5HT1A Serotonin Receptor Agonists Inhibit *Plasmodium falciparum* by Blocking a Membrane Channel

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To identify new leads for the treatment of *Plasmodium falciparum* malaria, we screened a panel of serotonin (5-hydroxytryptamine [5HT]) receptor agonists and antagonists and determined their effects on parasite growth. The 5HT1A receptor agonists 8-hydroxy-N-(di-n-propyl)-aminitetralin (8-OH-DPAT), 2,5-dimethoxy-4-iodoamphetamine, and 2,5-dimethoxy-4-bromophenylethylamine inhibited the growth of *P. falciparum* in vitro (50% inhibitory concentrations, 0.4, 0.7, and 1.5 μM, respectively). In further characterizing the antimalarial effects of 8-OH-DPAT, we found that this serotonin receptor agonist did not affect the growth of *Leishmania infantum*, *Trypanosoma cruzi*, *Trypanosoma brucei brucei*, or *Trichstrongylus colubriformis* in vitro and did not demonstrate cytotoxicity against the human lung fibroblast cell line MRC-5. 8-OH-DPAT had similar levels of growth inhibition against several different *P. falciparum* isolates having distinct chemotherapeutic resistance phenotypes, and its antimalarial effect was additive when it was used in combination with chloroquine against a chloroquine-resistant isolate. In a patch clamp assay, 8-OH-DPAT blocked a *P. falciparum* surface membrane channel, suggesting that serotonin receptor agonists are a novel class of antimalarials that target a nutrient transport pathway. Since there may be neurological involvement with the use of 8-OH-DPAT and other serotonin receptor agonists in the treatment of falciparum malaria, new lead compounds derived from 8-OH-DPAT will need to be modified to prevent potential neurological side effects. Nevertheless, these results suggest that 8-OH-DPAT is a new lead compound with which to derive novel antimalarial agents and is a useful tool with which to characterize *P. falciparum* membrane channels.

Discovery of drugs for the treatment of malaria is essential because of the widespread resistance of *Plasmodium falciparum* to chloroquine and other drugs. Drug resistance contributes importantly to the 1 to 2 million deaths caused by malaria every year (25, 37). Natural products from traditional medicinal plants have formed the basis of new synthetic antimalarial analogues with potent activity, and numerous other lead compounds have been identified, including alkaloids, quinones, terpenes, and flavonoids (17, 26). During the screening of plant extracts used in traditional Polynesian medicine and other natural products for antiviral and antimicrobial activity (19), we found that some serotonin (5-hydroxytryptamine [5HT]) receptor agonists have antimalarial properties. The activity of these agonists was accompanied by the blocking of a membrane channel on parasitized erythrocytes. Serotonin agonists may therefore be useful for the characterization of the membrane transport properties of the malaria parasite and may provide new lead candidates for the treatment of malaria.

**MATERIALS AND METHODS**

**Growth inhibition assays.** The *P. falciparum* isolates used were Uganda-Palo Alto (FUP; mefloquine resistant) (10), Falciparum Vietnam Oak Knoll (FVO; chloroquine resistant) (29), Indochina (chloroquine resistant) (31), Thailand (chloroquine, quinine, and mefloquine [multidrug] resistant) (32), GHA (sensitive to all tested drugs), and W-2 CDC Indochina III (resistant to chloroquine, quinine, and pyrimethamine but susceptible to mefloquine). Parasites were cultured by standard methods as previously described (30).

Inhibition of *P. falciparum* growth was assessed with two different assays. In the first assay, synchronous parasites at the schizont stage (0.1% parasitemia, 1% hematocrit) were plated onto 96-well tissue culture plates in triplicate at a final hematocrit of 1%. [3H]hypoxanthine incorporation was used to measure parasite growth. Other parasite growth inhibition assays that were evaluated in accordance with standard World Health Organization drug screening protocols included *Leishmania infantum* [isolate MHOM/MA (BE)/67], *Trypanosoma cruzi* (Tulahen C2C4, a nitrofurantoin-sensitive strain), and *Trichstrongylus colubriformis* (Dowell, Hoechst; an albenzadole-sensitive strain) (12). To evaluate mammalian cell cytoxicity, the MRC-5 human lung fibroblast cell line was cultured in minimal essential medium Rega 3 medium supplemented with 20 mM glucose, 16.5 mM NaHCO3, and 5% fetal calf serum. The test compounds were routinely tested at four concentrations (32, 8, 2, and 0.5 μM) in 384-well microtiter plates. If the IC50 was higher than 16 μM, the compound was classified as nontoxic; if it was between 16 and 1 μM, the compound was classified as moderately toxic, and if it was less than 1 μM, the compound was classified as highly toxic.

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TABLE 1. Inhibitory effects of serotonin receptor agonists and antagonists on P. falciparum growth*

<table>
<thead>
<tr>
<th>Serotonin receptor ligand</th>
<th>Serotonin receptor ligand specificity</th>
<th>Ligand function</th>
<th>Serotonin receptor $K_r$ (nM)</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-DPAT</td>
<td>5HT1A</td>
<td>Agonist</td>
<td>2.0</td>
<td>0.38</td>
</tr>
<tr>
<td>DOI</td>
<td>5HT2</td>
<td>Agonist</td>
<td>10.0</td>
<td>0.70</td>
</tr>
<tr>
<td>2-CB</td>
<td>5HT2</td>
<td>Agonist</td>
<td>90.0</td>
<td>1.52</td>
</tr>
<tr>
<td>Spiroperone</td>
<td>5HT1A</td>
<td>Antagonist</td>
<td>1,000</td>
<td>3.16</td>
</tr>
<tr>
<td>Ritanerzine</td>
<td>5HT2</td>
<td>Antagonist</td>
<td>1.0–2.0</td>
<td>6.32</td>
</tr>
<tr>
<td>Ketanserin</td>
<td>5HT2</td>
<td>Antagonist</td>
<td>0.8–1.0</td>
<td>12.65</td>
</tr>
</tbody>
</table>

* Percent inhibition of P. falciparum (Falciparum Uganda Palo Alto, FUP, isolate) growth was determined by measuring [3H]hypoxanthine incorporation (counts per minute) in vitro. Infected erythrocytes at the schizont stage (0.1% parasitemia) were plated onto 96-well tissue culture plates in triplicate at a final hematocrit of 1%, and [3H]hypoxanthine incorporation was used to measure parasite growth. Growth inhibition was determined over a 72-h period by comparing the parasitemia change in test cultures with cultures containing cell culture medium as follows: % growth inhibition = cpm of receptor ligand – cpm of background/cpm of culture medium control – cpm of background.

Results

Serotonin receptor agonists inhibit growth of malaria parasites. We first evaluated the antimalarial activity of test compounds with a standard [3H]hypoxanthine assay and cultured P. falciparum parasites. Three serotonin receptor agonists, 8-OH-DPAT, DOI, and 2-CB, markedly inhibited the growth of P. falciparum (FUP isolate) and had IC$_{50}$s of 0.4, 0.7, and 1.5 µM, respectively (Table 1). The extent of P. falciparum growth inhibition correlated with the affinity of these serotonin receptor agonists to the 5HT1A receptor, as determined by measuring the cAMP induced by serotonin in synaptosomal membrane-enriched fractions. In contrast, serotonin receptor antagonists generally had substantially lower antimalarial activity and this activity was not correlated with serotonin receptor affinity (Table 1). Nevertheless, the growth-inhibitory activity of each of the serotonin receptor agonists was dose dependent for the P. falciparum FUP isolate.

Since P. falciparum isolates are known to differ in sensitivity to chemotherapeutic compounds, we next determined the IC$_{50}$ of 8-OH-DPAT against four other P. falciparum isolates with different antimalarial resistance patterns. We found that the chloroquine-resistant parasite isolate from Indochina and a multidrug-resistant parasite isolate from Thailand were less sensitive to growth inhibition by 8-OH-DPAT than were the chloroquine-resistant isolate, FVO, and the mefloquine-resistant parasite isolate, FUP (Fig. 1).

Serotonin receptor ligands are specific for the human malaria parasite P. falciparum. We next evaluated the activities of serotonin receptor agonists and antagonists against other parasites. As shown in Table 2, the antiparasitic activity of 8-OH-DPAT was specific for P. falciparum (with a chloroquin-sensitive isolate from Ghana, the IC$_{50}$ was 0.36 µM). Marginal antimalarial activity was also observed with the 5HT1A receptor agonist buspirone (an IC$_{50}$ of 22 µM). No activity was observed against the protozoan parasite Trypanosoma brucei brucei, Trypanosoma cruzi, or Leishmania infantum or against the nematode Trichostrongylus colubriformis. Moreover, no cytotoxicity of any of these 5HT1A receptor agonists and antagonists for MRC-5 human lung fibroblast cells was observed.

The antimalarial activity of 8-OH-DPAT is additive in combination with that of chloroquine. Since calcium channel blockers are known to reverse chloroquine resistance in P. falciparum, we determined whether 8-OH-DPAT also reverses chloroquine resistance. We conducted an isobologram analysis with a chloroquine-resistant P. falciparum isolate (W-2 CDC Indochina III strain). We found that the effects of 8-OH-DPAT and chloroquine were additive. Thus, 8-OH-DPAT did not act as a chloroquine resistance reversal agent, but when combined, the two drugs were active at nanomolar concentrations against chloroquine-resistant parasites (data not shown).
8-OH-DPAT blocks a malaria parasite membrane channel. To determine if 8-OH-DPAT targets a membrane channel on the malaria parasite, a patch clamp assay was performed with erythrocytes infected with the FUP isolate. Patch recordings in phosphate-buffered saline revealed channel transitions between (at least) two states. When 8-OH-DPAT was added to the bath, these transitions were no longer recorded after about 2 min, indicating blocking of the channel (Fig. 2). Those few transitions that were observed were in the same direction as the ion control, suggesting that these are channel openings. Since the patch clamp recordings were performed in the cell-attached mode, blocking of the membrane channel may be mediated by a secondary messenger cascade.

**DISCUSSION**

This study shows that the 5HT1A receptor agonist 8-OH-DPAT has activity against the human malaria parasite *P. falciparum*. This study also demonstrates that 8-OH-DPAT targets a membrane channel on *P. falciparum*-infected erythrocytes. 8-OH-DPAT inhibited *P. falciparum* growth at about 10-fold lower concentrations than did the serotonin receptor antagonists tested (Table 1) and was specific for *P. falciparum* and not cytotoxic (Table 2). The lack of correlation between the growth inhibition activity and the serotonin receptor affinity of agonists and antagonists suggests that the serotonin-like receptor of *P. falciparum* differs from the receptor found on neurons.

8-OH-DPAT inhibited an ion membrane channel on the surface of *P. falciparum*, as demonstrated with a patch clamp assay (Fig. 2). The ion membrane channel characterized in this study may be identical to the nutrient channel that was described with a patch clamp assay (6, 7). *P. falciparum* modifies the membrane permeability of its host erythrocyte (16) and controls calcium levels with a plasma membrane transport pump (1, 33). 8-OH-DPAT is known to reduce the influx of sodium and calcium into rat synaptosomes (22) and disrupt noncognitive performance in rats (27). In addition, there is evidence that 8-OH-DPAT may function as an antidepressant (15). Other antidepressants, such as desipramine and imipramine (23), have modest antimalarial activity alone and have also been found to facilitate the reversal of chloroquine resistance (3, 4, 18, 34). Nevertheless, since desipramine has not had success in clinical trials for the treatment of chloroquine-resistant malaria (35), new leads and structure-function relationships need to be explored (2).

We conclude that 8-OH-DPAT may be a valuable lead compound with which to build new combinatorial libraries for antimalarials. 8-OH-DPAT is structurally similar to thebaine-like molecules and pain management pharmaceuticals such as morphine; therefore, selective compounds lacking neurological activity are needed.

![FIG. 2. Blocking of an ion channel on the parasitophorous vacuole membrane of *P. falciparum* by a 5HT1A agonist, as shown by patch clamp recordings of parasitized erythrocytes. (A) Control baseline recordings showing noncontinuous sweeps of current recorded with the pipette potential clamped at −100 mV (transmembrane potential not known). Each line shows every third sweep from a series of 50 sweeps. The channel is open when the amplitude of the recording is increased and closed (blocked) when the recording is flat. (B) Recordings 2 min after the 5HT1A agonist 8-OH-DPAT (0.1 mg/ml) was added to the bath under the same conditions as the control (A).](http://aac.asm.org/)

**TABLE 2. Activities of 5HT1A serotonin receptor ligands against different parasitic organisms**

<table>
<thead>
<tr>
<th>Ligand</th>
<th><em>T. brucei brucei</em></th>
<th><em>T. cruzi</em></th>
<th><em>T. colubriformis</em></th>
<th><em>L. infantum</em></th>
<th><em>P. falciparum</em></th>
<th>Conco (µM) showing cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-OH-DPAT</td>
<td>&lt;32</td>
<td>≥32</td>
<td>&gt;32</td>
<td>≥32</td>
<td>0.36</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Buspirone</td>
<td>≥32</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>≥32</td>
<td>22.0</td>
<td>≥32</td>
</tr>
<tr>
<td>NAN-190</td>
<td>≥32</td>
<td>≥32</td>
<td>&gt;32</td>
<td>≥32</td>
<td>≥32</td>
<td>≥32</td>
</tr>
</tbody>
</table>

*Parasite growth inhibition was determined in colorimetric assays or by direct staining of parasite cultures as described in Materials and Methods. Each compound was first tested in fourfold titrations ranging from 32 to 0.5 µM, and the IC50 was determined by comparison with control cultures. If the IC50 was below 0.5 µM, the compound was classified as highly active and was evaluated in a secondary screen. Values shown are means of results from assays performed in triplicate. Positive control compounds had the following IC50 (micromolar) in each assay: suramin, 0.09 for *T. brucei brucei*; nifurtimox, 0.25 for *T. cruzi*; albendazole, 0.02 for *T. colubriformis*; pentostam, 8.0 for *L. infantum*; chloroquine, 0.02 for *P. falciparum*.

* Cytotoxicity was determined with the MRC-5 human lung fibroblast cell line.

* NAN-190, hydrobromide 1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine.
activity would need to be selected. The use of 8-OH-DPAT directly for the treatment of falciparum malaria is unlikely because of possible neurological side effects, such as the serotonin syndrome (11, 21). The avoidance of neurological effects would need to be addressed in the design and evaluation of related compounds. For example, compounds that are predicted to have neurological effects could be screened in animal models of the serotonin syndrome (24, 28).

These data indicate that 8-OH-DPAT may target a 5HT1A-like receptor present in P. falciparum and thus may be used to identify this membrane channel by affinity purification or as a biotinylated probe for quantitative proteomic analysis (36). This receptor may be a nutrient channel critical for parasite development. Thus, this P. falciparum receptor may be an important target for malaria chemotherapeutic intervention.

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