Serotonin Receptor Signaling and Hallucinogenic Drug Action

Charles D. Nichols and Elaine Sanders-Bush*

Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter found in a variety of organisms from the worm to vertebrates. This diverse presence indicates that the serotonergic system is evolutionarily an ancient one.

In lower organisms 5-HT mediates simple behaviors, for example, egg laying in C. elegans (Waggoner et al, 1998). In humans, 5-HT mediates complex behaviors ranging from sleep and appetite to mood and aggression. Abnormalities in the serotonergic system have been implicated in depression, anorexia and schizophrenia, among others.

All serotonin neurons within the brain of vertebrates are found in specific regions of the brain called the raphe nuclei. Fibers project from the raphe to almost every region of the brain including the cortex, hippocampus, cerebellum and midbrain (Figure 1).

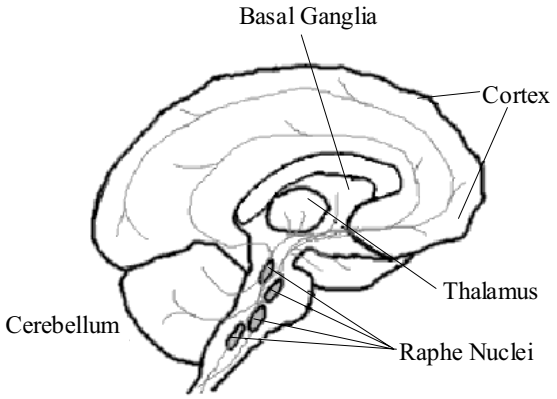

Figure 1. All serotonin within the brain originates from neurons whose cell bodies lie within the raphe nuclei. The axon fibers project to almost every structure in the brain and into the spinal cord.

While serotonin is nearly ubiquitous within the brain, the diversity and specificity of serotonin signaling and function arises from the molecules that receive the signals in the various target regions. These target molecules are proteins called receptors.

There are seven known families of serotonin receptors, named 5-HT_1 through 5-HT_7, encompassing at least 15 subtypes. The classification of receptors relies upon sequence similarity as well as second messenger pathways coupled to receptor activation (reviewed in Gerhardt and Heerikhuizen, 1997). Hallucinogens all have a high affinity for certain serotonin receptor subtypes, namely, the 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Glennon et al, 1984; Sanders-Bush et al, 1988). The relative hallucinogenic potencies of various drugs can be extrapolated by their affinities for these receptors (Glennon et al, 1984).

Some hallucinogenic drugs, such as DOI, bind exclusively to the 5-$HT_{2A/2C}$ receptors and have their behavior mediated primarily through the 5-HT_{2A} receptor pathway (Johnson et al, 1987; Krebs-Thomson et al, 1998). Other drugs, such as lysergic acid diethylamide (LSD), bind to a variety of receptors including the 5-$HT_{2A/2C}$, 5-HT_{1A}, 5-HT_6, 5-HT_7, dopamine D_1 and D_2 and adrenergic receptors and have aspects of behavior mediated through these multiple receptors (Teitler at al, 1988; Burris et al, 1991; Marona-Lewicka and Nichols, 1995; Watts et al, 1995; Giacomelli et al, 1998; Krebs-Thomson and Geyer, 1996; Krebs-Thomson et al, 1998). The core structures of many hallucinogens resemble the 5-HT molecule (Figure 2). This review will focus on the 5-HT_{1A} and the 5-HT_2 subtypes.

All 5-HT receptors, except the 5-HT_3 subtype, which is a ligand gated ion channel, belong to the large family of seven transmembrane spanning, guanine

* Department of Pharmacology and Center for Molecular Neuroscience
Vanderbilt University School of Medicine
Nashville, Tennessee

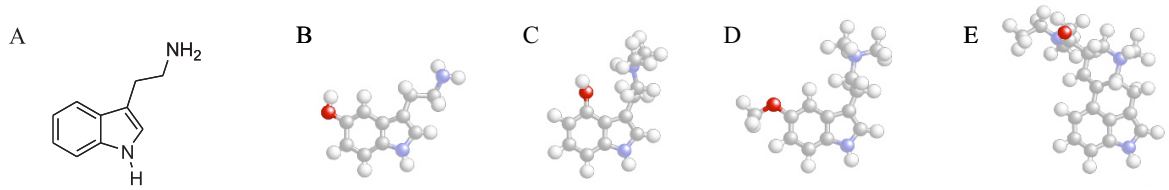

Figure 2. The structures of many hallucinogens are similar to serotonin and have a tryptamine core. A) The structure of tryptamine. B) Serotonin (5-hydroxytryptamine). C) Psilocin. D) 5-methoxy dimethyltryptamine. E) LSD.

nucleotide triphosphate (GTP)-binding protein (G-protein) coupled receptors. When ligand binds to these receptors, the associated heterotrimeric G-protein complex dissociates into α and βγ subunits. These subunits then activate downstream effector pathways until GTP hydrolysis occurs and the subunits reassociate with the receptor (Figure 3) (reviewed in Wess, 1997). There are multiple isoforms of α and βγ subunits, leading to a wide variety of possible combinations. Table 1 shows a summary for G-proteins associated with 5-HT receptors and the effector pathway regulated.

The 5-HT$_{2A}$ Receptor

5-HT$_{2A}$ receptor activation is necessary for the mechanism of action of hallucinogens. This receptor is expressed in regions of the brain believed to be involved in cognitive processes such as the prefrontal cortex, specifically in pyramidal neurons and interneurons. It is primarily coupled to G_q and activates various isoforms of phospholipase C (PLC) (Sanders-Bush et al, 1988; Apud et al, 1992).

PLC is a membrane bound enzyme that catalyzes the degradation of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-triphosphate (IP_3)

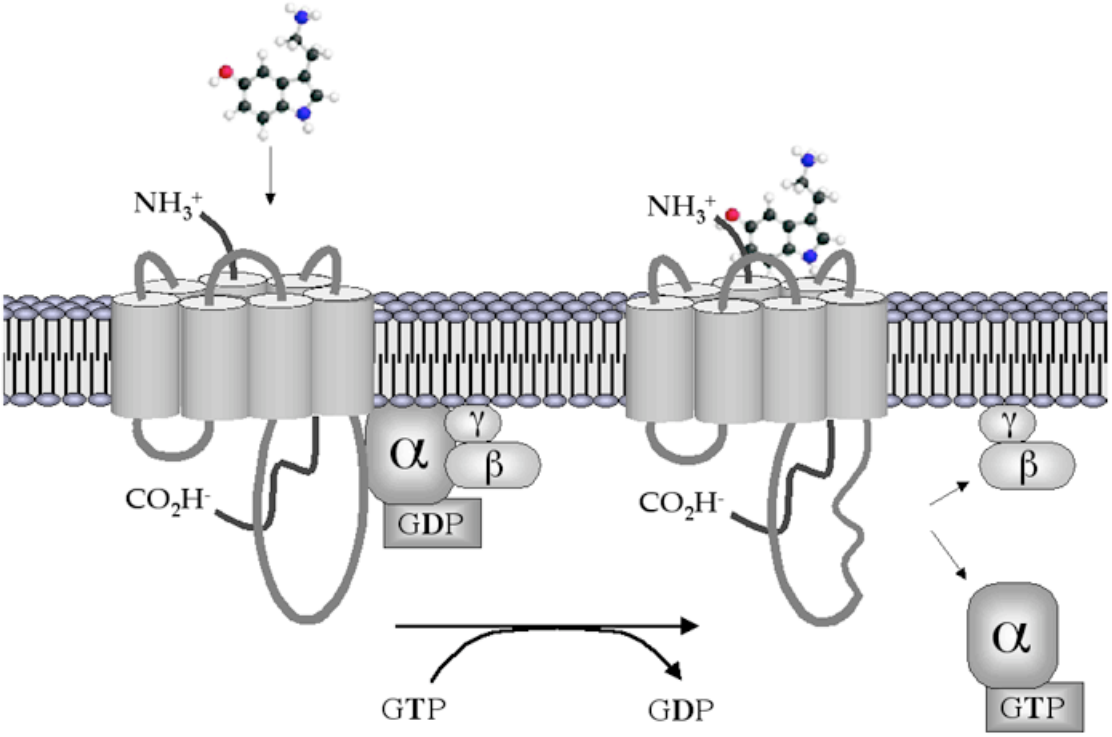

Figure 3. Heterotrimeric G protein complexes are made of an alpha, beta and gamma subunit that together associate with a seven transmembrane spanning receptor protein. In the inactive state, the alpha subunit is bound to GDP. Upon ligand binding, a change in conformation of the receptor is induced, coupling occurs, and the alpha and beta/gamma subunits are freed. GTP replaces GDP and the alpha subunit switches to the active conformation.

Receptor	G-protein	Effector
5-HT_{1A}	G_i/G_o	Inhibition of Adenylate Cyclase
5-HT_{1B}	G_i	"
5-HT_{1D}	G_i	"
5-HT_{1E}	G_i	"
5-HT_{2A}	G_q	Activation of Phospholipase Cβ
5-HT_{2B}	G_q	"
5-HT_{2C}	G_q	"
5-HT_3	-	(Ligand Gated Ion Channel)
5-HT_4	G_s	Activation of Adenylate Cyclase
5-HT_{5A}	G_i	Inhibition of Adenylate Cyclase
5-HT_{5B}	?	?
5-HT_6	G_s	Activation of Adenylate Cyclase
5-HT_7	G_s	Activation of Adenylate Cyclase

Table 1. This table lists subtypes of mammalian serotonin receptors, the G-proteins primarily coupled to them and the effector pathway activated. G_i inhibits adenylate cyclase, G_s activates adenylate cyclase, G_q activates phospholipase C. The 5-HT_3 receptor is an ion channel and is not known to couple to G-proteins.

and diacyglycerol (DAG). IP_3 mobilizes Ca^{2+} from intracellular stores, and Ca^{2+} goes on to activate calcium/calmodulin dependent kinases (Figure 4). The kinases are enzymes that phosphorylate other proteins that regulate cellular functions. The state of phosphorylation of a protein often reflects its activity. DAG can activate a different kinase, protein kinase C (PKC). In addition, DAG leads to the production of

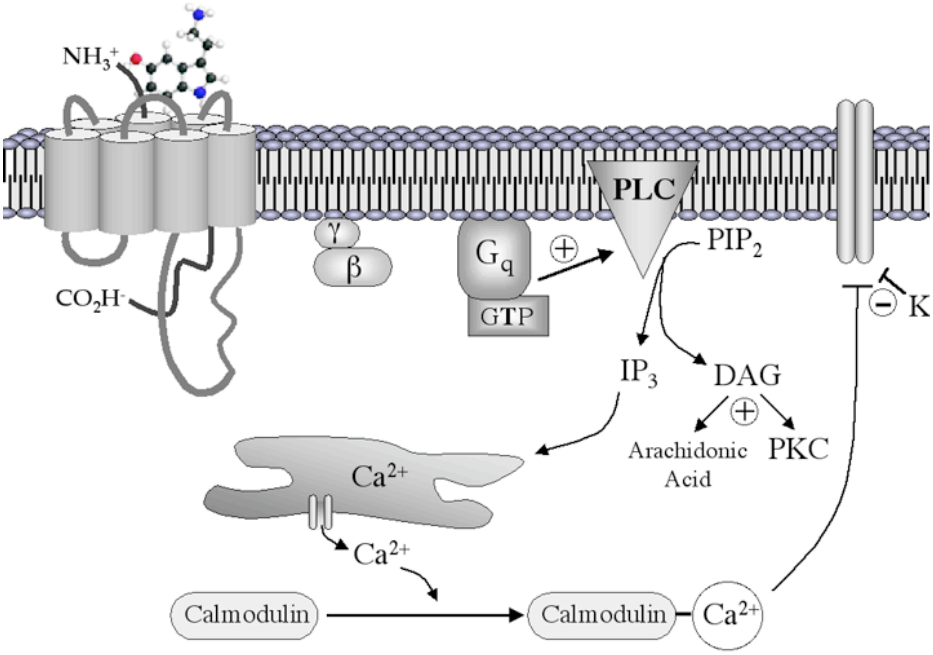

Figure 4. The 5-HT_2 receptor is coupled to G_q. Upon activation, G_q induces phospholipase C to hydrolyze PIP_2 to IP_3 and DAG. IP_3 leads to the release of calcium from intracellular stores while DAG leads to activation of PKC and the formation of arachidonic acid. A rise in intracellular calcium activates calmodulin, which closes potassium channels.

arachidonic acid, which in turn results in the formation of prostaglandins and prostacyclins that alter various cellular processes.

The 5-HT_{2A} receptor demonstrates multistate ligand binding (Branchek et al, 1990; Teitler at el. 1990). This means that there are two conformations of the receptor, each with a different affinity for ligand. These two states exist in equilibrium. The active, high affinity state activates effector pathways while the inactive, low affinity state does not. Agonists bind to the high affinity state, stabilize its conformation and increase effector activity. Another class of ligand called inverse agonists binds preferentially to the low affinity state and stabilizes it. This actually leads to a decrease in spontaneous activity. A third class of ligand called neutral antagonists can bind to either state, and as neutral antagonists, do not change the equilibrium state between the two conformations and do not alter effector activity.

The 5-HT_{2C} Receptor

The 5-HT_{2C} receptor is very similar to the 5-HT_{2A} receptor. It primarily couples to G_q and positively regulates PLC activity resulting in IP_3/DAG production (Sanders-Bush et al, 1988; Chang et al, 2000). 5-HT_{2C} receptor mRNA is expressed in a wider variety of brain areas than 5-HT_{2A} receptor mRNA. These include the cortex, thalamus, and hippocampus (Mengod et al, 1990a; Mengod et al, 1990b). It has been hypothesized that there are multistate affinities for this receptor, as there are for the 5-HT_{2A} receptor. These two receptor subtypes are so similar that it has been very difficult pharmacologically to distinguish between them. There are few antagonists that have preference for one over the other and no preferential agonists for the 5-HT_{2A} vs. the 5-HT_{2C} receptor have been developed. As is the case for the 5-HT_{2A} receptor, 5-HT_{2C} receptor ligands have been differentiated based on preferential affinities for active and inactive conformations of the receptor.

It was originally thought that there was only one effector pathway for each receptor subtype. As research progressed, another layer of complexity was added by showing that multiple effectors couple to a given receptor. Recently, yet another layer of complexity was added to serotonin receptor signaling. The 5-HT_{2C} receptor was discovered to undergo RNA editing of the primary transcript (Burns et al, 1997). Within the human gene there are five adenosine nucleotides that can separately be modified by deaminase enzymes to form an inosine within the region coding for the second intracellular loop. These events can lead to changes in the amino acid sequence of the protein for a total of 16 possible protein isoforms.

It has recently been demonstrated that these isoforms are spatially distributed within the brain and that there are functional differences between them (Niswender et al, 1998; Niswender et al, 1999). For

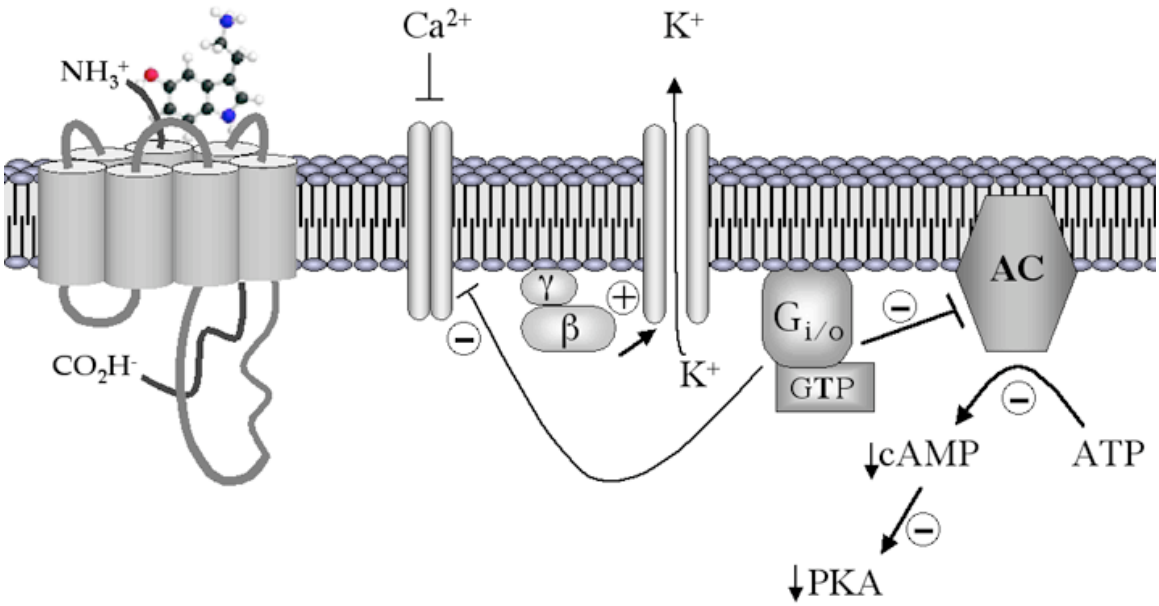

Figure 5. The 5-HT_{1A} receptor couples to $G_{i/o}$. Activated G_i leads to an inhibition of adenylate cyclase via the α_i subunit and opening of potassium channels via the $\beta\gamma$ subunit. Activated G_o leads to a closing of calcium channels via the a_o subunit.

example, LSD binds equally well to the unedited and fully edited isoforms. However, LSD acts as an agonist at the unedited isoform, but as an antagonist at the fully edited isoform. While these editing events have only been shown in the 5-HT_{2C} receptor transcript, they are likely to occur within other G-protein coupled receptors. Although the 5-HT_{2A} and the 5-HT_{2c} receptors are very similar, editing of the transcript has been shown not to occur in the 5-HT_{2A} receptor transcript within the region encoding for the second intracellular loop (Niswender et al, 1998).

The 5-HT_{1A} Receptor

The 5-HT_{1A} receptor is involved in the action of some hallucinogenic drugs. For example, some of the behavioral effects of LSD are mediated via the 5-HT_{1A} receptor (Krebs-Thomson et al, 1998). Another hallucinogenic drug, 5-methoxy-N,N-dimethyl-tryptamine, has recently been shown to produce a substantial portion of its stimulus cue in rats via the 5-HT_{1A} receptor (Winter et al, 2000). A high density of the 5-HT_{1A} receptor protein is localized presynaptically to the cell bodies of the serotonin neurons in the raphe nuclei. The cell body 5-HT_{1A} receptor functions as an autoreceptor, sensing the extracellular serotonin concentration and modulating the firing rate of the neurons of the raphe nuclei (Hamon et al, 1990).

When activated, 5-HT_{1A} autoreceptors inhibit firing and consequently inhibit subsequent release of serotonin from distal axon terminals. The 5-HT_{1A} receptor couples to $G_{i/o}$, with G_i activation leading to an inhibition of adenylate cyclase, an intracellular enzyme that catalyzes the formation of cyclic AMP (cAMP) from ATP (reviewed in Fargin et al, 1991). The cAMP molecule is a second messenger that can influence a variety of cellular processes including kinases. In addition to inhibiting adenylate cyclase, activation of the receptor leads to closing of calcium channels via the G_o subunit and opening of potassium channels via the $\beta\gamma$ subunits (Figure 5).

The serotonin signaling system is very complex. 5-$HT_{2A/2C}$ receptors alone couple to multiple pathways. Each pathway in turn can activate multiple second messengers, such as IP_3 and DAG. Furthermore, these same receptors are edited at the level of mRNA to produce spatially restricted isoforms, each with a different activity. The almost limitless number of these signaling possibilities emphasizes the variety and complexities of behaviors influenced by serotonin.

Serotonin and Hallucinogens

As previously mentioned, hallucinogens require agonist activity at 5-$HT_{2A/(2C)}$ receptors as illustrated in animal models and very recently in humans (Vollenweider et al, 1998).

How do these drugs alter behavior? The effects are not simply the result of over-stimulation of the serotonin system because excess serotonin alone does not produce hallucinogenic behavior.

Instead, excess serotonin produces what is known as the 'serotonin syndrome,' which is mediated by the 5-HT_{1A} receptor on the cell bodies of neurons in the raphe nuclei (Sternbach, 1991). There are multiple mechanisms of action of hallucinogens that involve serotonin receptors. The first is at the receptor/effector level. It has been demonstrated that some hallucinogens, such as LSD, activate different signaling cascades than 5-HT. For example, at the 5-HT_{2C} receptor, 5-HT binding produces a strong phosphoinositide hydrolysis response, a rise in intracellular calcium levels and phosphorylation of the receptor itself. LSD produces robust phosphoinositide hydrolysis, however there is no concomitant rise in intracellular calcium and only limited phosphorylation of the receptor (Backstrom et al, 1999).

One possible conclusion from these results is that LSD and 5-HT are activating different sets of effectors via different signaling cascades. Perhaps the LSD molecule produces a slightly different conformation of receptor when it binds, thereby altering the effector coupling profile (Almaula et al, 1996). Another possibility is that the rate of binding and conformation change alters the signaling profile. The net result of these differentially activated effectors may be to alter the physiological properties of the neurons on which these receptors are located from what would normally occur with 5-HT. Recent work has in fact demonstrated changes in the electrophysiological properties of neurons in slices of brain treated with either 5-HT or a hallucinogen.

This brings us to the next question. How might the differential intracellular signaling properties of hallucinogens as opposed to serotonin alter neuronal signaling?

The following is an example of how hallucinogens may alter a specific cortical process. A high concentration of 5-HT_{2A} receptor protein is localized postsynaptically, specifically on the apical dendrites of pyramidal neurons in the cerebral cortex in primates (Jakab and Goldman-Rakic, 1998). Expression of the 5-HT_{2A} receptor is also seen within large and medium sized interneurons of the cortex. Stimulation of this cortical region with 5-HT results in an increase in spontaneous (non-evoked) glutamatergic excitatory postsynaptic currents (EPSCs) within the apical dendrites (Marek and Aghajanian, 1999; Aghajanian and Marek, 1999).

The function of serotonin in these cortical regions

may also be to modulate the synaptic signals coming into pyramidal neurons. Excitatory transmission in the cortex has two components, an early synchronous (linked temporally to an action potential) and a late asynchronous component. Aghajanian and co-workers have recently shown that in evoked EPSCs the late asynchronous component is enhanced by the hallucinogen DOI, but to a much lesser extent by serotonin. This late asynchronous component is mediated by a late, slow release of glutamate from the axon terminals innervating pyramidal neurons. The net effect of the presynaptic and postsynaptic actions of DOI appears to be a general enhancement of cortical pyramidal cell activity.

As these specific cortical regions are involved in higher order cognitive and perceptual processes, hyperactivity of these sites may be part of a common mechanism for drugs similar to DOI in producing hallucinogenic effects such as altered perception. A more direct interaction, in the case of LSD, would be that, in addition to interacting with postsynaptic 5-$HT_{2A/2C}$ receptors, the drug would directly inhibit firing of the serotonin neurons themselves via its actions at the raphe 5-HT_{1A} receptors. This would lead to alterations in the endogenous serotonin input to the already altered postsynaptic receptor signals.

The cortex is not the only area of the brain that is affected by hallucinogens. Serotonin receptors and projections from the raphe nuclei are present in many other brain regions. Ultimately, changes in effector pathways and neuronal signaling initiated by the binding of hallucinogens to serotonin receptors may lead to transient changes in gene expression within serotonergic target systems or other cortical systems influenced by the serotonergic system. These changes in gene expression may contribute to alterations in the firing properties of neurons. Recent studies from our laboratory have shown that LSD does indeed induce changes in gene expression within many regions of the brain (unpublished data), however the relation of these genes and their products to serotonin receptor signaling is thus far unknown and it remains to be seen if they are involved in the behavior elicited by LSD.

Conclusion

Hallucinogenic drugs interact with the serotonergic system at many levels, from changing intracellular signaling pathways to altering neuron firing patterns to altering gene expression. Due to the complexity of the serotonin system and its multiple behavioral roles, it is perhaps not surprising that hallucinogens can elicit their effects at exquisitely low doses. Given the number of cognitive processes and behaviors in which serotonin is involved, these drugs are in a unique position to be used as tools in understanding the serotonin system.

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