INTRODUCTION

Several years ago antimicrobial activity was described for psychotropic drugs of the phenothiazine and thioxanthene groups (Brown, 1975). Since then, several non-antibacterial substances have been examined and it has been reported that selective serotonin re-uptake inhibitors (SSRIs) influence the in vitro viability of bacteria (Cederlund & Mardh, 1993; Munoz-Bellido et al., 1996, 2000) and may reverse chloroquine resistance in Plasmodium falciparum (Coutaux et al., 1994). These drugs have significant antimicrobial activity, mainly against Gram-positive bacteria, yet they are inactive against most enteric Gram-negative bacteria (Munoz-Bellido et al., 2000).

Recently, we found that sertraline, a typical SSRI has in vivo and in vitro antifungal activity (Lass-Flörli et al., 2001a, b). Since fungicidal effects were observed at high concentrations, immunomodulatory effects or several modifications of fungal virulence by SSRIs were more likely to explain the in vivo outcome in our patients. In humans, SSRIs modify the behaviour of 5-hydroxytryptamine (5 HT) and act primarily on the 5 HT transporter protein (SERT) (Schloss & Williams, 1998). A block in the re-uptake process of 5 HT causes an increase in 5 HT during therapy with SSRIs (Dimmock et al., 2000). This fact and the clinical phenomenon found in our patients (Lass-Flörli et al., 2001a) led us to examine the potential fungicidal role of 5 HT. We determined the direct influence of 5 HT on the viability of clinical isolates of Candida spp. and studied whether delayed regrowth as a post-antifungal effect results following short exposure to 5 HT.

METHODS

Strains. The in vitro tests were performed on clinical isolates of Candida albicans (n = 11), Candida glabrata (n = 9), Candida tropicalis (n = 10) and Candida parapsilosis (ATCC 22019). Isolates were maintained as suspensions in sterile water at room temperature and subcultures were grown on Sabouraud glucose agar (Merck) incubated at 35 °C for 2 days.

Drug. According to the manufacturer’s instructions, 5 HT (M, 212) (Sigma) was dissolved and further diluted in sterile water (Fresenius); final concentrations were 0.47 M–0.22 mM.

Broth microdilution test. Isolates were tested using the microbroth dilution method according to the National Committee for Clinical Laboratory Standards (1997) guidelines. A fungal inoculum size of 1–6 × 10⁶–6 × 10⁸ c.f.u. ml⁻¹ was used. A total of 100 µl of each of the drug dilutions was inoculated with 100 µl of the fungal suspensions, and the mixture was incubated at 35 °C and evaluated after 24 h for growth. The minimal inhibitory concentration (MIC) end-point criterion was the lowest drug concentration showing no visible growth after 24 h of incubation. To obtain the minimal fungicidal concentration (MFC), 100 µl volumes were taken from every well and spread on Saboraud Glucose Agar (Merck). The number of c.f.u. was counted after 24 h of incubation.

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Abbreviations: 5 HT, 5-hydroxytryptamine; MFC, minimal fungicidal concentration; MIC, minimal inhibitory concentration; SSRI, selective serotonin re-uptake inhibitor.
incubation of the plates at 35 °C for 48 h until growth of subcultures from the growth control well was apparent. MFC was defined as the lowest drug concentration at which 99% of the inoculum was killed.

Lag in regrowth. Lag in regrowth was assessed using a modification of the procedure of Nagl et al. (1999). 5HT concentrations equipotent, one dilution above and one below to the MFC for each isolate were investigated. Fungal suspensions were prepared as described above and incubated with 5HT for 1 and 3 h at 35 °C. Afterwards, these suspensions were centrifuged at 4000 g for 2 min and the supernatants were aspirated. Then, the fungi were washed twice with sterile water and reffilled with RPMI 1640. Quantitative cultures of non-diluted samples and 1:100 and 1:1000 dilutions in water were spread on Sabouraud glucose agar and incubated at 35 °C. With a magnifier we visually examined the plates for growth of fungi and investigated colony size and colony counts every 12 h. We compared the time required for colony regrowth of untreated and treated isolates and examined the cultures for delayed growth. Each experiment was done twice and performed in duplicate.

RESULTS

Broth microdilution test

5HT was effective towards the tested fungi, as shown in Table 1. The MIC and MFC ranges at 24 h for Candida spp. were 0·91–7·34 mM and 1·83–14·68 mM, respectively.

Table 1. Antifungal concentrations of 5HT against Candida species

<table>
<thead>
<tr>
<th>Species</th>
<th>5HT concn (mM)</th>
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<tr>
<td></td>
<td>MIC range</td>
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<tr>
<td>C. albicans (&lt;i&gt;n&lt;/i&gt; = 11)</td>
<td>1·83–7·34</td>
</tr>
<tr>
<td>C. glabrata (&lt;i&gt;n&lt;/i&gt; = 9)</td>
<td>0·91–3·67</td>
</tr>
<tr>
<td>C. tropicalis (&lt;i&gt;n&lt;/i&gt; = 10)</td>
<td>1·83–3·67</td>
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<tr>
<td>C. parapsilosis (ATCC 22019)</td>
<td>3·67–7·34</td>
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Table 2. In vitro effects of Candida spp. (<i>n</i> = 30) treated with different concentrations of 5HT

<table>
<thead>
<tr>
<th>Exposure time to 5HT (h)</th>
<th>Species</th>
<th>No. of isolates with certain effects (% in parentheses) *</th>
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<tbody>
<tr>
<td></td>
<td>C. albicans (&lt;i&gt;n&lt;/i&gt; = 11)</td>
<td>5 (45), 8 (73), 11 (100)</td>
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<tr>
<td></td>
<td>C. glabrata (&lt;i&gt;n&lt;/i&gt; = 9)</td>
<td>5 (55), 9 (100), 9 (100)</td>
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<tr>
<td></td>
<td>C. tropicalis (&lt;i&gt;n&lt;/i&gt; = 10)</td>
<td>4 (40), 8 (80), 10 (100)</td>
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<tr>
<td>3</td>
<td>C. albicans (&lt;i&gt;n&lt;/i&gt; = 11)</td>
<td>0 (0), 0 (0), 8 (73)</td>
</tr>
<tr>
<td></td>
<td>C. glabrata (&lt;i&gt;n&lt;/i&gt; = 9)</td>
<td>0 (0), 0 (0), 7 (78)</td>
</tr>
<tr>
<td></td>
<td>C. tropicalis (&lt;i&gt;n&lt;/i&gt; = 10)</td>
<td>0 (0), 0 (0), 8 (90)</td>
</tr>
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</table>

* Data are presented in the order (i) 5HT concentration one dilution above the MFC for each isolate, (ii) 5HT concentration equivalent to the MFC for each isolate and (iii) 5HT concentration one dilution below the MFC for each isolate. NE, No effects; LAG, lag in regrowth of 8–12 h; c.f.u., 80–90% killing of inoculum.

DISCUSSION

Our study revealed that 5HT can inhibit and kill isolates of C. albicans, C. glabrata and C. tropicalis. In addition, a delay in fungal regrowth depended on the concentration tested. There is a surprising coincidence of an increased rate of infection and low 5HT levels in certain diseases, e.g. AIDS (Larsson et al., 1989), Down’s syndrome (Tu & Zellweger, 1965) and Chediak–Higashi syndrome (Rendu et al., 1983). A possible role of 5HT in antifungal host defence has been suggested by a few other studies. Christin et al. (1998) reported that platelets, which contain 5HT attach to the cell wall of Aspergillus fumigatus and damage this organism. Furthermore, we observed that 5HT is able to kill conidia and hyphae of Aspergillus spp. in vitro (Lass-Florl et al., 2002). Even so, it is known that several antimycotic drugs interfere with platelets (Helmeste et al., 1998): miconazole and econazole inhibit platelet uptake of 5HT and could therefore...
contribute synergistic effects in the defence against fungal infections. Recently, it was reported that incubation of neutrophils with 5 HT results in a modulation of their bactericidal efficacy (Schuff-Werner & Splettstoesser, 1999).

However, since our in vitro effects were observed at high 5 HT concentrations the relevance for direct antifungal host defence remains unclear. In vivo, 5 HT levels occur under physiological and pathophysiological conditions (Harenbe et al., 2000). Base levels in several tissues is probably determined by platelets present in those tissues rather than by 5 HT localized in parenchymal cells. Similar considerations apply to 5 HT levels during inflammation. 5 HT content in in vitro preparations is quite variable (range about 748·3 ng per 10^9 cells; Kumar et al., 1990) whereas the serum 5 HT levels can exceed 7987 ng ml^{-1} in several circumstances (Harenbe et al., 2000). It is possible that these levels may be sufficient to modify fungal virulence. A lag in regrowth was observed after exposure to 5 HT and the extent of this effect depended on the concentration of 5 HT and the incubation time. The maximum duration of lag in regrowth was observed at incubation times lower than required for killing, yet at concentrations similar to the MFC.

In conclusion, 5 HT acts against Candida spp. in at least two steps: reversible attenuation and, if incubation is prolonged, irreversible changes, resulting in loss of viability. The data encourage us to focus on the relationship between Candida spp. and 5 HT and to define the role of 5 HT in antifungal host defence. It can be imagined that 5 HT acts in several ways, both directly on the fungi and indirectly on the defence system. At any rate, 5 HT could contribute synergistic antifungal effects when SSRIs are administered. Identification of the mode of action of 5 HT would be of great help in the research and development of new antifungal drugs.

REFERENCES


