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Bifunctional peptide-based opioid agonist/nociceptin antagonist ligand for dual treatment of nociceptive and neuropathic pain

Camille Lagard[a,b,c], Lucie Chevillard[a,b,c], Karel Guillemyn[d], Patricia Risède[a,b,c], Jean-Louis Laplanche[a,b,c,e], Mariana Spetea[f], Steven Ballet[d], Bruno Mégarbane[a,b,c,g,*]

Abstract

Drugs able to treat both nociceptive and neuropathic pain effectively without major side effects are lacking. We developed a bifunctional peptide-based hybrid (KGNOP1) that structurally combines a mu-opioid receptor agonist (KGOP1) with antinociceptive activity and a weak nociceptin receptor antagonist (KGNOP3) with anti-neuropathic pain activity. We investigated KGNOP1-related behavioral effects after intravenous administration in rats by assessing thermal nociception, cold hyperalgesia in a model of neuropathic pain induced by chronic constriction injury of the sciatic nerve, and plethysmography parameters including inspiratory time (T_I) and minute ventilation (V_M) in comparison to the well-known opioid analgesics, tramadol and morphine. Time-course and dose-dependent effects were investigated for all behavioral parameters to determine the effective doses 50% (ED_{50}). Pain-related effects on cold hyperalgesia were markedly increased by KGNOP1 as compared to KGNOP3 and tramadol (ED_{50}: 0.0004, 0.32, and 12.1 μmol/kg, respectively), whereas effects on thermal nociception were significantly higher with KGNOP1 as compared to morphine (ED_{50}: 0.41 and 14.7 μmol/kg, respectively). KGNOP1 and KGOP1 produced a larger increase in T_I and deleterious decrease in V_M in comparison to morphine and tramadol ($ED_{50}(T_I)$: 0.63, 0.52, 12.2, and 50.9 μmol/kg; $ED_{50}(V_M)$: 0.57, 0.66, 10.6, and 50.0 μmol/kg, respectively). Interestingly, the calculated ratios of anti-neuropathic pain/antinociceptive to respiratory effects revealed that KGNOP1 was safer than tramadol (ED_{50} ratio: 5.44×10^{-3} vs 0.24) and morphine (ED_{50} ratio: 0.72 vs 1.39). We conclude that KGNOP1 is able to treat both experimental neuropathic and nociceptive pain, more efficiently and safely than tramadol and morphine, respectively, and thus should be a candidate for future clinical developments.

Keywords: Opioid-nociceptin hybrid, Nociceptive pain, Neuropathic pain, Morphine, Tramadol, Respiratory depression, ED_{50}, Toxicity

1. Introduction

Nociceptive pain occurs in response to noxious stimuli mediated by a specialized high-threshold sensory system, from the periphery through the spinal cord, brainstem, and thalamus to the cerebral cortex.[54] If intense, treatment is usually based on mu-opioid receptor (MOR) agonists like morphine, which are able to attenuate nociceptive transmission but at high risk of significant adverse reactions, including respiratory depression.[18,41] Neuropathic pain is a spontaneous pain and hypersensitivity to pain that develops after nerve injury, when deleterious changes occur in injured neurons and along nociceptive and descending modulatory pathways in the central nervous system.[9] In contrast to nociceptive pain, neuropathic pain tends to be refractory to conventional analgesics including opioids. Among patients suffering from chronic pain, one-fifth are thought to have predominantly neuropathic pain.[6] Various mechanisms have been reported to explain the development of neuropathic pain,[9,54] but only limited treatments have been shown to be efficient.[10] Tramadol, a MOR agonist and serotonin/norepinephrine reuptake inhibitor, is currently recommended as first-line treatment,[14] although there is risk of opioid-related side effects such as those seen with morphine, in addition to seizures and serotonin syndrome.[3] Interestingly, nociceptin (NOP) receptors, previously called opioid receptor-like-1 (ORL1), modulate nociceptive transmission when activated by its endogenous ligand, nociceptin/orphanin FQ (N/OFQ).[34] Both NOP receptor agonists and antagonists have been reported to reduce neuropathic pain and potentiate morphine effects, thus potentially representing effective drugs for the future.[25,39,54]

Previously, efforts were focused at developing peptidic hybrid compounds combining MOR agonism and NOP receptor antagonism in order to treat both nociceptive and neuropathic pain with reduced opioid side effects.[13,19,51] The targets of these 2 pharmacophores are usually collocated,[40,53] and consequently, such hybrids may serve as a dual treatment for both

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[a] Inserm, UMR-S 1144, Paris, France, [b] Paris-Descartes University, UMR-S 1144, Paris, France, [c] Paris-Diderot University, UMR-S 1144, Paris, France, [d] Research Group of Organic Chemistry, Departments of Chemistry and Bio-engineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium, [e] Assistance Publique—Hôpitaux de Paris, Lariboisière Hospital, Laboratory of Biochemistry and Molecular Biology, Paris, France, [f] Department of Pharmaceutical Chemistry, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innsbruck, Austria, [g] Assistance Publique—Hôpitaux de Paris, Lariboisière Hospital, Department of Medical and Toxicological Critical Care, Paris, France

**Corresponding author. Address: Réanimation Médicale et Toxicologique, Hôpital Lariboisière, 2 Rue Ambroise Paré, 75010 Paris, France. Tel.: +33 1 49 95 89 61; fax: +33 1 49 95 65 78. E-mail address: bruno-megarbane@wanadoo.fr (B. Mégarbane).*

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kinds of pain. The recently developed KGNOP1 combines 2 pharmacophores: a mu-, delta-, and kappa-OR peptide agonist called KGOP1 aimed at acting on nociception and a weak NOP receptor antagonist called KGNOP3 made to develop anti-neuropathic pain activity (**Table 1**).[13] Furthermore, KGNOP1 is able to penetrate the blood–brain barrier and has stability against enzymatic degradation.[13] Potential benefits of co-targeting MOR and NOP receptors were additionally supported by the recent clinical development of cebranopadol as novel opioid analgesic.[26,28,47] In contrast to our newly discovered MOR/NOP hybrid ligand, KGNOP1, showing NOP antagonism, cebranopadol is a full agonist at the NOP receptor. Besides, as agonist of the kappa-OR, cebranopadol may have the capacity to produce psychotomimetic effects and other adverse reactions at sufficiently high doses, a property that could potentially limit its practical clinical dosage range.[42]

Thus, since MOR agonist/NOP receptor antagonist hybrids potentially represent a major advance for pain research, we studied the respective KGNOP1-related behavioral effects in the rat for each type of pain in comparison to the 2 clinically relevant opioid analgesics, morphine for nociceptive pain and tramadol for neuropathic pain. Furthermore, we investigated whether KGNOP1 is capable of developing substantially reduced respiratory toxicity in order to improve the benefit-to-risk ratio of the currently available opioids.

2. Materials and methods

All the experimental protocols used in this study were carried out within the ethical guidelines established by the National Institutes of Health, the French Minister of Agriculture, and the European Union Legislation. Protocols were approved by the local ethics committee for animal experimentation of Paris-Descartes University.

2.1. Animals

Male Sprague-Dawley rats (Janvier Labs, Le Genset-Saint-Isle, France) were used, weighing between 250 and 350 g at the time of the experiment. Animals were housed for 7 days before the experiment in an environment maintained at 21°C ± 0.5°C with controlled humidity and light–dark cycle (lights on between 08:00 and 20:00 hours). Food and tap water were provided ad libitum.

2.2. Chemicals and drugs

Morphine sulfate (molecular weight [MW]: 285.3 g/mol) was purchased from Francopia, Paris, France. Tramadol hydrochloride (MW: 263.4 g/mol) was purchased from Grünenthal Gmbh, Aachen, Germany. KGNOP1 (H-Dmt-D-Arg-Aba-βAla-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2; MW: 1474.8 g/mol), KGOP1 (H-Dmt-D-Arg-Aba-βAla-NH_2; MW: 594.7 g/mol), and KGNOP3 (Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2; MW: 939.1 g/mol) were produced in the Research Group of Organic Chemistry at the Vrije Universiteit Brussels, Belgium, according to previously described procedures.[13] All drugs were diluted in 0.9% NaCl to obtain the different required concentrations.

2.3. Jugular catheterization

One week before the testing, the rat jugular vein was catheterized using 20-cm silastic tubing with external and internal diameters of 0.94 and 0.51 mm, respectively (Dow Corning Co, Midland, MI), and under ketamine (70 mg/kg) and xylazine (10 mg/kg) anesthesia. Catheters were tunneled subcutaneously and fixed at the back of the neck. Heparinized saline was injected into the catheter to avoid thrombosis and catheter obstruction. Rats were then returned to their individual cages for 7 days, allowing complete anesthesia washout and complete recovery. On the day of the experiment, rats were placed in horizontal Plexiglas cylinders (6.5 cm internal diameter, up to 20 cm adjustable length) (Harvard Apparatus, Inc, Holliston, MA), modified by the addition of several holes at the cephalic end to avoid CO_2 rebreathing.[8] Before drug administration, the catheter was exteriorized, purged, and its permeability checked.

2.4. Model of neuropathic pain induced by a chronic constriction injury of the sciatic nerve

We used the chronic constriction injury of the sciatic nerve (SN-CCI) model to induce neuropathic pain in rats.[4] The rat's right sciatic nerve was exposed under anesthesia using ketamine (70 mg/kg intraperitoneally [IP]) and xylazine (10 mg/kg IP). Subsequently, the nerve was ligated proximally to the sciatic trifurcation with four 4-0 chromic gut sutures (Ethicon, Issy-les-Moulineaux, France). Rats were then returned to their individual cages for a recovery period of 14 days, allowing complete anesthesia washout and neuropathic pain development.

2.5. Cold-plate test

The individual SN-CCI rats were kept inside a transparent cylinder on the cooled surface of the plate (4.0°C ± 0.2°C; Panlab, Barcelona, Spain).[15] The total leg lift counts of the right hind paw were counted for 5 minutes. Counts were excluded when the hind paw was lifted while walking. The first measurement was performed after a 10-minute period of accommodation while the animal was quiet. Drugs were intravenously (IV) administered at

Table 1

In vitro properties of the different investigated peptides, KGNOP1 (the hybrid, Dmt-D-Arg-Aba-β-Ala-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2), KGOP1 (the opioid receptor agonist, Dmt-D-Arg-Aba-β-Ala-NH_2), and KGNOP3 (the nociceptin receptor antagonist, Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2).

Compound	Binding affinity (K_i, nM)				Opioid activity (IC_{50}, nM)		Antagonism
	μ-opioid receptor	δ-opioid receptor	κ-opioid receptor	NOP receptor	GPI*	MVD*	pA_2 NOP
KGNOP1	5 ± 1.7	99 ± 4	33 ± 15	42 ± 6	6.1 ± 0.1	5.3 ± 1.4	5.23
KGOP1	1.34 ± 0.05	17 ± 0.3	nd	nd	0.8 ± 0.1	0.24 ± 0.02	nd
KGNOP3	nd	nd	nd	1.5	nd	nd	nd

Values represent mean ± standard error of the mean.
Data were obtained from Guillemyn et al.[13]
* The functional guinea pig ileum assay is representative of mu-opioid receptor activation, whereas the mouse vas deferens assay is a delta-opioid receptor representative assay.
GPI, guinea pig ileum; MVD, mouse vas deferens; nd, not determined, NOP, nociceptin.

T_0. Cold hyperalgesia was recorded at −45, −30, −15, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 420 minutes (study 1A). Dose–effect relationships were investigated at the time corresponding to the observed peak effect (T_{max}) (study 1B). The percentage of decrease in leg lift counts was calculated based on the following equation:

$$\frac{\text{control leg lift counts} - \text{leg lift counts after injection}}{\text{control leg lift counts}} \times 100.$$

2.6. Hot-plate test

The individual rats were kept inside a transparent cylinder on the heated surface of the plate (52.0°C ± 0.2°C; Panlab).[27] Licking, brisk shaking of the hind paw, and jumping were considered as signs of thermal nociception. The cut-off time was set at 60 seconds. The first measurement was performed after a 10-minute period of accommodation while the animal was quiet. Drugs were intravenously (IV) administered at T_0. Thermal nociception was recorded at −30, −15, −5, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, and 420 minutes (study 2A). Dose–effect relationships were investigated at the time corresponding to the observed peak effect (T_{max}) (study 2B). The maximum possible effect (MPE%) was calculated based on the following equation:

$$\text{MPE\%} = \frac{\text{test latency} - \text{control latency}}{\text{cut-off time} - \text{control latency}} \times 100.$$

2.7. Whole-body plethysmography

One week before the testing, temperature transmitters (TA-F10; DSI, Chatillon, France) were implanted in the peritoneal cavity of the rat. Ventilatory parameters were recorded in a whole-body plethysmograph by the barometric method described and validated in the rat.[2] On the day of experimentation, animals were placed in a rectangular Plexiglas (Harvard Apparatus, Inc) chamber with a 3-L volume connected to a reference chamber of the same size by a high-resistance leak to minimize the effect of pressure changes in the experimental room. The animal chamber was flushed continuously with humidified air at a 5 L/min rate. During the recording periods, the inlet and outlet tubes were temporarily clamped and pressure changes associated with each breath were recorded using a differential pressure transducer (45 ± 3 cm·H_2O; Validyne MP, Northridge, CA) connected to the animal and reference chambers. The spirogram was recorded and stored on a computer with an acquisition data card (PCI-DAS 1000; Dipsi, Chatillon, France) using a respiratory acquisition software (Acquis1 Software; CNRS, Gif-sur-Yvette, France) for off-line analysis. This technique was daily validated with a series of leak tests (leak was signaled if diminution of the signal amplitude exceeded 33% in 5 seconds). Before each measurement, the temperature of the enclosure, the temperature of the animal, the volume of calibration, and the atmospheric pressure were noted to permit the calculation of plethysmography parameters. The first measurement was performed after a 30-minute period of accommodation, while the rat was quiet and not in deep or rapid eye movement sleep, as roughly estimated from their behavior, response to noise, and pattern of breathing. Then, the rat was gently removed from the chamber for IP injection at T_0 and replaced in the chamber for the remaining measurements. Ventilation was recorded at −30, −15, −5, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes (each record lasting about 60 seconds, study 3A). Dose–effect relationships were investigated at the time corresponding to the observed peak effect (T_{max}) (study 3B). The following parameters were measured: the tidal volume (V_T), the inspiratory time (T_I), and the expiratory time (T_E). Additional parameters were calculated, including the respiratory frequency (f) and the minute volume ($V_M = V_T \times f$). In the study of dose–effect relationships, we considered for each plethysmography parameter the normalized variable as follows: Parameter (T_{max}) − Parameter (T_0).

2.8. Dose–effect relationships modelling

For each test drug, the relationships between the effects (E) on respiratory, thermal nociception, and cold hyperalgesia as a function of the drug dose (D) were described using the sigmoidal E_{max} model, as follows:

$$E = E_0 + \frac{E_{max} \times D^{\gamma}}{ED_{50}^{\gamma} + D^{\gamma}} \quad (1)$$

where E_0 is the baseline value of the measured parameter, E_{max} represents the maximum effect, ED_{50} represents the median effective dose, ie, the dose of the drug associated with the half-maximum effect, and γ (or Hill coefficient) determines the steepness of the dose vs response curve. The pharmacological model was applied to the investigated drug effects using the maximum likelihood expectation maximization algorithm implemented in WinNonlin (Version 5.1; Pharsight, Princeton, NJ). A standard error variance model was defined as:

$$Var_i = {}^{1}/_{y_i^2} \quad (2)$$

where y_i is the ith predicted value from the pharmacological model. All model parameters were assumed to be log-normally distributed. Model selection was based on goodness-of-fit criteria, which included the convergence criterion (CV%), the Akaike information criterion, the estimation criterion value for the maximum likelihood method, and the visual inspection of predicted vs observed and residual plots. Estimated parameters for dose–effect relationships are expressed as mean (CV%).

2.9. Study design

2.9.1. Study 1: cold hyperalgesia in SN-CCI rats

To investigate the time-course (study 1A), SN-CCI rats were randomized into 4 groups (N = 6 per group) to receive IV 0.9% NaCl (control group), 17.62 μmol/kg tramadol, 0.34 μmol/kg KGNOP1, or 0.34 μmol/kg KGNOP3. Cold hyperalgesia was assessed during 420 minutes after the drug administration. T_{max} was determined for each drug.

To investigate the dose–effect relationships (study 1B), SN-CCI rats were randomized (N = 6 per group) to receive IV tramadol at 4 different doses (4.40, 8.81, 44.04, and 88.08 μmol/kg) or IV KGNOP1 at 5 different doses (6.78×10^{-5}, 6.78×10^{-4}, 6.78×10^{-2}, 0.51, and 0.68 μmol/kg) or IV KGNOP3 at 5 different doses (0.11, 0.22, 1.06, 1.60, and 5.32 μmol/kg). Cold hyperalgesia was performed to assess the ED_{50} at the T_{max} previously observed in study 1A.

2.9.2. Study 2: thermal nociception in rats

To investigate the time-course (study 2A), rats were randomized into 3 groups (N = 6 per group) receiving IV 0.9% NaCl (control group), 17.52 μmol/kg morphine, or 0.34 μmol/kg KGNOP1. Thermal nociception was measured during 420 minutes after the drug administration. T_{max} was determined for each drug.

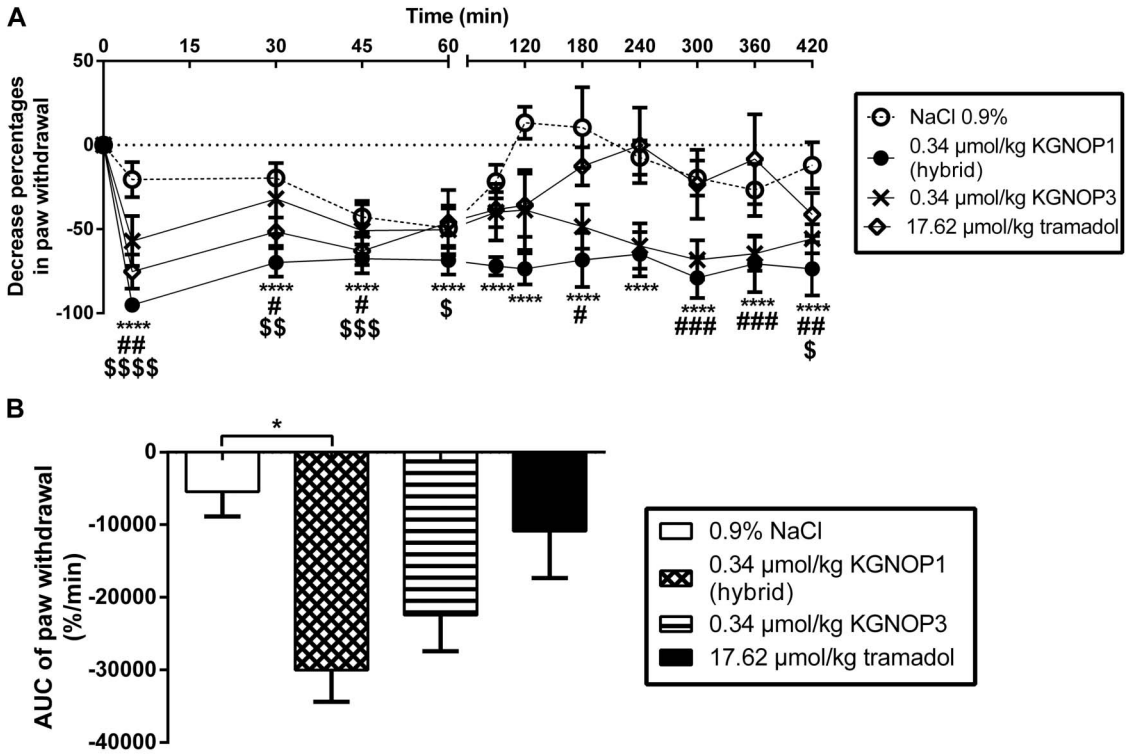

Figure 1. Drug effects on cold hyperalgesia in SN-CCI Sprague-Dawley rats. The effects on cold hyperalgesia of intravenous 0.9% NaCl (control), 0.34 µmol/kg KGNOP1, 0.34 µmol/kg KGNOP3, and 17.62 µmol/kg tramadol using the cold-plate test were tested (N = 6 per group). (A) The percentage of decrease in leg lift counts was measured as a function of time. Results are expressed as mean ± SEM. Comparisons to the baselines were performed using 2-way analysis of variance. ****$P < 0.0001$ for KGNOP; #$P < 0.05$, ##$P < 0.05$, ###$P < 0.001$ for KGNOP3; \$$P < 0.05$, \$\$$P < 0.01$, \$\$\$$P < 0.001$, \$\$\$\$$P < 0.0001$ for tramadol. (B) Areas under these curves (AUC) were represented. Results are expressed as mean ± SEM. Comparisons were performed using Kruskal–Wallis test. *$P < 0.05$. SEM, standard error of the mean; SN-CCI, chronic constriction injury of the sciatic nerve.

To investigate the dose–effect relationships (study 2B), rats were randomized (N = 6 per group) to receive IV morphine at 5 different doses (1.75, 5.26, 8.76, 14.02, and 35.05 µmol/kg) or IV KGNOP1 at 4 different doses (0.07, 0.17, 0.51, and 0.68 µmol/kg). Thermal nociception was performed to assess the ED_{50} at the T_{max} previously observed in study 2A.

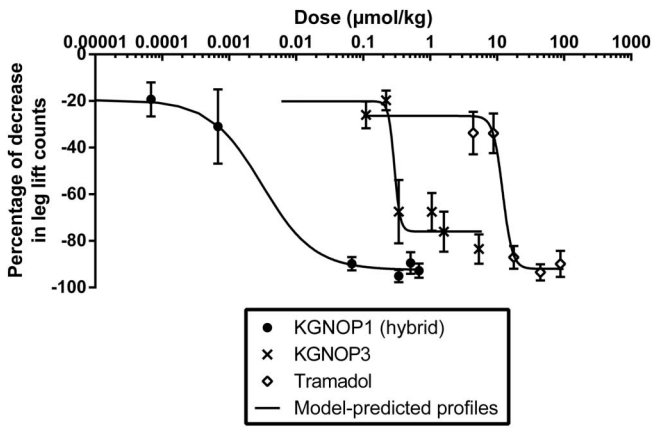

Figure 2. Dose–effect relationships on cold hyperalgesia in SN-CCI Sprague-Dawley rats. The effects on cold hyperalgesia using the cold-plate test as a function of intravenous dose of KGNOP1, KGNOP3, and tramadol were tested (N = 6 per group). The relationships were well-described by the sigmoidal E_{max} model. Solid lines represent the mean model-predicted profiles compared to the mean experimental data ± standard error of the mean. SN-CCI, chronic constriction injury of the sciatic nerve.

2.9.3. Study 3: respiratory effects in rats

To investigate the time-course (study 3A), rats were randomized (N = 6 per group) to receive IV 0.9% NaCl (control group), 17.62 µmol/kg tramadol, 17.52 µmol/kg morphine, 0.34 µmol/kg KGNOP1, or 0.34 µmol/kg KGOP1. The respiratory effects were assessed using plethysmography during 360 minutes after drug administration. T_{max} was determined for each drug.

To investigate the dose–effect relationships (study 3B), rats were randomized (N = 6 per group) to receive IV tramadol (44.04, 66.06, 88.08, and 94.91 µmol/kg), IV morphine (8.76, 13.14, 43.81, 87.61, 131.42, and 175.23 µmol/kg), IV KGNOP1 (0.17, 0.68, 1.02, and 1.19 µmol/kg) or IV KGOP1 (0.67, 1.01, 1.35, and 1.68 µmol/kg). The plethysmography effects were studied to assess the ED_{50} at the T_{max} previously observed in study 3A.

2.9.4. Study 4: benefit-to-respiratory toxicity ratio

Using the ED_{50} values of each drug effects on V_M and cold hyperalgesia, we calculated the benefit-to-respiratory toxicity ratio of KGNOP1 in comparison to tramadol in the SN-CCI rat as follows:

$$\frac{ED_{50}(V_M)}{ED_{50}(\%)}.$$

Using the ED_{50} values of each drug effects on V_M and thermal nociception, we calculated the benefit-to-respiratory toxicity ratio of KGNOP1 in comparison to morphine in the rat as follows:

$$\frac{ED_{50}(V_M)}{ED_{50}(MPE\%)}.$$

Table 2

Modeling of dose–effect relationships.

Studied parameters	Drugs				
	KGNOP1	KGOP1	KGNOP3	Morphine	Tramadol
Cold hyperalgesia					
ED_{50}, μmol/kg	0.0031 (49.7)		0.29 (9)		12.1 (16.6)
E_0, %	−19.48 (10.1)		−20.1 (20.7)		−26.4 (21.6)
E_{max}, %	−92.52 (2)		−76 (5.1)		−92 (5.6)
γ	1.12 (31)		10*		6.1 (45.8)
T_{max}, min	5		5		5
Thermal nociception					
ED_{50}, μmol/kg	0.41 (4.4)			14.7 (2.3)	
E_0, %	0*			0*	
E_{max}, %	100 (4.5)			100 (5.1)	
γ	11.9 (21.3)			15.6 (33.7)	
T_{max}, min	180			30	
Inspiratory time					
ED_{50}, μmol/kg	0.63 (13.6)	0.52 (20.2)		12.2 (5.7)	50.9 (43.9)
E_0, s	0.06 (13.1)	0.029 (9.7)		0.006 (11.5)	0.006 (14.7)
E_{max}, s	0.16 (16.1)	0.15 (15.1)		0.16 (5.9)	0.17 (34.6)
γ	4.3 (43.2)	2.4 (32.7)		4.4 (16.7)	2 (27.5)
T_{max}, min	180	240		30	30
Minute volume					
ED_{50}, μmol/kg	0.57 (29.6)	0.66 (4.01)		10.6 (15)	50 (15.1)
E_0, mL/s	20,268.5 (76.7)	16,517.9 (75.1)		27,449.3 (41.7)	13,350 (24.8)
E_{max}, mL/s	−126,733.3 (29.8)	−88,561.3 (21.1)		−74,007.6 (16.4)	−42,717.4 (27.4)
γ	2.9 (68.4)	2.2*		10*	3*
T_{max}, min	180	240		30	30

Parameters of the sigmoidal E_{max} models representing the dose–effect relationships, including ED_{50}, E_0, E_{max}, and γ, are presented. Measurements were carried out at the observed T_{max} determined in a preliminary experiment for each studied effect.

Data are expressed as mean (CV%).

* Fixed value.

E_0, baseline effect; E_{max}, maximum effect; ED_{50}, median effective dose; T_{max}, time since drug administration; γ, Hill coefficient.

2.10. Data analysis

Results are expressed as mean ± standard error of the mean. For cold hyperalgesia, thermal nociception, and plethysmography parameters, T_0 was the mean of the 3 baseline measurements. Regarding the time-course of each pharmacological effect, comparisons were performed using 2-way analysis of variance followed by multiple comparison tests using Sidak correction (studies 1A, 2A, and 3A). To permit the simultaneous analysis of the effects of time and treatment on the rat behavioral parameters, we calculated for each animal and for each studied parameter, the area under the curve (AUC) from T_0 to the completion of the measurement using the trapezoid method. For each parameter, we compared the AUCs using Kruskal–Wallis tests (studies 1A, 2A, and 3A). All tests were analyzed using Prism version 6.0 (GraphPad Software, Inc, San Diego, CA), and *P*-values of less than 0.05 were considered as significant.

3. Results

3.1. Cold hyperalgesia in SN-CCI rats

Intravenous administration of KGNOP1, KGNOP3, and tramadol to SN-CCI rats significantly decreased the percentage of leg lift counts in comparison to the baseline at T_0, peaking at 5 minutes after drug injection ($P < 0.0001$, $P < 0.01$, and $P < 0.0001$, respectively). The effects of KGNOP1 and KGNOP3 persisted up to 420 minutes ($P < 0.0001$ and $P < 0.01$, respectively), whereas tramadol-related effects declined after 60 minutes ($P < 0.05$). Using the AUC method, the percentage of leg lift counts was significantly decreased in KGNOP1-treated rats compared to the controls ($P < 0.05$; **Fig. 1**). Using the sigmoidal model, the ED_{50} values of KGNOP1-, KGNOP3-, and tramadol-induced effects on cold hyperalgesia were 0.0004, 0.32, and 12.1 μmol/kg, respectively (**Fig. 2** and **Table 2**).

3.2. Thermal nociception in rats

Morphine significantly increased the MPE% in rats during the first hour after IV injection in comparison to the baseline at T_0, peaking at 30 minutes ($P < 0.0001$). KGNOP1-related effects were delayed at 180 minutes ($P < 0.01$). Using the AUC method, the MPE% was significantly increased in morphine-treated rats compared to the controls ($P < 0.05$), whereas KGNOP1-related effects on thermal nociception did not significantly differ from those of morphine (**Fig. 3**). Using a sigmoidal model, the ED_{50} values of KGNOP1- and morphine-induced effects on thermal nociception were 0.41 and 14.7 μmol/kg, respectively (**Fig. 4** and **Table 2**).

3.3. Respiratory effects in rats

Significant increase in T_I started at 5 minutes after IV morphine injection ($P < 0.0001$), whereas a delay of 120 minutes was observed after IV KGNOP1 injection ($P < 0.05$) and a delay of 180 minutes after IV KGOP1 and tramadol injection ($P < 0.05$) (**Fig. 5**). Effects on T_I persisted up to 360 minutes for the 4 drugs ($P < 0.001$). KGNOP1 significantly decreased T_E from 5 minutes ($P < 0.05$) to 240 minutes after the injection ($P < 0.01$). KGOP1 significantly decreased T_E from 15 minutes ($P < 0.05$) to 120 minutes after the injection ($P < 0.05$). Morphine and tramadol did

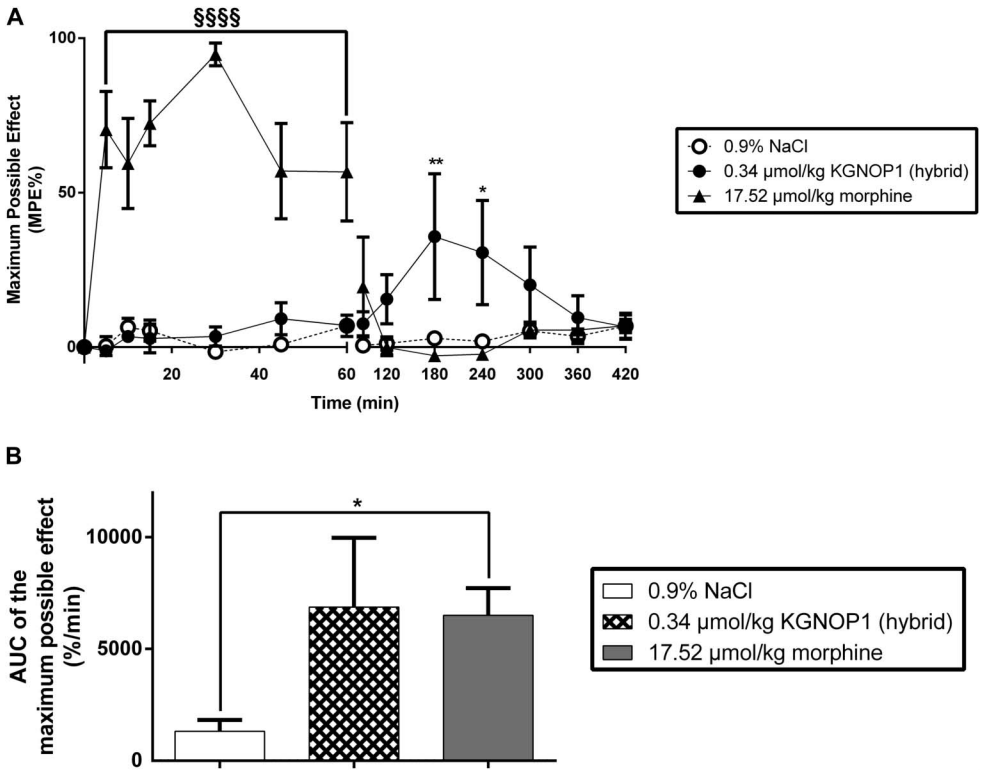

Figure 3. Drug effects on thermal nociception in Sprague-Dawley rats. The effects on thermal nociception of intravenous 0.9% NaCl (control), 0.34 μmol/kg KGNOP1, and 17.52 μmol/kg morphine using the hot-plate test were tested (N = 6 per group). (A) The maximum possible effect (MPE%) was measured as a function of time. Results are expressed as mean ± SEM. Comparisons to the baselines were performed using 2-way analysis of variance. *$P < 0.05$, **$P<0.01$ for KGNOP1, §§§§$P < 0.0001$ for morphine. (B) Areas under these curves (AUC) were represented. Results are expressed as mean ± SEM. Comparisons were performed using Kruskal–Wallis test. *$P < 0.05$. SEM, standard error of the mean.

not exhibit significant effects on T_E in comparison to baseline at T_0. Regarding V_T, only morphine exhibited significant effects, with an observed reduction at 5, 90, 120, 180, 240, 300, and 360 minutes after the injection ($P < 0.01$). Morphine decreased V_M at 30 minutes after the injection ($P < 0.05$), whereas KGNOP1 increased V_M from 5 to 45 minutes ($P < 0.01$) and KGOP1 increased V_M at 15 minutes after the injection ($P < 0.05$). Peaks of respiratory effects were observed at 30 minutes with morphine and tramadol, at 180 minutes with KGNOP1, and at 240 minutes with KGOP1.

Using the AUC method, T_I was significantly increased in morphine-treated rats in comparison to controls ($P < 0.05$) but not in rats receiving KGNOP1, KGOP1, or tramadol (**Fig. 6**). T_E was not significantly altered by the different treatments in comparison to the control group; however, T_E was significantly decreased in KGNOP1-treated rats in comparison to morphine- and tramadol-treated rats ($P < 0.05$ and $P < 0.01$, respectively). Similarly, V_T was not significantly altered in comparison to the controls; however, V_T was significantly decreased in KGNOP1-treated rats in comparison

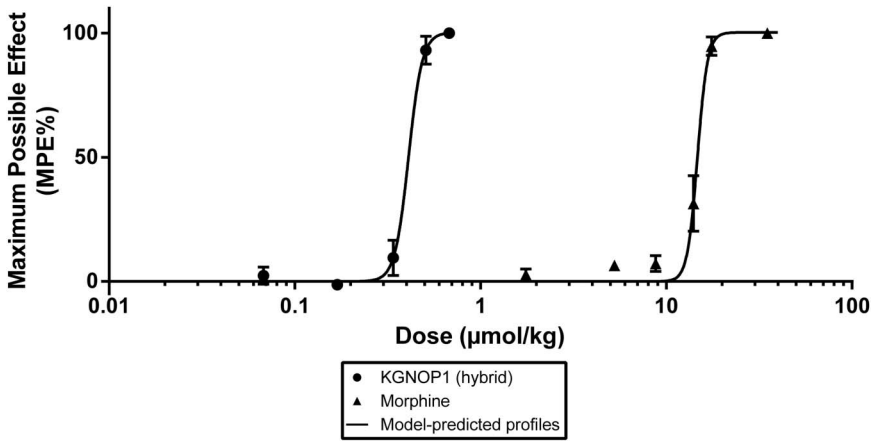

Figure 4. Dose–effect relationships on thermal nociception in Sprague-Dawley rats. The effects on thermal nociception using the hot-plate test as a function of the intravenous dose of KGNOP1 and morphine were tested (N = 6 per group). The relationships were well-described by the sigmoidal E_{max} model. Solid lines represent the mean model-predicted profiles compared to the mean experimental data ± standard error of the mean.

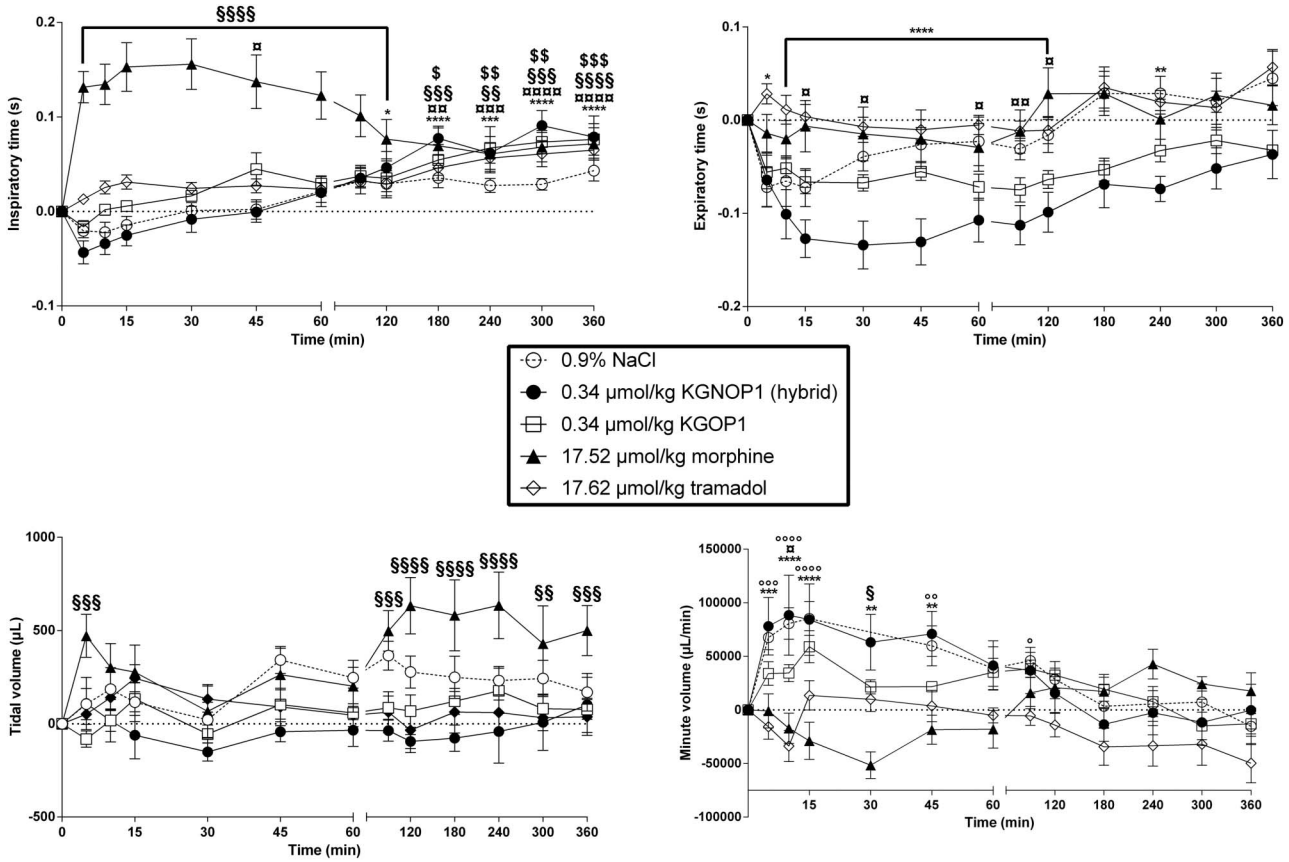

Figure 5. Drug effects on plethysmography parameters in Sprague-Dawley rats. The effects on plethysmography parameters, including inspiratory time, expiratory time, tidal volume, and minute volume, of intravenous 0.9% NaCl (control), 0.34 µmol/kg KGNOP1, 0.34 µmol/kg KGOP1, 17.52 µmol/kg morphine, and 17.62 µmol/kg tramadol were tested (N = 6 per group). The plethysmography parameters were measured as a function of time. Results are expressed as mean ± standard error of the mean. Comparisons to the baselines were performed using 2-way analysis of variance. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ for KGNOP1; ¤$P < 0.05$, ¤¤$P < 0.05$, ¤¤¤$P < 0.001$, ¤¤¤¤$P < 0.0001$ for KGOP1; §$P < 0.01$, §§$P < 0.01$, §§§$P < 0.001$, §§§§$P < 0.0001$ for morphine; \$$P < 0.05$, \$\$$P < 0.01$, \$\$\$$P < 0.001$ for tramadol; °$P < 0.05$, °°$P < 0.01$, °°°$P < 0.001$, °°°°$P < 0.0001$ for control group.

to morphine-treated rats ($P < 0.05$). Consequently, only V_M was significantly decreased in tramadol-treated rats compared to controls ($P < 0.05$).

Using the sigmoidal model, the ED_{50} values of KGNOP1-, KGOP1-, morphine-, and tramadol-induced effects on T_I were 0.63, 0.52, 12.2, and 50.9 µmol/kg, respectively (**Fig. 7** and **Table 2**). The ED_{50} values of KGNOP1-, KGOP1-, morphine-, and tramadol-induced effects on V_M were 0.57, 0.66, 10.6, and 50.0 µmol/kg, respectively.

3.4. Benefit-to-respiratory toxicity ratios

Concerning the neuropathic pain treatment, the calculated benefit-to-respiratory toxicity ratio was 7.02×10^{-4} for KGNOP1 in comparison to 0.24 for tramadol. Regarding nociceptive pain treatment, benefit-to-respiratory toxicity ratio was 0.72 for KGNOP1 in comparison to 1.39 for morphine.

4. Discussion

In the present study, we report on the new bifunctional peptide MOR agonist/NOP receptor antagonist, KGNOP1, as exhibiting greater activity on cold hyperalgesia and thermal nociception compared to the clinically used opioid analgesics, tramadol and morphine, respectively, paralleled by a superior benefit-to-respiratory toxicity ratio.

Treatment of nociceptive pain is currently based on opioid analgesics; however, at the risk of the occurrence of numerous unwanted side effects, including dependence and respiratory depression.[41] Management of neuropathic pain is more difficult since well-defined and specific mechanisms involved are still lacking.[9] As mechanism-based treatments are superior to disease-based or cause-based treatments, hybrids have been developed to act on multiple receptors involved in pain modulation. For instance, targeting 2 distinct pathways to produce analgesia may potentiate the overall analgesic effects and reduce adverse effects by requiring lower doses. Additionally, agonism or antagonsim of distinct targets can be achieved by combination therapy (ie, using drug cocktails) or by the use of designed multiple ligands,[38] single chemical entities able to bind 2 or more well-chosen receptor types. Both strategies have proven their efficiency, but major advantages of designed multiple ligands consist of an early onset, less expensive optimization in the drug discovery process, and less complex pharmacokinetics.[38]

Several hybrids have been developed to improve opioid action, to treat both nociceptive and neuropathic pain or to reduce opioid-related adverse effects.[23] Studies investigated opioid agonist/NOP receptor antagonist compounds and hybrids for their efficacy.[17,19,51,52,56] Studies addressing the function of N/OFQ, the NOP receptor endogenous ligand, in pain and analgesia remain contentious. The NOP receptor agonism is modulated by the administration route (intracerebroventricular vs intrathecal)

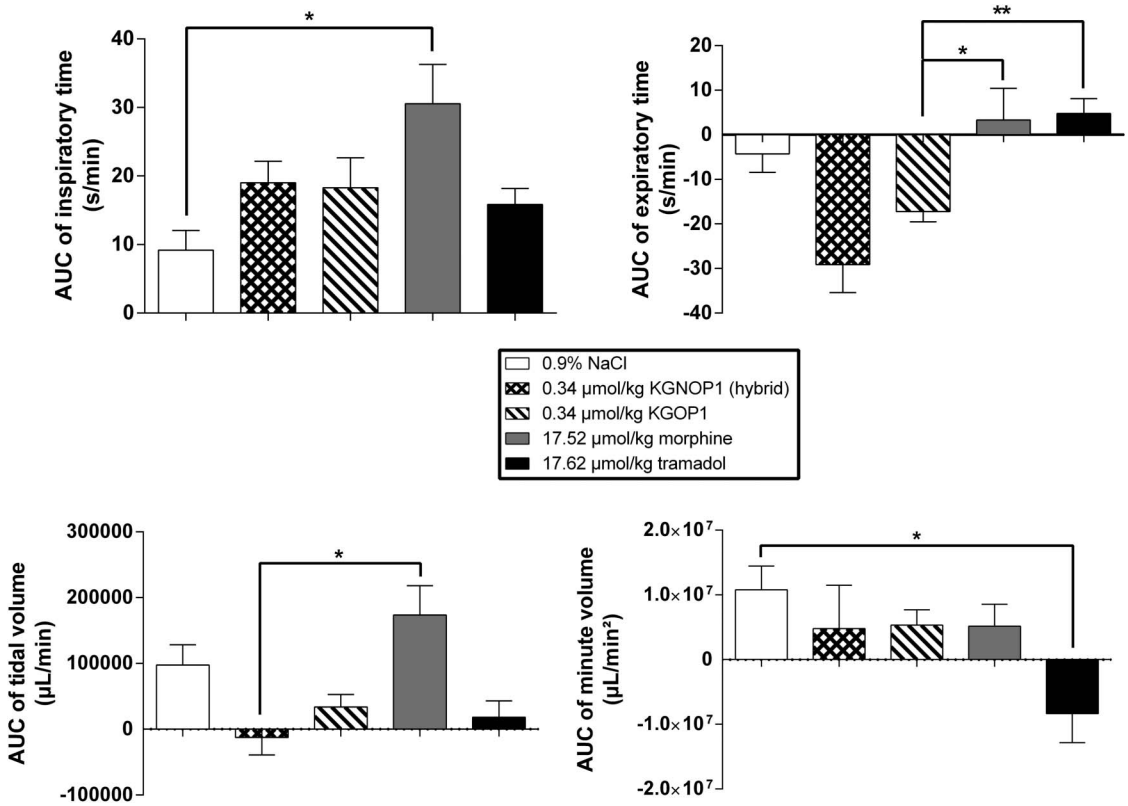

Figure 6. Drug effects on plethysmography parameters in Sprague-Dawley rats. The effects on plethysmography parameters, including inspiratory time, expiratory time, tidal volume and minute volume, of intravenous 0.9% NaCl (control), 0.34 μmol/kg KGNOP1, 0.34 μmol/kg KGOP1, 17.52 μmol/kg morphine, and 17.62 μmol/kg tramadol were tested (N = 6 per group). The plethysmography parameters were measured as a function of time. Areas under these curves (AUC) were represented. Results are expressed as mean ± standard error of the mean. Comparisons were performed using Kruskal–Wallis test. $^{*}P < 0.05$, $^{**}P < 0.01$.

and the doses (high doses producing analgesia[21,24,45,46,49,55] while low doses producing hyperalgesia and anti-opioid effects).[7,16,20,32,33,37,44,46,50] However, most of the studies assigned a pronociceptive activity to N/OFQ and thus to NOP receptor agonism,[34] explaining why NOP receptor antagonists in combination with MOR agonists were chosen to develop novel hybrids.[19]

The new MOR agonist/NOP receptor antagonist KGNOP1 was developed to treat both nociceptive and neuropathic pain to reduce opioid-related adverse effects. In our current study, effects on cold hyperalgesia in SN-CCI rats were early onset, as early as 5 minutes after KGNOP1 IV injection, and maintained for 420 minutes, whereas effects on thermal nociception were delayed up to 180 minutes. As previously shown,[13] delayed antinociceptive effects of KGNOP1 are not related to limitations in its brain distribution by the blood–brain barrier. The in vitro cell-based human model together with the blood–brain barrier–parallel artificial membrane permeability assay suggested that KGNOP1, KGOP1, and KGNOP3 are transported by active mechanisms and not through passive diffusion.[13] KGNOP1 was more efficient on cold hyperalgesia by ca 4000-fold than tramadol (ED_{50}: 0.0031 vs12.1 μmol/kg) and ca 100-fold than KGNOP3, its parent NOP receptor antagonist pharmacophore (ED_{50}: 0.0031 vs 0.29 μmol/kg), suggesting that combining the 2 pharmacophores has contributed to the observed enhancement in KGNOP1-related anti-neuropathic pain activity. Notably, KGNOP1 displayed similar potency in our rat model of neuropathic pain after IV administration when compared to the recently developed opioid analgesic, cebranopadol (KGNOP1 ED_{50} = 0.0031 μmol/kg vs cebranopadol ED_{50} = 0.8 μg/kg corresponding to 0.002 μmol/kg).[26,28,41] Similarly to cebranopadol, KGNOP1 was shown to be more potent in the model of chronic neuropathic than acute nociceptive pain. KGNOP1 is only a weak antagonist of NOP receptor (K_i = 42 nM and pA_2 = 5.39).[13] One possible hypothesis to explain its increased efficacy on neuropathic pain in comparison to its parent compound KGNOP3 could be related to interactions with heterodimerized opioid receptors. Emerging data support the hypothesis that heterodimerization of distinct G-protein-coupled receptor arises spontaneously, altering the pharmacological properties of the individual receptors.[1,11] Interestingly, the NOP receptor has also been shown to form heterodimers with MOR in different cell lines.[30,40,53]

We also established that KGNOP1 acted more efficiently than morphine on thermal nociception, being ca 35-fold more potent (ED_{50}: 0.41 vs 14.7 μmol/kg). This could be explained by its higher affinity to MOR in comparison to morphine (K_i: 5 vs 14 nM).[13,43] Our group has recently described one opioid/neurotensin hybrid developed to improve opioid-related antinociceptive activity.[22] Neurotensin induces both pronociceptive at low doses and antinociceptive effects at high doses. The opioid/neurotensin hybrid showed greater antinociceptive effects in comparison to saline and morphine after intrathecal administration in rats. Similarly, the antinociceptive activity of this opioid/neurotensin hybrid was greater after IV administration in mice in comparison to saline and morphine.

The opioid/NOP peptide hybrid KGNOP1 investigated in this study had a better profile than an opioid agonist/neurokinin1

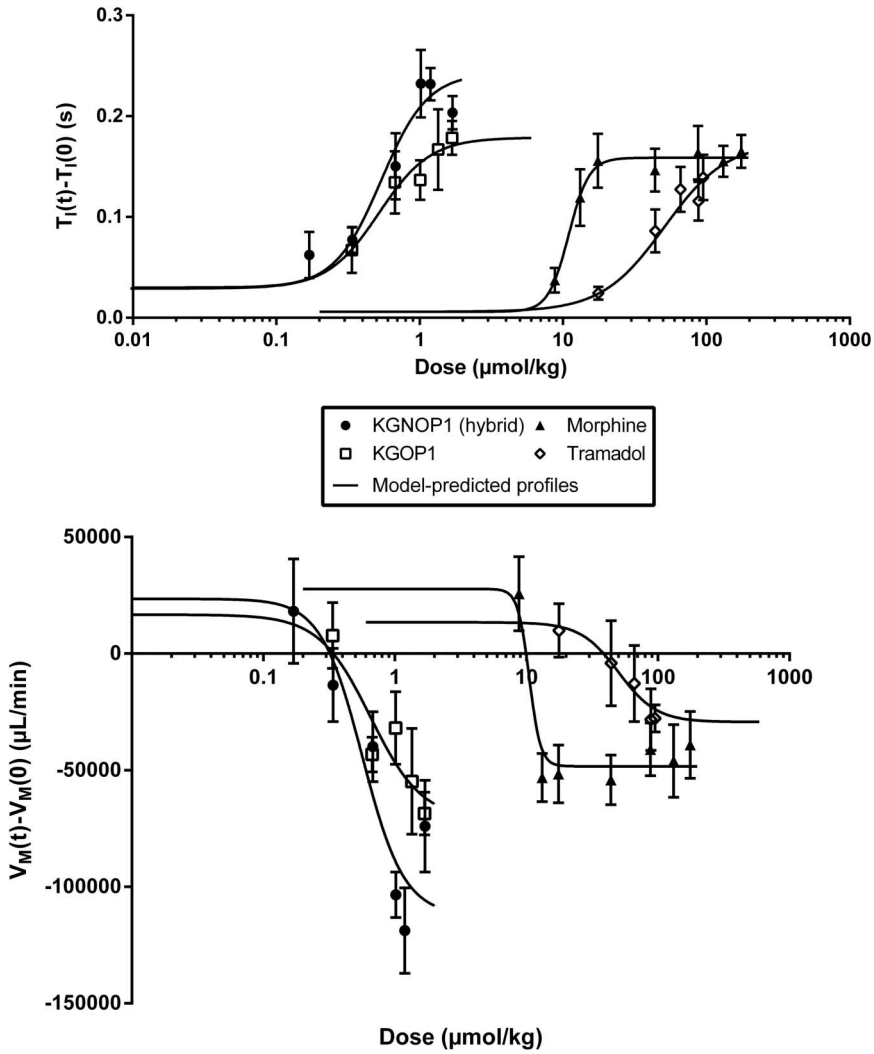

Figure 7. Dose–effect relationships on inspiratory time (T_I) and minute volume (V_M) in Sprague-Dawley rats. T_I (T_{max}) − T_I (T_0) and V_M (T_{max}) − V_M (T_0) as a function of the intravenous dose of KGNOP1, KGOP1, morphine, and tramadol were tested (N = 6 per group). The relationships were well-described using the sigmoidal E_{max} model. Solid lines represent the mean model-predicted profiles compared to the mean experimental data ± standard error of the mean.

(NK1) antagonist[22] whose parent compounds remained more efficient in the treatment of nociceptive pain than the hybrid, whereas in neuropathic pain, the hybrid was more efficient than the parent compounds and morphine.[13] This hybrid has been found to prolong opioid antinociceptive action, prevent pain worsening, and treat neuropathic pain via its NK1 antagonist part.[12] In chronic pain conditions, prolonged opioid treatment leads to increase substance P secretion and NK1 receptors expression. NK1 antagonists block the signal induced by substance P, an endogenous neurotransmitter that is a pronociceptive peptide involved in pain signaling.[29,48] To date, KGNOP1 appears to have a favorable efficacy in both nociceptive and neuropathic pain when compared to the hybrids previously developed.[19,51,52]

However, KGNOP1 and KGOP1 were responsible for non-significant distinct opioid-induced effects on ventilation ($ED_{50}(T_I)$: 0.63 vs 0.52 µmol/kg), resulting in comparable deleterious respiratory depression ($ED_{50}(V_M)$: 0.57 vs 0.66). The KGNOP1 was responsible for inducing more marked opioid-induced effects on ventilation than morphine and tramadol ($ED_{50}(T_I)$: 0.63 vs 12.2 and 50.9 µmol/kg, respectively). KGNOP1-related respiratory depression was more severe than that attributed to morphine and tramadol ($ED_{50}(V_M)$: 0.57 vs 10.6 and 50.0 µmol/kg, respectively). The NOP receptor involvement in respiratory depression is not proven, contrasting with the well-established relationship between respiratory depression and MOR agonists.[41] We assume that the NOP receptor antagonism may enhance MOR activity and thus could explain enhanced KGNOP1-related respiratory toxicity. Accordingly, N/OFQ, the endogenous agonist ligand of NOP receptor, functionally antagonizes MOR.[43] Additionally, MOR/NOP heterodimerization and its resulting impairment in MOR-activated signalling pathways may contribute to NOP-mediated anti-opioid effects in the brain.[51–53]

As a result of the calculated ratio of anti-neuropathic pain/antinociceptive to respiratory effects, KGNOP1 appears to be much safer when considering it for the treatment of neuropathic pain than tramadol (ED_{50} ratio: 5.44×10^{-3} vs 0.24, ca 40-fold safer than tramadol). KGNOP1 also has a better safety profile with respect to nociceptive pain than morphine (ED_{50} ratio: 0.72 vs 1.39, ca 2-fold safer than morphine). The benefit-to-respiratory toxicity ratios attributed to KGNOP1 were less than 1 meaning that its antinociceptive and anti-neuropathic pain effects appeared at lower doses than its deleterious effects on ventilation. Interestingly,

steepness of the dose–effect relationships of KGNOP3 and tramadol (anti-hyperalgesia effects, **Fig. 2**), KGNOP1 and morphine (antinociception effects, **Fig. 4**), and morphine (respiratory effects, **Fig. 7**) was relatively marked as previously established with morphine.[31,36] However, steepness may be variable, depending on the rat model, including the strain, the age, the gender, and the development opioid tolerance as well as the test system used to investigate antinociception, including the experimental conditions and the delay between the measurement and the drug administration.

In the current study, we did not perform pharmacokinetic investigations. Comparing the effects of drugs with different pharmacokinetics and ke0 could thus be questioned; however, our ED_{50} values were calculated at one given time initially determined at one given dose and should thus be interpreted only under these conditions. We determined the benefit-to-respiratory toxicity ratio using the well-established and generally accepted system, the ED_{50} side effect vs ED_{50} analgesia. Since the therapeutic and toxic effects of our tested drugs involve different mechanisms, the ED_{50} is clearly the best parameter to use as representing the point of maximal slope and therefore greatest precision.[5] Although this approach may have some limitations related to the conditions of ED_{50} determination, more importantly when the ke0 differs amongst the tested drugs, the benefit-to-respiratory toxicity ratio as determined in our study is reasonable when comparing doses at a specific level of response. Otherwise, our hybrid may be less efficient if administered orally or subcutaneously, thus limiting its extensive use in humans. To date, when the hybrid was administered orally to mice at doses of 61, 122, and 196 nmol, no analgesic effects were observed even at such high doses, suggesting limited oral bioavailability.[13] Further investigations are also needed to better characterize KGNOP1-related effects on tactile hyperalgesia and tactile and thermal allodynia. Tolerance development should also be a major concern as previously demonstrated for morphine in mice: repeated administration of an analgesic dose of morphine induced more marked tolerance to antinociceptive than to respiratory effects.[35]

In conclusion, our new bifunctional MOR agonist/NOP receptor antagonist peptide hybrid KGNOP1 exhibited greater dual activity on cold hyperalgesia and thermal nociception compared to tramadol and morphine, respectively. KGNOP1-related effects on ventilation are marked but these deleterious effects appear at significantly higher doses than its analgesic effects, resulting in an improved benefit–risk index in comparison to the conventional opioids. Thus, KGNOP1 appears promising for the dual treatment of nociceptive and neuropathic pain and it should be an excellent candidate for future clinical development.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

[1] Angers S, Salahpour A, Bouvier M. Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. Annu Rev Pharmacol Toxicol 2002;42:409–35.
[2] Bartlett DJ, Tenney S. Control of breathing in experimental anemia. Respir Physiol 1970;10:384–95.
[3] Beakley BD, Kaye AM, Kaye AD. Tramadol, pharmacology, side effects, and serotonin syndrome: a review. Pain Physician 2015;18:395–400.
[4] Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. PAIN 1988;33:87–107.
[5] Boom M, Olofsen E, Neukirchen M, Fussen R, Hay J, Groeneveld GJ, Aarts L, Sarton E, Dahan A. Fentanyl utility function: a risk-benefit composite of pain relief and breathing responses. Anesthesiology 2013;119:663–74.
[6] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. PAIN 2008;136:380–7.
[7] Calo' G, Bigoni R, Rizzi A, Guerrini R, Salvadori S, Regoli D. Nociceptin/orphanin FQ receptor ligands. Peptides 2000;21:935–47.
[8] Chevillard L, Mégarbane B, Baud FJ, Risède P, Declèves X, Mager D, Milan N, Ricordel I. Mechanisms of respiratory insufficiency induced by methadone overdose in rats. Addict Biol 2010;15:62–80.
[9] Cohen SP, Mao J. Neuropathic pain: mechanisms and their clinical implications. BMJ 2014;348:f7656.
[10] Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, Dworkin RH, Gilron I, Haanpää M, Hansson P, Jensen TS, Kamerman PR, Lund K, Moore A, Raja SN, Rice AS, Rowbotham M, Sena E, Siddall P, Smith BH, Wallace M. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol 2015;14:162–73.
[11] Gomes I, Jordan BA, Gupta A, Trapaidze N, Nagy V, Devi LA. Heterodimerization of mu and delta opioid receptors: a role in opiate synergy. J Neurosci 2000;20:RC110.
[12] Gonzalez MI, Field MJ, Hughes J, Singh L. Evaluation of selective NK(1) receptor antagonist CI-1021 in animal models of inflammatory and neuropathic pain. J Pharmacol Exp Ther 2000;294:444–50.
[13] Guillemyn K, Starnowska J, Lagard C, Dyniewicz J, Rojewska E, Mika J, Chung NN, Utard V, Kosson P, Lipkowski AW, Chevillard L, Arranz-Gibert P, Teixidó M, Megarbane B, Tourwé D, Simonin F, Przewlocka B, Schiller PW, Ballet S. Bifunctional peptide-based opioid agonist-nociceptin antagonist ligands for dual treatment of acute and neuropathic pain. J Med Chem 2016;59:3777–92.
[14] Hollingshead J, Dühmke RM, Cornblath DR. Tramadol for neuropathic pain. Cochrane Database Syst Rev 2006:CD003726.
[15] Hsu HC, Tang NY, Lin YW, Li TC, Liu HJ, Hsieh CL. Effect of electroacupuncture on rats with chronic constriction injury-induced neuropathic pain. ScientificWorldJournal 2014;2014:129875.
[16] Inoue M, Shimohira I, Yoshida A, Zimmer A, Takeshima H, Sakurada T, Ueda H. Dose-related opposite modulation by nociceptin/orphanin FQ of substance P nociception in the nociceptors and spinal cord. J Pharmacol Exp Ther 1999;291:308–13.
[17] Journigan VB, Polgar WE, Khroyan TV, Zaveri NT. Designing bifunctional NOP receptor-mu opioid receptor ligands from NOP-receptor selective scaffolds. Part II. Bioorg Med Chem 2014;22:2508–16.
[18] Kalso E, Edwards JE, Moore RA, McQuay HJ. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. PAIN 2004;112:372–80.
[19] Kawano S, Ito R, Nishiyama M, Kubo M, Matsushima T, Minamisawa M, Ambo A, Sasaki Y. Receptor binding properties and antinociceptive effects of chimeric peptides consisting of a micro-opioid receptor agonist and an ORL1 receptor antagonist. Biol Pharm Bull 2007;30:1260–4.
[20] King M, Chang A, Pasternak GW. Functional blockade of opioid analgesia by orphanin FQ/nociceptin. Biochem Pharmacol 1998;55:1537–40.
[21] King MA, Rossi GC, Chang AH, Williams L, Pasternak GW. Spinal analgesic activity of orphanin FQ/nociceptin and its fragments. Neurosci Lett 1997;223:113–6.
[22] Kleczkowska P, Kosson P, Ballet S, Van den Eynde I, Tsuda Y, Tourwé D, Lipkowski AW. PK20, a new opioid-neurotensin hybrid peptide that exhibits central and peripheral antinociceptive effects. Mol Pain 2010;6:86.

[23] Kleczkowska P, Lipkowski AW, Tourwé D, Ballet S. Hybrid opioid/non-opioid ligands in pain research. Curr Pharm Des 2013;19:7435–50.
[24] Kolesnikov YA, Pasternak GW. Peripheral orphanin FQ/nociceptin analgesia in the mouse. Life Sci 1999;64:2021–8.
[25] Lambert DG. The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential. Nat Rev Drug Discov 2008;7:694–710.
[26] Lambert DG, Bird MF, Rowbotham D. Cebranopadol: a first in-class example of a nociceptin/orphanin FQ receptor and opioid receptor agonist. Br J Anaesth 2015;114:364–6.
[27] Lilius TO, Jokinen V, Neuvonen MS, Niemi M, Kalso EA, Rauhala PV. Ketamine coadministration attenuates morphine tolerance and leads to increased brain concentrations of both drugs in the rat. Br J Pharmacol 2015;172:2799–813.
[28] Linz K, Christoph T, Tzschentke TM, Koch T, Schiene K, Gautrois M, Schröder W, Kögel BY, Beier H, Englberger W, Schunk S, De Vry J, Jahnel U, Frosch S. Cebranopadol: a novel potent analgesic nociceptin/orphanin FQ peptide and opioid receptor agonist. J Pharmacol Exp Ther 2014;349:535–48.
[29] Longmore J, Hill RG, Hargreaves RJ. Neurokinin-receptor antagonists: pharmacological tools and therapeutic drugs. Can J Physiol Pharmacol 1997;75:612–21.
[30] Mandyam CD, Altememi GF, Standifer KM. Beta-Funaltrexamine inactivates ORL1 receptors in BE(2)-C human neuroblastoma cells. Eur J Pharmacol 2000;402:R1–37.
[31] Meert TF, Vermeirsch HA. A preclinical comparison between different opioids: antinociceptive versus adverse effects. Pharmacol Biochem Behav 2005;80:309–26.
[32] Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B, Mazarguil H, Vassart G, Parmentier M, Costentin J. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 1995;377:532–5.
[33] Mogil JS, Grisel JE, Zhangs G, Belknap JK, Grandy DK. Functional antagonism of mu-, delta- and kappa-opioid antinociception by orphanin FQ. Neurosci Lett 1996;214:131–4.
[34] Mogil JS, Pasternak GW. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. Pharmacol Rev 2001;53:381–415.
[35] Mohammed W, Alhaddad H, Marie N, Tardy F, Lamballais F, Risède P, Noble F, Baud FJ, Mégarbane B. Comparison of tolerance to morphine-induced respiratory and analgesic effects in mice. Toxicol Lett 2013;217:251–9.
[36] Morgan MM, Fossum EN, Stalding BM, King MM. Morphine antinociceptive potency on chemical, mechanical, and thermal nociceptive tests in the rat. J Pain 2006;7:358–66.
[37] Morgan MM, Grisel JE, Robbins CS, Grandy DK. Antinociception mediated by the periaqueductal gray is attenuated by orphanin FQ. Neuroreport 1997;8:3431–4.
[38] Morphy R, Rankovic Z. Designed multiple ligands. An emerging drug discovery paradigm. J Med Chem 2005;48:6523–43.
[39] Mustazza C, Bastanzio G. Development of nociceptin receptor (NOP) agonists and antagonists. Med Res Rev 2011;31:605–48.
[40] Pan YX, Bolan E, Pasternak GW. Dimerization of morphine and orphanin FQ/nociceptin receptors: generation of a novel opioid receptor subtype. Biochem Biophys Res Commun 2002;297:659–63.
[41] Pattinson KT. Opioids and the control of respiration. Br J Anaesth 2008; 100:747–58.
[42] Pfeiffer A, Brantl V, Herz A, Emrich HM. Psychotomimesis mediated by kappa opiate receptors. Science 1986;233:774–6.
[43] Raynor K, Kong H, Chen Y, Yasuda K, Yu L, Bell GI, Reisine T. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. Mol Pharmacol 1994;45:330–4.
[44] Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ Jr, Civelli O. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 1995;270:792–4.
[45] Rossi GC, Leventhal L, Pasternak GW. Naloxone sensitive orphanin FQ-induced analgesia in mice. Eur J Pharmacol 1996;311:R7–8.
[46] Rossi GC, Leventhal L, Bolan E, Pasternak GW. Pharmacological characterization of orphanin FQ/nociceptin and its fragments. J Pharmacol Exp Ther 1997;282:858–65.
[47] Schunk S, Linz K, Hinze C, Frormann S, Oberbörsch S, Sundermann B, Zemolka S, Englberger W, Germann T, Christoph T, Kögel BY, Schröder W, Harlfinger S, Saunders D, Kless A, Schick H, Sonnenschein H. Discovery of a potent analgesic NOP and opioid receptor agonist: cebranopadol. ACS Med Chem Lett 2014;5:857–62.
[48] Takaya T. Discovery of neurokinin antagonists. Pure Appl Chem 1996; 68:875–80.
[49] Taylor F, Dickenson A. Nociceptin/orphanin FQ. A new opioid, a new analgesic? Neuroreport 1998;9:R65–70.
[50] Tian JH, Xu W, Fang Y, Mogil JS, Grisel JE, Grandy DK, Han JS. Bidirectional modulatory effect of orphanin FQ on morphine-induced analgesia: antagonism in brain and potentiation in spinal cord of the rat. Br J Pharmacol 1997;120:676–80.
[51] Toll L. The use of bifunctional NOP/mu and NOP receptor selective compounds for the treatment of pain, drug abuse, and psychiatric disorders. Curr Pharm Des 2013;19:7451–60.
[52] Toll L, Bruchas MR, Calo' G, Cox BM, Zaveri NT. Nociceptin/Orphanin FQ receptor structure, signaling, ligands, functions, and interactions with opioid systems. Pharmacol Rev 2016;68:419–57.
[53] Wang HL, Hsu CY, Huang PC, Kuo YL, Li AH, Yeh TH, Tso AS, Chen YL. Heterodimerization of opioid receptor-like 1 and mu-opioid receptors impairs the potency of micro receptor agonist. J Neurochem 2005;92: 1285–94.
[54] Woolf CJ. Pain: moving from symptom control toward mechanism-specific pharmacologic management. Ann Intern Med 2004;140:441–51.
[55] Xu XJ, Hao JX, Wiesenfeld-Hallin Z. Nociceptin or antinociceptin: potent spinal antinociceptive effect of orphanin FQ/nociceptin in the rat. Neuroreport 1996;7:2092–4.
[56] Zaveri NT, Jiang F, Olsen C, Polgar WE, Toll L. Designing bifunctional NOP receptor-mu opioid receptor ligands from NOP receptor-selective scaffolds. Part I. Bioorg Med Chem Lett 2013;23:3308–13.