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Mefloquine and Psychotomimetics Share Neurotransmitter Receptor and Transporter Interactions *In Vitro*

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Abstract

Rationale—Mefloquine is used for the prevention and treatment of chloroquine-resistant malaria, but its use is associated with nightmares, hallucinations, and exacerbation of symptoms of post-traumatic stress disorder. We hypothesized that potential mechanisms of action for the adverse psychotropic effects of mefloquine resemble those of other known psychotomimetics.

Objectives—Using *in vitro* radioligand binding and functional assays, we examined the interaction of (+)- and (–)-mefloquine enantiomers, the non-psychotomimetic anti-malarial agent, chloroquine, and several hallucinogens and psychostimulants with recombinant human neurotransmitter receptors and transporters.

Results—Hallucinogens and mefloquine bound stereoselectively and with relatively high affinity ($K_i = 0.71\text{--}341$ nM) to serotonin (5-HT) 2_A but not 5-HT $_{1A}$ or 5-HT $_{2C}$ receptors. Mefloquine but not chloroquine was a partial 5-HT $_{2A}$ agonist and a full 5-HT $_{2C}$ agonist, stimulating inositol phosphate accumulation, with similar potency and efficacy as the hallucinogen dimethyltryptamine (DMT). 5-HT receptor antagonists blocked mefloquine's effects. Mefloquine had low or no affinity for dopamine D $_1$, D $_2$, D $_3$, and D $_{4.4}$ receptors, or dopamine and norepinephrine transporters. However, mefloquine was a very low potency antagonist at the D $_3$ receptor and mefloquine but not chloroquine or hallucinogens blocked [^3H]5-HT uptake by the 5-HT transporter.

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Authorship Contributions:

Participated in research design: Janowsky, Smilkstein, Hinrichs, and Riscoe;

Contributed new reagents or analytical tools: Yang, and Zabriskie;

Performed experiments and conducted data analysis: Johnson, Wolfrum, Yang, and Eshleman;

Wrote or contributed to the writing of the manuscript: Janowsky, Smilkstein, Riscoe, Eshleman, and Zabriskie.

Conclusions—Mefloquine but not chloroquine shares an *in vitro* receptor interaction profile with some hallucinogens and this neurochemistry may be relevant to the adverse neuropsychiatric effects associated with mefloquine use by a small percentage of patients. Additionally, evaluating interactions with this panel of receptors and transporters may be useful for characterizing effects of other psychotropic drugs and for avoiding psychotomimetic effects for new pharmacotherapies, including antimalarial quinolines.

Keywords

mefloquine; chloroquine; quinine; malaria; LSD; psychotomimetic; neurotransmitter; transporter; serotonin receptor; dopamine receptor

Introduction

Mefloquine ([*(R**, *S**)-2,8-bis(trifluoromethyl)quinolin-4-yl]-(2-piperidyl)methanol; LariamTM) is a synthetic derivative of quinine (*(R)*-(6-Methoxyquinolin-4-yl)((2*S*,4*S*,8*R*)-8-vinylquinuclidin-2-yl)methanol) that has been used by millions of people world-wide to prevent and to treat symptoms of malaria following exposure to *Plasmodium falciparum* and other *Plasmodium* species. The antimalarial mechanism of action of mefloquine is not completely understood, but may include alteration of heme-iron transport, disposition across the parasite digestive vacuole and cytoplasm, and inhibition of cellular crystalline hemozoin formation (Haynes et al., 2012; Combrinck et al., 2013). The drug is used in lower doses, once weekly for prophylaxis, and in higher, more frequent doses to treat acute infections. Side effects after prophylactic and, particularly, therapeutic use has precluded more widespread use of mefloquine (Kennedy, 2009). Although some side effects are common among antimalarials, psychotropic effects similar to those of mefloquine are not usually found in subjects taking other antimalarial agents such as chloroquine (*(RS)*-*N'*-(7-chloroquinolin-4-yl)-*N*, *N*-diethyl-pentane-1,4-diamine)), which is in the 4-aminoquinoline class. Unwanted effects of mefloquine include sleep and dream disturbances (Toovey, 2009), depression (but see Schlagenhauf et al., 2009), hallucinations, and anxiety in a relatively large proportion of patients, and some of the deleterious side effects continue after discontinuation of drug treatment (van Riemsdijk et al., 2005), suggesting that the drug has neurotoxic effects (see AlKadi et al., 2007 for review). The severe neuropsychiatric side effects of the drug have resulted in the very recent inclusion of a “black box” warning by the United States Food and Drug administration, and discussion of the severity and frequency of the side effects has reached the popular press (MacLean, 2013).

Recent evidence suggests that mefloquine may cause oxidative stress, alter neuronal morphology (Hood et al., 2010), and exert apoptotic effects that are intimately involved in neurotoxicity *via* interaction with non-receptor tyrosine kinase 2 (Pyk2) (Milatovic et al., 2011). Additionally, a number of receptor-based neuropharmacological etiologies have been invoked to explain the psychiatric effects of mefloquine. However, few if any of these pharmacological effects resemble those of other psychotomimetic agents. For instance, mefloquine does not appear to interact with glutamate receptors (Caridha et al., (2008), but the psychotropic effects of lysergic acid diethylamide (LSD) may include indirect changes in the regulation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors

(Marona-Lewicka et al., 2011), and the psychotomimetic, phencyclidine, is an NMDA receptor antagonist (Thomson et al., 1985). In addition, there is literally no evidence concerning the interactions of LSD and other psychotomimetics with gap junction elements, but mefloquine (25 μ M) blocks a number of connexins (Cruikshank et al., 2004; Iglesias et al., 2008; Wang et al., 2010) and is now commonly used as a research tool to block gap junction channels (Sarihi et al., 2012). Mefloquine also interacts with gamma-aminobutyric acid A (GABA_A) receptors (Amabeoku and Farmer, 2005; Thompson and Loomis, 2008), and interacts with peripheral benzodiazepine receptors (Dzierszinski et al., 2002), adenosine A₁ and A_{2A} receptors (Weiss et al., 2003; Gillespie et al., 2008), and 5-HT₃ receptors (Thompson et al., 2007; Thompson and Loomis 2008). However, there is little or no information available concerning the interaction of LSD and related psychotomimetic agents with many of these receptors (for a compendium of values and references, see <http://pdsp.med.unc.edu/kidb.php>).

Drugs with differing chemical structures including LSD, 2,5-dimethoxy-4-methylamphetamine (DOM), DMT, and other hallucinogens share a number of common neurochemical effects that may be related to their psychotropic activity. For example, some hallucinogens bind to and stimulate specific 5-HT receptors (Nichols et al., 2002; Rabin et al., 2002; Kanagarajadurai et al., 2009; for review see Halberstadt and Geyer, 2011) and LSD interacts with specific dopaminergic and noradrenergic receptors (Minuzzi and Cumming, 2010). In contrast, many first generation antipsychotic agents block dopamine D₂ receptors, and second generation antipsychotic drugs block D₂ and (or) 5-HT₂ receptors (Miyake et al., 2012). Additionally, effects of these drugs on neurotransmitter transporters vary depending on chemical structure, and may account for differences in the behavioral properties of the drugs. However, despite its world-wide therapeutic use and its unwanted behavioral effects, relatively little is known about the receptor pharmacology of mefloquine.

We hypothesized that mefloquine's pharmacological profile at neurotransmitter receptors and transporters *in vitro* would resemble the effects of some known psychotomimetic agents, and tested this in *in vitro* models using recombinant neurotransmitter receptors and transporters. In addition, we speculated that these results might make mefloquine a useful tool to more fully characterize the pharmacological profile required for psychotomimetic activity. Lastly, as in our previous work (Kelly et al., 2009), we anticipate that exploiting neurotransmitter receptor and transporter interaction profiles may add useful information with which to develop chemotherapeutic agents with potentially fewer psychotropic effects. The results indicate that mefloquine has affinity for specific 5-HT and dopamine receptors, and in assays of receptor function, is a partial agonist at the 5-HT_{2A} receptor, a full agonist at 5-HT_{2C} receptors, and an antagonist at the dopamine D₃ receptor. Additionally, mefloquine binds to and blocks recombinant human 5-HT transporters (hSERT), and may increase synaptic 5-HT availability and stimulate specific aspects of serotonergic function. Thus, mefloquine shares pharmacodynamic effects with other psychotomimetic agents.

Materials and Methods

Materials

Racemic mefloquine, its individual enantiomers, and other anti-malarial agents were obtained from Walter Reed Army Medical Center or were synthesized, and structures verified in our laboratories (MZ, MR). Briefly, racemic mefloquine (BioBlocks) and (–)-3-bromo-8-camphosulfonic acid ammonium salt (Acros Organics) were mixed in aqueous methanol to form the (+)-mefloquine-(–)-3-bromo-8-camphosulfonate salt, which was further purified via recrystallization from aqueous methanol. Neutralization of this salt with 1 N NaOH, followed by recrystallization from aqueous methanol gave the pure (+)-mefloquine. (–)-Mefloquine was obtained from the above (–)-sulfonate filtrate after repeated recrystallizations as previously described (Carroll and Blackwell, 1974).

LSD, DMT, DOM and cocaine were obtained from the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD) or from commercial sources. [³H]8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), [¹²⁵I]2,5-dimethoxy-4-iodoamphetamine (DOI), [³H]7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol (SCH23390), [³H]*N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-(methylamino)benzamide (YM-09151-2, nemonapride), [¹²⁵I]methyl (1*R*,2*S*,3*S*)-3-(4-iodophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (RTI-55), [³H]dopamine, [³H]5-HT, and [³H]norepinephrine were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). Commonly used reagents were obtained from commercial sources except where specified below.

Tissue Culture

Human embryonic kidney cells (HEK-293) were cultured and transfected with the respective recombinant human receptor or transporter using modifications of our previously described methods (Eshleman et al., 1999, Eshleman et al., 2013).

Receptor binding assays

Radioligand binding experiments were conducted by modifications of our previously described methods (Eshleman et al., 1999; Gatch et al., 2011, Eshleman et al., 2013) using some of the receptor and transporter characterization panels that have been validated as part of the National Institute on Drug Abuse/Department of Veterans Affairs Interagency Agreement “*In Vitro* Receptor, Transporter and Release Assays for NIDA Medications Discovery and Abuse Liability Testing.”

Serotonin receptors—Human embryonic kidney (HEK-293) cells expressing the recombinant human 5-HT_{1A} (HEK-h5-HT_{1A}), 5-HT_{2A} (HEK-h5-HT_{2A}) or 5-HT_{2C} (HEK-h5-HT_{2C}) receptors were used. The methods for membrane preparation, [³H]8-OH-DPAT binding to HEK-h5-HT_{1A}, [¹²⁵I]DOI binding to HEK-h5-HT_{2A} and HEK-h5-HT_{2C} cell membranes and data analysis was conducted as previously described (Knight et al., 2004; Gatch et al., 2011; Eshleman et al., 2013). For comparison of agonist affinities at [¹²⁵I]DOI and [³H]ketanserin binding to HEK-5-HT_{2A} cells, the method was adapted from Sleight et al. (1996). In brief, well-washed membranes from HEK-h5-HT_{2A} cells were resuspended in

binding buffer (50 mM Tris-HCl, pH 7.4 at 25°C, 5 mM MgCl₂, 10 μM pargyline, 0.1% ascorbic acid). Assays were identical for each radioligand and consisted of 50 μl membrane preparation, 25 μl radioligand, 50 μl of displacing compound, or buffer, in a final volume of 250 μl. The reaction was incubated at 25°C for 1 h and terminated by filtration through FiltermatA filters (Perkin Elmer) presoaked in 0.05% polyethylenimine. Nonspecific binding was defined with 10 μM 5-HT. IC₅₀ values were converted to K_i values (Cheng and Prusoff, 1973). For [³H]ketanserin and [¹²⁵I]DOI, the K_d values were 0.52 and 3.62 nM and the final concentrations in the binding assays were 1–1.5 nM and 0.04–0.06 nM, respectively.

For dopamine receptor binding assays, mouse fibroblast cells expressing the recombinant human D₁ receptor at high density (LhD₁ cells) and Chinese hamster ovary cells expressing the recombinant human D₂ or D₃ receptor (CHOp-D₂ or CHOp-D₃) were obtained from Stanford Research Institute (SRI). HEK cells co-expressing the human D_{4.4} receptor with adenylate cyclase type 1 (HEK-D_{4.4}-AC1 cells) were a gift from Dr. Kim Neve (Portland, OR). Radioligand binding assays for dopamine receptors were conducted as described previously (Toll et al., 1998; Eshleman et al., 2013).

Recombinant Human Transporter Binding and neurotransmitter uptake assays

HEK cells expressing the recombinant human dopamine transporter (HEK-hDAT), SERT (HEK-hSERT) or norepinephrine transporter (HEK-hNET) were used as described previously (Eshleman et al., 1999). Specific binding and uptake were defined as the difference in binding or uptake observed in the presence and absence of mazindol (5 μM, HEK-hDAT and HEK-hNET) or imipramine (5 μM, HEK-hSERT). For all receptor binding and uptake assays, three or more independent competition experiments were conducted with duplicate determinations, unless the IC₅₀ value for a drug was consistently greater than 10 μM, and then only two experiments were conducted.

Inositol-1-phosphate (IP-1) formation

HEK-h5-HT_{2A} or -h5-HT_{2C} cells were used to determine agonist activation of the recombinant receptor as measured by accumulation of inositol-1-phosphate (IP-1) resulting from stimulation of the phospholipase C pathway. Assays were conducted as described previously (Gatch et al., 2011, Eshleman et al., 2013). Agonist effects were normalized to maximal stimulation by 5-HT above basal, and antagonists were tested in the presence of 100 nM 5-HT and normalized to inhibition by 30 μM ketanserin (5-HT_{2A}) or 1 μM SB 242084 (5-HT_{2C}).

Dopamine D₂ and D₃ mitogenesis assays

CHOp-D₂ and CHOp-D₃ cells were maintained in alpha-MEM with 10% fetal bovine serum (FBS, Atlas Biologicals, Fort Collins, CO), penicillin-streptomycin, and 200 μg/ml of G418. The assays were adapted from previous methods (Toll et al., 1998). The cells were seeded in 96 well plates at a density of 10,000 cells/well. After ~60 h, the cells were rinsed twice and then incubated for 24 h at 37°C with serum-free alpha-MEM. Serial dilutions of test compounds were made in serum-free alpha-MEM. The medium was removed from the plate and replaced with 100 μl of test compound. After 16 h (D₃) or 24 h (D₂), [³H]thymidine

(0.25 μ Ci) in alpha-MEM supplemented with 10% FCS was added to each well and the plates were incubated for 2 h at 37°C. The cells were trypsinized by addition of 1% trypsin solution and harvested using a Tomtec 96 well harvester (Hamden, CT) and radioactivity remaining on filters was counted using a Perkin Elmer microbeta scintillation counter.

Dopamine D₄ adenylate cyclase assay

HEK-D₄-AC1 cells were plated at a density of 375,000 cells per well in 48 well plates in DMEM supplemented with 5% FetalClone (HyClone, Logan, UT), 5% bovine calf serum and penicillin-streptomycin. After ~36 h, the medium was changed to DMEM supplemented with 10% charcoal-stripped FetalClone. The medium was removed ~18 hr later. For agonist assays, 0.8 ml EBSS (116 mM NaCl, 22 mM glucose, 15 mM HEPES, 8.7 mM NaH₂PO₄, 5.4 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 1 mM ascorbic acid, 0.5 mM IBMX [3-isobutyl-1-methyl-xanthine] and 2% BCS, pH 7.4 at 37°C) was added, cells were incubated 20 min, agonists were added, and, after 20 min incubation, 10 μ M forskolin was added in a final volume of 1 ml. After 20 min incubation with forskolin, the reaction was terminated by aspiration, and 0.1 ml trichloroacetic acid was added. Plates were incubated for 2 hr on a rotator. Adenylate cyclase activity was measured using a cyclic AMP EIA kit (Cayman, Ann Arbor, MI). Aliquots (9 μ l) of each well were diluted to 200 μ l with EIA buffer from the kit, and 50 μ l of the dilution was added to the EIA plate. After addition of tracer and monoclonal antibody, the EIA plates are incubated for 18 hr at 4°C. The reaction was aspirated, plates were washed 5 $\times$ 300 μ l with wash buffer, and Ellman's reagent was added. After 2 h, the plates were read at 410 nm. Basal cAMP was subtracted from all values. The maximal receptor effect is defined with 1 μ M quinpirole.

Data analysis

For radioligand binding, data were normalized to the binding in the absence of a competitive (mefloquine, etc.) drug. Three or more independent competition experiments were conducted with duplicate determinations. GraphPAD Prism was used to analyze the subsequent data, with IC₅₀ values converted to K_i values using the equation ($K_i = IC_{50} / (1 + ([drug^*] / K_d drug^*))$), where [drug*] is the concentration of the labeled ligand used in the binding assays (Cheng and Prusoff, 1973). The K_d values used in the equations are listed in Eshleman et al. (2013). Differences in affinities were assessed by one way ANOVA using the logarithms of the K_i values for test compounds. Tukey's multiple comparison test was used to compare the potencies and efficacies of test compounds.

For functional assays, GraphPAD Prism is used to calculate either EC₅₀ (agonists) or IC₅₀ (antagonists) values using data expressed as % 5-HT-stimulation for IP-1 formation and % quinpirole-stimulation for mitogenesis assays. For functional assays, one way ANOVA was used to assess differences in efficacies using normalized maximal stimulation, and differences in potencies using the logarithms of the EC₅₀ values for test compounds. Tukey's multiple comparison test was used to compare test compounds with significance set at $p < 0.05$.

RESULTS

5-HT Receptors

In a relatively small percentage of patients, the symptoms of mefloquine intoxication resemble those of some hallucinogens, including LSD, DMT and DOI. These drugs are relatively potent agonists at various 5-HT receptors, and so we examined the ability of (+)- and (–)-enantiomers of mefloquine to displace radiolabeled agonist binding from the recombinant h5-HT_{1A}, _{2A}, and _{2C} receptors. The results in Figure 1 and Table 1 indicate that the enantiomer that is active against the plasmodium parasite, (+)-mefloquine, has higher affinity for the h5-HT_{2A} receptor, but equal affinity for the h5-HT_{1A} and h5-HT_{2C} receptor as compared to the (–)-enantiomer. The enantiomers displayed similar rank orders of affinity across receptors, and had highest affinity for the [¹²⁵I]DOI binding site on the recombinant h5-HT_{2A} receptor and lowest affinity for the [³H]8-OH-DPAT binding site on the recombinant h5-HT_{1A} receptor. Compared to the mefloquine enantiomers, chloroquine had higher affinity for the h5-HT_{1A} receptor ($p < 0.05$, one way ANOVA followed by Tukey's multiple comparison test), similar affinity for the h5-HT_{2C} receptor ($p > 0.05$), and lower affinity for the h5-HT_{2A} receptor ($p < 0.05$). (+)-Mefloquine and DMT had similar affinity for the h5-HT_{2A} receptor ($p > 0.05$). Psychotropic compounds LSD and DOM had higher affinity for the h5-HT_{2A} and h5-HT_{2C} receptors compared to the mefloquine enantiomers ($p < 0.001$). Additionally, LSD had high affinity for the [³H]8-OH-DPAT binding site on the h5-HT_{1A} receptor ($K_i = 2.78$ nM). Subsequently, experiments were conducted to determine if mefloquine is an agonist at recombinant h5-HT receptors.

5-HT Receptor Agonist-Stimulated IP-1 Formation

IP-1 formation by cells expressing h5-HT receptors was measured following treatment of cells with various drugs. The data in Figure 2 and Table 2 indicate that the rank order of potency for psychotropic agents was LSD $\gg$ DOM $>$ DMT at both h5-HT_{2A} and h5-HT_{2C} receptors. DMT was only a partial agonist at the h5-HT_{2A} receptor ($p < 0.001$, Tukey's multiple comparison test), but a full agonist at the h5-HT_{2C} receptor ($p > 0.05$). LSD and DOM approached full efficacy at both receptors. Interestingly, (+)-mefloquine was significantly more potent (about two orders of magnitude) at stimulating h5-HT_{2A} and h5-HT_{2C} receptors, as compared to (–)-mefloquine. Further, both enantiomers were significantly more potent at stimulating h5-HT_{2C} receptors than h5-HT_{2A} receptors ($p < 0.01$, two tailed t-test). The EC₅₀ values for (+)-mefloquine at the receptors (224 nM and 1990 nM at h5-HT_{2C} and h5-HT_{2A} receptors, respectively) indicated that it had similar h5-HT_{2C} receptor potency as the psychoactive tryptamine, DMT ($p > 0.05$, Tukey's multiple comparison test), but lower potency than the other psychotropic drugs at these receptors ($p < 0.01$). The (+)- and (–)-enantiomers had similar efficacy at the h5-HT_{2C} receptor as compared to the full agonist, 5-HT ($p > 0.05$). However, (+)-mefloquine was only a partial agonist at the h5-HT_{2A} receptor ($p < 0.001$), and the (–)-enantiomer had almost no effect on h5-HT_{2A} receptor-mediated IP-1 formation. In contrast to the other drugs, chloroquine had no effect on IP-1 formation in either cell type. For comparison, the h5-HT₂ receptor antagonists ketanserin and SB242087 were tested. Both drugs potently inhibited 5-HT-mediated IP-1 formation (Table 2).

To verify that the effect of mefloquine on 5-HT receptor-mediated IP-1 accumulation was not a non-specific effect, we characterized the effects of mefloquine and other drugs in nontransfected HEK-293 cells. (+)Mefloquine (100 μ M), 5-HT (1 μ M), and LSD (10nM) had no effect on IP-1 accumulation in nontransfected cells, but all produced a robust response in cells expressing the h5-HT_{2A} or _{2C} receptor. As can be seen in Fig 2c, there is little to no response to mefloquine in nontransfected, wildtype (wt) cells, as opposed to transfected cells, in which mefloquine caused a 3–5 fold increase above basal activity. To further verify that mefloquine is an agonist, we examined the concentration-dependent effect of the antagonist ketanserin on mefloquine-induced IP-1 accumulation in HEK- h5-HT_{2A} cells, and the effect of the antagonist SB202084 on mefloquine-induced IP-1 accumulation in HEK- h5-HT_{2C} cells. The data in Fig 3 indicate that mefloquine is a partial agonist at h5-HT_{2A} receptors as compared to 5-HT and LSD, and that ketanserin dose dependently blocks the agonist effect of each drug. Additionally, mefloquine is a full agonist at h5-HT_{2C} receptors, as compared to LSD and 5-HT, and the effects of all drugs on IP-1 accumulation are blocked in a dose-dependent and complete manner by the antagonist SB202084.

Sleight and coworkers (1996) examined the ability of drugs to displace antagonist ([³H]ketanserin), partial agonist ([³H]-4-bromo-2,5-dimethoxyphenylisopropylamine, DOB), and agonist ([³H]5-HT) radioligand binding to the 5-HT_{2A} receptor. Additionally, Egan and coworkers (2000) used [³H]ketanserin [³H]DOB, [³H]5-HT and [³H]mesulergine to examine agonist affinity ratios (K_L/K_H) for a number of agonists at 5-HT_{2A} and 5-HT_{2C} receptors. The authors reported that agonists but not antagonists (Sleight et al., 1996) had higher affinity for [³H]agonist-labeled sites. We have now examined the ability of mefloquine and a number of other drugs to displace agonist and antagonist ligands in our cell lines (Fig 4, Table 3). As indicated in Fig. 4 and Table 3, the agonists 5-HT, DOM and DMT have higher affinity for the agonist labeled site, however LSD did not have a significantly higher affinity for the [¹²⁵I]DOI-labeled site. Additionally, (+)mefloquine did not have a statistically significant higher affinity for the agonist-labeled site, although there was a large trend. (–)Mefloquine tended to favor the antagonist-labeled site. As opposed to the previous report that antagonists do not have a significantly different affinity for agonist- or antagonist-labeled h5-HT_{2A} receptors (Sleight et al., 1995), the antagonist MDL 100,907 had a significantly higher affinity for the antagonist-labeled site. Thus, this assay did not allow us to contrast agonists versus antagonists based on differential affinities and the question of agonist activity was addressed using functional assays, above.

Transporters

Results in Table 4 indicate that the (+)- and (–)-enantiomers of mefloquine are very weak at the [¹²⁵I]RTI-55 binding site of the hNET and hDAT. However, (+)-mefloquine had similar affinity to cocaine for the hSERT ($p>0.05$, two tailed t-test). In assays measuring the ability of drugs to block the uptake of [³H]neurotransmitter by each transporter, (+)-mefloquine was equipotent with cocaine at blocking [³H]5-HT uptake by the hSERT ($p>0.05$), and therefore could increase 5-HT availability in the synapse. LSD and chloroquine had no effect on [³H]neurotransmitter uptake by any of the recombinant human transporters (Table 4).

Dopamine Receptors

The data in Table 5 indicate the effects of drugs on radioligand binding to the recombinant dopamine hD₁, hD₂, hD₃, and hD_{4.4} receptors. (+)-Mefloquine was selective, and blocked [³H]YM 09151-2 binding to the hD₃ receptor at a concentration that approaches its therapeutic concentration in blood ($K_i = 1960$ nM), but had no effect on radioligand binding to the hD₁, hD₂, or hD_{4.4} receptors at concentrations up to 10 μ M. Chloroquine and (–)-mefloquine had no measurable effect on radioligand binding at any of the dopamine receptors. LSD had moderate, mid-nanomolar affinity for the hD₁, hD₂, hD₃, and hD_{4.4} receptors. To determine if (+)-mefloquine is an agonist or antagonist at the hD₂ or hD₃ receptor, its effects on hD₂ and hD₃ receptor-mediated mitogenesis were characterized. Effects of quinpirole, a D₂/D₃ receptor agonist, were compared to the effects of the other drugs. (+)-Mefloquine had no agonist activity at the hD₂ or hD₃ receptors (Fig 5a and b; Table 6). LSD was a partial agonist at the D₂ receptor and a full agonist at the D₃ receptor, as compared to quinpirole, with mid-nanomolar EC₅₀ values. (+)-Mefloquine also had no antagonist activity at the D₂ receptor, but fully antagonized the effect of quinpirole at D₃ receptors with an IC₅₀ value of 3210 nM (Fig 5c and d; Table 6). The ability of drugs to inhibit hD_{4.4} receptor-mediated forskolin-stimulated adenylate cyclase activity was also examined. LSD was a full agonist and inhibited $93 \pm 6\%$, compared to the maximal inhibition by quinpirole, of forskolin-stimulated adenylate cyclase activity with an EC₅₀ value of 0.58 ± 0.20 nM. However, (+)- and (–)-mefloquine and chloroquine had no effect (data not shown).

Discussion

The results of experiments described above indicate that both (+)- and (–)-enantiomers of mefloquine interact with specific recombinant human neurotransmitter receptors and transporters. These effects appear to differ from those of the anti-malarial agent, chloroquine, and from quinine, a structural analogue, and the effects of mefloquine are stereoselective. The relevance of these findings is particularly intriguing, given the far higher incidence of serious mefloquine-induced psychotomimetic adverse effects following high-dose therapeutic use, a setting in which 10-fold higher mefloquine concentrations have been reported (Simpson et al., 1999). Comparing free to whole mouse brain concentrations following a single i.v. dose, Dow et al., 2011) calculated a ratio of 4.9/1807, or 0.0027, with a whole brain concentration of about 4 μ M, and about 18 nM free brain mefloquine in mice. However, soldiers taking the drug prophylactically and who were killed in the line of duty had brain levels of 8.7 to 14 mg/kg (Jones et al., 1994). This should translate to about 100 to 135 nM free mefloquine. Pham and others (1999) measured mefloquine levels in brains from individuals who were taking the drug acutely (750 mg, 37 to 70 hours before death), and found 51.5 nmol/g tissue, which should reach about 137 nM free drug. It is likely that humans taking repeated doses over time would have a much different ratio (more free drug), and so the whole brain/free ratio of 0.0027 may be a conservative estimate. Neuropsychiatric effects are reported by a relatively small percentage of patients who take mefloquine and so usual human brain concentrations should not exceed the concentrations that have effects on transporters and receptors *in vitro* that are reported here. Tissue sample mefloquine levels in the 50–150nM range are consistent with the finding that only a small number of patients,

predisposed by dose, pharmacogenetics, liver function, size, co-ingestion of selective serotonin reuptake inhibitors, over/underexpression of some neurotransmitter signaling intermediates, etc., would develop behavioral problems.

Because many psychotomimetic agents are agonists at h5-HT receptors, we hypothesized that mefloquine stimulates h5-HT receptors. The affinity of (+)-mefloquine for the [¹²⁵I]DOI binding site of recombinant h5-HT_{2A} receptors is similar to the affinity of DMT (K_i = 210 nM), a psychotomimetic, but DMT and mefloquine have much lower affinity than LSD and DOM at this receptor (Table 1). The K_i value for mefloquine (341 nM), is below the minimum anti-plasmodial therapeutic concentration of the drug in blood (1.6 μM; see Schlagenhauf et al., 2011; C_{max} = 1018 μg/L ≈ 2.6 μM, but varies by preparation, see Wiedekam et al., 1998). However, free concentrations of mefloquine in brain would be much smaller, as indicated above. Likewise, (–)-mefloquine also binds to the h5-HT_{2A} receptor, but its affinity (K_i = 1510 nM) is lower than the affinity of the (+)-enantiomer, and much lower than any of the psychotropic drugs that exert effects *via* the h5-HT_{2A} receptor. Although the K_i value for (–)-mefloquine is also close to the minimum therapeutic drug concentration, free brain levels would again be minimal by comparison. Thus it is possible that both enantiomers contribute to any side effects in patients presenting with neuropsychiatric symptoms by their action at h5-HT_{2A} receptors. Both enantiomers of mefloquine also displaced [¹²⁵I]DOI binding from recombinant h5-HT_{2C} receptors but the K_i values for the antimalarials are much higher. Many psychotomimetics, including LSD, DOM, and DMT also interact with the h5-HT_{2C} receptor (Tables 1 and 2). Thus, it is possible that some of the psychotropic effects of mefloquine, like those of the psychotomimetic drugs LSD, DMT and DOM, are mediated by interactions with both h5-HT_{2A} and h5-HT_{2C} receptors (Nichols, 2004; Passie et al., 2008; Halberstadt and Geyer, 2011).

The 5-HT receptors are coupled to multiple signal transduction systems (Gatch et al., 2011), and we examined the ability of mefloquine and other drugs to stimulate IP-1 formation. The data in Table 2 indicate that (+)-mefloquine is a full agonist at recombinant h5-HT_{2C} receptors, as compared to the effects of 5-HT, and stimulates IP-1 formation in a dose-dependent manner. However, (+)-mefloquine is weaker than LSD, DOM, or DMT. In addition, (+)-mefloquine stimulates h5-HT_{2A} receptor-mediated IP-1 formation. However, (+)-mefloquine is only a partial agonist at this receptor, as compared to 5-HT. On the other hand the psychotomimetic, DMT, is also a partial agonist at the h5-HT_{2A} receptor, while LSD and DOM are full agonists. Interestingly, (–)-mefloquine weakly stimulates h5-HT_{2C} receptors, but appears to have no effects on h5-HT_{2A} receptor-mediated IP-1 formation. Thus, the psychotropic effects of a racemic mixture of mefloquine analogues may reside with the (+) enantiomer. The finding that mefloquine is a partial to full agonist at h5-HT_{2C} receptors is interesting, but could be the result of receptor over-expression, which can uncover partial agonist properties (Gazi et al., 1999). However, the other psychotomimetic drugs that were examined in these assays had properties that were consistent with previous reports (see <http://pdsp.med.unc.edu/kidb.php>), suggesting that the finding is not an artifact of cell expression levels.

Multiple reports detail the differences in the affinity of agonists and antagonists at displacing agonist and antagonist radioligands from 5-HT receptors (Sleight et al. 1996; Egan et al., 2000). Our data (Table 3, Fig 4) from the use of agonist and antagonist radioligands are interesting but do not appear to decisively define agonists or antagonists. In agreement with the previous report, the agonist, 5-HT, had significantly higher affinity for the [125 I]DOI-labeled site. In addition, DOM, and DMT (Egan et al., 2000), had significantly higher affinity for the agonist-labeled site. However, the agonist LSD did not have higher affinity for the [125 I]DOI-labeled h5-HT_{2A} receptor. Interestingly, LSD had a relatively low K_L/K_H ratio in the previous report (Egan et al., 2000). Differences in the findings could be explained by potential differences in the concentration of guanine nucleotides across assays, as well as differences in the definition and use of full and partial agonists. The over-expression of receptors that has been shown to uncover partial agonist properties of antagonists (Gazi et al., 1999) could also be important since K_i values across agonist and antagonist binding sites are being compared. However, the question of agonist activity for mefloquine and other drugs used here are examined in assays of receptor function, as opposed to radioligand binding, and indicate significant agonist activity *in vitro*.

The (+) enantiomer of mefloquine also interacts with hSERT (Table 4). (+)-Mefloquine displaced [125 I]RTI-55, a cocaine analogue, from hSERT with a K_i value of 229 nM, and blocked [3 H]5-HT uptake with an IC_{50} value of 341 nM. The (–) enantiomer and quinine were much weaker at blocking transporter function, and chloroquine was essentially inactive. These data, and the results of experiments describing the effects of mefloquine at 5-HT receptors, above, suggest that mefloquine increases 5-HT availability in the synapse and also directly stimulates 5-HT receptors. Thus, the combined pre- and post-synaptic effects on serotonergic function are substantial and could play a role in its psychotomimetic effects. Chloroquine also interacted weakly (K_i = 1052 nM) with the [125 I]RTI-55 binding site on the hNET, but had no effect on transporter function. At pharmacologically relevant concentrations the anti-malarial drugs did not affect radioligand binding to, or the function of the hDAT (Table 4). Likewise, none of the hallucinogens/psychotropic agents that were tested had an effect on the dopamine transporter.

Because some psychotomimetic agents interact with dopamine receptors, we examined the effects of (+)- and (–)-mefloquine in dopamine D₁, D₂, D₃, and D_{4.4} receptor binding assays using cells that express the respective recombinant human receptors (Table 5). (+)- but not (–)-mefloquine displaced radioligand binding from the D₃ receptor while neither enantiomer displaced radioligand binding from the D₁, D₂, or D_{4.4} receptors. LSD and known dopamine receptor ligands also bound to D₁ and D₂-like receptors with affinities that resemble those described in previous reports (Neve and Neve, 1997). Because LSD is an agonist at dopamine D₂-like receptors, we examined the effects of LSD and mefloquine on dopamine D₂ and D₃ receptor-mediated signal transduction (Fig 5). The antimalarial had no efficacy at stimulating dopamine D₂- and D₃-receptor-mediated mitogenesis, as compared to LSD and quinpirole. It was a weak D₃ receptor antagonist with an IC_{50} value of 3.21 μ M. Dopamine disposition and function play integral roles in reinforcement behaviors (Glimcher 2011), and so in contrast to other psychotomimetics, mefloquine would not be expected to have reinforcing properties.

Mefloquine's behavioral effects differ from those of many of the psychotomimetic drugs that were examined here. However, stimulation of serotonin receptors is shared by all of the drugs, and is part of the etiology of the behavioral effects that are shared by these drugs. Thus, profiling of new antimalarial drugs against the receptor panel described here could help to identify therapeutic candidates with fewer psychotomimetic effects.

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Fig 1.

Displacement of radiolabeled agonist binding to h5-HT receptors was conducted as described in Methods. Assays were conducted with duplicate determinations, and were repeated at least three times. K_i values were derived from the Cheng-Prusoff correction and used radioligand K_d values as described in Methods. DOM, DMT, and LSD are psychotomimetic drugs with relatively high affinity for 5-HT receptors and were included for purposes of comparison. The K_i values are described in Table 2. *Abbreviations:* 5-HT serotonin; LSD lysergic acid diethylamide; DMT N,N-dimethyltryptamine; DOM 2,5-Dimethoxy-4-methylamphetamine; 8OH-DPAT 8-hydroxy-2-(di-n-propylamino) tetralin; DOI 2,5-dimethoxy-4-iodoamphetamine.

Displacement of radiolabeled agonist binding by mefloquine and other drugs at the h5-HT_{1A}, h5-HT_{2A}, and h5-HT_{2C} receptors.

Fig 2.

Stimulation of IP-1 formation was conducted as described in Methods. Assays were conducted with duplicate determinations and experiments were repeated at least three times. a, b: IP-1 formation is expressed as a percentage of IP-1 accumulation in response to the maximal effect of 5-HT. c. Data are expressed as nM IP1 to allow comparison between HEK wild-type (HEK-wt), HEK-h5-HT_{2A} and HEK-h5-HT_{2C} cells. The drug concentrations were 1 μ M 5-HT, 100 μ M (+)mefloquine and 10 nM LSD. *Abbreviations:* 5-HT serotonin; LSD lysergic acid diethylamide; DMT N,N-dimethyltryptamine; DOM 2,5-Dimethoxy-4-methylamphetamine; DOI 2,5-dimethoxy-4-iodoamphetamine; IP1 inositol-1-phosphate.

Stimulation of h5-HT_{2A} and h5-HT_{2C} receptor-mediated inositol-1-phosphate accumulation by mefloquine and other drugs.

Fig 3. 5-HT_{2A} and 5-HT_{2C} receptor antagonists inhibit (+)mefloquine, serotonin and LSD stimulated IP-1 formation. a. h5-HT_{2A} cells were preincubated with ketanserin for 10 min before the addition of agonists. n=3–4. b. h5-HT_{2C} cells were preincubated with SB 242084 for 10 min before the addition of agonists. n=3

h5-HT_{2A} and h5-HT_{2C} receptor antagonists inhibit (+)mefloquine-, serotonin- and LSDstimulated IP-1 formation.

Fig 4.

Displacement of $[^3\text{H}]\text{ketanserin}$ and $[^{125}\text{I}]\text{DOI}$ binding from $h5\text{-HT}_{2A}$ receptors by agonists and antagonist. Binding assays were conducted as described in Methods. a, b, c, d, e: (+)-Mefloquine, LSD, DOM, 5-HT and ketanserin concentration response curves, respectively. $n=4-9$

Displacement of radiolabeled agonist and antagonist binding by mefloquine and other drugs.

Fig 5.

Mefloquine is an antagonist of quinpirole-stimulated mitogenesis in CHO-D₃ cells. Assays were conducted with duplicate determinations. The number of independent experiments are given in Table 6. Mitogenesis activity is expressed in terms of $[^3\text{H}]$ thymidine incorporation. a, b: For agonists, the data is normalized to the maximal stimulation by quinpirole. c, d: For antagonists, the data is normalized to the stimulation by 30 nM quinpirole. Abbreviation: quin, quinpirole.

Mefloquine is an antagonist of quinpirole-stimulated mitogenesis in CHO-D₃ cells.

Table 1

Affinities of Mefloquine and other drugs for recombinant human serotonin receptors

Drug	h5-HT _{1A} [³ H]8OH-DPAT	h5-HT _{2A} [¹²⁵ I]DOI	h5-HT _{2C} [¹²⁵ I]DOI
	<u>K_i (nM) ± SEM</u>		
(+)-Mefloquine	>9,100 [#]	341 ± 67	5,730 ± 770
(-)-Mefloquine	>10 μM	1,510 ± 260	3,870 ± 620
Chloroquine	6,000 ± 1,600	>6,300 [#]	6,200 ± 2,200
5-HT	2.67 ± 0.33	9.1 ± 1	4.31 ± 0.93
LSD	2.78 ± 0.51	0.71 ± 0.17	2.91 ± 0.75
DMT	450 ± 150	210 ± 43	166 ± 50
DOM	14,200 ± 4,600	4.3 ± 1.2	25.7 ± 4.2
Ketanserin	6,510 ± 320	7.6 ± 1.9	117 ± 34
Ro60-0175	6,680 ± 430	13.1 ± 2.1	7.3 ± 1.6
SB242087	>9,300 [#]	120 ± 32	11.4 ± 4.0
WAY 100635	1.21 ± 0.16	331 ± 54	3,000 ± 1,100

Radioligand binding assays were conducted as described in the text. Each K_i value represents the mean of at least 3 independent experiments, each conducted with duplicate determinations.

[#] If some experiments yielded IC₅₀ or K_i values less than 10 μM and other experiments yielded IC₅₀ or K_i values greater than 10 μM, the latter experiments were assigned a value of 10 μM and averages calculated. The actual value is greater than that average and no standard error is reported. *Abbreviations:* 8OH-DPAT 8-hydroxy-2-(di-n-propylamino) tetralin, DOI 2,5-dimethoxy-4-iodoamphetamine; DMT N,N-dimethyltryptamine; DOM 2,5-Dimethoxy-4-methylamphetamine.

Table 2Effects of Mefloquine and other drugs on h5-HT_{2A}- and h5-HT_{2C}-receptor-mediated IP1 formation

Drug	h5-HT _{2A} IP1 formation	h5-HT _{2C} IP1 formation
	EC ₅₀ (nM) ± SEM % stimulation*	
(+)–Mefloquine	1990 ± 820	224 ± 100
	40.8 ± 4.2%	81.0 ± 9.7%
(–)–Mefloquine	>100 µM	12,000 ± 3,700
	<12%	71 ± 20%
Chloroquine	>100 µM	>100 µM
	<5%	<10% effect
5-HT	51 ± 14	1.52 ± 0.58
	99.7 ± 1.6%	93.2 ± 3.0%
LSD	0.242 ± 0.057	0.85 ± 0.26
	84.2 ± 5.2%	79 ± 10%
DMT	227 ± 64	96 ± 34
	41.0 ± 6.2%	96.1 ± 9.3%
DOM	24.7 ± 8.5	4.6 ± 2.6
	98.2 ± 7.7%	98 ± 19%
IC ₅₀ (nM) ± SEM % inhibition		
Ketanserin		
SB242087		
5.1 ± 1.5		
0.167 ± 0.061		
96.9 ± .2.1%		
93.7 ± 5.5%		

Functional assays were conducted as described in the text. Each EC₅₀ value represents the mean of at least 3 independent experiments, each conducted with duplicate determinations.

* % stimulation- for each experiment, the maximal stimulation for each drug is normalized to the maximal stimulation above baseline for serotonin. IC₅₀ values for antagonists are included to validate the assay. *Abbreviations:* 5-HT serotonin; *IP1* inositol-1-phosphate; *LSD* lysergic acid diethylamide; *DMT* N,N-dimethyltryptamine; *DOM* 2,5-Dimethoxy-4-methylamphetamine.

Table 3

Comparison of K_i values of 5-HT_{2A} agonists and antagonists at [³H]ketanserin and [¹²⁵I]DOI binding sites.

	K_i [¹²⁵ I]DOI	K_i [³ H]ketanserin	Ratio ketanserin/DOI K_i
<u>Agonists</u>			
5-HT	3.734 ± 0.92	12.6 ± 1.8	3.37^{**}
(+)mefloquine	2400 ± 290	2940 ± 770	1.23
(-)mefloquine	4260 ± 840	3280 ± 1100	0.77
chloroquine	39980 ± 5700	18310 ± 4800	0.46^*
LSD	0.72 ± 0.17	0.475 ± 0.094	0.66
DOM	2.06 ± 0.52	13.7 ± 6.0	6.65^{**}
DMT	53 ± 10	175 ± 42	3.3^*
<u>Antagonists</u>			
Ketanserin	4.1 ± 1.7	0.77 ± 0.26	0.19
MDL100,907	0.67 ± 0.24	0.048 ± 0.17	0.07^{**}

K_i values were calculated from the IC₅₀ values derived from the data in Fig 4.

*
p<0.05.

**
p<0.01.

Two tailed t-test for each compound comparing the log K_i values for inhibition of [¹²⁵I]DOI and [³H]ketanserin binding. 5-HT, DOM and DMT have higher affinity for the [¹²⁵I]DOI binding site; chloroquine and MDL100,907 have higher affinity for the [³H]ketanserin binding site; and ketanserin, (+)mefloquine, (-)mefloquine and LSD have similar affinity for both binding sites. n=3–8 independent experiments.

Table 4

Interaction of Mefloquine and other drugs with recombinant human neurotransmitter transporters

Dopamine transporter		Norepinephrine transporter	Serotonin transporter
A. Inhibition of [¹²⁵ I]RTI-55 binding			
<u>K_i (nM) ± SEM</u>			
(+)-Mefloquine	>6,400 [#]	5,300 ± 710	229 ± 39
(-)-Mefloquine	6,500 ± 930	5,280 ± 59	2,900 ± 310
Chloroquine	>8,000 [#]	1,052 ± 81	>8,300 [#]
Quinine	>8,300 [#]	>5,500 [#]	>4,000 [#]
LSD	>10 μM	5,600 ± 260	>10 μM
Cocaine	416 ± 59	410 ± 120	450 ± 140
B. Inhibition of [³ H] neurotransmitter uptake			
<u>IC₅₀ (nM) ± SEM</u>			
(+)Mefloquine	>10 μM	>10 μM	341 ± 64
(-)Mefloquine	>10 μM	>10 μM	>6,500 [#]
Chloroquine	>10 μM	>10 μM	>10 μM
Quinine	>10 μM	>10 μM	1,650 ± 420
LSD	>10 μM	>10 μM	>10 μM
Cocaine	272 ± 71	194 ± 21	295 ± 44

Specific [¹²⁵I]RTI-55 binding to HEK-293 cells expressing each of the neurotransmitter transporters was assessed as described in the text. Each K_i value represents the mean of at least 3 independent experiments, each conducted with duplicate determinations. Cocaine, a neurotransmitter transporter blocker, was included for purposes of comparison.

[#] If some experiments yielded IC₅₀ or K_i values less than 10 μM and other experiments yielded IC₅₀ or K_i values greater than 10 μM, the latter experiments were assigned a value of 10 μM and averages calculated. The actual value is greater than that average and no standard error is reported. *Abbreviations:* RTI-55 Methyl (1*R*,2*S*,3*S*)-3-(4-iodophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate; LSD lysergic acid diethylamide

Table 5

Interaction of Mefloquine and other drugs with recombinant human dopamine receptors

Drug	D ₁	D ₂	D ₃	D _{4.4}
	[³ H]SCH23390	[³ H]YM 09151-2	[³ H]YM 09151-2	[³ H]YM 09151-2
	<u>K_i (nM) ± SEM</u>			
(+)-Mefloquine	>10 μM	>10 μM	1960 ± 230	>10 μM
(-)-Mefloquine	>10 μM	>10 μM	>10 μM	>10 μM
Chloroquine	>10 μM	>10 μM	>10 μM	>10 μM
LSD	273 ± 89	38.8 ± 1.8	27.1 ± 1.3	330 ± 120
DA	4300 ± 1000	1710 ± 150	61 ± 20	630 ± 180
SKF 38393	258 ± 51	4800 ± 2600	4300 ± 1800	28000 ± 13000
SCH23390	0.491 ± 0.087	887 ± 17	1920 ± 580	9700 ± 1100
Quinpirole	>10 μM	2780 ± 510	52.8 ± 6.1	262 ± 66
Butaclamol	4.1 ± 2.0	2.04 ± 0.40	4.0 ± 1.3	255 ± 63

Radioligand binding assays were conducted as described in the text. Each K_i value represents the mean of at least 3 independent experiments, each conducted with duplicate determinations. For purposes of comparison, agonists and antagonists for each receptor were included in the assays (D₁ receptor: SKF 38393 and SCH 23390; D₂, D₃ and D_{4.4} receptor: quinpirole and butaclamol). *Abbreviations:* SCH23390 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; YM-09151-2 nemonapride *N*-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-(methylamino)benzamide; LSD lysergic acid diethylamide; DA dopamine; SKF38393 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol.

Table 6Dopamine D₂ and D₃ receptor-mediated mitogenesis

Drug	D ₂ mitogenesis	D ₃ mitogenesis
	EC ₅₀ (nM) ± SEM (n) % stimulation*	
(+)Mefloquine	NC (7)	NC (4)
LSD	18.1 ± 7.4 (6)	48 ± 14 (7)
	47.9 ± 6.2%	104.4 ± 6.1%
Quinpirole	27.7 ± 8.0 (7)	2.31 ± 0.47 (4)
	99.1 ± 2.0%	99.4 ± 5.1%
Dopamine	56 ± 13 (3)	1.0 ± 0.40 (3)
	110 ± 0%	117.5 ± 2.5%
IC ₅₀ (nM) ± range % inhibition		
(+)-Mefloquine	NC (6)	3210 ± 470 (6)
		97.3 ± 2.7%
Butaclamol	0.028 ± 0.011 (4)	0.78 ± 0.22 (4)
	98.1 ± 1.9%	96.4 ± 2.8%

Assays were conducted in duplicate as described in Methods. Data represent the mean ± SEM, (n) is the number of independent experiments. NC: the data did not converge. For purposes of comparison, the dopamine D₂-like receptor agonist, quinpirole, and the endogenous agonist dopamine were included. The dopamine D₂-like receptor antagonist, butaclamol, was included for comparison, when investigating the effects of drugs on antagonism of quinpirole (30 nM)-stimulated mitogenesis.