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Agonist-Directed Signaling of Serotonin 5-HT_{2C} Receptors: Differences Between Serotonin and Lysergic Acid Diethylamide (LSD)

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For more than 40 years the hallucinogen lysergic acid diethylamide (LSD) has been known to modify serotonin neurotransmission. With the advent of molecular and cellular techniques, we are beginning to understand the complexity of LSD's actions at the serotonin 5-HT_2 family of receptors. Here, we discuss evidence that signaling of LSD at 5-HT_{2C} receptors differs from the endogenous agonist serotonin. In addition, RNA editing of the 5-HT_{2C} receptor dramatically alters the ability of LSD to stimulate phosphatidylinositol signaling. These findings provide a unique opportunity to understand the mechanism(s) of partial agonism.
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The serotonin receptor superfamily is composed of 14 members to date which have recently been re-classified based on gene structure, amino acid sequence homology, and intracellular signaling cascades (Hoyer et al. 1994). All but one (5-HT_3) of the serotonin receptors couples to G proteins, producing second messengers that regulate cellular functions via phosphorylation/dephosphorylation of intracellular proteins. The five families of G-protein-coupled receptors (5-HT_1, 5-HT_2, 5-HT_4, 5-HT_5, and 5-HT_7) regulate the two major intracellular second messenger pathways, adenylate cyclase and phospholipase C (for review see Sanders-Bush and Canton 1995). The 5-HT_2 receptor family is composed of three subtypes (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) linked via the Gq/11 family of G proteins to activation of phospholipase C with the generation of two second messengers, IP_3 which releases intracellular stores of calcium, and diacylglycerol, which activates protein kinase C. Recently, it has become clear that the 5-HT_2 receptor family also has the ability to activate other intracellular signaling pathways. For example, the 5-HT_{2C} receptor has been shown to activate phospholipase A_2 (Berg et al. 1996), regulate adenylate cyclase (Lucaites et al. 1996) and increase cyclic GMP (Kaufman et al. 1995). Whether these various signaling pathways are parallel or converging is not yet known, nor is it known what are the relative contribution of these pathways to in vivo function of 5-HT_{2C} receptors in choroid plexus epithelia and neurons.

The ergoline hallucinogenic drug lysergic acid diethylamide (LSD) binds with high affinity to serotonin receptors. Indeed, the early finding that LSD blocks the action of serotonin in smooth muscle (Gaddum 1953; Wooley and Shaw 1954) fueled interest in brain serotonin. Although LSD has been linked to serotonin for

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more than 40 years, we still cannot explain why LSD has such potent and profound effects on perception, mood, and thought processes. Of the many subtypes of serotonin receptors, the action of LSD at the 5-HT_2 family of G-protein-coupled receptors has been most convincingly implicated in the behavioral effects of LSD and other hallucinogenic agents (Titeler et al. 1988). LSD behaves as a partial agonist at 5-HT_{2A} (Burris et al. 1991; Marek and Aghajanian 1996) and 5-HT_{2C} receptors (Sanders-Bush et al. 1988; Egan et al. 1998). Because of its partial agonist proterties, LSD has the potential to partially block the effect of serotonin. It is possible that this 5-$HT_{2A/2C}$ receptor partial agonist property explains the unique behavioral properties of LSD; however, other partial agonists at 5-$HT_{2A/2C}$ receptors, such as m-CPP and lisuride, do not produce LSD-like behavior in humans (Grotewiel et al. 1994; Titeler et al. 1988). Therefore, there must be something unique about LSD versus serotonin that is responsible for its potent hallucinogenic properties (Marek and Aghajanian 1998). This article highlights the differences between LSD and serotonin at the level of receptor signal transduction.

Signaling Properties of the 5-HT_{2C} Receptor when Activated by LSD versus Serotonin

To compare signaling properties of LSD with serotonin, we utilized an NIH 3T3 fibroblast cell line expressing the cloned rat 5-HT_{2C} receptor (3T3/2C cells). In these cells, LSD behaves as a fully efficacious agonist, eliciting a phosphoinositide hydrolysis response that is comparable to serotonin (Figure 1A) (for methodology, see Barker et al. 1994). Because receptor reserve in phosphoinositide hydrolysis could account for the similar responses, we examined phosphorylation of the 5-HT_{2C} receptor using an immunoblot assay that we developed with our antibodies (Backstrom et al. 1995; Backstrom and Sanders-Bush 1997). In this assay, unglycosylated 5-HT_{2C} receptors have a mass of 40 kDa, and after treatment of 3T3/2C cells with serotonin, an additional band appears with a mass of 41 kDa (Figure 1B). The bandshift could be blocked with antagonists in the presence of serotonin (Backstrom et al., unpublished observation), demonstrating a receptor-mediated event. Extensive studies, including [^{32}P]-incorporation experiments, have unequivocally shown that the 41-kDa band represents a phosphorylated form of the 5-HT_{2C} receptor (Backstrom et al., unpublished observation). At a concentration of 1 μM, LSD was unable to stimulate maximal phosphorylation of the 5-HT_{2C} receptor whereas the hallucinogenic amphetamines DOI and DOB as well as the anxiogenic agonist m-CPP showed equal efficacy relative to serotonin at promoting phosphorylation of the 5-HT_{2C} receptor (Backstrom et al., unpublished observation). To determine if phosphoinositide hydrolysis enhances serotonin-mediated receptor phosphorylation, 3T3/2C cells were pre-treated with the phospholipase C inhibitor U-73122 or the inactive analog U-73343 (Figure 1B). The phospholipase C inhibitor, but not the inactive analog, attenuated serotonin-mediated phosphorylation of the 5-HT_{2C} receptor, demonstrating a critical role for phosphoinositide hydrolysis. We also examined events downstream from phosphoinositide hydrolysis (Figure 2) including translocation of immunoreactive protein kinase C to the membrane fraction as an indirect measurement of protein kinase C activation and inositol (1,4,5)-trisphosphate (IP_3)-mediated release of intracellular calcium. Both LSD and serotonin promoted translocation of immunoreactive protein kinase C to the membrane fraction, but surprisingly, only serotonin caused detectable calcium release (Backstrom et al., unpublished observation). Furthermore, other partial agonists such as DOI and m-CPP promoted calcium release comparable to serotonin. These observations demonstrate that LSD differentially activates the two

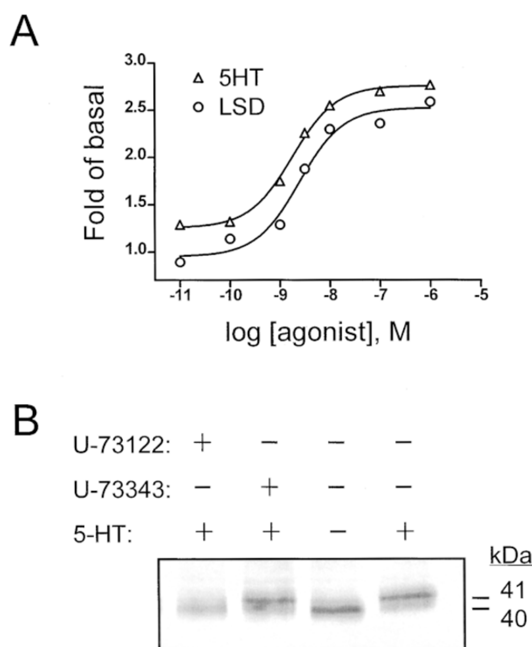

Figure 1. 5-HT_{2C} receptor-mediated responses measured by phosphoinositide hydrolysis and receptor phosphorylation. (A) Phosphoinositide hydrolysis dose-response curve for serotonin and LSD. NIH 3T3 cells stably expressing the rat unedited (INI) 5-HT_{2C} receptor (3000 fmol/mg protein; 3T3/2C) were labeled overnight with [^{3}H]-inositol. The ability of serotonin to increase [^{3}H]-inositol monophosphate was measured as described previously. LSD is a fully efficacious agonist with respect to phosphoinositide hydrolysis in this cell line. (B) The phospholipase C inhibitor U-73122 (15 μM, lane 1), but not the negative control analog U-73343 (15 μM, lane 2), attenuates serotonin-mediated phosphorylation of the 5-HT_{2C} receptor. The unphosphorylated and phosphorylated forms of the 5-HT_{2C} receptor have masses of 40 and 41 kDa, respectively.

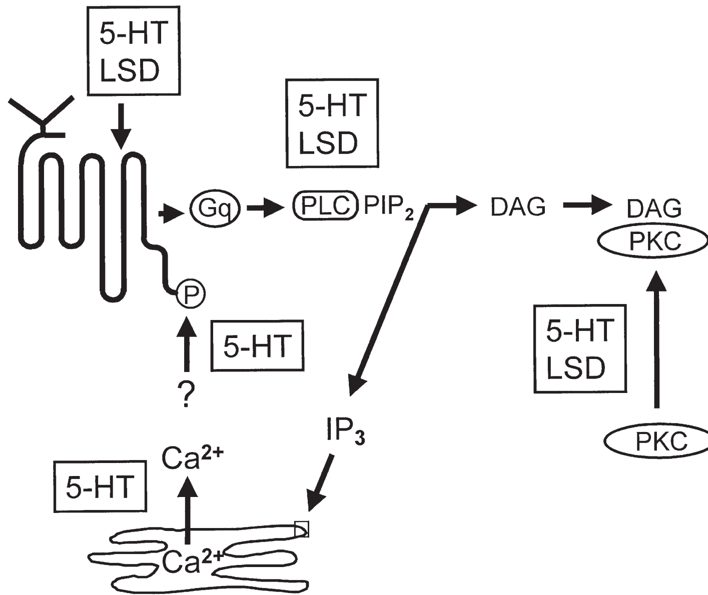

Figure 2. Schematic of 5-HT$_{2C}$ receptor signal transduction and the sites of action of serotonin (5-HT) and lysergic acid diethylamide (LSD). LSD and serotonin are equipotent at promoting phosphoinositide hydrolysis through phospholipase C (PLC). LSD and serotonin also cause comparable translocation of immunoreactive protein kinase C (PKC) to the membrane. Whereas serotonin stimulates mobilization of intracellular calcium, LSD-mediated calcium release was not detected. LSD-mediated phosphorylation of the 5-HT$_{2C}$ receptor was substantially lower than the serotonin signal.

arms of the phosphoinositide hydrolysis pathway, as illustrated in Figure 2. Whereas LSD is a fully efficacious agonist at phosphoinositide hydrolysis and translocation of protein kinase C to the membrane, LSD appears to be a partial agonist at calcium release and receptor phosphorylation. If the differential effects of LSD solely reflect its partial agonist properties, then it should promote phosphorylation at the same sites of the 5-HT$_{2C}$ receptor as does serotonin, but with less efficiency. Thus, we are currently determining which amino acids of the 5-HT$_{2C}$ receptor are necessary for agonist-mediated phosphorylation to test the hypothesis that serotonin promotes phosphorylation at sites in addition to those elicited by LSD. These results may provide molecular clues to some 5-HT$_{2C}$ receptor-mediated behavioral effects of LSD that are not observed with DOI (Krebs-Thomson et al. 1998).

Properties of LSD and Serotonin at 5-HT$_{2C}$ Receptor Isoforms Created by RNA Editing

Our laboratory published the first report showing that the 5-HT$_{2C}$ receptor is subject to post-transcriptional regulation by RNA editing (Burns et al. 1997). RNA editing, defined as an alteration in the coding potential of primary RNA transcripts by mechanisms other than RNA splicing, of a mammalian protein was discovered a decade ago. So far, RNA editing appears to have major functional consequences as illustrated by the profound alterations in the gating properties of the ligand-gated GluRB subunit of AMPA receptors (for review see Simpson and Emeson 1996). The 5-HT$_{2C}$ receptor, the first G-protein-coupled receptor found to be modified by RNA editing, is edited in rat brain at four principal sites yielding 11 RNA isoforms predicting six new 5-HT$_{2C}$ receptors isoforms (Niswender et al. 1998). The RNA isoforms are differentially expressed in brain regions, suggesting different roles for the protein isoforms in these brain regions. Editing at all four positions generates a receptor isoform, referred to as the 5-HT$_{2C-VSV}$ receptor, which has the amino acid sequence in the predicted second intracellular loop of the receptor changed

A. Rat

editing site:			**A B**		**C**		**D**		
	V	**A**	**I**	**R**	**N**	**P**	**I**	**E**	**H**
genomic:	GTA	GCA	ATA	CGT	AAT	CCT	ATT	GAG	CAT
cDNA:	GTA	GCA	GTG	CGT	AGT	CCT	GTT	GAG	CAT
	V	**A**	**V**	**R**	**S**	**P**	**V**	**E**	**H**

B. Human

editing site:			**A B**		**EC**		**D**		
	V	**A**	**I**	**R**	**N**	**P**	**I**	**E**	**H**
genomic:	GTA	GCA	ATA	CGT	AAT	CCT	ATT	GAG	CAT
cDNA:	GTA	GCA	GTG	CGT	GGT	CCT	GTT	GAG	CAT
	V	**A**	**V**	**R**	**G**	**P**	**V**	**E**	**H**

Figure 3. Principal RNA editing sites in the (A) rat and (B) human 5-HT$_{2C}$ receptors. Nucleotide and predicted amino acid sequence alignments between 5-HT$_{2C}$ receptor genomic and cDNA sequences. A to G nucleotide discrepancies and predicted alterations in amino acid sequence are indicated.

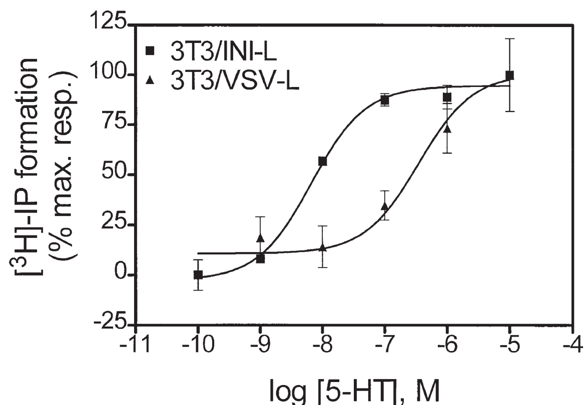

Figure 4. RNA editing of the rat 5-HT_{2C} receptor decreases the efficiency of activating phosphoinositide hydrolysis. Cell lines expressing either the 5-$HT_{2C\text{-}INI}$ receptor (60 fmol/mg protein; 3T3/INI-L) or 5-$HT_{2C\text{-}VSV}$ receptor (44 fmol/mg; 3T3/VSV-L) were labeled overnight with [^{3}H]-inositol. The data is expressed as percent of the maximal signal produced by 100 nM serotonin in each cell line. The average EC_{50} values (n = 3) were 7 ± 0.5 and 273 ± 47 nM for 3T3/INI-L and 3T3/VSV-L, respectively.

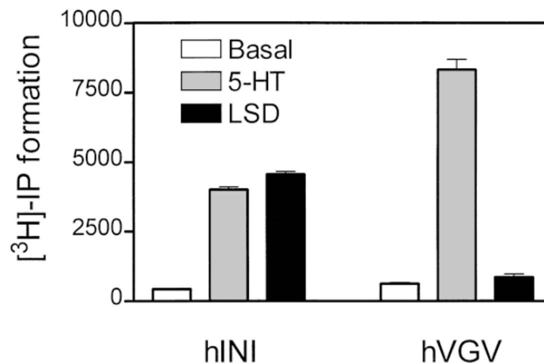

Figure 5. LSD has profoundly lower efficiency to activate phosphoinositide hydrolysis when interacting with the human fully edited isoform, 5-$HT_{2C\text{-}VGV}$ receptor. Cells expressing either the human 5-$HT_{2C\text{-}INI}$ receptor (250 fmol/mg protein; 3T3/hINI) or human 5-$HT_{2C\text{-}VGV}$ receptor (1200 fmol/mg protein; 3T3/hVGV) were labeled overnight with [^{3}H]-inositol. A maximum concentration (1 μM) of serotonin or LSD was added for 15 minutes and the formation of [^{3}H]-inositol monophosphate (IP) was measured. The bars show mean ± standard error for four independent determinations.

from ile^{157}-asn^{159}-ile^{161} (5-$HT_{2C\text{-}INI}$) to val^{157}-ser^{159}-val^{161} (5-$HT_{2C\text{-}VSV}$), as illustrated in Figure 3A. The rat 5-$HT_{2C\text{-}VSV}$ receptor isoform has reduced ability to signal through the principal signal transduction pathway, phospholipase C activation (Figure 4). Based on indirect evidence, we hypothesized that the 5-$HT_{2C\text{-}VSV}$ receptor isoform couples less efficiently to G-proteins and this explains its altered function (Burns et al. 1997).

We have recently found that the profile of receptor isoforms formed in human (h) brain differs from rat with the generation of a new isoform, h5-$HT_{2C\text{-}VGV}$ (Figure 3B) which also has reduced G-protein-coupling efficiency (Niswender et al. 1999). While studying the pharmacological properties of the isoforms expressed in clonal cell lines, we found a fascinating difference for LSD. In cells expressing the nonedited human isoform h5-$HT_{2C\text{-}INI}$ (hINI cells), LSD behaved as a partial or nearly full agonist as was found for the rat 5-$HT_{2C\text{-}INI}$ isoform, while at the fully edited human isoform h5-$HT_{2C\text{-}VGV}$ LSD had markedly attenuated ability to activate the phosphoinositide hydrolysis pathway compared to serotonin (Figure 5). If LSD is a partial agonist, then it would have greater efficacy in cells that express a high density of receptors due to the phenomena of receptor reserve. However, the receptor density of cells expressing the h5-$HT_{2C\text{-}INI}$ receptor is 5-fold lower than that of cells expressing the h5-$HT_{2C\text{-}VGV}$ receptor. Thus, the ability of LSD to elicit phosphoinositide hydrolysis is inversely related to receptor density, a finding that does not support the argument that functional differences between serotonin and LSD reflect partial agonist properties of LSD.

PERSPECTIVE

Signaling of LSD at the 5-HT_{2C} receptor differs from that of serotonin. First, although both agonists promote phosphoinositide hydrolysis and translocation of protein kinase C, LSD is unable to promote calcium release. Second, RNA editing of the 5-HT_{2C} receptor creates isoforms with differential sensitivities to LSD- and serotonin-mediated phosphoinositide signaling. If these observations were solely due to partial agonism, then LSD should behave as a partial agonist in other effector pathways. However, Berg et al. (1998) demonstrated that LSD is a more efficacious agonist than serotonin at activation of phopholipase A_2 whereas the opposite was found for activation of phospholipase C. Although differential activation of multiple G proteins by the two agonists could account for preferential effector activation (Berg et al. 1998), the addition of our findings of differences within the same pathway raise the possibility that the differences reflect other receptor–protein interactions. Possibilities include proteins that interact with defined protein modules such as the 5-HT_{2C} receptor PDZ binding motif. Because receptor phosphorylation has been shown to play a vital role in receptor–protein interactions (Pawson and Scott 1997; Krupnick and Benovic 1998), we hypothesize that the differences in receptor phosphorylation that we described earlier in this article may be a mechanistic key for explaining the differential actions of LSD and serotonin. Differential interactions between the 5-HT_{2C} receptor and co-activators or signal attentuators may in fact clarify the phenomenon of partial agonism, which after all is merely

an operational definition to explain different agonist efficiencies at activating a response.

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