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Neurokinin-1 receptor-based bivalent drugs in pain management: The journey to nowhere?

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ABSTRACT

Hybrid compounds (also known as chimeras, designed multiple ligands, bivalent compounds) are chemical units where two active components, usually possessing affinity and selectivity for distinct molecular targets, are combined as a single chemical entity. The rationale for using a chimeric approach is well documented as such novel drugs are characterized by their enhanced enzymatic stability and biological activity. This allows their use at lower concentrations, increasing their safety profile, particularly when considering undesirable side effects.
In the group of synthetic bivalent compounds, drugs combining pharmacophores having affinities toward opioid and neurokinin-1 receptors have been extensively studied as potential analgesic drugs. Indeed, substance P is known as a major endogenous modulator of nociception both in the peripheral and central nervous systems. Hence, synthetic peptide fragments showing either agonism or antagonism at neurokinin 1 receptor were both assigned with analgesic properties. However, even though preclinical studies designated neurokinin-1 receptor antagonists as promising analgesics, early clinical studies revealed a lack of efficacy in human. Nevertheless, their molecular combination with enkephalin/endomorphin fragments has been considered as a valuable approach to design putatively promising ligands for the treatment of pain.
This paper is aimed at summarizing a 20-year journey to the development of potent analgesic hybrid compounds involving an opioid pharmacophore and devoid of unwanted side effects. Additionally, the legitimacy of considering neurokinin-1 receptor ligands in the design of chimeric drugs is discussed.

Contents

1. Introduction

The treatment of acute and chronic pain remains one of the critical challenges in modern medicine. The most frequently used and most potent analgesics are opioids. They provide strong analgesia through activation of G protein-coupled opioid receptors (μ, δ and κ), which are located both in the central- and peripheral nervous systems (CNS and PNS). Opioids are used in the treatment of acute pain and cancer

Abbreviations: CNS, central nervous system; DMLs, designed multiple ligands; DOR, delta opioid receptor; i.c.v., intracerebroventricular;; i.m., intramuscular; i.t., intrathecal; i.v., intravenous; Ki, inhibition constant; MOR, mu opioid receptor; MPE, maximal possible effect; NK1R, neurokinin-1 receptor; NMDA, *N*-methyl-D-aspartate receptor; PNS, peripheral nervous system; SAR, structure-activity relationship; SP, substance P.

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pain, but are also considered for chronic non-malignant pain, for example in rheumatoid arthritis (Vadivelu, Schermer, Kodumudi, & Berger, 2016). Nevertheless, the efficacy of morphine and its derivatives in neuropathic pain is inconsistent and opioids should not be considered as first line medicines in these conditions (McNicol, Midbari, & Eisenberg, 2013). Hence, the long-term use of opioids presents several limitations, mainly related to the occurrence of adverse reactions, such as persistent constipation, nausea and vomiting or opioid-induced pain (i.e. hyperalgesia) and the risk of respiratory depression. Moreover, repeated administration of morphine and other opioid-derived drugs is associated with the development of tolerance as well as psychological and physical dependence (Kleczkowska, Lipkowski, Tourwé, & Ballet, 2013).

The widespread use of opioids resulting from their high clinical efficacy in several pain conditions has motivated researchers in designing innovative approaches preserving or improving their efficacy while minimizing their associated adverse effects. The simplest approach consists in co-administrating opioids with other molecules tackling the pain signal transmission at distinct sites. However, the *co*-administration of two medicines (polytherapy) also brings disadvantages, e.g. it is poorly convenient for the patients, leading to errors or decreased compliance. Also, administrating two separate drugs raises a significant risk of drug-drug interactions, which commonly result in unexpected side effects and/or toxicity.

The aforementioned reasons led to the development of newly designed multiple ligands (also known as DMLs) – chimeric compounds – that combine two pharmacophores but act as a single chemical entity (Kleczkowska et al., 2013). In the context of pain handling, DMLs commonly combine an opioid receptor agonist activity with the activity of other molecules acting as functional modulators of pain signalling or perception, such as neurotensin (Kleczkowska et al., 2010), substance P (SP) (Yamamoto et al., 2010), cholecystokinin or cannabinoids (Kleczkowska et al., 2013; Smith, 2008). Both pharmacophores of such chimeric molecules are thought to interact with their respective receptors at the same time, supporting an improved therapeutic outcome while showing more predictable pharmacokinetic and pharmacodynamic profiles. So far, the molecular mechanism supporting the benefit of bivalent ligands remains largely debated. Bivalent ligands have already been considered with many drugs and authors have suggested that these bivalent ligands interact with receptors that are co-expressed on target cells. Thus, an optimized spacer between the two active entities would support a cooperative binding of the bivalent ligand for two receptors located in a close vicinity (Portoghese, 1989). This concept has been reinforced with the demonstration that several receptors combine as homo or heterodimers where bivalent ligands could interact with both protomers simultaneously (Milligan, 2004; Prinster, Hague, & Hall, 2005). For example, the A_{2A} adenosine receptor is known for its ability to form heterodimeric A_{2A}/D_2 receptor complexes. As antagonists at A_{2A} receptor could be considered as promising drug for the treatment of Parkinson's disease (Armentero et al., 2011; Fuxe et al., 2005), the bivalent approach combining the A_{2A} antagonism and D_2 agonism could provide further benefit (Soriano et al., 2009). Nevertheless, only few papers, in particular the paper of Yamamoto and co-workers (Yamamoto et al., 2011) as well as Largent-Milnes (Largent-Milnes et al., 2013), indicate that the biological effect exerted by the administration of a chimera (i.e. opioid-NK1R) results from the entire molecule, and not from its two pharmacophores working independently. Similar mutual interactions were observed for hybrid compounds targeting both MOR and CB_1R (i.e. cannabinoid-1 receptor) (Le Naour et al., 2013; Mollica et al., 2017). Thereby, it is expected that these therapeutic tools can be used in the treatment of complex diseases, as they target the signalling network at different levels (Keith, Borisy, & Stockwell, 2005).

In this review, we focus on bivalent ligands that combine opioid and SP moieties and discuss their pharmacological potential for human use in clinical settings. In fact, the majority of newly presented DMLs combining an opioid receptor agonist pharmacophore and a SP receptor antagonist pharmacophore were reported to exert significant pain-relieving effects in several animal pain models. However, based on some recent clinical research, antagonists of SP (antagonists at neurokinin-1 receptors, NK1R) failed to exert antinociceptive activity in several pain conditions. Therefore, it appears that hybridizing opioid- and SP-related pharmacophores may not bring the expected attenuation of pain perception and reduction of opioid-induced unwanted side effects.

In order to bring the readers closer to the issue, we herein describe the importance of SP and the family of tachykinin receptors in pain processing. Both the development and the possible role of SP agonists and antagonists, including non-peptide derivatives, are summarized, highlighting some paradoxical data obtained in diverse experimental settings. The promises and limitations of the hybrid approach targeting opioid receptors and NK1R will then be developed in the light of recent preclinical studies.

2. Substance P and tachykinin receptors

The biologically active peptide SP was first isolated in 1931 by von Euler and Gaddum from the equine brain and intestines as it was crystallized as a white powder, hence its name where P stands for powder (von Euler & Gaddum, 1931). Later SP was also described in most mammalian species including humans. SP belongs to the tachykinin peptide family, which includes neurokinin A (NKA), neurokinin B (NKB), neuropeptide K and neuropeptide-γ (Maggi, Patacchini, Rovero, & Giachetti, 1993; O'Connor et al., 2004) (Table 1). As established in the early seventies by Chang et al., SP (Table 1 and Fig. 1) is a highly conserved peptide that comprises 11 amino acids (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2) (Chang, Leeman, & Niall, 1971). Positively charged amino acid residues are predominant on the N-terminus of the molecule, while the C-terminus contains more hydrophobic residues. This structure confers amphiphilic properties to the peptide which can thereby interact directly with the lipid bilayer of cell membranes.

SP is widespread throughout the CNS and the PNS, as well as the enteric nervous system (Hokfelt, Elfvin, Schultzberg, Goldstein, & Nilsson, 1977) where it mainly co-localizes with other classical transmitters, including serotonin and glutamate. In the brain it has been detected in the telencephalon, rhinencephalon, diencephalon, mesencephalon, metencephalon, myelencephalon and spinal cord, basal ganglia, hippocampus, amygdala, septal areas, hypothalamus and pons (Ribeiro-da-Silva & Hokfelt, 2000). In peripheral tissues, SP has been detected in the respiratory and urinary tracts, in the immune system, in the intestines, in the blood and in blood vessels (Severini, Improta, Falconieri-Erspamer, Salvadori, & Erspamer, 2002). It is expressed by a large variety of cells, such as neurons, astrocytes, microglia, epithelial and endothelial cells (Hokfelt, Johansson, Ljungdahl, Lundberg, & Schultzberg, 1980; Michel, Sakamoto, Bouvier, Tommasi, & Pearson, 1986). Also, it has been found in many types of immune cells, including T cells, macrophages, dendritic cells and eosinophils (Lai, Douglas, & Ho, 1998). This large

Table 1
Amino acid sequences of tachykinin family members and their receptor preference. Several mammalian neuropeptide interact with the 3 tachykinin receptors with different affinities. These peptides share a common F-GLM signature at their C-terminus as indicated in bold.

Tachykinin	Amino acid sequence	Receptor preference
Substance P	H-RPKPQQ**FFGLM**-NH_2	NK1R > NK2R > NK3R
Neurokinin A	H-HKTDS**FVGLM**-NH_2	NK2R > NK3R > NK1R
Neurokinin B	H-DMHDF**FVGLM**-NH_2	NK3R > > NK2R = NK1R
Neuropeptide K	H-DADSSIEKQVALLKALYGHGQI SHKRHKTDS**FVGLM**-NH_2	NK2R > NK1R > > NK3
Neuropeptide γ	H-DAGHGQISHKR HKTDS**FVGLM**-NH_2	

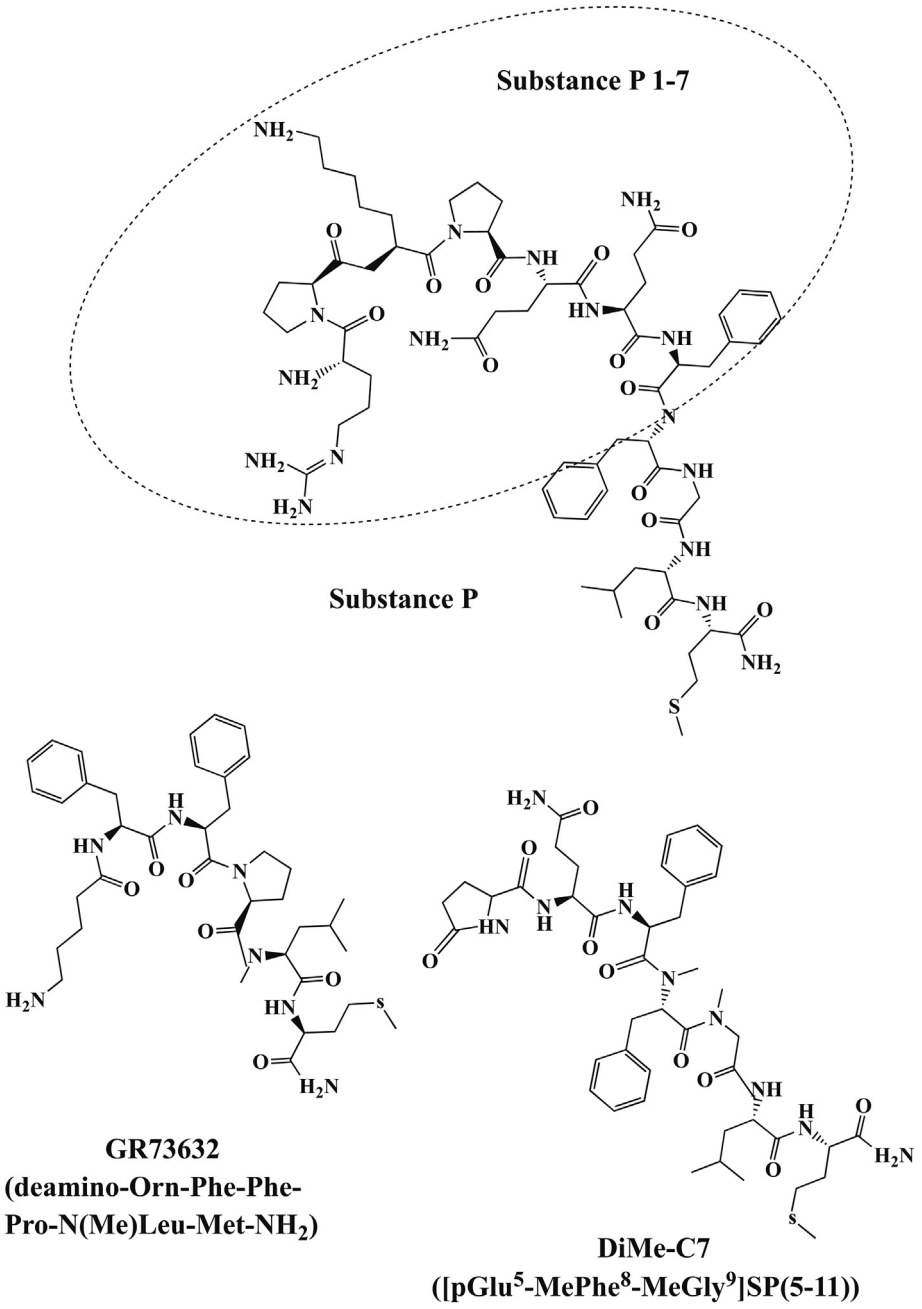

Fig. 1. Representative skeletal formulas of neurokinin-1 receptor agonists.

distribution of SP throughout tissues and cell types highlights its important role in both physiology and pathophysiology.

The activities of SP are mediated through the activation of several neurokinin receptors (Henry et al., 1987) that belong to the rhodopsin family of seven-transmembrane G protein-coupled receptors. To date, three such receptors are known, i.e. NK1R, NK2R and NK3R, often co-expressed by the same cell. Tachykinins bind to each of these receptors with different affinities. SP binds preferentially to NK1R, while NKA and NKB show the highest affinity for NK2R and NK3R, respectively (Table 1) (Mantyh, 2002). The amino acid sequence of NK1R is highly conserved with only subtle differences among mammalian species. It naturally occurs in two isoforms which recognize SP with distinct affinities. The full-length version (NK1R-F), which consists of 407 amino acid residues, is recognized with a nanomolar affinity by SP whereas a 10-fold lower affinity is displayed for the truncated version (NK1R-T) lacking 96 amino acid residues at the C-terminus (Fong, Anderson, Yu, Huang, & Strader, 1992; Lai et al., 2008). The expression of NK1R has been reported on neurons and glial cells, in smooth muscle cells, in endothelial cells and in fibroblasts. Moreover, as reported for SP, this receptor is expressed by a wide variety of immune and inflammatory cells, including T and B lymphocytes, natural killer cells and macrophages, among others (Schaffer, Beiter, Becker, & Hunt, 1998). Importantly, SP shows pronociceptive effects through NK1R densely expressed in the dorsal horn of the spinal cord. The full-length form of NK1R is predominant, especially in the CNS whereas the truncated isoform is detected throughout both the CNS (mainly within the substantia nigra and cerebellum) and some peripheral tissues, particularly in the heart, lungs, liver, spleen and bones (Caberlotto et al., 2003). In several cells, NK1R is believed to act as an autoreceptor and, as such, is involved in the regulation of SP release (Malcangio & Bowery, 1999).

Substance P is characterized by a short half-life in tissues, but is relatively stable in the plasma. Several factors influence its stability,

namely the kinetics of chemical or enzymatic degradation in the extracellular matrix, the binding to cells and the dynamics of cellular internalization (McGregor & Bloom, 1983). The binding of SP is followed by clathrin-dependent internalization of the SP-NK1R complex into the cytoplasm via endosomes. SP is then released after acidification of the endosomal compartment and targeted to lysosomes for degradation. At the same time the NK1R is recycled to the cell membrane (Mantyh, 2002). After its interaction with NK1R, SP may be cleared and degraded by several proteolytic enzymes, such as neutral endopeptidase (NEP), angiotensin-converting enzyme (ACE), SP-degrading enzyme, post-proline endopeptidase, cathepsin-D, cathepsin-E, SP-hydrolysing enzyme, aminopeptidase P and dipeptidyl aminopeptidase IV (Freed, Cooper, Davies, & Lunte, 2001). Although these enzymes have all been demonstrated to be effective in degrading SP in vitro, it is believed that ACE and NEP are primarily involved in the cleavage of SP in vivo (Harrison & Geppetti, 2001). Both of these enzymes inactivate this tachykinin by degrading the hydrolytic bonds of SP and cutting off several amino acid residues from the C-terminus, thus making the peptide unable to bind to its receptor. It has been shown that ACE degrades SP in plasma, cerebrospinal fluid and brain, especially in substantia nigra, while NEP acts within the spinal cord, brain and peripheral tissues (Harrison & Geppetti, 2001).

SP plays a significant role in a broad variety of biological processes. For instance, it is involved in the development of the nervous tissue and it plays a modulatory role in wound healing, airway contraction, vasodilation and salivary secretion. Moreover, it regulates some higher functions of the CNS, including emotional behaviour and memory formation. However, SP participates in the development of numerous diseases as well. Indeed, SP was shown to be involved in asthma, inflammatory bowel disease, psoriasis, rheumatoid arthritis, depression and emesis, among many others (Turner & Vink, 2013). It can also promote tumour cell growth. Several of the listed diseases are of inflammatory nature, as SP stimulates cytokine release by various cell types and modulates immune response in some peripheral tissues, including gastrointestinal and respiratory tracts (O'Connor et al., 2004). Hence, some immune cells, particularly lymphocytes and macrophages, show increased NK1R expression during infection (Mashaghi et al., 2016).

SP has been found to play a significant role in neurogenic inflammation which specifically refers to inflammation that operates at afferent C-fibers upon intense stimulation. Many factors contribute to neurogenic inflammation, such as prostanoids, leukotrienes, histamine and serotonin, as well as a low pH and an increased osmolarity in the extracellular environment (Harrison & Geppetti, 2001). Neurogenic inflammation manifests itself by local vasodilation, increased vascular permeability, mastocyte degranulation and the release of neuropeptides such as SP and calcitonin gene-related peptide (CGRP) (Samsam et al., 2001). As a potent initiator of neurogenic inflammation, SP is considered as one of the most important factors involved in this immune response (Foran et al., 2000b).

SP is however best known for its role as a sensory neurotransmitter participating in nociception (Young, Anklin, & Hicks, 1994). Released together with glutamate at spinal terminals after peripheral noxious stimulation, SP alters the properties of potassium channels and sensitizes excitatory transmission (Khasabov et al., 2002). SP also contributes to the development of inflammatory pain (O'Connor et al., 2004) and contributes to central sensitization and associated hyperalgesia. Beside these roles in pain transmission, SP in the CNS also regulates cardiovascular and respiratory functions and it participates in activating the emetic reflex. Hence, in the periphery it can be found in the primary sensory nerve cells and the neurons located within the respiratory, gastrointestinal and genitourinary tracts (O'Connor et al., 2004).

3. SP-related ligands as attractive tool for pain management

Considering the large distribution and the diversity of roles of SP, there is abundant experimental data presenting SP analogues, particularly antagonists, as potent attractive tools in the treatment of diverse disorders and/or diseases. Hence, aprepitant (Fig. 2) and its derivatives are the first NK1R antagonists approved for human, as antiemetic drugs used in the prevention of chemotherapy or postsurgical nausea or vomiting. Besides, the well described involvement of SP in the modulation of pain transmission prompted researchers to focus on NK1R ligands as a possible solution for intractable pain. Interestingly, although NK1R, NK2R and NK3R may sometimes be expressed in the same areas and structures, little attention was paid in the pain-relieving properties of NK2R as well as NK3R. Yet NK2R antagonists reduced abdominal contractions induced by acetic acid (Julia & Bueno, 1997). Similarly, in acute visceral pain, an NK3R antagonists reduced both the number of abdominal contractions and responses of pelvic nerve afferents to noxious colonic distension (Julia, Su, Bueno, & Gebhart, 1999). Considering that NK2R are barely present in the adult human brain, these responses evidenced in animal models could be absent in human (Dietl & Palacios, 1991; Saffroy, Torrens, Glowinski, & Beaujouan, 2001). Taking into consideration aforementioned, in this section we focus on both preclinical and clinical data showing the efficacy (if any) of NK1R-related ligands, either agonists or antagonists, in the management of several well-known pathological pain states.

3.1. SP and NK1 receptor agonists

It seems odd to propose that NK1R related agonists could support analgesia as SP is known as a pain mediator at the primary synapse in the pain transmission circuitry. Also, only blockade of the NK1R is

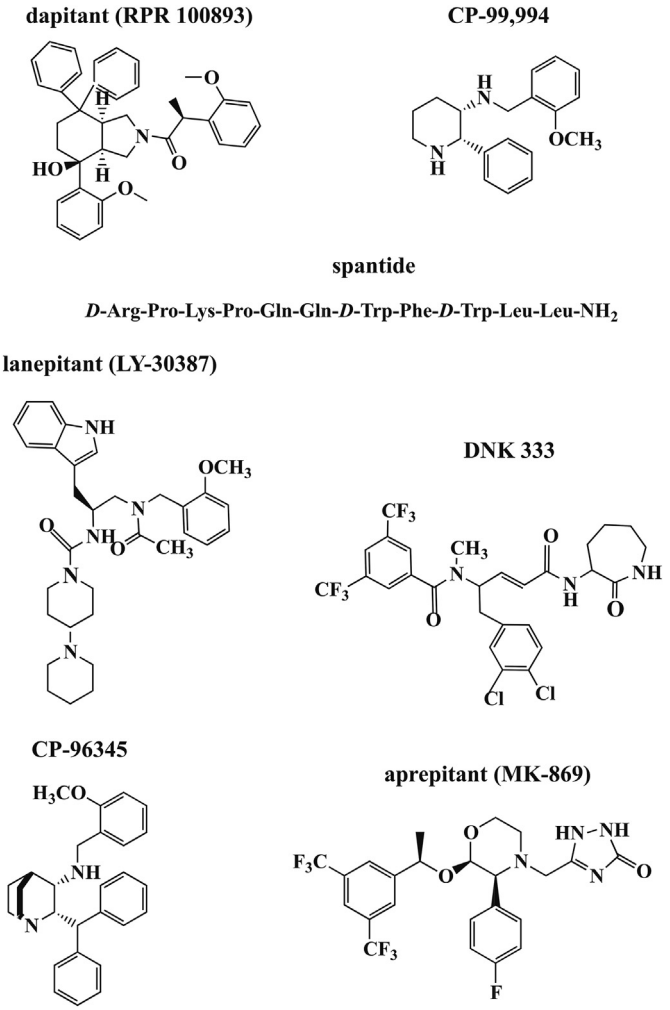

Fig. 2. Chemical structures of neurokinin-1 receptor antagonists studied in pain research.

known to be responsible for the reduction of pain sensation. However, it was reported that both endogenous SP and its metabolites as well as genes for encoding NK1R appear crucial for the antinociceptive effect induced by several compounds (e.g., botulinum toxin type A, acid and capsaicin) (Matak, Tékus, Bölcskei, Lacković, & Helyes, 2017; Mousseau, Sun, & Larson, 1994; Wei-Nan & Chih-Cheng, 2014). Also when given alone at specific doses, SP was shown to induce either nociceptive or antinociceptive responses.

Almost 20 years ago, Altier & Stewart (1997) demonstrated that GR-73632, a selective NK1R agonist (Fig. 1), efficiently attenuated formalin-induced tonic pain after its microinjection into the ventral tegmental area (VTA). The desirable pain-relieving activity of SP agonism in the formalin pain model was later recapitulated for DiMe-C7, another SP analogue (Altier & Stewart, 1993) (Fig. 1). Also, the amphibian peptide PG-SPI preferentially activating the NK1R, was shown to produce time- and dose-dependent analgesia after intracerebroventricular (i.c.v.) administration in rats (Improta & Broccardo, 2000). SP itself, when microinjected into the ventrolateral periaqueductal gray matter in a range of doses between 1 and 5 nmol, was shown to exert antinociceptive effect. This was confirmed by the increased hindpaw withdrawal latencies (HWL) to both thermal and mechanical stimuli in the study of Rosén and co-workers (Rosén, Zhang, Lund, Lundeberg, & Yu, 2004). The SP antinociceptive activity was thus revisited (Improta & Broccardo, 2000) after initial studies from the early 80ies (Lecci, Giuliani, Patacchini, Viti, & Maggi, 1991; Mohrland & Gebhart, 1979; Naranjo, Sanchez-Franco, Garzon, & del Rio, 1982), revealing that SP exerts modest but efficient pain-relieving effect in tests using thermal stimuli (tail flick and hot-water tests) in both mice and rats. Indeed, in the first paper mentioned (Improta & Broccardo, 2000), SP was injected supraspinally at a dose of 10 μg, and the antinociceptive effect occurred within 20 min after administration. However, at that time the maximum peak response reached the value of approx. 60% MPE (Maximal Possible Effect calculated using the following formula: %MPE = (pdr-br)/(co-br) × 100%, where pdr, br and co refer to post drug response, baseline response and cut-off value, respectively). Also, others indicated SP as a potent analgesic, even though the antinociception produced by this neurotransmitter appeared largely dose-dependent. Some reports suggested that the observed response was strictly dependent on the route of administration as well as on the analgesiometric procedures employed. For example, SP applied intraperitoneally exerted a potent pain-relieving action at a dose range of 0.25–1 mg/kg, whereas lower doses revealed no analgesia in response to thermally induced pain (radiant heat). Importantly, when the procedure was repeated and another thermal pain test was used (i.e. hot-plate), no analgesia was reported (Mohrland & Gebhart, 1979).

Metabolites of SP were found to exert diverse biological activities in behavioural tests, including diverse pain models. Indeed, this was especially noted for SP1–7 (Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7; Fig. 1) and SP7–11 (Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}), but not for SP4–11 (Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}). The N-terminal part of SP, i.e. SP1–7 (Fig. 1) was reported to produce dose- and time-dependent antinociception in various animal models of pain (Goettl & Larson, 1996; Kreeger, Kitto, & Larson, 1994; Skogh et al., 2017), particularly in neuropathic pain models, which activity was blocked by an opioid ligand, [D-Ala^2,$NMePhe^4$,Gly-ol] enkephalin (DAMGO), but neither by NK1R, NK2R nor NK3R agonists (Hall & Stewart, 1983). Worth mentioning, this N-terminal SP fragment was also found to significantly modify opioid-induced undesirable effects such as the development of tolerance with putative benefit on withdrawal symptoms (Kreeger & Larson, 1996). Similarly, different SP analogues, including constrained, amidated and truncated ligands exerted anti-allodynic action after systemic injection (Carlsson-Jonsson et al., 2014; Jonsson et al., 2015; Skogh et al., 2017). Importantly, opposite responses were reported with a C-terminal fragment of SP (Hall & Stewart, 1983 and 1984), suggestive of a distinct molecular mechanism of action.

Considering the modest affinity of the N-terminal fragments of SP for NK1R, it has been postulated that the desensitization to the behavioural effects of SP1–7 is mediated by an action at an independent binding site (Mousseau, Sun, & Larson, 1992; Yukhananov & Larson, 1994). In this respect, it is worth noting that promoting NK1R internalization may contribute to pain relief, as evidenced for capsaicin (see below).

It is also widely known that SP, together with its metabolites, influences the release of excitatory amino acids (EAA) aspartate and glutamate into the dorsal spinal cord extracellular fluid (Kangrga, Larew, & Randic, 1990; Skilling & Larson, 1993), thus increasing the pain transmission. This action is however absent for the N-terminal fragment SP1–7, here again suggesting the existence of a distinct SP1–7–sensitive receptor system that would support the unexpected physiological and pharmacological properties of this ligand (Igwe, Kim, Seybold, & Larson, 1990). In fact, binding sites recognizing SP1–7 and endomorphins have been described in the rat brain that appear unrelated to conventional NK receptors (Botros et al., 2008).

Nevertheless, to date there is no validated data supporting SP and its agonist analogues as clinically active analgesics. One exception is capsaicin, the pungent extract of Cayenne pepper, which does not possess structural analogy with SP. Indeed, it is known for decades that capsaicin dose- and time-dependently affects the release of SP, thus acting as an indirect agonist, but also as a potent blocker of SP transmission when all SP is depleted and receptors are desensitized (Dalsgaard et al., 1983; Hayes & Tyers, 1980).

3.2. NK1R antagonists

Based on the robust evidence for a role of SP in the spinal processing of pain signals, ligands endowed with a NK1R antagonist profile have received increasing attention as they could pave the way for the development of effective pain therapies. Moreover, recent studies have also indicated that such compounds may prove beneficial in preventing the risk of opioid abuse.

3.2.1. Development of peptide and non-peptide ligands of the NK1R

Several groups have conducted structure-activity relationship (SAR) studies aimed at defining the molecular determinants of SP and related peptides that support the interaction with the NK1R and its activation or blockade. Indeed, early studies on SP indicated that the minimal structural fragment of SP which is essential for NK1R agonist activity corresponds to its C-terminal partial sequence (hexapeptide) (Huang & Korlipara, 2010; Regoli, Escher, and Mizrahi, 1984). Further work showed that

- deamidation at the C-terminus inhibits SP activity (Werge, 2007);
- the two aromatic groups of Phe^7-Phe^8 are essential to support high affinity for the NK1R (Ofner, Hauser, & Schilling, 1996);
- incorporation of a Trp residue instead of Leu at position 10 decreases the intrinsic activity, thereby generating antagonist peptides (Regoli, Escher, and Mizrahi, 1984);
- similarly, combined substitutions with Trp in positions 7 and 9 or 7 and 10 or in position 7, 9 and 10 of octa- or undecapeptides effectively support NK1R antagonism (Regoli, Escher, and Mizrahi, 1984);
- substitution of Phe at position 8 with Val in the octapeptide molecule results in the reduction of NK1R binding affinity (Regoli, Escher, Drapeau, D'Orléans-Juste, and Mizrahi, 1984);
- peptide ligands containing both aromatic or aliphatic side chains at their C-terminus are recognized by the receptor. This suggested the existence of distinct functional sites for SP-related peptides (Regoli, Escher, Drapeau, et al., 1984 and Regoli et al., 1985);

Noteworthy, the binding affinity of diverse SP-related antagonist compounds at NK1R was found to depend on the smooth muscle examined (e.g., rabbit mesenteric vein, guinea-pig ileum, guinea-pig trachea,

rat urinary bladder). Indeed, some receptors located on smooth muscles were found to selectively recognize selected amino acid residues naturally occurring in the SP sequence, particularly Gln^5 and Gln^6 (Mathison, Escher, Huggel, Mizrahi, & Regoli, 1985). The design of synthetic ligands recognizing the tachykinin receptors revealed that the incorporation of a 3,5-bis-trifluoromethylphenyl group (−bis-CF_3 or $(CF_3)_2$) favoured the interaction with the His265 residue of the receptor, considerably enhancing the binding affinity (Humphrey, 2003) and yielding potent and selective NK1R antagonists (Lewis et al., 1995). The importance of this bis-aromatic motif has prompted many authors to design small NK1R ligands containing only two aromatics rings (e.g., constrained Phe and Trp amino acid mimics) (Ballet et al., 2011). These studies demonstrated that substituting the ester bound in the Trp-based potent ligand (i.e. Ac-Trp-O-3′,5′-$(CF_3)_2$Bn) into an amide bound may result in the loss of its antagonist activity (Ballet et al., 2011). Moreover, a conformationally constrained 4-amino-2-benzazepin-3-one (Aba) scaffold was found to serve as a potent core of compounds effective in terms of NK1R antagonism (Betti et al., 2015).

The molecular cloning of the NK1R in the early nineties (Takeda, Chou, Takeda, Sachais, & Krause, 1991) has opened the possibility to conduct large pharmacological screenings aimed at further identifying non-peptide ligands, acting as either agonists or antagonists at the cloned receptor (Garret et al., 1992; Pradier et al., 1995). SAR studies have herein revealed that pyridine-based analogues, among which netupitant and befetupitant, potently inhibited NK1R agonist-mediated responses (Hoffmann, Bos, & Stadler, 2006). Similar results were obtained for piperidine-containing derivatives (e.g., R116301 and rolapitant) (U.S. Patent No. 6,251,894, Janssen et al., 2001; Romerio, Linder, & Haefeli, 1999; World Patent No. WO03051840, Paliwal et al., 2003. The design of potent non-peptide NK1R antagonists is still ongoing and a variety of active ligands with unrelated structural backbones have been characterized. In fact, NK1R ligands with a core of tetrahydropyrans (World Patent No. WO00056727, Owen et al., 2000), hydropyrano[3,4-*c*]pyrroles (World Patent No. WO06065711, Devita et al., 2006), γ-lactam (World Patent No. WO06060344, Bunda et al., 2006) as well as tetrahydroquinolines (Ballet et al., 2011) or cyclic urea (World Patent No. WO06060344, Bunda et al., 2006; Shue et al., 2005) have been described.

Since the discovery of the antinociceptive activity of the endogenous N-terminal metabolite of SP (see above) was reported, a large number of peptide analogues of the SP1–7 fragment with preserved affinity at NK1R were synthesized and characterized. Based on SAR modifications it was reported that

- the C-terminal sequence of SP1–7, particularly the -Pro-Ala-Gln-Phe- sequence, appears essential for high affinity binding (Fransson et al., 2008; Geraghty & Burcher, 1993);
- replacement of Phe^7 for Ala is deleterious as the K_i value dramatically increases from 1.60 ± 0.06 to >10,000 nM (Fransson et al., 2008);
- the amidation of SP1–7 sequence results in a reinforced pain-relieving effect in the spared nerve injury animal model of chronic pain and improved binding affinity toward the SP1–7 binding site compared to the native compound (Fransson et al., 2008; Jonsson et al., 2015);
- the C-terminal rigidification of SP1–7 derived dipeptide (H-Phe-Phe-NH_2) using a *cis*-3-phenylpyrrolidine moiety yields ligands with preserved binding affinity for the SP1–7 binding site and endowed with similar potent anti-allodynic effect in the spared nerve injury model as the native heptapeptide (Jonsson et al., 2015);
- N-terminal truncation of SP1–7 is without influence on the NK1R binding affinity (Fransson et al., 2008) suggesting minor involvement of the N-terminal part.

In view of the panel of synthetic peptide and non-peptide ligands characterized at the NK1R, it is proposed that the binding pocket of the receptor provides a large opportunity for the development of original and selective pharmacological tools. However, it turns so far difficult to formulate strict rules on what should be fulfilled in order to predict strong NK1R antagonism. Also, the CNS penetration of the peptide analogues will inevitably influence the outcome of in vivo experiments and their interpretation in terms of SAR.

3.2.2. Efficacy in preclinical studies

The efficacy in preclinical experiments of various NK1R ligands with an antagonist profile that additionally show selectivity over the NK2R and NK3R subtypes has been discussed in several reports. These include peptide and non-peptide compounds as well as peptidomimetics. In 2014, Greenwood-Van Meerweld and co-workers reported that netupitant significantly reduced spinal nerve ligation-induced somatic hypersensitivity in rats (Greenwood-Van Meerveld et al., 2014). This effect was dose-dependent as an increased threshold for animal paw withdrawals in von Frey filaments testing was observed only at subcutaneous doses of 1 and 10 mg/kg but not at a dose 0.1 mg/kg. Also, the non-peptide NK1R antagonist, SR 140333 was characterized as a potent analgesic, especially after systemic administration in rats (Jung et al., 1994). Similarly, spantide I ([D-Arg^1, D-$Trp^{7,9}$, Leu^{11}]-substance P; Fig. 2) as well as spantide II (Nic-Lys^1–3-Pal^3-Cl_2-Phe^5-Asn^6-$Trp^{7,9}$-Nle^{11}-substance P) were identified as potent analgesics that produce dose-dependent antinociceptive response in several pain states. This is true for the formalin-induced inflammatory pain (Sakurada et al., 1993; Tan-No, Sakurada, Yamada, Sakurada, & Kisara, 1995), as well as for ocular and palpebral (eyelid) pain (U.S. Patent No. 5,730,998, De Lacharriere and Breton, 1998).

Meanwhile, NK1R antagonists were also demonstrated to impair peripheral antinociception (e.g., stress-induced analgesia), and this was found to be strictly associated with a decrease in the local recruitment of leukocytes that secrete opioids (Rittner et al., 2007), the most prominent of which is β-endorphin. Similar results were obtained for CP-96345 compound (Fig. 3) which failed to induce potent anti-pain effect after its supraspinal administration (Garces, Rabito, Minshall, & Sagen, 1992). These contradictory reports suggest that the activity of NK1R-prefering ligands is highly dependent on the route of administration. Furthermore, differential involvement of tachykinin receptor subtypes according to the pain model used can be observed, as some NK1R antagonists revealed inconsistent potency in two distinct models of neuropathic pain (diabetic and sciatic nerve ligature) (Coudoré-Civiale, Courteix, Eschalier, & Fialip, 1998).

3.2.3. Efficacy in clinical studies

While studies conducted on animal models have generated great hope, the use of NK1R antagonists (of both peptide and non-peptide nature) failed to produce the expected analgesic activity in clinical studies. This was reported for instance in a double-blind, placebo-controlled, two period study by Willert et al. in 2007 where one of the most potent NK1R antagonist (named NK1RA) failed to reduce the hydrochloric acid-induced oesophageal allodynia (Willert et al., 2007). Similarly, the lack of clinical efficacy for NK1R antagonism was also reported in somatic pain (Boyce & Hill, 2000) as well as in diabetic neuropathic pain (Sindrup, Graf, & Sfikas, 2006). Likewise, lanepitant (Fig. 2) was found ineffective either in patients with osteoarthritis pain (Goldstein et al., 2000) and painful diabetic neuropathy (Goldstein, Wang, Gitter, & Iyengar, 2001) or with acute migraine (Goldstein et al., 1997).

Intriguingly, several authors explained this failure simply by the lack of involvement of tachykinins and cognate receptors, particularly NK1R, in the pain states that have been considered. According to this assumption, it would appear that SP does not play a role in human pain at all. On the other hand, it was also suggested that these inconsistent results in pharmacological studies rely on interspecies variations in the tachykinin receptors (Beresford, Hagan, & Ireland, 1991). The poor blood-brain barrier penetration may also constitute a major limitation for the majority of NK1R antagonists. Hence, Hietala et al. provided evidence for a relatively good CNS penetration of the NK1R antagonist

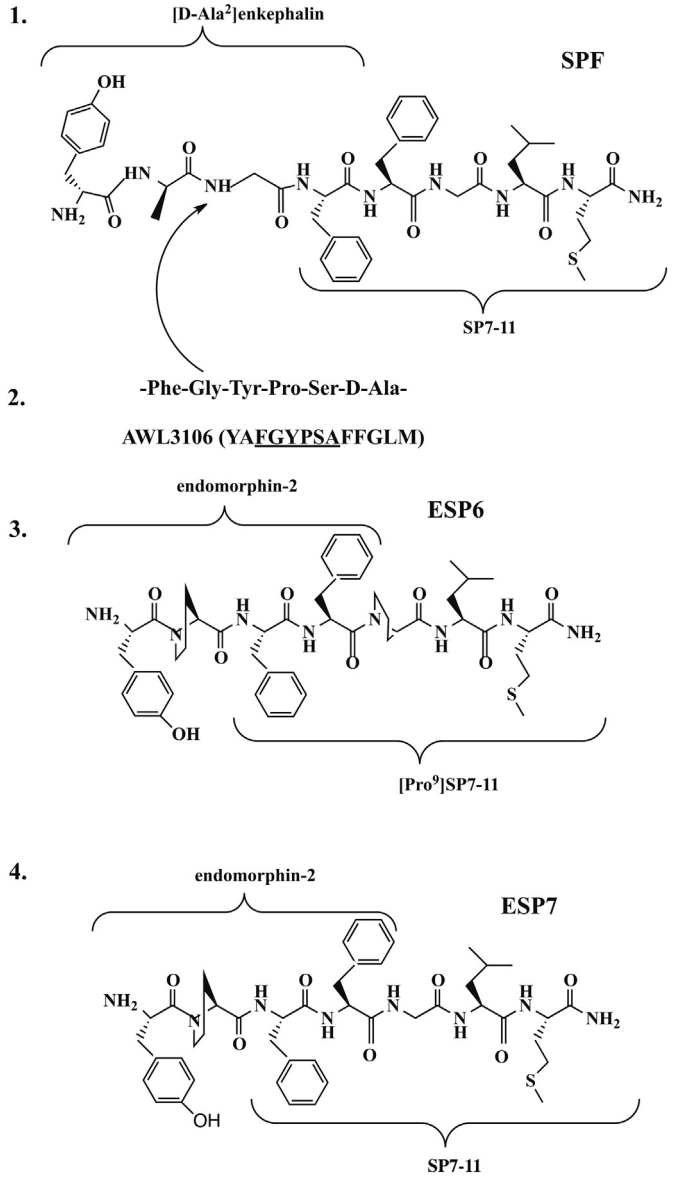

Fig. 3. Lipkowski's endomorphin-2 (enkephalin)/SP chimeric peptides. The structures presented contain either modified fragment of enkephalin (i.e. SPF and AWL 3106) or endomorphin-2 (i.e. ESP 6 and ESP7) hybridized with C-terminal fragment of SP (i.e. SP7–11). Phe is used as an overlap domain between the two pharmacophores.

MK-869, which was endowed with potent pain-relieving activity (Hietala, 2000). An additional confirmation of the lack of clinical efficacy of tachykinin antagonists in human pain was provided by the group of Diener (Diener & RPR100893 Study Group, 2003) reporting on the testing of various oral doses of RPR100893 (Fig. 2), also known as dapitant, in people with headache that was related to migraine. Similarly to previous reports, this NK1R blocker did not induce a desirable pain-relief when compared to placebo in this type of pain.

Together, this review of the literature shows that only little information is available with respect to the putative benefits resulting from the application of SP antagonists in terms of anti-pain effects. The paper of Dionne et al. describing the activity of CP-99,994 ((2*S*, 3*S*) - *N* - [(2 – methoxyphenyl)methyl] - 2 - phenyl - 3 - piperidinamine dihydrochloride; Fig. 2) is one of the few where this non-peptide compound relieved postoperative pain at doses devoid of side effects (Dionne et al., 1998). However, this effect was weaker than in the case of the non-steroid anti-inflammatory drug ibuprofen. Another research involved intravenous fosaprepitant (which serves as a prodrug for the well-known NK1R antagonist aprepitant; Fig. 2) administrated to patients who had undergone either gynaecologic abdominal surgery (Soga et al., 2015) or lower limb surgery (Kakuta et al., 2015). In these trials, fosaprepitant (150 mg) revealed similar effectiveness as ondansetron (4 mg) in the context of visual analogue pain score. However, both studies primarily focused on its antiemetic properties rather than its antinociceptive profile.

Potent pain-relieving activity was also suggested for DNK333 (*N*-[(*R*, *R*)-(*E*)-(3,4-dichlorobenzyl) – 3 - (2 – oxoazepan – 3 - yl)carbamoyl] allyl – *N* – methyl - 3, 5 -bis(trifluoromethyl)benzamide; Fig. 2), a novel dual tachykinin NK1R/NK2R antagonist, in women with irritable bowel syndrome, in particular the diarrhoea-predominant form (IBS-D) (Zakko, Barton, Weber, Dunger-Baldauf, & Rühl, 2011). Nonetheless, the benefit was stated after combined analysis of data from both trials, while no significant differences were seen between drug-given and placebo groups in each trial alone (that consisted in 2 or 4 weeks of administration, respectively). Therefore, in light of this information, presented results should not be treated unequivocally as they do not support a benefit of NK1R antagonism in abdominal pain prevention.

4. Opioid receptor-neurokinin-1 receptor hybrid ligands in pain therapy

The chimeric approach is essentially designed as a possible alternative to polytherapies. In fact, combination therapy, defined as a therapy that comprises more than one medication is frequently associated with the occurrence of undesirable side effects. The use of several drugs is often justified for patients suffering from two or more diseases and pathological states. However, the simultaneous administration of many drugs favours the emergence of both pharmacodynamic and pharmacokinetic drug-drug interactions. This is particularly common for treatments involving opiates of which effects can be amplified when combined with a variety of substances (e.g., benzodiazepines, antidepressants, gabapentinoids, alcohol, etc.), leading to potentially life-threatening complications. Hence, opiate use is also concerned by the development of tolerance and addiction, fear of which is usually the main reason for discontinuing therapy or prescribing suboptimal doses to patients.

In light of the experimental demonstration that antagonism at NK1R may protect against addiction in patients receiving opiates (Sandweiss et al., 2017) and considering the documented benefit of using hybrid compounds in diverse contexts, one may expect that coupling opioids and NK1R-related structures could pave the way to innovative drugs with added values for pain treatment. The rationale for such approach also relies in the documented colocalization of opioid receptors and NK1R in nervous structures involved in nociceptive transmission. A large body of pharmacological evidence are supportive of a strong relationship between these two classes of receptors. Thus Hylden and Wilcox (Hylden & Wilcox, 1983) reported that high doses of SP results in hyperalgesia as well as scratching and biting behaviours, which can be prevented by opioid agonists. Also, opioids are known to inhibit SP release in both in vitro and in vivo studies (Jessel & Iversen, 1977; Yaksh et al., 1980). In turn, the increased release of SP in the spinal cord observed after administration of the opioid receptor antagonist naloxone correlates with an intensification of pain in the second stage of the formalin test. Hence, Aicher and colleagues demonstrated that in the rat spinal cord, MOR-immunoreactive dendrites in the dorsal horns contain NK1R and that conversely, NK1-immunoreactive dendrites contain MOR (Aicher, Punnoose, & Goldberg, 2000). While these observations suggest that opioid-NK1R bivalent ligands may directly interact with both receptors, another possible scenario is proposed where the molecule that would preferentially bind MOR can alter the release of endogenous SP (Cano, Arcaya, Gómez, Maixner, & Suarez-Roca, 1999). Indeed, for decades, the opioid-mediated analgesia was accredited to an inhibition of the release of SP from the presynaptic afferent terminals in the spinal dorsal horn (Yaksh et al., 1980). This effect is also assigned

to other opioid receptor members, i.e. DOR and KOR (Kouchek, Takasusuki, Terashima, Yaksh, & Xu, 2013; Zachariou & Goldstein, 1996.)

4.1. Dual opioid - NK1R agonists

Knowing that SP may exhibit a bimodal activity (i.e. pronociceptive and antinociceptive) depending on the fragment used or the concentration tested (Cridland & Henry, 1988; Stewart et al., 1976), SP-related peptides have been commonly considered as promising candidates for combination into chimeric drugs. Furthermore, even though NK1R antagonists have initially received more credit for the development of pain treatment, examples of hybrids containing SP analogues with agonist activity at NK1R have also been described.

The first chemical combinations of opioids and SP or its agonist analogues in single chimeric entities were described by the group of Lipkowski in the early 80ies (Lipkowski, Osipiak, & Gumulka, 1983). In particular, a novel peptide with the sequence Tyr-*D*-Ala-Gly-Phe-Phe-Gly-Leu-Met-NH_2 and further designated as SPF (SP fusion analogue) was characterized. Unfortunately, this combination did not enhance the relief of pain experienced by mice in comparison with native SP. Hence, the SP pronociceptive properties were preserved in this peptide (Lei, Lipkowski, & Wilcox, 1991). Together with Kream, Lipkowski (Foran et al., 2000a and 2000b) subsequently developed two bivalent compounds encompassing the endomorphin-2 (Tyr-Pro-Phe-Phe) pharmacophore and the SP7–11 moiety by a so-called "merge strategy" (Kleczkowska et al., 2013) where a shared Phe is used as an overlap domain between the two structures. These two novel drugs, named ESP6 and ESP7 respectively, although showing subtle difference in their structure (ESP6 has Pro incorporated in the C-terminal pharmacophore instead of Gly in ESP7; Fig. 3), showed marked differences in terms of affinity ($K_i^{MOR} = 92$ nM and $K_i^{NK1R} = 305$ nM for ESP6 vs. $K_i^{MOR} = 218$ nM and $K_i^{NK1R} = 289$ nM for ESP7, respectively) (Lipkowski, Carr, Bonney, & Kosson, 2013) and agonist potency at the MOR (1900 vs. 95 nM for ESP6 and ESP7, respectively). Consistently, both compounds were endowed with antinociceptive activity in rats (Foran et al., 2000b). ESP7 was shown to support a significant and long-lasting pain-relieving effect which ranged between 20 and 40% MPE value (Foran et al., 2000a) while ESP6 produced analgesia reaching only 10% MPE value (Foran et al., 2000b). Interestingly, intrathecal (i.t.) administration of morphine with ESP6 leads to a prolonged analgesia indicating its putative relevance as adjuvant therapy for maintaining opioid supported analgesia during prolonged treatments.

Based on the previous results, Lipkowski et al. designed and synthesized AWL3106, another opioid/SP chimeric compound, (Tyr-*D*-Ala-Phe-Gly-Tyr-Pro-Ser-*D*-Ala-Phe-Phe-Gly-Leu-Met-NH_2; Fig. 3), with a spacer that enables the two pharmacophores to act independently (Lipkowski et al., 2013). The incorporation of a distance between the endomorphin and SP moieties contributed to a strong dose-dependent antinociceptive response after spinal as well as peripheral (i.v.) application. More importantly, the high efficacy of this drug after its peripheral administration, which putatively attests its ability to cross the blood-brain barrier, was observed from 15 to 60 min post-injection and reached nearly 100% MPE with a dose of 2 μmol/kg (Lipkowski et al., 2013).

Similar endomorphins-based chimeras were later developed by Kream et al. (2007)) and Varamini et al. (Varamini, Hussein, Mansfeld, & Toth, 2012). The later successfully designed a series of endomorphin-1-based chimeric compounds containing SP8–11 and SP7–11 domains of which agonist activities were improved through an N-terminal modification with a C10-carbon lipoamino acid (C10LAA) (Fig. 4). Although these original compounds were not validated in terms of analgesic potency, they showed improved resistance against enzymatic degradation and 8 to 10-fold enhanced permeability through the blood-brain barrier as compared to the less lipophilic parent hybrids. However, their affinities at the MOR were found

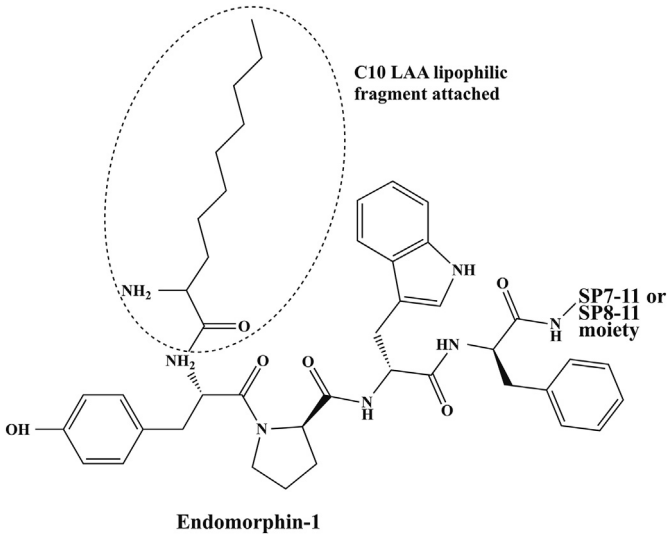

Fig. 4. Representative structure of C10 lipoamino acid-modified opioid agonist (YPWF)-NK1R agonist (FGLM and GLM) hybrid peptides developed by Varamini et al. (Varamini et al., 2012).

significantly reduced in comparison to C10LAA-unmodified peptides (K_i^{MOR} 132.0 ± 14.8 nM vs. 1.60 ± 0.15 nM and 3.87 ± 0.51 nM vs. 0.73 ± 0.20 nM, respectively).

In a distinct but complementary approach, Kream and colleagues (Kream et al., 2007) focused their efforts on opioid-NK1R ligand comprising morphine and SP3–11, the high potency agonist domain with the sequence of Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2. A succinic acid linker was introduced between the opioid and SP pharmacophores as this 4-carbon spacer can positively influence the receptor binding by preventing reciprocal steric inhibition. This compound, designated MSP9, was reported to produce a strong pain-relieving effect in a large range regimen (0.01–10 mg/kg intramuscularly, i.m.). Maximal efficacy was obtained at a dose of 0.2 mg/kg, at which the analgesic response in the tail flick test expressed as a percent increase over baseline latency reached approximately 42% (Kream et al., 2007).

More recently, a novel chimeric peptide was designed and synthesized by Lipkowski, was characterized with a promising analgesic profile (Kowalczyk et al., 2016). AA3052 (H-Tyr-*D*-Arg-Phe-Lys-*D*-Phe-Phe-*D*-Phe-Leu-Leu-NH_2) consisting of DALDA (a potent peptide MOR agonist (Schiller, Nguyen, Chung, Dionne, & Martel, 1990)) linked to a modified SP7–11 moiety. In fact, the naturally existing Phe in position 7 and Gly in position 9 were substituted by *D*-Phe, whereas Met^{11} was replaced with leucine. Although these features recapitulate those present in compound AWL-60, a chimeric opioid receptor agonist-NK1R antagonist with a sequence of (Tyr-Pro-*D*-Phe-Phe-*D*-Phe-*D*-Trp-Met-NH_2) (Lipkowski et al., 2013, Lipkowski & Misterek, 1992), the amino acid substitutions as well as further shortening and combination with a MOR-ligand resulted in a switch from NK1R antagonism to a partial agonism profile ($E_{max} = 118.2\%$). Also, the SP pharmacophore had a strong impact on the opioid moiety as the DALDA element did not bind (K_i of 729.8 nM) nor activate ($E_{max} = 130.9\%$) MOR (Kowalczyk et al., 2016). Nevertheless, the unexpected activity of this hybrid peptide was confirmed in behavioural studies where AA3052, at variance with DALDA, did not cause tolerance after subchronic i.c.v. administration.

Another study where a NK1R agonist was combined in a chimeric ligand was published in 2013 by Brown and Agnello (Brown & Agnello, 2013). The designed compound differed in its structure in comparison to the aforementioned ligands at the level of its second active pharmacophore partner. Instead of an opioid moiety, a recombinant version of the ribosome-inactivating protein, saporin (SAP) was attached to SP. With this approach, the cell toxicity of saporin, isolated

form the seeds of *Saponaria officinalis*, was specifically directed toward cells expressing NK1R. Thus, after injection in lamina 1 of the spinal cord, the SP-SAP conjugate is expected to enter the cell through NK1R mediated internalization, where SAP could interfere with protein synthesis, causing eventual death of the cell (Nichols et al., 1999). Such mechanism was proposed to explain the reduced degree of pain perception in dogs suffering from bone cancer pain after i.t. administration of SP-SAP (Brown & Agnello, 2013).

4.2. Opioid receptor agonists - NK1R antagonists

Considering the growing knowledge about the role of SP in various diseases (e.g., anti-inflammatory and anti-cancer properties) and the clinical benefit obtained with NK1R antagonists, efforts have been dedicated to the development of therapeutically useful hybrid drugs combining pharmacophores with opioid receptor agonism and NK1R antagonism. Indeed, the observation that simultaneous administration of an opioid agonist and a NK1R antagonist results in enhanced antinociceptive response in acute pain states, while preventing opioid-induced tolerance after chronic application (see below) (Misterek et al., 1994) has prompted the design of chemical entities combining these activities. Nowadays, it constitutes the largest group of opioid-based chimeric compounds that have been generated and new candidates are still under development and characterization. This group encompasses not only simple peptides composed of opioid and SP analogues but also peptidomimetics with complex structures, typically containing a substituted (-OMe or - bistrifluoromethyl) phenyl group.

Synthetic molecules containing both an opioid pharmacophore and a NK1R ligand with an antagonist profile were originally described in the early 90's in the pioneering work of the group of Lipkowski (Lipkowski, Carr, Misicka, & Misterek, 1994). The first compound characterized in this family (AWL-60), comprised casomorphin, a milk protein-derived opioid peptide, hybridized to an antagonist fragment of SP. This combination exhibited strong and long-lasting antinociceptive properties, even though the binding affinity at MOR was rather moderate, with an IC_{50} of 210 nM. Since then, a number of structurally related potent pain-relieving chimeras were developed. Thus, AA501 - the second opioid-NK1R hybrid peptide to be generated - encompassed both pharmacophores of Tyr-*D*-Ala-Gly-Phe and the carboxybenzyl-tryptophan moiety (Cbz-Trp) hybridized head-to-head with an additional hydrazide linker (-NH-NH-) (Maszczynska Bonney, Foran, Marchand, Lipkowski, & Carr, 2004). This chimera induced dose-dependent antinociceptive effect when given spinally (i.t.) in both acute and formalin pain tests. Furthermore, in spinal nerve ligation-induced neuropathic pain, AA501 produced significant blockade of mechanical allodynia (Maszczynska Bonney et al., 2004).

Based on the initially described hybrid peptide, a large variety of chemical modifications have been tested with the objective to increase the affinity for the two receptor targets (see Table 2). Many of the listed compounds also contain a 3′,5′-(bistrifluoromethyl)-benzyl (stated as 3′,5′-$(CF_3)_2$-benzyl or 3′,5′-$(CF_3)_2$-Bzl) motive, an important functional group that considerably promotes NK1R binding (Cascieri et al., 1994; Lewis et al., 1995). When incorporated as a C-terminal 3′,5′-$(CF_3)_2$-benzyl ester group, it was found to be subjected to rapid enzymatic hydrolysis and several analogues have been developed with an amide bond connecting the 3′,5′-$(CF_3)_2$-benzyl moiety. In fact, the C-terminally amidated chimeric compound TY027 with an amino acid sequence of H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl] was characterized by improved half-life in the rat plasma (over 4 h) in comparison with its analogue with C-terminal ester group (i.e. TY005; H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl] for which the half-life value reached approximately 1 min (Largent-Milnes et al., 2013; Yamamoto et al., 2007). Also, in the case of these two compounds it was observed that the replacement of the ester bond for an amide increased the affinity at MOR by a factor of 2, though both chimeric ligands exhibited analgesic efficacy following systemic (i.v.) and central (i.t.) administration in spinal nerve ligation-induced neuropathic as well as acute pain models with blood brain barrier permeability. Also, no development of either antinociceptive tolerance or reward liability and lack of impact on locomotor activity (even at a dose of 100 μg i.t.) were observed (Largent-Milnes et al., 2010 and 2013). Noteworthy, the removal of two trifluoromethyl groups while leaving the -NH-benzyl fragment, also resulted in an increased affinity for MOR, but with a substantial decline in NK1R-binding affinity (see compounds TY027 vs. TY025).

Although Table 2 mostly includes compounds carrying a N-terminal Tyr residue, known to be critical for the binding at opioid receptors, other Tyr-substituted chimeric ligands were developed. Thus, this residue is frequently replaced with an unnatural hydrophobic and conformationally restricted analogue 2′,6′ -dimethyltyrosine (Dmt). Such substitution enhances the MOR binding affinity and potency of several opioid peptides and peptidomiemtics (Hansen Jr. et al., 1992; Li et al., 2005; Szeto, Soong, Wu, Qian, & Zhao, 2003; Varamini & Toth, 2013). The Tyr/Dmt substitution is exemplified in the work of Hruby's lab as compound TY027 analogue 2 (H-Tyr-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]) modified into compound No 3 (H-Dmt-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]; Table 2), was found to display improved binding for MOR (K_i of 32 nM vs. 1.2 nM, respectively) (Yamamoto, Nair, Jacobsen, et al., 2009 and Yamamoto et al., 2011). Importantly, Dmt incorporation (such as in TY032) also results in an enhanced plasma stability when compared to corresponding Tyr^1-containing parent peptide TY027 (Yamamoto et al., 2011). These properties were translated into improved analgesic properties when tested in vivo. Thus, in comparison with TY027, TY032 produced stronger pain-relief after i.t. administration to rats which was additionally shifted in time (the maximal antinociception was observed at 60 min post-injection, a time at which TY027 showed no significant residual efficacy) (Yamamoto et al., 2011).

Intriguingly, further substitution of Met^5 with norleucine (Nle) in TY027, which resulted in compound (termed in the Table 2 as No 3) increased the nanomolar affinity at MOR (from $K_i = 16$ nM to $K_i = 1.2$ nM), while maintaining antagonist activity at NK1R (Yamamoto, Nair, Ma, et al., 2009 and Yamamoto et al., 2011). The unexpected importance of this fifth position and its Nle substitution was also presented in case of the ligand TY018 in comparison with TY005 (Table 2). The position appeared determinant for the affinities as well as activities at both MOR and NK1R. Surprisingly, the introduction of neither *N*-methylated α-amino acids (e.g., NMePhe for compound TY019) nor *D*-amino acids (e.g., TY007, TY024) resulted in substantial changes in terms of binding at NK1R, even though the impact on MOR was modest.

Other modifications also included peptide cyclization together with amino acids replacements. Some of the modifications in the chain resulted in a reinforced analgesic potency that correlated with enhanced MOR- and NK1R-binding affinities. They also included the reduction of the chain length (e.g., compounds NP43 and NP66) as well as O-glycosylation at selected serine residues. In addition to ameliorate the pharmacological profile, these modifications also influenced the biophysical properties of the peptides. Indeed, long peptides and peptide mimetics commonly show limited permeability through the blood-brain barrier. Finally, shorter peptides are easier to synthesize, with a positive outcome in terms of production costs.

Despite some rational rules that could be followed when designing putatively optimized drug, it remains difficult to predict how truncation of the peptide sequence will influence the affinities at MOR and NK1R, respectively. Furthermore, additional studies are required to confirm the MOR agonist/NK1R antagonist character of the compound evaluated. Regarding the interaction with the receptors, the chimera designated NP44 (H-Tyr-*D*-Ala-Gly-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]) (Nair et al., 2015) effectively meets the requirements concerning the balanced pharmacological profile, at variance to its shorter analogue NP43 (H-Tyr-*D*-Ala-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]). Thus NP44 binds to both receptors with affinities in a similar range (K_i^{MOR} of 49 nM and $K_i^{NK1\ R}$ of 15 nM,

Table 2
Representative structures and receptor affinities of some opioid receptor agonist – neurokinin-1 receptor antagonist hybrid compounds designed and synthesized by the group of Hruby.

		Amino acid sequence	Radioligand binding assay		Ref.
			MOR_{rat} Ki [nM]	$NK1R_{rat}$ Ki [nM]	
TY001		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	180	1.6	(Yamamoto et al., 2007 and Yamamoto, Nair, Vagner, et al., 2008)
TY003		H-Tyr-*D*-Ala-Gly-Phe-Phe-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	28	0.88	(Yamamoto et al., 2007)
TY004		H-Tyr-*D*-Ala-Gly-Phe-Leu-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	23	0.80	(Yamamoto et al., 2007)
TY005		H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	1.8	700	(Yamamoto, Nair, Jacobsen, et al., 2008)
TY006		H-Tyr-*D*-Ala-Gly-Phe-Gly-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	270	1.0	(Yamamoto et al., 2007)
TY007		H-Tyr-*D*-Ala-Gly-Phe-*D*-Phe-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	380	3.0	(Yamamoto et al., 2007)
TY018		H-Tyr-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	9.7	0.6	(Yamamoto et al., 2007)
TY019		H-Tyr-*D*-Ala-Gly-Phe-N-Me-Nle-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	140	0.71	(Yamamoto et al., 2007)
TY020		H-Tyr-*D*-Ala-Gly-Phe-Met-Ala-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	150	1.1	(Yamamoto et al., 2007)
TY021		H-Tyr-*D*-Ala-Gly-Phe-Met-CLeu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	110	7.8	(Nair et al., 2013)
TY022		H-Tyr-*D*-Ala-Gly-Phe-Met-Aib-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	63	9.8	(Nair et al., 2013)
TY023		H-Tyr-*D*-Ala-Gly-Phe-Met-(O)-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	5.5	0.20	(Nair et al., 2013)
TY024		H-Tyr-*D*-Ala-Gly-Phe-Met-*D*-Pro-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	76	3.6	(Nair et al., 2013)
TY025		H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-Bzl	1.8	700	(Yamamoto, Nair, Jacobsen, et al., 2008)
TY027		H-Tyr-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	16	7.3	(Yamamoto, Nair, Ma, et al., 2009)
TY035		H-Tyr-c[*D*-Cys-Gly-Phe-Nle-Pro-Cys]-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	79	30	(Yamamoto, Nair, Ma, et al., 2009)
TY037		H-Tyr-c[*D*-Cys-Gly-Phe-*D*-Cys]-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	52	45	(Yamamoto, Nair, Ma, et al., 2009)
TY038		H-Tyr-c[*D*-Cys-Gly-Phe-Nle-Pro-*D*-Cys]-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	160	7.1	(Yamamoto, Nair, Ma, et al., 2009)
TY039		H-Tyr-c[*D*-Cys-Gly-Phe-Cys]-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	200	560	(Yamamoto, Nair, Ma, et al., 2009)
TY046		H-Tyr-c[*D*-Pen-Gly-Phe-Pen]-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	2300	10	(Yamamoto et al., 2010)
TY047		H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-Pen]-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	2000	160	(Yamamoto et al., 2010)
TY048		H-Tyr-c[*D*-Pen-Gly-Phe-Nle-Pro-*D*-Pen]-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	1000	26	(Yamamoto et al., 2010)
TY049		H-Tyr-c[*D*-Pen-Gly-Phe-*D*-Pen]-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	2100	4.5	(Yamamoto et al., 2010)
TY027 analogs	2	H-Tyr-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	32	6.8	(Yamamoto, Nair, Jacobsen, et al., 2009)
	3	H-Tyr-*D*-Ala-Gly-Phe-Ser(Glc)-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	260	1.5	(Yamamoto, Nair, Jacobsen, et al., 2009)
	4	H-Tyr-*D*-Ala-Gly-Phe-Nle-Ser(Glc)-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	3400	23	(Yamamoto, Nair, Jacobsen, et al., 2009)
	5	H-Tyr-*D*-Ala-Gly-Phe-Nle-Pro-Ser(Glc)-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	8	14	(Yamamoto, Nair, Jacobsen, et al., 2009)
	6	H-Tyr-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Ser(Glc)-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	30	34	(Yamamoto, Nair, Jacobsen, et al., 2009)
No. 3		H-Dmt-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	1.2	13	(Yamamoto et al., 2011)
No. 4		H-Dmt-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NMe-[3′,5′-$(CF_3)_2$-Bzl]	1.8	11	(Yamamoto et al., 2011)
No. 5		H-Dmt-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3'-CF_3-Bzl]	0.74	140	(Yamamoto et al., 2011)
No. 6		H-Dmt-*D*-Ala-Gly-Phe-Nle-Pro-Leu-Trp-NH-[3′,4′-$(OMe)_2$-Bzl]	034	320	(Yamamoto et al., 2011)
No. 7		H-Dmt-*D*-Ala-Gly-Phe-Met-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	2.0	2.3	(Yamamoto et al., 2011)
NP30		H-Tyr-*D*-Ala-Gly-Phe-Gly-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	0.29	4.2	(Nair et al., 2015)
NP32		H-Tyr-*D*-Ala-Gly-Phe-β-Ala-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	11	1.6	(Nair et al., 2015)
NP35		H-Tyr-*D*-Ala-Gly-Phe-Nle-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	33	0.58	(Nair et al., 2015)
NP36		H-Tyr-*D*-Ala-Gly-Phe-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	37	0.15	(Nair et al., 2015)
NP37		H-Tyr-*D*-Ala-Gly-Phe-Met-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	23	0.29	(Nair et al., 2013)
NP38		H-Tyr-*D*-Ala-Gly-Phe-Leu-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	33	0.089	(Nair et al., 2013)
NP43		H-Tyr-*D*-Ala-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	180	2.9	(Nair et al., 2015)
NP44		H-Tyr-*D*-Ala-Gly-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	49	15	(Nair et al., 2015)
NP45		H-Tyr-*D*-Ala-Gly-pFPhe-Gly-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	0.05	0.042	(Nair et al., 2015)
NP46		H-Tyr-*D*-Ala-Gly-pClPhe-Gly-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	0.2	4.9	(Nair et al., 2015)
NP48		H-Dmt-*D*-Ala-Trp-O-[3′,5′-$(CF_3)_2$-Bzl]	0.30	0.28	(Nair et al., 2015)
NP62		H-Tyr-*D*-Ala-Gly-pFPhe-O-[3′,5′-$(CF_3)_2$-Bzl]	3.2	230	(Nair et al., 2015)
NP66		H-Dmt-*D*-Ala-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	1.4	92	(Nair et al., 2015)
2		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-O-Bzl	29	270	(Yamamoto, Nair, Vagner, et al., 2008)
3		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-NH-Bzl	0.65	-	(Yamamoto, Nair, Vagner, et al., 2008)
4		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-NMe-Bzl	4.6	-	(Yamamoto, Nair, Vagner, et al., 2008)
5		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-NH-[3′,5′-$(CF_3)_2$-Bzl]	9.5	33	(Yamamoto, Nair, Vagner, et al., 2008)
6		H-Tyr-*D*-Ala-Gly-Phe-Pro-Leu-Trp-NMe-[3′,5′-$(CF_3)_2$-Bzl]	6.8	6.1	(Yamamoto, Nair, Vagner, et al., 2008)

respectively) while compound NP43 lacking the Gly residue show moderate binding affinity at MOR but a strong affinity at NK1R (K_i^{MOR} of 180 nM and K_i^{NK1R} of 2.9 nM, respectively). The characterization of NP48 and NP66 provides additional insights into the optimized models of hybrid derivatives of SP (Nair et al., 2015). These short peptides contain the same H-Dmt-*D*-Ala-Trp structure, but differ in the coupling to a 3′,5′-$(CF_3)_2$-Bzl moiety through either an ester or an amide bound, respectively. As shown in the Table 1, the amidation of 3′,5′-$(CF_3)_2$-Bzl ester confers a substantial loss in binding affinity to both types of receptors.

The effect of halogen substitution in designed opioid agonist - NK1R antagonist bivalent compound was also determined by the group of Hruby (compounds NP45 and NP46, respectively; Table 2). This replacement was tested at the 4′ position of Phe and was varied. Increasing the size of the halogen substituent from F to Cl resulted in a decreased affinity of the chimeras for MOR (K_i^{MOR} = 0.05 nM vs. K_i^{MOR} = 0.2 nM, respectively). Worth mentioning is the fact that when Phe was substituted with a pF-Phe (compound NP45) the binding affinity for the rat NK1R was also increased (Nair et al., 2015). However, there is no data demonstrating in vivo biological properties of these ligands in terms of antinociception.

Apart from the long list of compounds that have been tested, where most are based on the sequence of biphalin (H-Tyr-*D*-Ala-Gly-Phe-NH-NH-Phe-Gly-*D*-Ala-Tyr-NH_2), Hruby et al. also designed and evaluated a

few functionalized fentanyl derivatives (Vardanyan et al., 2011) with pharmacophores bound either covalently or by ionic bonds. These compounds, however, exhibited low affinities at the rat MOR, thus probably resulting in a rather weak analgesic activity. Such differences in the affinities estimated either for biphalin- or fentanyl-based chimeric molecules are rather obvious and can be explained by the fact that biphalin, which consists in a dimer of tetrapeptide fragments derived from enkephalins linked through a hydrazide linker (Lipkowski, Konecka, and Sroczyńska, 1982), displays higher binding affinities and induces stronger antinociception than fentanyl (Horan et al., 1993). Indeed, biphalin's binding at MOR was reported to reach the K_i value of 2.6 nM (Lipkowski, Konecka, and Sroczyńska, 1982; Yamamoto et al., 2011), while fentanyl is estimated at 5.9 nM (Weltrowska et al., 2010) (determined in vitro bioassays using guinea pig ileum). Furthermore, biphalin also possesses affinity at delta opioid receptors, being a mixed MOR/DOR agonist ($K_i^{DOR} = 1.4$ nM) (Lipkowski, Konecka, and Sroczyńska, 1982; Yamamoto et al., 2011); for fentanyl the K_i was equal 568 nM (Weltrowska et al., 2010). Therefore, hybridizing fentanyl or its analogues with NK1R-related moieties may not necessarily results in compounds with pronounced analgesic activity but in a relatively improved safety profile. Hence, compounds of the fentanyl's family show reduced propensity to induce histamine release.

Using a totally distinct approach, several opioid receptor agonist-NK1R antagonists were described by Ballet and co-workers. Their peptidomimetics, based either on the [Dmt]^1-DALDA (Dmt-*D*-Arg-Phe-Lys-NH_2) peptide or the dermorphin sequence (H-Tyr-*D*-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) as opioid pharmacophore, are chemically conjugated to a variety of NK1R antagonist pharmacophores (e.g., piperidine derivatives). Many of the newly synthetized compounds possess the 4-amino-1,2,4,5-tetrahydro-2-benzazepin-3-one (Aba) scaffold that serves also as part of the NK1R-related fragment. This step was obviously privileged as the Aba moiety was found to preserve the affinity toward MOR (Ballet et al., 2005).

Chimeric compounds combining an opioid agonist and NK1R antagonist appeared to be effective not only in attenuating pain sensation but were also found to provide benefits in the context of opioid-withdrawn as initially observed in animal models. In 2012, Tumati et al. reported that TY027, an opioid agonist - NK1R antagonist bivalent compound, was able to normalize the spinal level of the pro-inflammatory mediator (TNFα) that is found elevated during opioid withdrawal (Tumati et al., 2012). Additionally, this novel chimera possesses several other advantages in terms of opioid-induced side effects. Thus, TY027 neither affected gastrointestinal motility nor induced retching/vomiting episodes compared with morphine. Furthermore, given systemically it exerts strong antihyperalgesic and anti-allodynic effects in rats with sciatic nerve ligation, thus being potentially useful in neuropathic pain (Tumati et al., 2012).

4.3. Opioid-substance P-based chimeric ligands in opioid tolerance prevention

The risk of tolerance to opioid pain medication, that manifested by the need to administer increasing amounts of the substance to achieve the desired effect, remains the main problem faced by patients requiring such a treatment for prolonged period of time. Several studies have shown that prolonged administration of opioid agonists results in the adaptation of diverse neuropeptide systems that in turn could participate in the development of both opioid tolerance and opioid mediated hyperalgesia. Hence, the inhibitory effects of SP on morphine tolerance development and withdrawal symptoms are strongly emphasized in the literature (Maszczynska, Lipkowski, Carr, & Kream, 1998; Sharpe & Jaffe, 1989). While raising the opportunity to combine drugs acting on SP transmission with opioids, this concept has paved the way for exploiting the hybrid approach in order to reduce opioid dosages and opioid associated side effects.

It was initially suggested that the use of a NK1R agonist as a C-terminal pharmacophore would preserve numerous SP releasing self-regulating mechanisms (i.e. activation of peptidases, impact on NMDA pathway) (Colin, Blondeau, & Baude, 2002; Lipkowski, Osipiak, Członkowski, and Gumułka, 1982). Hence, the advantage of combining NK1R and MOR agonisms in order to limit the development of tolerance was best exemplified by the previously introduced chimeric compounds ESP6 and ESP7 (see the Section 4.1). Their strong and long lasting antinociceptive properties was shown to partly rely on such mechanism since the benefit was lost upon co-administration with the selective NK1R antagonist RP67580, highlighting the importance of the SP moiety in preventing tolerance (Foran et al., 2000a and 2000b). Another hybrid peptide that appeared devoid of tolerance induction after subchronic i.c.v. administration is AA3052 presented by Kowalczyk et al. (2016). It is however worth to propose that the inability to trigger opioid-related actions/side effects might result from the weak interactions of this chimera with MOR (Kowalczyk et al., 2016).

As an alternative to the use of NK1R agonists, the use of SP antagonists has also been considered but only few chimeric molecules were reported with the desired pharmacological profile when considering the prevention of tolerance development. Blocking the NK1R is expected to promote the release of SP as compensatory mechanisms that additionally determine the excitatory amino acid release (such as glutamate). Hence, via activation of NMDA receptors, excitatory amino acids have been involved in some opioid-mediated processes, in particular tolerance and dependence. Thus, several behavioural studies have shown that NMDA receptor antagonists, not agonists, attenuate the development of opiate tolerance (Mao, Price, Caruso, & Mayer, 1996). In the small series of compounds targeting opioid receptors while blocking the NK1R, the chimeric drug TY005 (Table 2) developed by Hruby et al. (Largent-Milnes et al., 2010) is probably the best example. Thus, repeated i.t. administration of TY005 did not result in the development of antinociceptive tolerance or sedation in rats with spinal nerve ligation. Besides, the above mentioned compound AA501 which combines NK1R antagonism and MOR agonism was shown to support potent analgesia in several pain models after i.t. injection, but nevertheless with the slow development of tolerance (Maszczynska Bonney et al., 2004). It is however noteworthy that when AA501 was co-administered with SP, in order to neutralize the blockade of NK1R, the tolerance was more important, reaching similar level as upon use of pure opioids.

5. The future of opioid receptor agonists – NK1R receptor antagonist hybrids

Based on decades of research on the implication of SP in diverse physiopathological conditions, therapeutic niches for pure NK1R antagonists have been identified and validated in preclinical studies. Some compounds were even reported to have beneficial outcomes in human experiments (e.g., spantide in anti-cancer therapy). However, their evaluation in clinical trials, particularly in the treatment of pain, so far systematically failed for reasons that remain to be clarified (Herbert & Holzer, 2002; Hill, 2000). Interspecies differences, observable mainly in terms of receptor affinities and plasma stability, were repeatedly reported. This is well exemplified for GR203040, CP-96345, and even CP-99,994 for which significant differences in binding affinities were noticed between human and rat NK1R (Beattie et al., 1995; Gitter et al., 1991; McLean et al., 1993). Similar interspecies differences were obtained for short NK1R-ligands, particularly the phenylalanine-based carbamate derivative N-α-carbobenzoxy-L-phenylalaninamide (*Z*-Phe-NH_2) which appeared extremely resistant to enzymatic degradation in humans, whereas its plasma instability was observed after administration into rats (Fransson et al., 2014).

Such differences may not solely result from differences the amino acid sequence of the NK1R proteins, but from diverse mode of interaction between NK1R ligands and the receptor, thus inducing distinct pharmacological effect. For instance, Maggi and co-workers (Maggi

et al., 1993) suggested that the residues of the NK1R involved in the binding of peptide agonists are mostly non-overlapping with those interacting with nonpeptide antagonists. Along with this it is also suggested that either key amino acid changes or different lipid environments within the transmembrane binding region of the receptor could account for the species NK1R discrepancy. Worth mentioning is that the reported species-related heterogeneity of NK1R is evidenced both with SP itself or other natural tachykinin agonists (Fong et al., 1992) as well as for some selective synthetic agonists at NK1R. At variance, Tousignant et al. reported that neither [Sar^{9},$Met(O_2)^{11}$]SP nor [beta Ala^{8}]NKA(4–10) discriminate between NK1R from different species (Tousignant, Chretien, Guillemette, & Regoli, 1992).

Nevertheless, the existence of interspecies differences, even subtle ones, may obviously explain how molecules encompassing both opioid agonist and NK1R antagonist activities that elicit desirable analgesia in preclinical studies, may not possess such properties in humans. In the combinatory approach focusing on hybrid compounds for the pharmacological management of pain, preserving the affinity for the two targets constitutes a major challenge and extrapolating animal data to the clinic settings may turn into a complete failure. With preclinical tests and optimization conducted exclusively on rodents that respond differently to one of the two pharmacophores, subsequent clinical studies may lead to unexpected outcome. Hence, with some hybrid compounds, the SP-derived pharmacophore has even been shown to bring pronociceptive activities.

Of particular interest is the observation that the chimeric approach may result in bivalent compounds that do not behave similarly as their single pharmacophores administered alone or even when tested in combination (drug mixture) (Kleczkowska et al., 2016). Many studies have thus illustrated that the peptide hybridization may lead to completely new and unpredictable activities of the final compound. A good example of aforementioned is the recent study conducted with a hybrid compound combining the neuropeptide neurotensin and an opioid moiety (termed PK20). When used as pure chemical entities, agonist analogues at the NTS1 neurotensin receptor are known to cause significant amplification of NMDA receptor signalling (Antonelli et al., 2004; St-Gelais, Jomphe, & Trudeau, 2006), thus, acting as potent pro-neurodegenerative agents since NMDA-overactivation leads to glutamate-induced excitotoxicity. However, the coupling of such an NTS1 receptor agonist fragment with an opioid moiety was shown to generate novel compounds causing opposite effect on glutamate transmission (Kleczkowska et al., 2015). Likewise, with respect to the development of SP-derived ligands endowed with analgesic properties, one could also anticipate that hybrid compounds would also show unpredicted activity with potentially positive outcomes.

Despite the abundant literature showing that NK1R antagonists were not able to produce the desired efficacy in clinical trials focusing on pain, there is still hope that the final picture would turn out differently when such NK1R ligands are combined into hybrid compounds with dual activity. Thus, the anti-pain and antiemetic activities of NK1R antagonists may be used in combination with opioids in chemotherapy patients experiencing cancer pain, but also vomiting as a result of their primary antitumor treatment (dos Santos, Souza, Brunetto, Sasse, & da Silveira Nogueira Lima, 2012; Hargreaves et al., 2011). Also, at variance with acute pain contexts, hybrid compounds where pharmacophores are strictly related to NK1R and opioid receptors may turn into efficient drugs for chronic pain conditions including fibromyalgia, a syndrome in which elevated levels of SP are found in the cerebrospinal fluid (Russell, 2002). In this respect, it must be emphasized that the design of the clinical trials is essential with respect to the objective to tackle chronic pain. In fact, most of the clinical studies with NK1R antagonists have so far been performed in acute pain conditions (Urban & Fox, 2000). Consistently, a large majority of preclinical data indicate their usefulness as analgesics in pain associated with inflammation or nerve injuries, conditions in which NK1R is overexpressed (Muñoz & Coveñas, 2013).

6. Conclusion

Neurokinin receptor ligands have been characterized with positive outcomes in several animal models of pain before being considered for testing in the clinic. Nevertheless, the results obtained from human studies systematically demonstrated their lack of effectiveness, reducing the general interest for this class of drugs. Unfortunately, this assumption remains largely based on studies concerning acute pain conditions. When more specifically considering chronic pain conditions (e.g. rheumatoid arthritis) or pain with psychogenic origin, SP-related ligands however remain promising pharmacological tools for future development mainly because of their additional role as modifying factor in pain-associated diseases. This could turn particularly true for hybrid compounds comprising these NK1R ligands. Therefore, the lack of effectiveness commonly reported in the literature should not totally exclude the possibility to develop and exploit preclinically potent SP-related hybrid compounds for their analgesic properties. Further studies conducted with appropriate models of pain remain essential to validate this concept and have it translated into the clinic.

Conflict of interest statement

The authors declare no conflict of interest.

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This paper is dedicated to the memory of Professor Andrzej W. Lipkowski (1946–2014).

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