Review TRENDS in Pharmacological Sciences Vol.25 No.9 September 2004

Functional, molecular and pharmacological advances in 5-HT_7 receptor research

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The 5-HT_7 receptor was among a group of 5-HT receptors that were discovered using targeted cloning strategies 12 years ago. This receptor is a seven-transmembrane-domain G-protein-coupled receptor that is positively linked to adenylyl cyclase. The distributions of 5-HT_7 receptor mRNA, immunolabeling and radioligand binding exhibit strong similarities, with the highest receptor densities present in the thalamus and hypothalamus and significant densities present in the hippocampus and cortex. The recent availability of selective antagonists and knockout mice strains has dramatically increased our knowledge about this receptor. Together with unselective agonists, these new tools have helped to reveal the 5-HT_7 receptor distribution in more detail. Important functional roles for the 5-HT_7 receptor in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling and sleep have also been established. Hypotheses driving current research indicate that this receptor might be involved in mood regulation, suggesting that the 5-HT_7 receptor is a putative target in the treatment of depression.

Although traditional pharmacological techniques had revealed the existence of multiple receptor subtypes for 5-hydroxytryptamine (5-HT), it was not until after the first cDNA cloning of a 5-HT receptor that the large size of the family of 5-HT receptors became clear [1]. In a flurry of studies using targeted analysis of cDNA libraries based on conserved sequences in the known receptors, the number of identified 5-HT receptors was expanded greatly. Fourteen different receptor subtypes for 5-HT, grouped in seven families, have been described [1], which does not include the multiple proteins generated by alternative splicing of the transcripts of single genes. Among known neurotransmitters, 5-HT acts on the most diverse group of receptors. Although 5-HT is synthesized by only a small group of neurons within the raphe nuclei of the brain stem, these cells send both ascending and descending projections to large parts of the CNS [2]. Because of this widespread innervation, 5-HT has been implicated in numerous important physiological and pathophysiological phenomena, including sleep–wakefulness cycles and several psychiatric disorders [1]. The large number of receptor subtypes underscores the importance of 5-HT and the need for fine-tuning of its actions.

In 1993, the 5-HT_7 receptor was discovered independently by researchers in three laboratories [3–5]. This G-protein-coupled receptor (GPCR) protein was expressed readily in transfected cells and found to stimulate cAMP production [3–5]. Using northern blots [3–5] and *in situ* hybridization [3,4], 5-HT_7 receptor mRNA was found in the brain, mainly in the hypothalamus, thalamus, hippocampus and cortex, and in the periphery, mainly in blood vessels and the intestines. Pharmacologically, the receptor showed high affinity for 5-carboxytryptamine (5-CT) and 5-HT, and relatively high affinity for the 5-HT_{1A} receptor agonist 8-hydroxy-2(di-*n*-propylamino)-tetralin (8-OH-DPAT) [3–5]. Ritanserin (a 5-HT_2 receptor antagonist), but not pindolol (a 5-HT_1 receptor antagonist), showed antagonistic properties at this receptor [4]. Since its discovery, the 5-HT_7 receptor has been found in numerous species, including humans. Recent additions to this list of species include *Caenorhabditis elegans* [6] and *Aedes aegypti* [7]. Different splice variants of the 5-HT_7 receptor have been detected in rats compared with those detected in humans, although all the splice variants identified do not seem to possess any functional differences [8,9].

One consequence of the vast expansion of the number of known receptors for 5-HT is that it casts doubt on studies that were interpreted before the extent of the list was appreciated. Much progress has been made in identifying the contributions of these several receptors to 5-HT-mediated signaling, which, in the case of the 5-HT_7 receptor, has been helped by the recent availability of selective antagonists and knockout mice. Recent studies suggest that the 5-HT_7 receptor is involved in thermoregulation, circadian rhythm, learning and memory, hippocampal signaling, sleep and endocrine regulation. Furthermore, hypotheses driving current research indicate a possible involvement of this receptor in mood regulation, suggesting that the 5-HT_7 receptor is a potential target for the treatment of depression.

Pharmacology and knockouts

The availability of selective ligands is of utmost importance to the elucidation of the characteristics and function of a receptor. However, a well-characterized selective agonist for the 5-HT_7 receptor is not yet available.

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Available online 28 July 2004

 0165-6147/$ - see front matter doi:10.1016/j.tips.2004.07.002

Furthermore, the fact that 8-OH-DPAT, previously considered to be the standard selective agonist for the 5-HT_{1A} receptor, has high affinity for the 5-HT_7 receptor adds to the confusion regarding which receptor subtype is involved in previously observed phenomena. Most attempts to synthesize an agonist for the 5-HT_7 receptor have resulted in compounds showing high affinity for both 5-HT_{1A} and 5-HT_7 receptors [10,11]. However, one thiopyridine is promising because it has relatively high selectivity for 5-HT_7 receptors compared with 5-HT_{1A} receptors [12].

A significant recent advance is the availability of selective antagonists for the 5-HT_7 receptor. Compounds that belong to two chemically distinct groups have been described [13,14], and work to characterize the chemical properties for optimal antagonism of the 5-HT_7 receptor is ongoing [11,15,16]. Using the antagonists SB269970 (see Chemical names) and SB656104 it has been possible to demonstrate that blockade of the 5-HT_7 receptor leads to inhibition of 5-HT-mediated hypothermia in guinea-pigs and an increased latency to onset of rapid eye movement (REM) sleep with less time spent in REM sleep in rats [17].

The endogenous amidated lipid oleamide has been described as a modulator of 5-HT_7 receptor affinity *in vitro* [18]. Oleamide acts at an apparent allosteric site on the receptor protein to modulate its function.

Using knockout mice strains that lack the 5-HT_7 receptor [19,20], the involvement of this receptor in thermoregulation has been confirmed and descriptions of receptor distribution have been enhanced. The knockout lines are now facilitating the evaluation of the role of this receptor in behavior.

5-HT_7 receptor signaling

Although coupling of the 5-HT_7 receptor to cAMP formation has been known since the discovery of the receptor [3–5], recent studies have elucidated the functional coupling of the 5-HT_7 receptor in more detail. At least when expressed in cell lines, the receptor is tightly associated with the G protein, regardless of agonist binding [21]. The receptor activates the extracellular signal-regulated kinase (ERK) through a mechanism that is dependent on a Ras monomeric GTPase [22]. Although ERK activation is normally believed to be mediated through protein kinase A (PKA), the 5-HT_7 receptor stimulates ERK through a PKA-independent pathway, possibly by using a cAMP-activated guanine nucleotide exchange factor, Epac [23]. Activation of the 5-HT_7 receptor directly stimulates ERK in hippocampal neurons [24], an effect that can be of importance for hippocampal function and mood regulation (see later).

Distribution of the 5-HT_7 receptor

Early and more-recent studies in guinea-pigs and rats have used *in situ* hybridization to determine with increasing resolution where the gene encoding the 5-HT_7 receptor is expressed [4,25–27]. The studies all show that 5-HT_7 receptor mRNA is most abundant in the thalamus, hippocampus and hypothalamus. It is noteworthy that 5-HT_7 receptor mRNA is present in all of the CA fields of the hippocampus and in the suprachiasmatic nucleus (SCN) of the hypothalamus. Mapping of the expression of the gene encoding the 5-HT_7 receptor is consistent among studies for the major regions of expression.

An increasing number of studies have described the distribution of the 5-HT_7 receptor protein in mice [28,29] and rats [27,30,31] using immunohistochemical techniques. Two of these reports show that the protein distribution is similar to that of the mRNA, with the highest abundance in the thalamus, hypothalamus and hippocampus. As with *in situ* hybridization, low levels of immunoreactivity have been detected in the rat striatum [27,30]. In the developing rat brain, 5-HT_7 receptor immunoreactivity was observed in a cytoplasmic inclusion termed a stigmoid body [30]. In neonatal animals these immuno-positive inclusions were most prominent within the hypothalamus. The stigmoid bodies have been linked to the development of sexual dimorphism, which could be of interest in relation to the involvement of 5-HT_7 receptors in endocrine regulation (see later) [30]. In the hippocampus, the pyramidal cell layer of all CA regions exhibits immunoreactivity for the 5-HT_7 receptor [27,28]. Within the mouse SCN, the 5-HT_7 receptor is located in both dendrites and axon terminals of mostly GABA-containing neurons [29]. In these neurons most of the receptors seem to be in the plasma membrane outside of synapses, as detected by electron microscopy. Within the cerebellum, the 5-HT_7 receptor protein is located exclusively in Purkinje cells [31].

An important issue for any receptor is, of course, whether the expressed protein exhibits functional binding of agonist. The availability of selective antagonists for the 5-HT_7 receptor has made them the logical candidates for receptor binding experiments to study receptor distribution. Indeed, studies using radiolabeled [3H]SB269970 have been performed; however, these studies all used tissue membrane preparations [32,33], which lack anatomical resolution. To date, only reports using nonselective ligands in combination with indirect methods to determine 5-HT_7 receptor binding distribution are available. These studies used either unlabeled antagonists or knockout mice or a combination of both to discriminate 5-HT_7 receptors from other receptor subtypes. Bonaventure and colleagues used 5-HT_{1A}, 5-$HT_{1A/1B}$ and 5-HT_7 receptor knockout mice in combination with [3H]5-CT and [3H]8-OH-DPAT to obtain a detailed map of 5-HT_7 receptor binding distribution [34,35]. Because both of these ligands bind to both 5-HT_{1A} and 5-HT_7 receptors, the knockout mice were used in combination with selective antagonists (SB269970 for 5-HT_7 receptors; WAY100135 and pindolol for 5-HT_{1A} receptors) to discriminate between the two receptor subtypes. These studies confirm the distribution pattern also observed using *in situ* hybridization and immunohistochemistry, showing highest binding densities in the thalamus, hypothalamus and hippocampus. A noteworthy observation is that 8-OH-DPAT not only binds to 5-HT_{1A} and 5-HT_7 receptors, but also to a significant degree to α_{2A}-adrenoceptors [35]. A similar binding pattern, but with some notable differences, was found in rats, guinea-pigs and humans when using [3H]mesulergine as the radioligand [36]. The most

striking difference was the observation of relatively high binding density in the caudate–putamen (striatum) in rats, guinea-pigs and humans. Although other studies have not reported binding in this region, it is consistent with findings using immunohistochemistry.

As 5-HT_7 receptor functions are reviewed later it becomes evident that there is a significant agreement between the localization of 5-HT_7 receptors in the brain and the functions in which they are implicated (Figure 1). For example, their presence in the hypothalamus correlates with the involvement of these receptors in circadian rhythm, thermoregulation and endocrine regulation. In addition, thalamic and cortical 5-HT_7 receptors might be important for sleep and mood regulation, and it has been suggested that thalamic 5-HT_7 receptors might be important in epilepsy [37]. Finally, 5-HT_7 receptors in the hippocampus are of interest in learning and memory.

Functional roles of the 5-HT_7 receptor

Thermoregulation

The involvement of 5-HT in thermoregulation is a well-known phenomenon. For example, injection of 5-CT or 8-OH-DPAT induces hypothermia in rodents. The hypothermic effect is similar regardless of whether the drug is administered peripherally or centrally, suggesting a central mechanism of action. Because both 5-CT and 8-OH-DPAT are 5-HT_{1A} receptor agonists, this receptor was generally considered to be the main mediator of the hypothermia, although some reports had suggested the involvement of other receptor subtypes. The first indication that the 5-HT_7 receptor is important in 5-HT-induced hypothermia was provided by the fact that the effect of 5-CT on body temperature was blocked by the selective antagonists SB269970 [38] and SB656104 [39] in guinea-pigs. Furthermore, 5-HT and 5-CT failed to induce hypothermia in 5-HT_7 receptor knockout mice [19,20]. A detailed analysis using 8-OH-DPAT in combination with selective antagonists and knockout mice to discriminate between 5-HT_{1A} and 5-HT_7 receptors revealed that both receptor subtypes are involved in 5-HT-mediated hypothermia [40]. The 5-HT_7 receptor seems to be most important at low agonist concentrations, thus contributing to the fine-tuning of temperature homeostasis, whereas the 5-HT_{1A} receptor comes into play at higher agonist concentrations, possibly providing a defense against hyperthermia [40].

Learning and memory-related behavior

The first attempt to assess the role of the 5-HT_7 receptor in behavior used antisense oligonucleotides to inhibit receptor synthesis in the rat [41]. In this study, such treatment had no effect on feeding, locomotor activity or anxiety-like behavior using an elevated plus maze. It has, however, been suggested that the 5-HT_7 receptor might be important for stress regulation because 5-HT_7 receptor mRNA is upregulated in the hippocampus after acute, but not chronic, stress in the rat [42]. A more comprehensive study used 5-HT_7 receptor knockout mice to evaluate the role of this receptor in various behavioral and learning tasks [43] and found that knockout mice exhibited a specific impairment in contextual fear conditioning. Contextual fear conditioning, in which the animal learns to associate the environment (context) with an aversive stimulus, is generally believed to depend on the hippocampus, as are other types of place learning. However, interestingly, in a Barnes maze test, in which the task is to learn how to escape an open area by locating a chamber using environmental cues and thus also depends on the hippocampus, there was no difference between the behavior of wild-type and knockout mice [43]. There was also no difference in three hippocampus-independent learning tasks: cued fear conditioning, operant food conditioning and motor learning (rotarod) [43]. The impairment seen in contextual fear conditioning was not

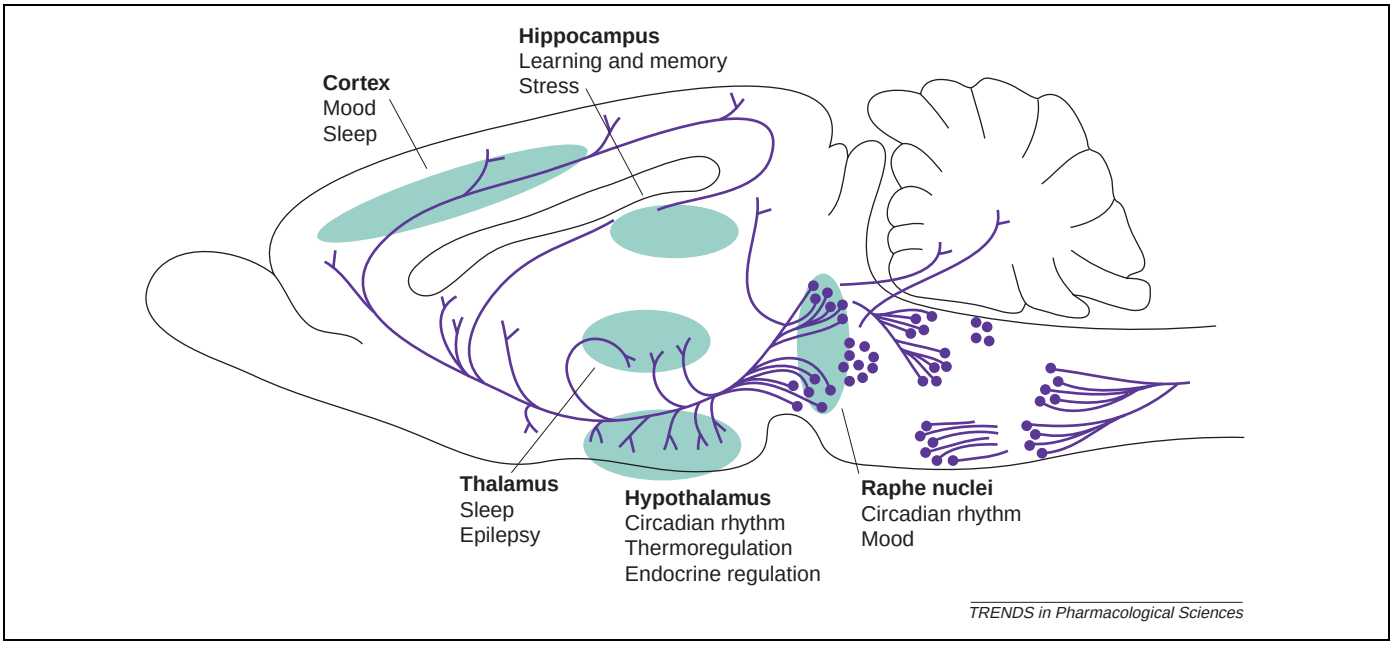

Figure 1. A sagittal view of the rodent brain, showing the 5-HT-producing neurons of the brain stem with their ascending and descending projections (purple). Regions that are relatively rich in 5-HT_7 receptor expression (green) and their putative correlation with 5-HT_7 receptor-mediated functions are indicated.

due to alterations in motor skills, visual acuity or anxiety level [43].

Role in the hippocampus

The above-mentioned studies suggest that the 5-HT_7 receptor has a role in the hippocampus, which is consistent with its identification in this brain region by *in situ* hybridization, immunohistochemistry and radioligand binding. Furthermore, as mentioned earlier, stress has been shown to induce the upregulation of 5-HT_7 receptor mRNA in the hippocampus, a brain region important in contextual learning. Electrophysiological studies have been used to examine the role of the 5-HT_7 receptor in the hippocampus [43–45]. 5-HT_7 receptor activation decreased the amplitude of slow afterhyperpolarizations in the CA3 region of the hippocampus by inhibiting Ca^{2+}-activated K^+ channels [44]. In the hippocampus, the selective antagonist SB269970 has been shown to inhibit bursting activity induced by 5-CT [45]. In the CA1 region of the hippocampus, 5-HT_7 receptor activation has been shown to modulate the excitability and intracellular signaling of pyramidal neurons [28,46]. Furthermore, there is a reduced ability to induce long-term potentiation (LTP) in the CA1 region of the hippocampus in 5-HT_7 receptor knockout mice [43]. The effect of 5-HT_7 receptors on ERK observed in hippocampal neurons might be important for these changes in LTP formation [23,24]. Together with the behavioral data, these findings suggest an important role for the 5-HT_7 receptor in hippocampus-dependent functions, including learning and memory [47].

In other electrophysiological studies, the 5-HT_7 receptor has been shown to be involved in the postnatal formation of synaptic connectivity in the prefrontal cortex [48], and in the development of neurons within the ventral pallidum [49].

Regulation of circadian rhythm and mood

Three closely linked physiological phenomena are circadian rhythms, sleep and mood. Since its discovery, the 5-HT_7 receptor has been implicated in the regulation of circadian rhythm following the demonstration in rat hippocampal slices that 8-OH-DPAT-induced phase resetting within the SCN (a brain region that is important in the regulation of circadian rhythms) is mediated by the 5-HT_7 receptor [4]. Recent studies have provided additional evidence for the involvement of the 5-HT_7 receptor in SCN function [50–55]. For example, the phase shift induced by 8-OH-DPAT is inhibited by the selective antagonists SB269970 [53] and DR4004 [54]. There is also evidence to suggest that the phase shifting induced by 8-OH-DPAT in hamsters involves the direct regulation of the homolog of the *Drosophila* SCN clock gene *Period* [51]. Phase shifting the SCN pacemaker neurons by 8-OH-DPAT is a non-photic stimulus involving serotonergic input from the dorsal and median raphe nuclei. In these nuclei, 5-HT_7 receptors have been shown to modulate SCN phase resetting [56,57], possibly through mechanisms involving GABA-containing interneurons [58]. The 5-HT_7 receptor is probably also involved in photic regulation of the SCN. For example, results of pharmacological profiling studies using unselective drugs [50] and DR4004 [54] suggest that 5-HT-mediated reduction of photic stimulation of SCN neurons is mediated by the 5-HT_7 receptor. The inhibition of spontaneous SCN activity by 8-OH-DPAT is also mediated by the 5-HT_7 receptor [52]. The above studies were performed in either rats or hamsters. In the mouse, however, the effects of 8-OH-DPAT, and hence the 5-HT_7 receptor, on SCN function are not as pronounced as in other species [55].

The direct involvement of the 5-HT_7 receptor in the regulation of sleep has been shown using selective antagonists. Both SB269970 and SB656104, when administered to rats at the beginning of the sleep phase, increased the latency to REM sleep and decreased the amount of time spent in REM sleep [39]. Other sleep parameters were not affected. These changes in sleep pattern are directly opposite to those seen in depressed patients.

Several antipsychotics and antidepressants have high affinity for the 5-HT_7 receptor [3,59,60]. Recently, it has been shown that antidepressants can exert at least some of their function through the 5-HT_7 receptor [61]. For example, several antidepressants, both tricyclic antidepressants and selective 5-HT reuptake inhibitors (SSRIs), induced c-FOS expression in rats in a manner consistent with 5-HT_7 receptor activation within the SCN [61]. The effect on c-FOS expression was attenuated after chronic treatment with antidepressants. Furthermore, chronic drug treatment led to a downregulation of 5-HT_7 receptor binding [61]. It is unclear how receptor blockade could lead to an antidepressant effect because antidepressants are generally believed to increase the levels of 5-HT. Part of the explanation might be the finding that 5-HT_7 receptors are localized close to, but outside, the synapse [29].

These findings are compatible with the hypothesis that the 5-HT_7 receptor is of considerable importance for regulating sleep, circadian rhythms and the overall mood of the individual. The direct actions of antidepressants on the 5-HT_7 receptor and the reversal of sleep disturbances observed in depressed patients following 5-HT_7 receptor blockade lead us to suggest that a 5-HT_7 receptor antagonist by itself can be sufficient to treat depression and might have advantages over currently available options.

Endocrine regulation

The endocrine system provides a transition between the CNS and the periphery. There is evidence to suggest a role for the 5-HT_7 receptor in both central and peripheral parts of this system. For example, the 5-HT_7 receptor is probably involved in 5-HT-mediated stimulation of both vasopressin and oxytocin release [62], and is involved in the regulation of luteinizing hormone (LH). Such regulation of LH appears to be complex: activation of the 5-HT_7 receptor stimulates the release of LH-releasing hormone in immortalized cells [63] but the receptor is also involved in terminating the pre-ovulatory LH surge [64]. Peripherally, 5-HT_7 receptors are present on granulose-lutein cells where they stimulate progesterone production [65]. Further studies are required to clarify fully the role of

Chemical names

DR4004: 2*a*-(4-(4-phenyl-1,2,3,6-tetrahydropyridyl)butyl)-2*a*,3,4,5-tetrahydrobenzo[*cd*]indol-2(1*H*)-one
SB269970: (*R*)-3-(2-(2-(4-methylpiperidin-1-yl)-ethyl)pyrrolidine-1-sulfonyl)phenol
SB656104: 6-((*R*)-2-{2-[4-(4-chloro-phenoxy)-piperidin-1-yl]-ethyl}-pyrrolidine-1-sulfonyl)-1*H*-indole
WAY100135: (*S*)-N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide

5-HT_7 receptors in these mechanisms. In addition, in the adrenal gland, the 5-HT_7 receptor has been shown to mediate 5-HT-induced aldosterone release [66]. In the hippocampus [67,68] and in cultures of hippocampal cells [69] 5-HT_7 receptors are involved in regulating the effects of glucocorticoids on their receptors. Glucocorticoids are known to be important in mood regulation. Together with the findings on sleep, these data support the hypothesis that 5-HT_7 receptors are relevant in depression.

Peripheral 5-HT_7 receptors

In peripheral tissues, the 5-HT_7 receptor has been found mainly on smooth muscle cells in blood vessels and other internal organs. In general, the receptor mediates relaxation of blood vessels, both arteries and veins [70,71], although a recent study excluded the 5-HT_7 receptor from involvement in the relaxation of the human occipital artery [72]. Because of its presence in blood vessels of the skull, it has been suggested that the receptor is a putative target for migraine treatment [70].

Other recent studies have reported that the 5-HT_7 receptor is involved in mediating the effects of 5-HT on ileum peristalsis [73], the micturition reflex [74] and relaxation of the oviduct [75]. These findings could be relevant in the treatment of irritable bowel syndrome and urine incontinence.

Concluding remarks

The availability of selective antagonists and knockout mice has led to an unprecedented activity in 5-HT_7 receptor research. As a result, the distribution and functional coupling of the receptor has been determined in more detail and many important roles for the receptor have been identified in thermoregulation, learning and memory, hippocampal activity, endocrine function, sleep, circadian rhythms and mood. Future studies and pharmaceutical development should determine whether a 5-HT_7 receptor antagonist is suitable as a pharmacological agent.

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