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Noribogaine is a G-protein biased κ-opioid receptor agonist

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ABSTRACT

Noribogaine is the long-lived human metabolite of the anti-addictive substance ibogaine. Noribogaine efficaciously reaches the brain with concentrations up to 20 μM after acute therapeutic dose of 40 mg/kg ibogaine in animals. Noribogaine displays atypical opioid-like components *in vivo*, anti-addictive effects and potent modulatory properties of the tolerance to opiates for which the mode of action remained uncharacterized thus far. Our binding experiments and computational simulations indicate that noribogaine may bind to the orthosteric morphinan binding site of the opioid receptors. Functional activities of noribogaine at G-protein and non G-protein pathways of the mu and kappa opioid receptors were characterized. Noribogaine was a weak mu antagonist with a functional inhibition constants (K_e) of 20 μM at the G-protein and β-arrestin signaling pathways. Conversely, noribogaine was a G-protein biased kappa agonist 75% as efficacious as dynorphin A at stimulating GDP-GTP exchange ($EC_{50} = 9$ μM) but only 12% as efficacious at recruiting β-arrestin, which could contribute to the lack of dysphoric effects of noribogaine. In turn, noribogaine functionally inhibited dynorphin-induced kappa β-arrestin recruitment and was more potent than its G-protein agonistic activity with an IC_{50} of 1 μM. This biased agonist/antagonist pharmacology is unique to noribogaine in comparison to various other ligands including ibogaine, 18-MC, nalmefene, and 6′-GNTI. We predict noribogaine to promote certain analgesic effects as well as anti-addictive effects at effective concentrations >1 μM in the brain. Because elevated levels of dynorphins are commonly observed and correlated with anxiety, dysphoric effects, and decreased dopaminergic tone, a therapeutically relevant functional inhibition bias to endogenously released dynorphins by noribogaine might be worthy of consideration for treating anxiety and substance related disorders.

1. Introduction

Noribogaine (Fig. 1) is the primary human metabolite of ibogaine (Obach et al., 1998), an alkaloid derived from the African shrub, *iboga* (*Tabernanthe iboga*). The therapeutic and oneirophrenic properties of *iboga* roots are known for centuries in

Non-standard abbreviations and acronyms: GPCR, G protein-coupled receptor; OPRM, μ-opioid receptor; OPRK, κ-opioid receptor; OPRD, δ-opioid receptor; Nor-BNI, nor-binaltorphimine; DAMGO, [D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin.

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Equatorial Africa where *iboga* continues to be used as natural medicine and for ceremonial purposes (Goutarel et al., 1993; Samorini, 1995). Naranjo, in collaboration with Bocher, issued a patent in 1969 based on 54 clinical cases featuring the usefulness of ibogaine for psychotherapy and anti-drug purposes (Bocher and Naranjo, 1969). A few decades later, the benefits of *iboga* (ibogaine) in the treatment of addiction for multiple drugs of abuse were highlighted by different groups (Alper et al., 1999; Mash et al., 1998; Sheppard, 1994). Preclinical studies show that ibogaine is a polypharmacological drug that can reduce self-administration to many drugs of abuse in rodents, including cocaine, morphine, heroin, alcohol, and nicotine; and further experimentation in humans supported its usefulness to treat addiction (Alper, 2001; Baumann et al., 2001a; Freedlander, 2003; Maciulaitis et al., 2008; Mash et al., 2000; Popik et al., 1995).

Noribogaine displayed a slow pharmacokinetic clearance rate in humans, being detected for several days in blood after ibogaine ingestion, and was proposed to be responsible for many of the human *in vivo* effects seen after ibogaine therapy (Mash et al., 2000). Noribogaine produced ibogaine-like anti-addictive effects in animals and the systemic administration of noribogaine induced long-lasting decrease of morphine and cocaine self-administration (Glick et al., 1996; Mash and Schenk, 1996). Noribogaine also decreased ethanol self-administration (Rezvani et al., 1995) and nicotine self-administration in rats (Chang et al., 2015). The brain levels of noribogaine in female/male rats were approximately 20/13, 10/7 and 0.8/0.1 μM at 1, 5, and 19 h after intra-peritoneal injection of 40 mg/kg ibogaine whereas plasma levels were 10–20 fold less (Pearl et al., 1997). This indicated that noribogaine had excellent drug permeability across the blood–brain barrier and reached high levels in the brain. Unlike ibogaine, noribogaine did not produce tremors and ataxia in rodents (Baumann et al., 2001b), suggesting that it is better tolerated than its parent compound and a better drug candidate for clinical development. Recently, a study performed in healthy volunteers indicated that single oral doses of noribogaine from 3 to 60 mg were safe and well tolerated (Glue et al., 2015).

Of particular interest with regards to their acknowledged roles in modulating opiate dependence are ibogaine and noribogaine's effects on the opioid system. In addition to effects on the pursuit and administration of drug of abuse, these drugs were shown to modulate the analgesic power and the development of physical tolerance to morphine. Pre-administration of noribogaine (40 mg/kg, 19 h) had a moderate but detectable effect of potentiation on both basal nociception and morphine-induced analgesia (Bagal et al., 1996). When noribogaine (40 mg/kg) was co-administered with 4 mg/kg morphine, it amplified the duration of morphine-induced analgesia (Bagal et al., 1996). Noribogaine (40 mg/kg) enhanced anti-nociception when added to morphine but did not enhance anti-nociception when combined with U50,488 (kappa opioid agonist) or DPDPE (delta opioid agonist) (Bhargava et al., 1997). At lower doses of 10–20 mg/kg, noribogaine has also been shown to greatly potentiate (or restore) morphine anti-nociceptive activity in morphine-tolerant mice but remained inactive in naïve mice (Sunder Sharma and Bhargava, 1998). Finally, noribogaine was shown to prevent the development of tolerance to the analgesic effects of morphine (Bhargava and Cao, 1997). The mode of action for these effects, as well as the anti-addictive effects, remained largely uncharacterized thus far.

Noribogaine has principal known binding affinities to the opioid receptors, the nicotinic acetylcholine receptors (Maillet et al., submitted manuscript), and the SERT and DAT transporters, but marginal affinities to NMDA, sigma 2 and 5-HT2 receptors in comparison to the parent compound ibogaine (Baumann et al.,

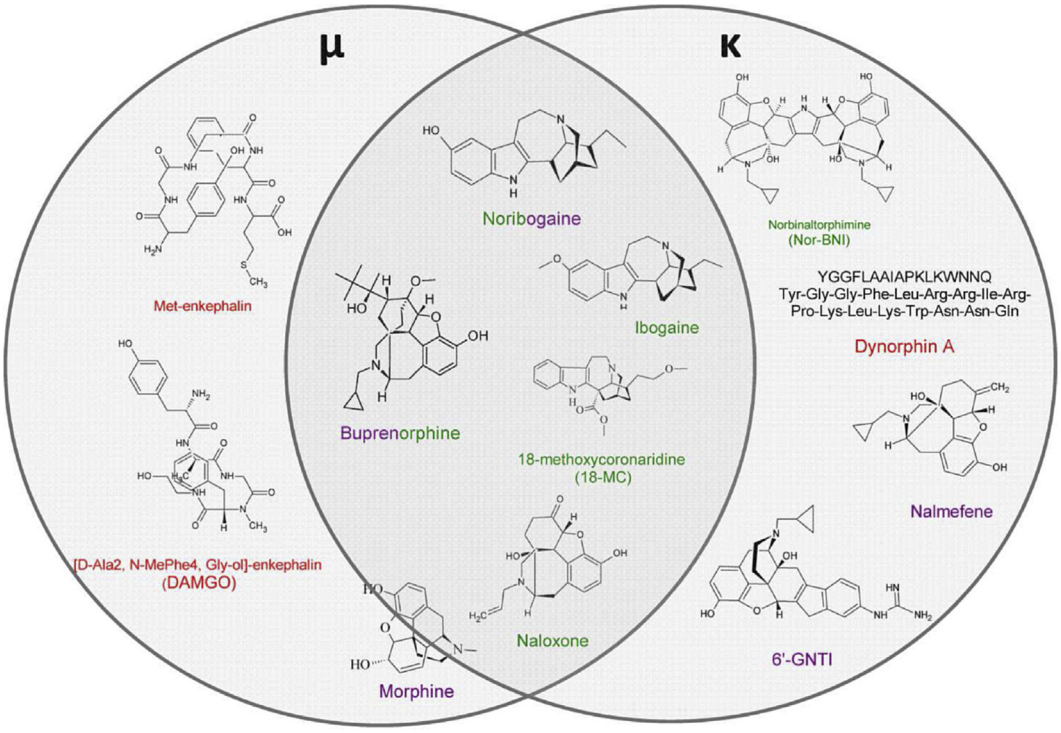

Fig. 1. Structures of noribogaine, and other mu and kappa opioids ligands tested in this study. [Met]-enkephalin, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin (DAMGO), buprenorphine, naloxone, noribogaine, ibogaine, morphine, norbinaltorphimine (nor-BNI), 18-methoxycoronaridine (18-MC), dynorphin A, nalmefene, 6'-guanidinonaltrindole (6'-GNTI). Agonists (red), partial agonists (purple), and antagonists (green) are arranged in the diagram according to their affinities for either the μ or κ opioid receptor. Structures in the overlapping region have affinity for both subtypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2001a; Bowen et al., 1995; Mash et al., 1995a, 1995b; Staley et al., 1996). Ibogaine and noribogaine were found to have potencies in the micro-to high nano-molar range for the opioid receptors (Pearl et al., 1995) but they do not appear to be conventional opioid agonists or antagonists. Noribogaine, and ibogaine to a lesser extent, were shown to induce naloxone-sensitive GTPγS binding in rat brain preparations (Pablo and Mash, 1998). These drugs however did not induce conditioned place preference like mu agonists or conditioned place aversion like kappa agonists or mu antagonists (Skoubis et al., 2001) in animals ((Parker et al., 1995) and unpublished results). In addition, neither morphine nor the kappa agonist U50,488 substituted for the discriminative stimulus of noribogaine treatment in rats (Helsley et al., 1998) (Zubaran et al., 1999). In a sense, noribogaine was neither a mu or a kappa opioid agonist or antagonist *in vivo* and it remained largely unclear what the specific roles and activities of noribogaine at the opioid receptors would be in relation to physiological outputs.

To address this gap of knowledge, we carried out a pharmacological study to characterize noribogaine to the mu (OPRM) and the kappa (OPRK) opioid receptors. We performed comprehensive *in vitro* and *in silico* experimental designs encompassing both binding and functional assays for G-protein signaling using GTPγS binding stimulation, β-arrestin signaling using protein recruitment assay, in addition to performing docking simulations. We then compared the pharmacology of noribogaine to its parent drug ibogaine, the synthetic indole alkaloid 18-methoxycoronaridine (18-MC) (Glick et al., 2000), and a series a specific opioid ligands including the kappa partial agonist nalmefene (Bart et al., 2005), and the biased kappa agonist 6′-GNTI (Rives et al., 2012) (Fig. 1). Our study reveals an atypical biased agonism of noribogaine at the kappa receptors and provides important pharmacological basis for its mechanism of action as a drug therapeutic.

2. Materials and methods

2.1. Materials

[Phenyl-3, 4-^{3}H]-U-69,593 (43.6 Ci/mmol), [Tyrosyl-3, 5-^{3}H(N)]-DAMGO ([D-Ala^2, N-MePhe^4, Gly^5-ol]-enkephalin) (50 Ci/mmol) and [35S]GTPγS (Guanosine 5′′-(gamma-thio)triphosphate) (1250 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). U69,593, naloxone, nor-binaltorphimine (nor-BNI), morphine, nalmefene, dynorphin A, DAMGO, GTPγS, GDP and all buffer constituents were purchased from Sigma–Aldrich Corp (St. Louis, MO). CHO–K1 cell lines expressing human opioid receptors were provided by Dr. Toll at Torrey Pines Institute (Port St. Lucie, FL). Ibogaine was provided by Dr. Mash at the University of Miami (Miami, FL). 18-methoxycoronaridine (18-MC) was purchased from Obiter Research LLC (Champaign, IL). Noribogaine hydrochloride was purchased from Sigma Aldrich Chemie GmbH (Buchs, Switzerland).

2.2. Membrane preparation

Membrane preparations of rat midbrain tissues were purchased from Chantest (Cleveland, OH). Membrane preparation of human OPRK CHO–K1 cells were purchased from PerkinElmer Life Sciences (Boston, MA) and membranes of human OPRM CHO–K1 cells were prepared as described below. Adherent cells were harvested on ice, with cold PBS and a cell scraper, pelleted and frozen at −80 °C overnight. Cell lysis was performed at 4 °C in 50 mM Tris (pH 7), 2.5 mM EDTA and cOmplete protease inhibitor cocktail (cOmplete, F. Hoffmann-La Roche Ltd). Cells were homogenized with a polytron, centrifuged at 2500 rpm for 10 min at 4 °C and the supernatant was recovered. The process was repeated once. Supernatant was centrifuged at 21,000 rpm for 90 min at 4 °C and pellets were re-suspended in 50 mM Tris (pH 7) and 0.32 M sucrose. Total protein concentration was determined using a ThermoScientific NanoDrop spectrophotometer or by Bradford assay. Membrane samples were stored at −80 °C at 1–5 mg/mL protein concentration. Membranes from brain tissues were stored in 50 mM Tris (pH 7), 1 mM EDTA and 0.32 M sucrose with protease inhibitor cocktail.

2.3. Radioligand binding

Competitive binding experiments were performed using conditions recommended by the supplier (Perkin Elmer). Membranes were thawed on ice and diluted in binding buffer 50 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$ at 5 μg of membrane per reaction. Competition binding assays were performed in 500 μL total volume containing [^{3}H]U69,593 (0.88 nM) for OPRK membranes or [^{3}H]DAMGO (0.75 nM) for OPRM membranes in the presence of increasing concentrations of each unlabeled drug (noribogaine, ibogaine, 18-MC, U69,593, morphine, DAMGO, naloxone) for 60 min at 25 °C. Nonspecific binding was defined in the presence of 1 μM naloxone. Bound and free radiolabeled ligands were separated by filtration using a MicroBeta FilterMate-96 Harvester and wash 6 × 1 mL with ice cold wash buffer (50 mM Tris-HCl pH 7.4) over GF/B filter (presoaked in 0.5% BSA) (Perkin Elmer, Waltham, MA). Radioactivity counts were determined using Perkin Elmer MicroBeta microplate counter with scintillation cocktail MicroScint-20™ according to manufacturer recommendations. Data were collected and the half maximal inhibitory concentration (IC_{50}) and apparent binding affinity (K_i) for all data sets were calculated with GraphPad Prism 5.04.

2.4. [^{35}S]GTPγS binding assay

[^{35}S]GTPγS binding to Gα proteins was determined using a procedure modified from (Toll et al., 1998) and carried out in a 96-well format. Cell membranes (10 μg per reaction) were incubated in a binding buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2x6H_2O$, 0.2% bovine serum albumin, and GDP 10 μM, pH 7.4) containing 80 pM [^{35}S]GTPγS and varying concentrations of opioid agonists (U69,593, DAMGO, morphine, dynorphin A, nalmefene, or noribogaine) in a total volume of 100 μL for 60 min at 25 °C. Membranes were pre-incubated with the GDP for 15 min on ice prior to the addition of ligands. Antagonists were added to the membrane solution 20 min prior the addition of the agonist, and [^{35}S]GTPγS was added 5 min after the agonist. Non-specific and basal levels of GTPγS binding were evaluated by using 10 μM cold GTPγS or binding buffer, respectively. Bound and free [^{35}S]GTPγS were separated by filtration using a MicroBeta FilterMate-96 Harvester and wash 4 × 1 mL with ice cold wash buffer (20 mM Tris, pH 7.4, and 2.5 mM $MgCl_2x6H_2O$, pH 7.4) over presoaked GF/B filter (Perkin Elmer, Waltham, MA). Radioactivity counts were determined using Perkin Elmer MicroBeta microplate counter with scintillation cocktail MicroScint-20™ according to manufacturer recommendations. Data were collected and the half maximal effective concentration (EC_{50}) and maximal responses (E_{max}) values were calculated using non-linear regression with GraphPad Prism 5.04.

2.5. β-Arrestin-2 recruitment assay

The PathHunter enzyme complementation Arrestin-2 Recruitment assays were performed at DiscoveRx Corporation (Fremont, CA) and at the DemeRx Laboratory (Miami, FL). This assay utilized CHO–K1 cells stably transfected to overexpress β-arrestin-2 fused

to a β-galactosidase fragment together with human OPRK gene (NM_000912.3, encoding human KOR) or human OPRM gene (NM_000914.3, encoding human MOR). Briefly, when β-arrestin-2 travels to active receptor, the complementary β-galactosidase fragments fused to the receptor and β-arrestin interact to form a functional enzyme with activity that is detected by chemiluminescence. For all *in vitro* assays, data were normalized as a percentage of control agonist responses, typically defined by dynorphin A stimulated activity in the OPRK assays, and [met]-enkephalin stimulated activity in the OPRM assays. For agonist concentration-response experiments, cells were treated with test compound for 180 min prior to assessment of enzyme complementation. For antagonist concentration-inhibition experiments, the cells were incubated with the test compound for 30 min prior to agonist addition.

2.6. Data analysis

The IC_{50} and K_i values for ligands in the radioactive binding assays were determined by fitting competition binding data of individual experiments normalized to buffer (total binding) and 1 μM naloxone (nonspecific binding) to a single site competition model in GraphPad Prism 5.04 using the transformation of Cheng and Prusoff (Cheng and Prusoff, 1973) (CFeq): $K_i = IC_{50}/(1 + [S]/K_m)$, where [S] is the concentration of agonist and K_m is the K_i value for [^{3}H]U69,593 and [^{3}H]DAMGO determined by homologous competition. The EC_{50} and E_{Max} values to agonists for [^{35}S]GTPγS binding and β-arrestin-2 translocation were determined by fitting data from individual experiments to sigmoidal concentration-response curves with variable slope in GraphPad Prism 5.04. I_{Max} was calculated as the % of inhibition from the E_{Max} of the full agonist U69,593 (OPRK) or DAMGO (OPRM) to the basal signal. Functional inhibitory potency (K_e) values for agonist concentration-response displacement experiments were calculated using the Gaddum/Schild EC_{50} shift calculation in GraphPad Prism or with the following equation: $K_e = [A]/(DR-1)$, where [A] is the concentration of antagonist and DR is the ratio of the EC_{50} for an agonist in the presence and absence of the antagonist. K_e values from concentration-inhibition experiments were calculated with a modified CFeq: $K_e = IC_{50}/(1+[S]/EC_{50})$ where [S] is the concentration of agonist, EC_{50} is the functional potency of the agonist, and IC_{50} is the half-maximal inhibition concentration of antagonist. Final mean and S.E. were calculated using individual values from each experiment.

Activation coupling efficiency (e-coupling) values describe the relationship between the apparent binding affinity K_i versus the apparent functional potency EC_{50} of a given agonist ligand and used the equation $pKi\text{-}pEC_{50}$ where "p" represents a −log10 transformation. For the functional inhibitory components of antagonists and partial agonists, e-coupling represents the relationship between the K_i versus the K_e of a given inhibitor against a given agonist ([ϘDyn-A] and used the equation $pKi\text{-}pK_e$. Efficacy efficiency (e-signal) values describe the ratio of the E_{Max} to a tested ligand versus the E_{Max} to reference full agonist dynorphin A (or U69,593) for OPRK and DAMGO or [met]-enkephalin for OPRM and used the equation E_{Max}(test compound)/E_{Max}(control agonist). For inhibitory ligands, e-signal was calculated using maximal level of inhibition (I_{max}) normalized from 0 (basal, buffer) to 1 (agonist without inhibitor).

Bias-coupling (quantification of pathway bias for the coupling efficiency) was calculated by dividing the EC_{50} (activation bias) or the K_e (inhibition bias) issued from the G-protein pathway assays by those issued from β-arrestin pathway assays for a given ligand, rectified by the intrinsic functional assay efficiency (defined as the average of the cluster values of functional coupling efficiency of typical full agonists and antagonists). The intrinsic functional assay efficiency is dependent of the assay and the experimental conditions (for instance the GDP content in the GTPγS assay, the temperature and time of incubation) and independent of the ligands binding affinity (the potency ranking of high to low affinity ligands remain constant regardless of the assay conditions). Bias-efficacy in favor of the G-protein pathway was evaluated by dividing the functional activation and the functional inhibition maximum responses (e-signal) from the G-protein pathway by the beta-arrestin pathway assays for a given ligand.

2.7. μ-Opioid receptor noribogaine binding model

We used the mouse μ-opioid receptor OPRM co-crystal structure available in the Protein Data Bank (PDB), PDB accession 4dkl, Uniprot accession P42866. The mouse OPRM has 94% (global) sequence identity to the corresponding human receptor (Uniprot accession P35372) and all residues in the binding site are identical. The receptor was crystallized as a fusion protein (OPRM–T4L) with an irreversible morphine antagonist ligand (bound to Lys233, PDB numbering). All simulations were performed using the Schrodinger 2014.2 and Desmond 2014.2 software suite. For initial docking studies we imported the PDB file into Maestro 9.5 (Schrodinger) and ran the standard protein preparation workflow to assign bond orders and clean up the structure including hydrogen bond optimization and constrained minimization. In the preparation process missing side chains were added using Prime. The fusion protein was manually cut and removed between residues Val262 and Glu270 to leave just the GPCR transmembrane domain; the cut residues were capped as primary amide (C-terminal) and acetate (N-terminal). A (non-covalent) ligand entry (separate from the chain) was manually created in Maestro. The resulting protein complex was again processed via the protein preparation workflow. A docking grid was created around the co-crystal ligand using Glide (standard settings). Several small molecules including the morphinan co-crystal ligand (unbound), ibogaine and noribogaine were imported as 2D SDF into Maestro and 3D structure representations were generated using LigPrep (default settings); two representations (inverted at the tertiary bridgehead nitrogen) were generated for each ligand. These were docked using Glide SP (standard settings except keeping 5 poses per compound out of 30 for post-minimization). The docked morphinan ligand reproduced the co-crystal almost perfectly. This docked complex was then optimized using Prime Refine Protein–Ligand complex (default settings). This complex was then used to generate another docking grid using Glide (default settings around the ligand) followed by Glide SP docking of the prepared ligands. In these results, the top poses of noribogaine and ibogaine aligned well the morphinan antagonist (hydrophobic ibogaine and noribogaine bicyclic system and ethyl substituent with morphinan cyclopropyl residues and the positively charged tertiary amines, which all form a hydrogen bond to the site chain of Asp147). The μ-OR noribogaine docking complex was then used in a 12 ns molecular dynamics (MD) simulation. The MD system generation and simulations were performed in Desmond using an all atom system with a membrane model and explicit water model (ASP). The Desmond software automatically sets up the systems (adjust charges, adds water molecules) and performs several rounds of minimization and short simulations before the 12 ns production run. MD was run on the Pegasus 2 cluster at the Center for Computational Science at the University of Miami (http://ccs.miami.edu/hpc/) using 48 processors and completed in less than 19 h. Simulation analysis was performed using the Desmond trajectory analysis software. A representative frame with these most prevalent interactions throughout the simulation was extracted from the trajectory, processed via protein preparation (including

constrained minimization) to remove overlapping atoms, and visualized using PyMol.

3. Results

3.1. Apparent binding affinities of noribogaine to OPRM and OPRK

Competitive inhibition of [^{3}H]-U69,593 to human OPRK and of [^{3}H]-DAMGO to human OPRM by noribogaine was conducted and compared to ibogaine, 18-MC, and various control ligands (Fig. 1, Table 1). Noribogaine exhibited the highest apparent affinity for OPRK with a K_i value of 720 ± 128 nM. At OPRK, ibogaine displayed a K_i of 3.68 ± 0.22 μM, while 18-MC had a K_i a 1.84 ± 0.12 μM. At the OPRM, noribogaine displayed a K_i of 1.52 ± 0.3 μM, while ibogaine and 18-MC K_i values were 6.92 ± 0.83 μM and 2.26 ± 0.35 μM respectively. Values of both noribogaine and ibogaine for the human OPRM/K receptors were comparable to that of the calf OPRM and OPRK receptors (1.52 and 0.96 μM, Table 1) where noribogaine was also previously shown to have ~30-fold less affinity at OPRD than at OPRK (Pearl et al., 1995). In our assays, 18-MC had no OPRM preferential binding with a ΔpK_i of 0.1, contrary to the previously reported 5-fold selectivity ($\Delta pK_i = -0.7$) for OPRM over OPRK (Glick et al., 2000). Experimental values, historical values from the literature, and control ligands, are displayed in Table 1 for agonists, partial agonists, and antagonists used in this study.

3.2. Noribogaine is agonist of the OPRK G-protein pathway

[^{35}S]GTPγS binding to membrane preparations of CHO cells stably transfected with OPRK was examined in response to noribogaine, ibogaine, morphine, and nalmefene drug treatment and the activation of the G-protein pathway by kappa agonists was measured (Fig. 2A). The prototypical full agonist, U69,593, and the endogenous ultra-potent agonist, dynorphin A, were used as controls for OPRK function. Calculated EC_{50} and E_{Max} values are enumerated in Table 2. Noribogaine was a partial agonist at stimulating [^{35}S]GTPγS binding to OPRK with an E_{Max} of 72 ± 3.8% of U69,593, and an EC_{50} of 8.75 ± 1.09 μM (Fig. 2B, Table 2A). Ibogaine displayed a notably lower agonist efficacy than noribogaine at OPRK with an E_{Max} of 18 ± 1.4%, while 18-MC failed to stimulate [^{35}S]GTPγS binding to OPRK. In our assays, morphine and dynorphin A displayed E_{Max} values of 91 ± 7% and 94 ± 7% respectively, and nalmefene, a partial agonist of OPRK, maximally stimulated at 35 ± 4.7%, which is similar to formally reported values (Bart et al., 2005). Noribogaine stimulation of [^{35}S]GTPγS binding was also observed in membrane preparations from rat midbrain tissues and this signal was nor-BNI-sensitive, confirming the agonist activity of noribogaine in native brain tissues (data not shown).

The apparent coupling efficiencies of agonists DAMGO, U69,593, morphine, dynorphin A, nalmefene, 6′-GNTI, noribogaine and ibogaine at the G-protein pathway were calculated ($pK_i - pEC_{50}$) and found to be congruent with EC_{50} values shifted by ~1 log in comparison to apparent affinity K_i for all agonists (Tables 2A and 3). The activation coupling efficiency of dynorphin A (0.6, this work) and 6′-GNTI (0.2, (Schmid et al., 2013)) were superior to other agonists in this assay, indicating that a lower fraction of bound receptors was sufficient to activate the pathway toward its maximal efficiency. Conversely, the hillslope for dynorphin A concentration response curves was below 1 (~0.7), indicating slower kinetics and probably a longer residence time in the receptor active conformations, producing the observed heightened coupling efficiency.

Table 1
Binding affinity of noribogaine and other drugs at the human mu (OPRM) and kappa (OPRK) opioid receptors. K_i values of noribogaine, ibogaine, and 18-MC (n ≥ 3). Values for control ligands morphine, naloxone DAMGO, U69,593, dynorphin A, [met]-enkephalin, nalmefene, and buprenorphine were determined and/or gathered from the literature. Specificity for the OPRK receptor was evaluated using the equation $\Delta pK_i = pK_i(OPRK) - pK_i(OPRM)$. Agonists (red), partial agonists (purple), and antagonists (green). (Huang et al., 2001, Li et al., 1993, Raynor et al., 1994, Sharma et al., 2001).

	OPRM ([^{3}H]-DAMGO)			OPRK ([^{3}H]-U69,593)			Spec.	References
Compound	pK_i	K_i (nM)	SE	pK_i	K_i (nM)	SE	ΔpK_i	
U69,593		n/q		9.2	0.59/0.87		> 3	Perkin Elmer/This work
DAMGO	9.1	0.6 0.5	0.2		n/q		< -3	(Toll et al., 1998) This work
Dynorphin A	8.1	7.7	2.2	8.8 10.3	1.7 0.05**	0.85 0.01**	0.7	(Toll et al., 1998) (Li et al., 1993)
[Met]-enkephalin	9.2	0.63		6.0	1000		< -3	(Raynor et al., 1994)
Morphine	9.0	1.1	0.05	7.3	46.9	4.5	-1.6	(Toll et al., 1998)
Nalmefene	9.0	1		10.1	0.083	0.0008	1.1	(Bart et al., 2005)
Buprenorphine	10.1	0.08	0.02	10	0.11	0.05	-0.1	(Huang et al., 2001)
6'-GNTI	7.1	82	21	8.9	1.15	0.39	1.84	(Sharma et al., 2001)
Noribogaine #	5.8 5.6	1520 2660*	300	6.1 6.0	720 960*	128	0.3 0.4	This work (Pearl et al., 1995)
Ibogaine	5.2 5.0	6920 11040*	830	5.4 5.4	3680 3770	220	0.3 0.5	This work (Pearl et al., 1995)
18-MC	5.6 6.0	2360 1100*	350 300	5.7 5.3	1840 5100*	120 500	0.1 -0.7	This work (Glick et al., 2000)
Naloxone	8.9	1.4 1.3	0.05	8.6	2.5	0.3	-0.3	(Toll et al., 1998) This work
Nor-BNI	7.7	21	5	9.7 10.4	0.2 0.04**	0.05 0.01**	2.0	(Toll et al., 1998) (Li et al., 1993)

Spec: Specificity. n/q non-quantifiable.: calf receptor. **: [^{3}H]diprenorphine binding. #: (OPRD=24720 nM) OPRD: human opioid receptor delta.*

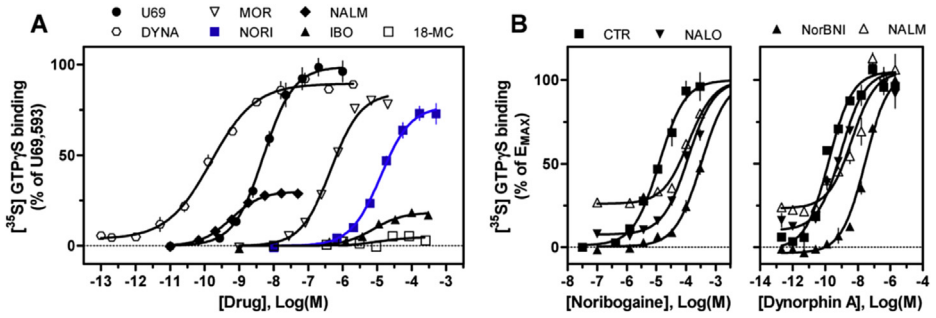

Fig. 2. Noribogaine G-protein agonist activity at the kappa opioid receptor (OPRK). (A) CHO–K1 cell membrane preparations expressing human OPRK receptors were stimulated with increasing concentrations of agonist (DAMGO, morphine: MOR, nalmefene: NALM) and test compound (noribogaine: NORI, ibogaine: IBO; 18-MC) in a [^{35}S]GTPγS binding assay. Mean ± SE of EC_{50}, E_{Max}, and K_e values from up to 10 experiments are shown in Table 2. (B) EC_{50} shifts of noribogaine and dynorphin A were produced by pre-incubating antagonists (naloxone: NALO; nalmefene: NALM; NorBNI) at 30, 3, and 5 nM respectively. Functional inhibition constants (K_e) of antagonists were calculated and are shown in Table 3. Data points used for the non-linear regression analysis figures (A and B) are shown as the mean ± SEM of each representative experiment(s).

3.3. Common kappa antagonists competitively compete with noribogaine

Inhibitory effects of kappa antagonists naloxone, nor-BNI, 18-MC, and the partial agonist nalmefene on the agonist-induced [^{35}S]GTPγS binding by full or partial agonists dynorphin A, U69,593, morphine, noribogaine and nalmefene were investigated. Concentration-response curve shifts of agonists were gathered in the absence and presence of fixed antagonist concentration (30 nM naloxone, 5 nM nor-BNI, 100 μM 18-MC, and 3 nM nalmefene) or in some instances with iterative concentrations of the antagonist (Fig. 2B, Table 3).

All antagonists tested right-shifted the concentration-response curves of noribogaine, dynorphin A, morphine or U69,593 in a concentration-dependent manner. This finding is consistent with these ligands being surmountable competitors of the noribogaine and other tested agonists' binding site. Functional inhibition constants (K_e) of the antagonists are shown in Table 3 with the assumption of ideal conditions of competitiveness and equilibrium. In all instances, the functional inhibition constants for these inhibitors were close to their K_i, regardless of the agonist they were competing with, indicating that noribogaine was no different than other agonists tested.

3.4. Noribogaine is an atypically weak functional competitor of other kappa agonists in the [^{35}S]GTPγS binding assay

Nalmefene is a partial agonist and can compete as a rival agonist against more efficacious agonists and inhibits their response up to the reduced efficacy of nalmefene itself, a pharmacologically

Table 2
A/B: Noribogaine modulation of [^{35}S]GTPγS binding (A) and β-Arrestin 2 recruitment (B) in CHO–K1 stably expressing human OPRK. The maximal stimulation (E_{Max}) and the functional activation constant (EC_{50}) of tested ligands dynorphin A (Dyn-A), U69,593, morphine, noribogaine, nalmefene, ibogaine, 18-MC, nor-BNI, and naloxone were measured using non-linear regression analysis. The functional inhibition constant (K_e) for GTPγS assay was calculated using EC_{50} shifts with the equation: $K_e = [Inhibitor]/(DR-1)$, where DR is the ratio of the agonist EC_{50} in the presence and the absence of inhibitor or from concentration-inhibition curves in the presence of agonist at EC_{80} using a modified Cheng-Prusoff equation: $K_e = [IC_{50}]/(1 + [agonist]/EC_{50})$. Coupling efficiency (e-coupling, e-cpl) indicated the relationship between the apparent binding affinity (K_i) versus the EC_{50} or the functional inhibition potency (K_e) of a given ligand and used the equations $p(Ki/EC_{50})$ and $p(Ki/K_e)$, where p represents a −log10 transformation. [ϘagonistΘ] represents the agonist used to test inhibitory compounds. Outliers in comparison to reference compounds dynorphin A and U69,593 (activation), and nor-BNI and naloxone (inhibition) are underlined. Data are shown as the mean ± SE of (n) experiments.

OPRK	pKi	[35S]-GTPγS binding				
		Activation			Inhibition [ϘDyn-AΘ]	
Ligands\outputs		E_{Max} in % ± s.e. (n)	EC_{50} in nM ± s.e. (n)	pEC_{50}/[e-cpl]	K_e in nM ± s.e. (n)	pK_e / [e-cpl]
Dynorphin A	10.3	94 ± 7 (3)	0.18 ± 0.04 (6)	9.7/[0.6]	n/q	n/q
U69,593	9.2	100 (9)	7.25 ± 0.9 (9)	8.1/[1.1]	n/q	n/q
Morphine	7.3	91 ± 7 (3)	434 ± 67 (4)	6.4/[0.9]	n/q	n/q
Noribogaine	6.1	72 ± 3.8 (14)	8749 ± 1092 (10)	5.1/[1.0]	39797 ± 15560 (2)	4.4/[1.7]
6′-GNTI[a]	8.9	37 ± 2	2.1 ± 0.5	8.7/[0.2]	0.18	9.7/[−0.8]
Nalmefene	10.1	35 ± 4.7 (3)	0.69 ± 0.14 (3)	9.2/[0.9]	0.077 ± 0.016 (4)	10.1/[0]
Ibogaine	5.4	18 ± 1.4 (2)	~12000 (2)	~4.9/[0.5]	6803 ± 250 (2)	5.2/[0.2]
18-MC	5.7	<5 (2)	n/q	n/q	2222 ± 257 (4)	5.6/[0.1]
Nor-BNI	10.4	<5	n/q	n/q	0.029 ± 0.004 (7)	10.5/[−0.1]
Naloxone	8.6	~10	n/q	n/q	4.85 ± 0.95 (5)	8.3/[0.3]
		PathHunter® β-arrestin recruitment				
Dynorphin A	10.3	100	11 ± 2 (3)	8.0/[2.3]	n/q	n/q
U69,593[a]	9.2	100	59	7.2/[2.0]	n/q	n/q
Noribogaine	6.1	13 ± 3 (1)	~110 (1)	~−7/[−0.9]	Non-competitive IC_{50} = 1000 ± 160 (5)	6/[0.1]
6′-GNTI[a]	8.9	12 ± 3	5.9 ± 3.3	8.2/[0.7]	0.56	9.3/[−0.4]
Nalmefene	10.1	31 ± 2 (1)	~0.3 (1)	−9.5/[0.6]	0.32	9.5/[0.6]
Ibogaine	5.4	~21 (1)			~11212	−5/[0.4]
18-MC	5.7	~−12 (1)		>	~10303	−5/[0.7]
Nor-BNI	10.4	<5	n/q	n/q	0.13 ± 0.006 (3)	9.9/[0.5]

n/q non-quantifiable. n/d not determined.
[a] 6′-GNTI values are from Schmid et al. (2013) and K_e was calculated from a [ϘU69,593Θ] IC_{50} value.

Table 3
Functional inhibition constants K_e of noribogaine and other ligands against agonist-induced [^{35}S]GTPγS binding in CHO–K1 stably expressing human OPRK. K_e values collected from the non-linear regression analysis of concentration-response EC_{50}-shift experimental designs of 3–7 experiments are shown as the mean ± SE in nM. [♀agonist] represents the agonist used to test inhibitory compounds. Italic value represents the estimate of a hypothetical functional activation constant of designated agonist in the presence of other agonists. Outliers are underlined. Graphical representation of K_e values against dynorphin A is shown in Fig. 3C.

Antagonists & rival agonists	[♀Agonists]						
	U69,593	Dynorphin A	Morphine	Noribogaine	Nalmefene	K_i	EC_{50}
U69,593	n/q	n/q	n/d	*0.4*	*4*	0.9	7.3
Dynorphin A	n/q	n/q	n/q	*0.003*	*0.1*	0.05	0.18
Morphine	n/d	n/a	n/q	*74*	*270*	47	434
Noribogaine	12e3 ± 0.8e3	40e3 ± 16e3	15e3 ± 4e3	n/q	24e3	700	8.7e3
Nalmefene	0.14 ± 0.04	0.077 ± 0.016	0.11 ± 0.005	0.33 ± 0.07	n/q	0.08	0.7
Naloxone	8.6 ± 1.3	4.8 ± 0.9	8.2 ± 1.2	4.2 ± 2.3	9.2	2.5	n/q
Nor-BNI	0.12 ± 0.04	0.029 ± 0.004	0.07 ± 0.013	0.075 ± 0.036	0.1 ± 0.09	0.04	n/q
18-MC	4.5e3 ± 1.4e3	2.8e3 ± 0.6e3	2.9e3 ± 0.7e3	4.3e3 ± 1.9e3	n/d	1.8e3	n/q

n/q non-quantifiable. n/d not determined.

coherent phenomenon also known as mixed agonism/antagonism property. Noribogaine was a partial agonist at OPRK in the [^{35}S]GTPγS binding stimulation assays (Fig. 2). Therefore, we set out to determine if noribogaine was also a rival agonist that can functionally compete with and reduce the activity of more efficacious agonists than itself.

Dynorphin A and morphine concentration-response curves were performed in the presence and absence of rival agonists nalmefene or noribogaine at concentrations of 36-fold and 79-fold their respective K_i (nalmefene 3 nM, noribogaine 50 μM) (Fig. 3A). Nalmefene readily shifted the EC_{50} of dynorphin A and morphine to the right, with a functional inhibition constant K_e of 0.077 ± 0.016 nM and 0.11 ± 0.005 nM. Calculated K_e were within a close range of the Ki (0.08 nM), similar to a competitive antagonist such as nor-BNI or naloxone (Table 3, Fig. 3A). Noribogaine, on the other hand, poorly shifted the EC_{50} of these agonists and the K_e estimates in these conditions were 40 ± 16 μM and 15 ± 4 μM respectively, about 40-fold its K_i (Table 3, Fig. 3A). Noribogaine against dynorphin A, U69,593, morphine, and nalmefene, was an outlier rival agonist in all instances compared to the typical partial agonist nalmefene and antagonists like naloxone, nor-BNI, and 18-MC (Table 3, underlined values, Fig. 3C). These data showed that noribogaine was an atypical partial kappa agonist because it was unable to functionally surmount receptor activation of other agonists of the OPRK G-protein pathway.

In another set of experiments (Fig. 3B), noribogaine and nalmefene concentration-inhibition curves were produced in the presence of more efficacious agonists such as U69,593, dynorphin A, or morphine. In the case of nalmefene, noribogaine was used as the agonist. Nalmefene readily reduced the signal of moderate to high concentrations of rival full (U69,593) or partial (noribogaine) agonists to its own reduced levels (30%) with an apparent IC_{50}

Table 4
Bias quantification at the kappa and mu opioid receptors. E-coupling for activation and inhibition is a measure of the coupling efficiency, using pKi-pEC_{50} and pKi-pK_e, where p is −log10 transformation. Bias-coupling represents the relative preference of coupling between the G-protein or β-arrestin pathways and used the difference in e-coupling corrected by the intrinsic assay bias (as measured with a known unbiased reference ligand). Underlined cells represent the reference compound for the values of the coupling bias. Bias-efficacy represents the relative efficacy to preferentially activate or inhibit the G-protein or β-arrestin pathways and used the ratio E_{Max}(G-protein)/E_{Max}(β-arrestin) for activation and I_{Max}(G-protein)/I_{Max}(β-arrestin) for inhibition. Italics are estimate only.

OPRK	G-protein Pathway		Beta-Arrestin2 Pathway		Bias G-protein Vs β-arrestin	
Activation	e-coupling	e-signal	e-coupling	e-signal	Bias-coupling	Bias-efficacy
Dynorphin A	0.6	0.94	2.3	1	6.3 vs 1	1 vs 1.06
U69,593	1.1	1	2	1	1 vs 1	1 vs 1
Morphine	0.9	0.91	n/d	n/d	n/d	n/d
Noribogaine	1.0	0.72	−0.9	0.13	1 vs 630	5.5 vs 1
6′-GNTI	0.2	0.37	0.7	0.12	1 vs 2.5	3.1 vs 1
Nalmefene	0.9	0.35	*0.6*	0.31	*1 vs 1.5*	1.13 vs 1
Ibogaine	0.5	0.18	n/q	0.21	n/d	1 vs 1.16
Inhibition	e-coupling	e-signal	e-coupling	e-signal	Bias-coupling	Bias-efficacy
Noribogaine	1.7	0.3	0.1	0.6	1 vs 158	1 vs 2
6′-GNTI	−0.8	0.32	−0.4	0.69	1 vs 1.6	1 vs 2.15
Nalmefene	0.0	0.7	*0.6*	0.7	*1 vs 1*	1 vs 1
Ibogaine	0.2	0.8	*0.4*	*1*	*1 vs 2.5*	*1.25 vs 1*
18-MC	0.1	1.0	*0.7*	*1*	*1 vs 1*	*1 vs 1*
Nor-BNI	−0.1	1.0	0.5	1	1 vs 1	1 vs 1

OPRM	G-protein Pathway		Beta-Arrestin2 Pathway		Bias G-protein Vs β-arrestin	
Activation	e-coupling	e-signal	e-coupling	e-signal	Bias-coupling	Bias-efficacy
[Met]-Enk	1.2	1.04	2.4	1	1 vs 1	1.04 vs 1
DAMGO	1.5	1	2.3	1.02	1 vs 2.5	1 vs 1.02
Morphine	1.5	0.8	2.6	0.79	1 vs 1.25	1.01 vs 1
Buprenorphine	n/d	0.26	n/q	n/d	n/q	n/d
Noribogaine	1	0.094	n/q	0.03	n/q	n/q
Inhibition	e-coupling	e-signal	e-coupling	e-signal	Bias-coupling	Bias-efficacy
Noribogaine	1.1	1	1.1	1	1 vs 1	1 vs 1

Table 5
Noribogaine modulation of [^{35}S]GTPγS binding (A) and β-Arrestin 2 recruitment (B) in CHO–K1 stably expressing human OPRM. The maximal stimulation E_{Max} and the functional activation constant EC_{50} of tested ligands [met]-enkephalin (Met-EnK), DAMGO, morphine, noribogaine, ibogaine, 18-MC, and naloxone were calculated using non-linear regression analysis. The functional inhibition constant K_e for GTPγS assays was calculated using EC_{50} shifts with the equation: $K_e = [Inhibitor]/(DR-1)$ where DR is the ratio of the agonist EC_{50} in the presence and the absence of inhibitor. For β-Arrestin assays, K_e was calculated from concentration-inhibition curves in the presence of agonist at EC_{80} using a modified Cheng-Prusoff equation: $K_e = [IC_{50}]/(1 + [agonist]/EC_{50})$. Coupling efficiency (e-coupling, e-cpl) indicated the relationship between the apparent binding affinity K_i versus the apparent functional activation potency (EC_{50}) or the functional inhibition potency (K_e) of a given ligand and used the equations $pKi\text{-}pEC_{50}$ and $pKi\text{-}pK_e$ where p represents a −log10 transformation.[♀agonist] represents the agonist that was used to produce concentration-response curves in the presence and the absence of tested inhibitory compounds noribogaine, ibogaine, naloxone, and 18-MC. Data are shown as the mean ± SE of (n) experiments.

OPRM	pKi	[35S]-GTPγS binding				
		Activation			Inhibition [♀Met-ENK]	
Ligands \ outputs		E_{Max} in % ± s.e. (n)	EC_{50} in nM ± s.e. (n)	pEC_{50}/[e-cpl]	K_e in nM ± s.e. (n)	pK_e/[e-cpl]
[Met]-Enk	9.2	104 ± 6 (3)	11 ± 3 (4)	8.0/[1.2]	n/q	n/q
DAMGO	9.1	100 (9)	27 ± 7 (9)	7.6/[1.5]	n/q	n/q
Morphine	9.0	80 ± 4.5 (4)	32 ± 1.2 (3)	7.5/[1.5]	n/d	n/d
Buprenorphine	10.1	26 ± 2.2 (2)	n/d.	n/d	n/d	n/d
Noribogaine[a]	5.8	9.4 ± 1.8 (4)	16050 ± 9409 (4)	4.8/[1]	19846 ± 3980 (4)	4.7/[1.1]
Ibogaine[b]	5.2	<5 (2)	n/q	n/q	7957 ± 2629 (4)	5.1/[0.1]
18-MC	5.6	<5 (2)	n/q	n/q	5831 ± 1418 (3)	5.2/[0.4]
Naloxone[c]	8.9	<5	n/q	n/q	1.2 ± 0.3 (2)	8.9/[0.0]
		PathHunter® β-Arrestin Recruitment				
[Met]-Enk	9.2	100	159 ± 38 (4)	6.8/[2.4]		
DAMGO[d]	9.1	102	162	6.8/[2.3]		
Morphine[d]	9.0	79	411	6.4/[2.6]		
Noribogaine	5.8	3 ± 0.5 (1)	n/q		20040 (1)	4.7/[1.1]

n/q non-quantifiable. n/d not determined.
[a] K_e values of noribogaine were [♀DAMGO] 19203 ± 5168 nM (n = 2) and [♀Morphine] 28467 ± 14439 nM (n = 2).
[b] K_e values of ibogaine was [♀DAMGO] 2614 ± 42.07 (n = 2).
[c] K_e value of naloxone was [♀DAMGO] 3.4 ± 0.75 (n = 5). Antonio et al. (2013) reported K_e [♀DAMGO] values of 38300 ± 1710 nM for noribogaine, 1940 ± 460 nM for ibogaine, and 19100 ± 2970 nM for 18-MC.
[d] PathHuner Assay Kit reference compounds.

proportional to the rival agonist concentration (including noribogaine) (Fig. 3B) and a K_e consistent with its K_i (Table 3). Noribogaine reduced the signal of more efficacious agonists to its own signal (70%), but the IC_{50} values were high (100–300 μM range) and the calculated K_e were consistent with poor functional inhibition. The apparent functional activation constant (apparent EC_{50}), of dynorphin A, U69,593 and morphine were then estimated in the presence of noribogaine or nalmefene (Table 3). Apparent EC_{50} for all agonists tested in the presence of nalmefene were close to their experimental EC_{50}. However, in the presence of noribogaine, apparent EC_{50} of common kappa agonists were significantly lower than their actual experimental EC_{50} (Table 3, underlined values). This atypical phenomenon may reflect the stabilization of a differential set of active receptor conformations by noribogaine compared to other kappa agonists tested.

Altogether, these data suggest a potential protean agonism of noribogaine, a predictive functional bias (Kenakin, 2007) that we set to assess further.

3.5. Noribogaine is a G-protein-biased kappa agonist

PathHunter β-Arrestin GPCR assays detecting the interaction of β-arrestin 2 with the activated receptor were used to measure non-G protein OPRK activity in CHO–K1 live cells (Violin et al., 2014). Concentration-response curves to noribogaine were compared to full endogenous agonist dynorphin A drug treatment (Fig. 4A). Calculated EC_{50} values, maximal responses and coupling efficiencies are shown in Table 2B. Dynorphin A displayed an EC_{50} of 11 ± 2 nM at OPRK. Noribogaine exhibited a profound functional bias at OPRK and was marginally efficacious at inducing β-arrestin recruitment with an E_{Max} of 13 ± 3% and an estimated EC_{50} of 110 nM. For comparative purposes, nalmefene was tested once and displayed an E_{Max} of 30% as expected for this unbiased partial agonist. EC_{50} and E_{Max} values from (Schmid et al., 2013) were collected for 6′-GNTI, and showed that noribogaine was activating at the same level as 6′-GNTI. Ibogaine and 18-MC were tested once; they did not display any bias and their pharmacological behavior was comparable to typical kappa ligands.

The functional activation coupling efficiencies in the β-arrestin assay were calculated in a similar manner as the [35S]GTPγS binding assay and showed that dynorphin A and U69,593 displayed a similar coupling efficiency of 2, reflecting the intrinsic assay efficiency (Table 2B, Fig. 4C). Noribogaine had a negative activation coupling efficiency of −0.9 for the β-arrestin pathway, indicating a profound coupling bias of 1:630 in favor of this pathway in comparison to the G-protein pathway (Table 4). In comparison, the biased ligand 6′-GNTI did not display activation coupling bias between the G-protein and the β-arrestin pathways (Table 4).

The activation efficacy bias between the G-protein pathway and the β-arrestin pathway was evaluated by comparing the maximal efficacy (E_{Max}) for each pathway in comparison to reference ligand dynorphin A or U69,593 (1–1 maximal efficacies ratio) (Table 4). Although nalmefene is a partial agonist, it did not display an activation efficacy bias, reflecting that partial agonism is not an indication of bias per se. Both noribogaine and 6′-GNTI displayed an activation efficacy bias in favor of the G-protein pathway and noribogaine was almost twice more biased than 6′-GNTI (5.5–1 versus 3 to 1).

3.6. Noribogaine is a β–arrestin-biased kappa antagonist

Because noribogaine was unable to induce the recruitment of β-arrestin following activation of the OPRK receptors, it was then tested for its ability to inhibit β-arrestin recruitment induced by efficacious agonists of this pathway (Fig. 4B). In these assays, β-arrestin recruitment was induced by the endogenous agonist dynorphin A sets at its EC_{80} concentration and challenged with increasing concentrations of noribogaine. Noribogaine inhibited dynorphin A-induced β-arrestin recruitment up to ~60%, with an IC_{50} of 1 ± 0.16 μM (Fig. 4B, Table 2B). In contrast, when a similar

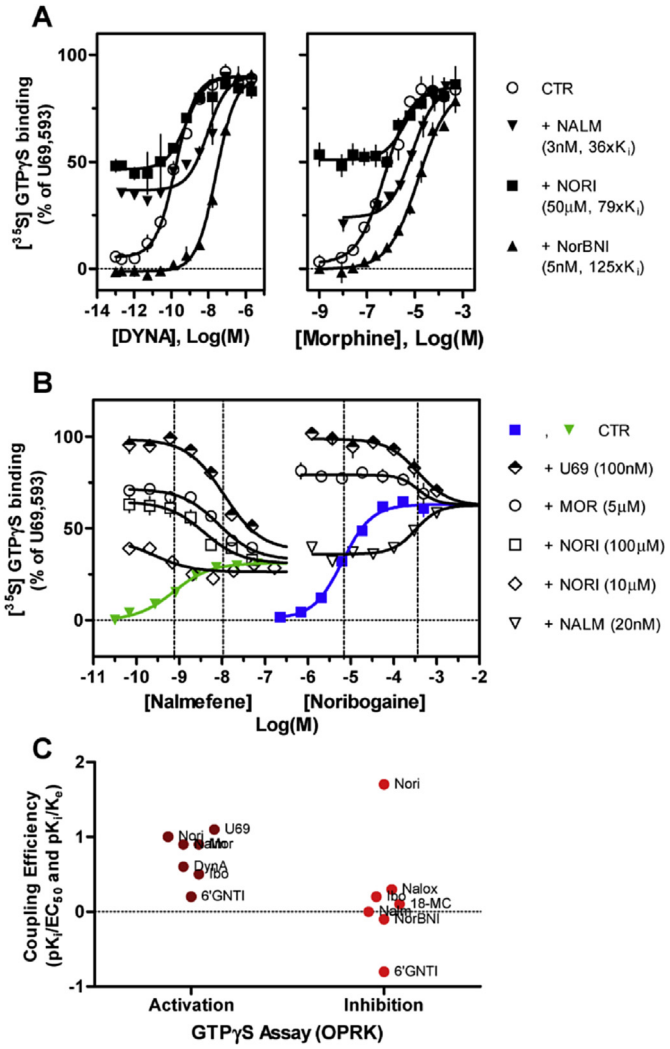

Fig. 3. Comparative inhibitory effects of partial agonists noribogaine and nalmefene on agonist-induced [35S]GTPγS binding at the kappa opioid receptors (OPRK). (A) CHO–K1 cell membrane preparations expressing the OPRK receptors were pre-incubated with the partial agonists nalmefene (NALM) and noribogaine (NORI), and the pure antagonist nor-BNI at 36-fold, 79-fold, and 125-fold their respective apparent affinity K_i. Membrane preparations were then stimulated with increasing concentrations of dynorphin A (DYNA) and morphine (MOR). The functional inhibition constants K_e were calculated and compared to the ligand's respective K_i and EC_{50} (GTPγS) as shown in Table 3. (B) Membrane preparations were stimulated by increasing concentrations of NALM or NORI in the presence of agonists U69,593 (U69- 100 nM), morphine (MOR- 5 μM), noribogaine (NORI- 10 and 100 μM) and nalmefene (NALM- 20 nM). Dotted lines indicate the interval between the EC_{50} and the IC_{50} of tested ligand (NALM and NORI) in the absence and the presence of 100 nM U69,593. Data are shown as the mean ± SEM of representative experiment(s) performed in triplicate. (C) Representation of the G-protein pathway coupling activation efficiency ($-\log(K_i/EC_{50})$) of a series of kappa agonists and of the coupling inhibition efficiency ($-\log(K_i/K_e)$) against dynorphin A (see Table 3 for other agonists) for a series of kappa antagonists and partial agonists. Clustering of data points represents the intrinsic assay efficiency in the current experimental conditions of [35S]GTPγS binding activation with CHO membranes expressing the human OPRK. Outlier data points indicate atypical pharmacology of certain ligands (noribogaine, 6′-GNTI).

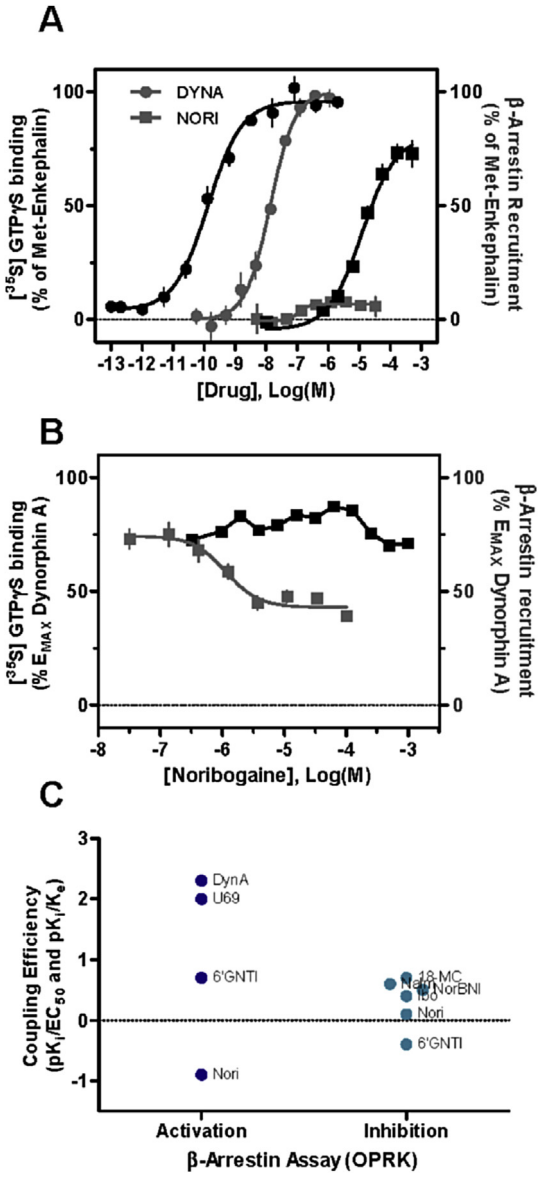

Fig. 4. Noribogaine is a G-protein biased agonist and a β-arrestin biased antagonist of the kappa opioid receptors (OPRK). CHO–K1 cell membrane preparations expressing the human OPRK receptors were used for [35S]GTPγS binding assays and live cells were used for PathHunter^R β-arrestin 2 recruitment assays. (A) Comparative concentration-response curves of dynorphin A (DYNA) and noribogaine (NORI) in [35S] GTPγS binding assay (black) and PathHunter^R β-arrestin 2 recruitment assay (grey). (B) Comparative noribogaine concentration-inhibition curves of DYNA set at its EC_{80} in [35S]GTPγS binding assay (black) and PathHunter^R β-arrestin 2 recruitment assay (grey). (C) Representation of the β-arrestin pathway coupling activation efficiency for a series of kappa agonists ($-\log(K_i/EC_{50})$) and of the coupling inhibition efficiency against DYNA for a series of kappa antagonists and partial agonists ($-\log(K_i/K_e)$). Clustering of data points represents the intrinsic assay efficiency in the current experimental conditions of β-arrestin recruitment assay with CHO cells expressing the human OPRK. Outlier data points (noribogaine, 6′GNTI) indicate atypical pharmacology of certain ligands.

noribogaine concentration-inhibition experiment was performed on the G-protein pathway with dynorphin A at its EC_{80}, noribogaine only inhibited agonist-induced activation of the receptor by ~5% with an IC_{50} of ~150 μM (Fig. 4B). Noribogaine was thus 150-fold more potent at inhibiting dynorphin A-induced β-arrestin 2 recruitment than at inhibiting dynorphin A-induced G-protein activation (Table 4). For comparison, typical partial agonist nalmefene inhibited dynorphin A-induced β-arrestin recruitment up to 70% with an approximate K_e of 0.32 nM, whereas it inhibited dynorphin A-induced GTP-binding with a K_e of 0.08 nM and was apparently equipotent with only a 4-fold difference. Notably, the apparent inhibition coupling efficiencies for antagonists of the β-arrestin pathway were all clustered in a 0.5 range (Fig. 4C) and indicated that the current experimental conditions were producing an assay slightly less sensitive to antagonists than the GTP-binding assay (coupling efficiency close to 0) with a robust assay's intrinsic

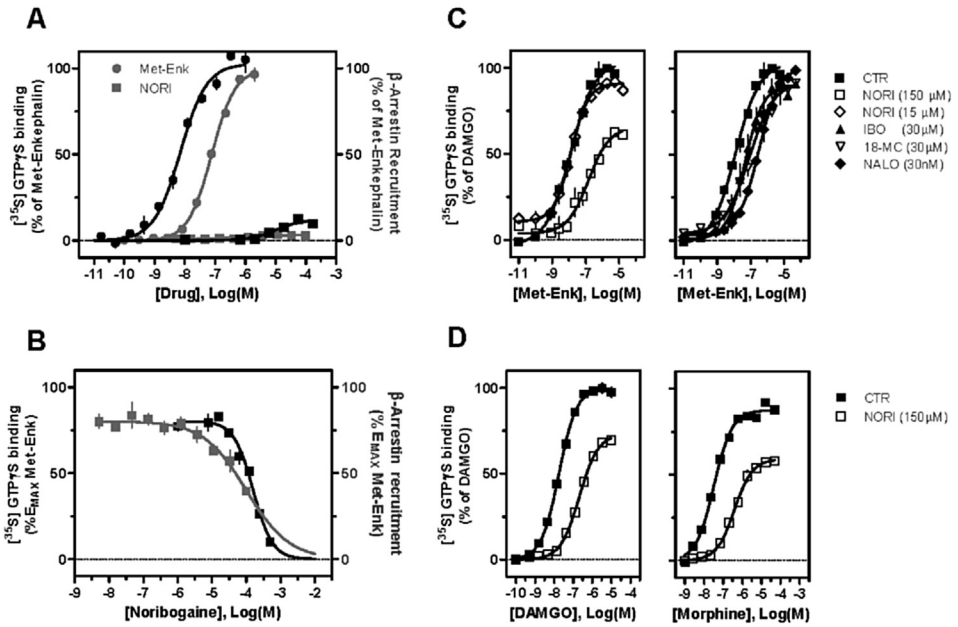

Fig. 5. Noribogaine is a weak antagonist of [^{35}S]GTPγS binding stimulation and β-arrestin recruitment at the mu receptor (**OPRM**). CHO–K1 cell membrane preparations expressing the human OPRM receptors were used for [^{35}S]GTPγS binding assays and live cells were used for PathHunter^{R} β-arrestin 2 recruitment assays. Receptors were stimulated by increasing concentration of agonists. (A) Comparative concentration-response curves of [met]-enkephalin (Met-Enk) and noribogaine (NORI) in [^{35}S]GTPγS binding assay (black) and PathHunter^{R} β-arrestin 2 recruitment assay (grey). (B) Comparative noribogaine concentration-inhibition curves of [met]-enkephalin set at its EC_{80} in [^{35}S]GTPγS binding assay (black) and PathHunter^{R} β-arrestin 2 recruitment assay (grey). (C, D) Concentration-response curves and EC_{50} shifts of [met]-enkephalin (Met-Enk), DAMGO and morphine in the presence and absence of 15 and 150 μM noribogaine (NORI), 30 μM ibogaine (IBO), 30 μM 18-MC, and 30 nM naloxone (NALO). (A–D) Data points used for the non-linear regression analysis are shown as the mean ± SEM of each representative experiment(s). Corresponding values of EC_{50}, E_{Max}, and K_e with mean ± SE for the entire set of experiments can be found in Table 5.

coupling efficiency reproducibility. Finally, the inhibition efficacy bias for noribogaine was 1:2 in favor of the β-arrestin pathway, which is similar to 6′-GNTI (Table 4), whereas other antagonists were unbiased.

3.7. Noribogaine has a marginal mu agonistic activity

[^{35}S]GTPγS binding to membrane preparations of CHO cells stably expressing human OPRM were examined in response to noribogaine, ibogaine, and morphine drug treatment (Fig. 5A, Table 5A). The prototypical full agonist DAMGO and the endogenous agonist [met]-enkephalin ([met]-Enk) were used as reference agonists. Morphine was a partial agonist with an E_{Max} of 80 ± 4.5% and an EC_{50} of 32 ± 1.2 nM. The partial agonist buprenorphine stimulated OPRM with an E_{Max} of 26 ± 2.2% in our assays and in previously reported assays (Saidak et al., 2006). Noribogaine marginally stimulated [^{35}S]GTPγS binding to OPRM, with an E_{Max} of 10% of the full agonist DAMGO or [met]-enkephalin (Fig. 5A, Table 5A) and comparable to the level of activation previously reported (Antonio et al., 2013). Ibogaine and 18-MC did not stimulate the OPRM G-protein pathway. Concentration-response curves of β-arrestin recruitment of noribogaine at the OPRM receptors were compared to full agonist [met]-enkephalin (Fig. 5A). [Met]-enkephalin displayed an EC_{50} of 159 ± 38 nM at OPRM. As expected given its marginal efficacy at the G-protein pathway, noribogaine was not a mu agonist of the β-arrestin pathway. Calculated EC_{50} values, maximal responses and coupling efficiencies are shown in Table 5.

3.8. Weak antagonistic properties of noribogaine at the mu opioid receptor

Noribogaine marginally stimulated [^{35}S]GTPγS binding via OPRM with an approximate EC_{50} of 16 μM (Fig. 5A). Therefore, we investigated if noribogaine was an antagonist of OPRM. [Met]-enkephalin, DAMGO and morphine dose responses were carried out in the presence and absence of 15 and 150 μM of noribogaine (Fig. 5C and D). Noribogaine was an inhibitor of all agonists tested and shifted their EC_{50} to the right by a magnitude of ~1–log. The calculated functional inhibition constant (K_e) values were ~20 μM (Table 5A, and Table 5 footer). In a similar design, naloxone displayed a K_e of 1.2 ± 0.3 nM, a value close to its K_i at OPRM, like its inhibition constant at OPRK (Tables 2 and 3). On the contrary, noribogaine was an outlier antagonist with an inhibitory coupling efficiency of 1.1, while ibogaine, naloxone, and 18-MC were still behaving like typical antagonists with clustered e-coupling values close to 0 (Fig. 5C and Table 5A). Noribogaine was then tested for its ability to inhibit [met]-enkephalin-induced β-arrestin-2 recruitment at OPRM (Fig. 5B and Table 5B). Noribogaine inhibited agonist responses by 80–100% with an IC_{50} of 100 ± 25 μM.

Regardless of the pathway tested (G-protein and β-arrestin pathways), noribogaine displayed similar functional inhibition constants and was an unbiased ligand of the mu opioid receptor (Table 4), albeit an inhibitory coupling efficiency outlier (Table 5A/B) in comparison to other mu antagonists. In fact, noribogaine also decreased the E_{Max} of [met]-enkephalin, DAMGO and morphine (Fig. 5C and D), indicating a degree of unsurmountable antagonism by noribogaine in functional assays. Apparent unsurmountable antagonism can encompass several distinct molecular mechanisms such as (a) irreversible competitive antagonism, (b) noncompetitive antagonism, or (c) functional antagonism; for review (Neubig et al., 2003).

3.9. Binding model of noribogaine and ibogaine to the inactive conformation of the mu opioid receptor

Noribogaine showed a profile of unbiased antagonist at the OPRM and stabilized the inactive conformation of the receptor.

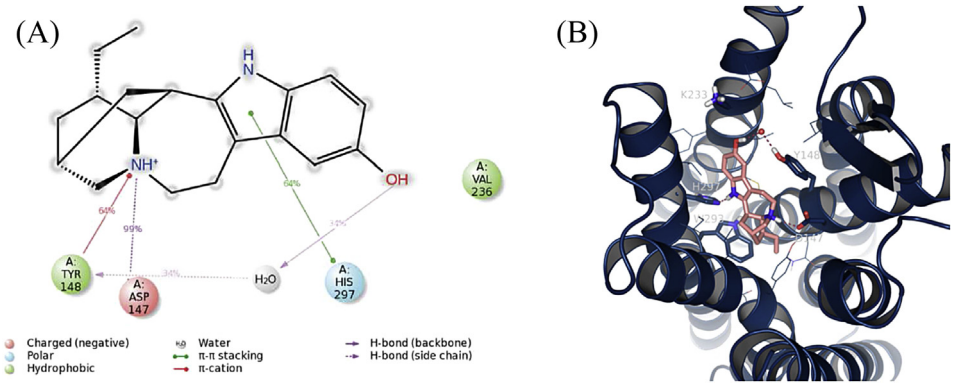

Fig. 6. Ligand-protein binding contacts of noribogaine with OPMR. (A) Schematic of detailed ligand atom interactions with the protein residues. Interactions that occur more than 30.0% of the simulation time in the selected trajectory (0.00 through 12.00 ns), are shown (Note: it is possible to have interactions with >100% as some residues may have multiple interactions of a single type with the same ligand atom. over a 12 ns molecular dynamics simulation). (B) Binding model of noribogaine in OPMR extracted from a molecular dynamics simulation (see text, compare to A).

Thus, it was possible to use an available crystal structure of the inactive form of the OPRM to perform *in silico* binding experiments with noribogaine.

We developed an *in silico* binding model based on the mouse OPRM co-crystal structure [PMID 22437502] (Manglik et al., 2012) as described in methods. The mouse and human OPRM share 94% (global) sequence identity and all binding site residues are identical. After initial optimization of the model, the top docking poses of noribogaine and ibogaine were pharmacophorically aligned with the co-crystal morphinan antagonist as one would expect: the hydrophobic ibogaine and noribogaine bicyclic system and ethyl substituent with morphinan cyclopropyl residues were spatially aligned and the positively charged tertiary amines were superimposed with each forming a hydrogen bond to the site chain of Asp147. Then, the noribogaine and ibogaine OPRM complexes were each used in a 12 ns all atom explicit water molecular dynamics simulation (see methods). Trajectory analysis revealed the most prevalent interactions of noribogaine (Fig. 6A) and ibogaine. Both ligands formed a stable hydrogen bond with Asp147 via their tertiary amine. Noribogaine and ibogaine formed pi-cation interaction with Tyr148 (64% and 56%, respectively), and hydrophobic interactions with His297 (64 and 93%, respectively). Further hydrophobic interactions were observed between Val236 (~40 and ~60%, respectively), Tyr326 (~20 and ~40% respectively), Met151 (~20% and ~30%, respectively) and also Trp293, Ile296, Val300. Characteristically, noribogaine, but not ibogaine, formed a water bridge with Tyr148 for 34% of the simulation time. Both ligands showed a hydrogen bond with His297 for about 20% of the simulation. Movies of the simulations were generated and are available as supporting material. A representative illustration frame of noribogaine in the OPRM was extracted from the simulation and is shown in Fig. 6B.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.neuropharm.2015.08.032.

4. Discussion

Historically, *in vivo* studies excluded the possibility of prototypical morphine-like agonistic mechanism of ibogaine and its metabolite noribogaine at the mu and/or kappa opioid receptors (see introduction). In the other hand, naloxone-like antagonistic mechanisms could not be demonstrated either. These ambiguous results lead to a gap of knowledge between the correlation of receptor-ligand mechanistics and the intriguing and beneficial effects of ibogaine and its metabolite noribogaine with opiate drugs and to the opioid system. Our current study now demonstrates that noribogaine is in fact a G-protein biased kappa agonist, and a weaker mu antagonist. The described potencies in our study closely match physiologically relevant brain concentrations of therapeutically effective doses of noribogaine. Thus, noribogaine's atypical modulatory properties of the kappa opioid system, and to a certain extend the mu opioid system, in the central nervous system (CNS) must be taken into account for its mechanism of action *in vivo*.

Our study shows that noribogaine is a kappa agonist. Intravenous noribogaine potently triggered the release of prolactin in rats and it was reported that this effect was centrally mediated (Baumann et al., 2001b). We propose that noribogaine-induced prolactin release is mediated by central OPRK, similar to what was described for the kappa agonist nalmefene (Bart et al., 2005). Our data also show that noribogaine was a moderately potent mu antagonist. Thus, noribogaine also belongs to the class of dual agonist/antagonist kappa-mu opioid ligands. In comparison, ibogaine is a more potent mu antagonist and a much weaker kappa agonist than noribogaine. Ibogaine is metabolized to noribogaine in mammals and, as a consequence, the acute and delayed biological effects of ibogaine treatment can be attributed to the ibogaine-noribogaine mixture rather than to ibogaine only (Zubaran et al., 1999). Intermediate levels of drug-related interoceptive stimuli generalization were observed with the mixed action opiates SKF10,047 (79%), pentazocine (74%), nalorphine (70%), and diprenorphine (75%) (Helsley et al., 1998). These results provide support to the notion that the ibogaine-noribogaine mixture could be recognized as a mixed opioid agonist-antagonist *in vivo*. 18-MC was a micro-molar non-specific competitive antagonist of the kappa and mu receptors; however it should remain clear that noribogaine belongs to a different class of opioid ligand than ibogaine or 18-MC. In consequence, the *in vivo* effects of 18-MC, experimentally demonstrated or hoped for, cannot rely on the pharmacological knowledge and demonstrated effects of noribogaine on the opioid system.

A series of studies (see introduction) described the biological effects of noribogaine treatment in reducing the stimulating and reinforcing properties of morphine, possessing a modest analgesic power on its own, and having the capability to potentiate morphine analgesia, especially in morphine tolerant animals. Our study shows that noribogaine inhibited β-arrestin-2 recruitment of both mu and kappa agonists. This could to a certain extent mimic the effects of a functional deletion of the β-arrestin-2 gene which resulted in remarkable potentiation and prolongation of the analgesic effect of morphine in mice (Bohn et al., 1999). Additionally, it

was shown that dynorphin A attenuated morphine tolerance at doses devoid of observable effects on nociception (Schmauss and Herz, 1987; Takemori et al., 1992). U50,488 also attenuated morphine tolerance and potentiated morphine analgesia in morphine tolerant animals at doses devoid of analgesic activity when co-administrated with morphine (Tao et al., 1994). Taken together, these studies show that low doses of OPRK agonists can reduce morphine tolerance and desensitization (Sharma and Mediratta, 2001). Future studies assessing the comparative activity of noribogaine in wild type and genetically modified opioid receptor animal models should provide further understanding of the contribution of the kappa receptors in the effects of noribogaine.

Ultra-low doses of opioid antagonists (naloxone and naltrexone), which selectively inhibit the excitatory effects of opioid agonists, have been reported to augment systemic morphine analgesia and inhibit the development of tolerance/physical dependence (Powell et al., 2002). *In vitro* assays demonstrated that these phenomena are reproducible at the cellular level where ultra-low concentrations of opioid ligand effectively synergized the binding and functional response of agonists via OPRM-OPRD heteromers (Gomes et al., 2011). In this regard, the moderate OPRM inhibitory activity of noribogaine at moderate to low therapeutic doses could be of biological relevance and warrants further investigation at the molecular level on OPRM-OPRD heteromers.

In line with their localization in the hippocampus, amygdala, hypothalamus, striatum and spinal cord, the function of the kappa opioid receptors are related to learning and memory, emotional control, stress response and pain (Bruchas et al., 2010; Butelman et al., 2012; Schwarzer, 2009). Kappa agonists hold therapeutic potential for mood and addiction disorders, psychiatric co-morbidities, and pain management, however they also induce undesirable on-target side effects such as place aversion, dysphoria and anhedonia; and hallucinations at high doses. On the other hand, kappa antagonists hold therapeutic potential as antidepressants and anxiolytics, but may induce hyperalgesic states. Thus, until recently, kappa ligands were not considered for therapeutic development due to their undesirable side effects. This view changed with the introduction of a more elaborate understanding of ligand-receptor pharmacology and the characterization of receptor drugs exhibiting functional selectivity, for review (Violin et al., 2014). As reviewed in (Kyle, 2013), certain opioid ligands were reported to be G protein-biased agonists, notably at the mu receptor, and exhibited interesting physiological properties (Rives et al., 2012).

Recent elegant studies in rodents have mechanistically linked the activation of p38 MAPK to stress-mediated OPRK stimulation via the β-arrestin mediated transduction pathway (Bruchas et al., 2007, 2006). In this frame, G-protein biased kappa agonists were described as hypothetical analgesic drugs without aversive and dysphoric components (Chavkin, 2011). In our study, noribogaine exhibited a profound functional bias and was not an agonist of the OPRK β-arrestin pathway. Therefore, noribogaine appears to carry the prerequisite pharmacological characteristics of an analgesic kappa opioid drug devoid of aversive and dysphoric effects which may become important during opiate detoxification and painful states.

Our study also shows that noribogaine is a β-arrestin biased kappa antagonist, more potent and efficacious at inhibiting agonist-induced signaling to this pathway than to the G-protein pathway. At a concentration corresponding to levels in the brain of rats (e.g. 0.5–5 μM) several hours after noribogaine dosing in animals (Pearl et al., 1997), or days after ibogaine treatment in humans (Mash et al., 2000), noribogaine tested *in vitro* preserved the signaling of dynorphin A to the G-protein pathway while markedly inhibiting β-arrestin recruitment. Thus noribogaine in the presence of dynorphin was able to modulate the functional selectivity of dynorphin. This peculiar pharmacological property could contribute to antagonist-like anti-depressive and anxiolytic activities against stress-induced and over-active dynorphin/kappa system, as seen during drug dependence, drug withdrawal and cravings. This functionally selective inhibitory kappa activity may also mediate positive effects against stress and anxiety, and atypical depression that will be considered in future studies.

Ligand-induced functional selectivity of otherwise unbiased agonists was previously demonstrated for some receptors of the GPCR family interactions with conformation-specific allosteric modulators of the receptors (i.e. the allosteric ligand LPI805 for the NK2 receptor (Maillet et al., 2007); for review (Kenakin, 2007)). However, in the present study noribogaine does not appear to be an allosteric ligand: 1) it directly competed with the binding of orthosteric radiolabeled agonists DAMGO, U69,593; 2) it displayed functional competitive behavior with opioid orthosteric antagonists in GTPγS assays; 3) it was docked to the morphinan orthosteric binding site of the OPRM inactive state *in silico* with good stringency: *in silico* binding experiments provide basis for noribogaine's interacting moieties with the inactive conformational state crystal structure of the mu receptor and *in silico* interaction scores of noribogaine and ibogaine are congruent to their affinity ranking in *in vitro* radioligand assays. In fact, our data suggest that noribogaine would induce functional selectivity to dynorphin A via the interplay of a set of active and inactive conformational states. Certain conformations would be easily accessible to other agonists (e.g. the inactive conformations and active G-protein conformations) and other conformations would be energetically challenging to populate in place of noribogaine (e.g. the non-recruiting β-arrestin conformation).

Multiple studies provide evidence for the existence of intermediate conformational states linking the inactive receptor to the fully active receptor. Agonist binding and activation of GPCRs has been proposed to occur through a multistep process; for review (Kobilka, 2004). The intermediate conformational states generated during multistep agonist binding may have unique functional properties as it is known that GPCR can couple to different G-proteins and also activate non-G protein dependent pathways depending on their conformational state(s); for review (Kenakin, 2007). Interestingly, recent investigations in drug design described an *allotropic* binding mode for certain OPRK agonists, which encompassed sequential drug–receptor interaction mechanisms (Munro et al., 2013). In this regard, noribogaine merits further investigation at a deeper molecular level using discrete drug–receptor interactions and conformational dynamic designs as well as cellular designs to assess further these potential allotropic binding modalities and their functional consequences.

5. Conclusion

This study shows that noribogaine is a dual ligand of both mu and kappa opioid receptors. Noribogaine exhibits a profound G-protein biased agonism at the opioid receptors and can modulate dynorphin signaling via the kappa receptor. This study clarifies the mechanisms of noribogaine at modulating the function of opioid receptors at the cellular level, providing ground for explanatory mechanisms at the opioid system *in vivo* as well as new avenues of therapeutic development and applicability.

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Declaration of conflict of interest

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Authors scientific contributions statement

E.L.M. and D.C.M. designed the study. E.L.M., S.C.S. wrote the manuscript. E.L.M., N.M., M.D.H. collected and analyzed data. N.M, E.L.M, M.D.H., J.F., N.G., performed experiments. S.C.S performed and analyzed computational simulations.

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References

Alper, K.R., 2001. Ibogaine: a review. Alkaloids Chem. Biol. 56, 1–38.
Alper, K.R., Lotsof, H.S., Frenken, G.M., Luciano, D.J., Bastiaans, J., 1999. Treatment of acute opioid withdrawal with ibogaine. Am. J. Addict. 8, 234–242.
Antonio, T., Childers, S.R., Rothman, R.B., Dersch, C.M., King, C., Kuehne, M., Bornmann, W.G., Eshleman, A.J., Janowsky, A., Simon, E.R., Reith, M.E., Alper, K., 2013. Effect of Iboga alkaloids on micro-opioid receptor-coupled G protein activation. PLoS One 8, e77262.
Bagal, A.A., Hough, L.B., Nalwalk, J.W., Glick, S.D., 1996. Modulation of morphine-induced antinociception by ibogaine and noribogaine. Brain Res. 741, 258–262.
Bart, G., Schluger, J.H., Borg, L., Ho, A., Bidlack, J.M., Kreek, M.J., 2005. Nalmefene induced elevation in serum prolactin in normal human volunteers: partial kappa opioid agonist activity? Neuropsychopharmacology 30, 2254–2262.
Baumann, M.H., Pablo, J., Ali, S.F., Rothman, R.B., Mash, D.C., 2001a. Comparative neuropharmacology of ibogaine and its O-desmethyl metabolite, noribogaine. Alkaloids Chem. Biol. 56, 79–113.
Baumann, M.H., Rothman, R.B., Pablo, J.P., Mash, D.C., 2001b. In vivo neurobiological effects of ibogaine and its O-desmethyl metabolite, 12-hydroxyibogamine (noribogaine), in rats. J. Pharmacol. Exp. Ther. 297, 531–539.
Bhargava, H.N., Cao, Y.J., 1997. Effects of noribogaine on the development of tolerance to antinociceptive action of morphine in mice. Brain Res. 771, 343–346.
Bhargava, H.N., Cao, Y.J., Zhao, G.M., 1997. Effects of ibogaine and noribogaine on the antinociceptive action of mu-, delta- and kappa-opioid receptor agonists in mice. Brain Res. 752, 234–238.
Bocher, D.P.M., Naranjo, C., 1969. Nouveau médicament agissant au niveau du système nerveux central utilisable dans les traitements psychothérapiques et comme antidrogue [A New CNS-active medication for use against Drugs]. Bull. Off. Propriété Ind. No. 35, France, P.V. n° 138.081, n° 7131M. Class. Int. A 61k. délivré le 21 juillet 1969.
Bohn, L.M., Lefkowitz, R.J., Gainetdinov, R.R., Peppel, K., Caron, M.G., Lin, F.T., 1999. Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 286, 2495–2498.
Bowen, W.D., Vilner, B.J., Williams, W., Bertha, C.M., Kuehne, M.E., Jacobson, A.E., 1995. Ibogaine and its congeners are sigma 2 receptor-selective ligands with moderate affinity. Eur. J. Pharmacol. 279, R1–R3.
Bruchas, M.R., Land, B.B., Aita, M., Xu, M., Barot, S.K., Li, S., Chavkin, C., 2007. Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. J. Neurosci. 27, 11614–11623.
Bruchas, M.R., Land, B.B., Chavkin, C., 2010. The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. Brain Res. 1314, 44–55.
Bruchas, M.R., Macey, T.A., Lowe, J.D., Chavkin, C., 2006. Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. J. Biol. Chem. 281, 18081–18089.
Butelman, E.R., Yuferov, V., Kreek, M.J., 2012. kappa-opioid receptor/dynorphin system: genetic and pharmacotherapeutic implications for addiction. Trends Neurosci. 35, 587–596.
Chang, Q., Hanania, T., Mash, D.C., Maillet, E.L., 2015. Noribogaine reduces nicotine self-administration in rats. J. Psychopharmacol. 29, 704–711.
Chavkin, C., 2011. The therapeutic potential of kappa-opioids for treatment of pain and addiction. Neuropsychopharmacology 36, 369–370.
Cheng, Y., Prusoff, W.H., 1973. Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.
Freedlander, J., 2003. Ibogaine: a novel anti-addictive compound. J. Drug Educ. Aware. 1, 79–98.
Glick, S.D., Kuehne, M.E., Maisonneuve, I.M., Bandarage, U.K., Molinari, H.H., 1996. 18-Methoxycoronaridine, a non-toxic iboga alkaloid congener: effects on morphine and cocaine self-administration and on mesolimbic dopamine release in rats. Brain Res. 719, 29–35.
Glick, S.D., Maisonneuve, I.M., Szumlinski, K.K., 2000. 18-Methoxycoronaridine (18-MC) and ibogaine: comparison of antiaddictive efficacy, toxicity, and mechanisms of action. Ann. N. Y. Acad. Sci. 914, 369–386.
Glue, P., Lockhart, M., Lam, F., Hung, N., Hung, C.T., Friedhoff, L., 2015. Ascending-dose study of noribogaine in healthy volunteers: pharmacokinetics, pharmacodynamics, safety, and tolerability. J. Clin. Pharmacol. 55, 189–194.
Gomes, I., Ijzerman, A.P., Ye, K., Maillet, E.L., Devi, L.A., 2011. G protein-coupled receptor heteromerization: a role in allosteric modulation of ligand binding. Mol. Pharmacol. 79, 1044–1052.
Goutarel, R., Golnhoffer, N., Siddens, R., 1993. Pharmacodynamics and therapeutic applications of iboga and ibogaine. (Translated from French by Gladstone WJ). Psychedelic Monogr. Essays 70–111.
Helsley, S., Filipink, R.A., Bowen, W.D., Rabin, R.A., Winter, J.C., 1998. The effects of sigma, PCP, and opiate receptor ligands in rats trained with ibogaine as a discriminative stimulus. Pharmacol. Biochem. Behav. 59, 495–503.
Huang, P., Kehner, G.B., Cowan, A., Liu-Chen, L.Y., 2001. Comparison of pharmacological activities of buprenorphine and norbuprenorphine: norbuprenorphine is a potent opioid agonist. J. Pharmacol. Exp. Ther. 297, 688–695.
Kenakin, T., 2007. Functional selectivity through protean and biased agonism: who steers the ship? Mol. Pharmacol. 72, 1393–1401.
Kobilka, B., 2004. Agonist binding: a multistep process. Mol. Pharmacol. 65, 1060–1062.
Kyle, J.D., 2013. Functionally Biased Agonism of Mu and Kappa Opioid Receptors. Research and Development of Opioid-related Ligands. American Chemical Society, pp. 177–197.
Li, S., Zhu, J., Chen, C., Chen, Y.W., Deriel, J.K., Ashby, B., Liu-Chen, L.Y., 1993. Molecular cloning and expression of a rat kappa opioid receptor. Biochem. J. 295 (Pt 3), 629–633.
Maciulaitis, R., Kontrimaviciute, V., Bressolle, F.M., Briedis, V., 2008. Ibogaine, an anti-addictive drug: pharmacology and time to go further in development. A narrative review. Hum. Exp. Toxicol. 27, 181–194.
Maillet, E.L., Pellegrini, N., Valant, C., Bucher, B., Hibert, M., Bourguignon, J.J., Galzi, J.L., 2007. A novel, conformation-specific allosteric inhibitor of the tachykinin NK2 receptor (NK2R) with functionally selective properties. FASEB J. 21, 2124–2134.
Manglik, A., Kruse, A.C., Kobilka, T.S., Thian, F.S., Mathiesen, J.M., Sunahara, R.K., Pardo, L., Weis, W.I., Kobilka, B.K., Granier, S., 2012. Crystal structure of the micro-opioid receptor bound to a morphinan antagonist. Nature 485, 321–326.
Mash, D.C., Kovera, C.A., Buck, B.E., Norenberg, M.D., Shapshak, P., Hearn, W.L., Sanchez-Ramos, J., 1998. Medication development of ibogaine as a pharmacotherapy for drug dependence. Ann. N. Y. Acad. Sci. 844, 274–292.
Mash, D.C., Kovera, C.A., Pablo, J., Tyndale, R.F., Ervin, F.D., Williams, I.C., Singleton, E.G., Mayor, M., 2000. Ibogaine: complex pharmacokinetics, concerns for safety, and preliminary efficacy measures. Ann. N. Y. Acad. Sci. 914, 394–401.
Mash, D.C., Schenk, S., 1996. Preclinical screening of an ibogaine metabolite (noribogaine) on cocaine-induced hyperlocomotion and cocaine self-administration. Soc. Neurosci. Abstr. 22, 1929.
Mash, D.C., Staley, J.K., Baumann, M.H., Rothman, R.B., Hearn, W.L., 1995a. Identification of a primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. Life Sci. 57, PL45–50.
Mash, D.C., Staley, J.K., Pablo, J.P., Holohean, A.M., Hackman, J.C., Davidoff, R.A., 1995b. Properties of ibogaine and its principal metabolite (12-hydroxyibogamine) at the MK-801 binding site of the NMDA receptor complex. Neurosci. Lett. 192, 53–56.
Munro, T.A., Xu, W., Ho, D.M., Liu-Chen, L.Y., Cohen, B.M., 2013. Studies toward bivalent kappa opioids derived from salvinorin A: heteromethylation of the furan ring reduces affinity. Beilstein J. Org. Chem. 9, 2916–2924.
Neubig, R.R., Spedding, M., Kenakin, T., Christopoulos, A., 2003. International Union of pharmacology committee on receptor nomenclature and drug classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. Pharmacol. Rev. 55, 597–606.
Obach, R.S., Pablo, J., Mash, D.C., 1998. Cytochrome P4502D6 catalyzes the O-demethylation of the psychoactive alkaloid ibogaine to 12-hydroxyibogamine. Drug Metab. Dispos. 26, 764–768.
Pablo, J.P., Mash, D.C., 1998. Noribogaine stimulates naloxone-sensitive [35S] GTPgammaS binding. Neuroreport 9, 109–114.
Parker, L.A., Siegel, S., Luxton, T., 1995. Ibogaine attenuates morphine-induced conditioned place preference. Exp. Clin. Psychopharmacol. 344–348.
Pearl, S.M., Herrick-Davis, K., Teitler, M., Glick, S.D., 1995. Radioligand-binding study of noribogaine, a likely metabolite of ibogaine. Brain Res. 675, 342–344.
Pearl, S.M., Hough, L.B., Boyd, D.L., Glick, S.D., 1997. Sex differences in ibogaine antagonism of morphine-induced locomotor activity and in ibogaine brain levels and metabolism. Pharmacol. Biochem. Behav. 57, 809–815.
Popik, P., Layer, R.T., Skolnick, P., 1995. 100 years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. Pharmacol. Rev. 47,

235–253.
Powell, K.J., Abul-Husn, N.S., Jhamandas, A., Olmstead, M.C., Beninger, R.J., Jhamandas, K., 2002. Paradoxical effects of the opioid antagonist naltrexone on morphine analgesia, tolerance, and reward in rats. J. Pharmacol. Exp. Ther. 300, 588–596.
Raynor, K., Kong, H., Chen, Y., Yasuda, K., Yu, L., Bell, G.I., Reisine, T., 1994. Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. Mol. Pharmacol. 45, 330–334.
Rezvani, A.H., Mash, D.C., Hearn, W.L., Lee, Y.W., Overstreet, D.H., 1995. Noribogaine, a primary ibogaine metabolite, reduces alcohol intake in P and Fawn-hooded rats. Research society on alcoholism. Alcohol Clin. Exp. Res. 19, 15A.
Rives, M.L., Rossillo, M., Liu-Chen, L.Y., Javitch, J.A., 2012. 6′-Guanidinonaltrindole (6′-GNTI) is a G protein-biased kappa-opioid receptor agonist that inhibits arrestin recruitment. J. Biol. Chem. 287, 27050–27054.
Saidak, Z., Blake-Palmer, K., Hay, D.L., Northup, J.K., Glass, M., 2006. Differential activation of G-proteins by mu-opioid receptor agonists. Br. J. Pharmacol. 147, 671–680.
Samorini, G., 1995. The Bwiti religion and the psychoactive plant Tabernanthe iboga (Equatorial Africa). Integration 5, 105–114.
Schmauss, C., Herz, A., 1987. Intrathecally administered dynorphin-(1-17) modulates morphine-induced antinociception differently in morphine-naive and morphine-tolerant rats. Eur. J. Pharmacol. 135, 429–431.
Schmid, C.L., Streicher, J.M., Groer, C.E., Munro, T.A., Zhou, L., Bohn, L.M., 2013. Functional selectivity of 6′-guanidinonaltrindole (6′-GNTI) at kappa-opioid receptors in striatal neurons. J. Biol. Chem. 288, 22387–22398.
Schwarzer, C., 2009. 30 years of dynorphins–new insights on their functions in neuropsychiatric diseases. Pharmacol. Ther. 123, 353–370.
Sharma, K.K., Mediratta, P.K., 2001. Psychotherapeutic implications of cooperation and control-operations among multiple opioid receptors. In: Gupta, S.K. (Ed.), Pharmacology and Therapeutics in the New Millennium.
Sharma, S.K., Jones, R.M., Metzger, T.G., Ferguson, D.M., Portoghese, P.S., 2001. Transformation of a kappa-opioid receptor antagonist to a kappa-agonist by transfer of a guanidinium group from the 5′- to 6′-position of naltrindole. J. Med. Chem. 44, 2073–2079.
Sheppard, S.G., 1994. A preliminary investigation of ibogaine: case reports and recommendations for further study. J. Subst. Abuse Treat. 11, 379–385.
Skoubis, P.D., Matthes, H.W., Walwyn, W.M., Kieffer, B.L., Maidment, N.T., 2001. Naloxone fails to produce conditioned place aversion in mu-opioid receptor knock-out mice. Neuroscience 106, 757–763.
Staley, J.K., Ouyang, Q., Pablo, J., Hearn, W.L., Flynn, D.D., Rothman, R.B., Rice, K.C., Mash, D.C., 1996. Pharmacological screen for activities of 12-hydroxyibogamine: a primary metabolite of the indole alkaloid ibogaine. Psychopharmacol. Berl. 127, 10–18.
Sunder Sharma, S., Bhargava, H.N., 1998. Enhancement of morphine antinociception by ibogaine and noribogaine in morphine-tolerant mice. Pharmacology 57, 229–232.
Takemori, A.E., Loh, H.H., Lee, N.M., 1992. Suppression by dynorphin A-(1-13) of the expression of opiate withdrawal and tolerance in mice. Eur. J. Pharmacol. 221, 223–226.
Tao, P.L., Hwang, C.L., Chen, C.Y., 1994. U-50,488 blocks the development of morphine tolerance and dependence at a very low dose in guinea pigs. Eur. J. Pharmacol. 256, 281–286.
Toll, L., Berzetei-Gurske, I.P., Polgar, W.E., Brandt, S.R., Adapa, I.D., Rodriguez, L., Schwartz, R.W., Haggart, D., O'Brien, A., White, A., Kennedy, J.M., Craymer, K., Farrington, L., Auh, J.S., 1998. Standard binding and functional assays related to medications development division testing for potential cocaine and opiate narcotic treatment medications. NIDA Res. Monogr. 178, 440–466.
Violin, J.D., Crombie, A.L., Soergel, D.G., Lark, M.W., 2014. Biased ligands at G-protein-coupled receptors: promise and progress. Trends Pharmacol. Sci. 35, 308–316.
Zubaran, C., Shoaib, M., Stolerman, I.P., Pablo, J., Mash, D.C., 1999. Noribogaine generalization to the ibogaine stimulus: correlation with noribogaine concentration in rat brain. Neuropsychopharmacology 21, 119–126.