Pharmacology, Biochemistry and Behavior 141 (2016) 28–41

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Antidepressant-like activity of aroxyalkyl derivatives of 2-methoxyphenylpiperazine and evidence for the involvement of serotonin receptor subtypes in their mechanism of action

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ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 24 November 2015
Accepted 27 November 2015
Available online 2 December 2015

Keywords:
Depression
Serotonin
5-HT1A
5-HT2A
5-HT3
5-HT7
2-methoxyphenylpiperazine
Forced swim test

ABSTRACT

Since serotonin (5-HT) is strongly involved in the etiology and pathophysiology of depression, the development of new antidepressants is still based on the serotonergic system. The complexity of serotonergic system provides an opportunity for the development of compounds with multiple and complementary mechanism of action. This study describes serotonin receptor profile, functional characterization, and pharmacological in vivo evaluation of new aroxyalkyl derivatives of 2-methoxyphenylpiperazine. The obtained results allowed for the identification of compound **3**, (1-[3-(2,6-dimethylphenoxy)propyl]-4-(2-methoxyphenyl)piperazine hydrochloride), a partial 5-HT_{1A} receptor agonist, and 5-HT_{2A} receptor antagonist, with high affinity toward 5-HT_7 receptors, showing antidepressant- and anxiolytic-like properties. Moreover, 5-HT_{1A} receptor activation is crucial for the antidepressant-like activity of compound **3**. The rest of the compounds (except compounds **1** and **9**) showed antidepressant but not anxiolytic-like properties, which did not result from 5-HT_{1A} receptors activation. Furthermore, the compounds are 5-HT_{1A} and weak 5-HT_3 receptors antagonists, and some of them 5-HT_{2A} antagonists. Moreover, none of the studied compounds impaired motor coordination at antidepressant-like doses. Since the studied compounds exhibited activity in behavioral assays and interacted with various receptors, the results of our experiments are very promising and require further studies.

1. Introduction

Major depressive disorder (MDD) is a serious psychiatric illness affecting nearly 20% of the general population. It is characterized by anhedonia, persistent low mood, low self-esteem, disturbed sleep and appetite, as well as suicidal tendencies. MDD and anxiety are often concomitant and associated with more severe clinical symptoms and reduced treatment response. Despite the increasing number of new drugs, their effectiveness is still unsatisfactory. Therefore, scientists are still looking for new antidepressants that are more effective, with fewer side effects and faster onset of action (Carr and Lucki, 2011; Willner et al., 2013; Mahar et al., 2014; Partyka et al., 2015; Pytka et al., 2015b, 2015c).

The etiology and pathophysiology of MDD is still not fully understood. However, one of the most widely accepted hypothesis states that there is a significant depletion of monoamines, mostly serotonin (5-hydroxytryptamine (5-HT)) (Mahar et al., 2014). In agreement with this hypothesis is the fact that reduced level of 5-HT was found in postmortem brain tissues of patients with depression or suicide victims (Owens and Nemeroff, 1994). Many studies indicate the important role of 5-HT, as well as 5-HT receptors, in the pathogenesis of MDD (Carr and Lucki, 2011; Pytka et al., 2015a). Moreover, it is noteworthy that the majority of currently available antidepressants act predominantly by targeting the serotonin transporter (SERT), but their therapeutic effects are a result of the interaction with multiple 5-HT receptors (Carr and Lucki, 2011). Both clinical and preclinical studies suggest that various 5-HT receptors are involved in the therapeutic effect of antidepressants (Carr and Lucki, 2011; Stahl et al., 2013; Partyka et al., 2015). It has been

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http://dx.doi.org/10.1016/j.pbb.2015.11.013
0091-3057/

demonstrated that 5-HT_{1A}, 5-HT_{2A}, 5-HT_3, 5-HT_6, and 5-HT_7 receptors are involved in MDD or anxiety, as well as in antidepressant- and antianxiety-like effects (Carr et al., 2011; Artigas, 2013; Fakhoury, 2015; Waszkielewicz et al., 2015).

5-HT_{1A} antagonists or partial agonists have been proposed as adjunctive therapies for selective serotonin reuptake inhibitors (SSRIs) because of their potential to accelerate the onset of SSRIs' action (Carr and Lucki, 2011; Artigas, 2013; Czopek et al., 2013; Waszkielewicz et al., 2015). In addition, selective 5-HT_{1A} receptor agonists were able to reproduce many of the effects of SSRIs (Carr and Lucki, 2011). Moreover, many studies suggest that 5-HT_{1A} receptor ligands such as buspirone themselves possess antidepressant- and/or anxiolytic-like activities (Celada et al., 2013; Fakhoury, 2015; Partyka et al., 2015; Waszkielewicz et al., 2015).

In the case of 5-HT_{2A} receptors, numerous postmortem studies have reported that a high density of these receptors can be found in suicide victims with depression (Shelton et al., 2009). Thus, 5-HT_{2A} receptors contribute to the pathogenesis of MDD. 5-HT_{1A} and 5-HT_{2A} receptors are coexpressed in the neocortex and it is postulated that the blockade of 5-HT_{2A} receptors enhances the 5-HT_{1A} receptor-mediated neurotransmission in cortical and limbic areas. This effect is probably linked to the antidepressant efficacy of 5-HT_{2A} antagonists. 5-HT_{2A} receptor antagonists may also produce antidepressant-like effects by modulating the release of other neurotransmitters (e.g., dopamine and noradrenaline) in the prefrontal cortex (Artigas, 2013; Celada et al., 2013; Fakhoury, 2015). Preclinical studies demonstrated antidepressant-like effects of 5-HT_{2A} receptor antagonists such as LY367265 and EMD-281014, and 5-HT_{2A} antagonism is partially responsible for the clinical efficacy of trazodone or nefazodone (Pandey et al., 2010).

Ionotropic 5-HT_3 receptors may also represent a potential target for depression treatment. Some 5-HT_3 receptor antagonists, such as bemesetron or tropisetron, showed antidepressant-like activity in rodent tests. Moreover, antidepressants, for example, fluoxetine or reboxetine, act as 5-HT_3 functional antagonists (Gupta et al., 2014).

5-HT_6 receptors, exclusively expressed in central nervous system (CNS), mainly in corticolimbic areas, may also be involved in MDD. It is noteworthy that several antidepressants (e.g., amitriptyline and mianserin) have high affinity for 5-HT_6 receptors. It has been demonstrated that both 5-HT_6 agonists and antagonists show antidepressant-like effects in preclinical studies (Wesołowska, 2010; Carr et al., 2011).

It has been reported that the blockade of 5-HT_7 receptors causes antidepressant-like effects in preclinical studies (Wesołowska and Kowalska, 2008). It is not surprising though that 5-HT_7 receptor antagonists were proposed as new pharmacotherapy agents for the treatment of MDD. Studies revealed that 5-HT_7 receptor antagonists alone or in combination with monoamine reuptake inhibitors exhibit antidepressant-like activity (Sarkisyan et al., 2010).

Some known drugs possess several simultaneous mechanisms of action, for example, mirtazapine with 5-HT_{2A}/5-HT_3/α_2 antagonism, vilazodon with 5-HT_{1A} partial agonism in addition to SERT inhibition or vortioxetine, combining partial agonism at 5-HT_{1A} with antagonism at 5-HT_3 and 5-HT_7 receptors and SERT inhibition (Stahl et al., 2013). It is postulated that such multitarget ligands may be more effective in the treatment of MDD.

An arylpiperazine moiety is present in the structures of many agents acting through G-protein coupled receptors (GPCRs). Arylpiperazines are found to be 5-HT receptor ligands, in particular 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7, as well as other GPCRs, that is, α-adrenoceptors. Alkyl derivatives of phenylpiperazine represent the class of compounds with high affinity for 5-HT_{1A} receptors (Zygmunt et al., 2014; Partyka et al., 2015; Waszkielewicz et al., 2015). Our former research within the group of piperazine derivatives focused on both receptor binding studies and in vivo central activity (Marona et al., 2011; Waszkielewicz et al., 2013; Pytka et al., 2015b, 2015c). A special moiety constituted methoxyphenylpiperazine was present in some of our previously synthesized derivatives (Marona et al., 2011; Waszkielewicz et al., 2013; Pytka et al., 2015b, 2015c). In terms of aroxyethyl and aroxypropyl derivatives of 1-(2-methoxyphenyl)piperazine, we have selected several compounds with beneficial receptor properties (Marona et al., 2011). In particular, substitution of the phenyl ring in positions 2,3; 2,5; 2,6; 2,3,5, or 2,4,6 with lipophilic substituents such as methyl or chlorine proved beneficial.

Herein the in vitro affinity evaluation for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors of the 1-[(phenoxy)alkyl]-4-(2-methoxyphenyl)piperazine derivatives, achieved in the form of hydrochlorides for their optimum bioavailability, based on their previously published chemistry and some pharmacological properties is reported (Marona et al., 2011; Waszkielewicz et al., 2013; Pytka et al., 2015b, 2015c). On the basis of receptor-binding studies, we performed functional experiments and evaluated the antidepressant- and anxiolytic-like activities of the investigated compounds by behavioral tests.

2. Materials and methods

2.1. Animals

The experiments were carried out using adult male Albino Swiss mice (CD-1, 18–21 g), male Wistar rats (Krf:(WI) WU), 200–250 g), male guinea pigs (outbred 300–400 g). The animals were housed in constant-temperature facilities exposed to 12:12 h light/dark cycles and were maintained on a standard pellet diet with tap water given ad libitum. All experiments were conducted between 9:00 AM and 2:00 PM. Each experimental group consisted of 6–10 animals and all animals were used only once. All procedures were conducted according to the Animal Care and Use Committee Guidelines and approved by the Local Ethics Committee of the Jagiellonian University in Krakow (resolution nos. 168/2012, 14.11.2012, 79/2013, 29.05.2013, 52/2014, 19.03.2014).

2.2. Drug administration

8-Hydroxy-2-(di-n-propylamino)tetralin (hydrobromide, 8-OH-DPAT, Tocris, Cookson Ltd., UK), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl) cyclohexanecarboxamide (trihydrochloride, WAY 100635), (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride ((±)DOI), and imipramine (hydrochloride, Polfa-Starogard, Poland) were dissolved in saline. Diazepam (Polfa-Poznan, Poland) was suspended in 1% aqueous solution of Tween 80 and the investigated compounds were dissolved in 0.9% NaCl. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc). Imipramine, diazepam, and the tested compounds were given intraperitoneally (ip). Injections were made in a volume of 10 ml/kg (mice) or 2 ml/kg (rats).

2.3. Radioligand binding experiments

Compounds were tested by competition binding experiments for 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors, according to the previously published procedures (Waszkielewicz et al., 2013, 2015). Binding experiments were conducted in 96-well microplates in a total volume of 200 μl of appropriate buffers. Reaction mix included 50 μl solution of test compound, 50 μl of radioligand, and 150 μl of diluted membranes or the tissue suspension. Specific assay conditions for each receptor are shown in Table 1. Recombinant human proteins were used for 5-HT_{2A}, 5-HT_6, and 5-HT_7 receptors. 5-HT_{1A} receptors were prepared from rat hippocampal tissue. Radioactivity was measured by MicroBeta TriLux 1450 Liquid Scintillation Counter (PerkinElmer, USA).

Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 4.0—San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation. The concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-5} M.