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Synthesis and pharmacological analysis of a morphine/substance P chimeric molecule with full analgesic potency in morphine-tolerant rats

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

We have previously explored the functional role of the tachykinin substance P (SP) in the mediation of opioid-dependent antinociception and now describe the formulation, synthesis, and initial pharmacological characterization of a hybrid chimeric molecule, designated MSP9, containing the mu opioid receptor (MOR) agonist morphine covalently attached through a succinic acid linker to the SP receptor (SPR) agonist domain SP3-11.

Material/Methods:

Pharmacological characterization of MSP9, administered by the intramuscular route, was achieved in naive and morphine-tolerant male rats utilizing the tail-flick test.

Results:

MSP9 produced significant antinociceptive responses across a wide concentration range and displayed an atypical bell-shaped analgesic dose response relationship with peak effect of 40±10% reached at 0.2 mg/kg. The antinociceptive responses achieved by very low concentrations of MSP9 were not obtained by administration of equivalent low doses of morphine, suggesting that kinetic and dynamic parameters may contribute to its unusual analgesic properties. Importantly, MSP9 produces a strong antinociceptive response when administered to morphine-tolerant rats, suggesting a significant activation of kappa and/or delta receptors (KORs and DORs, respectively) in the presence of functionally down regulated MORs.

Conclusions:

Analyses employing selective, blood brain barrier (BBB) permeable, opioid and SP antagonists administered alone or in combination, indicate an obligate requirement for coincident activation of populations of CNS opioid and SP receptors. These combined data suggest that MSP9 activates multiple opioid- and SPR-expressing systems functionally linked to CNS analgesic responses, designating this class of hybrid chimeric molecules as prime candidates for therapeutic development for a wide range of clinical indications.

key words:

morphine • substance P • hybrid chimeric molecules • antinociception • opioid tolerance • drug abuse

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BACKGROUND

Spinal and supraspinal neurons utilizing the undecapeptide SP and endogenous opioids as neuromodulators are intimately involved in the regulation of acute and chronic pain transmission [1–6]. Previous pharmacological data from our group strongly suggest that SP released in the spinal dorsal horn plays an important role in antinociception by modulating secondary responsiveness of postsynaptic opioid systems [7–10]. Pico-molar concentrations of SP co-administered with marginally effective doses of morphine sulfate (MS) into the rat subarachnoid space promote markedly enhanced analgesic responses and mediate a novel inhibitory effect on the development of MS tolerance. In subsequent work, MOR- and SPR-activating domains were combined as linear 7 amino acid chimeric peptide constructs with the MOR-selective opioid endomorphin-2 at the N-terminus and a SP fragment at the C-terminus [8,9].

Our underlying rationale for the development of opioid/SP chimeric peptides was that the intrinsic design of these molecules, designated ESP6 and ESP7, avoided a major confound by limiting pharmacokinetic parameters to one compound, thereby maximizing bioavailability, distribution, and targeting of appropriate populations of CNS MORs and SPRs.

Operationally, however, it is clear that the chemical nature of opioid/SP chimeric peptides will restrict their therapeutic development as parenterally administered analgesics. Limiting factors include significant metabolism in the blood stream, difficulties encountered in crossing the mammalian BBB [11], and poor absorption at the luminal surface of the gastrointestinal tract after oral administration [12].

Accordingly, we have developed a prototype hybrid opioid/SP chimeric molecule, designated MSP9, consisting of an alkaloid core (morphine) covalently attached through a succinic acid linker to a biologically active 9 amino acid SP C-terminal fragment SP3-11. Morphine is a highly efficacious MOR-selective opioid analgesic and will cross the human BBB, as will its active metabolite morphine 6-glucuronide to a limited degree [13,14]. Strategically, chemically modified morphine contained within the structure of MSP9 will mediate at least two independent pharmacological roles: 1) to activate populations of MORs within the CNS functionally linked to antinociceptive responses and 2) to act as a BBB transport vehicle for the biologically active SP3-11 moiety to activate CNS populations of SPRs intimately associated with spinal and supraspinal MORs.

We now present the formulation, synthesis, and initial pharmacological characterization of MSP9 parenterally administered to naive male rats. Intramuscular administration of MSP9 produces a significant antinociceptive response via the tail-flick test across a wide concentration range (0.1–10 mg/kg BW). MSP9's pharmacological effects display an atypical bell-shaped analgesic dose response relationship with peak effect of $40 \pm 10\%$ reached at 0.2 mg/kg. The antinociceptive response is markedly reduced by prior treatment of animals with BBB permeable opioid and SP antagonists supporting our contention that MSP9 is promoting coincident activation of opioid and SP receptors. Importantly, MSP9 produces a strong antinociceptive re-

sponse when administered to tolerant rats chronically exposed to morphine sulfate, distinguishing MSP9 as a novel candidate for therapeutic development for a wide range of clinical indications.

MATERIAL AND METHODS

Chemical synthesis

The opioid/SP hybrid chimeric compound MSP9 was custom synthesized by Commonwealth Biotechnologies, Inc, 601 Biotech Dr, Richmond, VA 23235, according to the following scheme (Figure 1). Initially, a morphine 6-hemisuccinate ester intermediate was synthesized by a relatively straight forward procedure utilizing succinic anhydride that does not require chemical blocking/de-blocking of the essential 3 phenolic OH of morphine [15]. Morphine 6-hemisuccinate was purified to 99% homogeneity by preparative reverse-phase HPLC and converted to a morphine 6-succinyl-N-succinimidyl ester intermediate [16] prior to coupling to the 9 amino acid C-terminal fragment SP3-11 (KPQQFFGLM-NH₂) recognized as a high affinity agonist at the SPR [17,18]. The SP C-terminal fragment SP3-11 (KPQQFFGLM-NH₂) was synthesized by a standard solid phase peptide synthesis procedure employing FMOC amino acids, similar to that previously described for the syntheses of the chimeric peptides ESP7 and ESP6 [8,9]. Prior to coupling, purified SP3-11 was acetylated at its primary amino group in order to utilize the epsilon amino group of Lys3 as the sole point of covalent attachment to morphine 6-hemisuccinate via pseudo-peptide bond formation. N-acetylated SP3-11 (Ac-KPQQFFGLM-NH₂) was reacted with at least a 2-fold excess of morphine 6-succinyl-N-succinimidyl ester to form morphine-6-succinyl-SP3-11 diester, here designated as MSP9. Synthesized MSP9 was purified to greater than 99% homogeneity as monitored and validated by reverse phase HPLC and MALDI TOF MASS SPEC.

Pharmacological agents

Morphine and morphine pellets are from NIDA. Naltrexone (NTX), nor-binaltorphimine (nor-BNI) and L733060 are from Tocris (Ellisville, MO). Other pharmacological compounds and chemical reagents were obtained from either Sigma (St. Louis, MO) or Bachem (San Carlos, CA).

Animal subjects

Male, Sprague-Dawley rats, 225–275 gm, were used in all experiments. All procedures fulfill the guidelines of the Ethical Standards of the IASP as assured by IUCAC approval number A3260-01. For all experimental animals, euthanasia was performed by deeply anesthetizing them with sodium pentobarbital followed by decapitation, as is consistent with recommendations of the American Veterinary Medical Association.

Antinociceptive testing: tail-flick assay

Stock solutions of MSP9 were prepared in DMSO and further diluted in DMSO to achieve each tested concentration. Tested concentrations of MSP9 were administered by intramuscular injection of 50–100 μ l. Control injections consisted of 50–100 μ l DMSO. All experimental subjects were habit-

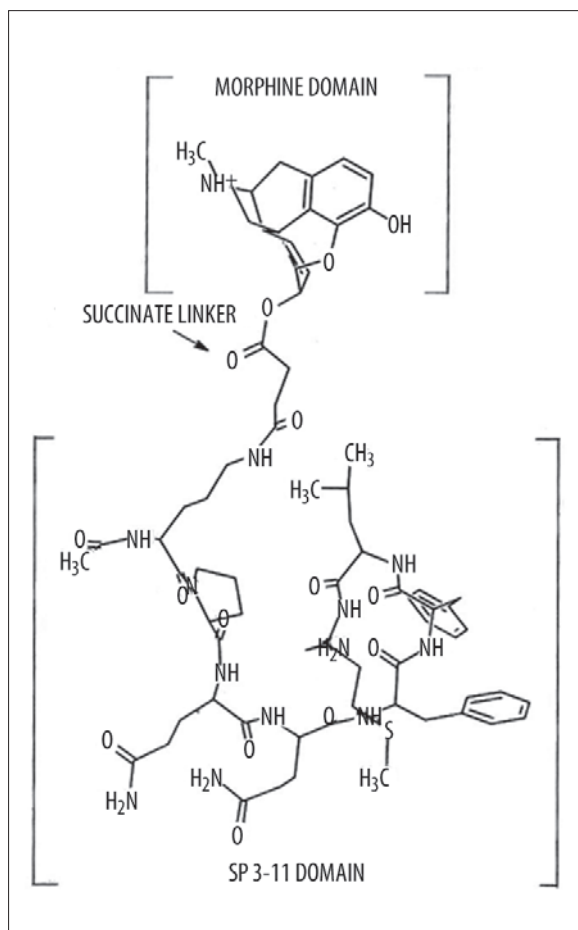


Figure 1. Chemical structure of MSP9. Morphine is covalently bonded to N-acetyl SP-11 through a succinic acid diester linkage (arrow). The 3D representation of MSP9 was realized using the ACD/ChemSketch 8.0 program provided by Advanced Chemistry Development, Inc.

uated to the laboratory environment and analgesic testing apparatus. For measurement of the thermal antinociceptive properties of MSP9, the tail-flick test was performed using an IITC Model 336 Analgesia Meter. Baseline latency was adjusted to approximately 3.0–3.5 sec and the cut-off time was set at 10 sec. Before each pharmacological trial, for each rat a series of three to five measurements was made to establish stable baseline latency. After injection, post-treatment latency measurements were recorded at set time points (10, 20, 30, 45, 60, 75, 90, 120 and 150 min). For each animal tested, the magnitude of the analgesic response was expressed as a percent increase over baseline latency. Statistical analyses of the data were performed using ANOVA followed by Bonferroni's multiple comparison tests.

For experiments employing opioid-tolerant rats, morphine was administered in either of two ways: 1) via subcutaneous implantation of one pellet containing 75 mg morphine base on day 1, two pellets on day 3 and three pellets on day 5, under sodium pentobarbital anesthesia (40 mg/kg, i.p., Anpro Pharmaceutical), or 2) via subcutaneous injection of increasing doses of morphine sulfate from 10 mg/kg up to 40 mg/kg, 2 times a day (08:00 h and 18:00 h) for 5 days [19].

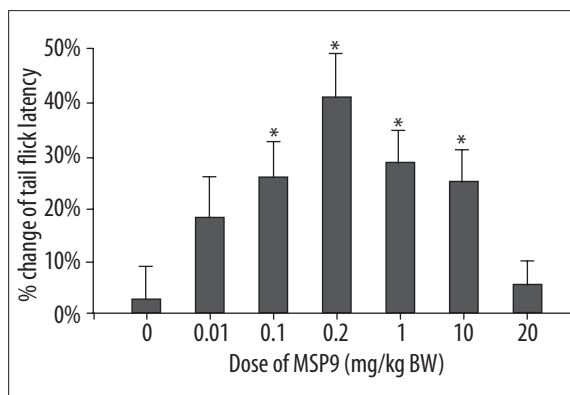


Figure 2. Atypical bell-shape or inverted-U analgesic dose-response relationship of MSP9. Ordinal values represent maximal analgesic responses reached at 75min following IM administration, expressed as % change over baseline of the tail-flick latency (mean \pm SEM; n=5 for DMSO vehicle and 20 mg/kg BW groups; n=7–12 for the other doses). Over a concentration range of 0.1–10 mg/kg BW, MSP9 engenders a moderately potent analgesic response (30–40%, $p < 0.001$). In contrast, the low (0.01 mg/kg) and the high (20 mg/kg) end of tested concentrations were observed to be without effect ($p > 0.05$).

RESULTS

Intramuscular administration of MSP9 over a concentration range of 0.1 to 10 mg/kg produced significant analgesic effects with an atypical bell-shaped or inverted-U dose response relationship and peak effect of $40 \pm 10\%$ reached at 0.2 mg/kg ($F_{6,41} = 10.9$, $p < 0.001$, Figure 2). For each pharmacological trial, the time effect curve of the analgesic response was observed to reach its maximal value at 75 min and slowly return to baseline between 2 and 3 hrs (data not shown). The bell-shaped or inverted-U dose response relationship of MSP9 is similar to our previous observations of the spinal analgesia produced by opioid/SP peptide chimeras [8,9], and most likely reflects the coincident and competing activation effects of MSP9s independently expressed opioid and SP agonist domains on interactive populations of CNS opioid and SP receptors, respectively. In parallel analyses, the antinociceptive responses achieved by very low concentrations of MSP9 were not obtained by administration of equivalent low doses of morphine, suggesting that a combination of kinetic and dynamic parameters may contribute to its unusual analgesic properties (data not shown). Control injections of DMSO vehicle were observed to produce no significant increases in latency to thermal nociceptive stimulation.

The magnitude of MSP9-mediated antinociceptive response achieved by a representative concentration of 0.1 mg/kg was markedly diminished by prior injection of animals with the BBB permeable KOR antagonist nor-BNI or the BBB permeable SPR antagonist L733060, respectively (Figure 3, $p < 0.001$ compared to the MSP9 group; $p > 0.05$ compared to the DMSO group for both treatments). Administration of equivalent doses of nor-BNI or L733060 without MSP9 was observed to produce no significant increases in latency to thermal nociceptive stimulation. Pretreatment of animals with intermediate doses of the broad spectrum opioid an-

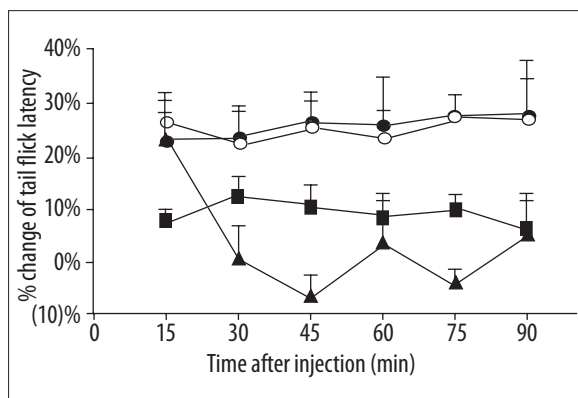


Figure 3. Effect of pretreatment with the KOR antagonist nor-BNI, broad spectrum opioid antagonist NTX, and SPR antagonist L733060 on the analgesic response engendered by MSP9 administration. In these analyses, a representative dose of 0.1 mg/kg MSP9 was utilized based on its stable and long lasting time-effect curve. Pretreatment with the SPR antagonist, L733060 (▲, 3 mg/kg), 30 min before MSP9 administration, is observed to markedly reduce the analgesic effect of MSP9 administered to naïve rats (○, 0.1 mg/kg). Chronic pretreatment with the KOR antagonist nor-BNI (■, 30 mg/kg), administered as previously described (31,32), is observed to markedly reduce the analgesic effect of MSP9 administered to naïve rats ($p > 0.05$ compared to DMSO-treated rats). Pretreatment with NTX (●, 5–15 mg/kg), 30 min before MSP9 administration, is observed to produce a minimal reduction of the analgesic effect of MSP9 administered to naïve rats ($p > 0.05$ compared to MSP9 administered to naïve rats and $p < 0.001$ compared to DMSO-treated rats), suggesting an interactive involvement of MORs in MSP9's analgesic responsiveness.

tagonist NTX was observed to have minimal effect on the magnitude of the analgesic response engendered by MSP9 ($p > 0.05$ compared to the MSP9 group).

MSP9 engendered a strong antinociceptive response when administered to tolerant rats chronically exposed to morphine via either implantation of morphine sulfate pellets or injection of morphine solution (Figure 4). Rats rendered partially or fully tolerant to morphine, induced by morphine sulfate pellets and morphine solution injection respectively, exhibited a very similar significant and long lasting analgesic effect as observed in naïve rats ($p < 0.001$ compared to the DMSO group). The ability of MSP9 to engender equivalent antinociceptive responses in naïve and in morphine tolerant animals indicates a potentially novel mode of action and suggests that the hybrid opioid/SP chimeric molecule promotes a significant activation of KOR and/or DOR systems in the presence of functionally down regulated MORs. MSP9-mediated secondary activation of other CNS antinociceptive systems may also be contributing factor to its analgesic potency in morphine tolerant rats [20].

DISCUSSION

We have described the development and initial pharmacological testing of a newly developed hybrid opioid/SP chimeric analgesic MSP9. Unlike previously described pure peptide opioid/SP chimeric molecules [8,9], MSP9 utilizes

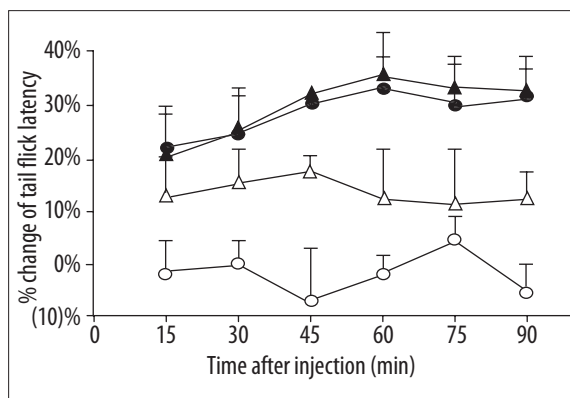


Figure 4. Analgesic responsiveness of MSP9 vs. morphine in morphine-tolerant rats. Intramuscular administration of morphine (2 mg/kg) produced a small but significant analgesic effect in rats implanted with morphine pellets (△, $p < 0.01$) was without effect in rats that had received daily injections of morphine (○, $p > 0.05$). In contrast, MSP9 produced significant, long-lasting, analgesic responses in rats rendered partially or fully tolerant to morphine, induced by morphine sulfate pellets (▲) and morphine solution injections (●), respectively ($p < 0.001$ compared to the DMSO group and $p > 0.05$ compared to naïve rats, as in Figure 3). The ability of MSP9 to engender equivalent antinociceptive responses in naïve and in morphine tolerant animals indicates a potentially novel mode of action and suggests that the hybrid opioid/SP chimeric molecule promotes a significant activation of KOR and/or DOR systems in the presence of functionally downregulated MORs.

the multi-ringed alkaloid core of morphine as its key chemical moiety and starting point of construction based on important pharmacological criteria.

From a well-established pharmacological perspective, and despite well-publicized adverse sequelae, morphine and morphine-related opiate alkaloids remain the key agents of choice for treatment of a wide variety of acute and chronic pain states via profound inhibition of neurons of the peripheral/sensory nervous system (PNS) and the CNS [21,22]. Preservation of the potent antinociceptive properties of morphine and related opiate alkaloids represents a key strategic issue underlying the design of chimeric opioid/SP molecules. Second, due to chemical nature of its protonated tertiary amino group, morphine will efficiently penetrate the mammalian BBB, as will its active metabolite morphine 6-glucuronide [11,13].

Design of chimeric hybrid molecules comprised of a chemically modified morphine moiety to activate CNS opioid receptors and an SP peptide fragment to activate SPRs incorporates the morphine alkaloid domain as a novel BBB transport domain for the molecule as a whole and in particular the SPR agonist 9 amino acid SP C-terminal fragment. Additional key structure/function relationships are incorporated into the design of MSP9. The free phenolic OH at position 3 of morphine (and at the N-terminal tyrosine side chain of enkephalins, endorphins, and endomorphins [23]) is essential for opioid activity and was preserved. Based on the potent MOR activity and potential for BBB

penetration of chemically modified morphine-6-glucuronide [13], the allylic OH group at position 6 of morphine, distal to the essential phenolic OH group on the A ring, was chosen as a logical point of conjugation to the succinic acid linker moiety. The dicarboxylic succinic acid provides a bi-functional cross-linking group between the OH at position 6 of morphine and the epsilon amino group found on the Lys3 side chain of SP3-11 via diester bonding. Additionally, the covalently bound succinic acid provides a necessary 4-carbon spacer domain between the pharmacologically distinct and spatially separate MOR- and SPR-agonist domains in order to optimize receptor binding without significant reciprocal steric inhibition. In sum, the structural design of MSP9 incorporates spatially separate, independently regulated, MOR- and SPR-agonist domains within one molecule, and contains only one charged functional group, the well-characterized tertiary amino group of morphine necessary for BBB penetration.

The pharmacological profile of parenterally administered MSP9 displays similarities to previous observations relating to the analgesia produced by intrathecal co-administration of morphine and SP and by pure peptide opioid/SP chimeric molecules [7–10]. MSP9 exhibited an atypical antinociceptive dose response relationship with bell-shaped or inverted-U configuration across a very wide concentration range of at least 2-orders of magnitude. The magnitude of the analgesic responses achieved at almost all tested concentrations was observed to reach a similar plateau at 30–50% above baseline latency. Similar atypical antinociceptive dose response relationships were observed in our initial pharmacological study of SP-mediated potentiation of morphine analgesia following intrathecal co-administration of both compounds to naive rats [7,10]. The analgesic dose response relationship of spinally co-administered SP and morphine appeared to be of a bell-shaped or inverted-U configuration, strongly indicating a concentration-dependent dissociation of opioid-potentiating and analgesic properties of SP from traditional hyperalgesic effects of SP realized at higher concentrations. Similarly, the magnitude of the analgesic responses achieved across a concentration of intrathecally administered chimeric peptide ESP7 was observed to reach plateau values of 30–40% maximal effect, presumably because of ESP7's ability to activate simultaneously both stimulatory and inhibitory systems within the spinal dorsal horn [8,9,24,25].

The observed similarities of the analgesic dose-response relationship displayed by parenterally administered MSP9 to those previously observed for spinally administered morphine and SP, or pure opioid/SP chimeric peptides, provide good presumptive evidence for significant BBB penetration of the hybrid chimeric molecule. This is further supported by the observation that the antinociceptive response engendered by MSP9 is markedly reduced by prior treatment of animals with a BBB permeable opioid-receptor or SPR antagonist. Furthermore, in a limited number of cases, intrathecal administration of the compound produces a similar pattern of analgesic responsiveness as observed for parenterally administered MSP9 (data not shown). Although these present data cannot definitively evaluate the potential contribution of peripheral SP and opioid receptor activation to MSP9's analgesic potency, our accumulated pharmacological data indicate a fundamental mechanism of action that

is dependent on coincident activation of functionally integrated populations of opioid-receptor and SPR-expressing neurons within the CNS.

In contrast to previous observations relating to intrathecal administration of opioid/SP peptide chimeric molecules, the MSP9-mediated antinociceptive response was observed to be dependent on pharmacological co-activation of MORs as well as KORs, with an obligate requirement for coincident SPR activation (Figure 3). Initial assessment of the binding affinity of MSP9 to the MOR and KOR yielded equivalent intermediate affinity values of approximately 10^{-7} M, with an apparent ten fold weaker affinity to the DOR (data not shown). Interestingly, initial assessment of MSP9 binding to the SPR indicated a high affinity value of approximately 10^{-9} M (data not shown). It is highly probable, therefore, that a potent functional coupling of SPRs with both MORs and KORs, is mediating the pharmacological/ antinociceptive effects of the compound. This is consistent with observations that the antinociceptive responses achieved by very low concentrations of MSP9 are not achieved by equivalently low doses of morphine, normalized on a molar basis, thereby indicating cooperative interactions of multiple opioid receptors with SPR activation. The inability of NTX to effectively block the MSP9-mediated analgesic response, however, also indicates a predominant high affinity coupling of SPRs and KORs in promoting MSP9's pharmacological effects, including its restorative antinociceptive functions in morphine tolerant rats.

Our proposed mechanism for MSP9's potent analgesic properties may also reside in the ability of SP to release opioid peptides within spinal and supraspinal environments [26]. Complementary anatomical studies indicating SP-containing sensory afferent terminals making synaptic contact with second order opioid-expressing neurons in the superficial dorsal horn provide additional validation for this proposed mechanism [23,27,28]. Because opioid and SPRs have been observed to be sparsely colocalized on individual neurons within the superficial layers of the spinal dorsal horn, parenterally administered MSP9 is probably not promoting coincident activation of both receptors on the same neuron, but rather acting on closely spaced populations of interactive neurons [29,30]. Following BBB penetration and distribution within CNS areas functionally linked to antinociceptive responses, MSP9 is most likely mimicking the role of spinally released SP to initiate an amplification mechanism for opioid action subsequent to painful stimuli with convergence through KORs linked to antinociceptive responses.

Pharmacological administration of MSP9 will also provide targeted delivery of SP to areas of the CNS containing high densities of opioid-expressing neurons expressing SPRs. MSP9 may serve two independent functions by providing direct activation of neural systems linked to antinociceptive processes, as well as targeting SPR-agonist domains to SPRs promoting evoked release of endogenous opioids. MSP9 produces a strong antinociceptive response when administered to tolerant rats chronically exposed to morphine sulfate, indicating a significant activation or recruitment of KORs and/or DORs, or possibly other analgesic systems, in the presence of functionally down regulated MORs. This is consistent with previous observations indicating a restoration of analgesic responsiveness following intrathecal ad-

ministration of chimeric opioid/SP peptides to morphine tolerant rats [8,9] and provides a compelling argument for the establishment of SP as an intrinsic homeostatic regulator of opioid-dependent antinociceptive processes at a systems level (anatomical organization of CNS populations of opioid- and SPR-expressing neurons) and as a pharmacological principle for construction of future hybrid chimeric analgesics. A recent publication describing SPR-mediated feed-forward inhibitory activity in the mammalian spinal cord, presumably via excitation of opioid inter-neurons, lends strong support to these contentions [31].

Development of a class of hybrid chimeric molecules based on the structure of MSP9 as future therapeutic agents for the widest range of pain indications in large subpopulations of patients necessitates significant BBB penetration with retention of pharmacological activity while inhibiting tolerance development and dependence formation. Based on our previous work, the high affinity of the SPR activating domain represents the major regulatory factor responsible for maintenance of opioid efficacy following repeated administration of the respective hybrid chimeric compound. It is also an intrinsic characteristic of this class of analgesics that opioid efficacy and safety are intimately connected by the internal regulatory domain contributed by the biologically active SP fragment. In practical terms, 90% metabolic degradation of the SP peptide moiety of the respective hybrid chimeric compound will still provide sufficient SPR activation to achieve the desired pharmacological endpoint: analgesia with marked diminution of tolerance and a high degree of safety from opioid-induced toxicity.

CONCLUSIONS

The predominance of a strong SPR-KOR coupling as an analgesic principle underlying MSP9's mode of action appears to be functionally linked to the prevailing high affinity binding of the SP3-11 agonist domain. For clinical pain indications requiring a more potent contribution from morphine's traditionally recognized MOR activation domain, it is highly feasible to design and synthesize MSP9 homologs with shorter less potent SPR activation domains. Conversely, insufficient MOR-mediated opioid analgesia due to a preponderance of SPR activation can be overcome by addition of chemically authentic morphine to form a pharmacological mixture of morphine and hybrid chimeric analgesic. In conclusion, to achieve the desired fixed stoichiometric relationship of morphine to SP within the structure of the hybrid chimeric analgesic, the chemical sequence of each activation domain is amenable to chemical modification to differentially target both receptors through altered affinity at each site, thereby optimizing analgesic endpoints with minimal tolerance development and dependence formation.

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MSP9 and related hybrid chimeric analgesics are described and protected by US Patent # 6,881,829.

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Effective search tool for collaborators worldwide. Provides easy global networking for scientists. C.V.'s and dossiers on selected scientists available. Increase your professional visibility.

IC Virtual Research Groups [VRG]

Web-based complete research environment which enables researchers to work on one project from distant locations. VRG provides:

- ⊗ customizable and individually self-tailored electronic research protocols and data capture tools,
- ⊗ statistical analysis and report creation tools,
- ⊗ profiled information on literature, publications, grants and patents related to the research project,
- ⊗ administration tools.

IC Journal Master List

Scientific literature database, including abstracts, full text, and journal ranking. Instructions for authors available from selected journals.

IC Patents

Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.

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Effective search tool for worldwide medical conferences and local meetings.

IC Grant Awareness

Need grant assistance? Step-by-step information on how to apply for a grant. Provides a list of grant institutions and their requirements.

IC Lab & Clinical Trial Register

Provides list of on-going laboratory or clinical trials, including research summaries and calls for co-investigators.