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COMMENTARY

Diverse Signalling by 5-Hydroxytryptamine (5-HT) Receptors

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ABSTRACT. Fourteen different receptor subtypes might be regarded as a diversity that is sufficient to accommodate the wide-ranging physiological roles of 5-hydroxytryptamine (5-HT). However, it is becoming clear that, for 5-HT as for other neurotransmitters, the concept of a receptor as a gatekeeper for a specific cellular process or event is too restrictive. Multiple receptor-mediated biochemical cascades can be activated in cells in response to an agonist by a number of mechanisms. Whereas it is well established that different agonists do not necessarily elicit the same magnitude of response, they probably also select between various possible signal transduction pathways. Receptor signalling may be diverse via a single receptor subtype as a consequence of specific agonist–receptor–G protein interactions. 5-HT receptors are even more heterogeneous when one considers that the amino acid sequence of these receptor subtypes may vary from individual to individual, and that there is an increasing number of receptor isoforms due to alternative splicing and RNA editing of 5-HT receptor transcripts. Activation, in particular constitutive, agonist-independent activation, of some of these receptor isoforms has been reported to be altered. This implies that ligands with similar binding affinities may display different pharmacological properties (partial agonist, antagonist, or inverse agonist) versus these receptor isoforms, depending on their activation state. Therefore, intervention with receptor ligands to modify hampered neurotransmission pathways is a difficult task, and one needs to consider the growing evidence of diversity in G protein-coupled receptor signalling. BIOCHEM PHARMACOL **60**;12:1743–1750, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. 5-HT receptor; receptor isoform; signal transduction pathway; differential signalling

A lot of progress has been made during the last decade on the structure and signalling properties of 5-HT† receptors. The 5-HT receptor superfamily is composed of fourteen members to date, which have been classified based on gene structure, amino acid sequence homology, and intracellular signalling cascades [1]. All except one (5-HT_3) of the 5-HT receptors couple to guanine nucleotide-binding proteins (G proteins), producing second messengers that regulate cellular functions via phosphorylation/dephosphorylation of intracellular proteins. Five families of G protein-coupled 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_4, 5-ht_6, and 5-HT_7) regulate two major intracellular second messenger pathways, adenylate cyclase and phospholipase C. Many reports put forward the idea that these receptor subtypes also have the ability to activate other intracellular signalling pathways [2]. It is not very clear whether these additional signalling pathways are parallel or converging. Although fourteen different receptor subtypes might be regarded as providing sufficient diversity to accommodate the wide-ranging physiological roles of 5-HT, it appears that for this as for other neurotransmitters, the concept of a receptor as a gatekeeper for a specific cellular process or event is far too restrictive [3]. Multiple receptor-mediated biochemical cascades can be activated in cells in response to an agonist by a number of mechanisms. Whereas it is well established that different agonists do not necessarily elicit the same magnitude of response, they probably also select among various signal transduction pathways [4]. Receptor signalling may be diverse via a single receptor subtype as a consequence of specific agonist–receptor–G protein interactions. Some members of the 5-HT receptor family with introns provide scope for additional diversity by virtue of splicing events that result in the formation of different receptor mRNAs and consequently distinct receptor isoforms (i.e. 5-HT_4 and 5-HT_7 receptors [5]). The 5-HT receptors are even more heterogeneous when considering the possibility that the amino acid sequences of these receptor subtypes may vary from individual to individual [6]. Whereas most of the available functional receptor data have been obtained in *in vitro* model systems, the challenge will be to unravel the *in vivo* functioning of this receptor diversity. The present commentary will focus on recent

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† *Abbreviations*: 5-HT, 5-hydroxytryptamine, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propyl-amino) tetralin; TMD, transmembrane domain; and LSD, lysergic acid diethylamide.

findings in molecular diversity of 5-HT receptors and consider potential pharmacological guidelines.

LIGANDS AND RECEPTOR ACTIVATION

A widely accepted model used to describe the activation of G protein-coupled receptors by agonists is the ternary complex model, which accounts for the cooperative interactions between agonist, receptor, and G protein [7]. This model has been extended recently to accommodate the observation that many receptors can activate G proteins in the absence of agonist and that mutations in different structural domains of receptors can enhance this constitutive, agonist-independent activity [8, 9]. The extended ternary complex model also accounts for the effects of different classes of ligands on receptor signalling [10]: full agonists, partial agonists, silent neutral antagonists (with intrinsic activity close to zero), and inverse agonists (also defined as negative antagonists). A reduced maximal response was the original definition of a partial agonist, but this nomenclature disguises the crucial fact that not all full agonists are the same [11]. In the case of G protein-coupled receptors, sensible inferences can be made about obviously partial agonists; however, there is no firm basis for distinguishing between different degrees of efficacy among agonists that can all produce a maximum response. By following [^{35}S]GTPγS binding responses as a measure of ligand–receptor activation at increasing GDP concentrations, it is possible to differentiate to a certain degree among agonists with high efficacy [12–14]. The lack of observation of a difference in ligand efficacy in a particular model system does not necessarily preclude the absence of such a difference, only that the system was inadequate to make it observable. Hence, most of the investigated agonists endowed with positive intrinsic activity probably have to be considered as partial agonists, differing from the efficacy of the native agonist 5-HT. The relevance of observed small to large variations in the efficacy of ligands may be important to an *in vivo* situation [15], but their relevance to pathophysiological conditions is less clear. Nonetheless, it is reasonable to expect that the difference between variations in ligand efficacy will be most important in conditions with poor receptor coupling [16].

SURMOUNTABLE 5-HT RECEPTOR ACTIVATION

Quite a lot is known about the tridimensional structure of G proteins [17], but very little is established about the conformational change in the receptor itself upon activation [11]. This is unfortunate, because it is probably in the receptor conformational changes that the main secrets of agonism (full, partial, and inverse) lie. In the case of an ion channel, the conformational change can be seen directly as channel opening. However, no such direct approach is available for a G protein-coupled receptor. Gether *et al.* [18] provided the first evidence for ligand-specific conformational changes occurring in a G protein-coupled β_2-adrenoceptor. Both agonists and inverse agonists induced, respectively, a decrease and an increase in baseline fluorescence used as a marker of receptor conformational changes. It is not clear how many G protein molecules the receptor can access easily for activation. The fusion protein approach by which the C-terminus of the receptor is covalently linked to the N-terminus of the G_α protein offers a novel strategy to investigate receptors and G_α proteins under controlled experimental conditions. The receptor and G_α protein are in spatial proximity following expression and have a defined receptor: G_α protein stoichiometry of 1.0 [19–21]. Although it is likely that this approach is not physiological, it allows one to perform quantitative pharmacological comparisons between either a single receptor subtype and diverse G_α protein subunits or one particular G_α protein subunit and diverse wild-type or mutant receptors. Wenzel-Seifert *et al.* [22] suggest that the 1:1 stoichiometry of G protein-coupled receptors and G_α obtained in fusion proteins may reflect the *in vivo* stoichiometry of receptor–G protein coupling more closely than was appreciated previously. Dupuis *et al.* [23] have demonstrated modulation of 5-HT$_{1A}$ receptor signalling by point mutation of cysteine^{351} in the C-terminus of the rat $G_{\alpha o}$ protein using this fusion protein approach. These data extend the hypothesis that the activity state of G proteins co-determines the magnitude of ligand responses [12–14], along with the receptor: G protein density ratio and the intrinsic ability of an agonist to activate the receptor. Remarkably, the wild-type $G_{\alpha i3}$ protein did not result in maximal 5-HT$_{1A}$ receptor activation by the agonists [5-HT, 8-OH-DPAT, and (−)-pindolol] being investigated [24]. Some of the mutant $G_{\alpha i3}$ proteins with a nonpolar amino acid at position 351 attained more than 300% of the agonist activation observed to be induced by the wild-type $G_{\alpha i3}$ protein. These data suggest that the wild-type $G_{\alpha i3}$ protein does not allow maximal activation of the 5-HT$_{1A}$ receptor; it can be exceeded substantially by agonists in the presence of mutant $G_{\alpha i3}$ proteins. While its physiological implications remain unclear, this finding opens perspectives for devising new ligands for 5-HT$_{1A}$ receptors, which may surmount the intrinsic activity observed with 5-HT.

The magnitude of agonist, partial agonist, and inverse agonist responses at the 5-HT$_{1A}$ receptor is highly dependent upon the nature of the amino acid (i.e. Gly < Cys < Ile) at position 351 of the $G_{\alpha i3}$ protein [24]. Both the degree of 5-HT$_{1A}$ receptor activation by 8-OH-DPAT and (−)-pindolol and its inhibition by spiperone strongly correlate with the octanol/water partition coefficient of the mutated amino acid at position 351 of the $G_{\alpha i3}$ protein [24]. Kellett *et al.* [25] also demonstrated that the alteration of a single amino acid (Ile^{351} instead of Gly^{351}) in the $G_{\alpha i1}$ protein regulates constitutive, agonist-independent activity of the G protein-coupled 5-HT$_{1A}$ receptor and that these fusion proteins can directly regulate adenylyl cyclase activity. Although this fusion protein approach seems to be functional for 5-HT$_{1A}$ receptors, less favorable results have

TABLE 1. Some examples of ligands, previously characterized as antagonists, behaving as either a partial agonist, a neutral antagonist, or an inverse agonist at 5-HT receptors

Receptor subtype	Partial agonist	Neutral antagonist	Partial inverse agonist	Inverse agonist	References
Wild-type h5-HT_{1A}		WAY 100635		Spiperone, methiothepin	[62-64]
Wild-type h5-HT_{1B}	GR 125743, GR 127935, 1-naphthylpiperazine			GR 55562, SB 224289, methiothepin	[65-67]
r5-HT_{2A} $Cys^{322}Lys$				Chlorpromazine, clozapine, haloperidol, loxapine, risperidone	[68]
Wild-type r5-HT_{2C}				Mianserin, spiperone, mesulergine, ketanserin, clozapine, cyproheptadine	[69, 70]
r5-HT_{2C} $Ser^{312}Lys$				Mianserin, mesulergine	[71]
Wild-type h5-HT_{4C}				ML 10375	[72]
Wild-type h5-$HT_{7\ long}$			SB-258719, mesulergine	Risperidone, methiothepin, olanzapine, clozapine	[73]

been obtained for α_{2A}-adrenoceptors. Burt *et al.* [26] demonstrated that agonist occupation of the α_{2A}-adrenoceptor–$G_{\alpha i1}$ fusion protein results in activation of both receptor-linked and endogenous G_i proteins. Therefore, caution should be taken to ensure that the ligand activates exclusively the fusion protein and not endogenous G_α proteins of the host cell. Fusion between the receptor and a $G_{\alpha 15}$ protein may be an alternative way to monitor receptor activation at the effector level (e.g. by Ca^{2+} measurements [27]). The $G_{\alpha 15}$ protein is absent in most cell types, as it is expressed only in a subset of hematopoietic cells [28]. Therefore, receptor coupling to endogenous $G_{\alpha 15}$ proteins can be excluded in most of the host cell types currently used for transfection experiments.

ANTAGONISTS, OR RATHER INVERSE AGONISTS, AT 5-HT RECEPTORS

Pure silent neutral antagonists (with intrinsic activity close to zero) are probably rare; many are actually inverse agonists or partial agonists (Table 1). Initially it was believed that the action of inverse agonists to decrease basal effector activity was due to competition between the ligand (acting as an antagonist) and an endogenous receptor agonist present in the system. The criterion currently used to conclude that a receptor system is constitutively active includes demonstration of effects of inverse agonists that can be blocked in a competitive fashion by neutral antagonists. However, this criterion is not fulfilled in many reports, often due to the lack of a relevant neutral antagonist. Typically, the inverse agonist properties of a ligand are most easily detectable in systems where a large degree of constitutive receptor activity exists, such as when receptors are overexpressed or are mutated (Table 1). Most likely, inverse agonism is determined by constitutive receptor activation by specific G protein subtypes. Consequently, ligands may demonstrate distinct pharmacological properties (i.e. neutral antagonist or inverse agonist) depending on which receptor–G protein–effector pathway is involved [29]. The concept that G protein-coupled receptors can couple to different G protein–effector pathways receives further support from receptor mutagenesis studies. A conserved threonine residue (Thr^{149}) in the second intracellular loop of the 5-HT_{1A} receptor is directly involved in $\beta\gamma$-mediated coupling to Ca^{2+} channels (via $G_{\alpha o}$) and to phospholipase C (via $G_{\alpha i2}$), but plays a minor role in the coupling to $G_{\alpha i}$-mediated inhibition of cyclic AMP accumulation [30]. Similarly, Asp^{79} (TMD II) and Arg^{322} (TMD VII) have been shown to be involved in the coupling of α_{2A}-adrenoceptors and prostaglandin EP3D receptors, respectively, to specific signal transduction pathways [31, 32]. Perez *et al.* [33] reported on an α_{1B}-adrenoceptor $Cys^{128}Phe$ (TMD III) mutation resulting in G protein coupling in the absence of agonist and constitutive activation of the phospholipase C pathway, but not of the phospholipase A_2 pathway. A similar mutation ($Cys^{116}Phe$) in the β_2-adrenoceptor causes selective constitutive activation of Na^+/H^+ exchange through a pathway not involving cyclic AMP [34]. These data suggest that the pharmacological profile of a receptor subtype may be co-determined by the effector pathway that is being considered. Pathway-dependent constitutive receptor activity may result from differences in the coupling efficiency between a receptor and its effector pathways, and/or there may be multiple active conformational states of the receptor, each of which has its own level of constitutive activity and couples to an effector pathway.

NOVEL ACTIONS OF INVERSE AGONISTS AT 5-HT RECEPTORS

Inverse agonists have novel actions that extend beyond simply reducing basal effector activity and/or antagonising the agonist response. Prolonged treatment with inverse agonists can lead to increased receptor density and enhanced responsiveness. Importantly, β_2-adrenergic receptor

up-regulation generally does not occur when neutral antagonists are used, and neutral antagonists have been shown to block the effects of inverse agonists [35, 36]. It is possible that constitutively active receptor systems, like ligand-dependent receptor activity, activate cellular effector pathways responsible for desensitization and down-regulation. Prolonged treatment with an inverse agonist, by reducing constitutive receptor activity, would permit the system to resensitize and up-regulate receptors. This also suggests that the prolonged treatment method may be a more sensitive measure of the inverse agonist properties of a ligand than the conventional measure of reduction of basal effector activity, especially in *in vivo* models. Berg *et al.* [37] suggest that actions of inverse agonists may also be mediated through effects on receptor systems that are not direct targets for these ligands. For instance, 24-hr exposure to inverse agonists acting at 5-HT_{2C} receptors selectively enhanced accumulation of inositol phosphates, but not arachidonic acid, elicited by activation of endogenous purinergic P_2 receptors in a CHO-1C19 cell line in which 5-HT_{2C} receptors are not overexpressed or mutated. Therefore, the therapeutic action of ligands previously thought to be simply antagonists, but which are in fact inverse agonists, may not be related solely to their properties as antagonists at their target receptors, but rather indirect actions on other co-expressed receptor systems may be involved as well.

PATHWAY-DEPENDENT LIGAND EFFICACY OF 5-HT RECEPTORS

Although traditional receptor theory allows for activation of multiple cellular effectors by agonists, it predicts that the relative degree of activation of each effector pathway by an agonist (relative efficacy) must be the same. Berg *et al.* [38] demonstrated that at low expression (about 200 fmol/mg protein) of the human 5-HT_{2A} and 5-HT_{2C} receptors, agonists differentially activate two signal transduction pathways independently coupled to these receptors (phospholipase C-mediated inositol phosphate accumulation and phospholipase A_2-mediated arachidonic acid release). The relative efficacies of agonists differed depending on which signal transduction pathway was measured. Some 5-HT_{2C} agonists [i.e. *m*-trifluoro-methyl-phenyl-piperazine (TFMPP) relative to 5-HT] preferentially activated the phospholipase C pathway, whereas others (i.e. LSD) favored the phospholipase A_2 pathway. These data support the hypothesis of agonist-directed trafficking of receptor stimulus as formulated by Kenakin [4]. The term "trafficking" may be confusing with regard to transport and addressing of proteins; differential signalling may better define the pathway-dependent agonist efficacy. Certain agonists may have the capacity to selectively activate a subset of the multiple signal transduction pathways that may be coupled to a single receptor subtype. Agonists would preferentially induce or select receptor conformational states that favour activation of one effector pathway over another. Computational simulations of ligand interactions with the 5-HT_{2A} receptor [39] and experimental evidence with the β_2-adrenergic receptor [18, 40] support the hypothesis of agonist-selective receptor states, although there is some debate as to the number of receptor conformational states [41]. The most likely mechanism by which agonists may preferentially direct a receptor stimulus to different effector mechanisms is via differential G protein coupling. The influence of the G protein subtype on ligand efficacy cannot be excluded, although there are to date few experimental data for this hypothesis. It has been suggested that each agonist may induce a different receptor conformation or set of conformations. The question about G protein-coupled receptors is not so much whether each ligand produces a distinct active state, but rather, are the differences in conformation for different agonists sufficiently great at the far end of the receptor molecule that interacts with the G protein for the G protein to know which agonist is bound [11]. Gettys *et al.* [42] suggested agonist-dependent coupling of the human 5-HT_{1A} receptor expressed in CHO cells to different $G_{\alpha i}$ proteins. Rauwolscine and ipsapirone yielded similar efficacy at the $G_{\alpha i3}$ protein, whereas rauwolscine was more effective than ipsapirone at the $G_{\alpha i2}$ protein. Yang and Lanier [43] demonstrated that signal transfer from the α_{2A}-adrenoceptor is achieved more readily with the $G_{\alpha o}$ protein than with the $G_{\alpha i2}$ and $G_{\alpha i3}$ proteins. Constitutive α_{2A}-adrenoceptor activity is observed by $G_{\alpha o}$ protein co-expression in contrast to the results with co-expressed $G_{\alpha i2}$ and $G_{\alpha i3}$ proteins [29]. Bahia *et al.* [44] did not report any evidence for $G_{\alpha i1}$ protein-dependent constitutive α_{2A}-adrenoceptor activity. Nonetheless, in the case of 5-HT_{1A} receptors, constitutive receptor activity is apparent with both $G_{\alpha i1}$ and $G_{\alpha i3}$ proteins [24, 25]. The molecular tools are available to search new ligands for 5-HT receptors that might activate only a single G_α protein subtype and have reduced collateral effects because of non-activation of other G_α protein subtypes. Partial agonists could be less efficacious than full agonists because they are unable to induce the optimal conformational change in the receptor that regulates contact with the same set of G proteins. In consequence, differential activation of G proteins by partial and full agonists may occur, as illustrated in Fig. 1. This may evoke different, and maybe distinct, receptor-mediated responses for ligands targeting the same receptor population. Therefore, it is likely that pharmacological diversity not only may be achieved between different receptor subtypes but may even occur for a single receptor subtype.

CONTRIBUTION OF 5-HT RECEPTOR SPLICING FORMS TO DIVERSE SIGNALLING

Alternative splicing of 5-HT receptor transcripts adds another level of complexity to our knowledge of serotonergic signal transduction. It is now clear that the existence of introns in genes encoding some members of a receptor family provides scope for additional diversity by virtue of

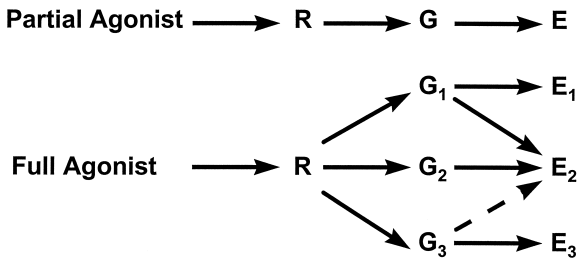

FIG. 1. Differential activation of G proteins by partial and full agonists. It is hypothesised that partial agonists will activate one set of G proteins submaximally, whereas full agonists will do this more efficaciously and with multiple distinct G proteins. Consequently, partial agonists may yield a more selective response, as they will only activate a single effector pathway, as opposed to full agonists, which may mediate diverse signalling responses. R, receptor; G, G protein; E, effector.

splicing events that result in the formation of different receptor mRNAs and consequently distinct receptor isoforms [5]. Alternative splicing serves as a molecular tool to introduce more diversity into gene expression, and thus it may have been generated as a more parsimonious alternative to gene duplication during evolution. Although species-homologues have been found for several receptor variants, in certain cases isoforms may be species-specific. For example, five C-terminus 5-HT_4 receptor variants have been discovered in the human, yet only three of them show similarity with the rat homologues [45]. Splice variants for several receptors are known to be differentially distributed; these include variants of the 5-HT_7 receptor that differ in their C-terminal intracellular tail [46]. The human and rat repertoires of 5-HT_7 splice variants are substantially different. But differential distribution is not universal. It had been thought that the rat 5-HT_4 receptor splice variants had different distributions, but *in situ* hybridization studies have revealed their distributions in the central nervous system to be similar [47]. The majority of distributional work has been conducted using *in situ* hybridization techniques, although definitive studies require the use of isoform-specific antibodies. Research in this area is limited by the marked lack of antisera. Given the predominance of splice variants in regions of receptors that appear to be unimportant for ligand binding, it seems unlikely that the attempt to develop ligands that are selective for particular variants will be successful [5]. Alternative strategies could offer some possibilities. Partial agonists might have selective actions at receptor variants that are more efficiently coupled to their second-messenger pathways. For instance, constitutive activity can vary amongst these receptor variants. Claeysen *et al.* [45] have shown that, whereas all the splice variants of the 5-HT_4 receptor are constitutively active, even at low receptor expression levels (<500 fmol/mg protein), those with a short C-terminal sequence (m5-HT_{4e} and m5-HT_{4f}) exhibit a higher activity than those with a longer C-terminal domain (m5-HT_{4a} and m5-HT_{4b}). This could suggest that the short variants have a higher capacity to isomerize the receptor from the inactive to the active conformation. A sequence within the C-terminal tail upstream of Leu^{348}, rich in serine and threonine residues, has been identified that plays a crucial role in maintaining 5-HT_4 receptors under its inactive conformation. There is also the possibility that variants at the C-terminus might influence receptor regulation, as receptor phosphorylation by G protein-coupled receptor kinases is generally believed to occur at the C-terminus of the receptor [48, 49].

RNA EDITING AND 5-HT RECEPTOR ACTIVITY

Burns *et al.* [50] have demonstrated in an elegant way that transcripts encoding the 5-HT_{2C} receptor undergo RNA editing events in which genomically encoded adenosine residues are converted to inosines by the action of double-stranded RNA adenosine deaminase. RNA editing, defined as a modification in the coding potential of primary RNA transcripts by mechanisms other than RNA splicing, of a mammalian mRNA was discovered a decade ago and has been shown to have major functional consequences in the gating properties of, for instance, the ligand-gated GluRB subunit of (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [51]. In the rat brain, tissue-specific expression has been found for seven major 5-HT_{2C} receptor isoforms encoded by eleven distinct RNA species. Editing of 5-HT_{2C} receptor mRNAs alters the amino acid coding potential of the predicted second intracellular loop of the receptor and can lead to a 10- to 15-fold reduction in the efficacy of the interaction between receptors and their G proteins. The rat 5-HT_{2C}-Val^{157}-Ser^{159}-Val^{161} (VSV) receptor isoform has reduced ability to signal through the principal signal transduction pathway, phospholipase C activation. Burns *et al.* [50] hypothesized that the 5-HT_{2C}-VSV receptor isoform couples less efficiently to G proteins, and this may explain its attenuated function. The profile of 5-HT_{2C} receptor isoforms in the human brain differs from that in the rat with the generation of a new isoform, h5-HT_{2C}-Val^{156}-Gly^{158}-Val^{160} (VGV), which also has reduced G protein-coupling efficiency [52]. In cells expressing the unedited human isoform h5-HT_{2C}-Ile^{156}-Asn^{158}-Ile^{160} (INI), LSD behaved as a partial or nearly full agonist as was found for the rat 5-HT_{2C}-Ile^{157}-Asn^{159}-Ile^{161} (INI) isoform, whereas for the fully edited human isoform h5-HT_{2C}-VGV, LSD has markedly attenuated ability to activate the phosphoinositide hydrolysis pathway compared with 5-HT [53]. Hence, RNA editing is another mechanism for regulating serotonergic signal transduction that may be critical for modulating different cellular functions.

5-HT_{2C} receptor RNA editing further alters receptor basal activity [54]. The importance of the N-terminal region of the second intracellular loop in G protein activation is known. Site-directed mutagenesis studies have shown that amino acid mutations within the DRY (Asp-Arg-Tyr) motif in the second intracellular loop can produce overactive α_{1B}-adrenoceptors [55]. Studies with m_1 and m_3

TABLE 2. Genetic variants of 5-HT receptors with modified properties

Receptor subtype	Coding region	Allele frequency (%)	Receptor properties	Reference
h5-HT_{1A}	$Gly^{22}Ser$ (N-term.)	2	Attenuation of 8-OH-DPAT-induced down-regulation and desensitization	[57]
h5-HT_{1B}	$Phe^{124}Cys$ (TMD III)	2	Higher binding affinity for 5-HT, sumatriptan, and SB-216641	[58]
h5-HT_{2A}	$His^{452}Tyr$ (C-term.)	9	Smaller peak amplitude in Ca^{2+} mobilization	[60]

N-term. = N-terminal portion; TMD III = transmembrane domain III; and C-term. = C-terminal portion.

muscarinic receptors and β_2-adrenoceptors have shown that amino acid substitutions within the DRYXX(I/V)XXPL motif, the same region affected by 5-HT_{2C} receptor editing, decrease receptor–G protein coupling and second messenger activation [56]. The unedited isoform (5-HT_{2C}-INI) displays the greatest basal activity, stimulating inositol phosphate production 4-fold over the fully edited isoform (5-HT_{2C}-VGV). RNA editing also decreases agonist affinity and potency, suggesting that this editing may play a role in response to drug therapy. These results imply that different brain regions may have different levels of serotonergic receptor basal activity, impaired by the different 5-HT_{2C} receptor isoforms, which would vary in sensitivity to endogenous 5-HT released from nerve terminals and perhaps even to drug therapy.

NATURALLY OCCURRING AMINO ACID SUBSTITUTION OF 5-HT RECEPTORS

Normal variation (i.e. sequence polymorphism) in 5-HT receptors in some cases can be associated with a difference in signal response [6]. Table 2 illustrates three 5-HT receptor variants that have been shown to yield modified receptor properties. 8-OH-DPAT-induced down-regulation and desensitization of the h5-HT_{1A} $Gly^{22}Ser$ receptor variant is attenuated compared with the wild-type 5-HT_{1A} receptor response in transfected Cos-7 cells [57]. Although this effect was monitored at a high concentration (100 μM), an altered sensitivity of the 5-HT_{1A} receptor variant may have consequences for drug action. Brüss *et al.* [58] found higher binding affinities (0.3 to 0.5 log units) for the agonists 5-HT and sumatriptan and the antagonist SB-216641 at the 5-HT_{1B} $Phe^{124}Cys$ receptor variant compared with the wild-type 5-HT_{1B} receptor. The observation with sumatriptan may be of interest. Sumatriptan-induced coronary vasospasm, which occurs at low incidence as a side-effect of its therapeutic action in migraine [59], may be due in part to the expression of the 5-HT_{1B} receptor variant in the susceptible individuals, leading to an increased affinity for the contraction-inducing 5-HT_{1B} receptor in the coronary artery [6]. The $His^{452}Tyr$ form of the 5-HT_{2A} receptor exhibits a blunted Ca^{2+} response to 5-HT in platelets of these individuals. This receptor variant may be in a state of relative desensitization, potentially as a result of phosphorylation of the Tyr^{452} residue [60]. Whereas a number of efforts have been made to link the occurrence of 5-HT receptor variants with certain pathological disorders, no clear profile is apparent. Most studies with positive correlations are controversial [61]. Otherwise, the recent progress in pharmacogenomics contributes to a mapping of single-nucleotide polymorphisms. This may eventually lead to better designed drugs with fewer side-effects. Importantly, this approach should allow us to address more specifically the right target population of patients, dependent on their genetic profile and susceptibilities to develop drug side-effects and resistance.

CONCLUSION

The more we discover about signalling via G protein-coupled receptors, the more we realise that this is a highly complex process. Two factors principally contribute to this complexity: the possibility that one single receptor subtype may govern multiple effector pathways, and the possibility that the receptor subtype may be present as a mutant form or as a different isoform due to alternative splicing and RNA editing of the transcript. A better understanding of the activation state(s) of the G protein-coupled receptor appears crucial to develop ligands that may either enhance, attenuate, block, or reverse a response as mediated by a given receptor–effector pathway. Efforts should not be limited to the study of the receptor under normal conditions, but also should extend to its behaviour under pathophysiological conditions. In particular, the mapping and the relevance of the recently discovered 5-HT receptor isoforms need further investigation.

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