Current Opinion in Pharmacology

5-HT_6 receptor and cognition

Xabier Codony[1], Jose Miguel Vela[1], Maria Javier Ramírez [2, 3]*

[1] Laboratorios Dr Esteve SA, Barcelona, Spain; [2] Dpto Farmacologia, Universidad de Navarra, Pamplona, Spain; [3] Division of Neurosciences, CIMA, Pamplona, Spain

Correspondence

Dr. María J. Ramírez
Department of Pharmacology
University of Navarra
C/ Irunlarrea, 1
31008 Pamplona
Spain
Tel.: +34 948425600
Fax.: +34 948425649
e-mail: mariaja@unav.es

Abstract

Since its discovery in 1993 and subsequent development of selective antagonists, a growing number of studies support the use of serotonin 5-HT_6 receptor antagonism as a promising mechanism for treating cognitive dysfunction. Lately, several studies with structurally different compounds have shown that not only antagonists, but also 5-HT_6 receptor agonists improve learning and memory in animal models. There is even an antagonist, SB-742457, that has completed phase II trials for the treatment of Alzheimer´s disease. In addition to describe pre-clinical and clinical evidence of the effect of 5-HT_6 receptor compounds on cognition, this article will also focus on the purported biochemical and neurochemical mechanisms of action by which 5-HT_6 receptor compounds could influence cognition in health and disease.

Introduction

The 5-HT_6 receptor is the most recently identified of serotonin (5-HT) receptor superfamily. Initially cloned from striatal tissue [1], the rat 5-HT_6 receptor gene encodes a protein of 438 amino acids and shares 89% homology to the human form ([[2] and [3]]. The 5-HT_6 receptor has no known functional isoforms and is a G-protein-coupled receptor that positively stimulates adenylate cyclase activity. A non-functional truncated splice variant of the 5-HT_6 receptor has been identified but appears not to have any physiological significance. Kohen et al. [2] identified a silent polymorphism at base pair 267 (C267T). Although there is evidence linking this polymorphism in several syndromes that affect cognition, including dementia, Alzheimer´s disease and schizophrenia, these findings have not always been replicated and their significance remains to be established [4].

In situ hybridization and northern blot studies revealed an exclusive distribution of 5-HT_6 mRNA in the rat central nervous system with the highest density found in the olfactory tubercle, followed by the frontal and entorhinal cortices, dorsal hippocampus (ie, dentate gyrus and CA1, CA2, and CA3 regions), nucleus accumbens, and striatum [5]. Lower levels were observed in the hypothalamus, amygdala, substantia nigra, and several diencephalic nuclei. These findings have been corroborated by immunolocalization studies and radioligand binding showing a similar distribution of 5-HT_6 receptor protein in the rat CNS [6].

The 5-HT_6 receptor has been implicated in affective disorders, anxiety and depression [7] epilepsy and obesity [8]. However, the greatest current interest is on the role that this receptor plays and the therapeutic potential of 5-HT_6 receptor compounds in learning and memory processes. In addition to describe the newly synthesized compounds active on this receptor, this article will focus on pre-clinical and limited clinical works describing the effect of 5-HT_6 receptor compounds on cognition, and the purported mechanism of action by which 5-HT_6 receptor compounds could influence learning and memory in healthy and disease.

5-HT_6 receptor ligands

Since the initial discovery of the first ligands in the late 1990s, using high throughput screening technologies on compound libraries, a growing number of scientific publications and patent applications have developed [9]. The synthesis of 5-HT_6 receptor ligands has been very successful, with a number of highly potent ligands being reported [[8], [10], [11] and [12]]. Some of them have been extensively used as pharmacological tools (i.e. Ro-04-6790 or SB-271046) but few 5-HT_6 receptor selective antagonists have reached clinical phases, being developed for the treatment of cognitive disorders (Table 1). There is also a radiolabeled compound, [11C]-GSK215083 (GlaxoSmithKline) being developed for PET radiotracer of 5-HT6 receptors in clinical phase I [13].

At preclinical level, 5-HT_6 receptor medicinal chemistry is taking benefit from the knowledge achieved since the discovery of the receptor, and a better understanding of different tools like pharmacophore modelling, 3D-molecular docking or structure similarity algorithms. As a result, increasing number and diversity of novel, highly selective 5-HT_6 receptor ligands of all functional types has been reported [12], although main efforts have been focused on antagonism [14]. Positive results in animal models of memory have been reported for lead compounds (i.e. L-483518, Ro-4368144, BGC20-761 or E-6801) further confirming the involvement of this receptor in cognitive processes and its therapeutic potential.

Experimental approaches to the role of 5-HT$_6$ receptors in cognition

Following the discovery of 5-HT$_6$ receptor ligands with good brain penetration, a growing body of preclinical evidence has supported the use of 5-HT$_6$ receptor antagonism for treating cognitive dysfunction (for review see [15]). Further support came from studies about how learning paradigms decrease 5-HT$_6$ receptor expression [[16], [17] and [18]], while 5-HT$_6$ receptor overexpression in the striatum impairs instrumental learning [19].

Different 5-HT$_6$ receptor antagonists have been reported to be active in the novel object discrimination test in rats and improved water maze retention, even in aged rats [20], although failing to alter acquisition of spatial learning. One of the more consistent data about the involvement of 5-HT$_6$ receptors in memory is the ability of 5-HT$_6$ receptor antagonist to reverse a scopolamine-induced. Interestingly, a combined treatment of SB-271046 with an acetylcholinesterase inhibitor produced an additive increase in passive avoidance, and significantly reversed scopolamine-induced amnesic effects [21]. Lu AE58054, a 5-HT$_6$ receptor antagonist, reversed cognitive impairment induced by subchronic phencyclidine in a novel object recognition test in rats [22], Ro 04-6790 also reversed impairment in learning consolidation produced by the NMDA receptor antagonist, MK-801and the antagonist PRX-07034 restored the impairment of novel object recognition in the social isolation rearing model, which show behavioral changes that resemble the core defects seen in schizophrenia [15]. SB271046 have been also shown to reverse memory disturbances in experimental models of stress-related psychiatric disorders that have been associated to an impairment of the HPA axis reactivity [23]. Altogether, it appears that 5-HT$_6$ receptor blockade is more consistently effective in alleviating memory deficits rather than increasing memory in normally functioning animals [21].

In contrast to the works cited above, Russell and Dias [24] Lindner et al. [25] failed to detect any effects of Ro 04-6790 or SB-271046, upon acquisition of an autoshaping task, scopolamine-induced deficits in contextual fear-conditioning, or retention of a water maze task. Even more, Fone [15] and Kendall et al. [26] reported that selective 5-HT$_6$ receptor agonists appear to restore memory impairments in the novel object discrimination paradigm. More intriguing were the results obtained when combining non-active doses of the 5-HT$_6$ receptor agonist E-6801 and the 5-HT$_6$ receptor antagonist SB-271046, which produced an improvement in novel object discrimination. In addition, E-6801, at a non-active dose by itself, was able to improve synergistically the activity of non-active doses of donezepil (an acetylcholinesterase inhibitor) and memantine (a NMDA receptor antagonist) [26]. Thus both 5-HT$_6$ receptor agonist and antagonist compounds show pro-cognitive activity in pre-clinical studies, although the explanation for their paradoxical analogous effect is currently unclear (see point Biochemical effects associated to 5-HT$_6$ receptors).

5-HT$_6$ receptors and Alzheimer´s disease

Significant reductions in 5-HT$_6$ receptor density in cortical areas of AD patients have been found, although reductions in 5-HT$_6$ receptor density were unrelated to cognitive status before death [27]. As 5-HT$_6$ receptor blockade induces acetylcholine release, reductions in 5-HT$_6$ receptors may represent an effort to restore acetylcholine levels in a deteriorated cholinergic system. In addition, it has also been described that a dysregulation of 5-HT$_6$ receptor activation by 5-HT in the temporal cortex may be related to behavioral symptoms in AD [[27] and [28]].

A number of 5-HT$_6$ receptor antagonists have successfully undergone phase I clinical studies (healthy volunteers) and some have been evaluated in clinical phase II studies (patients) for the treatment of AD (Table 1). In a phase I study, PRX-07034 showed positive results when assessing cognitive function as secondary endpoint. For another compound, SB-742457, two phase II trials have recently been completed in subjects with mild-to-moderate AD. Preliminary data from these trials shows that SB-742457 produced an improvement in both

cognitive and global function in AD [[29], [30] and [31]]. Other clinical phase II studies are being performed, either alone or as add-on therapy with the acetylcholine esterase inhibitor, donepezil. This is the case for SB-742457, Lu-AE-58054 (SGS-518) or PF-05212365 (SAM-531) (www.ClinicalTrials.gov). Besides those selective compounds, it is worth to mention dimebon (latrepirdine, also known as dimebolin) originally developed as an anti-histamine drug. This compound antagonizes the 5-HT_6 receptor with higher affinity than other targets characterized to date [32]. Dimebon has received widespread publicity as a potential therapy for Alzheimer's disease following a very positive phase 2 study [33]. A more recent multinational phase 3 study, however, showed no improvements [34].

Localization of 5-HT_6 receptors. Neurochemical mechanisms mediating 5-HT_6 receptor functions.

Lesioning studies have described that 5-HT_6 receptors are present within 5-HT projection fields and not in serotonergic neurons of the raphe, indicating a probable postsynaptic role for these receptors [35]. A purported localization of 5-HT_6 receptors on cholinergic neurons was discharged as a selective cholinergic lesion, induced by injection of the selective immunotoxin 192-IgG-Saporin, failed to alter the density of 5-HT_6 receptor mRNA or protein expression in the deafferentated frontal cortex [36]. However, it has been consistently described that the influence of 5-HT_6 receptors on memory is mediated at least partially by increased cholinergic neurotransmission. 5-HT_6 receptor antagonists increase acetylcholine release [[36] and [37]]. In addition, behavioural studies have shown that blockade of the 5-HT_6 receptor led to an increase in the number of yawns or stretches in rats. These behaviours are highly dependent on the cholinergic system as were reversed by muscarinic antagonists [38].

Other neurotransmitter systems have also been shown to be associated with 5-HT_6 receptor function, such as the glutamatergic and dopaminergic system [[39] and [40]. Treatment with a 5-HT_6 receptor antagonist or atypical antipsychotics with high affinities for 5-HT_6 receptors, such as clozapine, enhance glutamate levels in the frontal cortex and hippocampus. On the other hand, 5-HT_6 receptor agonism attenuated stimulated glutamate levels elicited by high KCl treatment [41].

It has also been shown that 5HT_6 receptors may be expressed on GABAergic spiny neurons of the striatum. The co-localisation of glutamic acid decarboxylase and 5-HT_6 receptors in rat cerebral cortex and hippocampus has also been demonstrated, and almost 20% of 5-HT_6-like immunoreactive neurons have shown to be GABAergic [42]. Based on these data on localisation of 5-HT_6 receptors, and data from releasing experiments [[41] and [43]], it can also be suggested that 5-HT_6 receptor agonists/antagonists may modulate cholinergic and/or glutamatergic systems via disinhibition of GABAergic neurons (Figure 1).

Biochemical mechanisms associated to 5-HT_6 receptors

It is well known that 5-HT_6 receptor activity leads to activation of cAMP signalling pathways through adenylate cyclase stimulation. In fact, activity on adenylate cyclase confers the classical definition as agonist/antagonist upon 5-HT_6 receptors.

Interestingly, as mentioned before, recent studies have suggested that both 5-HT_6 receptor agonists and antagonists may have pro-cognitive activities, suggesting that both activation and inhibition of this receptor could evoke similar responses. The mechanism for this apparently paradoxical effect could be related to the existence of alternative biochemical pathways activated by 5-HT_6 receptors.

5-HT_6 receptor coupling to Gαs has been widely described, but coupling of 5-HT_6 receptors to other Gα protein subunits (Gαi/o or Gαq) has also been recently reported using a SPA/antibody-immunocapture technique [44]. In addition, the coupling of 5-HT_6 receptors to Ca2+ signalling using a chimeric G-protein has been reported [45].

On the other hand, it has also been described that the carboxyl-terminal region of 5-HT_6 receptors interacts with Fyn-tyrosine kinase, a member of the Src family of non-receptor protein-tyrosine kinases (Figure 1), and even more, 5-HT_6 receptors activated the extracellular signal regulated kinase1/2 (ERK1/2) via Fyn-dependent pathway [[46] and [17]]. Recently, a physical interaction between 5-HT_6 receptor and the Jun activation domain-binding protein-1 (Jab-1), using different experimental approaches, has also been described [47].
Other alternative mechanisms [15] have been described, including that agonists and antagonists could act on receptors located on distinct neuronal populations. Even more, the 5-HT_6 receptor agonist, LY-586713, has been found to increase expression of cortical BDNF and the immediate early gene Arc that were not antagonised by the antagonist SB-271046 [48], consistent with a potential differential mechanism.

Conclusions

During the last decade, the 5-HT_6 receptor has gained increasing attention, and it has become a promising target for improving cognition. Overall, consistent effects have been demonstrated with 5-HT_6 receptor antagonists in preclinical models of cognition. Currently, 5-HT_6 receptors have an obvious pharmaceutical potential in terms of related patents. There are several of 5-HT_6-targeted compounds, agonists and antagonists that are reasonably regarded as powerful drug candidates for the treatment of a range of neuropathological disorders, including Alzheimer's disease [9].
However, 5-HT_6 receptor functionality is being revealed to be much more complex than initially defined. Based on the existing data, and depending on the drug used, different cellular pathways may be activated. However, the full characterization of the functional profile of 5-HT_6 receptor is still pending. The drug discovery process may be highly benefited from this complexity, in terms of quantity and quality of new molecules.

Conflict of interest

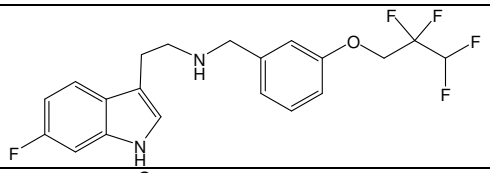
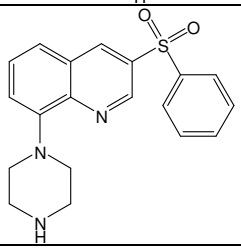
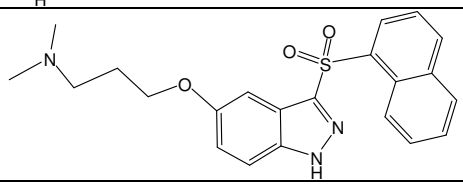
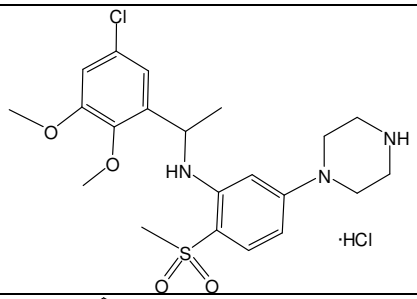
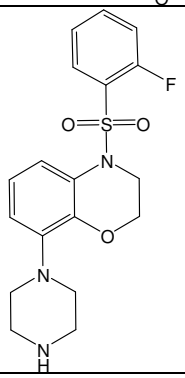
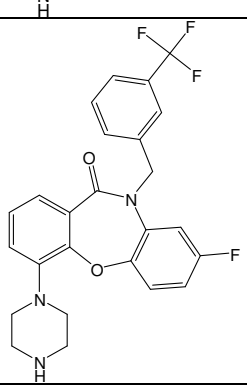
MJR declare that there is no conflict of interest.

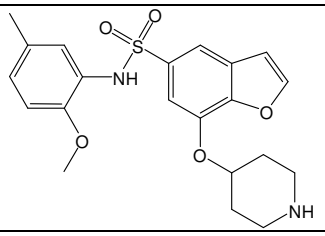
XC is Head of *In vivo* Safety Pharmacology and Comorbidities, and JMV is Director of Drug Discovery and Preclinical Development, at ESTEVE, Barcelona, Spain.

Acknowledgements

Authors thank Sandra Lizaso and Mari Luz Muro for technical work.

Table 1. 5-HT_6 receptor ligands under development for cognition disorders.

Name	Structure	Being developed by	Clinical Phase
Lu-AE-58054; SGS-518		Lundbeck	II
SB-742457		GlaxoSmithKline	II
PF-05212365; SAM-531		Pfizer	II
AVN-211	Not available	Avineuro Pharmaceuticals	II
PRX-07034		Epix Pharmaceuticals, Inc.	I
R-1485		Roche	I
SYN-114		Synosia Therapeutics	I

BVT-74316		Swedish Orphan Biovitrum	I
SUVN-502	Not available	SuvenLife Sciences	I
SYN-120	Not available	Synosia Therapeutics	I
AVN-322	Not available	Avineuro Pharmaceuticals	I

Source: Thomson Reuters Integrity[SM] database.

Legend to Figure

Figure 1. Neurochemical and biochemical mechanisms mediating 5-HT_6 receptor functions. In addition to the activation of cAMP signalling pathways, 5-HT_6 receptors activate the extracellular signal regulated kinase1/2 (ERK1/2) via Fyn-dependent pathway. The proposed neurochemical circuitry for 5-HT_6 receptors to influence cognition involves modulation of cholinergic and/or glutamatergic activity through GABAergic interneurons. AC: adenylate cyclase; ACH: acetylcholine; Glut: glutamate. (+): stimulation; (-): inhibition.

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Papers of particular interest have been highlighted as:

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- • • of outstanding interest

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5-HT$_6$ receptor is a serotonergic receptor localized almost exclusively in the CNS, predominating in brain regions associated with cognition and behavior. In this study it was shown a dense immunostaining in the frontal, entorhinal and piriform cortices, the nucleus accumbens, the cerebellum,the caudate-putamen and the hippocampus (particularly the dentate gyrus and the CA1 area of Ammon's horn).

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This is a comprehensive search through several key patent collections. The study covers compounds currently evaluated in different clinical trials as well as drugs available in the pharmaceutical market.

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This review compares the specific approaches of different institutions to discovering, optimising and exploiting 5-HT_6 receptor ligands. The focus of the paper is on scientific publications and patent applications describing new chemical series published since 2005.

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Excellent review on the effects of 5-HT_6 receptor agonists and antagonists on cognition both in normal adult rats and in rodent models of psychiatric disorders, as well as some data on clinical studies.

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Training in the morris water maze, a hippocampal-dependant learning task, induces a down-regulation of the 5-HT_6 receptor protein and mRNA receptor expression. However, there seems to be a discrepancy between the data on decreased levels of 5-HT_6 receptors associated to learning, and the use of 5-HT_6 receptor antagonists to facilitate learning. Once again, supporting the notions that 5-HT_6 receptor agonists

might be useful compounds and the necessity to characterise the biochemical pathways involved in 5-HT_6 receptor activation. In this sense, in this paper it is described that the improvement in learning associated to the use of the 5-HT_6 receptor antagonist SB-271046 was associated to increased pERK1/2 levels.

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Regarding the role of 5-HT_6 receptors in cognition, it could be argued that the beneficial effects of 5-HT_6 receptor antagonists are compound-related effects rather than effects associated to the antagonism upon the receptor. Supporting the notion that 5-HT_6 receptors are associated to cognition, this paper shows that increased levels of 5-HT_6 receptors in the striatum, achieved by targeted gene delivery, lead to cognition impairments, in a reward-based instrumental learning task, a striatum-dependent learning model.

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Review on 5-HT6 receptor antagonists, and their effects at a preclinical and clinical level. In this paper it is reviewed the effects of SB-742457 on two phase II trials in subjects with mild-to-moderate AD. The first was reportedly a dose-ranging trial comparing SB-742457 with placebo, and the second was an exploratory study with SB-742457 and donepezil arms. Overall, these studies demonstrated that SB-742457 is well tolerated in AD patients. SB-742457 produced an improvement in both cognitive and global function in AD as assessed by ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale) and CIBIC+ (Clinician's Interview-Based Impression of Change-plus Caregiver Input), respectively.

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It is widely accepted that the effects of 5-HT$_6$ receptor antagonists in cognition could be mediated, at least in part, by a modulation of the cholinergic activity. In this study it was shown that a selective cholinergic lesion, induced by injection of the immunotoxin 192-IgG-Saporin into the nucleus basalis magnocellularis, failed to alter the density of 5-HT$_6$ receptor mRNA or protein expression in the deafferentated frontal cortex, suggesting that 5-HT$_6$ receptors are not located on cholinergic neurons.

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