0026-895X/90/050622-09$02.00/0

MOLECULAR PHARMACOLOGY, 37:622-630



# Serotonin-2 Receptors Coupled to Phosphoinositide Hydrolysis in a Clonal Cell Line

KATHRYN J. IVINS and PERRY B. MOLINOFF

*Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084*

Received July 20, 1989; Accepted January 8, 1990

**SUMMARY**

A permanent line of cells has been established from the transplantable rat pituitary tumor 7315a. P11 cells have been cloned repeatedly, and after more than 60 passages their growth and characteristics are stable. Results of radioligand binding studies with $^{125}$I-lysergic acid diethylamide ($^{125}$I-LSD) indicate that P11 cells express serotonin-2 (5-HT$_2$) receptors. Analysis of the binding of $^{125}$I-LSD to membranes prepared from P11 cells revealed the presence of a single class of high affinity sites ($K_d$ = 1.6 nM; $B_{max}$ = 211 fmol/mg of protein). The pharmacological profile of the inhibition of the binding of $^{125}$I-LSD by a panel of drugs was consistent with the expected profile of these drugs at 5-HT$_2$ receptors. The affinity of the site for serotonin was in the low micromolar range and was decreased by GTP. Phosphoinositide hydrolysis in P11 cells, measured in the presence of lithium, was stimulated by serotonin. Increasing concentrations of the 5-HT$_2$-selective antagonist ketanserin blocked phosphoinositide hydrolysis stimulated by serotonin, and Schild analysis was consistent with a simple competitive interaction. The $K_i$ for ketanserin derived from Schild analysis was comparable to the $K_i$ for ketanserin at the binding site for $^{125}$I-LSD. These results suggest that stimulation of phosphoinositide hydrolysis in P11 cells by serotonin is mediated by 5-HT$_2$ receptors. Pretreatment of P11 cells with pertussis toxin caused ADP-ribosylation of $G_i$ and $G_o$, but did not affect the ability of serotonin to stimulate phosphoinositide hydrolysis. Therefore, the guaninine nucleotide-binding protein involved in the coupling of 5-HT$_2$ receptors to phospholipase C in P11 cells is unlikely to be either $G_i$ or $G_o$. P11 cells expressing 5-HT$_2$ receptors coupled to phosphoinositide hydrolysis will be a useful model system for future studies of the regulation and function of 5-HT$_2$ receptors on cultured cells.

Receptors for serotonin in the central nervous system were originally classified, on the basis of radioligand binding studies, into two subclasses, 5-HT$_1$ receptors with a nanomolar affinity for serotonin and 5-HT$_2$ receptors with a micromolar affinity for serotonin (1). A third major subclass of serotonin receptors, 5-HT$_3$ receptors, was identified and characterized in the periphery. More recently, these receptors have been localized in the central nervous system (2). On the basis of their pharmacology, at least four subtypes of 5-HT$_1$ receptors have been identified (3), and subtypes of 5-HT$_3$ receptors may also exist (4). The existence of subtypes of 5-HT$_2$ receptors has been postulated (5), but the possibility of multiple states of a single subtype of 5-HT$_2$ receptors has not been ruled out (6). 5-HT$_2$ receptors have a high affinity for antagonists including ketanserin, pipamperone, and mianserin. They can be labeled with [$^3$H]ketanserin and $^{125}$I-LSD (7, 8).

High densities of 5-HT$_2$ receptors are found in brain regions including the frontal cortex, mesolimbic areas, and striatum. Behavioral responses associated with activation of these receptors include head twitches in rats (9) and discriminative stimulus effects of serotonin agonists (10). Activation of 5-HT$_2$ receptors in the hypothalamus is associated with an increase in prolactin secretion from the anterior pituitary (11). 5-HT$_2$ receptors are also found on vascular smooth muscle, where they mediate contraction (12), and on platelets, where their activation is associated with platelet aggregation and increased calcium flux (13, 14). In platelets (15), blood vessels (16), and the cerebral cortex (17, 18), 5-HT$_2$ receptors are coupled to phosphoinositide hydrolysis.

The regulation of the density and function of 5-HT$_2$ receptors in the central nervous system has been investigated in studies involving drug treatments and lesions of neuronal pathways. Unlike the results of studies of the regulation of adrenergic receptors, the regulation of 5-HT$_2$ receptors cannot be explained by an adaptive response of receptors, in which chronic receptor blockade causes a compensatory up-regulation of re-

This work was supported by USPHS Grant (NS 18591) and a National Science Foundation fellowship to K.J.I.

**ABBREVIATIONS:** 5-HT, 5-hydroxytryptamine (serotonin); LSD, lysergic acid diethylamide; IAP, islet-activating protein (pertussis toxin); *m*CPP, 1-(3-chlorophenyl)piperazine; 8-OH-DPAT, (±)-8-hydroxy-dipropylaminotetralin HBr; DOI, (±)-2,5-dimethoxy-4-iodoamphetamine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle medium; PBS, phosphate-buffered saline; $B_{max}$, maximal number of binding sites; IBZM, (*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide; G protein, guanine nucleotide-binding protein; DOB, 4-bromo-2,5-dimethyoxphenylisopropylamine.

ceptors and chronic receptor activation causes a compensatory down-regulation of receptors. Instead, ligands that are potent antagonists at 5-HT$_2$ receptors, including ketanserin, mianserin, methysergide, and setoperone, cause a paradoxical decrease in the density of receptors that cannot be explained by the presence of residual drug in binding assays (19–22). Lesions of serotonergic neurons with 5,7-dihydroxytryptamine deplete endogenous serotonin but do not cause a compensatory increase in the density of 5-HT$_2$ receptors (23). Furthermore, fluoxitene and citalopram, selective inhibitors of serotonin uptake, do not cause a decrease in the density of receptors (24, 25).

Studies of the regulation of receptors are facilitated by the availability of cell lines expressing high densities of receptors. With the use of clonal cell lines, it is possible to study the regulation of receptors in homogeneous populations of cells under controlled conditions. However, no cell lines are available that are known to express high densities of 5-HT$_2$ receptors. Serotonin stimulates phosphoinositide hydrolysis in A7r5 smooth muscle cells (26), WRK1 mammary tumor cells (27), C6 glioma cells (28), and NRK normal rat kidney cells (29). The results of experiments using selective antagonists suggested that this stimulation was mediated by 5-HT$_2$ or 5-HT$_2$-like receptors. However, direct binding assays or a more extensive pharmacological analysis of phosphoinositide hydrolysis would be necessary to confirm that these cells express 5-HT$_2$ receptors.

We have used the prolactin-secreting transplantable rat pituitary tumor 7315a (30) as a tissue source to establish permanent cell lines. We report here that one of the cell lines isolated expresses a high density of 5-HT$_2$ receptors measured by the specific binding of $^{125}$I-LSD. Activation of 5-HT$_2$ receptors in these cells caused a marked increase in phosphoinositide hydrolysis that was insensitive to IAP.

## Experimental Procedures

**Materials.** Buffalo rats carrying the 7315a tumor were a gift from R. M. MacLeod (University of Virginia, Charlottesville, VA). $^{125}$I-LSD and [$^{32}$P]NAD were purchased from New England Nuclear. *myo*-[$^3$H]Inositol was purchased from American Radiochemicals. IAP was from List Biologicals. Ketanserin, spiroperidol, pipamperone, and domperidone were donated by Janssen Pharmaceutica. Prazosin was donated by Pfizer; haloperidol was donated by MacNeil Laboratories. Mianserin, *m*-CPP, quipazine, 8-OH-DPAT, and DOI were purchased from Research Biologicals. Other drugs were from Sigma.

**Tissue culture.** Clonal cell lines were established from the rat pituitary tumor 7315a maintained subcutaneously in female Buffalo rats. Animals carrying the tumor were sacrificed by decapitation. Tumors were dissected from adhering tissue and incubated at room temperature for 30 min in L15 medium (GIBCO) buffered with 15 mM HEPES (pH 7.4) and supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin (penicillin/streptomycin). The tissue was then minced with a razor blade and incubated for 15 min at 37° in L15 medium containing 15 mM HEPES (pH 7.4), 0.4% collagenase, 0.001% DNAse, 0.125% hyaluronidase, 1% bovine serum albumin, and penicillin/streptomycin. After centrifugation for 5 min at 180 × *g*, the tissue was resuspended in DMEM (GIBCO) supplemented with 10% fetal bovine serum (HyClone) and penicillin/streptomycin and was filtered through a Millipore Swinnex-25 filter to obtain a suspension of individual cells. Cells were centrifuged again for 5 min at 180 × *g*, resuspended in the same medium, plated onto protamine-coated (2.5 mg/ml) tissue culture plates, and grown in an atmosphere of 10% $CO_2$/90% air at 37°. After several days, cells were detached by exposure to 0.1% trypsin/0.02% EDTA in PBS (138 mM NaCl, 4 mM KCl, 5 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, 11 mM glucose, pH 7.4). Clonal lines of cells were established by colony selection. Cells were subcultured at low density onto uncoated tissue culture plates in medium A [DMEM supplemented with 10% charcoal-treated fetal bovine serum (31), 2 mM glutamine, 150 μg/ml oxaloacetate, 50 μg/ml pyruvate, 0.2 units/ml insulin, and penicillin/streptomycin]. Individual colonies of cells were isolated using cloning rings and expanded. One of the cell lines obtained in this way, P11, was selected for use in subsequent experiments because results of radioligand binding experiments suggested that P11 cells expressed a high density of $^{125}$I-LSD binding sites. The P11 cell line was recloned twice and used in the experiments described below.

P11 cells were grown in monolayer culture in medium A. Cells were detached from plates with 0.1% trypsin/0.02% EDTA in PBS and were subcultured at densities of 5000–20,000 cells/cm$^2$. The medium was changed every third day until cells were harvested or subcultured. Contrast-enhanced phase contrast photomicrographs of cells were produced using a Nikon Diaphot microscope equipped with a Dage Newvicon camera, an Image 1/AT digital image processor, and a Mitsubishi image printer.

**Radioligand binding.** To prepare crude membranes for binding assays, cells were washed with PBS, lysed by incubation with ice-cold hypotonic buffer (5 mM HEPES, 5 mM EDTA, pH 7.5) for 10 min, detached with a rubber policeman, and centrifuged for 10 min at 20,000 × *g*. The resulting pellet was resuspended using a Brinkmann Polytron (10 sec at speed 6) in buffer (20 mM HEPES, 154 mM NaCl, 10 mM EDTA, pH 7.5) and centrifuged for 10 min at 20,000 × *g*. The pellet obtained from this centrifugation was resuspended in 50 mM Tris buffer (pH 7.4) for use in binding assays.

Assays were initiated by the addition of 50 μl of tissue (10–40 μg of protein) to 50 μl of 50 mM Tris buffer (pH 7.4) containing $^{125}$I-LSD (0.1–6 nM) and appropriate competing ligands. In experiments using agonists, 1 mM $MgCl_2$ with or without 300 μM GTP was included in the reaction mixture. Specific binding was defined as binding that was inhibited by 1 μM ketanserin. Following incubation for 60 min at 37°, reactions were terminated by the addition of 5 ml of ice-cold 10 mM Tris buffer (pH 7.5). Samples were filtered through glass-fiber filters (No. 30; Schleicher and Schuell) coated with 3% polyethylenimine (pH 9.5). Filters were washed three times with 5 ml of the same buffer and radioactivity retained by the filters was determined. Saturation binding data were transformed by the method of Scatchard (32). $K_d$ and $B_{max}$ values were estimated by unweighted linear regression analysis. Competition binding data were analyzed using the NEWFITSITES program of the PROPHET software package available through the National Institutes of Health (33). Competition curves were modeled to one-site and two-site equations, and $IC_{50}$ values and Hill coefficients were obtained as described by Lin *et al.* (33). In most cases, data were best fit by a one-site model with a Hill coefficient not significantly different from 1. $IC_{50}$ values were then estimated after constraining the Hill coefficient to 1.0. Protein was determined by the method of Lowry *et al.* (34).

**Phosphoinositide hydrolysis.** Measurement of phosphoinositide hydrolysis in cultured cells was carried out essentially as described by Lee *et al.* (35). P11 cells were plated in enriched medium in six-well cluster dishes (Costar), at a density of 10,000 cells/cm$^2$. After 24 hr, the medium was replaced by medium containing *myo*-[$^3$H]inositol (3.5 μCi/well). Assays were carried out 2–3 days later, when cells approached confluency. Cells were washed twice with DMEM and then equilibrated in 2 ml of DMEM for 1 hr, in an atmosphere of 10% $CO_2$/90% air. LiCl was added in a volume of 250 μl, and 10 min later assays were started by the addition of 250 μl of drug. Unless otherwise stated, the final concentration of LiCl was 20 mM. Incubations were carried out for 40 min and were stopped by the addition of 250 μl of perchloric acid (final concentration, 4.5%). The soluble contents of each well were transferred into tubes and 0.5 M KOH/9 mM Na tetraborate was added to achieve a pH of 8–9. After centrifugation for 5 min at 10,000 × *g*, inositol phosphates in the supernatant were isolated by column chromatography. Briefly, supernatants were loaded onto 0.5 ml of Bio-Rad

AG 1-x8 resin (200–400 mesh, formate form) and, after being washed eight times with 5 ml of $H_2O$, inositol phosphates were eluted with 6 ml of 0.1 M formic acid containing 0.2 M ammonium formate. Radioactivity was determined with Ecolite scintillation fluid (ICN). Cell membranes remaining in each well were solubilized with 1 ml of 0.1 N NaOH and radioactivity was determined. Data were expressed as the percentage of [$^3$H]phospholipids converted to [$^3$H]inositol phosphates. In some experiments, individual inositol phosphates were chromatographically resolved, according to the method of Berridge *et al.* (36).

**Detection of pertussis toxin substrates.** Membranes from control cells and from cells treated with 200 ng/ml IAP were harvested as described for radioligand binding studies. Membranes (25 μg of protein) were incubated with 5 μM [$^{32}$P]NAD (3 μCi), 1 mM EDTA, 5 mM dithiothreitol, 10 mM thymidine, 200 μg/ml bovine serum albumin, 0.025% SDS, and 20 μg/ml activated IAP in 10 mM HEPES (pH 8) buffer, for 60 min at 30° (37). Incubations were stopped with sample buffer (10% glycerol, 1% β-mercaptoethanol, 2% sodium dodecyl sulfate, 0.01% bromophenol blue, and 65 mM Tris, pH 6.8) and samples were boiled for 5 min before analysis by one-dimensional polyacrylamide gel electrophoresis. [$^{32}$P]ADP-ribosylated proteins were detected by autoradiography, and the amount of radioactivity present was determined by densitometry.

## Results

Cells were cultured from collagenase-dispersed 7315a tumors, as described in Experimental Procedures. Several cell lines were isolated using cloning rings. One of these lines, P11, was subcloned twice and used in the experiments described below. P11 cells grow in monolayer culture on untreated plastic tissue culture plates. They attach tightly to plates, and little phase contrast is evident. At low densities, the cells have a round flat morphology (Fig. 1A). As they approach confluency, the cells appear elongated (Fig. 1B). Vacuoles are sometimes apparent in the cells. Under the culture conditions used, the doubling time of the cells was approximately 25 hr. P11 cells have been in culture for over 1 year and have been subcultured more than 60 times. Their characteristics have been stable over this period. Furthermore, the cells are viable after storage in liquid nitrogen for 6 months.

Radioligand binding studies were carried out to detect receptors for monoamines on P11 cells. The specific binding of $^{125}$I-LSD (0.1–6 nM) to membranes prepared from P11 cells was saturable when nonspecific binding was defined by the inclusion of 1 μM ketanserin (Fig. 2). Nonlinear regression analysis of untransformed saturation data indicated that the data were best fit by a one-site model. The site had a high affinity for $^{125}$I-LSD and was present at high density ($K_d$ = 1.6 ± 0.3 nM; $B_{max}$ = 211 ± 5 fmol/mg of protein; mean ± SE from five separate experiments). Scatchard transformation of the data resulted in a linear plot (Fig. 2, *inset*).

The binding site for $^{125}$I-LSD on P11 cell membranes was characterized in competition experiments. Inhibition of the binding of $^{125}$I-LSD by serotonin resulted in a biphasic competition curve best fit by a two-site model (Fig. 3). The high and low affinity components of binding were present in a ratio of approximately 1:1. In the presence of 300 μM GTP, the competition curve produced by serotonin was monophasic and best fit by a one-site model. A high affinity component of binding was not observed when assays were carried out in the presence of GTP (Fig. 3). Inhibition of the binding of $^{125}$I-LSD by other agonists at serotonin receptors was studied in the presence of 300 μM GTP (Fig. 4A). Under these conditions, the competition curves generated by all drugs studied, including

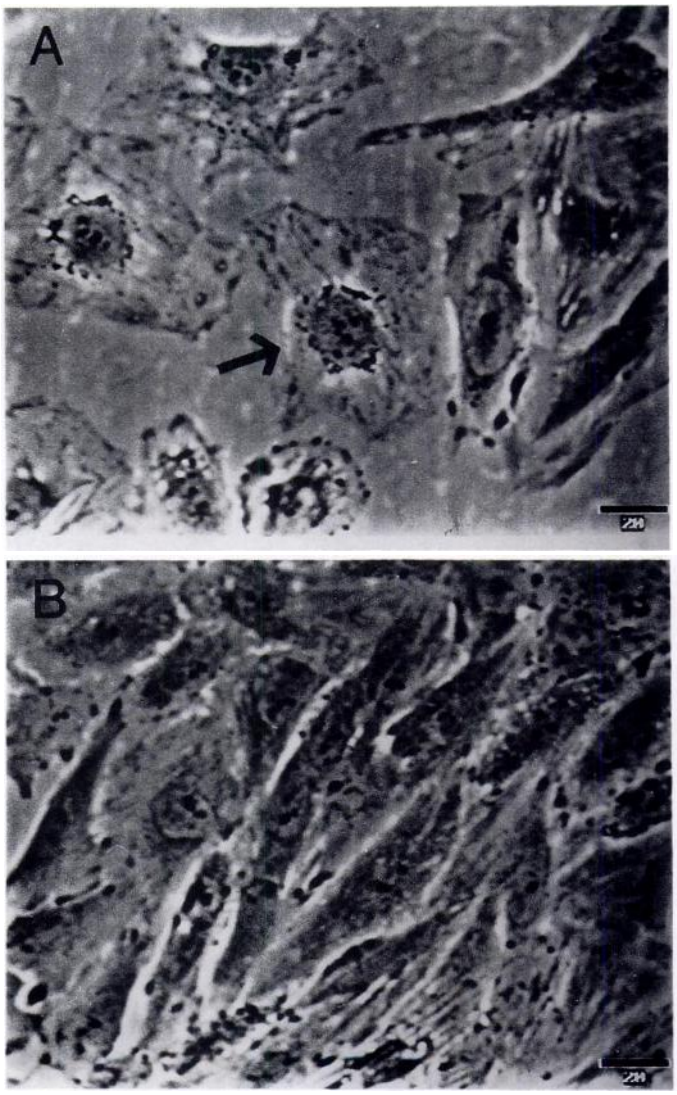


**Fig. 1.** Photomicrograph of P11 cells in monolayer culture. A, P11 cells were subcultured at a low density (approximately 3000 cells/cm$^2$) on an untreated tissue culture plate. The cells were allowed to attach and were grown for 2 days. Note the round, flat appearance of an individual cell (*arrow*). B, After 6 days, the cells approached confluency. *Calibration bar*, 20 μm.

*m*CPP, DOI, quipazine, and 8-OH-DPAT, were best fit by a one-site model. Dissociation constants for these drugs at the $^{125}$I-LSD binding site were calculated (Table 1).

Inhibition of the binding of $^{125}$I-LSD by 5-HT$_2$-selective antagonists including ketanserin, pipamperone, and mianserin resulted in monophasic competition curves best fit by a one-site model (Fig. 4B). Inhibition of the binding of $^{125}$I-LSD by haloperidol, an antagonist with a high affinity for dopamine D-2 receptors, was of lower potency. In some tissues, $^{125}$I-LSD labels $H_1$-histaminergic and $\alpha_1$-adrenergic receptors as well as the 5-HT$_2$ and 5-HT$_{1c}$ subtypes of serotonin receptors. Ligands selective for $H_1$-histaminergic and $\alpha_1$-adrenergic receptors, tripolidine and prazosin, respectively, inhibited the binding of $^{125}$I-LSD to membranes prepared from P11 cells only at micromolar concentrations. Furthermore, although 5-HT$_2$ and 5-HT$_{1c}$ receptors have a similar affinity for some antagonists, including mianserin, spiroperidol is 1000-fold more potent at 5-HT$_2$ receptors than at 5-HT$_{1c}$ receptors (38). Spiroperidol

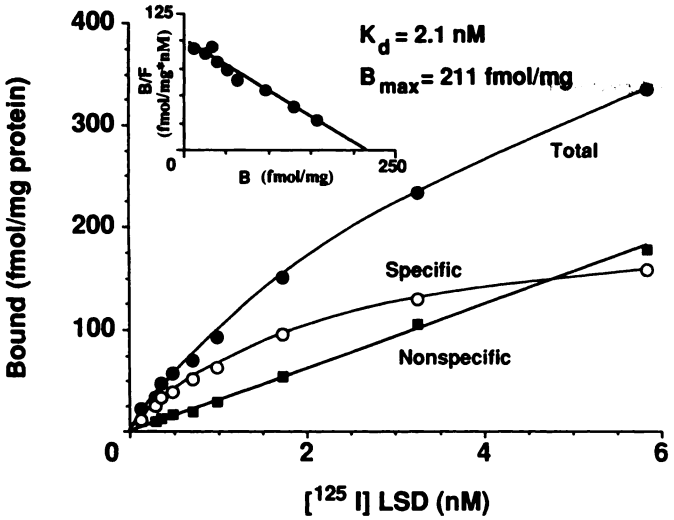


**Fig. 2.** Saturation binding of $^{125}$I-LSD to membranes prepared from P11 cells. Increasing concentrations of $^{125}$I-LSD (0.1–6 nM) were bound to membranes prepared from P11 cells. Nonspecific binding was defined using 1 $\mu$M ketanserin. Specific binding was saturable and best fit by a one-site model ($K_d$ = 2.1 nM, $B_{max}$ = 211 fmol/mg of protein). Data shown are means of triplicate determinations in a representative experiment. ●, Total binding; ■, nonspecific binding; ○, specific binding. *Inset*, Scatchard transformation of the same data.

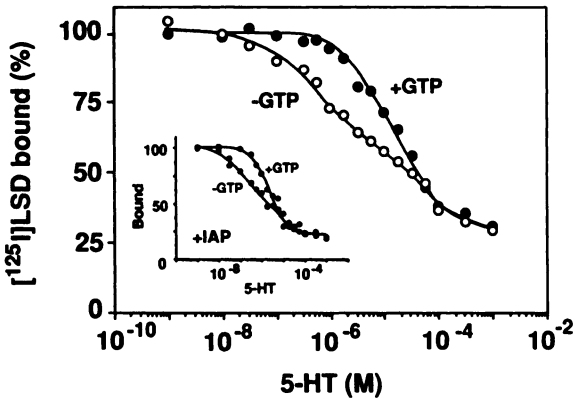


**Fig. 3.** Inhibition of the binding of $^{125}$I-LSD to membranes from P11 cells by 5-HT. The binding of 0.1 nM $^{125}$I-LSD to membranes prepared from P11 cells was inhibited by increasing concentrations of 5-HT. In the absence of GTP, the competition curve generated was best fit by a two-site model (○). The high affinity component ($IC_{50}$ = 550 nM) and low affinity component ($IC_{50}$ = 47 $\mu$M) were present in a ratio of approximately 1:1. In the presence of 300 $\mu$M GTP (●), the competition curve was best fit by a one-site model ($IC_{50}$ = 16 $\mu$M) with a Hill coefficient of 0.9. Data shown are representative of three similar experiments. *Inset*, results of a similar experiment carried out using membranes prepared from P11 cells pretreated with IAP (200 ng/ml for 24 hr).

inhibited the binding of $^{125}$I-LSD to membranes prepared from P11 cells with subnanomolar affinity, consistent with the affinity of spiroperidol at 5-HT$_2$ receptors. $K_i$ values calculated for antagonists at the $^{125}$I-LSD binding sites on P11 cells were consistent with reported $K_i$ values of these antagonists at 5-HT$_2$ receptors (Table 1). The correlation coefficient between the $K_i$ values obtained with P11 cells and reported $K_i$ values was 0.97.

The coupling of serotonin receptors on P11 cells to the phosphoinositide second messenger system was investigated. The accumulation of inositol phosphates stimulated by 20 $\mu$M serotonin was maximal in the presence of 20 mM LiCl (Fig. 5A) and was linear for at least 50 min (Fig. 5B). In subsequent

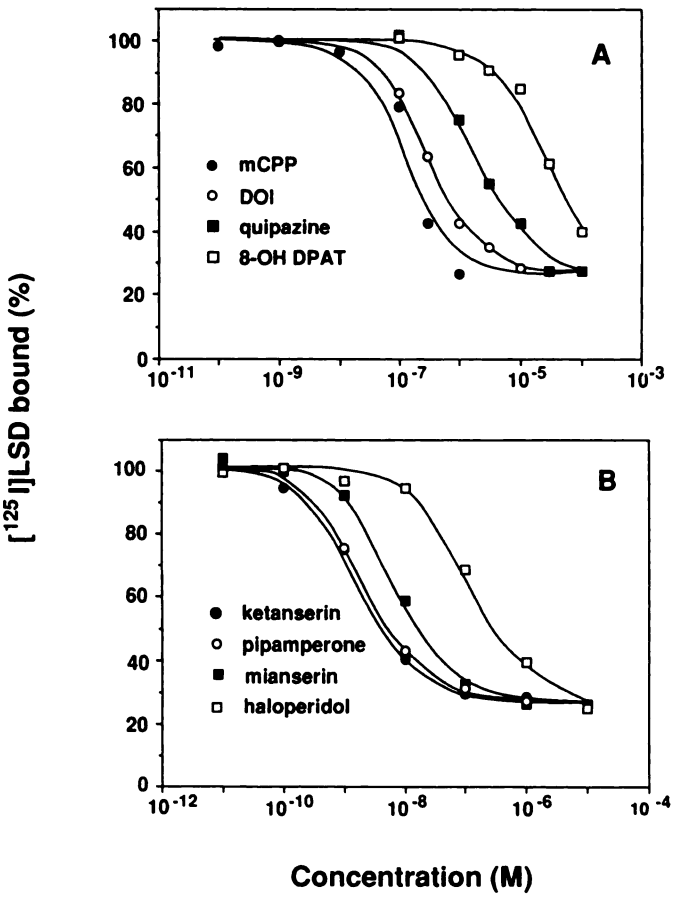


**Fig. 4.** Inhibition of the binding of $^{125}$I-LSD to membranes from P11 cells by competing drugs. A, The binding of 0.4 nM $^{125}$I-LSD to membranes prepared from P11 cells was inhibited by agonists in the presence of 300 $\mu$M GTP. Competition curves generated were best fit by a one-site model. B, The binding of 0.1 nM $^{125}$I-LSD to membranes prepared from P11 cells was inhibited by antagonists. Competition curves generated were best fit by a one-site model. Data shown are representative of three similar experiments.

experiments, phosphoinositide hydrolysis was measured in the presence of 20 mM LiCl, using a 40-min incubation with drugs. In some experiments, individual products of phosphoinositide hydrolysis were isolated (Fig. 6). Inositol monophosphate was the predominant form of inositol phosphate measured under these conditions. Levels of both inositol monophosphate and glycerol phosphoinositol increased in cells treated with serotonin (Fig. 6).

Serotonin caused a dose-dependent 4- to 6-fold increase in phosphoinositide hydrolysis in P11 cells (Fig. 7). The $EC_{50}$ for serotonin was 640 ± 180 nM (mean ± SE of seven determinations). When increasing concentrations of the 5-HT$_2$-selective antagonist ketanserin were included, parallel shifts in the dose-response curve were observed (Fig. 7). Schild analysis of the data resulted in a linear plot with a slope of approximately 1, consistent with a simple competitive interaction (Fig. 7, *inset*). The $K_i$ value for ketanserin derived from Schild analysis was 0.73 ± 0.19 nM (mean ± SE from three experiments). This value was in good agreement with the $K_i$ of the receptor for ketanserin, as determined in binding assays (1.7 ± 0.1 nM; five experiments).

The ability of serotonin to stimulate phosphoinositide hydrolysis in P11 cells was blocked in a dose-dependent manner

TABLE 1

**Pharmacological profile of ligands at the 5-$HT_2$ site on P11 cells**

Competing ligands inhibited the binding of $^{125}$I-LSD to homogenates of P11 cells. Inhibition curves using agonists were generated in the presence of 300 μM GTP and 1 mM $MgCl_2$. All of the inhibition curves were best fit by a one-site model of binding. Hill coefficients ($n_H$) of the competition data are shown. $K_i$ values were calculated from $IC_{50}$ values using the Cheng and Prusoff equation (61). Data are expressed as mean ± standard error. The number of separate determinations is given in parentheses. Published $K_i$ values of these drugs at cortical 5-$HT_2$ receptors are included for comparison.

| | $K_i$ values | $n_H$ | Reported $K_i$ values | $n_H$ |
|---|---|---|---|---|
| | nM | | nM | |
| Agonists | | | | |
| DOI | 420 ± 84 (4) | 1.11 ± 0.16 | | |
| 5-HT | 33,600 ± 6,400 (5) | 1.00 ± 0.09 | 928[a,b] | 0.70[a,b] |
| 8-OH-DPAT | 22,200 ± 1,400 (3) | 0.99 ± 0.12 | 5350[a,b] | 0.83[a,b] |
| 5-Methoxytryptamine | 141,000 ± 35,000 (4) | 1.45 ± 0.25 | 305[a,b] | 0.75[a,b] |
| *m*CPP | 330 ± 90 (4) | 1.03 ± 0.05 | | |
| Quipazine | 1,206 ± 434 (3) | 1.13 ± 0.13 | 228[a,b] | 0.74[a,b] |
| Tryptamine | 163,000 ± 13,000 (4) | 1.20 ± 0.27 | 2005[a,b] | 0.81[a,b] |
| Antagonists | | | | |
| Spiroperidol | 0.69 ± 0.19 (5) | 0.90 ± 0.14 | 0.53[c] | |
| Domperidone | 309 ± 56 (3) | 1.10 ± 0.00 | 481[d] | |
| Haloperidol | 173 ± 34 (4) | 0.96 ± 0.09 | 153[d] | |
| Ketanserin | 1.7 ± 0.1 (5) | 1.05 ± 0.07 | 3.8[d] | |
| Mianserin | 8.3 ± 1.8 (4) | 1.02 ± 0.09 | 8.1[d] | |
| Pipamperone | 2.1 ± 0.1 (4) | 0.91 ± 0.04 | 0.78[c] | |
| Prazosin | 48,900 ± 6,700 (5) | 0.89 ± 0.06 | >10,000[a] | |
| Tripolidine | 4,410 ± 670 (4) | 1.01 ± 0.20 | | |

[a] From Lyon *et al.* (6).
[b] Measured in the absence of GTP.
[c] From Leysen *et al.* (7).
[d] From Kaden *et al.* (8).

by 5-$HT_2$ antagonists including spiroperidol, mianserin, and haloperidol, with a rank order of potency consistent with their order of potency at 5-$HT_2$ receptors (Fig. 8). Spiroperidol and mianserin inhibited phosphoinositide hydrolysis, in a monophasic manner, to basal or near-basal levels. Tripolidine (10 μM) and prazosin (10 μM) did not affect phosphoinositide hydrolysis stimulated by serotonin (data not shown).

The effect of IAP on phosphoinositide hydrolysis in P11 cells was investigated. Cells were treated with IAP (200 ng/ml for 24 hr) or vehicle, and dose-response curves for the stimulation of phosphoinositide hydrolysis by serotonin were generated. Pretreatment of P11 cells with IAP had no effect on the ability of serotonin to stimulate phosphoinositide hydrolysis (Fig. 9). IAP pretreatment of cells also had no effect on the ability of serotonin to inhibit the binding of $^{125}$I-LSD to membranes prepared from P11 cells (Fig. 3, *inset*). To confirm that substrates for IAP in the cells had been ADP-ribosylated by this treatment, membranes from control cells and from cells pretreated with IAP were prepared and incubated *in vitro* with [$^{32}$P]NAD and IAP. [$^{32}$P]ADP-ribosylated substrates for IAP with molecular weights of approximately 41,000 and 39,000, presumably corresponding to the α-subunits of $G_i$ and $G_o$, respectively, were identified in membranes prepared from control cells (Fig. 10). These substrates were not detectable in membranes prepared from treated cells. The absence of labeling in membranes prepared from cells pretreated with IAP indicates that $G_i$ and $G_o$ had been quantitatively ADP-ribosylated by the drug treatment.

## Discussion

The 7315a pituitary tumor was originally induced by trimethylaniline and has been maintained subcutaneously in female Buffalo rats (30). It has a lactotroph origin, demonstrated by the spontaneous and stimulated secretion of prolactin from the tumor and from cells dispersed from the tumor (30, 39). Dopamine interacts with D-2 receptors on normal lactotrophs to inhibit prolactin secretion (40). Previous studies have shown that the 7315a tumor is an appropriate model system to study some of the properties of lactotrophs, because the tumor contains dopamine D-2 receptors coupled to the inhibition of adenylate cyclase activity (33, 41) and to the inhibition of prolactin secretion (39). In preliminary experiments using $^{125}$I-LSD, we determined that the 7315a tumor also expresses a low density of 5-$HT_2$ receptors (data not shown).

The P11 cell line described here was isolated from cells dispersed from a 7315a tumor. P11 cells attached to untreated tissue culture plates, in contrast to other cells that grew in suspension. P11 cells do not appear to express detectable levels of dopamine D-2 receptors, as measured by the specific binding of [$^{125}$I]IBZM, or to spontaneously secrete prolactin.[1] The origin of these cells is, therefore, ambiguous. There is a possibility that the cells were not derived from tumor cells. However, the appearance of the cells does not resemble that of fibroblasts, and the stability of P11 cells in culture over an extended time period suggests that the cell line is immortal. Therefore, it seems likely that the P11 cell line was derived from lactotrophs of the 7315a tumor. These tumor cells may normally express 5-$HT_2$ receptors or their expression of receptors may have changed as the cells were adapted to culture. The question of whether P11 cells can be stimulated to secrete prolactin is currently under investigation.

When P11 cells were characterized in terms of their expression of receptors for monoamines, a binding site for $^{125}$I-LSD was detected that was present at high density. The pharmacological profile of ligands at this site was consistent with their profile at 5-$HT_2$ receptors. Furthermore, the ability of serotonin to stimulate phosphoinositide hydrolysis in P11 cells was consistent with the reported coupling of 5-$HT_2$ receptors to the

[1] K. J. Ivins. Unpublished observations.

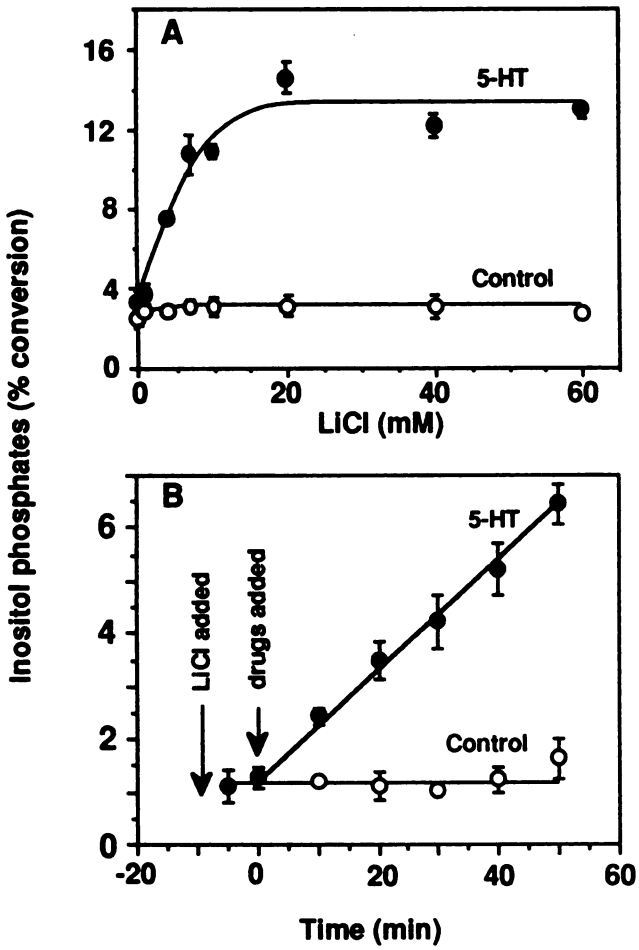


**Fig. 5.** LiCl dependence and time course of the accumulation of inositol phosphates in P11 cells. A, Phosphoinositide hydrolysis in P11 cells was measured in the presence of increasing concentrations of LiCl. Cells were incubated with 20 $\mu$M 5-HT (●) or vehicle (○) for 40 min, and the accumulation of inositol phosphates was determined. B, The accumulation of inositol phosphates in P11 cells stimulated with 20 $\mu$M 5-HT (●) or vehicle (○) was measured in the presence of 20 mM LiCl after various times of incubation. Data shown are means ± standard deviations of triplicate determinations in a representative experiment.

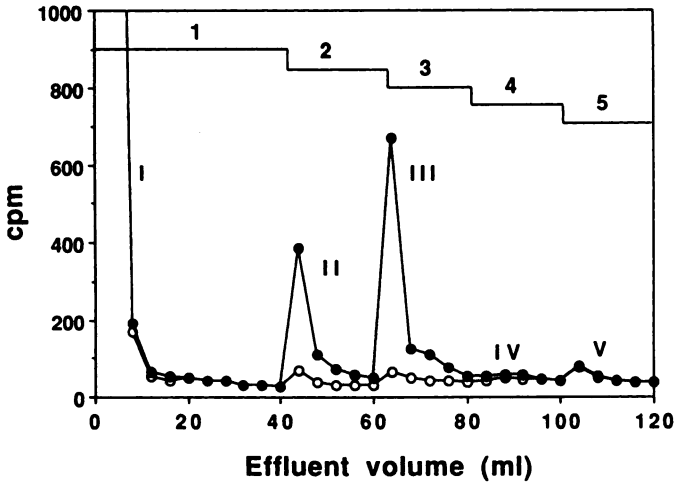


**Fig. 6.** Elution profile of [$^3$H]inositol-derived products. Phosphoinositide hydrolysis was measured in P11 cells in the presence of 20 mM LiCl after a 40-min incubation with 20 $\mu$M 5-HT (●) or vehicle (○). Stepwise gradients were used to elute tritiated products of phosphoinositide hydrolysis, as described by Berridge *et al.* (36). Steps were 1) $H_2O$; 2) 50 mM sodium formate/5 mM sodium tetraborate; 3) 0.2 M ammonium formate/0.1 M formic acid; 4) 0.4 M ammonium formate/0.1 M formic acid; 5) 1.0 M ammonium formate/0.1 M formic acid. The peaks of radioactivity represent the following products: *I*, inositol; *II*, glycerol phosphoinositol; *III*, inositol monophosphate; *IV*, inositol diphosphate; and *V*, inositol trisphosphate.

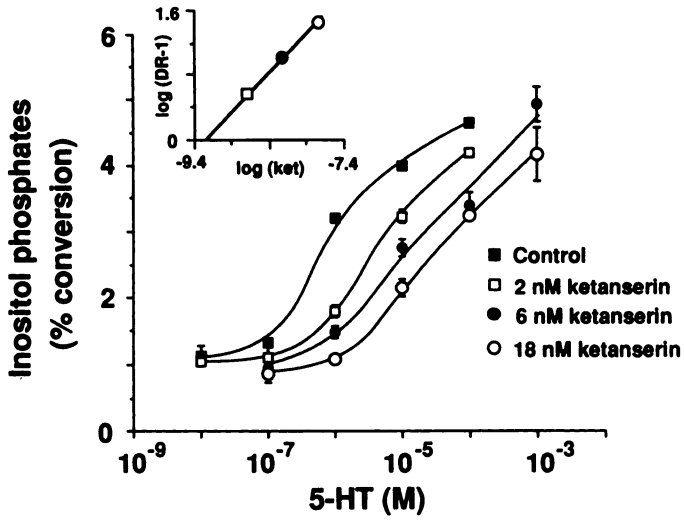


**Fig. 7.** Schild analysis of the stimulation of phosphoinositide hydrolysis by 5-HT in P11 cells. Phosphoinositide hydrolysis in P11 cells was measured in the presence of increasing concentrations of 5-HT. The $EC_{50}$ for the stimulation of phosphoinositide hydrolysis by 5-HT was 260 nM. Dose-response curves for the stimulation of phosphoinositide hydrolysis by 5-HT were also generated in the presence of increasing concentrations of the 5-HT$_2$-selective antagonist ketanserin. *Inset*, Schild transformation of the data resulted in a linear plot with a slope of 0.96 and a derived $K_i$ value for ketanserin of 0.40 nM. Data shown are means ± standard deviations of triplicate determinations and are representative of three similar experiments.

phosphoinositide second messenger system. The response to serotonin in P11 cells seemed to be mediated by 5-HT$_2$ receptors, because the 5-HT$_2$-selective antagonist ketanserin inhibited the response with an affinity similar to its affinity at 5-HT$_2$ receptors. Antagonists at $\alpha_1$-adrenergic and $H_1$-histaminergic receptors, which also have a relatively high affinity for ketanserin (7), were ineffective in blocking phosphoinositide hydrolysis stimulated by serotonin. Furthermore, the ability of nanomolar concentrations of spiroperidol to completely inhibit the response to serotonin suggested that the observed phosphoinositide hydrolysis was mediated by 5-HT$_2$ receptors and not by 5-HT$_{1c}$ receptors (42). 5-HT$_{1c}$ receptors bind $^{125}$I-LSD with high affinity and stimulate phosphoinositide hydrolysis (38, 43), but spiroperidol has only a micromolar affinity for these receptors.

Together, these results suggest that P11 cells express a high density of 5-HT$_2$ receptors coupled to phosphoinositide hydrolysis. A discrepancy was observed between the $EC_{50}$ of serotonin in the activation of phosphoinositide hydrolysis and the $K_i$ of serotonin at the 5-HT$_2$ receptor. A similar discrepancy in the $EC_{50}$ and $K_i$ of serotonin at cortical 5-HT$_2$ receptors has been described by other investigators (38). Differences in assay conditions may account for the greater potency of serotonin in the activation of phosphoinositide hydrolysis than in binding to 5-HT$_2$ receptors. Assays of phosphoinositide hydrolysis were carried out using intact cells in isotonic medium. In contrast, radioligand binding assays were carried out using homogenates of cells in Tris buffer. Another possible explanation for the greater potency of serotonin in assays of phosphoinositide

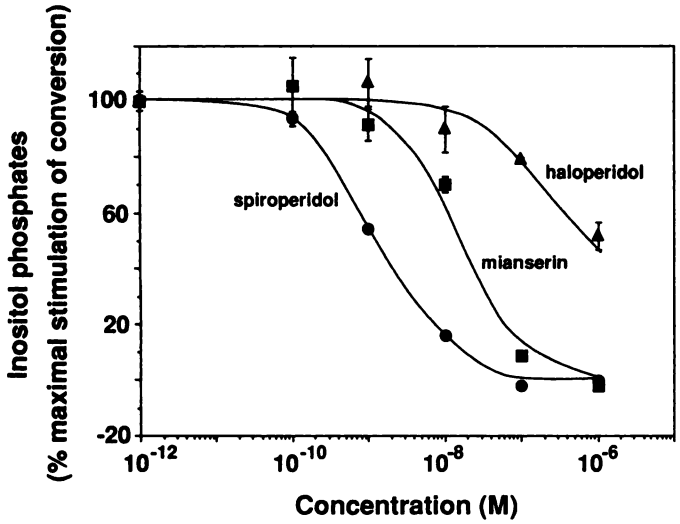


**Fig. 8.** Inhibition of the effect of 5-HT on phosphoinositide hydrolysis by antagonists. Phosphoinositide hydrolysis stimulated by 20 $\mu$M 5-HT in P11 cells was inhibited by increasing concentrations of spiroperidol (●), mianserin (■), and haloperidol (▲). Data shown are means ± standard deviations of triplicate determinations and are representative of three experiments.

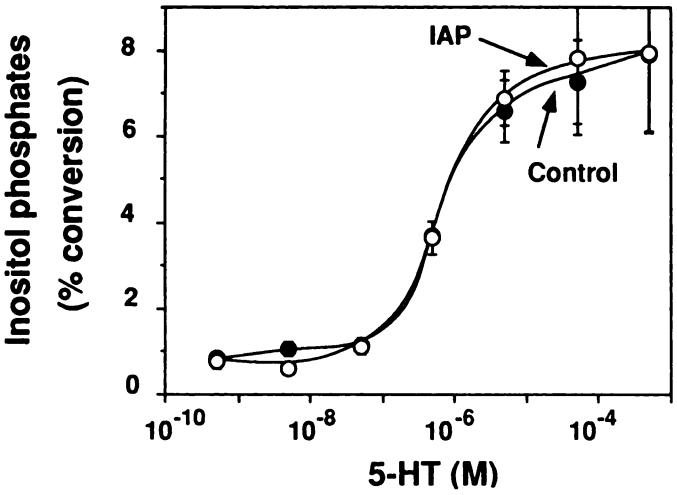


**Fig. 9.** Stimulation of phosphoinositide hydrolysis by 5-HT following treatment of P11 cells with IAP. P11 cells were treated with 200 ng/ml IAP (○) or vehicle (●) for 24 hr, and phosphoinositide hydrolysis was measured in the presence of increasing concentrations of 5-HT. Data shown are means ± standard deviations of triplicate determinations and are representative of three experiments.

hydrolysis than in radioligand binding assays would be the presence of spare 5-$HT_2$ receptors on P11 cells.

These results also raise questions as to whether 5-$HT_2$ receptors are normally expressed on lactotrophs. Because an increase in intracellular calcium in lactotrophs is associated with an increase in prolactin secretion (39), and because phosphoinositide hydrolysis is accompanied by an increase in intracellular calcium, it might be expected that stimulation of 5-$HT_2$ receptors on lactotrophs would cause an increase in the secretion of prolactin. Administration of the serotonin precursor 5-hydroxytryptophan or intraventricular injection of serotonin stimulates secretion of prolactin *in vivo*, but this stimulation is widely believed to be due to the ability of serotonin to cause the release of a secretagogue for prolactin from the central nervous system, rather than to a direct action at the pituitary (44, 45). In fact,

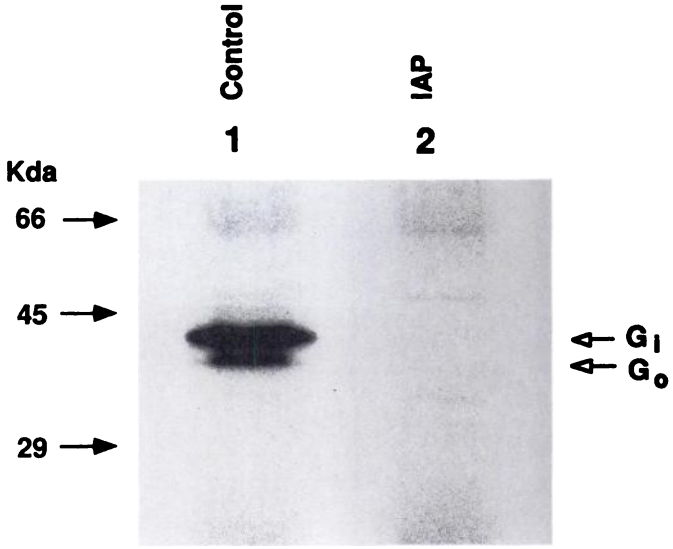


**Fig. 10.** Detection of substrates for IAP in P11 cells. Proteins in P11 cell membranes were [$^{32}$P]ADP-ribosylated *in vitro* in the presence of IAP, solubilized in sodium dodecyl sulfate, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and visualized by autoradiography. The experiment has been repeated two times with similar results. *Lane 1*, membranes from control cells; *lane 2*, membranes from cells pretreated for 24 hr with 200 ng/ml IAP.

several studies using isolated pituitary glands have failed to detect stimulation of prolactin secretion by serotonin (46, 47). However, it was recently suggested that 5-$HT_2$ receptors are present on normal lactotrophs and their activation causes a rapidly desensitized increase in prolactin secretion (48). These results raise the intriguing possibility that serotonin binds to 5-$HT_2$ receptors on lactotrophs and directly stimulates the secretion of prolactin at the level of the anterior pituitary.

Results of several types of experiments have led to the conclusion that receptor-stimulated phosphoinositide hydrolysis is mediated by G proteins (for review, see Ref. 49). In P11 cells, phosphoinositide hydrolysis stimulated by serotonin appeared to be mediated by a G protein, because the high affinity component of binding of serotonin to 5-$HT_2$ receptors was eliminated by GTP. This is consistent with the observation that the affinity of 5-$HT_2$ receptors in the frontal cortex for agonists, including serotonin, tryptamine, and 5-methoxytryptamine, was decreased by GTP (50). It has been reported that the proportion of sites with a high affinity for agonist depended on the agonist studied (50). It will be interesting to identify compounds that are full or partial agonists at the 5-$HT_2$ receptor on P11 cells and to determine whether they bind to the same number of sites with high affinity.

Several investigators have studied the effect of IAP on the coupling of 5-$HT_2$ receptors to phosphoinositide hydrolysis. Kavanaugh *et al.* (51) reported that IAP did not affect phosphoinositide hydrolysis stimulated by serotonin in primary cultures of vascular smooth muscle from calf aorta. Experiments designed to document the effectiveness of the exposure to IAP were not reported. In contrast, Go *et al.* (52) reported that IAP blocked the ability of serotonin to stimulate phosphoinositide hydrolysis in primary cultures from rabbit aorta. In P11 cells, a concentration of IAP that caused quantitative ADP-ribosylation of substrates for IAP in the cells did not affect phosphoinositide hydrolysis stimulated by serotonin, suggesting that the G protein that couples 5-$HT_2$ receptors to

phospholipase C in P11 cells is not a substrate for ADP-ribosylation by IAP and is not $G_i$ or $G_o$. Previous reports have shown that IAP does not have a consistent effect on phosphoinositide hydrolysis; in some cells, including mast cells (53) and NG108-15 neuroblastoma-glioma cells (54), IAP blocks receptor-stimulated phosphoinositide hydrolysis. In others, including 3T3 fibroblasts (55) and $GH_3$ pituitary cells (56), IAP has no effect. These findings have been interpreted as evidence that multiple G proteins are involved in coupling receptors to phosphoinositide hydrolysis. Ashkenazi *et al.* (57) recently presented evidence suggesting that multiple G proteins within a cell couple selectively to different receptors and activate phospholipase C. Therefore, the 5-HT$_2$ receptor may belong to a class of receptors that selectively couples to an IAP-insensitive G protein to activate phospholipase C. One candidate for the IAP-insensitive G protein is $G_z$, a G protein identified on the basis of sequence homology to $G_o$ and $G_t$ (58, 59). The predicted amino acid sequence of $G_z$ lacks the cysteine residue, present in $G_i$, $G_o$, and $G_t$, that is ADP-ribosylated by IAP. The concentration of mRNA encoding $G_z$ is highest in brain and retina but is also detectable in endocrine tissues and in $GH_3$ pituitary cells (58, 59).

Previous reports have identified cell lines in which serotonin stimulates phosphoinositide hydrolysis (26–29). However, neither a detailed pharmacological analysis of phosphoinositide hydrolysis nor results of direct binding studies have been reported. Therefore, P11 cells represent the first cell line in which 5-HT$_2$ receptors have been unambiguously identified. 5-HT$_2$ receptors on P11 cells can be assayed with radioligands, and they are coupled to phosphoinositide hydrolysis by a G protein that is neither $G_i$ nor $G_o$. The relatively high density of receptors on P11 cells should facilitate studies of 5-HT$_2$ receptors and the regulation of receptor density. A current issue in studies of 5-HT$_2$ receptors is whether binding sites for the hallucinogens DOI and DOB represent the agonist-preferring high affinity state of the 5-HT$_2$ receptor, as postulated by Titeler and coworkers (6), or whether they represent a subtype of 5-HT$_2$ receptors, as postulated by Pierce and Peroutka (5). This issue might be addressed using P11 cells, by correlating the density of [$^3$H]DOB binding sites in homogenates of P11 cells with the density of the component of $^{125}$I-LSD binding with high affinity for serotonin. Recently, the sequence of a cDNA encoding the rat 5-HT$_2$ receptor was reported (60), and probes constructed from this sequence will allow detailed study of the regulation of 5-HT$_2$ receptors at the level of gene transcription. Because peptide fragments of the receptor can be synthesized, antibodies to the 5-HT$_2$ receptor should become available for examination of receptor synthesis and sequestration or internalization. With the use of these molecular biological probes, as well as radioligand binding studies and assays for second messenger production, we believe that P11 cells will be a useful model system for future studies of the regulation and function of 5-HT$_2$ receptors coupled to phosphoinositide hydrolysis in cultured cells.

**References**


1. Peroutka, S. J., and S. H. Snyder. Multiple serotonin receptors: differential binding of [$^3$H]5-hydroxytryptamine, [$^3$H]lysergic acid diethylamide, and [$^3$H] spiroperidol. *Mol. Pharmacol.* **16**:687–699 (1979).
2. Kilpatrick, G. J., B. J. Jones, and M. B. Tyers. Identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. *Nature* (*Lond.*) **330**:746–748 (1987).
3. Heuring, R. E., and S. J. Peroutka. Characterization of a novel $^3$H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J. Neurosci.* **7**:894–903 (1987).
4. Fozard, J. R. MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **326**:36–44 (1984).
5. Pierce, P. A., and S. J. Peroutka. Evidence for distinct 5-hydroxytryptamine$_2$ binding site subtypes in cortical membrane preparations. *J. Neurochem.* **52**:656–658 (1989).
6. Lyon, R. A., K. H. Davis, and M. Titeler. [$^3$H]DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT$_2$ receptors. *Mol. Pharmacol.* **31**:194–199 (1987).
7. Leysen, J. E., J. E. Niemegeers, J. M. Van Nueten, and P. M. Laduron. [$^3$H] Ketanserin (R 41 468), a selective $^3$H-ligand for serotonin$_2$ receptor binding sites. *Mol. Pharmacol.* **21**:301–314 (1982).
8. Kadan, M. J., A. M. Krohn, M. J. Evans, R. L. Waltz, and P. R. Hartig. Characterization of $^{125}$I-lysergic acid diethylamide binding to serotonin receptors in rat frontal cortex. *J. Neurochem.* **43**:601–606 (1984).
9. Peroutka, S. J., R. M. Lebovitz, and S. H. Snyder. Two distinct central serotonin receptors with different physiological functions. *Science* (*Wash. D. C.*) **212**:827–829 (1981).
10. Glennon, R. A., R. Young, and J. A. Rosencrans. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT$_2$ antagonists. *Eur. J. Pharmacol.* **91**:189–196 (1983).
11. Kertesz, E., G. M. Somoza, J. L. D'Eramo, and C. Libertun. Further evidence for endogenous hypothalamic serotonergic neurons involved in the cimetidine-induced prolactin release in the rat. *Brain Res.* **413**:10–14 (1987).
12. Cohen, M. L., R. W. Fuller, and K. S. Wiley. Evidence for 5-HT$_2$ receptors mediating contraction in vascular smooth muscle. *J. Pharmacol. Exp. Ther.* **218**:421–425 (1981).
13. De Clerck, F., B. Xhonneux, J. Leysen, and P. A. J. Janssen. Evidence for functional 5-HT$_2$ receptor sites on human blood platelets. *Biochem. Pharmacol.* **33**:2807–2811 (1984).
14. Affolter, H., P. Erne, E. Burgisser, and A. Pletscher. $Ca^{2+}$ as messenger of 5-HT$_2$-receptor stimulation in human blood platelets. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **325**:337–342 (1984).
15. de Chaffoy de Courcelles, D., J. E. Leysen, F. De Clerck, H. Van Belle, and P. A. J. Janssen. Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S$_2$ receptor sites. *J. Biol. Chem.* **260**:7603–7608 (1985).
16. Roth, B. L., T. Nakaki, D.-M. Chuang, and E. Costa. Aortic recognition sites for serotonin (5-HT) are coupled to phospholipase C and modulate phosphatidylinositol turnover. *Neuropharmacology* **23**:1223–1225 (1984).
17. Kendall, D. A., and S. R. Nahorski. 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J. Pharmacol. Exp. Ther.* **233**:473–479 (1985).
18. 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).
19. Blackshear, M. A., R. L. Friedman, and E. Sanders-Bush. Acute and chronic effects of serotonin (5HT) antagonists on serotonin binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **324**:125–129 (1983).
20. Gandolfi, O., M. L. Barbaccia, and E. Costa. Different effects of serotonin antagonists on $^3$H-mianserin and $^3$H-ketanserin recognition sites. *Life Sci.* **36**:713–721 (1985).
21. Leysen, J. E., P. Van Gompel, W. Gommeren, R. Woestenborghs, and P. A. J. Janssen. Down regulation of serotonin-S$_2$ receptor sites in rat brain by chronic treatment with the serotonin-S$_2$antagonists: ritanserin and setoperone. *Psychopharmacology* **88**:434–444 (1986).
22. Sanders-Bush, E., M. Breeding, and M. Roznoski. 5-HT$_2$ binding sites after mianserin: comparison of loss of sites and brain levels of drug. *Eur. J. Pharmacol.* **133**:199–204 (1987).
23. Quik, M., and E. Azmita. Selective destruction of the serotonergic fibers of the fornix-fimbria and cingulum bundle increases 5-HT$_1$ but not 5-HT$_2$ receptors in rat midbrain. *Eur. J. Pharmacol.* **90**:377–384 (1983).
24. Peroutka, S. J., and S. H. Snyder. Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. *Science* (*Wash. D.C.*) **210**:88–90 (1980).
25. Hyttel, J., K. Fredericson Overo, and J. Arnt. Biochemical effects and drug levels in rats after long-term treatment with the specific 5-HT-uptake inhibitor citalopram. *Psychopharmacology* **83**:20–27 (1984).
26. Doyle, V. M., J. A. Creba, U. T. Ruegg, and D. Hoyer. Serotonin increases the production of inositol phosphates and mobilises calcium via the 5-HT$_2$ receptor in A7r5 smooth muscle cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **333**:98–103 (1986).
27. Cory, R. N., B. Rouot, G. Guillon, F. Sladeczek, M. Balestre, and J. Bockaert. The 5-hydroxytryptamine (5-HT$_2$) receptor stimulates inositol phosphate formation in intact and broken WRK1 cells: determination of occupancy-response relationships for 5-HT agonists. *J. Pharmacol. Exp. Ther.* **241**:258–267 (1987).
28. Ananth, U. S., U. Leli, and G. Hauser. Stimulation of phosphoinositide hydrolysis by serotonin in C6 glioma cells. *J. Neurochem.* **48**:253–261 (1987).
29. Hamamori, Y., M. Hoshijima, T. Ohmori, T. Ueda, and Y. Takai. Serotonin as a major serum factor inducing the phospholipase C-mediated hydrolysis of phosphoinositides in normal rat kidney cells. *Cancer Res.* **48**:6697–6702 (1988).

30. Bates, R. W., M. M. Garrison, and H. P. Morris. Comparison of two different transplantable mammotropic pituitary tumors: hormone content and effect on host. *Proc. Soc. Exp. Biol. Med.* **123**:67–70 (1966).
31. Lamberts, S. W. J., and T. Verleun. Differences in the mechanism of the inhibitory actions of catecholestrogens, tamoxifen and high concentrations of estrogens on prolactin release by cultured rat pituitary tumor cells. *Eur. J. Cancer Clin. Oncol.* **23**:1117–1123 (1987).
32. Scatchard, G. The attractions of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.* **51**:660–672 (1949).
33. Lin, C., P. McGonigle, and P. B. Molinoff. Characterization of D-2 dopamine receptors in a tumor of the rat anterior pituitary gland. *J. Pharmacol. Exp. Ther.* **242**:950–956 (1987).
34. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
35. Wax, M. B., and M. Coca-Prados. Receptor-mediated phosphoinositide hydrolysis in human ocular ciliary epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **30**:1675–1679 (1989).
36. Berridge, M. J., R. M. C. Dawson, C. P. Downes, J. P. Heslop, and R. F. Irvine. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. *Biochem. J.* **212**:473–482 (1983).
37. Woolkalis, M. J., M. T. Nakada, and D. R. Manning. Alterations in components of adenylate cyclase associated with transformation of chicken embryo fibroblasts by Rous sarcoma virus. *J. Biol. Chem.* **261**:3408–3413 (1986).
38. Conn, P. J., E. Sanders-Bush, B. J. Hoffman, and P. R. Hartig. A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. *Proc. Natl. Acad. Sci. USA* **83**:4086–4088 (1986).
39. Judd, A. M., K. Koike, G. Schettini, I. S. Login, E. L. Hewlett, T. Yasumoto, and R. M. MacLeod. Dopamine decreases 7315a tumor cell prolactin release induced by calcium mobilization. *Endocrinology* **117**:1215–1221 (1985).
40. Caron, M. C., M. Bealieu, V. Raymond, B. Gagne, J. Drouin, R. J. Lefkowitz, and F. Labrie. Dopaminergic receptors in the anterior pituitary gland. *J. Biol. Chem.* **253**:2244–2253 (1978).
41. Cronin, M. J., C. A. Valdenegro, S. N. Perkins, and R. M. MacLeod. The 7315a pituitary tumor is refractory to dopaminergic inhibition of prolactin release but contains dopamine receptors. *Endocrinology* **109**:2160–2166 (1981).
42. Hoyer, D., S. Srivatsa, A. Pazos, G. Engel, and J. M. Palacios. [$^{125}$I]LSD labels 5-HT$_{1c}$ recognition sites in pig choroid plexus membranes: comparison with [$^{3}$H]mesulergine and [$^{3}$H]5-HT binding. *Neurosci. Lett.* **69**:269–274 (1986).
43. Yagaloff, K. A., and P. R. Hartig. $^{125}$I-Lysergic acid diethylamide binds to a novel serotonergic site on rat choroid epithelial cells. *J. Neurosci.* **5**:3178–3183 (1985).
44. Clemens, J. A., M. E. Roush, and R. W. Fuller. Evidence that serotonin neurons stimulate secretion of prolactin releasing factor. *Life Sci.* **22**:2209–2214 (1978).
45. Krulich, L., E. Vijayan, R. J. Coppings, A. Giachetti, S. M. McCann, and M. A. Mayfield. On the role of the central serotoninergic system in the regulation of the secretion of thyrotropin and prolactin: thyrotropin inhibiting and prolactin releasing effects of 5-hydroxytryptophan and quipazine in the male rat. *Endocrinology* **105**:276–283 (1979).
46. Birge, C. A., L. S. Jacobs, C. T. Hammer, and W. H. Daughaday. Catecholamine inhibition of prolactin secretion by isolated rat adenohypophyses. *Endocrinology* **86**:120–130 (1970).
47. Lamberts, S. W. J., and R. M. MacLeod. Effects of cyproheptadine on prolactin synthesis and release by normal and suppressed pituitary glands and by dispersed pituitary tumor cells. *Endocrinology* **103**:287–295 (1978).
48. Apfelbaum, M. E. Effect of serotonin on basal and TRH-induced release of prolactin from rat pituitary glands *in vitro*. *Acta Endocrinol. (Copenh.)* **114**:565–571 (1987).
49. Fain, J. N., M. A. Wallace, and R. J. Wojcikiewicz. Evidence for involvement of guanine nucleotide-binding regulatory proteins in the activation of phospholipases by hormones. *FASEB J.* **2**:2569–2574 (1988).
50. Battaglia, G., M. Shannon, and M. Titeler. Guanyl nucleotide and divalent cation regulation of cortical $S_2$ serotonin receptors. *J. Neurochem.* **43**:1213–1219 (1984).
51. Kavanaugh, W. M., L. T. Williams, H. E. Ives, and S. R. Coughlin. Serotonin-induced deoxyribonucleic acid synthesis in vascular smooth muscle cells involves a novel, pertussis toxin-sensitive pathway. *Mol. Endocrinol.* **2**:599–605 (1988).
52. Go, M., M. Yokoyama, H. Akita, and H. Fukuzaki. Phorbol ester modulates serotonin-stimulated phosphoinositide breakdown in cultured vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* **153**:51–58 (1988).
53. Nakamura, T., and M. Ui. Simultaneous inhibitions of inositol phospholipid breakdown, arachidonic acid release, and histamine secretion in mast cells by islet-activating protein, pertussis toxin. *J. Biol. Chem.* **260**:3584–3593 (1985).
54. Higashida, H., R. A. Streaty, W. Klee, and M. Nirenberg. Bradykinin-activated transmembrane signals are coupled via $N_o$ or $N_i$ to production of inositol 1,4,5-trisphosphate, a second messenger in NG108-15 neuroblastoma-glioma hybrid cells. *Proc. Natl. Acad. Sci. USA* **83**:942–946 (1986).
55. Murayama, T., and M. Ui. Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts: selective susceptibility to islet-activating protein, pertussis toxin. *J. Biol. Chem.* **260**:7226–7233 (1985).
56. Martin, T. F. J., D. O. Lucas, S. M. Bajjalieh, and J. A. Kowalchyk. Thyrotropin-releasing hormone activates a $Ca^{2+}$-dependent polyphosphoinositide phosphodiesterase in permeable $GH_3$ cells: GTP$\gamma$S potentiation by a cholera and pertussis toxin-insensitive mechanism. *J. Biol. Chem.* **261**:2918–2927 (1986).
57. Ashkenazi, A., E. G. Peralta, J. W. Winslow, J. Ramachandran, and D. J. Capon. Functionally distinct G proteins selectively couple different receptors to PI hydrolysis in the same cell. *Cell* **56**:487–493 (1989).
58. Fong, H. K. W., K. K. Yoshimoto, P. Eversole-Cire, and M. I. Simon. Identification of a GTP-binding protein *alpha*-subunit that lacks an apparent ADP-ribosylation site for pertussis toxin. *Proc. Natl. Acad. Sci. USA* **85**:3066–3070 (1988).
59. Matsuoka, M., H. Itoh, T. Kozasa, and Y. Kaziro. Sequence analysis of cDNA and genomic DNA for a putative pertussis toxin-insensitive guanine nucleotide-binding regulatory protein $\alpha$ subunit. *Proc. Natl. Acad. Sci. USA* **85**:5384–5388 (1988).
60. Pritchett, D. B., A. W. J. Bach, M. Wozny, O. Taleb, R. Dal Toso, J. C. Shih, and P. H. Seeburg. Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *EMBO J.* **7**:4135–4140 (1988).
61. Cheng, Y. C., and W. H. Prusoff. Relationship between the inhibition constant ($K_i$) and the concentration of inhibitor which causes 50 percent inhibition ($IC_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).

**Send reprint requests to:** Kathryn J. Ivins, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6084.