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Review Article

Nociceptin receptor agonists

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CONTENTS

Introduction

Opioid receptors consist of a family of G-protein-coupled receptors termed μ, δ, κ and the more recently discovered nociceptin/orphanin FQ receptor, also termed orphan opioid receptor-like (ORL1) in the case of the human homologue (1). With the more recent nomenclature of the opioid receptor family recommended by IUPHAR, these receptors are now termed OP_1 (δ), OP_2 (κ), OP_3 (μ) and OP_4 (nociceptin receptor or ORL1) (2). This nomenclature will be used henceforth. Nociceptin has been identified as a naturally occurring agonist of OP_4 and is a heptadecapeptide structurally similar to dynorphin A, but lacking the N-terminal tyrosine essential for activation of traditional (OP_1-OP_3) opioid receptors (3, 4). At the cellular level, OP_4 activation has been associated with decreases in cAMP formation, closing of voltage-gated calcium channels and opening of inwardly rectifying potassium channels (5, 6). This is a classic feature of a G-protein-coupled receptor of the inhibitory autoreceptor type which when activated, in general terms, leads to decreased synaptic transmission. The OP_4 is localized at high levels in cortical and limbic areas including the cerebral cortex, thalamus, amygdala, hypothalamus and hippocampus (6). Clear expression of OP_4 has also been observed in brain stem and spinal cord in areas (*e.g.*, periaqueductal gray, raphe nuclei, superficial layer of the dorsal horn) associated with sensory and pain transmission (6).

Nociceptin has been frequently used to study the physiological roles of OP_4 and it is now clear that this receptor is implicated in a variety of functions in the periphery (*e.g.*, vascular contraction, water retention, pain perception) and in the brain (*e.g.*, memory and learning, emotions, pain sensation, control of appetite). These roles will be discussed in more detail with respect to the possible therapeutic implications of OP_4 agonism. Despite the fact that much information regarding the physiological roles of OP_4 has been obtained using i.v. injection or local central infusion of nociceptin, the recent availability of potent and selective nonpeptide OP_4 agonists is likely to greatly facilitate the understanding of the functioning of this receptor and its therapeutic relevance.

Development of nonpeptide OP_4 agonists

Three strategies have been applied to develop nonpeptidergic agonists for OP_4. One approach was to develop nonpeptide mimetics based on a pharmacophore model generated via critical motifs in nociceptin or in shorter high-affinity hexapeptides such as ac-RYYRWK-NH_2 (7). This has, so far, not proven successful. A second strategy involved traditional high-throughput screening using membranes prepared from cells expressing OP_4 and either ligand binding (using [^{125}I]-nociceptin) or functional [^{35}S]GTP-γ-S binding assays. The group at F. Hoffmann-LaRoche identified a triazaspiro[4.5]decan-4-one analogue (Fig. 1) based on such a high-throughput screening effort using receptor binding to OP_4 (8). This hit was subjected to chemical modification leading to a series of OP_4 agonists (8-11) of which most data is available on the lead structure, [(1*S*,3a*S*)-8-(2,3,3a,4,5,6-hexahydro-1*H*-phenalen-1-yl)-1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one] (Ro 64-6198) (Fig. 2). This compound is a high-affinity (K_i = 0.4 nM) full agonist of OP_4 (with an efficacy similar to nociceptin) with over 100-fold less affinity for related receptors such as OP_3 (12). Ro 64-6198 has poor (4%) oral bioavailability but crosses the blood-brain barrier (12). The *in vivo* effects of this compound, particularly in anxiety models, is described below.

The third approach to develop nonpeptide OP_4 agonists was to use pan opioid agonists as leads to improve the selectivity for OP_4 (13). In general, agonists at opioid receptors such as morphine or fentanyl show negligible affinity for OP_4. However, a few nonpeptide opioid agonists have been reported to have affinity for OP_4 as well, including lofentanil (K_i = 24 nM), etorphine (K_i = 530 nM)

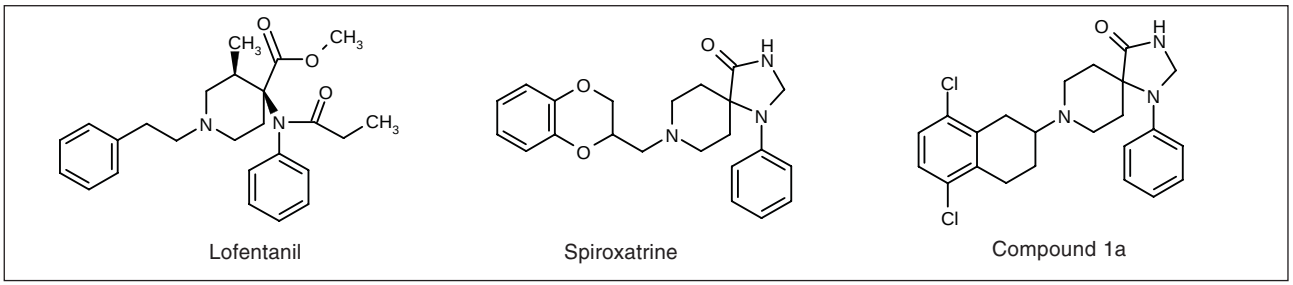

Fig. 1. Chemical structures of leads for developing nonpeptide OP_4 agonists: lofentanil, spiroxatrine and the high-throughput screening hit Compound 1a: 8-(5,8-dichloro-1,2,3,4-tetrahydronaphtalen-2-yl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one.

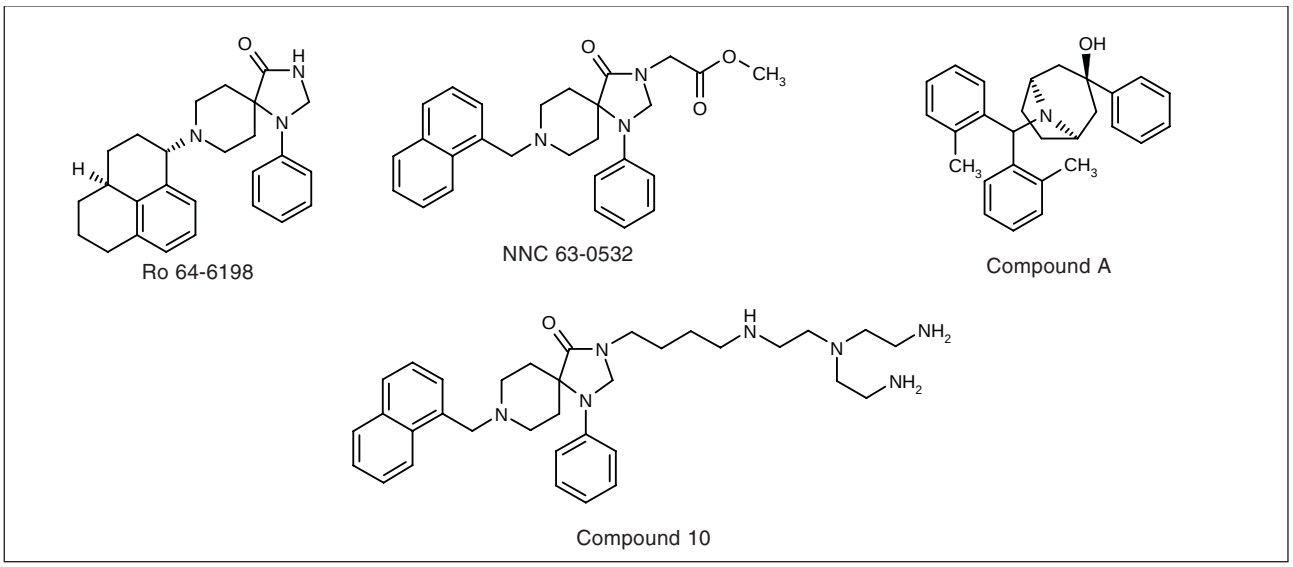

Fig. 2. Chemical structures of selected nonpeptide OP_4 agonists: Ro 64-6198, NNC 63-0532, Compound A (8-[bis(2-methylphenyl)methyl]-3-phenyl-9-azabicyclo[3.2.1]octan-3-ol) and Compound 10 (3-[4-[2-[bis-(2-aminoethyl)amino]ethylamino]butyl])-8-(naphtalen-1-ylmethyl)-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one).

(13) and buprenorphine (EC_{50} = 8.4 nM) (14). All of these compounds display a quite unfavorable selectivity ratio for OP_4 over other opioid receptors such as OP_3 (their respective K_i values for the OP_3 are 0.023 nM, 0.18 nM and 0.51 nM) (15, 16). Nevertheless, these compounds served as templates for generating novel nociceptin agonists with an improved selectivity ratio for OP_4 over OP_3 receptors. It should be noted that the high-throughput screening hit identified at F. Hoffmann-LaRoche (Fig. 1) showed structural similarities to lofentanil (Fig. 1). In parallel, the group at Novo Nordisk performed a 3D-search on lofentanil which predicted that the 5-HT_{1A} agonist spiroxatrine (Fig. 1) should have affinity for OP_4. This was subsequently confirmed in receptor binding experiments to both rat and human OP_4, showing that spiroxatrine had moderate affinity for OP_4 (K_i = 118 nM) in addition to its potent affinity for 5-HT_{1A} (K_i = 4 nM) and opioid receptors (17).

Spiroxatrine was then subjected to chemical modification to afford a series of OP_4 agonists (18) of which most information is available on the lead, 2-[8-(naphthalen-1-ylmethyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl])-acetic acid methyl ester (NNC 63-0532) (Fig. 2) (17). Using solid phase synthesis, a number of chemical modifications of spiroxatrine were prepared keeping the triazaspiro[4.5]decanone structure constant but varying other substituents (18). A 1-naphtylmethyl group seemed optimal with regards to high nociceptin and low OP_3 affinity (18). NNC 63-0532 is a high-affinity (K_i = 7 nM) full agonist at OP_4 (with an efficacy similar to nociceptin) with about 20-fold selectivity over *e.g.*, OP_3 and dopamine receptors. NNC 63-0532 has about 20% oral bioavailability and readily crosses the blood-brain barrier (17). However, from unpublished pharmacological *in vivo* studies it was clear that NNC 63-0532 was not sufficiently selective for OP_4 over OP_3.

More recently, a number of analogues were published with modifications in the ester position of NNC 63-0532 (19). These compounds contained carbon chains with basic amino moieties of variable size, which for the most promising compounds resulted in a large improvement both in the OP_4 affinity and also in the selectivity ratio

over OP_3 (19). Owing to the relative hydrophilic nature of these compounds, they are not immediate candidates for CNS drugs but are more likely to have potential for the peripheral indications relevant for OP_4 agonism (see below). Also on the basis of the triazaspiro[4.5]decan-4-one structure, the group at Schering Plough has subsequently synthesized a number of analogues which are OP_4 agonists, as recently published in the patent literature (20). An example is shown in Figure 2 and this compound has high nM affinity for OP_4, is a full OP_4 agonist in a functional [^{35}S]GTP-γ-S binding assay and has efficacy in a model of cough following oral administration (20) (see further discussion below).

Therapeutic prospects for OP_4 agonists

Inflammatory pain

As evident from the nomenclature of the peptide, the earliest indication for nociceptin was its role in the central mechanisms of pain perception (3, 4). However, in subsequent studies using different routes of administration the role of nociceptin in pain became more controversial. In summary, most studies have demonstrated hyperalgesia following central (supraspinal) administration while analgesia has been observed following intrathecal and peripheral administration of higher doses of nociceptin. Accordingly, when nociceptin is administered at the spinal level a marked analgesic effect has been found in several studies (6, 21, 22) which is independent of activation of classic opioid receptors and involves inhibition of the release of inflammatory substances (23). In addition to acute analgesic effects, it has been shown that intrathecal nociceptin alleviates hyperalgesic and allodynic responses in rats after inflammation or partial peripheral nerve injury (22). Electrophysiological studies have also indicated that the antinociceptive potency of spinal nociceptin is maintained or even enhanced after nerve injury (21, 22). Interestingly, nociceptin is about 10-fold more potent as an analgesic in diabetic mice (following intrathecal administration and tested in the tail flick test) as compared to experimental control mice (24). This effect is dependent upon activation of capsaicin-sensitive c-fibers and may involve regulation of substance P-mediated pain transmission (24). Thus, in the spinal cord the predominant action of nociceptin appears to be inhibitory. Despite these encouraging studies it should be noted that lower doses of nociceptin also produced hyperalgesia following intrathecal and peripheral application in some studies (6, 22, 25, 26). Proinflammatory effects of nociceptin have been observed following local intradermal application, which involved mast cell-mediated histamine release (27). Thus, the effects of nociceptin on inflammation are biphasic (26).

Given these opposing effects of OP_4 activation on inflammatory pain, overall this indication does not seem attractive for OP_4 agonists. Adding to the scepticism for using OP_4 agonists for the indication of pain is that the nonpeptide OP_4 agonist Ro 64-6198 has been reported to be inactive in the tail flick assay in rats and failed to have effect in a test for inflammatory pain (12).

Anorexia/wasting

The dense localization of OP_4 in the hypothalamus may suggest that the receptor is involved in the regulation of food intake. Indeed, nociceptin was shown to stimulate acute feeding behavior in rats (28) which may suggest that OP_4 agonists could be useful for treating anorexia and/or progressive wasting which is common in many types of cancer. In addition, nociceptin (infused i.c.v.) has been shown to block stress- and CRF-induced anorexia in rats but food consumption in food-deprived rats was not affected (29). Furthermore, in normal rats no changes in body weight have been observed with rats treated with the OP_4 agonist Ro 64-6198 for 15 days (30). However, no detailed studies on food consumption or preference have been reported with this compound. On the negative side is that centrally acting OP_4 agonists used in chronic treatment for other indications may induce weight gain as a side effect in nonanorexic patients but this remains to be clarified in clinical trials.

Substance dependence

While nociceptin and the nonpeptide OP_4 agonist Ro 64-6198 show no reinforcing properties *per se* (12), they have been shown to reduce the rewarding properties of morphine and alcohol (31). Furthermore, nociceptin has been shown to reduce ethanol consumption in alcohol-preferring rats and to inhibit stress-induced alcohol-seeking behavior (31). Thus, in this paradigm nociceptin functions as an antiopioid agent similar to observations with central blockade of morphine-induced analgesia (32). However, the development of tolerance to the analgesic effects of morphine was attenuated in OP_4 knockout mice (33) and these mice showed reduced physical signs of morphine dependence (34). On the other hand, mice genetically deficient in the nociceptin peptide showed increased physical signs of morphine dependence but no changes in tolerance to morphine (35).

In conclusion, as for the indication of pain (6, 25), it does not seem consistent from the literature whether an agonist or an antagonist should be used for treating dependence on abuse substances and withdrawal symptoms.

Anxiety

The original cloning of the OP_4 gene and the analysis of its pattern of distribution led to the suggestion that the receptor was involved in learning/memory and emotional behavior (1). This was very convincingly demonstrated by the group at Hoffman LaRoche, who showed that

nociceptin was anxiolytic in several rodent models and this effect was resistant to the opioid antagonist, naloxone (36). Subsequently, the nonpeptide agonist Ro 64-6198 was shown to possess anxiolytic effects at doses between 1-3 mg/kg i.p. in rodents when tested in the elevated plus maze, against fear-potentiated startle reflex and in the conditioned conflict test (12). Furthermore, OP_4 knockout mice have increased susceptibility to stress and display less adaptation than littermate control mice (37). Unlike agonists at OP_3, tolerance to the anxiolytic effects of Ro 64-6198 (3 mg/kg/day i.p.) is not produced when the compound is administered for up to 15 days (30).

While these data appeared very promising for the development of OP_4 agonists as novel anxiolytics, a recent publication also by the F. Hoffmann-LaRoche group has shown that in doses similar to the effective doses in anxiety tests severe side effects were noted (38). Accordingly, Ro 64-6198 caused loss of motor coordination and balance (from 0.3 mg/kg i.p.), catalepsy and general immobility at higher doses (1-3 mg/kg i.p.) and at the 10 mg/kg dose severe neurological impairment was observed (38). Because these effects of Ro 64-6198 were not observed in OP_4 knockout mice (38) it seems to be a class effect (*i.e.*, directly related to central OP_4 agonism and not to the particular compound via other receptors, enzymes or through metabolites). It should be noted that no detailed data on the side effect potential of competing nonpeptide OP_4 agonists is available. But obviously, if these effects are indeed general to OP_4 agonism and are translated to similar clinical side effects in humans, the therapeutic potential of centrally acting OP_4 agonists is very limited.

Cough and asthma

In isolated tracheae and bronchi from guinea pigs or rats nociceptin inhibited both the electric field stimulation-induced bronchoconstriction as well as the release of inflammatory mediators, for example, substance P and calcitonin gene-related peptide (CGRP) (39, 40). Central antitussive effects of nociceptin have also been observed in guinea pigs when exposed to capsaicin and this effect was blocked by the selective OP_4 antagonist J113397 but not by the OP_1-OP_3 antagonist naltrexone (41). The group at Schering Plough has prepared a series of nonpeptide OP_4 agonists and claims these compounds specifically as antitussive agents (20). Thus, the potential of OP_4 agonists as a novel therapeutic approach for the treatment of cough is likely to be clarified in clinical trials within the next years.

Vasomotor disturbances

The rationale for suggesting OP_4 agonists for the treatment of vasomotor disturbances is based on two sets of observations, which are actually in opposition to each other. The first set shows that nociceptin causes vasodilation and decreases in blood pressure as well as heart rate in anesthetized or awake rodents (42-44). Whether this is generalized to higher species is, however, unclear at present because opposite effects have been observed in sheep where nociceptin causes vasoconstriction and increases in heart rate (45). Depending on the outcome of OP_4 activation with regard to blood pressure and heart rate in man, such agents may be used to regulate blood pressure in conditions involving hypertension.

The second set of observations is more circumstantial and may involve interaction with CGRP, which is the most potent endogenous vasodilating substance known (46). Nociceptin has been shown to inhibit the release of CGRP (23, 47) and, at least in theory, nociceptin could thereby prevent the local vasodilating effects of CGRP in conditions where the levels of this peptide are elevated. There is evidence from human studies to suggest that CGRP can cause hot flush-type reactions when injected subcutaneously (48) and that a positive correlation between the incidence of menopausal hot flushes and the serum levels of CGRP (49) has been demonstrated. Based on this, OP_4 agonists have been claimed for the indication of menopausal hot flushes (19). Whether this concept is indeed valid remains to be seen in clinical trials, particularly because more recent evidence suggests that nociceptin can cause local vasodilation *per se* possibly via activation of potassium channels (27, 50).

Incontinence and water retention

Nociceptin has been shown to depress the micturition reflex in rats in a naloxone-independent manner (51). Peripherally, nociceptin interacts with capcaicin-sensitive afferent fibers and thereby diminishes the bladder activity (51). This effect is mimicked by nonpeptide nociceptin agonists such as NNC 63-0532. Nociceptin has been shown to inhibit the micturition reflex both at a peripheral and a central site, possibly the pontine micturition center (52). Particularly if OP_4 agonists block the micturition reflex both at central and peripheral sites, such agents may show promise for treating incontinence in man. In rats, nociceptin has also been shown to increase urinary secretion and lower its sodium content when administered both peripherally and centrally (53, 54). These data suggest that OP_4 agonists could be useful as novel diuretics for treating water-retaining diseases, for which there is still a clear unmet need for more efficacious drugs.

Conclusions

From the cloning of OP_4 and the identification of its endogenous ligand, the field has now taken another significant step forward with the development of selective nonpeptide OP_4 agonists and antagonists. These OP_4 agonists offer either sufficiently long half-life in plasma to verify their preclinical potential for OP_4's peripheral

indications and, for some analogues, have the capability to cross the blood-brain barrier which allows for exploring the central indications. Centrally acting OP_4 agonists are still of potential interest as novel anxiolytics or beneficial for cancer patients (*e.g.*, attenuation of tolerance to the analgesic effects of morphine as well as stimulating appetite) if the neurological impairment associated with OP_4 agonism in rodents is not seen in higher species and if the overall safety profile is acceptable. Peripherally acting OP_4 agonists are obviously devoid of neurological side effects but with such agents the impact on the cardiovascular system should be carefully evaluated. Nevertheless, the peripheral effects of OP_4 agonists may offer new treatments for persistent cough and/or asthmatic conditions. Furthermore, if peripherally acting OP_4 agonists devoid of any significant side effect potential are developed, these agents may provide symptomatic relief for the undesired manifestations of the postmenopausal state in women (*e.g.*, hot flushes and/or incontinence).

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