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Regulation of Serotonin-Stimulated Phosphoinositide Hydrolysis: Relation to the Serotonin 5-HT-2 Binding Site

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The hypothesis that serotonin (5-HT)-stimulated phosphoinositide hydrolysis is mediated by the 5-HT-2 binding site in cerebral cortex was tested by comparing antagonist K_d values determined by Schild analyses with K_i values at the 5-HT-2 binding site. A significant correlation was found between K_d values and K_i values at competing for ^{3}H-ketanserin binding ($R = 0.98$), suggesting that the phosphoinositide-linked receptor and the 5-HT-2 site are identical. The 5-HT-2-mediated phosphoinositide response was then used as a measure of 5-HT-2 receptor sensitivity after *in vivo* treatments that alter the availability of 5-HT. Chronic treatment with the 5-HT-2 antagonist mianserin resulted in a significant decrease (52%) in the density of 5-HT-2 binding sites and a significant decrease (49%) in the maximal phosphoinositide hydrolysis response to 5-HT. Depletion of 5-HT levels with para-chlorophenylalanine or chemical denervation of serotonergic neurons with 5,7-dihydroxytryptamine had no significant effect upon 5-HT-2 receptor density or upon the phosphoinositide response to 5-HT. These data suggest that changes or lack of changes in 5-HT-2 receptor density following *in vivo* manipulations reflect the functional state of the receptor.

Central serotonin (5-HT) receptors are divided into 2 broad subclasses based upon radioligand binding data (Leysen et al., 1982; Peroutka and Snyder, 1979). The 5-HT-1 site is labeled with ^{3}H-5-HT, whereas the 5-HT-2 site is labeled with the 5-HT antagonists, ^{3}H-spiroperidol and ^{3}H-ketanserin. Recent evidence suggests that the 5-HT-1 site can be further divided into at least 3 subtypes (Pazos et al., 1984; Pedigo et al., 1981; Yagaloff and Hartig, 1985). For the most part, the 5-HT-1 site responds predictably to *in vivo* manipulations that alter 5-HT availability. Chemical denervation induces an increase in the density of ^{3}H-5-HT binding sites in the hippocampus (Nelson et al., 1978; Seeman et al., 1980), and inhibition of 5-HT inactivation with monoamine oxidase inhibitors (Savage et al., 1980b), or by chronic reuptake blockade (Dumbrille-Ross and Tang, 1983; Wong and Bymaster, 1981), reduces the number of 5-HT-1 binding sites. Furthermore, chronic treatment with 5-HT receptor agonists, or antagonists, decreases, or increases, the density of 5-HT-1 binding sites, respectively (Samanin et al., 1980; Savage et al., 1980a). The 5-HT-2 site, on the other hand, does not respond predictably to *in vivo* manipulations. It does not develop increased density after denervation (Blackshear et al., 1981; Quik and Azmita, 1983; Seeman et al., 1980) and is not down-regulated after chronic 5-HT reuptake blockade (Dumbrille-Ross and Tang, 1983; Peroutka and Snyder, 1980). Paradoxically, chronic treatment with putative 5-HT antagonists results in a down-regulation of the 5-HT-2 binding site (Blackshear and Sanders-Bush, 1982; Blackshear et al., 1983).

It is possible that 5-HT-2 receptor sensitivity does respond predictably to *in vivo* manipulations and that the observed changes (or lack of changes) in 5-HT-2 receptor density are not accompanied by parallel changes in the responsiveness to 5-HT. Studies of behavioral models of 5-HT-2 activation, although not entirely consistent, lend support to this hypothesis. For instance, chemical denervation of 5-HT neurons has no effect on 5-HT-2 receptor density, but it does induce supersensitivity in the head twitch model of 5-HT-2 receptor activation (Nakamura and Fukushima, 1978; Yamamoto and Ueki, 1981). Futhermore, there are reports that chronic administration of putative 5-HT-2 antagonists induces supersensitivity in behavioral models of 5-HT-2 activation (Friedman et al., 1983; Mogilnicka and Klimek, 1979; Stoltz et al., 1983). Other groups have failed to confirm this; instead, they found subsensitivity after chronic antagonists (Blackshear and Sanders-Bush, 1982; Lucki et al., 1985). Although such behavioral responses are clearly mediated by the 5-HT-2 binding site, nonserotonergic neuronal systems undoubtedly come into play, making interpretation of the behavioral results complicated. For instance, behavioral supersensitivity to a 5-HT-2 agonist could represent the loss of inhibitory input from another system rather than 5-HT-2 receptor supersensitivity. Furthermore, it is conceivable that changes in the density of the 5-HT-2 site, which are usually measured in frontal cortex, do not occur on cells involved in mediating the behavioral responses. For these reasons, it would be advantageous to study a 5-HT-2-mediated response that is closely linked to the receptor. This would allow a direct measurement of the relationship between 5-HT-2 receptor density and 5-HT-2 receptor sensitivity.

One response that could serve this purpose is 5-HT-stimulated phosphoinositide hydrolysis. Phosphoinositide hydrolysis is a multifunctional transmembrane transducing mechanism that results in release of at least 2 second messengers (inositol-1,4,5-trisphosphate and diacylglycerol). These second messengers elicit a number of cellular responses, including neurotransmitter release, lysosomal enzyme release, glycogenolysis, DNA synthesis, photoreception, platelet aggregation, and heterologous receptor desensitization (Berridge, 1984). Recently, it has been suggested that 5-HT-stimulated phosphoinositide hydrolysis in human platelets (de Chaffoy de Courcelles et al., 1985), rat aorta (Roth et al., 1984, 1985), and rat cerebral cortex (Conn and

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Sanders-Bush, 1984, 1985) is mediated by the 5-HT-2 binding site. In cerebral cortex, however, a poor correlation between antagonist potencies at blocking 5-HT-stimulated phosphoinositide hydrolysis and competing for 5-HT-2 binding prevented an unequivocal conclusion.

In the present study, we further test the hypothesis that 5-HT-stimulated phosphoinositide turnover in cerebral cortex is mediated by the 5-HT-2 binding site by using Schild analyses to estimate absolute potencies (K_d values) of 5-HT-2 antagonists for the phosphoinositide-linked receptor. Comparison of these values with affinities at the 5-HT-2 binding site gave a significant correlation, supporting this hypothesis. The 5-HT-2-mediated phosphoinositide response was then used to measure 5-HT-2 responsiveness after chronic administration of the 5-HT antagonist, mianserin, or after 5-HT depletion or destruction of serotonergic neurons with *para*-chlorophenylalanine (PCPA) or 5,7-dihydroxytryptamine (5,7-DHT), respectively.

Materials and Methods

Drugs

PCPA methyl ester HCl, 5-HT creatinine sulfate, 5,7-DHT creatinine sulfate, phosphatidylinositol phosphate (PIP) and phosphatidylinositol bisphosphate (PIP2) were purchased from Sigma Chemical Co. (St. Louis, MO); phosphatidylinositol (PI) and Lyso PI from Avanti Polar Lipids Inc. (Birmingham, AL); pentobarbital sodium from Abbott Laboratories (North Chicago, IL); and mianserin HCl from Research Biochemicals Inc. (Wayland, MA). The following drugs were kindly donated by the indicated manufacturers: ketanserin tartrate, spiperone, and pipamperone HCl from Janssen Pharmaceutica (Beerse, Belgium); promethazine HCl from Wyeth Laboratories (Philadelphia, PA); desipramine HCl from Merrell Dow Research Institute (Cincinnati, OH); clozapine and pizotifen from Sandoz, Inc. (East Hanover, NJ); cinanserin HCl from E. R. Squibb and Sons (Princeton, NJ); pargyline HCl from Abbott Laboratories (North Chicago, IL); trazadone HCl from Mead Johnson and Co. (Evansville, IN). ^{3}H-ketanserin HCl (78.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA) and ^{3}H-myo-inositol (14 Ci/mmol) from American Radiolabeled Chemicals (St. Louis, MO).

Levels of phosphoinositides

The levels of radioactivity in PIP and PIP2 and the specific radioactivity of PI were determined by established methodologies. Cerebral cortices from male Sprague-Dawley rats were dissected and sliced as described previously (Conn and Sanders-Bush, 1985). Slices were incubated for 30 min (37°C) in a gently shaking bath in Krebs bicarbonate buffer containing 10 mM glucose (KRBG). Slices were then washed with warm KRBG and 300 μl aliquots of gravity-packed slices were added to vials containing 12 μCi ^{3}H-inositol in 3.3 ml KRBG and incubated for an additional 3 hr (37°C). Free ^{3}H-inositol was washed away with warm KRBG containing 10 mM myo-inositol and 200 μl aliquots of gravity-packed slices were transferred to conical centrifuge tubes and rapidly homogenized using a Brinkman polytron. The lipids were immediately extracted as described by Schacht (1981). The lipid extract was divided into 4 fractions and evaporated under a stream of nitrogen while kept on ice.

The method of Yavin and Zutra (1977) was used to separate PI from other phospholipids. This method employs 2-dimensional thin-layer chromatography (TLC) and gives excellent separation of PI from phosphatidylserine compared with other TLC methods. Briefly, fractions were spotted on Brinkman silica gel G plates and developed in the first dimension using chloroform/methanol/40% methylamine (13:6:1.5). The plates were dried under warm air and exposed to fumes of HCl to neutralize the methylamine. The plates were then developed in a solvent system consisting of chloroform/acetone/methanol/acetic acid/water (10:4:2:3:1). The dried plates were sprayed with Enhance (New England Nuclear) and exposed to X-ray film for 2–3 d. Only 1 radioactive spot was visible. This corresponds to PI as determined by TLC of standards run simultaneously. The spot corresponding to PI was scraped into acid-washed test tubes for the determination of phosphate. Phosphate content was determined by the method of Van Dongen et al. (1985), using KH_2PO_4 as a standard. Two of the 4 fractions were analyzed in this manner and the values were averaged.

Radioactivity in all 3 phosphoinositides was determined in the second pair of fractions. Samples were spotted on silica gel 60 plates (American Scientific) and run in 1-dimension using chloroform/methanol/4 N NH_4OH as the solvent system (Gonzalez-Sastre and Folch-Pi, 1968). Standard nonlabeled phosphoinositides were run simultaneously, and the spots were visualized with iodine vapor. The R_f values of PI, PIP, and PIP2 were 0.59, 0.34, and 0.12, respectively. Sections, 1 cm, were scraped into counting vials and the radioactivity quantitated by liquid scintillation counting. Only 4 radioactive peaks were found, 3 of which agree with the 3 phosphoinositide standards. The fourth radioactive peak (which represented less than 10% of the radioactivity spotted) had an R_f value (0.47), which corresponded with a lyso PI standard.

Radioligand binding

For competition binding studies, the binding of ^{3}H-ketanserin was measured in buffer containing physiological salts as described previously (Conn and Sanders-Bush, 1985). IC_{50} values were determined from Hill plots of competition binding data. K_i values were calculated by the method of Cheng and Prusoff (1973). Scatchard analyses of ^{3}H-ketanserin binding were performed as previously described (Conn and Sanders-Bush, 1985), except that physiological salts were omitted.

Phosphoinositide hydrolysis

Measurement of agonist-induced phosphoinositide hydrolysis was as described previously (Conn and Sanders-Bush, 1985) except that 25 μl aliquots of gravity-packed slices were labeled for various times in tubes containing 1–2 μCi ^{3}H-inositol and 200 μl KRBG. Drugs were added directly to these tubes, and subsequent incubations were in the continuous presence of ^{3}H-inositol. Pargyline (10 μM) and LiCl (10 mM) were routinely added to the incubation medium. Pargyline shifts the 5-HT concentration response curve leftward and eliminates the nonspecific effects of high concentrations of 5-HT (Conn and Sanders-Bush, 1985). Lithium inhibits the metabolism of inositol phosphate (IP) and allows the direct measurement of ^{3}H-IP released from labeled phosphoinositides (Berridge et al., 1982). ^{3}H-inositol was stored with a small amount of Dowex-1 anion-exchange resin in the formate form in order to maintain purity.

The specific radioactivity of PI increases linearly with increasing labeling incubation time, and the ratio of radioactivity in PIP and in PIP2 relative to PI remains constant (data not shown). Furthermore, the radioactivity present in ^{3}H-IP increases linearly with increasing labeling time, but the percentage-response to 5-HT is independent of labeling time (data not shown). Thus, different labeling times (1–3 hr) were used depending on the particular needs of the experiments. For Schild analyses, it was important to have maximum labeling of IP in order to increase the accuracy of EC_{50} estimates. For this reason, slices were labeled for 3 hr. After lesioning with 5,7-DHT, it was necessary to reduce incubation time to 1 hr in order to preserve the integrity of the tissue. In these samples, 2 μCi of ^{3}H-inositol was added rather than the usual 1 μCi. For all other experiments, labeling was for 2 hr.

Schild analyses

Antagonist K_d values at the phosphoinositide-linked receptor were estimated using the method of Arunlakshana and Schild (1959). Briefly, the concentration response curve of 5-HT was determined in the presence of various concentrations of antagonists. The 5-HT concentration–response curves were progressively shifted to the right with increasing concentrations of antagonists. EC_{50} values of 5-HT were determined at each antagonist concentration, and dose ratio (DR) values were calculated by dividing the EC_{50} of 5-HT in the absence of antagonist by the EC_{50} of 5-HT in the presence of antagonist. If the regression analysis of $\log(DR-1)$ versus $-\log$ antagonist concentration was consistent with a straight line with a slope of unity, this was interpreted as indicative of competitive antagonism (Arunlakshana and Schild, 1959). In cases where competitive antagonism was found, extrapolation of the regression line to the X-axis gave a value theoretically equal to the negative log of the K_d value of that antagonist for the phosphoinositide-linked receptor (pA2).

Monoamine levels

Monoamine levels in hippocampus were determined by high-pressure liquid chromatography with electrochemical detection (HPLC-EC). The

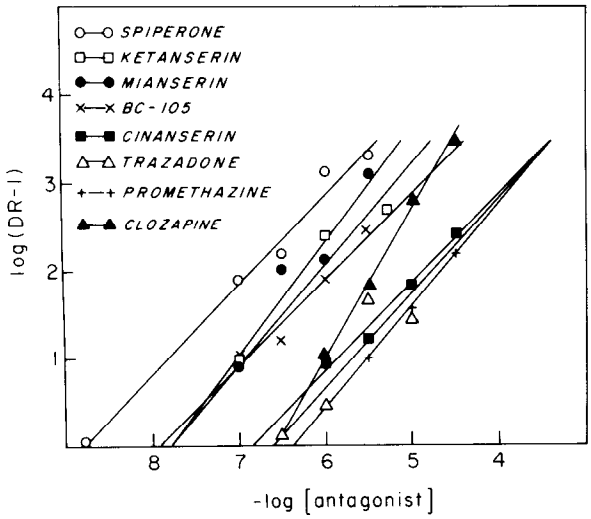

Figure 1. Schild regressions of the effects of antagonists at the phosphoinositide-linked 5-HT receptor. EC_{50} values for 5-HT at stimulating phosphoinositide hydrolysis were determined in the presence or absence of various concentrations of antagonists. The dose ratio (*DR*) was determined by dividing 5-HT's EC_{50} value in the presence of antagonist by the EC_{50} value in the absence of antagonist. Log (DR − 1) is plotted as a function of −log antagonist concentration. Each regression represents values determined from 4–8 5-HT concentration–response curves.

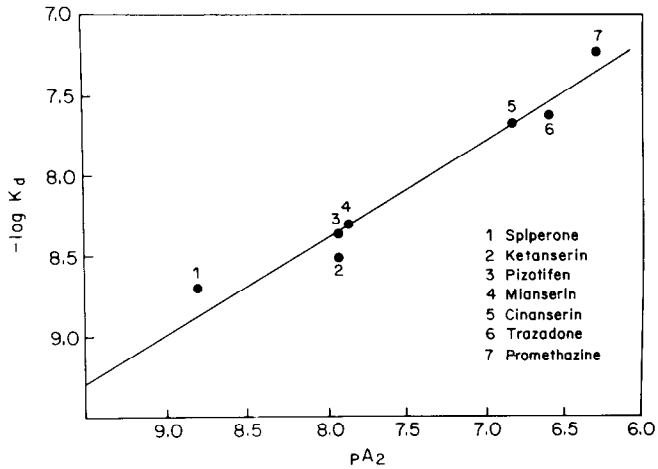

Figure 2. Comparison of Schild-determined K_d values versus K_i values determined from radioligand competition binding. Data are taken from Table 1. The correlation coefficient obtained from linear-regression analysis was 0.98 ($p < 0.001$).

HPLC apparatus consisted of a Bioanalytical Systems (West Lafayette, IN) model LC-304 liquid chromatograph equipped with an electrochemical detector and a glassy carbon electrode set at +0.75 V. A modification of the method of Mayer and Shoup (1983) was employed using a mobile phase of 6% acetonitrile in buffer, a column temperature of 25°C, and a single electrode. *N*-Methylserotonin was routinely added as an internal standard.

In vivo *drug treatments*

Male Sprague-Dawley rats were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Three different *in vivo* manipulations were used to alter 5-HT availability. For each of these treatments, a control group of animals was injected with vehicle only.

Mianserin-treated rats were injected subcutaneously with mianserin (5 mg/kg) daily for 10 consecutive days. Animals were sacrificed 48 hr after the last injection, and cerebral cortices were dissected and used for measurement of phosphoinositide hydrolysis, ^{3}H-ketanserin binding, or phospholipid levels.

PCPA-treated animals were injected intraperitoneally with 200 mg/kg daily for 4 consecutive days. Rats were sacrificed 48 hr after the last injection, and cerebral cortices were dissected and used for measurement of ^{3}H-ketanserin binding, phosphoinositide hydrolysis, or phospholipid levels. Hippocampi were dissected as described by Glowinski and Iversen (1966) for the determination of monoamine levels.

The neurotoxin 5,7-DHT was injected intraventricularly according to the method of Janowsky et al. (1982). Sixty minutes before injection of the neurotoxin, desipramine (25 mg/kg, i.p.) was administered to protect noradrenergic neurons (Björklund et al., 1975). 5,7-DHT, 150 μg dissolved in 20 μl of 0.9% saline containing 0.1% ascorbic acid, was injected into the lateral ventricle over a period of 5 min. Control rats received desipramine and an intraventricular injection of vehicle. Nine to 12 d after surgery, animals were sacrificed, and cerebral cortices were dissected and used for ^{3}H-ketanserin binding or for the measurement of phosphoinositide hydrolysis. The levels of monoamines in hippocampi were determined as an index of the effect of the lesion.

Table 1. Relationship between potencies at phosphoinositide-linked receptor and 5-HT-2 binding site

Antagonist	Schild plot				5-HT-2 binding,
	Slope	r	pA_2	K_d (nM)	K_d (nM)
Spiperone	1.0	0.97	8.80	2	2.0 ± 0.6
Ketanserin	1.1	0.96	7.93	12	3.1 ± 0.8
Pizotifen	1.0	0.97	7.92	12	4.4 ± 1.5
Mianserin	1.2	0.98	7.86	14	5.0 ± 0.4
Cinanserin	1.0	0.99	6.83	148	21 ± 6.2
Trazadone	1.0	0.91	6.60	240	24 ± 5.0
Clozapine	1.5	1.0			34 ± 6.4
Promethazine	1.2	1.0	6.30	500	52 ± 5.8

Binding of 0.6 nM ^{3}H-ketanserin to broken membranes was measured in buffer identical to that used in the phosphoinositide hydrolysis assays except that 25 mM sodium bicarbonate was replaced with 25 mM Tris. The K_d values at the phosphoinositide-linked receptor were determined by taking the antilog of the *X* intercept of the Schild regressions shown in Figure 1. The binding values represent the means ± SEM of 4–9 separate experiments each done in duplicate or triplicate.

Results

Schild analyses

The 5-HT antagonists—spiperone, ketanserin, pizotifen, mianserin, cinanserin, trazadone, clozapine, and promethazine—caused concentration-dependent rightward shifts of the 5-HT concentration response curves. The Schild regression lines of these data gave slopes close to unity for all antagonists except clozapine (Fig. 1, Table 1). The Schild slope of clozapine was 1.5, indicating that this drug may not interact with the phosphoinositide-linked receptor in a simple competitive manner. Thus, a K_d value of clozapine at the phosphoinositide-linked receptor could not be determined.

K_i values at the ^{3}H-ketanserin labeled 5-HT-2 binding site were estimated from competition binding data (Table 1). The rank order of the antagonists' potencies at the phosphoinositide-linked receptor was identical to the rank order for inhibition of binding to the 5-HT-2 site. Furthermore, regression analysis of the K_d values at the phosphoinositide-linked receptor versus K_i values at the 5-HT-2 site (Fig. 2) gave a correlation coefficient of 0.98 ($p < 0.001$). K_d values estimated by Schild analyses were generally higher than those determined in radioligand binding assays. Different tissue preparations (slices versus homogenates) were used for the two assays, which may explain the observed differences in potencies.

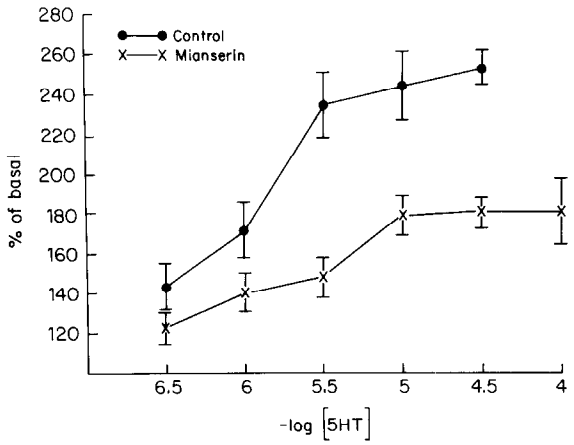

Figure 3. Effect of chronic mianserin treatment on 5-HT-stimulated phosphoinositide hydrolysis. Labeled cerebral cortical slices were incubated with increasing concentrations of 5-HT and the amount of radioactivity present in ^{3}H-IP was measured as described in Materials and Methods. Data are presented as the percentage of basal radioactivity (427 ± 42 cpm for control; 269 ± 45 cpm for treated). The values are the means of 4 separate experiments, each done in triplicate. Each experiment included a dose–response for 1 control and 1 treated animal. *Vertical bars,* SEM.

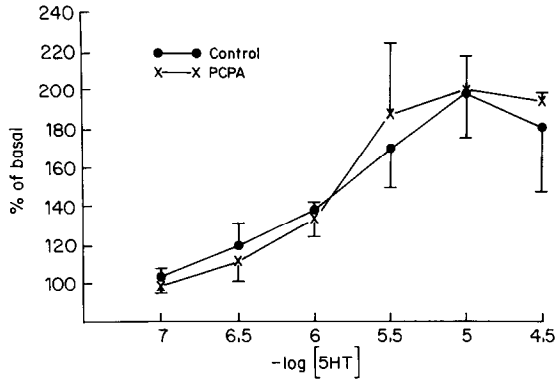

Figure 4. Effect of chronic PCPA on 5-HT-stimulated phosphoinositide hydrolysis. Phosphoinositide hydrolysis was measured as described in Figure 3 in cerebral cortical slices from rats that had received chronic administration of either PCPA or water. Data are presented as the percentage of basal radioactivity (303 ± 51 cpm for control; 460 ± 67 cpm for treated). The values are the means of 3 separate experiments each done in triplicate. Each experiment included a dose–response for 1 control and 1 treated animal. *Vertical bars,* SEM.

Chronic treatments

Scatchard analyses of ^{3}H-ketanserin binding in cerebral cortices of rats treated chronically with mianserin showed a significant reduction (48% of control) in the density of 5-HT-2 binding sites with no effect upon the K_d value (Table 2). Two-way analysis of variance (ANOVA) revealed a significant main effect of mianserin treatment ($p < 0.001$) upon 5-HT-stimulated phosphoinositide hydrolysis in cerebral cortical slices (Fig. 3). Mianserin treatment caused a significant reduction (51% of control) in the maximal phosphoinositide response elicited by 5-HT, with no change in the EC_{50} value. The EC_{50} values of 5-HT in control and treated rats were 1.3 ± 0.4 and 2.4 ± 1.0 μM, respectively. The specific radioactivity of PI and the relative radioactivity in the polyphosphoinositides were not altered by the treatment (Table 2).

Chronic administration of PCPA had no significant effect upon the density of 5-HT-2 binding sites or upon the affinity of ^{3}H-ketanserin at the 5-HT-2 binding site (Table 2). Furthermore, this treatment did not change the cerebral cortical phosphoinositide response to 5-HT (Fig. 4) or the radioactivity present in the phosphoinositides (Table 2). PCPA caused a greater than 97% reduction in the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) with no statistically significant effect upon levels of norepinephrine (data not shown).

Chemical denervation with 5,7-DHT resulted in a 90% depletion of 5-HT and 5-HIAA with no significant effect upon NE levels (data not shown). Treatment with 5,7-DHT did not change the density of 5-HT-2 binding sites or the K_d value of ^{3}H-ketanserin (Table 2). Furthermore, this treatment had no significant effect upon the maximum phosphoinositide response to 5-HT (Fig. 5). The 5-HT concentration response curve was slightly shifted to the left in lesioned animals but 2-way ANOVA revealed that the main effect of lesions was not statistically significant ($p = 0.09$). The EC_{50} value of 5-HT was less in 6 of the 7 lesioned rats compared with controls analyzed simultaneously: mean EC_{50} values in control and lesioned rats of 0.61 ± 0.1 and 0.38 ± 0.1, respectively. However, the difference in EC_{50} values did not reach statistical significance ($p = 0.1$). 5,7-DHT treatment had no effect upon the incorporation of ^{3}H-inositol into any of the phosphoinositides (Table 2).

Table 2. Effect of chronic treatments on ^{3}H-ketanserin binding and ^{3}H-phosphoinositides

	^{3}H-ketanserin binding		^{3}H-phosphoinositides					
			PI		PIP		PIP2	
Group	K_d (nM)	B_{max} (fmol/mg protein)	nCi	nCi/nmol	nCi	PI/PIP	nCi	PI/PIP2
Vehicle	1.1 ± 0.24	209 ± 25	30 ± 2.2	2.4 ± 0.18	6.2 ± 0.5	4.8	5.8 ± 0.84	5.2
Mianserin	2.2 ± 0.40	100 ± 1[a]	56 ± 1.8	1.9 ± 0.13	5.4 ± 0.7	4.8	4.0 ± 0.68	6.5
Vehicle	0.93 ± 0.07	172 ± 17	7.5 ± 0.96	0.38 ± 0.05	4.0 ± 0.4	1.9	2.5 ± 0.48	3.0
PCPA	0.89 ± 0.07	191 ± 26	8.9 ± 0.32	0.45 ± 0.02	4.4 ± 1.0	2.0	2.6 ± 1.10	3.4
Vehicle	0.61 ± 0.08	227 ± 19	43 ± 6.3	1.2 ± 0.18	8.0 ± 1.6	5.4	6.5 ± 1.4	6.6
5,7-DHT	0.67 ± 0.04	256 ± 18	51 ± 5.1	1.8 ± 0.18	10.0 ± 1.3	5.1	8.0 ± 1.2	6.4

Scatchard analyses of ^{3}H-ketanserin binding to the 5-HT-2 site were performed in drug-treated and vehicle-treated rats. Data for the mianserin/vehicle treatment are means ± SEM for 3 rats/group; for PCPA/vehicle, 4 rats/group; and for the 5,7-DHT/vehicle treatment, 7 rats/group. For phosphoinositide determinations, cerebral cortical slices (200 μl) were incubated with ^{3}H-inositol as described in Materials and Methods. Lipids were extracted and separated by TLC. Spots were scraped, and radioactivity present in each phosphoinositide was measured. Phosphorus content in PI was measured and specific radioactivity was calculated. The values are means ± SEM of 3–4 separate determinations.

[a] $p < 0.01$.

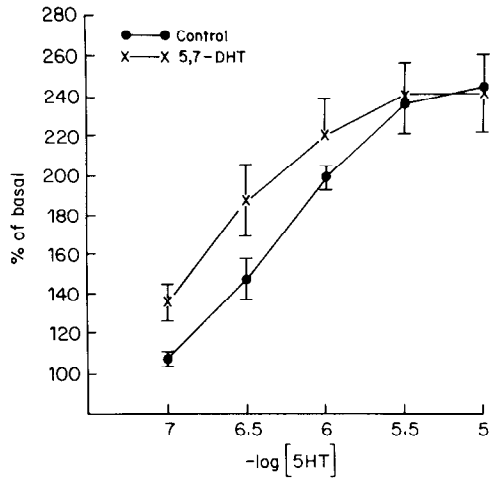

Figure 5. Effect of 5,7-DHT lesions on 5-HT-stimulated phosphoinositide hydrolysis. Phosphoinositide hydrolysis was measured as described in Figure 3 in cerebral cortical slices from rats that had received intraventricular injection of either 5,7-DHT or vehicle. Data are presented as the percentage of basal radioactivity (657 ± 62 cpm for control; 628 ± 97 cpm for treated). The values are the means of 7 separate experiments each done in triplicate. Each experiment included a dose–response for 1 control and 1 treated animal. *Vertical bars,* SEM.

Discussion

The present results confirm previous reports that 5-HT-2 antagonists inhibit 5-HT-stimulated phosphoinositide hydrolysis (Conn and Sanders-Bush, 1984, 1985; de Chaffoy de Courcelles et al., 1985; Kendall and Nahorski, 1985; Roth et al., 1984) and extend those findings by showing that a significant correlation exists between Schild analysis determined K_d values at the cerebral cortical phosphoinositide-linked receptor and K_i values at the 5-HT-2 binding site. The phosphoinositide response to some agonists does not reflect a direct coupling of a receptor to phosphoinositide hydrolysis but is secondary to agonist-induced increases in neurotransmitter release (Bone and Michell, 1985) or arachidonate metabolism (Rittenhouse, 1984; Watson et al., 1985). We have previously shown that 5-HT's effect on phosphoinositide hydrolysis in cerebral cortical slices apparently is not secondary to either of these indirect mechanisms (Conn and Sanders-Bush, 1986a). Taken together, these data provide strong evidence that phosphoinositide hydrolysis is the transducing mechanism linked to the 5-HT-2 receptor.

5-HT stimulates phosphoinositide hydrolysis in rat choroid plexus with a pharmacology similar to that of the 5-HT-1c binding site (Conn et al., 1986). This recently described binding site is present in high density in the choroid plexus (Pazos et al., 1984; Yagaloff and Hartig, 1985). The 5-HT-1c-mediated phosphoinositide response apparently is not mediated by 5-HT-stimulated arachidonate metabolism or neurotransmitter release (Conn and Sanders-Bush, 1986b). Thus, at least 2 5-HT receptor subtypes appear to utilize phosphoinositide hydrolysis for signal transduction. The properties of the phosphoinositide response to activation of these 2 receptors has recently been reviewed (Sanders-Bush and Conn. 1986a, b).

Given the evidence that the 5-HT-2 site is directly coupled to phosphoinositide hydrolysis, we employed this response as a measure of 5-HT-2 receptor activation in order to determine the effect of *in vivo* manipulations of 5-HT availability upon 5-HT-2 receptor responsiveness. Consistent with previous reports (Blackshear and Sanders-Bush, 1982; Blackshear et al., 1983), chronic administration of the 5-HT-2 antagonist, mianserin, resulted in a marked decrease in the density of 5-HT-2 binding sites (48% of control), with no effect upon the K_d of ^{3}H-ketanserin. This was accompanied by a corresponding decrease in the maximum phosphoinositide response to 5-HT (52% of control), with no effect upon the EC_{50} value of 5-HT. The excellent agreement between the percentage decrease in the density of the 5-HT-2 binding site and the maximum phosphoinositide response to 5-HT further supports the hypothesis that the 5-HT-2 site is linked to phosphoinositide hydrolysis. Furthermore, this finding demonstrates that chronic mianserin-induced down-regulation of the 5-HT-2 site is accompanied by 5-HT-2 receptor subsensitivity. It is unclear why 5-HT-2 antagonists induce this paradoxical desensitization, and it remains a challenge to determine the mechanism by which mianserin and other 5-HT-2 antagonists induce such changes.

Also consistent with previous reports is the finding that chemical lesioning of 5-HT neurons with 5,7-DHT or the administration of PCPA results in profound, selective depletion of 5-HT and its metabolite but has no effect upon the density of the 5-HT-2 binding site (Blackshear et al., 1981; Quik and Azmita, 1983; Seeman et al., 1980). Destruction of noradrenergic neurons has a similar lack of effect upon α_1-adrenergic receptor density. However, noradrenergic denervation induces a supersensitive α_1-mediated phosphoinositide response (Akhtar and Abdel-Latif, 1986; Janowsky et al., 1984; Kendall et al., 1985; Zatz, 1985). This suggests that the α_1-receptor is regulated by alterations in receptor–effector coupling rather than alterations in receptor density. Given the possibility that the 5-HT-2 receptor system is regulated in a similar manner, it was of interest to determine the effect of serotonergic denervation or depletion upon 5-HT-2-mediated phosphoinositide hydrolysis. The current results show that 5-HT-stimulated phosphoinositide hydrolysis was unchanged following these treatments, suggesting that regulation of the 5-HT-2 receptor system is not analogous to regulation of the α_1-adrenergic receptor.

Lesioning of 5-HT neurons with 5,7-DHT does result in supersensitive 5-HT-2-mediated behavioral responses (Nakamura and Fukushima, 1978; Yamamoto and Ueki, 1981), and some reports indicate that chronic administration of 5-HT-2 antagonists does the same (Friedman et al., 1983; Mogilnicka and Klimek, 1979; Stolz et al., 1982). Evidently, this supersensitivity reflects a change that occurs distal to the receptor/effector complex, since neither the binding nor the transducing mechanism is altered. An example of such regulation exists in 1321N1 astrocytoma cells in which sensitivity to muscarinic agonists is regulated by changes in sensitivity of the cell to inositol trisphosphate, a second messenger released upon stimulation of phosphoinositide hydrolysis. This is not accompanied by alterations in muscarinic binding properties or in carbachol-induced phosphoinositide hydrolysis (Masters et al., 1985). Another possible mechanism of regulation, which exists in the CNS, is an alteration of the activity of a neuronal system that is antagonistic to the serotonergic systems involved in these 5-HT-2-mediated behavioral responses. If activity of such a system is decreased following 5-HT denervation, it could result in supersensitive behavioral responses to 5-HT.

Since the assay system used in this study measures the release of radioactivity in IP as an index of phosphoinositide hydrolysis, it is conceivable that *in vivo* manipulations could alter the specific radioactivity of the phosphoinositide precursors of IP and thus compromise interpretation of the results. However, none of the *in vivo* drug treatments altered the specific radioactivity of PI or the relative radioactivity in PIP or PIP2. These results suggest that the radioactivity present in IP has the same relation to IP mass in treated and in untreated rats. However, these data do not rule out the possibility that the manipulations specifically alter small phosphoinositide pools that are directly accessible to the 5-HT-2 receptor.

In conclusion, the present study provides further evidence that phosphoinositide hydrolysis is the transducing system of

the 5-HT-2 receptor. Furthermore, this study shows that antagonist-induced 5-HT-2 receptor down-regulation is accompanied by 5-HT-2 receptor subsensitivity and that denervation has no effect upon the 5-HT-2 binding site or upon 5-HT-2 receptor sensitivity. Future studies should be aimed at determining the mechanism of development of supersensitivity to 5-HT-2 activation seen in behavioral models of 5-HT-2 activity.

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