SYNTHESIS AND SAR STUDIES OF 1,2,3,4-TETRAHYDRO-9-CARBOLINE DERIVATIVES AS NEW 5-HT₁/5-HT₁A RECEPTOR LIGANDS

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Introduction

Since its discovery in 1993, the 5-HT₁ receptor is gaining increasing interest as a potential drug target. Studies utilizing recently developed selective antagonists revealed that 5-HT₁ receptors play a role in thermoregulation, learning and memory, hippocampal activity, sleep, circadian rhythms and mood. Due to a relatively limited number of papers describing structure-activity relationship (SAR) studies of 5-HT₁ receptor ligands further research in this field is of particular interest.

It is known that pharmacophoric arylpiperazine fragment is well recognized by 5-HT₁A, 5-HT₁B as well as 5-HT₁C receptors. Indeed, 1-(2-methoxyethyl)piperazine (oMPP) derivatives were among the most active 5-HT₁ receptor ligands identified by the screening of our compounds library. Those compounds, however, were usually several times more active at 5-HT₁A sites (e.g. compounds 1, 2 and 4-6). Interestingly, indoline derivative 3 was equally potent, dual 5-HT₁A/5-HT₁B receptor ligand.

In the case of 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives it was found that they were less active 5-HT₁ ligands than the respective oMPP analogues, but more significant decrease was observed for their 5-HT₁B affinity. Compounds with indoline and 8-azaspiro[4,5]decane-7,9-dione fragments (9 and 11, respectively) showed the highest 5-HT₁ affinity.

In order to search for a new 5-HT₁ ligands with increased selectivity for 5-HT₁A receptors two new series of compounds were designed based on the results presented by Kikuchi C. et al. [1]. In the structure of oMPP or THIQ derivatives, amine fragment was replaced with a 1,2,3,4-tetrahydro-β-carboline (THBC) or 9-methylcarbamoylmethyl-THBC moiety (Scheme, Table). For all those compounds binding affinity for 5-HT₁ and 5-HT₁B receptors was measured, and next, functional profile at 5-HT₁ receptors for two selected derivatives was determined.

Figure 1. Concentration-response curve of 5-CT in the absence (a) and in the presence B1013 (b), B1032 (c) for stimulation of cAMP accumulation in H4 cells. Data points represent the mean ± S.E.M.

SEROTONIN 5-HT₄ AND 5-HT₁A BINDING ASSAYS

Radioligand binding studies with native 5-HT₁ receptors used rat hypothalamic membranes, [³H]5-HT-CT (102.0 Ci/mmol, Amersham) and serotonin for nonspecific binding, whereas for 5-HT₁B assays rat hippocampal membranes, [³H]8-OH-DPAT (170 C/mmol, NEN Chemicals) and 5-HT for nonspecific binding were used.

The new THBC derivatives exhibited moderate to low 5-HT₁ affinity ranging from 80 nM to 2600 nM for B1013 and 19, respectively. All the new compounds were less active at 5-HT₁ receptors, than their oMPP analogues, however, some of them (12, 20 and 21) showed higher affinity than THIQ derivatives. Again, the presence of indoline and 8-azaspiro[4,5]decane-7,9-dione moieties in the ligand structure was beneficial to 5-HT₁ receptor activity.

Table: Structure and 5-HT₁ (red) and 5-HT₁B (blue) binding affinities of the investigated compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>5-HT₁ IC₅₀ [nM]</th>
<th>5-HT₁B IC₅₀ [nM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1013</td>
<td>6.4 ± 0.6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>B1032</td>
<td>10 ± 2</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>11M199</td>
<td>11M199</td>
<td></td>
</tr>
<tr>
<td>200⁹</td>
<td>6.3 ± 0.2</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>1A</td>
<td>9.0 ± 0.1</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>3</td>
<td>13 ± 0.4</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>20 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>25 ± 4</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>6</td>
<td>30 ± 5</td>
<td>36 ± 6</td>
</tr>
</tbody>
</table>

<sup>9</sup> Compounds are listed in Table 1 with affinities in nM. Corresponding to the structure of 5-HT₁ receptors shown in Figure 1. (Fig. 2)

Cyclic AMP measurements

5-HT₁ receptor is positively coupled to adenylate cyclase and the stimulation of this receptor results in the increase of cAMP level. It was previously shown that 5-CT (5-carboxamidotryptamine maleate)–stimulated cAMP accumulation in H4 (ATCC HTB-178) human glioblastoma cell line was selectively blocked by SB269970 in a dose dependent manner [2]. Similar experiments were used to determine functional profile of the investigated compounds at 5-HT₁ receptor. First it was found that neither B1013 nor B1032 used in different concentrations influenced cAMP accumulation.

Next the ability of B1013 and B1032 to inhibit 5-CT (with pEC₅₀ 7.2 ± 0.12) stimulated cAMP accumulation in H4 cell line was investigated. The dose–response curves representing 1 M of B1013 (with pEC₅₀ 5.7 ± 0.042) or 1 M of B1032 (with pEC₅₀ 6.1 ± 0.013) in the presence of 5-CT in a concentration of 1 x 10⁻⁸ to 1 x 10⁻⁴ M are shown in Figure 1. In accordance with the measured 5-HT₁ affinities, antagonistic potency of B1013 was higher than that of B1032.

Electrophysiological studies

In addition to the above experiments, our potent 5-HT₁A antagonist with anxiolytic-like activity – MM77, displaying comparable 5-HT₁ affinity to that of B1013, was electrophysiologically characterized and compared with a potent 5-HT₁ antagonist SB269970.

After desipation, the rat hippocampus was cut into transverse slices (400 µm thick) using a vibrating microtome. Spontaneously occurring epileptiform bursts were recorded within 15–30 minutes of perfusion of the slices with nominally Mg²⁺ -free ACSF. Buzding events, representing primary bursts, consisted of a prominent initial population spike-like waveform, reaching 2–3 mV in amplitude, which was followed by smaller afterdischarges, superimposed on a slower, positive-going wave, lasting 60–100 ms (Fig. 2).

The application of 5-CT for 10 min in the presence of 1 M WAY 100635, a selective 5-HT₄ receptor antagonist, resulted in an increase in the bursting frequency (Fig. 3). The excitatory effect of 5-CT was dose-dependent. The SB269970, a specific antagonist of the 5-HT₄ receptor, in dose dependent manner inhibited the excitatory effect of 5-CT (Fig. 3).

The application of MM77 decreased the excitatory action of 5-CT in similar manner to SB269970 but with smaller efficacy (Fig. 3). Neither SB269970 nor MM77 applied alone exerted any effect on the bursting frequency.

Summary

14 new tetrahydro-β-carboline derivatives with tetramethylene linker and different (cyclic imide/amine, benzotriazole) termini were synthesized and evaluated for 5-HT₁ and 5-HT₁A receptor activity. Compounds with indoline and 8-azaspiro[4,5]decane-7,9-dione fragments were among the best 5-HT₁ ligands and two of them (B1013 and 1032) showed antagonistic activity in adenylyl cyclase assay. Electrophysiological studies with MM77 revealed that this potent antagonist of postsynaptic 5-HT₁A receptors behaved also like antagonist at 5-HT₁ receptors, however, of slightly lower efficacy than SB269970.

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References
