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5-HT_4 Receptor Agonists for the Treatment of Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (*AD*) *is a progressive neurological disorder primarily affecting new memory formation as well as retrieval of previously acquired memories. According to World Health Organization, current global population suffering from cognitive impairment is estimated to* 37 *million. The number is projected to double in next one and half decade. Half of the population afflicted with dementia is represented by AD patients. Current therapies, which provide marginal symptomatic relief to AD patients, are effective only in half of the patient population. In depth understanding of the molecular mechanism of the disease is urgently required to develop more effective therapies. Therapies in clinical development may either offer symptomatic relief to patients or provide pure disease modifications, thus limiting benefit to patients.* 5-HT_4 *receptor agonists offer an attractive option for the treatment of AD patients. Activation of* 5-HT_4 *receptor under preclinical conditions is demonstrated to improve neurotransmission and enhance the release of acetylcholine resulting in the memory formation. In various cell based and animal models, partial* 5-HT_4 *receptor agonists are demonstrated to promote the release of soluble amyloid precursor protein alpha and block the release of amyloid beta peptide offering suitable candidates as disease modification agents. Remarkably,* 5-HT_4 *receptor agonists are also reported to induce neurogenesis in hippocampus as well as enteric system through the activation of cyclic AMP response element binding protein in rodents. Taken together,* 5-HT_4 *agonists address all major facets of Alzheimer's disease and may provide therapeutic potential for other neurological disorders.*

***Keywords*:** 5-HT_4 *Receptor*, *Cognition*, *Neurogenesis*, *Alzheimer's Disease*

1. Introduction

5-HT_4 receptor is a G protein coupled receptor (GPCR) which belongs to serotonin receptor family and is coupled to G protein containing Gαs subunit [1]. The receptor, upon activation by an agonist, leads to the generation of intracellular cyclic AMP (cAMP) which in turn activates Protein kinase A. A cascade of signaling events result in the phosphorylation of cAMP response element binding protein (CREB) which binds to its response element leading to the expression of a number of genes involved in cell survival. Many excellent review articles were published recently highlighting the role of 5-HT_4 receptor agonists for treatment of gastro-intestinal (GI) spectrum of diseases [2-4]. Contributions of 5-HT_4 receptor along with other 5-HT receptors in learning and memory were also reviewed recently in a series of in depth articles [5,6]. An impressive article highlighting the role of 5-HT_4 receptor in AD was written with specific emphasis on beta amyloid peptide secretion [7].

In the present article, we focus on the role of 5-HT_4 receptor agonists in the treatment of AD with current understanding of their role in cognitive improvement, disease modification and neurogenesis. We further list enormous challenges that need to be overcome to realize the potential of the target and offer possible future directions that may help in overcoming some of the challenges.

2. Receptor Splice Variants

A larger number of 5-HT_4 receptor splice variants were identified both in human and rodents with no significant splice specific tissue expression [8]. **Figure 1** provides an image of various 5-HT_4 receptor splice variants based on sequence comparison. All the identified human splice variants exhibited identical amino acid sequence up to Leucine 358 with differences emerging in the short carboxyl terminal tail [9]. Such an observation led to the conclusion that ligand binding properties of various receptor isoforms may not differ, as entire extracellular and

transmembrane domain remained conserved across various receptor subtypes. Indeed 5-HT as well as 5-HT$_4$ receptor antagonist GR-113808 did not demonstrate any significant differences in their binding affinity to human 5-HT$_{4(a)}$ and 5-HT$_{4(b)}$ receptors [10]. In the same set of studies, a number of compounds were evaluated for their ability to induce generation of cAMP and they all showed similar functional affinities for both the receptor variants. Activation of 5-HT$_4$ receptor was reported to induce influx of calcium ions in the host cell by blocking the potassium channels. Surprisingly, a differential effect in calcium mobilization was observed with various compounds acting through 5-HT$_{4(a)}$ receptor but not through 5-HT$_{4(b)}$ receptor [10]. However, 5-HT did not exhibit any differential effect on the calcium mobilization between two receptor variants. Above observations suggested interaction of diverse effector proteins with different receptor subtypes as expected from the diversity of the carboxyl terminal tail. Furthermore, it appears that interaction of an effector protein with the receptor may be modulated by the type of ligands and their mode of interactions.

However, it was also reported that a partial 5-HT$_4$ receptor agonist SL65.0155 as well as 5-HT showed significant differences in their functional affinities to human 5-HT$_{4(b)}$ and 5-HT$_{4(e)}$ receptors [11]. Above observation suggested that carboxyl terminal tail of the 5-HT$_4$ receptor variants may modulate the affinity of certain ligands to the receptor apart from its ability to differentially transmit the intracellular signaling. Such a differential affinity of a ligand for various 5-HT$_4$ receptor variant may be more prominent between specific variants.

3. Cognitive Enhancement

Positive impact of cAMP in acquisition and consolidation of memory is well accepted. Activation of CREB protein by cAMP dependent protein kinase is an important mediator of memory formation [12,13]. A number of signaling pathways leading to cAMP accumulation in neurons are being explored as possible candidates for therapeutic interventions of cognitive deficits associated with various neurological disorders.

5-HT$_4$ receptor is expressed at high level in limbic system of CNS. The receptor is coupled to G protein containing Gαs subunit [1]. Thus, activation of the receptor by an agonist leads to cAMP formation which through CREB phosphorylation is proposed to help in new memory formation. CREB mediated memory formation is likely mediated through the expression of brain derived neurotrophic factor (BDNF) and other trophic and procognitive factors. An illustration of 5-HT$_4$ receptor's role in memory formation is provided in **Figure 2**. In addition, activation of the 5-HT$_4$ receptor is proposed

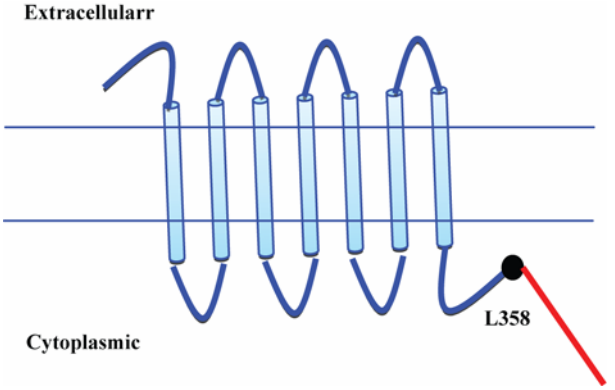

Figure 1. 5-HT$_4$ receptor splice variants. The human 5-HT$_4$ receptor sequence is conserved up to leucine 358 with differences appearing at amino acid 359 onwards depicted in red. Sequence of different variants downstream of Leu358 is shown. The blue line represents the conserved sequence with light blue barrels as the transmembrane domain. 5-HT$_{4(a)}$ RYTVLHRGHHQELEKLPIHNDPESLESCF; 5-HT$_{4(b)}$ RDAVECGGQWESQCHPPATSPLVAAQPSDT; 5-HT$_{4(c)}$ SSGTETDRKKLWNKEEKIDQTIQMPKRKRKK-KASLSYEDLILLGRKSCFREGK; 5-HT$_{4(d)}$ RF; 5-HT$_{4(e)}$ S-FPLLFCNRPVPV; 5-HT$_{4(f)}$ SPVPV; 5-HT$_{4(g)}$ SGCSPVSS-FLLLFCNRPVPV.

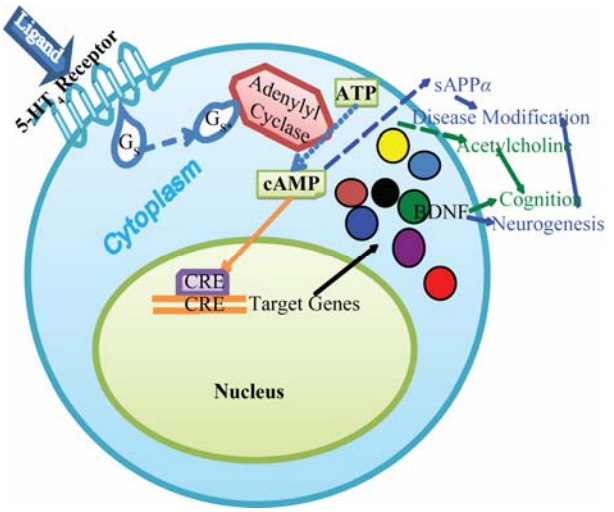

Figure 2. A cartoon representation of 5-HT$_4$ receptor activation leading to various cellular events. Activation of 5--HT$_4$ receptor leads to acetylcholine release, which coupled with the release of BDNF, may help in memory formation. The activation of the receptor is also reported to enhance the release of sAPPα, which along with BDNF-induced neurogenesis offers disease modifying potential for AD patients.

to facilitate the release of various neurotransmitters by blocking potassium channels and subsequent mobilization of calcium ions. Indeed a number of 5-HT$_4$ receptor agonists were reported to promote learning tasks in various animal models. 5-HT$_4$ receptor partial agonist SL-65.0155 demonstrated pro-cognitive effect in a Novel

Object Recognition Task (NORT) in a dose dependent manner. The compound also reversed scopolamine-induced amnesia in rats when evaluated in a water maze assay. The pro-cognitive effect of the compound was blocked by 5-HT$_4$ receptor antagonist SDZ 205,557 in the NORT assay [11]. SL65.0155 was also reported to reverse amnesia in rodents induced by diverse class of agents such as amyloid beta peptide 1 - 42, carbon monoxide and methylazoxymethanol acetate suggesting that the target offers a great therapeutic potential [14]. In a model of attention, five choice serial reaction time task, SL65.0155 reduced incorrect choices and promoted correct choices [15]. This observation suggested that compounds developed through 5-HT$_4$ receptor may also find therapeutic utility in the treatment of ADHD patients apart from their potential in therapeutic interventions in cognitive deficits associated with AD.

In a passive avoidance paradigm, icv injection of SC 53116, a 5-HT$_4$ receptor agonist reversed scopolamine induced amnesia [16]. A number of studies were reported in various international conferences with a 5-HT$_4$ partial agonist PRX03140 (formerly VRX03011). The compound reached up to Phase IIb of clinical development for the treatment of AD. PRX-03140 demonstrated high functional affinity to various human 5-HT$_4$ receptor splice variants with no apparent binding to other serotonin receptors. The compound demonstrated activity in a four arm cross maze assay in delayed mode response [17]. In a recently published study, various 5-HT$_4$ receptor agonists were demonstrated to reverse scopolamine induced amnesia in a Water Maze paradigm [18]. The dose dependent effect of the ligands was blocked by selective 5-HT$_4$ receptor antagonist GR125487. Taken together, all the above studies and reports provide a strong support for the development of 5-HT$_4$ receptor agonists as therapeutic agents for the treatment of various dementia related disorders.

4. Neurotransmitter Release

In order to better understand the mode of action of 5-HT$_4$ receptor agonists leading to memory formation, specific and targeted studies were conducted using various neurochemistry and electrophysiology based approaches. Role of 5-HT$_4$ receptor in synaptic plasticity was demonstrated in freely moving rats with implanted microelectrodes. Activation of the receptor by specific agonist RS67333 led to neurotransmission in hippocampus [19]. Similar neurotransmission was demonstrated in CA1 region of hippocampus in anesthetized rats using another 5-HT$_4$ selective agonist SC 53116 [16]. As mentioned earlier, activation of 5-HT$_4$ receptor leads to the release of various neurotransmitters mediated through calcium influx, as a result of blockade of potassium channels. A 5-HT$_4$ receptor agonist, methoxytryptamine was reported to increase the level of acetylcholine in prefrontal cortex of rats. It was further demonstrated that PRX-03140 enhanced the release of acetylcholine upon trial challenge in hippocampus of rats but not during resting period [17]. One of the mechanistic roles of extracellular acetylcholine is to enhance cholinergic neurotransmission which plays central role in memory formation. Thus, 5-HT$_4$ receptors may improve memory formation by enhancing the synaptic release of acetylcholine in the brain which would in turn enhance the cholinergic transmission.

5. APP Processing

A major proposed advantage of using 5-HT$_4$ receptor agonist for the treatment of AD patients is its ability to shift the equilibrium of amyloid precursor protein (APP) processing from amyloidogenic to non-amyloidogenic form [7, **Figure 2**]. Aβ peptide either in soluble oligomer or aggregated multimer format in the brain is a major contributing factor of AD pathology [20]. The peptide is generated by the action of beta and gamma secretases which either produce 42 or 40 amino acid peptides [21]. Both these peptides are amyloidogenic and implicated in the progression of the disease with 42 amino acid peptide having higher potential to aggregate. Another enzyme reported to cleave APP within the amyloidogenic peptide sequence is less known alpha secretase [21,22]. Activation of the alpha secretase pathway is shown to shift the balance of APP cleavage from amyloidogenic to non-amyloidogenic form. The first product of alpha secretase pathway is soluble APP alpha (sAPPα) protein. sAPPα acts as a neurotrophic factor and helps in neuronal survival. The protein gets further cleaved by other proteases and eliminated.

Major efforts to develop disease modifying drugs for AD treatment focused on identifying potent and selective inhibitors of beta or gamma secretases [23,24]. The blockade of these enzymes would not only prevent generation of amyloidogenic form of the peptide but may also shift the equilibrium of APP processing towards alpha secretase pathway. Highly involved effort for the identification of potent and selective beta secretase inhibitors was hampered due to large and complex substrate binding pocket of the enzyme [24]. In addition, compounds which demonstrated acceptable *in vitro* properties exhibited poor pharmacokinetic and brain penetration profile. Development of gamma-secretase inhibitor was more successful with molecules reaching up to phase III of clinical development [23]. As gamma secretase was reported to cleave other critical signaling proteins, development of compounds which block the APP processing activity without affecting the critically required enzyme action on other substrates had offered initial challenge which was overcome with smart chemistry efforts [23, 25].

Activation of alpha secretase pathway, despite offering distinct advantages as a therapeutic target for AD treatment, was overlooked for too long [22]. Such reluctance to develop alpha-secretase activator was probably due to strong and valid historical reason. It is much easier to identify a compound which can bind to the active site of an enzyme and inhibit its activity. For synthesis of an enzyme activator, an allosteric site needs to be first characterized which can bind to specific compounds. To overcome the challenge of an allosteric site, activation of an upstream signaling pathway may be exploited to enhance the enzyme activity. Agonism of 5-HT$_4$ receptor is reported to activate alpha-secretase pathway offering an attractive mechanism to develop new therapy for AD (**Figure 2**). The shifted equilibrium of APP processing from amyloidogenic to non-amyloidogenic form generates sAPPα which is reported to provide neuroprotective effect. Activation of alpha-secretase pathway by 5-HT$_4$ agonists was first reported in a recombinant as well as a neuronal cell line [26] and subsequently demonstrated in other cell lines as well as in mouse brain [10,27]. Treatment with 5-HT$_4$ receptor agonist RS67333 is also reported to inhibit the generation of Aβ peptide from the cortical neurons of Tg 2576 mice under culture conditions [28].

Most of the investigators utilized 5-HT$_{4(e)}$ receptor variant for the study of APP processing under artificial conditions [17,26,29]. However, 5-HT$_{4(d)}$ receptor activation was also demonstrated to enhance sAPPα level with concomitant decrease in Aβ peptide level [7,18]. It is not yet clear whether other variants of 5-HT$_4$ receptor would also exhibit similar kind of differential APP processing. 5-HT$_{4(e)}$ receptor is not restricted to CNS but is expressed in other peripheral tissues [8]. In addition as mentioned earlier, all differences in various 5-HT$_4$ receptor variants are restricted at the carboxy terminal cytoplasmic domain while their extracellular and transmembrane domains are absolutely conserved [9]. Majority of the compounds are not expected to show significant differences in their binding affinity to various receptor variants based on absolute conservation of the sequence in the ligand binding domain. However, due to differences in the carboxyl terminal domain of the receptor, these variants may interact with distinct or overlapping signaling machinery leading to differential intracellular responses. Blockade of specific 5-HT$_4$ activated pathway is reported to reduce the sAPPα level in the medium under cell culture conditions [28]. Despite identification of potent and selective 5-HT$_4$ receptor agonists long ago [30], their development for CNS indications moved at a slow pace. Potential impedance for the development of such compounds for neurological disorders could likely be their poor pharmacokinetic and brain penetration profile coupled with a concern for cardiovascular safety. The safety concern was further compounded due to withdrawal of marketed drugs acting through this target for their cardiovascular liability. However, most of the compounds which were withdrawn from the market demonstrated poor selectivity and their cardiovascular liability could have been independent of the target. Indeed, few 5-HT$_4$ agonists are still in market for the treatment of GI conditions. In addition, Prucalopride, a selective 5-HT$_4$ receptor agonist with excellent cardiovascular safety profile from Movetis is already approved for treatment of chronic constipation in women in Europe offering a proof of concept for the target.

6. Neurogenesis

One of the most remarkable features of 5-HT$_4$ receptor agonists is their ability to induce neurogenesis in hippocampus as well as enteric system in rodents [31,32]. This feature of 5-HT$_4$ receptor agonists truly offers disease modifying potential, as the compounds may be able to replace the degenerated cells by inducing production of new neurons (**Figure 2**). Partial 5-HT$_4$ receptor agonist RS67333 demonstrated capacity to induce neurogenesis in hippocampus of treated rats resulting in faster onset of the antidepressant activity [32]. Due to abundant expression of 5-HT$_4$ receptor in enteric system, neurogenesis mediated by the receptor was investigated extensively in enteric system. A number of 5-HT$_4$ receptor agonists are reported to induce the survival of enteric neurons and induce the neurite outgrowth under culture conditions which can be blocked by 5-HT$_4$ receptor specific antagonist [31]. In the same study, incorporation of bromo-deoxyuridine was reported *in vivo* in enteric cells expressing neuronal markers upon treatment with 5-HT$_4$ receptor agonists. Mouse model with targeted disruption of 5-HT$_4$ gene was explored to investigate any role of the receptor in neurogenesis. While the number of neurons in the enteric system were comparable between wild type and gene knock out mice at birth, the number of neurons declined over a period of time in the mutant mice [31]. Above observations strongly support the role of 5-HT$_4$ receptor and its agonists in neurogenesis in brain and peripheral tissues.

7. Major Challenges

5-HT$_4$ receptor agonists are used in clinic for the treatment of various GI indications. Some of the drugs targeting 5-HT$_4$ receptor such as Cisapride were withdrawn from the market due to cardiovascular liability. As the receptor is also expressed in heart tissue, a major and justifiable concern of on-target cardiovascular liability of 5-HT$_4$ receptor agonists is valid. However, more selective compounds targeting 5-HT$_4$ receptor are still in market. Prucalopride, a selective 5-HT$_4$ receptor agonist with excellent cardiovascular safety profile is approved

in Europe for treatment of chronic constipation in women. Above molecules offer a clinical proof of concept for exploration of 5-HT$_4$ receptor agonists as drugs. A partial 5-HT$_4$ agonist PRX-03140 advanced to phase II of clinical development for the treatment of AD. However, overall development of 5-HT$_4$ receptor agonists for CNS disorders was likely hampered due to poor pharmacokinetic profile or inadequate brain penetration of the molecules. While 5-HT$_4$ partial agonists RS67333 and SL65.0155 were identified more than a decade ago and demonstrated excellent efficacy in various animal models, they could not be developed as drugs possibly due to selectivity or cardiovascular liabilities. RS67333 exhibited strong binding to sigma receptors [30]. SL65.0155 reached to phase II of clinical development but was abandoned for undisclosed reasons. Another major challenge was to develop molecules which offer strong neurological effect with minimum activity at GI level. Identification of CNS specific molecules is highly challenging as most of the receptor variants show common expression pattern in various tissues. For example, there is no CNS specific 5-HT$_4$ receptor isoform.

8. Future Direction

Majority of the 5-HT$_4$ receptor ligands developed for GI indications (Cisapride, Tegaserod, Prucalopride etc.) were full agonist whereas compounds with partial agonism to the receptor (PRX-03140, RS67333 and SL 65.0155) exhibited remarkable CNS effect in various animal models. RaQualia (www.raqualia.com) is developing a 5-HT4 receptor partial agonist RQ00000009 for treatment of AD. The compound has completed human Phase I clinical trial and ready to move to Phase II. Identification of a partial agonist with acceptable oral absorption, good pharmacokinetic profile and high brain penetration would be a significant start in this direction. Such molecules are expected to have minimal GI effect as the equilibrium of drugs would shift from systemic circulation to the CNS tissue.

5-HT$_4$ receptor agonists appear to recapitulate all desired features of an ideal treatment for AD patients. Based on their procognitive properties coupled with neurogenesis, they may offer potential treatment for other neurodegenerative diseases. Partial 5-HT$_4$ receptor agonists also offer excellent differentiation from current SSRI based treatment for depression by having quick onset of action [32].

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