Neurochemical Evidence That Hallucinogenic Drugs Are 5-HT_{1C} Receptor Agonists: What Next?

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INTRODUCTION

Behavioral, electrophysiological, and neurochemical evidence points to the serotonin$_2$(5-HT_2) receptor as an important site of action for hallucinogenic drugs (Glennon, this volume). Several years ago, the hypothesis was developed that hallucinogenic drugs might also be 5-HT_{1C} receptor agonists. This hypothesis was based on the significant sequence homology in the deduced amino acid structure of the 5-HT_2 and 5-HT_{1C} receptors, their pharmacological similarities, and the evidence that both receptors are linked to the same signal transduction pathway: phosphoinositide hydrolysis. In the past 4 years, the author and colleagues at Vanderbilt University have accumulated compelling evidence in support of this hypothesis.

A more profound question remaining unanswered is the relative roles of the 5-HT_2 and 5-HT_{1C} receptors in the various actions of these drugs, in particular, hallucinations. Studies of this question are just beginning and are based on three strategies: a neurochemical effect important to hallucinations should be a common property of all hallucinogenic drugs; close structural analogs of hallucinogenic drugs that do not elicit hallucinations should also not elicit the neurochemical response; and tolerance should develop to the behavioral effects of hallucinogenic drugs. Results of these studies are summarized in this chapter.

IS 5-HT_{1C} RECEPTOR ACTIVATION A COMMON PROPERTY OF HALLUCINOGENIC DRUGS?

In the choroid plexus, 5-HT_{1C} receptors are positively coupled to the phosphoinositide hydrolysis signaling cascade (Conn et al. 1986). The phosphoinositide hydrolysis response was therefore used to characterize the properties of hallucinogenic drugs at 5-HT_{1C} receptors. The first study examined (+) lysergic acid diethylamide (LSD), the most potent

hallucinogen known. (+)LSD potently activated phosphoinositide hydrolysis in the choroid plexus (median effective concentration [EC_{50}] = 10 nanomolars [nM]), although the maximum response was less than that produced by serotonin (figure 1). (+)LSD produced an incomplete blockade of the effect of serotonin, suggesting that it is a partial 5-HT_{1C} receptor agonist (Sanders-Bush et al. 1988).

Consistent with this interpretation, the 5-HT_2 and 5-HT_{1C} antagonists mianserin and ketanserin antagonized the agonist effect of LSD on phosphoinositide hydrolysis whereas spiperone, a selective 5-HT_2 antagonist, was without effect (figure 2). Since these original studies, a number of hallucinogens of different chemical classes have been examined, and all were found to act as agonists—some full, some partial—at 5-HT_{1C} receptors (Sanders-Bush and Breeding 1991). Comparison of the properties at 5-HT_{1C} and 5-HT_2 receptors shows that the hallucinogenic drugs are nearly equally effective at the two receptors.

The studies of Sanders-Bush and Breeding (1991) were the first to show that hallucinogenic drugs are 5-HT_{1C} receptor agonists. Because this property is common to all hallucinogens tested, this action may be important in the behavioral effects of these drugs. The challenge now is to formulate testable hypotheses, given the limitations of studies of hallucinogenic properties in animals. The most obvious and straightforward approach is to determine whether 5-HT_{1C} receptor antagonists block the behavioral effects of hallucinogenic drugs in animal models that are predictive of human activity (e.g., the drug discrimination [DD] paradigm). Such studies are not feasible, however, because there are no suitable antagonists (i.e., drugs that block 5-HT_{1C} but not 5-HT_2 receptors or vice versa). In vitro studies have relied on spiperone, which blocks 5-HT_2 but not 5-HT_{1C} receptors. However, behavioral studies of spiperone are not possible because of its potent neuroleptic action. Definitive behavioral studies await the development of specific 5-HT_2 and 5-HT_{1C} receptor antagonists.

WHAT ARE THE PROPERTIES OF CLOSELY RELATED STRUCTURAL ANALOGS OF LSD THAT ARE NOT HALLUCINOGENIC?

Recent studies have focused on three drugs: (-)LSD, 2-bromo LSD (BOL), and lisuride, all of which are close chemical relatives of (+)LSD

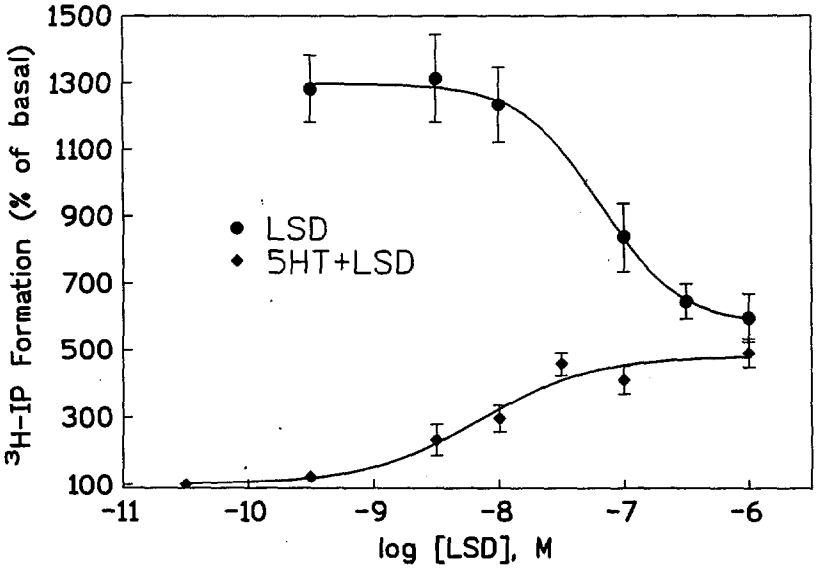

FIGURE 1. *Partial agonist effect of LSD at 5-HT$_{1C}$ receptors in rat choroid plexus. [^{3}H]-Inositol-labeled choroid plexi were incubated for 45 minutes with lithium chloride, pargyline, and increasing concentrations of (+)LSD with and without 100 nM serotonin. [^{3}H]-Inositol-monophosphate (IP) formation is expressed as percentage of the basal radioactivity. The circles represent a dose-response for LSD alone (n=4). The diamonds show the effect of 100 nM serotonin plus increasing concentrations of LSD alone (n=4). The maximum [^{3}H]-IP formation in the presence of 1 µM serotonin was 1363 ± 143% of basal. Mean basal [^{3}H]-IP formation was 729 ± 117 cpm.*

SOURCE: Sanders-Bush et al. 1988.

(figure 3) but apparently do not mimic its effects in humans (White 1986). The properties of these drugs at both 5-HT$_2$ and 5-HT$_{1C}$ receptors were examined. (-)LSD and BOL were pure antagonists of these receptors, with no evidence of agonist properties (Burris et al. 1991).

The effects of lisuride on the 5-HT$_2$ and 5-HT$_{1C}$ receptors are illustrated in figure 4. As shown, lisuride had no apparent 5-HT$_{1C}$ receptor agonist activity, and it completely blocked the response to 100 nM serotonin. In

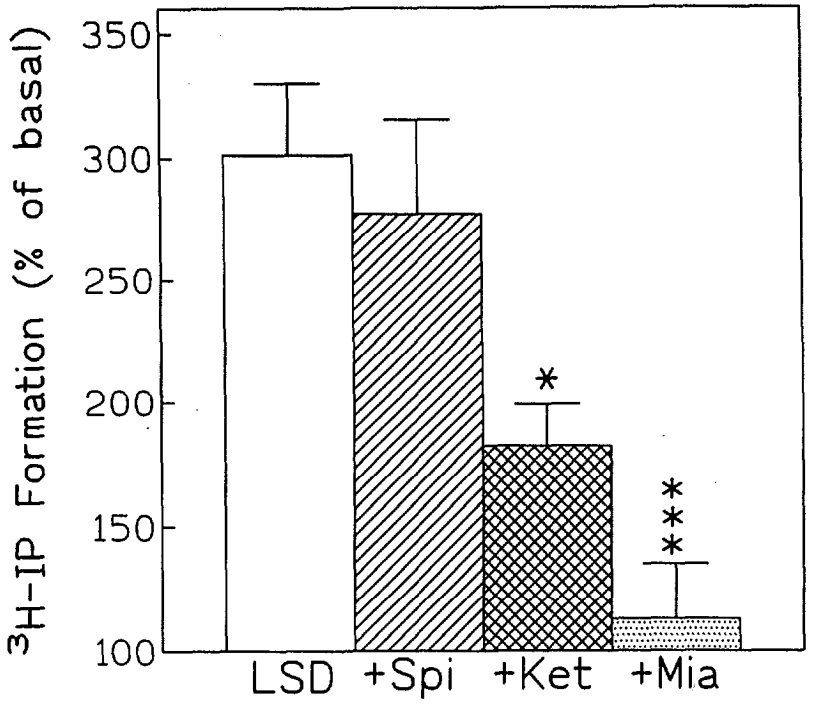

FIGURE 2. *Antagonism of (+)LSD-stimulated phosphoinositide hydrolysis in rut choroid plexus. Antagonists (1 µM) were added 15 minutes before the addition of 20 nM (+)LSD. Results are expressed as percent of basal, which was 2,751±219 cpm (n=21). Antagonists alone did not alter basal. *p<0.05, ***p<0.001. Spi = Spiperone; Ket = Ketunserin; Mia = Mianserin.*

contrast, lisuride was an agonist at the 5-HT_2 receptor, eliciting a strong phosphoinositide hydrolysis signal that was somewhat less than that of serotonin. Like LSD, lisuride partially blocked the effect of serotonin consistent with a partial agonist action at 5-HT_2 receptors. Therefore, lisuride, a nonhallucinogenic congener of LSD, appears to mimic LSD's agonist properties at the 5-HT_2 receptor. Lisuride does not, however, reproduce the effects of LSD at the 5-HT_{1C} receptor, consistent with the hypothesis that the 5-HT_{1C} receptor may be involved in the hallucinogenic action of LSD. On the other hand, the finding that 5-HT_2 receptor activation is a property shared by (+)LSD and lisuride is not consistent with a critical role for the 5-HT_2 receptor in the production of such au action.

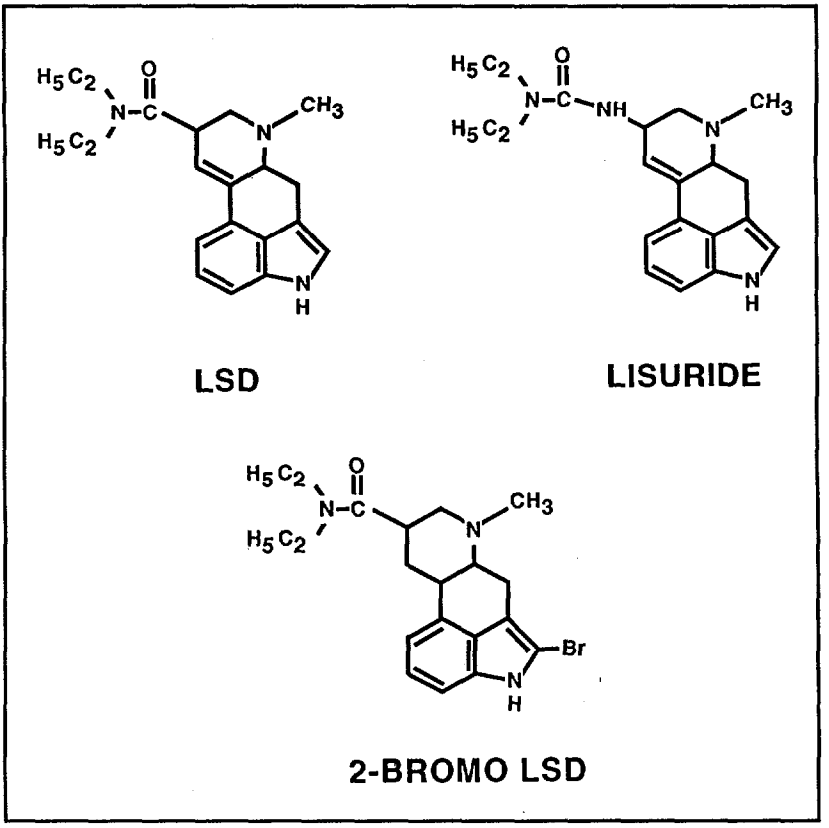

FIGURE 3. *Chemical structures of LSD, lisuride, and 2-bromo-lysergic acid diethylamide.*

DOES TOLERANCE DEVELOP TO ACTIVATION OF 5-HT $_{1C}$ RECEPTORS BY HALLUCINOGENS?

Early clinical studies showed that a profound and rapid tolerance occurred after the administration of LSD in humans (Abramson et al. 1956; Isbell et al. 1959). This tolerance has since been confirmed innumerous animal studies of hallucinogenic drugs (Adams and Geyer 1985; Carvey et al. 1989; Darmani et al. 1990; Leysen et al. 1989). One of the most common mechanisms of tolerance is a reduction in the density of receptors for the drug (i.e., down-regulation). Down-regulation of 5-HT_2 receptors is found in rat brain after chronic administration of LSD and hallucinogenic amphetamines (Buckholtz et al. 1990; Leysen et al. 1989; McKenna et al. 1989). This neurochemical

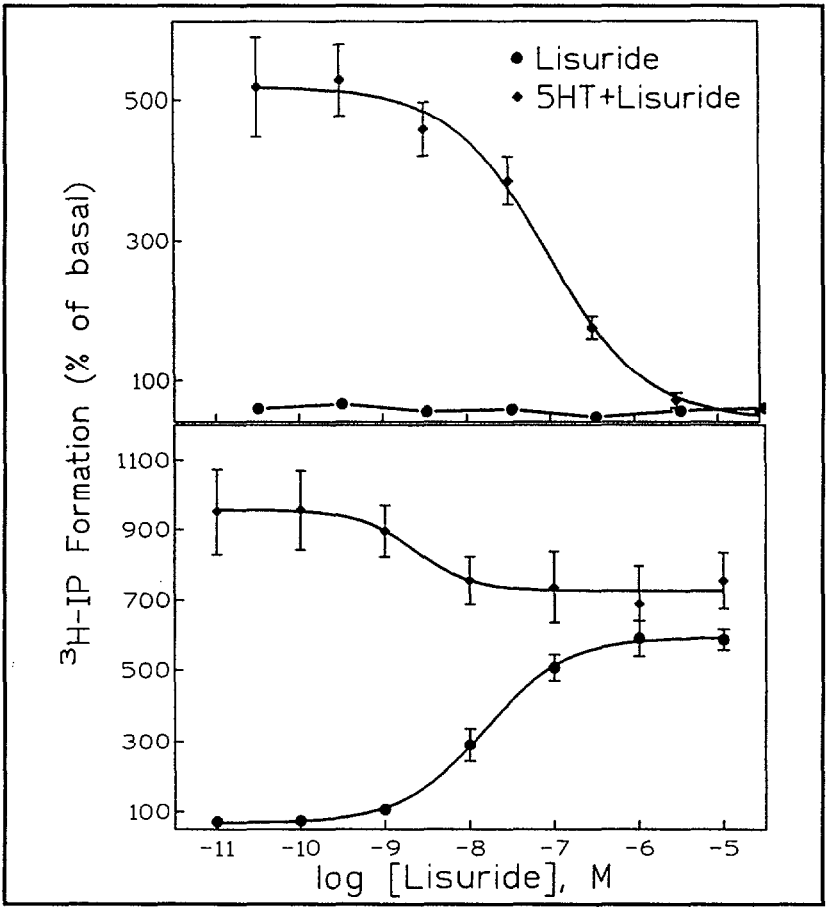

FIGURE 4. *Effect of lisuride at 5-HT_{1C} receptors in choroid plexus (upper panel) and 5-HT_2 receptors in transferred cells (lower panel). [^{3}H]-Inositol-labeled tissue was incubated for 45 minutes with lithium chloride, pargyline, and increasing concentrations of lisuride in presence and absence of 100 nM serotonin. [^{3}H]-Inositol-monophosphate (IP) formation is expressed as percentage of the basal radioactivity. The circles represent a dose-response for lisuride alone (n=6). The diamonds show the effect of 100 nM serotonin plus increasing concentrations of lisuride. Mean basal [^{3}H]-IP formation was 195±21 cpm in choroid plexus and 257±36 cpm infibroblasts transfected with 5-HT_2 receptor cDNA. Julius et al. 1990*

response may be involved in the tolerance that occurs to the behavioral effects of hallucinogens.

Recent studies have begun to explore the question of whether a down-regulation of 5-HT_{1C}, receptors is also produced after chronic treatment with hallucinogenic drugs. These studies have been done in choroid plexus epithelial cells that express the 5-HT_{1C} receptor. Chronic exposure to 1 micromolar (μM) of (-)1(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB) elicited a delayed decrease'in the density of 5-HT_{1C} receptors (Barker and Sanders-Bush 1993). This loss of receptors presumably will blunt the response to 5-HT_{1C}, receptor agonists such as hallucinogenic drugs; however, this effect has not yet been demonstrated. A blunted response would be consistent with the interpretation that 5-HT_{1C} receptors may be involved in the behavioral effects of hallucinogenic drugs.

WHAT IS DIFFERENT ABOUT 5-$HT_{2/1}$ AGONISTS THAT ARE HALLUCINOGENIC VERSUS THOSE THAT ARE NOT?

This author advocates the opinion that actions shared by lisuride and LSD, such as 5-HT_2 receptor activation, are probably not important in mediating hallucinations. This is not a new idea; for example, it was eloquently defended several years ago by White (1986). An alternative interpretation is that although LSD and lisuride, as well as other nonhallucinogenic 5-HT_2 agonists (e.g., quipazine), are capable of eliciting a receptor signal, they do so by different mechanisms (e.g., by interacting with different functional domains of the receptor). This possibility is especially interesting in light of the suggestion that LSD is an allosteric antagonist of the 5-HT_2 receptor (Kaumann 1989; Xu and Purdy 1989). Since the antagonist and agonist properties of a partial agonist such as LSD presumably reflect the same binding event, this may indicate that LSD has unique agonist properties (i.e., it may interact with a domain that is different from the serotonin binding domain). Conversely, lisuride and other nonhallucinogenic 5-HT_2/5-HT_{1C} agonists may not interact in an allosteric fashion.

Recent investigations have explored the putative allosteric interactions of LSD in cells transfected with the 5-HT_2 receptor (Burris and Sanders-Bush 1992). LSD produced a noncompetitive blockade of 5-HT-mediated phosphoinositide hydrolysis; high concentrations of serotonin were not able to overcome the LSD blockade. One possible

interpretation of these results is that LSD interacts at a site on the 5-HT_2 receptor that is different from the site where serotonin binds (i.e., an allosteric site). An alternative interpretation is that LSD binds tightly to the active site of the receptor in an irreversible or pseudoirreversible manner. To investigate the latter possibility, the dissociation rate of LSD was determined in membranes isolated from cells expressing the cloned 5-HT_2 receptor carrier deoxyribonucleic acid (cDNA).

In the initial studies of ^{3}H-LSD binding by Bennett and Snyder (1975), ^{3}H-LSD dissociated slowly, but since a whole brain was used and LSD is nonspecific, it is not clear which 5-HT receptor was involved. This is not a problem with fibroblasts transfected with the 5-HT_2 receptor cDNA, since only one 5-HT receptor subtype is expressed: the 5-HT_2 receptor (Julius et al. 1990). In membranes from these cells, ^{3}H-LSD dissociated slowly, with a half life (t l/2) of 20 minutes. Consistent with this slow off-rate, LSD caused an apparent loss of receptors in the usual ^{3}H-ketanserin binding assay, where the incubation time was 30 minutes. However, this receptor loss was not found when ^{3}H-ketanserin was incubated with the LSD-pretreated membranes for 60 minutes (Burris and Sanders-Bush 1992). Therefore, it is likely that the apparent blunting of the phosphoinositide hydrolysis response, as well as the blunting of serotonin-elicited contractions in smooth muscle (Kaumann 1989; Xu and Purdy 1989), are explained by the fact that LSD dissociates slowly from the 5-HT_2 receptor. With short incubation times, equilibrium is not reached.

These data suggest that LSD is not an allosteric agonist/antagonist, but rather that it binds tightly to the 5-HT_2 receptor and dissociates very slowly. This property of slow dissociation could be important. It would be interesting to compare the rates of dissociation of hallucinogenic versus nonhallucinogenic agonists from 5-HT_2 and 5-HT_{1C} receptors.

CONCLUSION

Hallucinogens of different chemical classes activate the 5-HT_{1C} receptor whereas nonhallucinogenic congeners interact with the receptor as antagonists. The numerous behavioral studies of hallucinogens that utilize 5-HT_2 receptor antagonists should be reevaluated in light of this finding, especially because currently available 5-HT_2 receptor antagonists also block 5-HT_{1C} receptors (Sanders-Bush and Breeding 1988). These neurochemical studies are consistent with a role for the 5-HT_{1C} receptor

in the psychoactive effects of hallucinogenic drugs, but they are far from conclusive. In the future new pharmacological tools that discriminate between 5-HT_2 and 5-HT_{1C} receptor subtypes may become available and can be used to explore this possibility in greater detail.

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