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Paradoxical trafficking and regulation of 5-HT_{2A} receptors by agonists and antagonists

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ABSTRACT: 5-Hydroxytryptamine$_{2A}$ (serotonin$_{2A}$, 5-HT_{2A}) receptors are important for many physiologic processes including platelet aggregation, smooth muscle contraction, and the modulation of mood and perception. A large number of pharmaceutical agents mediate their actions, at least in part, by modulating the number and/or activity of 5-HT_{2A} receptors. Drugs with action at 5-HT_{2A} receptors are used in the treatment of many disorders, including schizophrenia, depression, and anxiety disorders. This review summarizes over two decades of research on the regulation of 5-HT_{2A} receptors and provides a comprehensive review of numerous *in vivo* studies describing the paradoxical phenomenon of 5-HT_{2A} receptor down-regulation by chronic treatment with antidepressants and antipsychotics. In addition, studies reporting antagonist-induced internalization of 5-HT_{2A} receptors and other G protein-coupled receptors will be highlighted as a possible mechanism to explain this paradoxical down-regulation. Finally, a review of the cellular and molecular mechanisms that may be responsible for agonist-mediated desensitization and internalization of 5-HT_{2A} receptors will be presented. © 2001 Elsevier Science Inc.

KEY WORDS: Down-regulation, Antipsychotic, Antidepressant, Internalization, Clozapine, Arrestin.

INTRODUCTION

Serotonin 5-HT_{2A} receptors are important for mediating a large number of physiologic processes both in the periphery and in the central nervous system. These processes include platelet aggregation, smooth muscle contraction, and the modulation of mood and perception. 5-HT_{2A} receptors belong to a family of serotonin receptors currently made up of more than 15 different receptors encoded by distinct genes which are divided into seven major classes: 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 [87]. Most of these classes have multiple subtypes, including the 5-HT_2 class that is divided into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} [56,84,90,91]. With the exception of 5-HT_3 receptors, which are ligand-gated ion channels [22,68], 5-HT receptors are members of the G protein-coupled receptor (GPCR) superfamily. A large number of drugs mediate their actions, at least in part, by interactions with 5-HT_{2A} receptors. These include hallucinogens, atypical antipsychotic drugs and antidepressants.

GPCRs comprise at least two percent of the estimated 30,000 genes in the human genome and are targets for a wide array of molecules ranging from hormones and neurotransmitters to odorants and even light. GPCRs are characterized by seven membrane-spanning helices with an extracellular amino-terminus, an intracellular carboxy-terminus, and three intracellular and three extracellular loops connecting each of the transmembrane segments. Binding of an agonist to its GPCR leads to conformational changes in the receptor that induce the dissociation and activation of a receptor-specific heterotrimeric G protein into its α- and $\beta\gamma$-subunits. These dissociated subunits can then activate or inhibit a number of downstream effectors, such as nucleotide cyclases, phospholipases, and kinases, resulting in a variety of downstream cellular effects.

In addition to initiating intracellular signal transduction cascades, agonist activation of GPCRs also triggers cellular and molecular mechanisms that lead to the attenuation of receptor signaling. Thus, GPCR responsiveness to agonist-induced stimulation wanes over time, a process termed desensitization. Other regulatory phenomena include resensitization, a recovery of receptor responsiveness following desensitization, and down-regulation, a reduction in receptor number. The past decade has seen much progress in elucidating the cellular events mediating GPCR regulation and these processes will be briefly reviewed. Specifically, receptor trafficking via the endocytic pathway will be discussed in relation to its role in mediating and modulating the pharmacologically defined patterns of GPCR regulation. This review will briefly describe the classical mechanistic models of receptor regulation as a prelude to a summary of the paradoxical behavior of 5-HT_{2A} receptors.

MECHANISMS OF SHORT-TERM 5-HT_{2A} REGULATION

5-HT_{2A} Desensitization and the Role of Kinases

Desensitization is an adaptive mechanism by which cells regulate receptor responsiveness to repetitive environmental stimuli. Two major patterns of rapid GPCR desensitization have been characterized, homologous or agonist-specific, and heterologous or agonist-nonspecific [18,33]. Homologous desensitization refers to the attenuation of a cell's response to only that agonist. For example, a cell exposed to a 5-HT_{2A} receptor agonist would, over time, become desensitized to repeated exposure to the same 5-HT_{2A} receptor agonist. In contrast, heterologous desensitization is the attenuation of the response to multiple distinct agonists acting at different receptor types following stimulation by a single agonist. Thus, exposure of a cell to a 5-HT_{2A} receptor agonist may result in the desensitization of the cells responsiveness to an

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agonist with activity at another GPCR. In both homologous and heterologous desensitization, phosphorylation of the intracellular domains of GPCRs is thought to be essential. G protein-coupled receptor kinases (GRKs) and arrestins are thought to be involved in mediating homologous desensitization while the second messenger-dependent kinases, protein kinase A (PKA) and protein kinase C (PKC), are typically involved in heterologous desensitization [28,33].

The mechanism of 5-HT_{2A} receptor desensitization is incompletely understood. For some time though, it has been clear that 5-HT_{2A} receptors may be desensitized following PKC activation [48,49,80,81,88], though cell-type specific effects have been noted. In platelets, accordingly, a selective PKC inhibitor was able to attenuate agonist-dependent desensitization [48]. Similarly, we have previously demonstrated that down-regulating various PKC isoforms by overnight treatment with phorbol esters had no effect on rapid (10 min-2 h) or delayed ($>$ 6 h) phases of agonist-induced desensitization, whereas the intermediate phase (2–4 h) was attenuated by down-regulating PKC [89]. Studies with chimeric 5-HT_{2A}/thrombin receptors expressed in HEK-293 cells have demonstrated that 5-HT_{2A} receptors are desensitized by PKC activation but without being phosphorylated [104]. Additionally, in HEK-293 cells, PKC-selective antagonists did not attenuate agonist-induced desensitization [104]. Together, these results imply that PKC may be involved in some aspects of 5-HT_{2A} desensitization, although other cellular processes are also involved (Fig. 1B).

Following receptor activation, downstream signaling cascades result in feedback phosphorylation of agonist-occupied receptors by specific GRKs (Fig. 1A) [33]. This phosphorylation results in attenuation of second messenger production in response to agonist binding. We have recently demonstrated that, in HEK-293 cells, 5-HT_{2A} receptor desensitization is unaffected by overexpression of GRK2 or GRK5 as well as a kinase deficient mutant of GRK2 (GRK2-K220R) [42]. Interestingly, however, overexpression of GRK2 and GRK2-K220R results in a significant reduction in the level of agonist-mediated phosphoinositide production [42]. Similar attenuation of phosphoinositide hydrolysis was seen with cotransfection of GRK2 with the 5-HT_{2C} receptor in HEK-293 cells [92]. These results suggest that the Gαq subunit may be regulated by direct interaction with GRK2 via its amino-terminal RGS homology domain [42,92]. Thus, while it seems that 5-HT_{2A} receptors may not be regulated by GRK phosphorylation, GRKs may play important roles in the regulation of receptor signaling through Gq (Fig. 1B).

Role of Arrestins in 5-HT_{2A} Desensitization and Internalization

Arrestins were initially described as proteins that function as co-factors for GRKs [66] and appear to mediate the desensitization of several GPCRs [25] (Fig. 1A). In 1996, Ferguson et al. [26,27] found that overexpression of arrestin-2 potentiated agonist-mediated internalization of β2-adrenergic receptors. Additionally, a dominant-negative mutant of arrestin-2 attenuated agonist-induced internalization in transiently transfected HEK-293 cells [26]. Subsequently, it was found that purified arrestin-2 bound directly to clathrin and clathrin-coated vesicles [39] and the domains of arrestin-2 that are essential for clathrin binding have been identified [38,39,57]. Recently, arrestin-2 has been shown to also interact with adaptor protein 2 (AP-2) [60], a protein that plays a critical role in the recruitment of clathrin and assembly of clathrin-coated pits (Fig. 1A). These studies suggest that arrestins may serve as scaffolding proteins that allow for the efficient targeting of GPCRs to coated pits [73,108].

The role of arrestins in regulating 5-HT_{2A} receptors is only partially known. We recently demonstrated that arrestin-2 and arrestin-3 can bind to the purified third intracellular loop of the 5-HT_{2A} receptor *in vitro* and that 5-HT_{2A} receptors are co-localized with arrestin-2 and arrestin-3 in some, but not all, cortical neurons [37]. That arrestins are not ubiquitously co-expressed with 5-HT_{2A} receptors could indicate that either arrestins are not obligatory regulatory proteins for 5-HT_{2A} receptors in neurons or that 5-HT_{2A} receptor regulation differs depending on the specific neuronal environment. Surprisingly, in transiently transfected HEK-293 cells, we found that agonist-induced internalization of 5-HT_{2A} receptors is dynamin-dependent, but arrestin-independent [6]. Additionally, arrestin-2 and arrestin-3 were sorted to membrane-bound compartments that were distinct from those containing 5-HT_{2A} receptors (Fig. 1B) [6]. Taken together, these results imply that arrestins are not necessary for mediating 5-HT_{2A} receptor internalization and that arrestins may have additional cellular functions. Intriguingly, we have evidence that agonist-induced desensitization is unaffected by coexpression of dominant negative forms of arrestin-2 and arrestin-3 in transiently transfected HEK-293 cells but potentiated in C6 glioma cells that endogenously express the 5-HT_{2A} receptor [42]. Thus, since arrestins have cell-type specific effects, additional modes of arrestin action may exist. Therefore, although arrestins are apparently intimately involved in the short-term regulation of many GPCRs, their role in 5-HT_{2A} receptor regulation and internalization is still unclear and is cell-type dependent.

MECHANISMS OF LONG-TERM GPCR REGULATION

In addition to short-term regulation of GPCRs by the processes of desensitization, resensitization, and internalization, receptors can be regulated on a longer time scale. Thus, following prolonged or repetitive activation of receptors by agonists, there is a measurable reduction in the number of receptors within cells or tissues. This results in a long-term attenuation of the receptor's ability to signal in the presence of new stimuli. This phenomenon, known as down-regulation, is traditionally defined as a reduction in total specific binding sites (B_{max}) without a change in apparent affinity (K_d) indicating a loss of total cellular receptors [54]. It is generally thought (though not proven) that the decrease in total receptor number is due to receptor degradation in the lysosome following agonist-induced internalization along the endocytic pathway (Fig. 1A). Thus, while GPCR desensitization is rapidly reversible following the removal of agonist, recovery following down-regulation is exceedingly slow and represents the synthesis of new receptors.

Role of Endocytosis in GPCR Down-regulation

It has been presumed for some time that most GPCRs are down-regulated by lysosomal degradation of endocytosed receptors (Fig. 1A). This lysosomal mechanism was suggested by early biochemical work on the delta opioid receptor [61] and has been recently supported by fluorescence microscopic data allowing a visual conformation of lysosomal trafficking in culture [53]. Similarly, lysosomal trafficking of the β2-adrenergic receptor in living cells has recently been observed [34,50]. Using a dominant inhibitory mutant of dynamin, dynamin K44A, it is possible to block the internalization of receptors that are internalized via clathrin-coated pits. Recently, this dominant negative dynamin has been shown to attenuate the internalization and down-regulation of the β2-adrenergic receptor [34]. These studies, as well as others, suggest a common mechanism of down-regulation by degradation in the lysosomes (Fig. 1A).

Recently, however, the role of endocytosis for GPCR down-regulation has been brought into question. Studies by Jockers et al.

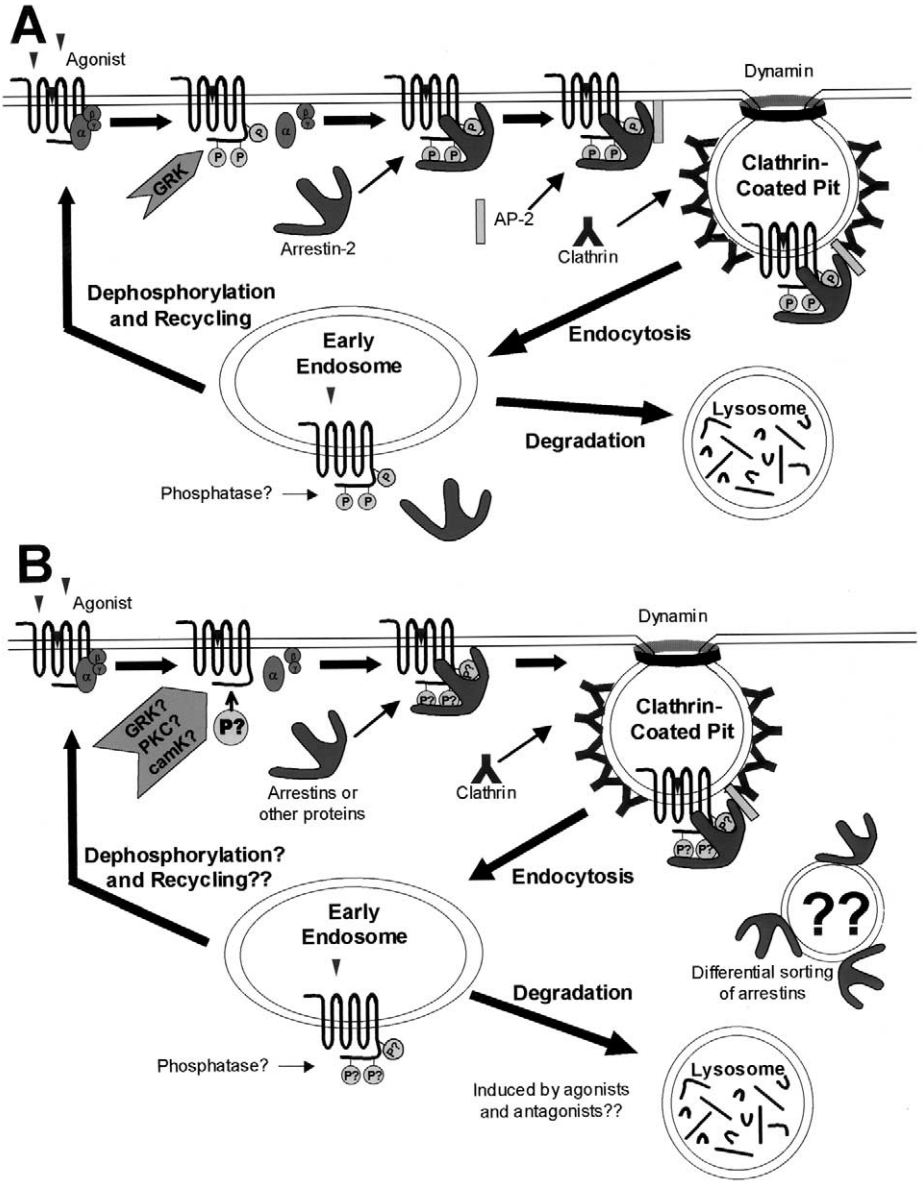

FIG. 1. (A) Current model of desensitization, internalization, resensitization, and degradation of the prototypical β2-adrenergic receptor. Following agonist-induced activation, the β2-adrenergic receptor is feedback phosphorylated by kinases such as G protein-coupled receptors kinases (GRKs) or protein kinase A (PKA), resulting in rapid desensitization. Arrestins bind to phosphorylated receptors potentiating desensitization. These bound arrestins act as scaffolding proteins interacting with AP-2 and clathrin, resulting in the targeting of desensitized β2-adrenergic receptors to clathrin-coated pits. The GTPase dynamin induces neck formation of coated pits and their release into the cytoplasm as clathrin-coated vesicles. These coated vesicles fuse with early endosomes where the receptors may be dephosphorylated by specific phosphatases and recycled back to the plasma membrane fully resensitized or targeted to lysosomes for degradation. (B) Differential trafficking and regulation of the 5-HT$_{2A}$ receptor. Following agonist-induced activation, 5-HT2A receptors are possibly feedback phosphorylated by a number of different kinases including GRKs, protein kinase C (PKC), or calmodulin-dependent kinases (camK). Subsequently, 5-HT$_{2A}$ receptors are internalized via clathrin-coated pits in a dynamin-dependent but arrestin-independent manner and targeted to the early endosomal compartment. Interestingly, arrestins have been found to be differentially sorted to membrane-bound compartments distinct from internalized 5-HT$_{2A}$ receptors. Internalization of 5-HT$_{2A}$ receptors also occurs following antagonist exposure and may mediate the paradoxical down-regulation of 5-HT$_{2A}$ receptors following antagonist exposure.

[47] in stable clones of L cells expressing β2-adrenergic receptors and in A431 cells that endogenously express β2-adrenergic receptors, demonstrated that blocking endocytosis by dynamin K44A and with multiple chemical inhibitors of endocytosis did not prevent agonist-mediated down-regulation. Additionally, chemical blockers of lysosomal degradation and of proteosome-mediated degradation were also ineffective at preventing β2-adrenergic receptor down-regulation [47]. These data indicated that receptor endocytosis is not necessarily a prerequisite for receptor down-regulation and that receptor loss may occur at the plasma membrane. Thus, other mechanisms have been proposed to mediate receptor down-regulation. These may include changes in mRNA stability, regulation of gene transcription or translation, or proteolysis at the cell surface [103].

Down-regulation of 5-HT$_{2A}$ Receptors by Agonists

Like most GPCRs, 5-HT$_{2A}$ receptors are down-regulated following prolonged agonist exposure. For instance, 5-HT$_{2A}$ receptors are down-regulated by daily LSD [9,10] and DOI [10,71,79] administration *in vivo*. Similar results have been found in some, but not all, *in vitro* systems. For instance, it was found that DOM caused a rapid 5-HT$_{2A}$ receptor down-regulation in smooth muscle cells *in vitro* [62–64,75] as well as in P11 cells [32,45]. The down-regulation in P11 cells was found to be due to a PKC-mediated change in 5-HT$_{2A}$ receptor mRNA stability [30,31]. However, in cultured cerebellar granule cells, agonist exposure induced an up-regulation of 5-HT$_{2A}$ receptors [1,16,17] apparently by activation calcium/calmodulin-dependent kinase [16]. In NIH 3T3 cells, 5-HT$_{2A}$ receptors are desensitized, but not down-regulated, by acute [89] and chronic [43] agonist administration. Though there are differences in 5-HT$_{2A}$ receptor regulation among various models systems, most cells exhibit a characteristic receptor down-regulation following agonist exposure (Fig. 1B). The precise mechanism of 5-HT$_{2A}$ receptor down-regulation by agonists is yet to be fully explained and thus the use of model *in vitro* systems and dominant inhibitory proteins that will block specific steps of receptor signaling, internalization, and trafficking with be extremely valuable for elucidating the details of these events.

Paradoxical Down-regulation of 5-HT$_{2A}$ by Antagonists

Unlike other GPCRs, 5-HT$_2$ class receptors have been shown to be down-regulated by many antagonists (Fig. 1B, Tables 1, 2, and 3). The paradoxical down-regulation of 5-HT$_{2A}$ receptors was first described in *in vivo* studies of rats chronically treated with antidepressants by Bergstrom and Kellar in 1979 [4] and Peroutka and Snyder in 1980 [76]. Bergstrom and Kellar [4] demonstrated that chronic treatment with the antidepressant desipramine caused a reduction in serotonin binding sites in the cerebral cortex. In more complete studies using a high affinity 5-HT$_{2A}$ radio-labeled ligand, spiroperidol, Peroutka and Snyder [76], described statistically significant down-regulation of 5-HT$_{2A}$ receptors in the frontal cortex of rats chronically treated with the tricyclic antidepressants amitryptiline, desipramine, and imipramine (Tables 1 and 2).

Subsequently, numerous follow-up studies have demonstrated this paradoxical antagonist-induced down-regulation by other antidepressants that are high affinity antagonists at 5-HT$_{2A}$ receptor (Tables 1 and 2). Though most tricyclic antidepressants and monoamine oxidase (MAO) inhibitors cause 5-HT$_{2A}$ down-regulation *in vivo,* this is likely not the final pathway of antidepressant drug action, as some antidepressants such as buproprion [29] do not cause down-regulation. Additionally, serotonin-selective uptake inhibitors (SSRIs) have varying effects on 5-HT$_{2A}$ down-regulation. For instance, depending on the study, fluoxetine has been reported to have either no effect on 5-HT$_{2A}$ receptor number [15,40,41,76], or actually increase receptor number [52]. Similarly, paroxetine has also been reported to increase [15] or have no effect [3] on 5-HT$_{2A}$ receptor numbers. By contrast, citalopram has been shown to down-regulate 5-HT$_{2A}$ [52,58]. It is likely that 5-HT2A receptor down-regulation is only one mode by which antidepressant drugs exert their therapeutic effects and that other downstream events are also involved in antidepressant drug action.

Possible mechanisms responsible for the antidepressant-induced 5-HT$_{2A}$ receptor down-regulation have been described [86]. These include increased degradation of 5-HT$_{2A}$ receptors or changes in gene transcription and/or translation. Roth and Ciaranello [85] found that chronic mianserin treatment did not alter 5-HT$_{2A}$ receptor mRNA levels in rat cortex. Similarly, Burnet et al. [12] also found no change in receptor mRNA levels in imipramine-treated rats while Butler et al. [13] found a small increase in 5-HT$_{2A}$ receptor mRNA in mianserin-treated rats. These *in vivo* studies stand in sharp contrast to results from *in vitro* studies. Toth and Shenk [102] described a decrease in 5-HT$_{2A}$ receptor mRNA in C6 glioma cells following exposure to mianserin, due to regulatory sequences on the 5′-untranslated region of the 5-HT$_{2A}$ gene [101]. It is likely that antagonist-induced down-regulation *in vivo* is primarily mediated by posttranslational mechanisms (e.g., endocytosis, proteolysis).

In addition to the effects of antidepressants on 5-HT$_{2A}$ down-regulation, other 5-HT$_{2A}$ antagonists, such as typical and atypical antipsychotics, have been shown to induce down-regulation of 5-HT$_{2A}$ receptors *in vivo* and *in vitro*. Clozapine was the first antipsychotic drug to be demonstrated to down-regulate 5-HT$_{2A}$ receptors [82]. In this study, the rats were treated for 1 year with clozapine administered in drinking water, and a 63% reduction in specific [^{3}H]ketanserin binding sites in the frontal cortex compared with control rats was found [82]. Since then, a large number of other studies have demonstrated down-regulation of 5-HT$_{2A}$ receptors following administration of typical and atypical antipsychotics (Table 3). The ability of antipsychotic drugs to induce 5-HT$_{2A}$ receptor down-regulation appears to be correlated with their high affinities for the 5-HT$_{2A}$ receptor [72]. However, this is not a perfect correlation since risperidone, which has high affinity for 5-HT$_{2A}$, does not induce receptor down-regulation [59] (Table 3). Therefore, it seems that a high affinity for 5-HT$_{2A}$ receptors is necessary but not sufficient to induce receptor down-regulation.

Like the antidepressants, the role of transcriptional regulation has been studied for antipsychotic-induced 5-HT$_{2A}$ receptor down-regulation. Burnett et al. [11] found no statistically significant change in 5-HT$_{2A}$ mRNA levels in the frontal cortex following chronic clozapine administration though there was a significant down-regulation of 5-HT$_{2A}$ receptor numbers. Thus, like the antidepressants, it appears unlikely that transcriptional regulation significantly contributes to antagonist-induced 5-HT$_{2A}$ down-regulation.

Antagonists can Induce Internalization of 5-HT$_{2A}$ Receptors: A Possible Mechanism of Antagonist-induced Down-regulation

In 1996, we first provided evidence that antagonist-induced internalization may be a mechanism by which antidepressants and antipsychotics cause down-regulation of 5-HT$_{2A}$ receptors [5]. In these studies, we discovered that exposure of NIH 3T3 fibroblasts stably expressing the 5-HT$_{2A}$ receptor to ketanserin induced internalization of 5-HT$_{2A}$ receptors as assessed by confocal laser microscopy [5]. The degree of ketanserin-induced internalization was statistically significant although ketanserin was much less efficient at internalizing 5-HT$_{2A}$ receptors than was quipazine, a 5-HT$_{2A}$ partial agonist [5]. Subsequently, Willins et al. [105,106] demonstrated that clozapine and other atypical antipsychotic drugs induce

TABLE 1

DOWN-REGULATION OF 5-HT$_{2A}$ BINDING SITES BY TRICYCLIC ANTIDEPRESSANTS AND SELECTIVE SEROTONIN REUPTAKE INHIBITORS *IN VIVO*

	% of control	Amount (mg/kg)	Per day	Length (days)	System	Tissue	[^{3}H] ligand	Cold ligand	Ref. #
Tricyclic antidepressants									
Amitriptyline	57*	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	60*	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[77]
	60*	10	1×	28	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[77]
	60*	10	1×	35	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[77]
	50*	10	1×	42	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[77]
	85*	10	1×	7	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[77]
	62*	10	1×	21	Rats	Cortex	Spiperone	1 uM LSD	[51]
	60*	20	1×	7	Rats	Cortex	Spiperone	1 uM cinanserine	[19]
	68*	10	2×	10	Rats	Cortex	Spiperone	25 uM ketanserin	[20]
	none	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	65*	10	1×	21	Rats	Frontal cortex	Ketanserin	methysergide	[97]
	81*	10	1×	21	Rats	Cortex	Spiperone	25 uM ketanserin	[96]
	80*	10	1×	21	Rats	Cortex	Spiperone	25 uM ketanserin	[96]
	95	10	2×	21	Rabbits	Frontal cortex	Ketanserin	methysergide	[95]
	109	10	2×	21	Rabbits	Frontal cortex	Imipramine	100 uM desipramine	[95]
	57*	10	1×	21	Rats	Frontal cortex	Imipramine	100 uM desipramine	[95]
	116	10	1×	21	Guinea pigs	Cortex	Ketanserin	1 uM methysergide	[15]
Amoxapine	21*	10	1×	28	Rats	Cortex	Spiperone	50 nM mianserin	[44]
	21*	10	1×	2	Rats	Cortex	Spiperone	50 nM mianserin	[44]
	19*	10	1×	7	Rats	Cortex	Spiperone	50 nM mianserin	[44]
Chlorimipramine	84*	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	93	10	1×	21	Rabbits	Frontal cortex	Ketanserin	methysergide	[95]
Desipramine	79*	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	65*	10	2×	15	Rats	Frontal cortex	Spiperone	1 uM LSD	[29]
	75*	5	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]
	65*	10	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]
	77*	5	1×	14	Rats	Frontal cortex	Ketanserin	10 uM mianserin	[40]
	73*	10	1×	14	Rats	Frontal cortex	Ketanserin	10 uM mianserin	[40]
	66*	15	1×	14	Rats	Frontal cortex	Ketanserin	10 uM mianserin	[40]
Dothiapine	68*	30	2×	24	Rats	Frontal cortex	Spiperone	1 uM LSD	[23]
Imipramine	60*	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	none	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	85	10	1×	5	Rats	Frontal cortex	Spiperone	100 uM cinanserine	[70]
	89	10	1×	21	Rats	Cortex	Ketanserin	10 uM cinanserin	[2]
	108	10	2×	21	Rabbits	Frontal cortex	Ketanserin	methysergide	[95]
	88*	20	1×	21	Rats	Cortex	Ketanserin	1 uM methysergide	[14]
	67*	15	1×	21	Rats	Frontal cortex	Ketanserin	10 uM methysergide	[13]
	54*	5	1×	56	Rats-Strain1	Cortex	Ketanserin	10 uM methysergide	[12]
	58*	5	1×	56	Rats-Strain2	Cortex	Ketanserin	10 uM methysergide	[12]
	67*	5	1×	56	Rats-Strain3	Cortex	Ketanserin	10 uM methysergide	[12]
Selective serotonin reuptake inhibitors									
Fluoxetine	87	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	73*	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	103	10	1×	21	Guinea pigs	Cortex	Ketanserin	1 uM methysergide	[15]
	106	10	1×	21	Rats	Frontal cortex	Ketanserin	10 uM methysergide	[13]
	98	10	1×	14	Rats	Frontal cortex	Ketanserin	10 uM mianserin	[40]
Paroxetine	96	10	1×	21	Guinea pigs	Cortex	Ketanserin	1 uM methysergide	[15]
Zimelidine	83	5	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]
	80*	10	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]

Loss of 5-HT$_{2A}$ binding sites is expressed as the percentage relative to vehicle treated animals.
* $p < 0.05$ versus vehicle treated animals.

TABLE 2

DOWN-REGULATION OF 5-HT_{2A} BINDING SITES BY MONOAMINE OXIDASE INHIBITORS, OTHER ANTIDEPRESSANTS AND OTHER DRUGS *IN VIVO*

	% of control	Amount (mg/kg)	Per day	Length (days)	System	Tissue	[3H] ligand	Cold ligand	Ref. #
Monoamine oxidase inhibitors									
Pargyline	65*	25	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
Phenelzine	96	46.8	1×	18	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[55]
Tranylcypramine	55*	5	1×	21	Rats	Cortex	Spiperone	1 uM LSD	[51]
	41*	10	1×	21	Rats	Frontal cortex	Ketanserin	10 uM methysergide	[13]
Other antidepressants									
Buproprion	88	12.5	3×	21	Rats	Frontal cortex	Spiperone	1 uM LSD	[29]
	87	25	3×	21	Rats	Frontal cortex	Spiperone	1 uM LSD	[29]
	105	50	3×	21	Rats	Frontal cortex	Spiperone	1 uM LSD	[29]
Iprindole	60*	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	60*	10	1×	21	Rats	Cortex	Spiperone	1 uM LSD	[51]
	77*	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	52*	5	2×	21	Rats	Frontal cortex	Mianserin	10 uM mianserin	[35]
	65*	5	2×	21	Rats	Frontal cortex	Ketanserin	10 uM spiroperidol	[35]
Mianserin	50*	5	1×	14	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[8]
	52*	10	1×	1	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[8]
	55*	5	1×	14	Rats	Frontal cortex	Ketanserin	10 uM LSD	[35]
	64*	5	1×	1	Rats	Frontal cortex	Ketanserin	10 uM LSD	[35]
	50*	15	1×	14	Rats	Frontal cortex	Ketanserin	1 uM mianserin	[85]
	45*	15	1×	21	Rats	Frontal cortex	Ketanserin	1 uM mianserin	[85]
	44*	15	1×	4	Rats	Frontal cortex	Ketanserin	1 uM mianserin	[85]
	23*	10	1×	10	Rats	Frontal cortex	Ketanserin	10 uM methysergide	[13]
Nefazadone	74*	50	1×	21	Rats	Cortex	Spiperone	25 uM ketanserin	[96]
Trazadone	102	40	1×	4	Rats	Cortex	Spiperone	10 uM LSD	[100]
Zimelidine	83	5	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]
	80*	10	2×	21	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[21]
Other drugs									
Cyproheptadine	42*	10	1×	1	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
	66*	10	1×	14	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
Metergoline	96	4	1×	14	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
	none	15	2×	14	Rats	Frontal cortex	Spiperone	100 uM 5-HT	[98]
	68*	4	1×	1	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
Reserpine	128*	0.5	1×	12	Rats	Frontal cortex	Ketanserin	methysergide	[97]
	none	5	1×	4	Rats	Cortex	Spiperone	25 uM ketanserin	[96]
Tetrabenazine	76*	20	1×	21	Rats	Cortex	Ketanserin	1 uM methysergide	[14]
Methysergide	90	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	76*	10	1×	14	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
	43*	10	1×	1	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
Pizotifen	65*	3	1×	14	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
	58*	3	1×	1	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[7]
Promethazine	69*	5	1×	1	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Pyrilamine	96	5	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]

Loss of 5-HT_{2A} binding sites is expressed as the percentage relative to vehicle treated animals.
Similar results seen in [36,67,93,94]. * $p < 0.05$ versus vehicle treated animals.

the internalization of 5-HT_{2A} receptors *in vitro* (Fig. 2) as well as the redistribution of 5-HT_{2A} receptors *in vivo* [106]. In these studies, internalization was quantified via fluorescent confocal microscopy as well as biochemically with a surface-biotinylation assay [106]. Additionally, the treatment of rats for 7 days with clozapine induced an increase in intracellular 5-HT_{2A} receptor-like immunoreactivity in pyramidal neurons, while causing a decrease in the labeling of apical dendrites in the medial prefrontal cortex

TABLE 3

DOWN-REGULATION OF 5-HT$_{2A}$ BINDING SITES BY ANTIPSYCHOTIC DRUGS *IN VIVO*

	% of control	amount (mg/kg)	per day	length (days)	system	tissue	[3H] ligand	cold ligand	Ref. #
Typical antipsychotics									
Butaclamol, d-	81*	10	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Butaclamol, l-	110	10	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Chlorpromazine	90	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	41*	10	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
	70*	5	1×	21	Rats	Cortex	Ketanserin	10 uM cinanserin	[2]
	87	10	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
	55*	15	1×	14	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[59]
Chlorpromazine sulfoxide	93	5	1×	21	Rats	Cortex	Ketanserin	10 uM cinanserin	[2]
Cis-flupenthixol	73*	1	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
	85*	1	2×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
	92	10	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Clothapine	59*	10	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Haloperidol	98	10	1×	21	Rats	Frontal cortex	Spiroperidol	1 uM LSD	[76]
	100	1	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
	108	2	1×	14	Rats	Frontal cortex	Ketanserin	10 uM 5-HT	[11]
	97	2	1×	14	Rats	Cingulate cortex	Ketanserin	10 uM 5-HT	[11]
	90	2	1×	14	Rats	Piriform cortex	Ketanserin	10 uM 5-HT	[11]
Loxapine	14*	5	1×	28	Rats	Cortex	Spiperone	50 nM mianserin	[44]
	18*	5	1×	2	Rats	Cortex	Spiperone	50 nM mianserin	[44]
	16*	5	1×	7	Rats	Cortex	Spiperone	50 nM mianserin	[44]
	44*	10	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Moperone	87*	1	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Perphenazine	90	1	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Spiroperidol	76*	0.5	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Atypical antipsychotics									
Amperozide	93	30	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
	74*	5	1×	21	Rats	Cortex	Ketanserin	1 uM methysergide	[99]
	70*	5	1×	14	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[59]
Clozapine	37*	25	water	365	Rats	Frontal cortex	Ketanserin	1 uM LSD	[82]
	70*	20	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
	50*	25	1×	14	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[59]
	30*	25	1×	14	Rats	Frontal cortex	Ketanserin	10 uM 5-HT	[11]
	32*	25	1×	14	Rats	Cingulate cortex	Ketanserin	10 uM 5-HT	[11]
	89	25	1×	14	Rats	piriform cortex	Ketanserin	10 uM 5-HT	[11]
Fluperlapine	72*	20	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Melperone	105	30	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
ORG 5222	60*	0.1	1×	14	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[59]
Risperidone	88	0.3	1×	14	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[59]
RMI-81582	75*	10	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Setoperone	83	10	bolus	1	Rats	Frontal cortex	Ketanserin	1 uM methysergide	[65]
	69*	5	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]
Sulpiride	98	10	1×	21	Rats	Cortex	Spiperone	10 uM cinanserin	[2]
Tiospirone	91	2.5	1×	1	Rats	Cortex	Ketanserin	2 uM methysergide	[69]

Loss of 5-HT$_{2A}$ binding sites is expressed as the percentage relative to vehicle treated animals.
Similar results seen in [36,65]. * $p < 0.05$ versus vehicle treated animals.

[106]. It had been previously shown that 5-HT$_{2A}$ receptors are localized primarily on the apical dendrites and cell body of pyramidal neurons in the cerebral cortex [46,107]. Taken together, these results suggest that antagonist-induced internalization, and possibly degradation in lysosomes, may be responsible for the paradoxical down-regulation of 5-HT$_{2A}$ receptors by antidepressants and antipsychotics with high affinity for 5-HT$_{2A}$ receptors.

Internalization of 5-HT$_{2A}$ receptors by antagonists is not entirely unique among GPCRs. Studies by Roettger et al. [83] reported that cholecystokinin (CCK) receptor antagonists caused the internalization of the CCK receptor *in vitro*. They found that multiple CCK antagonists induced the internalization of CCK receptors without resulting in CCK receptor phosphorylation [83]. Similarly, the human vasopressin V_2 receptor was found to be

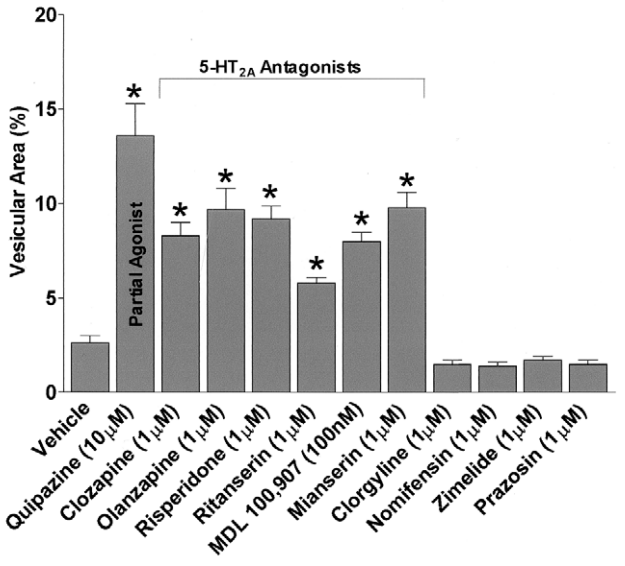

FIG. 2. Antagonist-induced internalization of 5-HT_{2A} receptors in stably transfected NIH 3T3 cells. In these studies, cells were exposed to vehicle, a partial 5-HT_{2A} agonist (quipazine), high affinity 5-HT_{2A} antagonists (as labeled), or other nonselective drugs for 30 min at 37°C, then fixed and viewed by immunofluorescent confocal laser microscopy. Results are expressed as the percent of total cellular immunofluorescence that was intracellular. *$p < 0.001$ vs. vehicle-treated control. Adapted from Willins et al. [106].

internalized by a peptide antagonist but not by a nonpeptide antagonist [78]. In this study, internalization only occurred to a significant extent, as determined by confocal microscopy, following 24 h of antagonist exposure [78]. More recently, the A_1 adenosine receptor was shown to undergo antagonist-induced desensitization and internalization [74], however, the mechanism by which this occurs remains to be determined.

As discussed earlier, many GPCRs have been shown to be internalized following receptor phosphorylation and arrestin-binding and that these events may be responsible for initiating internalization. However, 5-HT_{2A} antagonists do not activate 5-HT_{2A} receptors as measured by any index of receptor activity (PI hydrolysis or arachidonic acid release) and do not induce cellular shape change [6]. In fact, clozapine has been demonstrated to be a potent antagonist with negative intrinsic activity at 5-HT_{2A} receptors [24]. Thus, based on the large number of studies reviewed here finding that 5-HT_{2A} antagonists can induce internalization and down-regulation, the current models of GPCR regulation must not be complete. Supporting this hypothesis, Roettger et al. [83] found that some receptors can be internalized following antagonist interaction without subsequent receptor phosphorylation. Thus, it is unlikely that receptor phosphorylation is a universal signal for internalization. Additionally, we have shown that agonists and antagonists cause 5-HT_{2A} receptors to internalize via a dynamin-dependent but arrestin-independent manner based on their sensitivities to the dominant negative mutants of these proteins [6]. Thus, it is likely that arrestin- and phosphorylation-independent mechanisms are involved in the cellular trafficking of GPCRs, and in particular the 5-HT_{2A} receptor.

CONCLUSIONS

As is clear from the studies reviewed here, 5-HT_{2A} receptors are paradoxically regulated by endogenous and exogenous factors. Though two decades have passed since the first report of 5-HT_{2A} receptor down-regulation by antidepressants, we still have only an elementary understanding of the molecular and cellular processes involved in mediating this phenomenon. As is becoming clear, studies of the regulation of prototypical GPCRs, such as the β2-adrenergic receptor, only provide the barest of hints as to how 5-HT_{2A} and other receptors may be regulated. Thus, no superfamily-encompassing conclusions can be drawn from studies with the β2-adrenergic receptor and no signaling, regulatory, or trafficking pathway can be assumed to apply to every GPCR. This is not surprising as cells have many different co-expressed receptors, and thus have evolved ways to differentially regulate each receptor. This adds to the level of biological complexity we are only now beginning to understand. Thus, continued diligence and creativity in the utilization of molecular and cellular approaches will push us ever closer to unlocking the secrets of this fascinating and clinically relevant set of phenomena.

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