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REVIEW

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Interplay between Serotonin 5-HT$_{1A}$ and 5-HT$_7$ Receptors in Depressive Disorders

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SUMMARY

Serotonin (5-hydroxytryptamine or 5-HT) is an important neurotransmitter regulating a wide range of physiological and pathological functions via activation of heterogeneously expressed 5-HT receptors. Besides the important role of 5-HT receptors in the pathogenesis of depressive disorders and in their clinical medications, underlying mechanisms are far from being completely understood. This review focuses on possible cross talk between two serotonin receptors, 5-HT$_{1A}$ and the 5-HT$_7$. Although these receptors are highly co-expressed in brain regions implicated in depression, and most agonists developed for the 5-HT$_{1A}$ or 5-HT$_7$ receptors have cross-reactivity, their functional interaction has not been yet established. It has been recently shown that 5-HT$_{1A}$ and 5-HT$_7$ receptors form homo- and heterodimers both *in vitro* and *in vivo*. From the functional point of view, heterodimerization has been shown to play an important role in regulation of receptor-mediated signaling and internalization, suggesting the implication of heterodimerization in the development and maintenance of depression. Interaction between these receptors is also of clinical interest, because both receptors represent an important pharmacological target for the treatment of depression and anxiety.

Introduction

The brain serotonergic system is known to be involved in the control of a wide range of physiological functions as well as of different kinds of behavior. Such polyfunctionality of serotonin (5-hydroxytryptamine or 5-HT) is mediated by the existence of large number of serotonin receptors. Currently, 14 different 5-HT receptor subtypes expressed in the mammals have been identified. To provide more detailed characterization of 5-HT receptors, three main criteria are normally used: (1) structural features, including primary amino acid sequence, (2) signal transduction characteristics, including specific signal pathways, and (3) pharmacological profile [1]. Based on these criteria, 5-HT receptors were classified in seven subfamilies, which include both G protein-coupled and ionotropic receptors [2,3].

Despite the great clinical interest and large body of investigations dedicated to 5-HT receptors, the mechanism regulating 5-HT receptor functions are far from being completely understood. This review focuses on two serotonin receptors: the 5-HT$_{1A}$ receptor representing a key player in the brain 5-HT system [4], and the 5-HT$_7$ receptor. The 5-HT$_{1A}$ receptor is known to be critically involved in major depression, anxiety, and suicide. More recently, functional studies have also implicated 5-HT$_7$ receptors in depression [5–7]. As 5-HT$_{1A}$ and 5-HT$_7$ receptors are co-expressed in majority of the brain structures and modulate the same second messenger systems (even though in an opposite way), one intriguing question is the possible functional cross talk between these receptors. Since both receptors represent a pharmacological target for the treatment of depression, receptor–receptor interaction is also of great clinical significance.

5-HT$_{1A}$ and 5-HT$_7$ Receptors: Genes, Signaling Pathways, and Distribution in the Brain

The 5-HT$_{1A}$ receptor is one of the most extensively characterized members of the serotonin receptor family. Increased interest to the 5-HT$_{1A}$ receptor is based on several reasons: (1) this receptor plays an important role in the regulation of neuronal development and plasticity [8,9], (2) presynaptic 5-HT$_{1A}$ autoreceptors are involved in the regulation of functions of 5-HT neurons [2], (3) numerous data demonstrate implication of 5-HT$_{1A}$ receptor in the control of various physiological functions [4], and (4) 5-HT$_{1A}$ receptors are involved in the mechanisms of depression, anxiety, suicide, and schizophrenia [10–14].

The 5-HT$_{1A}$ receptor gene was cloned in 1987 [15]. One year later, it has been demonstrated that the protein product of this

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genomic clone possesses all characteristics of the 5-HT_{1A} receptor [16]. In following studies, the 5-HT_{1A} receptor gene was cloned from rat [17] and mouse [18]. It was shown that 5-HT_{1A} receptor gene is localized at 5th chromosome in human [15] and at 13th chromosome in mice [19], and does not contain introns in its coding sequence [17]. Mouse 5-HT_{1A} receptor gene demonstrates 94% homology with rat and 88% homology with human 5-HT_{1A} receptor gene (Ensemble Genome Browser, http://www.ensembl.org/index.html). The detailed structure of 5-HT_{1A} receptor gene promoter, including position of selective and nonselective enhancer as well as selective silencer, was also determined [20,21]. In addition, several rare single-nucleotide polymorphisms (SNPs) were described for 5-HT_{1A} receptor gene [22]. Between those, the SNP C(-1019)G localized in the repressor region of promoter is of particular interest because of its association with depression, suicidal behavior, and schizophrenia [13] as well as sensitivity to the antidepressant drug treatment [23].

The 5-HT_{1A} receptor is known to activate a variety of effectors via $G_{i/o}$ proteins, and receptor stimulation leads to inhibition of adenylyl cyclase (AC). The 5-HT_{1A} receptor is also involved in $G_{\beta\gamma}$-mediated activation of a K^+ current, inhibition of a Ca^{2+} current, and stimulation of the phospholipase C, as well as an activation of the mitogen-activated protein kinase Erk2 [2,24]. One important mechanism regulating 5-HT_{1A} receptor function is receptor desensitization and internalization [10]. Interestingly, that 5-HT_{1A} receptor activation leads to effective internalization of 5-HT_{1A} autoreceptors in the *nucleus raphe dorsalis*, but not of heteroreceptors resided in hippocampal neurons [25], indicating the difference in the regulation of pre- and postsynaptic 5-HT_{1A} receptors activity.

5-HT_{1A} receptors are widely distributed within the brain. In rodents, high receptor density was detected in limbic areas; in hippocampus, septum, and cortical areas; and in the midbrain dorsal and median raphe nuclei [2,26]. Similar distribution was reported in human by postmortem autoradiography [27] as well as in the living human brain using positron emission tomography (PET) [28]. Several experimental data suggested correlation between depressive disorders and changes in the distribution of 5-HT_{1A} receptor in the brain, although results of such studies are often contradictory. For example, an increase in 5-HT_{1A} autoreceptors was reported in postmortem brain from depressed suicide victims [29,30]. In contrast, PET analysis in depressed (submissive) monkeys revealed a reduction in 5-HT_{1A} receptor binding throughout the all brain areas [31]. At the same time, PET analysis of 5-HT_{1A} receptor binding in the brain of depressed patients before and after electroconvulsive therapy demonstrated a strong reduction in 5-HT_{1A} receptor binding in regions consistently reported to be altered in major depression [32].

The 5-HT_7 receptor is one of the most recently described members of the 5-HT receptor family [33–35]. This receptor is coupled to G_s protein and activates AC. In addition, the 5-HT_7 receptor is coupled to the G_{12} protein to activate small GTPases of the Rho family (i.e., Cdc42 and RhoA), leading to enhanced neurite outgrowth, synaptogenesis, and neuronal excitability [36–38]. It has been also demonstrated in cell lines that the 5-HT_7 receptor can stimulate intracellular calcium release [39].

The gene encoding the 5-HT_7 receptor is localized at 19th chromosome in mouse and at 7th chromosome in human. Comparison of amino acid sequence of the transmembrane regions of 5-HT_7 receptor from different species showed homology between 39% and 50%. Interestingly, the homology of transmembrane domains of 5-HT_7 receptor with those for 5-HT_{1A} receptor gene is relatively high (about 40%), which can partly explain the fact that most agonists developed for the 5-HT_{1A} and/or 5-HT_7 receptors have cross-reactivity between these receptors [40] (see below). The human 5-HT_7 receptor gene contains three introns within the coding region [41,42]. Detailed analysis of human 5-HT_7 receptor gene revealed existence of eight SNPs. Noteworthy that two of these polymorphisms (rs3808932 and rs12412496) were shown to be associated with schizophrenia [43]. A number of 5-HT_7 receptor splice variants including 5-$HT_{7(a)}$, 5-$HT_{7(b)}$ 5-$HT_{7(c)}$ 5-$HT_{7(e)}$ and 5-$HT_{7(a)}$, 5-$HT_{7(b)}$ and 5-$HT_{7(d)}$, differing in the amino acid sequence of their C-terminus, but possessing similar pharmacological profile were cloned from rat and human, respectively [41,42,44,45]. Noteworthy that the human 5-$HT_{7(d)}$ receptor isoform possesses a differential pattern of receptor internalization, which can affect receptor-mediated signaling [46]. The 5-HT_7 receptors are abundantly expressed in brain limbic structures. Significant density of 5-HT_7 receptor was also observed in hippocampus and in raphe nuclei area. Moderate or relatively low receptor density was obtained in cortex, caudate putamen, and cerebellum [47].

The 5-HT_7 receptor is associated with a number of physiological and pathological responses, including serotonin-induced phase shifting of the circadian rhythm [34] and age-dependent changes of the circadian timing [48,49]. There are some data implicating 5-HT_7 receptor in the control of memory [50], emotionality, locomotor, and exploratory activity [51,52]. A large body of evidence indicates involvement of 5-HT_7 receptor in the anxiety and depression, and recent studies suggest that the 5-HT_7 receptor can be highly relevant for the treatment of major depressive disorders [53].

Pharmacological Properties of 5-HT_{1A} and 5-HT_7 Receptors

Over the years, several agonists and antagonists for 5-HT_{1A} and 5-HT_7 receptors have been developed and applied to investigate the pharmacology of these receptors. Most frequently used molecules along with their binding affinities at 5-HT_{1A} and 5-HT_7 receptors are summarized in Table 1. The 5-HT_{1A} receptor was first identified in 1983 pharmacologically as a distinct binding site for 8-OH-DPAT [54], which was considered as the selective 5-HT_{1A} receptor reference agonist [55]. The affinity of 8-OH-DPAT for 5-HT_{1A} receptors in rat hippocampal membranes resides in the low nanomolar range (K_i = 1.78 nM) [56]. 8-OH-DPAT demonstrated the properties of a full 5-HT_{1A} receptor agonist in the forskolin-stimulated cAMP assay for both native and cloned 5-HT_{1A} receptors [56]. However, when the 5-HT_7 receptor was cloned in 1993, it became apparent that 8-OH-DPAT possesses affinity also for this receptor (K_i = 52 nM) [57] and behaves as a partial agonist (E_{max} = 62% of 5-CT response; EC_{50} = 2345 nM).

Several antagonists of 5-HT_{1A} receptor became available in early 1990. One of them, WAY-100635, is the prototypical silent 5-HT_{1A} receptor antagonist, which has been widely used as a pharmacological probe to investigate the distribution and function

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Table 1 Binding affinity values of agonists and antagonists acting on 5-HT$_{1A}$ or 5-HT$_7$ receptors

	5-HT$_{1A}$ receptor			5-HT$_7$ receptor		
Compound	Binding affinity	System	Reference	Binding affinity	System	Reference
8-OH-DPAT	K_i = 1.78 nM	Rat hippocampal membranes	52	K_i = 52 nM	Rat cloned receptor	53
WAY-100635	IC_{50} = 1.35 nM	Rat hippocampal membranes	54	K_i > 10000 nM	Human cloned receptor	59
WAY-100135	IC_{50} = 34 nM	Rat hippocampal membranes	55	Not available		
NAN-190	pK_i = 9.2	Rat hippocampal membranes	126	K_i > 1000 nM	Rat cloned receptor	60
NAD-299	K_i = 0.59 nM	Rat hippocampal membranes	58	K_i > 1000 nM	Rat cloned receptor	58
5-CT	K_i = 0.53 nM	Human cloned receptor	127	IC_{50} = 0.83 nM	Rat cloned receptor	31
AS-19	K_i = 89.7 nM	Human cloned receptor	66	K_i = 0.6 nM	Human cloned receptor	66
E-55888	K_i = 700 nM	Human cloned receptor	66	K_i = 2.5 nM	Human cloned receptor	66
LP-211	K_i = 15 nM	Human cloned receptor	67	K_i = 379 nM	Human cloned receptor	67
SB-269970	pK_i < 5	Human cloned receptor	128	pK_i = 8.9	Human cloned receptor	128
SB-258719	pK_i < 5.1	Human cloned receptor	129	pK_i = 7.5	Human cloned receptor	129
SB-656104	pK_i = 6.25	Human cloned receptor	130	pK_i = 8.70	Human cloned receptor	130

of 5-HT$_{1A}$ receptors [58]. WAY-100635 displaces specific [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in the rat hippocampus with IC_{50} = 1.35 nM, and it is more then 100-fold selective for the 5-HT$_{1A}$ site in comparison with a range of other 5-HT receptors. However, WAY-100635 also display affinities for 5-HT$_{2B}$ (K_i = 24 nM), dopamine D_2 (K_i = 16.4 nM), and adrenergic α_{1A} (K_i = 19.9 nM) receptors. In addition, WAY-100135 was initially considered as a silent 5-HT$_{1A}$ antagonist [59], but it showed partial agonist properties in a subsequent study [60]. Noteworthy that WAY-100635 together with other 5-HT$_{1A}$ receptor antagonists NAN-190 [61] and NAD-299 [62] did not reveal any affinity for 5-HT$_7$ receptors [62–64].

In case of the 5-HT$_7$ receptors, 5-CT has been identified as a high-affinity receptor agonist (IC_{50} = 0.83 nM, EC_{50} = 13 nM, 100% maximal efficacy) that is widely used to study the receptor pharmacology and functions [34]. However, due to its high affinity for 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors [65], analysis of 5-HT$_7$ receptor functions by 5-CT requires parallel application of WAY-100635 (5-HT$_{1A}$ receptor antagonist) and GR-127935 (5-HT$_{1B/1D}$ receptor antagonists) [66]. Interestingly, 8-OH-DPAT has also been used to study the functional effects of 5-HT$_7$ receptor in combination with application of the 5-HT$_{1A}$ antagonists WAY-100635, NAN-190, and NAD-299 [67–69].

During the last decade, various selective 5-HT$_7$ receptor agonists became available in addition to 5-CT. AS-19 (K_i of 0.6 nM for the human 5-HT$_7$ receptor) was reported to display high selectivity for 5-HT$_7$ receptor (more then 100-fold) in comparison with the other 5-HT receptor subtypes with exception of 5-HT$_{1D}$ receptors (only 11-fold higher selectivity). AS-19 was found to behave as a potent (EC_{50} = 9 nM), but partial 5-HT$_7$ receptor agonist with a maximal effect reaching 77% of the cAMP response evoked by the 5-HT [70]. Another selective and potent 5-HT$_7$ receptor agonist E-55888 [70] shows high affinity for 5-HT$_7$ receptors (K_i = 2.5 nM) and very low affinity for 5-HT$_{1A}$ (K_i = 700 nM) with no significant affinity for other 5-HT receptors. E-55888 behaves as a full agonist with efficacy and potency (E_{max} = 99%; EC_{50} = 16 nM) to induce receptor-mediated cAMP formation in HEK-293F cells in a range similar to that obtained for 5-HT [70]. Selective 5-HT$_7$ receptor agonist LP-211 displays high affinity for rat and human 5-HT$_7$ receptors (K_i = 0.58 and 15.0 nM, respectively) and moderate to low affinity for other 5-HT receptors, including the 5-HT$_{1A}$ receptor [71]. LP-211 possesses agonistic properties *in vivo* inducing hypothermia in 5-HT$_7^{+/+}$, but not in 5-HT$_7^{-/-}$ mice.

Recently, several specific 5-HT$_7$ receptor antagonists were developed. The prototype of 5-HT$_7$ receptor antagonist SB-269970 demonstrates a high binding affinity for human (pK_i = 8.9) and guinea pig (pK_i = 8.3) native 5-HT$_7$ receptors. This compound possesses very high selectivity for 5-HT$_7$ receptors (more than 100-fold) in comparison with other G protein-coupled receptors (GPCRs), except the human 5-HT$_{5A}$ receptor for which selectivity was 50-fold. SB-269970 acts as a competitive antagonist of the human 5-HT$_7$ receptor in AC assay (pA_2 = 8.5) as well as in the guinea pig native tissue assay (pK_B = 8.3). Interestingly, SB-269970 produces an inhibition of basal AC activity in the absence of any agonist, suggesting an inverse agonism [72]. Other selective 5-HT$_7$ receptor antagonists are SB-258719 [73], that is less potent than SB-269970 (pK_i = 7.5; pA_2 = 7.2), and SB-656104 [74]. Although the latter has similar pharmacological properties with SB-269970 (pK_i = 8.7; pA_2 = 8.4), it demonstrates only 10-fold selectivity over the 5-HT$_{1D}$ receptor.

Functional Cross Talk Between 5-HT$_{1A}$ and 5-HT$_7$ Receptors

There is a set of studies suggesting functional cross talk between 5-HT$_7$ and 5-HT$_{1A}$ receptors at the level of thermoregulation. Involvement of 5-HT$_{1A}$ receptor in the regulation of the body temperature has been demonstrated more than 25 years ago by the observation that 5-HT$_{1A}$ receptor agonist 8-OH-DPAT produced considerable hypothermic response [75,76]. This effect has been used as a test for 5-HT$_{1A}$ receptor functional activity [75–78]. We have also shown that changes in the expression of 5-HT$_{1A}$ receptor gene are linked to natural hibernation and associated hypothermia [79].

Based on the absence of 5-HT-induced hypothermic response in 5-HT$_7^{-/-}$ mice, Hedlund and co-authors suggested that 5-HT-induced hypothermia is mainly mediated via 5-HT$_7$ receptor [80]. However, in this study, 5-HT was applied peripherally. Because of

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the poor ability of 5-HT to cross the blood–brain barrier [81,82], the importance of the central 5-HT$_7$ receptor in thermoregulation suggested in this study should be therefore considered more carefully.

It was shown that at lower doses (0.3–0.6 mg/kg, i.p.), 8-OH-DPAT decreased body temperature in 5-HT$_7^{+/+}$ mice but not in 5-HT$_7^{-/-}$ mice. At a higher dose (1 mg/kg, i.p.), 8-OH-DPAT induced hypothermia in both 5-HT$_7^{-/-}$ and 5-HT$_7^{+/+}$ mice. At the same time, it was found that pretreatment of mice with a 5-HT$_{1A}$ receptor selective antagonist WAY-100135 completely blocked the hypothermic response of 8-OH-DPAT in 5-HT$_7^{+/+}$ mice [83]. Moreover, we have shown that intracerebroventricular, but not intraperitoneal administration of selective 5-HT$_7$ receptor agonist LP44 produced considerable hypothermic response in mice, indicating involvement of central rather then peripheral 5-HT$_7$ receptors in thermoregulation. The comparison of 5-HT$_7$ and 5-HT$_{1A}$ receptor-induced hypothermia in eight mouse strains did not reveal any correlation, suggesting the low functional interaction between 5-HT$_7$ and 5-HT$_{1A}$ receptors in the control of the body temperature. Together with the observation that the 5-HT$_7$ receptor selective antagonist did not affect 8-OH-DPAT-induced hypothermia, these data suggest that central 5-HT$_7$ and 5-HT$_{1A}$ receptors can be independently involved in thermoregulation [84].

Antidepressants effect of selective serotonin reuptake inhibitors (SSRIs) partly depends on 5-HT$_{1A}$ autoreceptor functions. Chronic treatment with SSRIs is associated with a progressive recovery of 5-HT neurons firing rate, correlating with clinical amelioration of depression [10]. It is noteworthy that 5-HT$_7$ receptors can also be involved in the therapeutic effects of antidepressants. For example, both tricyclic antidepressants as well as SSRIs induced c-Fos expression in rats in a manner consistent with the 5-HT$_7$ receptor activation within the suprachiasmatic nucleus. Moreover, chronic antidepressants treatment led to a decreased 5-HT$_7$ receptor binding [85]. These data suggest possible interplay between 5-HT$_7$ and 5-HT$_{1A}$ receptors during antidepressant drug action. One possible mechanism of such cross talk can be selective and regulated heterodimerization (see below).

Oligomerization of 5-HT$_{1A}$ and 5-HT$_7$ Receptors

Results of multiple biochemical, structural, and functional studies collected during the last decade clearly indicate that GPCRs can exist as oligomeric complexes [86,87]. Moreover, a growing body of evidence points to the functional importance of GPCR oligomers for receptor trafficking, receptor activation, and G protein coupling in native tissues [88]. The clinical significance of GPCR oligomerization has also become more evident during recent years, leading to identification of oligomeric complexes as novel therapeutic targets [89,90].

Oligomerization can occur between identical receptor protomers (homomerization) or between different receptors belonging to the same or different GPCR families (heteromerization). Heteromerization may result in changed receptor pharmacology either by affecting the ligand binding on individual monomers or by the formation of new binding sites [91,92]. More importantly, heteromerization may also influence signaling pathways regulated by a given receptor monomer [93–97], which can lead to a switch in G protein coupling [98]. Thus, heteromerization provides an additional mechanism regulating cellular processes through the fine tuning of receptor-mediated signaling.

Using a novel Förster resonance energy transfer (FRET) technique based on the spectral analysis, we have recently demonstrated that 5-HT$_{1A}$ receptors can form homodimers at the plasma membrane [99,100]. We also showed that FRET efficiency measured for the 5-HT$_{1A}$ receptor oligomers significantly decreased in response to agonist stimulation. Combined results of our studies suggest that this decrease was mediated by accumulation of FRET-negative complexes rather than by dissociation of oligomers to monomers. Formation of 5-HT$_{1A}$ homomers (including the higher-order oligomers) was further confirmed by several recent publications [101,102]. By combination of computational protein–protein docking, site-directed mutagenesis, and FRET-based analysis, we have recently demonstrated that transmembrane domains TM4/TM5 can be involved in the formation of 5-HT$_{1A}$ receptor dimers and indicated that specific amino acids interactions (e.g., W175$^{4.64}$, Y198$^{5.41}$, R151$^{4.40}$, and R152$^{4.41}$) maintain the interaction interface [103].

The 5-HT$_7$ receptor has been shown to form homooligomers as well. This was demonstrated in recombinant system by pharmacological studies using the antagonist risperidone, which binds in a pseudo-irreversible manner to one protomer of a dimer as well as by the Bioluminiscence resonance energy transfer approach [104]. Recently, homooligomerization of 5-HT$_7$ receptor was confirmed using two different FRET assays [101]. Existence of 5-HT$_7$ receptor homodimers has been also shown under *in vivo* like condition in primary cultures of rat cortical astrocytes [105].

Heterooligomerization between 5-HT$_{1A}$ and 5-HT$_7$ receptors has been demonstrated by combined application of biochemical (co-immunoprecipitation) and biophysical (FRET) approaches [106]. In the same study, it has been also demonstrated that hippocampal neurons express both 5-HT$_{1A}$ and 5-HT$_7$ receptors and that these receptors are highly co-localized at the plasma membrane. Moreover, co-immunoprecipitation studies in mouse brain provided direct evidence that these receptors can form heteromers in neurons *in vivo*. Functional analysis of oligomerization between 5-HT$_{1A}$ and 5-HT$_7$ receptors revealed that heterooligomers decreased the 5-HT$_{1A}$ receptor-mediated activation of G_i protein without affecting 5-HT$_7$ receptor-mediated G_s protein activation. In addition to reduced G_i protein coupling of 5-HT$_{1A}$ receptor, heterodimerization affected 5-HT$_{1A}$ receptor-mediated activation of G protein-gated potassium (GIRK) channels, an effect mediated through the $G_{\beta\gamma}$-subunits of G_i proteins [107,108]. Noteworthy that inhibitory effect of heterooligomerization on GIRK currents was preserved in hippocampal neurons, suggesting a physiological relevance of heteromerization *in vivo* [106].

We have also demonstrated that heterodimerization can initiate agonist-mediated internalization of 5-HT$_{1A}$ receptor, which is highly resistant to internalization when expressed alone. Once internalized, 5-HT$_{1A}$ receptor can activate G protein-independent signaling pathways such as a β-arrestin-mediated coupling to mitogen-activated protein kinase (MAPK) [106]. Thus, dependent on the relative amount of 5-HT$_{1A}$ receptors participating in

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heterooligomers, the same ligand (serotonin) can activate distinct ERK-mediated pathways (i.e., G protein dependent or β-arrestin dependent). This also raises the possibility that conditions selectively promoting or inhibiting heterodimerization could have a significant physiological relevance.

Possible Role of Heterodimerization between 5-HT$_{1A}$ and 5-HT$_7$ Receptors in Anxiety and Depression

Modulation of receptor signaling by heterodimerization, in particular enhancement of 5-HT$_{1A}$ receptor internalization, is also likely to play an important role in pathophysiological processes in CNS. The 5-HT$_{1A}$ receptor is expressed as postsynaptic receptor in multiple brain regions including hippocampus and cortex [109,110]. In addition, the 5-HT$_{1A}$ receptor is highly expressed as a presynaptic autoreceptor in 5-HT neurons of raphe nuclei, where it controls serotonin release through feedback inhibition [111,112]. The role of 5-HT$_{1A}$ autoreceptor in the regulation of presynaptic serotonin release has led to the hypothesis that its selective and progressive desensitization is a key element responsible for the therapeutic action of the SSRIs. Although widely accepted, this hypothesis still cannot explain the fact that the chronic *in vivo* SSRI treatment results in preferential functional desensitization of only 5-HT$_{1A}$ autoreceptors without affecting the postsynaptic 5-HT$_{1A}$ receptors [113,114]. We propose that higher amount of heterodimers produced in presynaptic versus postsynaptic neurons might represent a mechanism responsible for the differential desensitization obtained for the 5-HT$_{1A}$ auto- and heteroreceptors (Figure 1). Such asymmetric distribution of heterodimers will result in effective co-internalization of 5-HT$_{1A}$ receptor within 5-HT$_{1A}$-5-HT$_7$ heterodimers upon 5-HT release, which can take place either under physiological conditions or as a consequence of SSRI treatment. It has been demonstrated that the expression level of postsynaptic 5-HT$_7$ receptors in hippocampus and forebrain progressively decreased during the postnatal development, while the expression of 5-HT$_{1A}$ receptor remained relatively constant [36,106,115]. This suggests that under physiological conditions, 5-HT$_{1A}$ receptor homodimers represent a dominant postsynaptic population in adulthood ([5-HT$_{1A}$-5-HT$_{1A}$] $\gg$ [5-HT$_{1A}$-5-HT$_7$]). As 5-HT$_{1A}$ receptor homodimers are resistant to the agonist-mediated internalization, the amount of the postsynaptic 5-HT$_{1A}$ receptor will not be affected by 5-HT. Different relative concentration of 5-HT$_{1A}$-5-HT$_7$ heterodimers between serotonergic presynaptic autoreceptors and target (postsynaptic receptors) neurons can explain not only the differences in desensitization between pre- and postsynaptic 5-HT$_{1A}$ receptors, but also suggests that the regulated and balanced ratio of homo- and heterodimerization on pre- and postsynaptic neurons may be critically involved in both the onset as well as the response to treatment of psychiatric diseases such as depression and anxiety (Figure 1). Moreover, differences in relative concentration of 5-HT$_{1A}$-5-HT$_7$ heterodimers in raphe and hippocampus represent an intrigue possibility to explain regional differences in the coupling of 5-HT$_{1A}$ receptor to G proteins. It has been shown that in raphe, Gα_{i3} is the predominat coupling partner of 5-HT$_{1A}$ receptor, while in hippocampus, receptor couples to both Gα_o and Gα_{i3} proteins [116]. Therefore, future investigations will be needed to more precisely evaluate to which extent the 5-HT$_{1A}$ and 5-HT$_7$ oligomerization can contribute to selective activation of different G proteins.

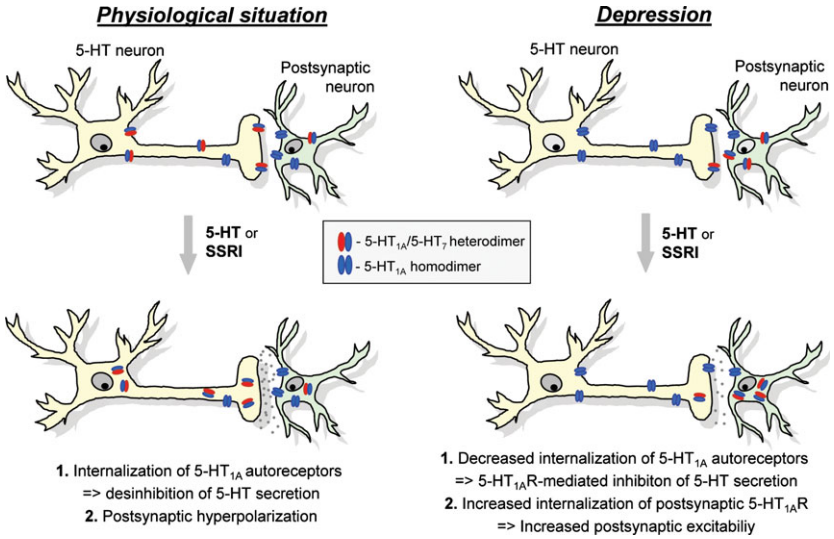

Figure 1 Hypothetical model explaining the functional role of 5-HT$_{1A}$-5-HT$_7$ heterodimerization. Under physiological conditions (*left*), the amount of 5-HT$_{1A}$-5-HT$_7$ heterodimers in presynaptic 5-HT neurons is higher than in postsynaptic neurons, representing a mechanism responsible for the differential 5-HT or SSRI-mediated internalization obtained for the 5-HT$_{1A}$ auto- versus heteroreceptors. By depression (*right*), the relationship between 5-HT$_{1A}$-5-HT$_{1A}$ homodimers and 5-HT$_{1A}$-5-HT$_7$ heterodimers in presynaptic 5-HT neurons becomes shifted toward 5-HT$_{1A}$-5-HT$_{1A}$ homodimers. This will result in decreased 5-HT or SSRI-mediated internalization of 5-HT$_{1A}$ autoreceptors, which in turn will lead to 5-HT$_{1A}$ receptor-mediated inhibition of 5-HT release. On the postsynaptic neurons, higher amount of heterodimers ([5-HT$_{1A}$-5-HT$_7$] > [5-HT$_{1A}$-5-HT$_{1A}$]) is expected during depression. Consequently, internalization of postsynaptic 5-HT$_{1A}$ receptors will be increased, leading to the increased neuronal excitability.

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Abnormalities in 5-HT$_{1A}$ receptors have been previously noted in depressed patients and implicated in the pathophysiology of depression. Analysis of the postmortem brains of depressed subjects in comparison with control samples revealed a specific up-regulation of 5-HT$_{1A}$ autoreceptors in the raphe area, with no changes in postsynaptic 5-HT$_{1A}$ receptor sites [30]. In addition, it has been recently shown that transgenic mice with low level of 5-HT$_{1A}$ autoreceptor possess increased resilience to chronic stress and faster response to SSRI treatment as compared to mice with the high level of presynaptic 5-HT$_{1A}$ receptor [117]. On the other hand, postmortem studies of major depression demonstrated a decrease in postsynaptic 5-HT$_{1A}$ receptor expression in hippocampus and prefrontal cortex, consistent with decreased postsynaptic 5-HT$_{1A}$ receptor-mediated signaling observed in depressed suicide tissue [118,119]. Decreased 5-HT$_{1A}$ receptor density in the prefrontal cortex was also found in multiple PET studies of human patients with major depression [120–122]. All these results demonstrate that in depression, the number of presynaptic 5-HT$_{1A}$ receptors increased and this is accompanied by the concomitant decrease in postsynaptic 5-HT$_{1A}$ receptor level.

Although the detailed analysis of 5-HT$_7$ receptor distribution in patients with depression is not available yet, recent studies in rats have shown that expression of 5-HT$_7$ receptor is up-regulated in the hippocampus after exposure to stress, and these changes were inhibited by treatment with fluoxetine [123]. Similar results were obtained in mice, where chronic fluoxetine treatment led to a down-regulation of 5-HT$_7$ receptor binding in hypothalamus [124]. Moreover, analysis of 5-HT$_7$ knockout mice revealed that those mice exhibit a behavioral phenotype similar to that of antidepressant-treated mice [6,125]. These data suggest that in depression, the relationship between 5-HT$_{1A}$ homodimers and 5-HT$_{1A}$-5-HT$_7$ heterodimers in presynaptic neurons may be shifted from [5-HT$_{1A}$-5-HT$_7$] > [5-HT$_{1A}$-5-HT$_{1A}$] to [5-HT$_{1A}$-5-HT$_{1A}$] > [5-HT$_{1A}$-5-HT$_7$], while in postsynaptic neurons, the opposite effect is expected (i.e., [5-HT$_{1A}$-5-HT$_7$] > [5-HT$_{1A}$-5-HT$_{1A}$]) (Figure 1). Functionally, an increased concentration of 5-HT$_{1A}$-5-HT$_7$ heterodimers in hippocampus (and consequently inhibition of 5-HT$_{1A}$ mediated signaling via its internalization) may cause depression or accentuate the effect of stress. In combination with decreased presynaptic activity due to a relative increase in concentration of 5-HT$_{1A}$ homodimers in presynaptic neurons, serotonergic transmission particularly through 5-HT$_{1A}$ receptor appears to be compromised in depression.

Conclusion

Serotonin receptors 5-HT$_{1A}$ and 5-HT$_7$ are co-expressed in multiple brain regions, where they are critically involved in regulation of various physiological and pathological brain functions. Recent studies demonstrated that these receptors can form heterodimers both *in vitro* as well as *in vivo*. From the functional point of view, heterodimerization decreases G_i protein coupling of 5-HT$_{1A}$ receptor without substantial changes in the coupling of the 5-HT$_7$ receptor to the G_s protein. In addition, heterodimerization facilitates internalization of 5-HT$_{1A}$ receptors as well as its ability to activate MAP kinase. Thus, differences in relative concentration of 5-HT$_{1A}$-5-HT$_7$ heterodimers on presynaptic serotonergic neurons and target (post-synaptic) neurons can explain the differences in desensitization patterns between pre- and postsynaptic 5-HT$_{1A}$ receptors. More importantly, regulated and balanced ratio of homo- and heterodimerization on pre- and postsynaptic neurons may be critically involved in both the onset as well as the response to the treatment of psychiatric diseases such as depression and anxiety. As heterodimerization can exist between other 5-HT receptor subtypes and between 5-HT receptors and nonserotonergic GPCRs, it would be therefore interesting to analyze in future studies relevance of such receptor complexes for the pathophysiology and treatment of depression.

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Conflict of Interest

The authors declare no conflict of interest.

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