host cell comprising one or more peptides according to the

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invention, polynucleotides according to the invention or vectors according to the invention.

A subject of the present invention is also a
5 pharmaceutical composition comprising one or more peptides according to the invention, polynucleotides according to the invention, vectors according to the invention or host cells according to the invention. A pharmaceutical composition according to the invention
10 may also comprise one or more pharmaceutically acceptable vehicles (calcium carbonate, starch, talc, lactose, magnesium stearate, acacia gum, etc.) and be in the form of a solution, suspension, paste, gel capsule, tablet, capsule, powder, granule,
15 lyophilisate, controlled-release system, microparticle, micro- or nanosphere, liposome, etc. A pharmaceutical composition according to the invention can be administered orally, intramuscularly, intravenously, subcutaneously, topically, via the pulmonary route,
20 intranasally, buccally, rectally, sublingually, intradermally, intraperitoneally, intrathecally, etc. The effective amount of active ingredient (peptide, polynucleotide, vector or host cell according to the invention), in a pharmaceutical composition according
25 to the invention, can vary such that an effective dose is obtained and that an analgesic amount is administered to a mammal. The dosage administered to a particular mammal depends on several factors: the route of administration, the duration of treatment, the size
30 and physical condition of the mammal, the potency of the active ingredient and the response of the mammal to said active ingredient. For example, an effective analgesic amount of active ingredient administered intrathecally generally ranges from approximately
35 5 ng/kg to 500 µg/kg of bodyweight of the mammal, preferably from approximately 50 ng/kg to 50 µg/kg of bodyweight of the mammal, most preferentially from approximately 500 ng/kg to 5 µg/kg of bodyweight of the

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mammal. The effective amounts of active ingredient can vary when other routes of administration are used. An effective analgesic amount can be estimated by testing the active ingredient in one or more pain tests mentioned hereinafter at a dose which can vary according to one or more criteria cited above, in order to determine the effective amount of active ingredient to be administered to a mammal.

10 A subject of the present invention is also a substance chosen from a peptide according to the invention, a polynucleotide according to the invention, a vector according to the invention, a host cell according to the invention or a pharmaceutical composition according to the invention, for application thereof as a medicament.

Preferably, said medicament is an analgesic, for example intended for the prevention or treatment of pain involving the activation of ASIC channels, in particular ASIC channels containing at least one subunit chosen from the group consisting of the ASIC1a and ASIC1b subunits, most particularly of the homomeric ASIC1a channels, homomeric ASIC1b channels, heteromeric ASIC1a+ASIC1b channels and/or heteromeric ASIC1a+ASIC2a channels. For example, the pain may (i) result from the activation of central or peripheral ASIC channels or (ii) induce activation thereof. For example, the pain is pain, in particular pain associated with ASIC channel activation, chosen from the group comprising inflammatory, neuropathic, cancer-related, post-operative, musculoskeletal, visceral, etc., pain.

Preferably, said medicament is intended for the prevention or treatment of a pathological condition involving the activation of ASIC channels, in particular of ASIC channels containing at least one subunit chosen from the group consisting of the ASIC1a

and ASIC1b subunits, most particularly the homomeric ASIC1a channels, homomeric ASIC1b channels, heteromeric ASIC1a+ASIC1b channels and/or heteromeric ASIC1a+ASIC2a channels. For example, it involves pathological
5 conditions chosen from the group comprising inflammations, cancers, fibromyalgia, irritable bowel syndrome, etc.

Preferably, said medicament is intended for the
10 prevention or treatment of a central neurological disease, for example chosen from the group comprising post-traumatic stress, depression, anxiety, strokes, epilepsy, central inflammations, multiple sclerosis, neurodegenerative diseases, etc.

15 Preferably, said medicament is administered parenterally, namely locoregionally or centrally (intraperitoneally, peridurally, intrathecally, intracerebroventricularly, intradermally, etc.) and
20 systemically (intramuscularly, intravenously, subcutaneously, etc.), orally, locally (transcutaneously, etc.) or via the respiratory route (inhalation, instillation, etc.). Most preferentially, said medicament is administered intrathecally,
25 peridurally, intracerebroventricularly, intra-peritoneally or subcutaneously.

A subject of the present invention is also a substance chosen from a peptide according to the invention, a
30 polynucleotide according to the invention, a vector according to the invention, a host cell according to the invention or a pharmaceutical composition according to the invention, for use thereof as a diagnostic tool.

35 A subject of the present invention is also a method for identifying a compound which mimics the analgesic activity of a peptide according to the invention, comprising the following steps:

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- a) determining the analgesic activity of a peptide according to the invention;
 - b) determining the analgesic activity of a candidate compound;
 - c) comparing the analgesic activities obtained in steps a) and b);
 - d) selecting the candidate compound which has an analgesic activity equivalent to or greater than that of a peptide according to the invention.
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A subject of the present invention is also a method for identifying a compound which mimics the analgesic activity of a peptide according to the invention, comprising the following steps:

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- a) bringing a peptide according to the invention into contact with a sample, and measuring the binding of said peptide with said sample;
 - b) adding a candidate compound, and evaluating the effect of said compound on the binding of said peptide with said sample;
 - c) selecting the candidate compound capable of modulating the binder of said peptide with said sample.
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For the purpose of the present invention, the expression "compound which mimics the analgesic activity of a peptide according to the invention" is intended to mean a candidate compound capable of inducing analgesia and of binding the ASIC channels to which the peptides according to the invention bind or else of acting in a physiological manner identical or similar to that of the peptides according to the invention, namely modulating (inhibiting or stimulating), reversibly and in a concentration-dependent manner, the current produced by at least one

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channel containing at least one subunit chosen from the group consisting of the ASIC1a and ASIC1b subunits, in particular by homomeric ASIC1a channels, homomeric ASIC1b channels, heteromeric ASIC1a+ASIC1b channels and/or heteromeric ASIC1a+ASIC2 channels. For example, it involves a peptide which, like PcTx1 [Escoubas et al., 2000, mentioned above] [25], is capable of inhibiting, at a concentration of 1 nM, 50% of the current produced by the homomeric ASIC1a channels, or which, like APETx2 [Diochot et al., 2004, mentioned above] [26], is capable of inhibiting, at a concentration of 2 μ M, 50% of the current produced by rat heteromeric ASIC1a+ASIC3 channels.

For the purpose of the present invention, the term "sample" is intended to mean cells or tissue isolated beforehand from the whole organism or from immortalized cell lines, of insects or mammals.

The term "analgesic activity" refers to the capacity of a peptide according to the invention to treat pain in a mammal, or to reduce pain, as demonstrated by one or more conventional laboratory models for testing pain or evaluating analgesia, such as those described hereinafter. Thus, the analgesic activity of a peptide according to the invention can be determined, for example, by means of one or more of the following *in vivo* tests: i) tail/paw withdrawal latency (measurement of thermal nociception) [Abott et al., 1982; Cridland and Henry, 1992] [29, 30], ii) hot/cold plate threshold (measurement of thermal nociception) [Woolfe and Macdonald, 1944, Ankier, 1974] [31, 32], iii) von Frey filament threshold or Randall-Selitto test or instrumented pincher test (mechanical nociceptive activity measurement) [Kim et al., 1993] [33], iv) dynamic weight distribution test (measurement of nociceptive activity linked to posture), v) measurement of spontaneous nociceptive behavior, and/or of one or

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more *in vitro* tests: for example, screening of candidate compounds by competition in which a peptide according to the invention which has been labeled (for example, with a radiolabel such as C^{14} , H^3 or I^{125} , an enzyme such as peroxidase or alkaline or acid phosphatase, a fluorescent label such as FITC or rhodamine, an antibody, an antigen, biotin, a paramagnetic ion, a latex particle, etc.) is brought into contact with a sample under conditions which allow it to bind to said sample, and the binding of said labeled peptide bound to the sample is measured, and in which the compounds which mimic the activity of said labeled peptide compete with said peptide for binding to the sites on the receptor (ASIC channels containing at least one subunit chosen from the group consisting of the ASIC1a and ASIC1b subunits). Thus, a lower amount of detectable labeling is measured when the test compounds mimic the activity of said peptide by binding to the receptor than when the test compounds do not mimic the activity of said peptide and do not bind the receptor, or bind with less affinity. As an alternative, the competition screening can be carried out with labeling of the candidate compound instead of labeling of the peptide according to the invention. Thus, a higher amount of detectable labeling will be measured when the test compounds mimic the activity of said peptide by binding to the receptor than when the test compounds do not mimic the activity of said peptide and do not bind the receptor, or bind with less affinity.

Examples of methods for identifying compounds which mimic the analgesic activity of peptides are described (without being limited by this description), for example in patent US 5 877 026 [34].

Other advantages may become further apparent to those skilled in the art on reading the examples below,

illustrated by the appended figures, which are given by way of illustration.

Brief description of the figures

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- Figure 1 represents (A) the amino acid sequence of the ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides isolated from the venom of the snake *Dendroaspis polylepis* (SEQ ID No: 2 and 3, respectively), (B) the nucleotide sequence of the complementary DNA (cDNA) encoding ASICalgin-1 (SEQ ID No: 5). The signal sequence (in italics) and the final peptide (in bold) (cf. SEQ ID No: 6), and the stop codon (*) are indicated. The sequence of the 5' noncoding end is not represented (corresponding to SEQ ID No: 9).

- Figure 2 represents the analgesic effect of the ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides *in vivo* after intrathecal injection in mice. Chemical pain (formol): (A) kinetics of spontaneous pain behavior measured in intervals of 5 minutes, (B) total duration of phase I (0-10 minutes) of acute pain and of phase II (10-45 minutes) of inflammatory pain. Acute thermal pain: (C) kinetics of tail withdrawal latency, (D) mean of the areas under the curve (AUC) measured for each animal. Inflammatory hyperalgesia: (E) kinetics of paw withdrawal latency. Significance of hyperalgesia relative to the control value: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; by ANOVA test followed by a Newman Keuls multiple comparison. (F) mean of the areas under the curve (AUC) measured for each animal, from the hyperalgesic level (T-7 min). The mean values $\pm$ sem (standard error of the mean) are represented and the number (n) of animals tested is mentioned in the legends. Significance relative to the injection of the vehicle $\pm$ naxolone (NAL) or else according to where indicated on the figure by a line: not significant *ns*,

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$p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; by ANOVA test followed by a Newman Keuls multiple comparison.

- Figure 3 represents the analgesic effect of the ASICalgin-1 (π -Dp1) peptide *in vivo* after intracerebro-ventricular injection in mice in the acute thermal pain test: (A) kinetics of tail withdrawal latency, (B) mean of the areas under the curve (AUC) measured for each animal. The mean values $\pm$ sem (standard error of the mean) are represented. The number (n) of animals tested is mentioned in the legends. Significance relative to the injection of the vehicle or else according to where indicated on the figure with a line: not significant *ns*, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; by ANOVA followed by a Newman Keuls multiple comparison.

- Figure 4 represents the analgesic effect of the ASICalgin-1 (π -Dp1) peptide *in vivo* after subcutaneous injection in the inflammatory hyperalgesia test: (A) kinetics of paw withdrawal latency. The ASICalgin-1 peptide is injected subcutaneously into the left rear paw at the same time as the carrageenan ($\square$) then again two hours later (times 0 or T0, $\square$). A similar procedure is carried out, but with injection of the ASICalgin-1 peptide only at T0, i.e. after the appearance of the inflammatory hyperalgesia ($\blacksquare$). (B) Mean of the areas under the curve (AUC) estimated for each animal, from the value at T-7 min. The mean values $\pm$ sem are represented. The number (n) of animals tested is mentioned in the legends. Significance relative to the injection of the vehicle: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; by ANOVA test followed by a Newman Keuls multiple comparison. Significance relative to the control value before the injection of carrageenan: #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$; by paired T-test.

- Figure 5 represents the inhibition, by the ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides,

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of the currents generated by the ASIC channels heterologously expressed in COS cells. Dose-response curve of the inhibition by ASICalgins-1 (π -Dp1) of the rat homomeric ASIC1a current, $IC_{50} = 49$ nM (A), of the human homomeric ASIC1a current, $IC_{50} = 127$ nM (B), of the rat homomeric ASIC1b current, $IC_{50} = 650$ nM (C), of the rat heteromeric ASIC1a+ASIC1b (ratio 1:1) current, $IC_{50} = 223$ nM (D), and of the rat heteromeric ASIC1a+ASIC2a (2:1 ratio) current, $IC_{50} = 308$ nM (E). The mean values $\pm$ sem are indicated, with n from 4 to 15 experiments. Original plots of currents inhibited by ASICalgins-1 (π -Dp1, 674 nM) and ASICalgins-2 (π -Dp2, 852 nM) are shown in miniature. (F) Original plots of current showing the inhibition of the human heteromeric ASIC1a+ASIC2a (2:1 ratio) current by ASICalgins-1 (π -Dp1, 674 nM). Resting potential: -60 mV, white bars: drop in pH from 7.4 to 5.5, black bars: application of the peptide.

- Figure 6 represents the inhibition, by the ASICalgins-1 (π -Dp1) and ASICalgins-2 (π -Dp2) peptides, of the native ASIC currents of murine sensory and central neurons in culture. (A) Mean inhibition of the ASIC current of sensory neurons of rat (n = 34) by ASICalgins-2 (π -Dp2, 852 nM, $\square$) and ASICalgins-1 (π -Dp1, 674 nM, $\blacksquare$). On the right, examples of original currents recorded at the resting potential of -50 mV and activated with a drop in pH of from 7.4 to 6 (white bar). In the sensory neurons, the total ASIC current results from a mixture of homomeric ASIC1a current, of current of ASIC1b type and of current of ASIC3 type. (B) Mean inhibition of the ASIC current of dorsal cord neurons of mice (n = 18) by ASICalgins-2 (π -Dp2, 852 nM, $\square$) and ASICalgins-1 (π -Dp1, 674 nM, $\blacksquare$). On the right, example of original currents recorded at the resting potential of -50 mV and activated with a drop in pH of from 7.4 to 6 (white bar). (C) Mean inhibition of the ASIC current of hippocampal neurons of mice (n = 26) by

- 20 -

ASICalgin-2 (π -Dp2, 293 nM, 852 nM and 2.9 μ M, $\square$) and ASICalgin-1 (π -Dp1, 224 nM and 2.2 μ M, $\blacksquare$). On the right, example of original currents recorded at the resting potential of -50 mV and activated with a drop in pH of from 7.4 to 5.5 (white bar). In the central neurons (mouse cord and hippocampus), the total ASIC current results from a mixture of homomeric ASIC1a current and of heteromeric ASIC1a+ASIC2a current. The mean values $\pm$ sem are indicated.

10

- Figure 7 represents the effect of the ASICalgin-1 (π -Dp1) and ASICalgin-2 (π -Dp2) peptides on the membrane potential of mouse dorsal spinal cord neurons. (A) Membrane potential of a neuron where the spontaneous action potentials reflect synaptic activity (dashed line: 0 mV). The activation of the ASIC current by a drop in pH of from 7.4 to 6 induces a depolarization of which the peak triggers an action potential (enlargement). The application of the ASICalgin-1 peptide (π -Dp1, 674 nM) does not modify the resting potential of the neuron nor the spontaneous activity. On the other hand, the depolarization induced by the acid pH drop is reduced and no longer induces an action potential. (B) Action potential evoked by an electric stimulation (square-wave currents represented above). Four responses are superimposed (left-hand panel): two subliminal depolarizations and two others triggering an action potential (dashed line: 0 mV). The application of ASICalgin-2 (π -Dp2, 852 nM, right-hand panel) does not modify the excitability of the neuron.

30

EXAMPLES

**EXAMPLE 1: ISOLATION OF TWO PEPTIDES, ASICALGIN-1
(π -Dp1) AND ASICALGIN-2 (π -Dp2), FROM THE VENOM OF THE
5 SNAKE *DENDROASPIS POLYLEPIS***

Venom and purification of the peptide toxins

The lyophilized venom comes from the company Latoxan
10 (Valence, France). The venom was diluted in acetic acid
(1%) and fractionated by passing it over a column
containing a Sephadex G50 resin. The peptide fraction
was recovered and subsequently fractionated on a High
Performance Liquid Chromatography (HPLC) system: 1) The
15 peptide fraction was loaded onto and eluted from a
cation exchange column with a pH gradient with the
solvents 1% acetic acid, pH 3.0, and 1M ammonium
acetate, pH 6.8. The fraction containing the ASICalgins
was eluted starting from 60% ammonium acetate. 2) This
20 peptide fraction was loaded onto a C18 reverse-phase
column and the constituents thereof were eluted with a
hydrophobicity gradient between the solvents water-0.1%
TFA and acetonitrile-0.1% TFA. The ASICalgin-1 and
ASICalgin-2 peptides were eluted pure at 26% and 27% of
25 acetonitrile, respectively.

Peptide characterizationAmino acid analysis

30 ASICalgin-1 and ASICalgin-2 are two basic isopeptides
which have a sequence of 57 amino acids containing 8
cysteines forming 4 disulfide bridges (figure 1A, SEQ
ID No: 2 and 3, respectively), and which is preceded by
35 a signal sequence of 21 amino acids (figure 1B, cf. SEQ
ID No: 6). They differ from one another only by virtue
of an amino acid in position 4 of SEQ ID No: 1, 2 or 3
(position 25 of SEQ ID No: 6).

Determination of the amino acid sequence by cloning

The complete sequence (figure 1B) of ASICalgIn-1 was
5 determined by cloning the cDNA by PCR, from the
messenger RNA (mRNA) present in trace amounts in the
crude venom. 25 mg of the venom of the snake
Dendroaspis polylepis (Sigma) were resuspended in lysis
buffer: 500 mM LiCl, 10 mM EDTA, 1% (w/v) LiDS, and
10 5 mM dithiothreitol in 100 mM of tris-HCl buffer, pH
7.5. The messenger RNA was captured by means of
magnetic beads grafted with dT25 oligonucleotides
(Dynal, UK). These oligonucleotides were directly used
as primers for carrying out the reverse transcription
15 of the mRNA to cDNA with the PrimeScript™ reverse
transcriptase (TaKaRa Bio Inc., Japan). The resulting
solid-phase cDNA library was used to carry out a
degenerate PCR with the sense (TGITTYCARCAYGGIAARGT,
SEQ ID No: 7) and antisense (YTTIARRTTICGRAAIGGCAT, SEQ
20 ID No: 8) oligonucleotides defined from the partial
protein sequence of the toxin determined by direct
N-terminal sequencing. In order to exclude any error
due to the polymerase, three independent PCRs were
carried out, which produced fragments at the hoped for
25 size (89 base pairs), that were subsequently subcloned
into the pGEMTeasy vector (Promega) and sequenced. By
virtue of these sequences, a specific sense oligo-
nucleotide with an added EcoRI restriction site was
synthesized (ACAC(GAATTC)GCTATCATAACACTGGCATG, SEQ ID
30 NO: 9), as was a nonspecific poly-dT30 antisense oligo-
nucleotide, also with an added EcoRI site
(ACAC(GAATTC)dT30, SEQ ID No: 10). These two oligo-
nucleotides made it possible to amplify by PCR the
entire 3'-coding and 3'-noncoding end for
35 identification purposes. Three independent PCRs
generated a band of approximately 400 base pairs and
the subcloning thereof into the pGEMTeasy vector at the

EcoRI restriction site made it possible to sequence it while excluding any error generated by the polymerase.

On the basis of the 3'- and 5'-noncoding sequences, a sense oligonucleotide (ACAC(GAATTC)TCCAGAGAAGATCGCAAGATG, SEQ ID No: 11) and an antisense oligonucleotide (ACAC(GAATTC)ATTTAGCCACTCGTAGAGCTA, SEQ ID No: 12) framing the coding sequence were synthesized. Four independent PCRs were carried out on the solid-phase cDNA library and the PCR products were again subcloned into the pGEMTeasy vector so as to obtain the complete nucleotide sequence of the ASICalgin-1 toxin precursor.

Peptide sequencing

The sequence was confirmed up to amino acid Asp40 by direct N-terminal sequencing of the reduced and alkylated peptides on an automatic sequencer (LC491, Applied Biosystems, USA).

Enzymatic cleavage

In order to confirm the sequences, the peptides were subjected to the following enzymatic treatments: (a) V8 protease with separation of the digested peptides on a C18 reverse phase HPLC column. The cleavage in position D15 and E43 and the separation on HPLC of two peptides of molecular weight 1786.9 Da (peptide 1-15), entirely resequenced, and 3194.5 Da (peptide 16-43), partial sequence, make it possible to confirm the N-terminal sequence. (b) Trypsin digestion with direct analysis by mass spectrometry. The tryptic fragments also confirm the N-terminal sequence and also other parts of the peptide sequence of the ASICalgins. The tryptic peptides detected and sequenced by MS/MS analysis are given in table 1 below.

Table 1

Position	Peptide sequence	Mw (Da)
3-8	CFQHGK (SEQ ID No: 13)	775.27
3-8	CYQHGK (SEQ ID No: 14)	791.27
3-14	CFQHGKVV TCHR (SEQ ID No: 15)	1527.58
3-14	CYQHGKVV TCHR (SEQ ID No: 16)	ND
9-14	VV TCHR (SEQ ID No: 17)	770.31
15-28	DMKFCYHNTGMPFR (SEQ ID No: 18)	1803.57
32-48	LILQGCSSSCSETENNK (SEQ ID No: 19)	1925.63
49-54	CCSTDR (SEQ ID No: 20)	797.24
32-54	LILQGCSSSCSETENNKCCSTDR (SEQ ID No: 21)	2705.78

5 Mass spectrometry (MALDI-TOF)

In all cases, the mass spectrometry analysis was carried out on a 4800 MALDI-TOF-TOF spectrometer (Applied Biosystems, USA).

10

The native proteins were analyzed in positive ion linear mode with a sinapinic acid matrix (Sigma Aldrich, USA) using an internal calibration with a mixture of proteins of known molecular weight.

15

The molecular weight measured is 6554.8 Da for ASICalgin-1 and 6538.4 for ASICalgin-2. It is in perfect agreement with the molecular weights calculated via the sequence, 6554.58 Da and 6538.58 Da, respectively, on the GPMW protein analysis software. These results suggest a free carboxylic acid C-terminal end. The molecular absorbance calculated at 280 nm is ϵ = 3040 (for ASICalgin-1) and ϵ = 1760 (for ASICalgin-2).

25

Database search for homologues

ASICalgin-1 and ASICalgin-2 do not appear in the databases and do not exhibit any total sequence identity with other already known peptides or toxins.

The BLAST sequence alignment program shows that ASICalgin-1 and ASICalgin-2 nevertheless exhibit 47% to 55% sequence identity (63% to 66% sequence homology) with the peptides CM-1b, CM-3 and CM-2a purified from the venoms of various species of cobra snakes (*Naja* and *Ringhals*), and also with an α -neurotoxin, OH-26, present in the venom of the king cobra *Ophiophagus hannah*. However, these CM and OH-26 neurotoxins are not described as acting on ASIC channels nor as exhibiting analgesic effects *in vivo*, but rather as exhibiting cytotoxic effects. It therefore appears that, below 55% sequence identity with ASICalgin-1 or ASICalgin-2, the biological properties exhibited by the peptides of the invention are lost. On the other hand, it is known from the art that isoform and/or orthologous toxins exhibit a preserved activity despite some differences in their protein sequences resulting in a sequence identity of about 70%. It therefore appears that isoform and/or orthologous toxins which exhibit a sequence identity of at least 56% to 70% with ASICalgin-1 or ASICalgin-2 will retain the properties of said peptides of the invention.

EXAMPLE 2: ANALGESIC EFFECT OF THE ASICALGIN-1 (π -Dp1) AND ASICALGIN-2 (π -Dp2) PEPTIDES *IN VIVO* AFTER INTRATHECAL INJECTION

The pain behavior of mice (C57B16J, 7- to 11-week-old males) was evaluated after intrathecal (IT) injection of the peptides between the L5 and L6 vertebrae, according to the protocol of Hylden and Wilcox [1980] [35]. The effects of an IT injection of 10 μ l of

solution containing 34 μ M of ASICalgin-1 (0.34 nmol) or 20 μ M of ASICalgin-2 (0.2 nmol) were compared with the effects of an IT injection of 10 μ l of solution containing 3.1 mM of morphine (31 nmol) or of 10 μ l of saline solution vehicle (145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM HEPES, pH 7.4, 0.05% bovine serum albumin). In certain series of experiments, naxolone, an inhibitor of opioid receptors (especially of μ type), was injected subcutaneously at 2 mg/kg in 50 μ l of 0.9% NaCl, ten minutes before the IT injection of the ASICalgin peptide.

Toxicity

The intrathecal injection of the ASICalgin-1 and ASICalgin-2 peptides induces no modification of behavior of motor problem, balance problem, apathy, paralysis or convulsion type, nor any deaths, in mice.

Acute chemical pain and inflammatory pain

The subcutaneous injection of formol (15 μ l at 2% in a 0.9% NaCl solution) into the arch of the hind paw of a mouse induces pain exhibiting two phases: a phase I of acute chemical pain for 10 minutes and a phase II of inflammatory pain for 15 to 45 minutes (figure 2A).

The pain is measured by observing the spontaneous pain behavior of the animal, i.e. by counting the time during which the mouse raises the injected paw, licks it, chews at it or shakes it (integrated voluntary behavior).

The IT injection of ASICalgin-1 significantly reduces the phase I pain by 40% and the phase II pain by 70%. This analgesic effect is similar to that of morphine (figure 2B).

The IT injection of ASICalgin-2 produces an effect identical to ASICalgin-1.

5 The pretreatment with naxolone, an opiate receptor inhibitor, has no effect on the analgesic effect of ASICalgin-1, whether it be on either of the two pain phases I and II (figure 2B).

10 The ASICalgin-1 and ASICalgin-2 peptides are the first analgesic peptides extracted from the venom of *Dendroaspis polylepis*.

Acute thermal pain

15 The thermal pain was studied in mice by measuring the latency of withdrawal of the tail immersed in water at 46°C (reflex movement, the animal is braced), with a limiting duration of 30 s.

20 The ASICalgin-1 peptide induces an analgesic effect which is as powerful as that of morphine (7 minutes after the IT injection), but which is more transient at the concentration used (figure 2C).

25 The analgesic effect of ASICalgin-1 is not significantly inhibited by a pretreatment with naxolone (figure 2D).

Inflammatory hyperalgesia

30

The subcutaneous injection of 2% carrageenan into the arch of the hind paw of the mice leads to the development of a tissue inflammation and of hyperalgesia, measured by the latency of withdrawal of the hind paw
35 immersed in water at 46°C (integrated voluntary movement, the animal is braced) with a limiting duration of 30 s, two hours after the injection of carrageenan (figure 2E, #).

The IT injection of ASICalgin-1 leads to rapid analgesia, comparable to that of morphine and persisting throughout the duration of the experiment
5 (67 minutes, figure 2E).

The analgesic effect of ASICalgin-1 is only slightly inhibited by 20% by a pretreatment with naxolone (figure 2F).

10

Motor activity

The effects of the ASICalgin-1 peptide on motor activity were evaluated by means of the accelerating
15 rotarod test. The mice were placed on an axle rotating at four rotations per minute (rpm) which undergoes a constant acceleration of 5 rpm per minute up to 40 rpm. The latency to fall is measured, with a maximum limit of 300 s.

20

The IT injection of the ASICalgin-1 peptide does not induce any significant effect (ANOVA test) on the performance levels of the mice with regard to motor activity compared with an injection of vehicle
25 solution.

EXAMPLE 3: ANALGESIC EFFECT OF THE ASICALGIN-1 PEPTIDE (π -Dp1) *IN VIVO* AFTER INTRACEREBROVENTRICULAR INJECTION

30 The intracerebroventricular (ICV) injection of the ASICalgin-1 peptide was carried out in the third ventricle of the brain of mice (C57B16J, 7- to 11-week-old males) anesthetized with isoflurane (1.5%) using the following stereotaxic coordinates: -2.4 mm under
35 the cortical surface, -0.5 mm in the anterior-posterior axis and +1.6 mm in the mediolateral axis relative to the bregma. The effects of an ICV injection of 5 μ l of solution containing 34 μ m of ASICalgin-1 (0.34 nmol)

were compared with the effects of 5 µl of saline solution vehicle (145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM HEPES, pH 7.4, 0.05% bovine serum albumin). naxolone, an inhibitor of opioid receptors (especially μ -type), was injected subcutaneously at 2 mg/kg in 50 µl of 0.9% NaCl, ten minutes before the ICV injection of the ASICalgin-1 peptide.

Toxicity

10

The ICV injection of the ASICalgin-1 peptide induces no modification of behavior of motor problem, balance problem, apathy, paralysis or convulsion type, nor any deaths in the mice, even after 3 days.

15

Acute thermal pain

The thermal pain was studied in mice by measuring the latency of withdrawal of the tail immersed in water at 46°C (reflex movement, the animal is braced) with a limiting duration of 30 s.

The ICV injection of the ASICalgin-1 peptide induces a powerful analgesic effect which is maintained throughout the duration of the experiment (67 min, figure 3), which effect is not modified by a pretreatment with naloxone. The analgesic effect persists for several hours before the value of the tail withdrawal latency returns to its control value.

30

EXAMPLE 4: ANALGESIC EFFECT OF THE ASICALGIN-1 PEPTIDE (π -Dp1) *IN VIVO* AFTER SUBCUTANEOUS INJECTION

The effect of a subcutaneous (SC) injection of the ASICalgin-1 peptide was tested on carrageenan-induced inflammatory hyperalgesia.

35

- 30 -

The SC injection of 2% carrageenan into the arch of the hind paw of the mice (C57B16J, 7- to 11-week-old males) leads to the development of a tissue inflammation and of hyperalgesia, measured by the latency of withdrawal of the hind paw immersed in water at 46°C (integrated voluntary movement, the animal is braced) with a limiting duration of 30 s, two hours after the injection of carrageenan.

When the ASICalgin-1 peptide (10 µl of solution containing 34 µM of ASICalgin-1 (0.34 nmol) in 0.9% NaCl, 0.05% bovine serum albumin) is coinjected with the carrageenan, no thermal inflammatory hyperalgesia is observed (figure 4, □). Under these conditions, a second SC injection of the ASICalgin-1 peptide two hours later produces no additional significant effect (figure 4, □).

If the ASICalgin-1 peptide was not coinjected with the carrageenan, the SC injection of said peptide when the inflammation is already established, two hours after the injection of carrageenan alone, reverses the inflammatory hyperalgesia and furthermore produces significant analgesia compared with the control condition, before the injection of carrageenan (figure 4, ■ and #).

EXAMPLE 5: ACTIVITY OF THE ASICALGIN-1 (π -Dp1) AND ASICALGIN-2 (π -Dp2) PEPTIDES ON THE ASIC CURRENTS

The ASICalgin-1 and ASICalgin-2 peptides inhibit the currents generated by the ASIC channels heterologously expressed in the COS cell line and recorded using the patch-clamp technique in "whole cell" configuration ("applied potential" mode).

The peptides are applied at pH 7.4, 30 seconds before the extracellular pH drop from 7.4 to 6 or 5.5.

The ASICalgin-1 and ASICalgin-2 peptides have the same effect, and inhibit the current generated by the rat homomeric ASIC1a channels with a concentration that produces 50% inhibition (IC_{50}) of 49 nM (figure 5A), and the current generated by the human homomeric ASIC1a channels with an IC_{50} of 127 nM (figure 5B). The current generated by the rat homomeric ASIC1b channels is inhibited with an IC_{50} of 650 nM (figure 5C), the rat and human heteromeric ASIC1a+ASIC2a channels with an IC_{50} of 308 nM (figure 5E), and the rat heteromeric ASIC1a+ASIC1b channels with an IC_{50} of 223 nM (figure 5D) (the ASIC1b channel was not described in humans).

The ASICalgin-1 and ASICalgin-2 peptides do not inhibit the rat and human homomeric ASIC2a and ASIC3 channels, nor the rat heteromeric ASIC1a+ASIC3 and ASIC1b+ASIC3 channels.

The effects of the ASICalgin-1 and ASICalgin-2 peptides are reversible once the application of the peptides is stopped (figure 5).

The ASICalgin-1 and ASICalgin-2 peptides are the only peptides described today that are capable of inhibiting the homomeric ASIC1b channels and the heteromeric ASIC1a+ASIC1b and ASIC1a+ASIC2a channels.

On the other hand, the ASICalgin-1 peptide (2 μ M) does not inhibit the current produced by rat TRPV1 channels activated with capsaicin (1 μ M), which are channels also involved in the transduction of pain by sensory nerve endings.

Without being limited to this explanation, the analgesic effects of the ASICalgin-1 and ASICalgin-2 peptides appear to involve the inhibition of the homomeric ASIC1a channels and heteromeric ASIC1a+ASIC2a

channels in central nervous system neurons (IT and ICV injections), and the inhibition of the homomeric ASIC1a and ASIC1b channels and heteromeric ASIC1a+ASIC1b channels in sensory neurons (subcutaneous injection, IT injection with the proviso that homomeric ASIC1a and ASIC1b and heteromeric ASIC1a+ASIC1b are present in the central endings of the sensory neurons at the level of the dorsal horn of the spinal cord).

EXAMPLE 6: ACTIVITY OF THE ASICALGIN-1 (π -Dp1) AND ASICALGIN-2 (π -Dp2) PEPTIDES ON THE NATIVE ASIC CURRENTS OF CENTRAL NEURONS AND OF SENSORY NEURONS

The ASICALgin-1 and ASICALgin-2 peptides reversibly inhibit the ASIC currents recorded using the patch-clamp technique in "whole cell" configuration ("applied potential" mode) in neurons in primary culture isolated from adult rat spinal ganglia (figure 6A), from mouse embryo dorsal spinal cord (figure 6B), and from newborn mouse (2 days) hippocampus (figure 6C).

The partial inhibitory effect of the ASICALgin-1 and ASICALgin-2 peptides on the total ASIC current of sensory neurons is explained by the large proportion of current of ASIC3 type (not inhibited by the ASICALgin-1 and ASICALgin-2 peptides) in sensory neurons, whereas the central (hippocampus and spinal cord) ASIC currents are composed of a mixture of homomeric ASIC1a and heteromeric ASIC1a+ASIC2a currents (both inhibited by the ASICALgin-1 and ASICALgin-2 peptides).

EXAMPLE 7: ACTIVITY OF THE ASICALGIN-1 (π -Dp1) AND ASICALGIN-2 (π -Dp2) PEPTIDES ON NEURONS

The ASICALgin-1 and ASICALgin-2 peptides applied to spinal neurons in culture do not induce any modification of the basal current level, nor any variations in resting potential (figure 7A) recorded

using the patch-clamp technique in "whole cell" configuration.

5 The application of the ASICalgins-1 and ASICalgins-2 peptides does not modify the spontaneous synaptic electrical activity of spinal neurons in culture, nor the shape or the threshold of action potentials evoked by an electrical stimulation ("applied current" mode of the patch clamp) (figure 7B).

10

The results show that the ASICalgins-1 and ASICalgins-2 peptides have no effect on the intrinsic excitability properties of neurons, on synaptic function or on potential-dependent currents involved in the generation (Na⁺ channels) or repolarization (K⁺ channels) of action potentials.

15

On the other hand, the specific inhibition of ASIC channels by the ASICalgins-1 and ASICalgins-2 peptides reduces the excitability of neurons in response to an acidic pH drop (from 7.4 to 6.0), the depolarization produced by the ASIC current then not being sufficient to trigger an action potential (figure 7A).

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CLAIMS

1. A peptide comprising the amino acid sequence
- 5 LKCX⁴QHGKVVVTCHRD⁴MKFCYHNTGMPFRNLKLILQGCSSSCSETENNKCCSTDRCNK (SEQ ID No: 1)
wherein X⁴ represents any amino acid; or a sequence exhibiting an identity of at least 56% with the sequence SEQ ID No: 1 and retaining the biological
10 properties of said peptide comprising the sequence SEQ ID No: 1.
2. The peptide as claimed in claim 1, wherein said peptide is a blocker of at least one ASIC channel
15 containing at least one subunit chosen from the group consisting of the ASIC1a and ASIC1b subunits.
3. The peptide as claimed in claim 2, wherein said peptide is a blocker of the homomeric ASIC1a channel,
20 homomeric ASIC1b channel, heteromeric ASIC1a+ASIC1b channel and/or heteromeric ASIC1a+ASIC2a channel.
4. The peptide as claimed in any one of claims 1 to 3, wherein X⁴ represents Y or F in the sequence SEQ ID
25 No: 1.
5. A polynucleotide comprising a nucleotide sequence encoding a peptide as defined in any one of claims 1 to 4.
30
6. The polynucleotide as claimed in claim 5, comprising the nucleotide sequence:
atgaaaactctgctgctgaccttgctggtggtgacaatcgtgtgcctagacttag
gatactccctgaaatgt⁷³txx⁷⁵caacatggttaaagttgtgacttgtcatcgaga
35 tatgaagttttgctatcataacactggcatgccttttcgaaatctcaagctcatc
ctacagggatgttcttcttcgtgcagtgaaacagaaaacaataagtgttgctcaa
cagacagatgcaacaaatag (SEQ ID No: 4); or
ctgaaatgt¹⁰txx¹²caacatggttaaagttgtgacttgtcatcgagatatgaagt

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tttgctatcataacactggcatgccttttcgaaatctcaagctcatcctacaggg
atgttcttcttctcgtgcagtgaaacagaaaacaataagtgttgctcaacagacaga
tgcaacaaatag (SEQ ID No: 22);

wherein ⁷³txx⁷⁵ and ¹⁰txx¹² represent tac, tat, ttt or
5 ttc.

7. The polynucleotide as claimed in claim 5, wherein
said polynucleotide hybridizes under stringent
conditions with the nucleotide sequence SEQ ID No: 4 or
10 SEQ ID No: 22, or a sequence complementary thereto.

8. The polynucleotide as claimed in claim 5 or 7,
wherein said polynucleotide comprises a natural or
synthetic sequence exhibiting an identity of at least
15 76% with the sequence SEQ ID No: 4 or the sequence SEQ
ID No: 22.

9. A vector comprising a polynucleotide as defined in
any one of claims 5 to 8.

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10. The vector as claimed in claim 9, wherein said
vector is an expression vector.

11. A host cell comprising one or more peptides as
25 defined in any one of claims 1 to 4, polynucleotides as
defined in any one of claims 5 to 8, or vectors as
defined in either one of claims 9 and 10.

12. A pharmaceutical composition comprising one or
30 more peptides as defined in any one of claims 1 to 4,
polynucleotides as defined in any one of claims 5 to 8,
vectors as defined in either one of claims 9 and 10, or
host cells as defined in claim 11.

35 13. A substance chosen from:

- a peptide as defined in any one of claims 1 to 4;

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- a polynucleotide as defined in any one of claims 5 to 8;
- a vector as defined in either one of claims 9 and 10; or
- 5 - a host cell as defined in claim 11;
- a pharmaceutical composition as claimed in claim 12;

for use thereof as a medicament.

10 14. The substance as claimed in claim 13, wherein said medicament is an analgesic.

15 15. The substance as claimed in claim 14, wherein the analgesic is intended for the prevention or treatment of pain or of a pathological condition involving the activation of ASIC channels.

20 16. The substance as claimed in claim 15, wherein the pain and the pathological condition involving the activation of ASIC channels are chosen from the group comprising inflammatory, neuropathic, cancer-related, post-operative, musculoskeletal and visceral pain, inflammations, cancers, fibromyalgia and irritable bowel syndrome.

25 17. The substance as claimed in claim 13, wherein said medicament is intended for the prevention or treatment of a central neurological disease chosen from the group comprising depression, anxiety, strokes, epilepsy, 30 central inflammations and neurodegenerative diseases.

35 18. The substance as claimed in any one of claims 13 to 17, wherein said medicament is administered centrally, subcutaneously, transcutaneously, systemically, orally or via the respiratory route.

19. A substance chosen from:

- 40 -

- a peptide as defined in any one of claims 1 to 4;
- a polynucleotide as defined in any one of claims 5 to 8;
- a vector as defined in either one of claims 9 and 10; or
- a host cell as defined in claim 11;
- a pharmaceutical composition as claimed in claim 12;

for use thereof as a diagnostic tool.

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20. A method for identifying a compound which mimics the analgesic activity of a peptide as defined in any one of claims 1 to 4, comprising the following steps:

- 15 a. determining the analgesic activity of a peptide as defined in any one of claims 1 to 4;
- b. determining the analgesic activity of a candidate compound;
- c. comparing the analgesic activities obtained in steps a. and b.;
- 20 d. selecting the candidate compound which has an analgesic activity equivalent to or greater than that of said peptide.

25 21. A method for identifying a compound which mimics the analgesic activity of a peptide as defined in any one of claims 1 to 4, comprising the following steps:

- a) bringing a peptide as defined in any one of claims 1 to 4 into contact with a sample, and measuring the binding of said peptide with said sample;
- b) adding a candidate compound, and evaluating the effect of said compound on the binding of said peptide with said sample;
- 30 c) selecting the candidate compound capable of modulating the binding of said peptide with said sample.

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