0026-895X/92/050826-05$03.00/0

MOLECULAR PHARMACOLOGY, 42:826-830

Unsurmountable Antagonism of Brain 5-Hydroxytryptamine$_2$ Receptors by (+)-Lysergic Acid Diethylamide and Bromo-lysergic Acid Diethylamide

KEVIN D. BURRIS[1] and ELAINE SANDERS-BUSH

Department of Pharmacology, School of Medicine, Vanderbilt University, Nashville, Tennessee 37232-6600

Received January 7, 1992; Accepted August 31, 1992

SUMMARY

Lysergic acid diethylamide (LSD) and its structural analogue 2-bromo-lysergic acid diethylamide (BOL) act as unsurmountable antagonists of serotonin-elicited contractions in smooth muscle preparations. Two different models, allosteric and kinetic, have been invoked to explain these findings. The present studies investigate the mechanism of antagonism of brain 5-hydroxytryptamine (5HT)$_2$ receptors, utilizing cells transfected with 5HT$_2$ receptor cDNA cloned from rat brain. A proximal cellular response, phosphoinositide hydrolysis, was examined in order to minimize possible postreceptor effects. Even though LSD behaved as a partial agonist and BOL as a pure antagonist, both drugs blocked the effect of serotonin in an unsurmountable manner, i.e., increasing concentrations of serotonin could not overcome the blocking effect of LSD or BOL. Radioligand binding studies showed that preincubation of membranes with either LSD or BOL reduced the density of [^{3}H]ketanserin binding sites, suggesting that the drugs bind tightly to the 5HT$_2$ receptor and are not displaced during the binding assay. Two additional experiments supported this hypothesis. First, the off-rate of [^{3}H]LSD was slow (20 min), relative to that of [^{3}H]ketanserin (≈4 min). Second, when the length of incubation with [^{3}H]ketanserin was increased to 60 min, the LSD-induced decrease in B_{max} was essentially eliminated. The possibility that LSD and BOL decrease [^{3}H]ketanserin binding by interacting with an allosteric site was rejected, because neither drug altered the rate of dissociation of [^{3}H]ketanserin. The most parsimonious interpretation of these results is that unsurmountable antagonism reflects prolonged occupancy of the receptor by slowly reversible antagonists.

LSD and its 2-bromo derivative, BOL, unsurmountably block peripheral 5HT$_2$ receptor-mediated effects, such as contraction of calf coronary artery (1) and rabbit thoracic artery (2) and phosphatidic acid formation in blood platelets (3). An allosteric model, originally proposed by Kaumann and Frenken (4), has been invoked to explain the unsurmountable blockade. This model proposes interconverting high and low activity states of the 5HT$_2$ receptor; unsurmountable antagonists bind at an allosteric site and drive the equilibrium toward a low activity state. Alternatively, a kinetic model has been proposed by Bond *et al.* (5), in which slowly reversible binding of the antagonist is responsible for unsurmountable blockade. The most direct way to test these models involves radioligand binding studies, which have not been possible in these peripheral systems.

LSD is a partial agonist and the nonhallucinogenic compound BOL is an antagonist at brain 5HT$_2$ receptors coupled to the phosphoinositide hydrolysis signaling pathway (6, 7). Unsurmountable antagonism of brain 5HT$_2$ receptors by these drugs has not been investigated. Functional studies of brain 5HT$_2$ receptors have been hampered by a low phosphoinositide hydrolysis response and the absence of other suitable model systems. Cell lines expressing a high density of the cloned 5HT$_2$ receptor from rat brain have been developed recently (8). 5HT$_2$ receptors in these cells are coupled to the hydrolysis of membrane phosphoinositides, and this response has been used to define the actions of drugs at brain receptors. Cell culture systems have many advantages over intact tissues, including a homogeneous population of cells expressing the receptor and greater control of the microenvironment. Additionally, the ability to analyze an event proximal to receptor activation minimizes artifacts produced by interaction with nonreceptor components of the response. The present study demonstrates that LSD and BOL block brain 5HT$_2$ receptors in an unsurmountable manner. Radioligand binding experiments were undertaken to address an allosteric versus a kinetic model. The results of these studies are consistent with a kinetic model of unsurmountable antagonism.

This work was supported by a research grant from the Alcohol, Drug Abuse, and Mental Health Administration (DA05181).
[1] Present address: Department of Pharmacology, University of Pennsylvania, 36th Street and Hamilton Walk, Philadelphia, PA 19104-6084.

ABBREVIATIONS: LSD, lysergic acid diethylamide; BOL, 2-bromo-lysergic acid diethylamide; 5HT, 5-hydroxytryptamine; IP, inositol monophosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Materials and Methods

Cell cultures. NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA (8) were grown in 11-mm wells (for phosphoinositide hydrolysis) or 100-mm plates (for radioligand binding) containing Dulbecco's modified Eagle medium with 10% bovine calf serum and 200 μg/ml levels of the neomycin analogue G-418 sulfate. Cells were maintained in an humidified incubator in an atmosphere of 8% CO_2/92% air at 37°.

Phosphoinositide hydrolysis. When the cells were at or near confluency, the growth medium was replaced with CMRL 1066 medium, which contains a low concentration of inositol. Agonist-induced phosphoinositide hydrolysis was determined by a modification of the method of Berridge *et al.* (9). The cells were incubated for 18–24 hr with 0.25 μCi of *myo*-[^{3}H]inositol. Lithium chloride and pargyline were added to give final concentrations of 10 mM and 10 μM, respectively; 15 min later, serotonin was added. The reaction was stopped after 30 min by aspiration of the medium and addition of 25 μl of methanol to each well. The plates were air dried and 0.5 ml of buffer (20 mM NaCl, 5 mM Na_2EDTA, 10 mM Tris, pH 7.4) containing 2.5% trypsin was added to each well. After standing for 10 min the mixture was sonicated. [^{3}H]IP formation was determined according to the method of Conn and Sanders-Bush (10). A 0.3-ml aliquot was transferred to an assay tube containing 0.9 ml of chloroform/methanol (1:2). After extraction, an aliquot of the aqueous phase, containing the inositol phosphates, was transferred to a Dowex-1 anion exchange resin column. Inositol was eluted and discarded. [^{3}H]IP was eluted with ammonium formate/formic acid directly into counting vials. Aquasol (DuPont NEN Products) was added and radioactivity was determined by liquid scintillation counting. Control experiments showed that serotonin elicited a linear increase in [^{3}H]IP accumulation for at least 45 min.

Radioligand binding. Crude membranes were prepared by a modification of the method of Ivins and Molinoff (11). Confluent cells were washed with ice-cold phosphate-buffered saline and then incubated for 10 min with ice-cold 5 mM HEPES buffer, pH 7.5, containing 5 mM EDTA. Cells were scraped off the plate and the mixture was centrifuged for 30 min at 20,000 × *g*. The supernatant was discarded and the pellet was homogenized in 20 mM HEPES buffer, pH 7.5, containing 154 mM NaCl and 10 mM EDTA, using a Brinkmann Polytron microprobe. The homogenate was centrifuged for 30 min at 20,000 × *g*. The final pellet was resuspended in 0.05 M Tris buffer (pH 7.6).

Ten-milliliter aliquots of the tissue (about 100 μg of protein/ml) were incubated with BOL, (+)-LSD, mianserin, or water for 30 min in a shaking water bath at 37°. After centrifugation (20,000 × *g* for 30 min at 5°), the pellet was washed by resuspension in fresh ice-cold Tris buffer followed by centrifugation as before. This wash was repeated once and the pellet was resuspended to a final concentration ranging from 10 to 30 μg of protein/ml. All solutions were ice-cold and the samples were kept cold during all of the manipulations.

One-milliliter aliquots of the crude membrane preparation were incubated at 37° for 30 min (unless otherwise stated) with [^{3}H]ketanserin (61 Ci/mmol; NEN Products, Boston, MA) or with [^{3}H]LSD (60 Ci/mmol; NEN Products). In saturation experiments, the concentrations of [^{3}H]ketanserin ranged from 0.065 to 10 nM. In dissociation experiments, the concentration of [^{3}H]ketanserin or of [^{3}H]LSD was 1.0 nM. Filtration was performed using a Brandel cell harvester, with GF/C filters that had been previously soaked in 3% polyethylenimine, pH 9.5. Nonspecific binding was defined by 10 μM methysergide. Protein concentrations were determined using the method of Bradford (12).

Results

Antagonism of $5HT_2$ receptors in cells transfected with rat brain $5HT_2$ receptor cDNA. Consistent with its action as a partial agonist at $5HT_2$ receptors (6, 7), (+)-LSD increased [^{3}H]IP formation in the presence of a low concentration of serotonin but blocked the maximum response to serotonin (Fig. 1). The blocking effect of (+)-LSD could not be reversed by higher concentrations of serotonin, suggesting that (+)-LSD behaved as an unsurmountable antagonist. Unsurmountable antagonism was also found with BOL. BOL blocked serotonin-mediated phosphoinositide hydrolysis, and this effect was not reversed by high concentrations of serotonin (Fig. 2).

Radioligand binding studies of unsurmountable antagonists. The possibility of slowly reversible binding of LSD and/or BOL to the $5HT_2$ receptor was examined using crude membrane preparations from cells transfected with rat brain $5HT_2$ receptor cDNA. Membranes were incubated with either LSD or BOL for 30 min (the same pretreatment time used in the phosphoinositide hydrolysis assays) and washed; ^{3}H-ketanserin binding was then measured in order to determine the density of $5HT_2$ receptors. If the drug binds to the receptor in a readily reversible manner, then the wash should remove it. Even if some residual drug remains, this should not decrease the density of [^{3}H]ketanserin binding sites but, rather, increase the K_d value. Conversely, if LSD and/or BOL bind to the receptor in a slowly reversible or irreversible manner, then the wash may not remove the drug and the density of [^{3}H]ketanserin binding sites would be decreased. Pretreatment with 33

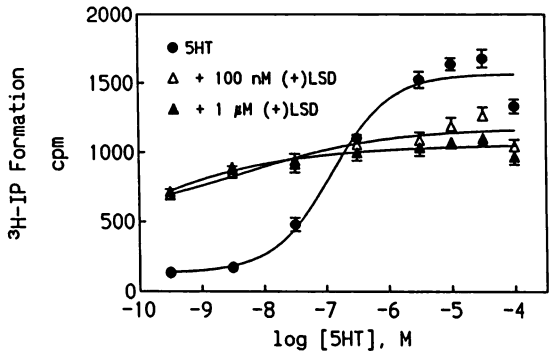

Fig. 1. Antagonism by (+)-LSD of serotonin-stimulated phosphoinositide hydrolysis in NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA. [^{3}H]Inositol-labeled cells were incubated with 10 mM LiCl and 10 μM pargyline for 15 min before the addition of increasing concentrations of serotonin (5HT). (+)-LSD was added 30 min before the addition of 5HT. Incubation with 5HT continued for 30 min and then the reaction was stopped. [^{3}H]IP was isolated by column chromatography as described in Materials and Methods and was quantitated by liquid scintillation counting. The values plotted are the mean of six determinations. *Vertical bars*, standard errors.

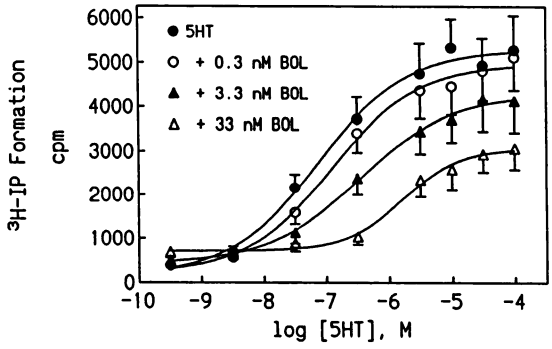

Fig. 2. Antagonism by BOL of serotonin-stimulated phosphoinositide hydrolysis in NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA. Conditions were the same as in Fig. 1. BOL was added 30 min before the addition of serotonin (5HT). The values plotted are the mean of six determinations.

nM BOL decreased the density but not the affinity of [^{3}H]ketanserin binding, indicating that BOL binds to the receptor in a slowly reversible or irreversible manner (Fig. 3). Similarly, pretreatment with 330 nM (+)-LSD resulted in a decrease in the density, but not the affinity, of [^{3}H]ketanserin (data not shown). On the other hand, pretreatment with mianserin, a $5HT_2$ receptor antagonist that dissociates rapidly from $5HT_2$ binding sites (13), did not decrease the density of [^{3}H]ketanserin binding sites (Fig. 3).

Three strategies were used to test the hypothesis that the reduction in binding site density by LSD and BOL reflects slowly reversible binding of these drugs. First, the dissociation of LSD was examined directly. Crude membranes from the transfected cells were incubated with [^{3}H]LSD for 30 min, to reach steady state binding, and then dissociation was initiated by addition of excess unlabeled LSD. [^{3}H]LSD dissociated with a $t_{1/2}$ of 20 min (Fig. 4), consistent with the original observations of Bennett and Snyder (14). The addition of 10 μM serotonin simultaneously with unlabeled LSD did not alter the dissociation rate of [^{3}H]LSD. Second, to determine whether the loss of [^{3}H]ketanserin sites was reversible, membranes were preincubated with LSD, washed, and incubated with [^{3}H]ketanserin

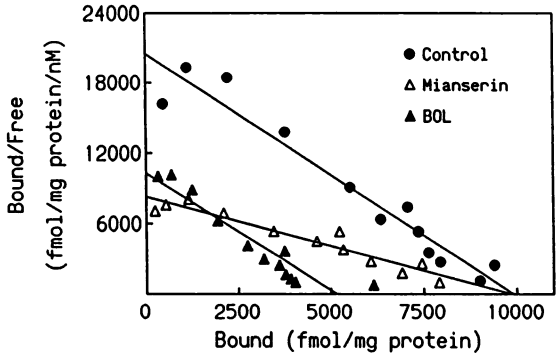

Fig. 3. Effect of antagonists on [^{3}H]ketanserin binding in crude membrane preparations of NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA. BOL (33 nM) or mianserin (330 nM) was incubated with crude membranes for 30 min and washed extensively before the addition of [^{3}H]ketanserin. The data are from a single experiment using 12 concentrations of [^{3}H]ketanserin, in duplicate, and are representative of three separate experiments. The mean values for these experiments were as follows: B_{max}, 63 ± 4% of control and 96 ± 2% of control for BOL and mianserin, respectively; K_d, 0.5 ± 0.03 nM and 0.8 ± 0.17 nM.

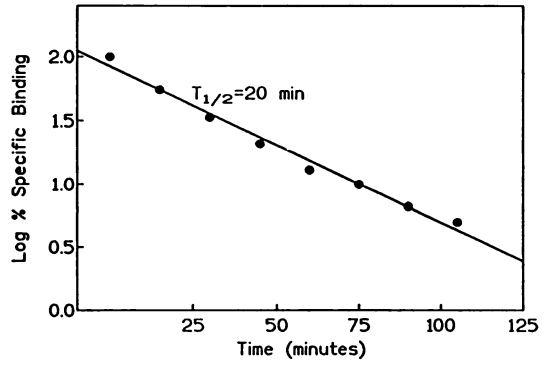

Fig. 4. Dissociation of [^{3}H]LSD from binding to the $5HT_2$ receptor. Crude membranes, prepared from fibroblasts transfected with the $5HT_2$ receptor cDNA, were incubated with 1 nM [^{3}H]LSD for 30 min to establish steady state binding. Nonlabeled LSD was added to a final concentration of 1 μM, in order to determine the rate of dissociation of the radioligand. The $t_{1/2}$ for dissociation of [^{3}H]LSD was 20 min.

for 20 or 60 min. Consistent with its slow off-rate, LSD decreased the density of sites by nearly 50% in the samples that were incubated with [^{3}H]ketanserin for 20 min; however, when this incubation was increased to 60 min the number of [^{3}H]ketanserin sites in control and LSD-treated membranes was not significantly different (Fig. 5). Third, in order to investigate the possibility that LSD or BOL binds to an allosteric site in a slowly reversible manner to decrease [^{3}H]ketanserin binding, the effect of the drugs on the dissociation of [^{3}H]ketanserin was examined. [^{3}H]Ketanserin was incubated with membranes at 37° for 30 min, to reach steady state binding, and then dissociation was initiated by the addition of an excess of nonlabeled ketanserin. To determine their effect on dissociation rate, LSD or BOL was added simultaneously with ketanserin. The half-life of dissociation of [^{3}H]ketanserin was 4–5 min, and neither BOL nor LSD altered the rate of dissociation (Fig. 6).

Discussion

Consistent with studies of peripheral $5HT_2$ receptors, the present results show that both (+)-LSD and BOL are unsurmountable antagonists of brain $5HT_2$ receptors. Much of the previous work on unsurmountable antagonism utilized smooth muscle contraction as the end point; however, antagonists may interact with components of the contractile machinery rather than the receptor itself to produce unsurmountable antagonism. The present studies show that a proximal response, phosphoinositide hydrolysis, was blocked in an unsurmountable manner by (+)-LSD and BOL.

The ability of LSD to block $5HT_2$ receptor-mediated contraction of calf coronary artery in an unsurmountable manner has been attributed to the presence of an allosteric binding site for LSD on the $5HT_2$ receptor (1). Similarly, BOL blockade of $5HT_2$ receptor-mediated contraction of rabbit thoracic artery has been attributed to the presence of an allosteric binding site (2). Kaumann and Frenken (4) proposed that the $5HT_2$ receptor exists in equilibrium between two interconvertible activity states, one with high activity and one with low activity. Binding of serotonin to its recognition site shifts the equilibrium to the high activity state. Conversely, drugs such as BOL and LSD that block the effects of serotonin in an unsurmountable manner are proposed to bind to an allosteric site, shifting the equilibrium to the low activity state. The most direct way to test for allosterism is to determine the kinetics of radioligand dissociation in the absence and presence of the putative allosteric antagonist. A supramaximal concentration of unlabeled ligand is added to occupy the primary site and, if the additional drug alters dissociation, it must do so by interacting with a different (allosteric) site. The radioligand binding studies in the present manuscript do not support an allosteric model, because neither BOL nor LSD altered [^{3}H]ketanserin dissociation. A more definitive study would be to determine the effects of LSD and BOL on the dissociation kinetics of [^{3}H]serotonin; however, the affinity of serotonin is so low that such studies are not feasible. The possibility remains, therefore, that ketanserin, BOL, and LSD interact with a domain of the $5HT_2$ receptor that is distinct from the serotonin recognition site.

Slowly reversible or irreversible binding of an antagonist to a receptor may also result in unsurmountable antagonism. By virtue of its slow dissociation rate, the antagonist limits the number of receptors accessible to agonist within the time period

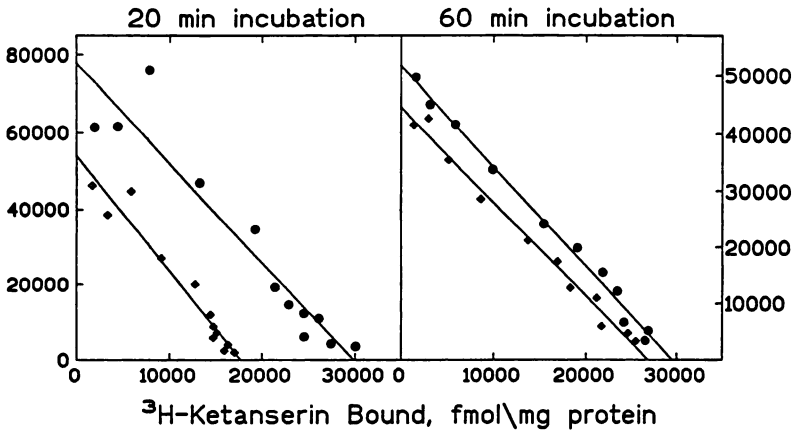

Fig. 5. Effect of (+)-LSD on [^{3}H]ketanserin binding in crude membrane preparations of NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA. Crude membranes were incubated with 330 nM (+)-LSD (♦) or no additions (●) for 30 min and were washed extensively before the addition of [^{3}H] ketanserin. The time of incubation with [^{3}H]ketanserin was either 20 min (*left*) or 60 min (*right*). The *y*-axis represents bound/free. The data are from a single experiment using 12 concentrations of [^{3}H]ketanserin, in duplicate, and are representative of three separate experiments. The mean B_{max} values in these experiments were 57 ± 1% of control and 97 ± 5% of control for the 20-min and 60-min incubations, respectively.

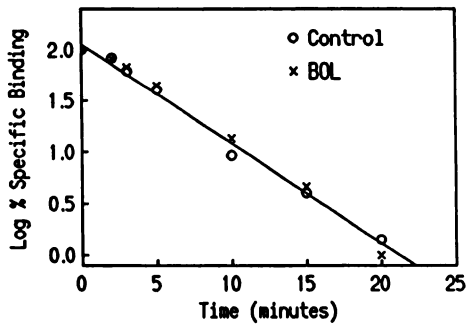

Fig. 6. Dissociation of specific [^{3}H]ketanserin binding to membrane preparations of NIH 3T3 fibroblasts transfected with rat brain $5HT_2$ receptor cDNA. The [^{3}H]ketanserin concentration was 1 nM. After incubation for 30 min to allow association of the [^{3}H]ketanserin, dissociation was initiated by addition of 100 μM ketanserin without or with 33 nM BOL. The samples were filtered at the indicated time. Specific binding was obtained as the difference between total binding and binding in the presence of 100 μM ketanserin. The values are the means of three experiments. The $t_{1/2}$ of [^{3}H]ketanserin was 4 min for both control and BOL-treated membranes. In a separate series of three experiments, LSD (330 nM) was added instead of BOL. The $t_{1/2}$ of [^{3}H]ketanserin in these studies was 5 min for both control and LSD-treated membranes.

for agonist stimulation. Paton and Rang (15) described a kinetic model known as "hemiequilibrium," referring to a condition in which "the agonist equilibrates with the available receptors and produces its measured response before any adjustment of antagonist occupancy has time to occur." For tissues with low receptor reserve, blockade of the agonist response by drugs with slow dissociation rates may appear unsurmountable. We found that (+)-LSD dissociates slowly from the cloned $5HT_2$ receptor binding sites prepared from transfected cells. Therefore, we hypothesized that the unsurmountable antagonism induced by (+)-LSD and its close structural analogue BOL may have resulted from the ability of these compounds to form high affinity, slowly reversible complexes with the $5HT_2$ receptor. This would result in a decrease in the $5HT_2$ receptor sites available for binding by serotonin and a concomitant decrease in the maximum phosphoinositide hydrolysis response to serotonin stimulation. Radioligand binding studies were consistent with this model. In crude membrane preparations from cells transfected with rat brain $5HT_2$ receptor cDNA, BOL and (+)-LSD decreased the density of [^{3}H]ketanserin binding sites without altering the affinity of the receptor for the radioligand. This decrease in maximum density was found when the incubation times (30 min or less) were comparable to those used in the functional assays. However, when the incubation was extended to 60 min, the effect of (+)-LSD on the density of sites was no longer evident. This finding is consistent with the slow dissociation rate of LSD. It was not possible to perform comparable temporal studies in the functional assay because the serotonin-induced phosphoinositide hydrolysis response desensitizes with longer incubations. The possibility remains that BOL and LSD were binding to an allosteric site in a slowly reversible manner to decrease the binding of [^{3}H]ketanserin. However, neither BOL nor (+)-LSD altered the dissociation of [^{3}H]ketanserin, arguing against such a mechanism. Thus, the most parsimonious interpretation of our data is that the apparent unsurmountable blockade of $5HT_2$ receptors by (+)-LSD and BOL is an artifact reflecting slow dissociation of these drugs and failure to establish equilibrium conditions.

Unsurmountable antagonism of $5HT_2$ receptors induced by (+)-LSD and BOL in other systems may also result from nonequilibrium conditions. In the studies of de Chaffoy de Courcelles *et al.* (3), $5HT_2$ receptor-mediated phosphatidic acid formation was examined in human platelets after pretreatment with antagonist for 20 min, followed by stimulation with agonist for 40 sec. Every $5HT_2$ receptor antagonist examined, including LSD, decreased serotonin-stimulated phosphatidic acid formation in an unsurmountable manner. Because drugs with either fast or slow receptor dissociation half-times decreased the maximum serotonin effect, the authors concluded that the unsurmountable blockade cannot be explained by a kinetic argument. However, given the short stimulation time with serotonin, it is unlikely that equilibrium is reached between serotonin and the antagonists, even those with relatively fast dissociation times. Unsurmountable antagonism of the effects of serotonin in calf coronary artery (1) and rabbit thoracic artery (2) after pretreatment with LSD or BOL may also result from hemiequilibrium conditions. In these preparations, attainment of equilibrium conditions with slowly reversible drugs is difficult to achieve due to multiple uncontrollable factors. It would seem, therefore, that functional studies of allosterism using slowly reversible antagonists will never be definitive.

In conclusion, (+)-LSD and BOL blocked $5HT_2$ receptor-mediated phosphoinositide hydrolysis in cultured cells in an unsurmountable manner. Radioligand binding studies showing a concomitant and slowly reversible decrease in $5HT_2$ binding sites were consistent with a kinetic rather than an allosteric model for the unsurmountable blockade. However, it is difficult to rule out the possibility that BOL and/or LSD interact in a

slowly reversible manner with a domain of the $5HT_2$ receptor distinct from the serotonin recognition site.

Acknowledgments

The authors wish to thank Dr. David Julius, University of California at San Francisco, for the gift of transfected cells and Ms. Ann Westphal and Ms. Antoinette Poindexter for expert technical assistance.

References

1. Kaumann, A. J. The allosteric $5HT_2$ receptor system, in *The Peripheral Actions of 5-Hydroxytryptamine* (J. R. Fozard, ed.). Oxford University Press, New York, 45–71 (1989).
2. Xu, Z., and R. E. Purdy. Evidence for allosteric blockade of serotonergic receptors in rabbit thoracic aorta. *J. Pharmacol. Exp. Ther.* **248**:1091–1095 (1989).
3. de Chaffoy de Courcelles, D., J. E. Leysen, P. Roevens, and H. Van Belle. The serotonin-S_2 receptor: a receptor-transducer coupling model to explain insurmountable antagonist effects. *Drug Dev. Res.* **8**:173–178 (1986).
4. Kaumann, A. J., and M. Frenken. A paradox: the $5HT_2$-receptor antagonist ketanserin restores the 5-HT-induced contraction depressed by methysergide in large coronary arteries of calf. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **328**:295–300 (1985).
5. Bond, R. A., A. G. Ornstein, and D. E. Clarke. Unsurmountable antagonism to 5-hydroxytryptamine in rat kidney results from pseudoirreversible inhibition rather than multiple receptors or allosteric receptor modulation. *J. Pharmacol. Exp. Ther.* **249**:401–410 (1989).
6. Sanders-Bush, E., K. D. Burris, and K. Knoth. Lysergic acid diethylamide and 2,5-dimethoxy-4-methylamphetamine are partial agonists at serotonin receptors linked to phosphoinositide hydrolysis. *J. Pharmacol. Exp. Ther.* **246**:924–928 (1988).
7. Burris, K. D., M. Breeding, and E. Sanders-Bush. (+)Lysergic acid diethylamide, but not its nonhallucinogenic congeners, is a potent serotonin $5HT_{1c}$ receptor agonist. *J. Pharmacol. Exp. Ther.* **258**:891–896 (1991).
8. Julius, D., K. N. Huang, T. J. Livelli, R. Axel, and T. M. Jessell. The $5HT_2$ receptor defines a family of structurally distinct but functionally conserved serotonin receptors. *Proc. Natl. Acad. Sci. USA* **87**:928–932 (1990).
9. Berridge, M. J., P. C. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**:587–595 (1982).
10. Conn, P. J., and E. Sanders-Bush. Serotonin-stimulated phosphoinositide turnover: mediation by the S_2 binding site in rat cerebral cortex but not in subcortical regions. *J. Pharmacol. Exp. Ther.* **234**:195–203 (1985).
11. Ivins, K. J., and P. B. Molinoff. Serotonin-2 receptors coupled to phosphoinositide hydrolysis in a clonal cell line. *Mol. Pharmacol.* **37**:622–630 (1990).
12. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
13. Peroutka, S. J., and S. H. Snyder. [^{3}H]-Mianserin: differential labeling of serotonin$_2$ and histamine$_1$ receptors in rat brain. *J. Pharmacol. Exp. Ther.* **216**:142–148 (1981).
14. Bennett, J. P., and S. H. Snyder. Stereospecific binding of D-lysergic acid diethylamide (LSD) to brain membranes: relationship to serotonin receptors. *Brain Res.* **94**:523–544 (1975).
15. Paton, W. D. M., and H. P. Rang. A kinetic approach to the mechanism of drug action. *Adv. Drug Res.* **3**:57–80 (1966).

Send reprint requests to: Elaine Sanders-Bush, Ph.D., Vanderbilt University School of Medicine, 432 Medical Research Building, Nashville, TN 37232-6600.