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Paradoxical Increase of 5-Hydroxytryptamine$_2$ Receptors and 5-Hydroxytryptamine$_2$ Receptor mRNA in Cerebellar Granule Cells after Persistent 5-Hydroxytryptamine$_2$ Receptor Stimulation

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SUMMARY

Rat cerebellar granule cells express 5-hydroxytryptamine (5-HT)$_2$ receptors that mediate phosphoinositide turnover by a pertussis toxin-sensitive mechanism. Prestimulation of these neurons with 10 μM 5-HT or (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane [(±)-DOI], a putative 5-HT$_2$ receptor agonist, resulted in a time-dependent desensitization of the phosphoinositide response to 5-HT. The desensitization was detected within 30 min after prestimulation and reached a maximum (about 80%) decrement at 8 hr. However, [^{3}H]ketanserin binding to 5-HT$_2$ receptors in crude membranes or intact cerebellar granule cells was increased by treatment with 5-HT or DOI, in a time- and concentration-dependent manner. The increase occurred after the onset of desensitization and was fully manifest (about 160–190%) at 4 hr after stimulation. Although the B_{max} and K_d were unchanged at 1 hr after 5-HT or DOI treatment, both parameters were significantly increased at 4 and 24 hr. The amount of 5-HT$_2$ receptor mRNA detected by Northern blot hybridization using a 5-HT$_2$ receptor-specific riboprobe was increased in parallel with the up-regulation of 5-HT$_2$ receptor binding sites. Thus, an increase in 5-HT$_2$ receptor mRNA was detected within 2 hr after 5-HT or DOI prestimulation, reached a maximum around 4 hr, and remained at a plateau for at least 24 hr. The levels of total RNA, m_3 muscarinic acetylcholine receptor mRNA, and β-actin mRNA were not significantly affected by these treatments. Our results demonstrated that 5-HT$_2$ receptor binding sites and their mRNA undergo a paradoxical induction during persistent agonist stimulation.

5-HT induces an array of physiological responses in the central and peripheral nervous systems. These include cognition, sleep, mood control, vascular smooth muscle contraction, gastrointestinal tone, and platelet aggregation (see Refs. 1–3 for reviews). 5-HT has also been implicated in the etiology of depression, migraine headache, vasospasm, and pulmonary hypertension (see Ref. 4 for review). There is ample evidence suggesting that 5-HT elicits its effects by interacting with multiple receptor subtypes linked to specific second messengers (2, 3). One receptor subtype, 5-HT$_2$, is coupled to PI turnover, which was first demonstrated in the brain (5, 6) and aortic smooth muscles (7).

Regulation of 5-HT$_2$ receptors has been studied in animals chronically treated with several putative 5-HT$_2$ receptor agonists (8–11). These treatments result in a decrease in cortical 5-HT$_2$ receptor binding sites (12) and 5-HT$_2$ receptor-mediated behavioral (head-shake) responses (13, 14). Molecular and biochemical mechanisms underlying the regulation of central 5-HT$_2$ receptors remain poorly understood, largely because of the complexity of neuronal networks and heterogeneity of cell types. Primary cultures of neurons and neuronal cell lines have proven to be useful as model systems to study 5-HT$_2$ receptor regulation. Initially, we demonstrated that primary cultures of rat cerebellar granule cells express 5-HT$_2$ receptors coupled to phospholipase C (15) and that prestimulation of these neurons produces a time-dependent homologous desensitization (16). Ivins and Molinoff (17), using the rat pituitary tumor cell line P11, reported a similar homologous desensitization of the 5-HT$_2$ receptor-mediated PI response after 5-HT pretreatment. A loss of [^{3}H]lysergic acid diethylamide binding to 5-HT$_2$ receptors appeared to follow the onset of desensitization in P11 cells. In this study we further characterized 5-HT$_2$ receptor-mediated PI turnover and its desensitization in cerebellar granule cells, using either 5-HT or DOI as the receptor agonist. Surprisingly, we found that pretreatment with either 5-HT$_2$ receptor agonist produces an increase in the density of [^{3}H]ketanserin binding to 5-HT$_2$ receptors. This up-regulation occurs after the initiation of desensitization and is associated with a selective increase of 5-HT$_2$ receptor mRNA detected by Northern blot hybridization using a labeled riboprobe directed against 5-HT$_2$ receptor mRNA.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); DOI, (±)-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; PI, phosphoinositide; PTX, pertussis toxin; IP$_1$, inositol monophosphate; PSS, physiological salt solution; mAChR, muscarinic acetylcholine receptor; G protein, guanine nucleotide-binding protein; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Experimental Procedures

Materials. Cell culture medium and fetal calf serum were purchased from GIBCO (Grand Island, NY). *myo*-[^{3}H]Inositol (16.5 Ci/mmol) and [^{3}H]ketanserin (60.0 Ci/mmol) were products of New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Primary cultures of cerebellar granule cells. Cerebellar granule cells were prepared and cultured as described (15). Briefly, cerebella were removed from 8-day-postnatal Sprague-Dawley rats, chopped into 0.4-mm cubes, washed, and trypsinized in Krebs-Ringer solution to dissociate the cells. The cells were then suspended in basal modified Eagle's medium containing 10% fetal calf serum, 2 mM glutamine, 50 μg/ml gentamicin, and 25 mM KCl and were plated onto polylysine-precoated 35- or 60-mm Petri dishes, at a density of approximately 3 or 10 × 10^6 cells/dish. The cells were maintained at 37° in the presence of 6% CO_2 in a humidified incubator. Cytosine arabinoside (10 μM) was added 18–24 hr later to arrest the replication of non-neuronal cells, particularly glial cells. Unless otherwise specified, cells were treated on the eighth day in culture by the addition of drugs dissolved in the growth medium. During this time, cerebellar granule cells differentiate in culture into glutamatergic neurons with a purity of 92–95% and with glial cell contamination of only 2–3% (18, 19). For time course studies, drugs were added sequentially, and the experiments were terminated at the same time to vary the time of treatment.

Measurements of PI hydrolysis. PI hydrolysis was measured by monitoring the accumulation of [^{3}H]IP_1 in the presence of 5 mM LiCl in cells that had been prelabeled with *myo*-[^{3}H]inositol, as described (15). Briefly, cells cultured in 35-mm dishes for 7 days were labeled with *myo*-[^{3}H]inositol (2.5 μCi/dish) for 24 hr, washed, and preincubated in PSS (118 mM NaCl, 4.7 mM KCl, 3.0 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, 0.5 mM EDTA, 10 mM glucose, 20 mM HEPES, pH 7.4) containing 5 mM LiCl. The indicated 5-HT receptor agonist was then added and the incubation was allowed to proceed for 45 min at 37°; during this time, [^{3}H]IP_1 was linearly accumulated. The reaction was terminated by addition of ice-cold methanol and the accumulation of [^{3}H]IP_1 was measured by anion exchange chromatography (AG-1 × 8, formate form, 100–200 mesh).

Northern blotting. Total RNA was isolated by the method of Chirgwin *et al.* (20), from cerebellar granule cells grown in 60-mm dishes. The RNA was then rinsed with 75% ethanol and dissolved without further manipulation in formaldehyde gel sample buffer. A one-tenth aliquot of the sample was used for the quantification of total RNA, and the remaining material was electrophoresed on 1% agarose-formaldehyde gels, blotted, and fixed to nitrocellulose by baking.

An *Alu*I fragment of the 5-HT_2 receptor genomic clone RR13 (base pairs 636–914), containing a portion of the first exon subcloned into the *Eco*RV site of the pBluescript KS$^-$ vector, was kindly provided by Dr. Bryan L. Roth (Laboratory of Biological Psychiatry, Case Western Reserve University, Cleveland, OH). The m_3-mAChR probe was made from a fragment of the cDNA clone designated Rm3 p8 and was generously provided by Dr. Tom I. Bonner (Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD). The β-actin probe was derived from a cDNA clone of the chicken β-actin gene (21) and was kindly supplied by Dr. Craig B. Thompson (Howard Hughes Medical Center, Ann Arbor, MI). The high stringency washes for hybridized blots were performed at 65° for 5-HT_2 and m_3-mAChR riboprobes and at 56° for the β-actin cDNA probe, in 0.1 × standard saline citrate (1× standard saline citrate is 0.15 M NaCl, 15 mM sodium citrate, pH 7) containing 0.1% sodium dodecyl sulfate.

Specific 5-HT_2 and m_3-mACh receptor mRNA bands were quantified by using a βetagen βetascope (Waltham, MA). Total RNA was quantified by image analysis of photographic negatives taken of ethidium bromide-stained conventional agarose gels on which all the samples had been electrophoresed (in parallel with RNA standards) only far enough to enter the gel. Specific hybridization of the probes was then normalized to total cellular RNA or β-actin mRNA in each sample before comparisons were made between the experimental and control groups. Estimation of the molecular size of 5-HT_2 mRNA was performed using RNA standards (BRL, Gaithersburg, MD) electrophoresed in parallel.

Measurement of [^{3}H]ketanserin binding. For assays of [^{3}H] ketanserin binding to crude membranes, cells grown in 100-mm dishes were washed three times with PSS at room temperature after agonist treatment, scraped off the dishes, and homogenized with a Polytron for 15 sec in 50 mM Tris·HCl, pH 7.4. The homogenates were centrifuged at 30,000 × *g* for 15 min and the pellets were resuspended using a Polytron homogenizer in a buffer containing 50 mM Tris·HCl, pH 7.4, 0.5 mM EDTA, and 10 mM $MgCl_2$. The crude membranes were

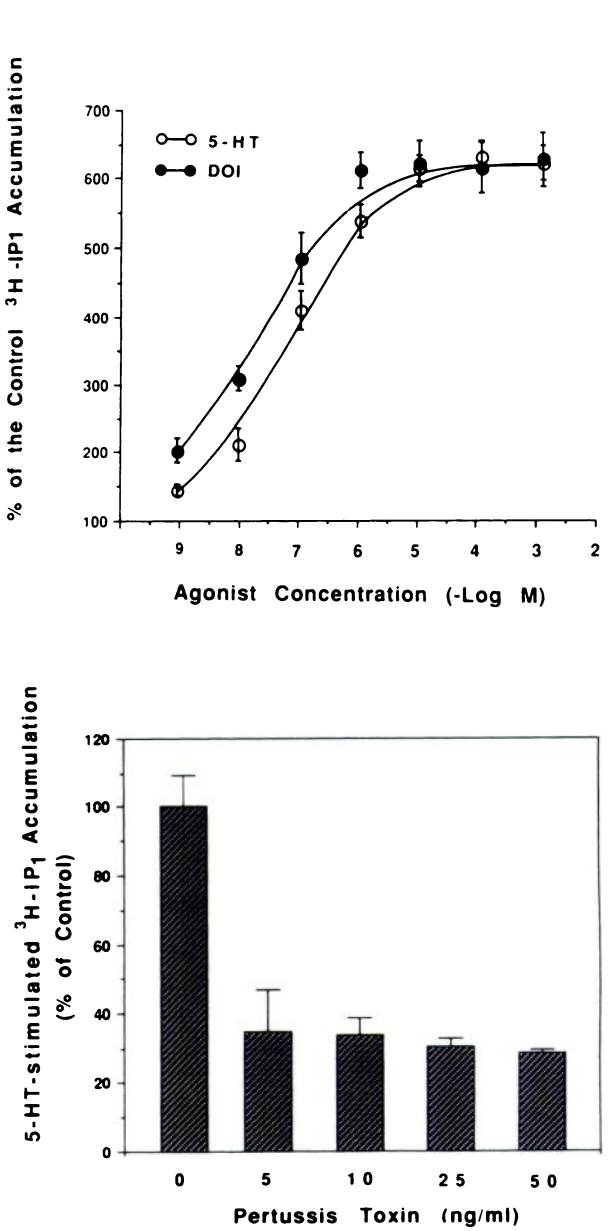

Fig. 1. 5-HT_2 receptor-mediated [^{3}H]IP_1 accumulation: agonist dose dependence and PTX sensitivity. A, Cells, after 8 days in culture, were prelabeled with *myo*-[^{3}H]inositol and then stimulated with various concentrations of 5-HT or DOI for the measurement of lithium-dependent accumulation of [^{3}H]IP_1 accumulation. B, Cells were pretreated with the indicated concentrations of PTX for 24 hr. [^{3}H]IP_1 accumulation was then measured in the presence of 100 μM 5-HT. The values are the mean ± standard error of a quadruplicate experiment that has been repeated three times with similar results. Basal (100%) [^{3}H]IP_1 accumulation in A was 435 ± 58 dpm/dish. The maximal 5-HT-stimulated (100%) activity in B was 2648 ± 219 dpm/dish.

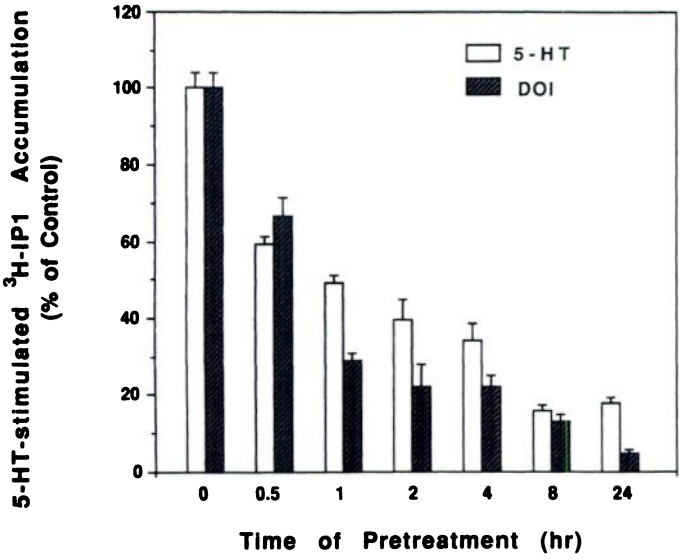

Fig. 2. Time course of 5-HT- and DOI-induced desensitization of 5-HT-induced PI turnover. Cells were pretreated 10 μM 5-HT or DOI for the indicated time periods and then washed three times with PSS. The pretreated cells were challenged with 100 μM 5-HT for induction of [^{3}H] IP$_1$ accumulation. Details of the experiments were as described in Experimental Procedures and Ref. 16. The values are the mean ± standard error of a quadruplicate experiment that has been repeated three times with similar results. At time 0, the values for 5-HT- and DOI-induced [^{3}H] IP$_1$ accumulation were 3031 ± 104 and 2961 ± 127 dpm/dish, respectively.

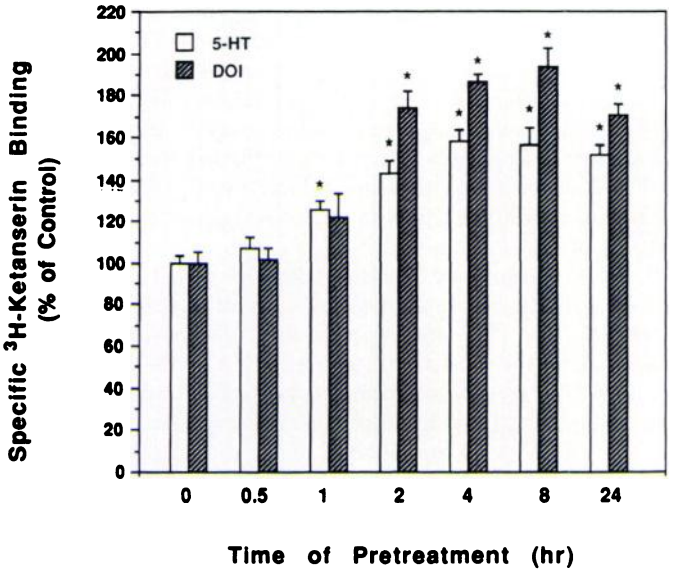

Fig. 3. 5-HT- or DOI-induced increase of [^{3}H]ketanserin binding to 5-HT$_2$ receptors in crude membranes. Cells were pretreated with 10 μM 5-HT or DOI for the indicated time periods and then washed three times with PSS at room temperature to remove the prestimulating agonist. Crude membranes were prepared and binding of [^{3}H]ketanserin (10 nM) to 5-HT$_2$ receptors was performed. The values are the mean ± standard error of a triplicate experiment that has been repeated three times with nearly identical results. The time 0 (100%) values were 116 ± 5 and 122 ± 7 fmol/mg of protein for 5-HT- and DOI-treated groups, respectively. The specific activity of [^{3}H]ketanserin was 169 ± 2 dpm/fmol. *, $p < 0.01$, compared with the respective time 0 control.

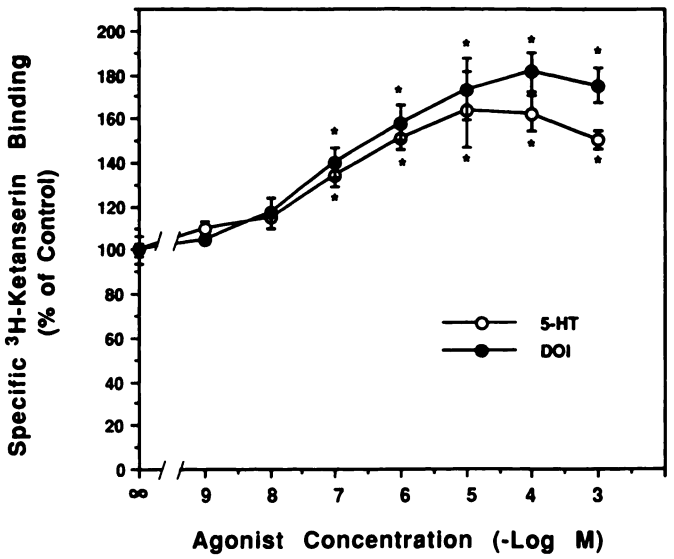

Fig. 4. Dose-dependent effect of 5-HT pretreatment on [^{3}H]ketanserin binding to 5-HT$_2$ receptors. Cells were pretreated with the indicated concentration of 5-HT or DOI for 24 hr and [^{3}H]ketanserin (10 nM) binding to membranes was then measured as described in Experimental Procedures. The data presented are mean ± standard error of a triplicate experiment that has been repeated three times with similar results. The untreated control (100%) value was 127 ± 4 fmol/mg of protein. *, $p < 0.01$, compared with the untreated control.

TABLE 1

Scatchard analysis of [^{3}H]ketanserin binding to membranes of cells treated with 5-HT or DOI

Cerebellar granule cells were pretreated with 10 μM 5-HT or DOI for 24 hr and then washed three times with PSS at room temperature to remove the prestimulating agonist. Membranes were then prepared and [^{3}H]ketanserin binding was performed as described in Experimental Procedures, using ligand concentrations varying from 0.5 to 8 nM. Nonspecific binding was determined in the presence of 1 μM methysergide. The B_{max} and K_d values were derived from the means of triplicate determinations of specific binding at each ligand concentration. The values presented are means ± standard errors from four independent experiments.

Treatment conditions	B_{max}	K_d
	fmol/mg of protein	*nM*
Control		
1 hr	122 ± 8	0.6 ± 0.1
5-HT	143 ± 11	0.7 ± 0.1
DOI	149 ± 15	0.8 ± 0.2
4 hr		
5-HT	185 ± 9*	1.5 ± 0.3*
DOI	248 ± 25*	1.7 ± 0.1*
24 hr		
5-HT	181 ± 17*	2.3 ± 0.2*
DOI	252 ± 31*	2.9 ± 0.3*

*$p < 0.01$, compared with the untreated control.

washed once by centrifugation and resuspended in the aforementioned buffer, and aliquots were used for binding assays. The binding mixtures (100–150 μg/ml membrane proteins) were incubated for 2 hr at 2° in 1 ml of the aforementioned buffer containing 0.1% ascorbate, 10 μM pargyline, and 10 nM [^{3}H]ketanserin. The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters, using a Brandel 48 R cell harvester. Dried filters were counted for tritium radioactivity in ACS scintillation fluid (New England Nuclear, Boston, MA). Nonspecific binding was determined in the presence of 1 μM methysergide and was subtracted from total binding to obtain specific binding, which represented approximately 65% of total binding. All experiments were performed in triplicate.

For binding to intact cells, monolayer cultures grown on 35-mm dishes were washed three times with 1 ml of PSS and then incubated with the indicated concentration of [^{3}H]ketanserin in 2 ml of PSS for 75 min at 37°. Nonspecific binding was subtracted from total binding to obtain specific binding, which was >60% of total binding. The binding reaction was terminated by aspiration of the binding mixture followed by three rapid washes with 3 ml of ice-cold PSS. The cells were solubilized with 1.0 ml of 3% Triton X-100, scraped, and trans-

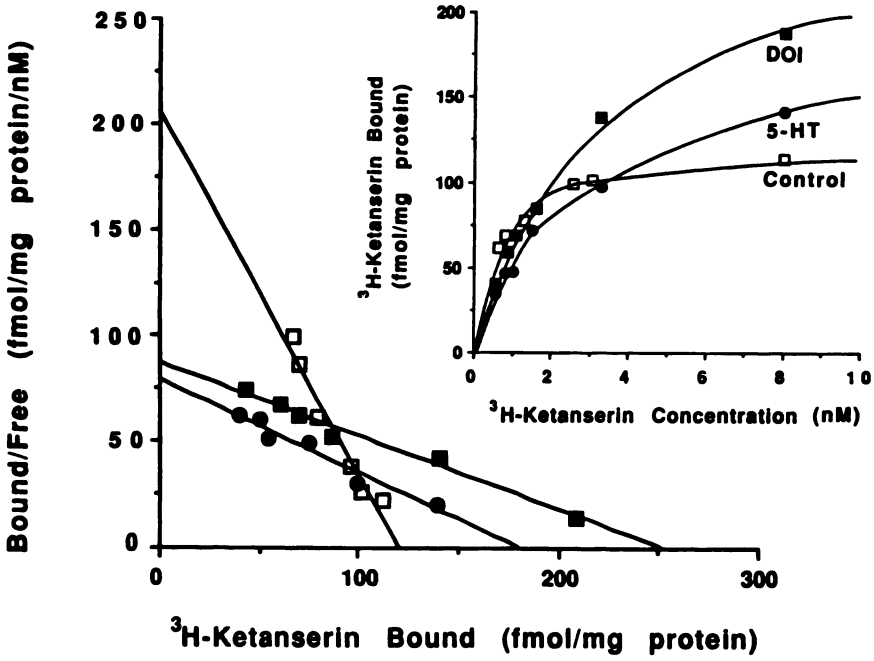

Fig. 5. Scatchard analysis of [^{3}H]ketanserin specific binding to 5-HT$_2$ receptors in membranes: effects of 5-HT or DOI pre-exposure for 24 hr. Cerebellar granule cells were pretreated with 10 μM 5-HT or DOI for 24 hr. Membranes were then prepared and [^{3}H]ketanserin binding was performed as described in the legend to Table 1 and Experimental Procedures. Nonspecific binding was determined in the presence of 1 μM methysergide. The values presented are means of triplicate determinations, from a typical experiment, of the saturation binding (inset) and Scatchard analysis of the specific binding data. The experiment has been repeated three times with similar results.

ferred to a vial for counting of radioactivity. Radioligand binding data were analyzed with the LIGAND program (22).

Statistical analysis. All data are expressed as means ± standard errors. Data from the experiments were analyzed by means of a one-way analysis of variance followed by Dunnett's *t* test. Protein was determined by the method of Smith *et al.* (23).

Results

Stimulation of prelabeled cerebellar granule cells with 5-HT or DOI, a putative 5-HT$_2$ receptor agonist, resulted in a 500–700% increase in [^{3}H]IP$_1$ accumulation. The effects of 5-HT and DOI were concentration dependent, with EC$_{50}$ values of 80 and 29 nM, respectively (Fig. 1A). The 5-HT-stimulated activity was inhibited by 24-hr pretreatment with PTX, in a dose-dependent manner (Fig. 1B), suggesting that a PTX-sensitive G protein is involved in the coupling of the 5-HT$_2$ receptor to phospholipase C. Prestimulation of granule cells with 10 μM 5-HT or DOI produced a time-dependent loss of [^{3}H]IP$_1$ accumulation in response to rechallenge with 5-HT. The desensitization was detectable within 30 min and was maximal (about 80% decrease) at 8 hr after prestimulation (Fig. 2). We have demonstrated that 5-HT-induced desensitization is homologous and associated with a loss in the maximal extent of [^{3}H]IP$_1$ accumulation (16).

To determine what changes in the binding characteristics of 5-HT$_2$ receptors occurred during the course of 5-HT-induced desensitization, specific binding of [^{3}H]ketanserin to 5-HT$_2$ receptors was compared in untreated and 5-HT- or DOI-pretreated cells. Surprisingly, specific binding of [^{3}H]ketanserin (10 nM) to a membrane preparation was increased after treatment with 10 μM 5-HT or DOI (Fig. 3). In both cases, the increase in binding was evident between 1 and 2 hr and was maximal (about 160–190% of the control) approximately 4 hr after pretreatment. At 24 hr, the increase was still evident in 5-HT- or DOI-pretreated cells. A similar time course for the 5-HT-induced increase of [^{3}H]ketanserin binding was observed when the binding was performed using a subsaturating concentration (2 nM) of [^{3}H]ketanserin in membranes or intact cerebellar granule cells (data not shown). The up-regulation of [^{3}H]ketanserin binding induced by 24-hr pretreatment with 5-HT or DOI was concentration dependent (Fig. 4). In both cases, the increase was significant at 0.1 μM and the maximal effect was seen at approximately 10 μM. Scatchard analysis revealed that treatment of cells with 10 μM 5-HT or DOI resulted in a time-dependent increase in both the B_{max} and the K_d for [^{3}H]ketanserin binding to membrane preparations (Table 1). The B_{max} values were unchanged at 1 hr but markedly increased at 4 and 24 hr after 5-HT or DOI stimulation. Similarly, in 5-HT- or DOI-treated cells the K_d values were unaltered at 1 hr but significantly increased at 4 hr and further increased at 24 hr. Fig. 5 shows a typical Scatchard analysis of [^{3}H]ketanserin binding to membranes derived from cells treated with 5-HT or DOI for 24 hr.

We then determined whether the level of 5-HT$_2$ receptor mRNA was changed in association with an apparent increase in the B_{max} of [^{3}H]ketanserin binding sites. Northern blot hybridization revealed that a labeled RNA probe specific for the rat 5-HT$_2$ receptors hybridized to a mRNA species with a size of approximately 5.2 kilobases. A similar size for 5-HT$_2$ receptor mRNA has been reported in rat brain cortex (24). The amount of 5-HT$_2$ receptor mRNA was increased to 130 ± 7% of the control at 1 hr after 5-HT treatment and further increased to 158 ± 1% at 4 hr and to 152 ± 1% at 24 hr (Fig. 6). Similarly, in cells treated with DOI the 5-HT$_2$ receptor mRNA level was increased to 149 ± 2% of the control at 2 hr and further increased to 179 ± 28% and 212 ± 15% at 4 and 24 hr, respectively (Fig. 7). The levels of total RNA and the amount of mRNA for m_3-mAChR and β-actin derived from the same blot were not significantly changed throughout the time course of treatment with 5-HT or DOI, indicating the selectivity of this modulation.

Discussion

In this report, we extended our previous studies on 5-HT$_2$ receptor-mediated PI turnover in cerebellar granule cells using 5-HT or DOI as the receptor agonist. Pretreatment with either agonist elicited a rapid (≤0.5 hr), time-dependent desensitization of the PI response. Unexpectedly, we found that 5-HT or

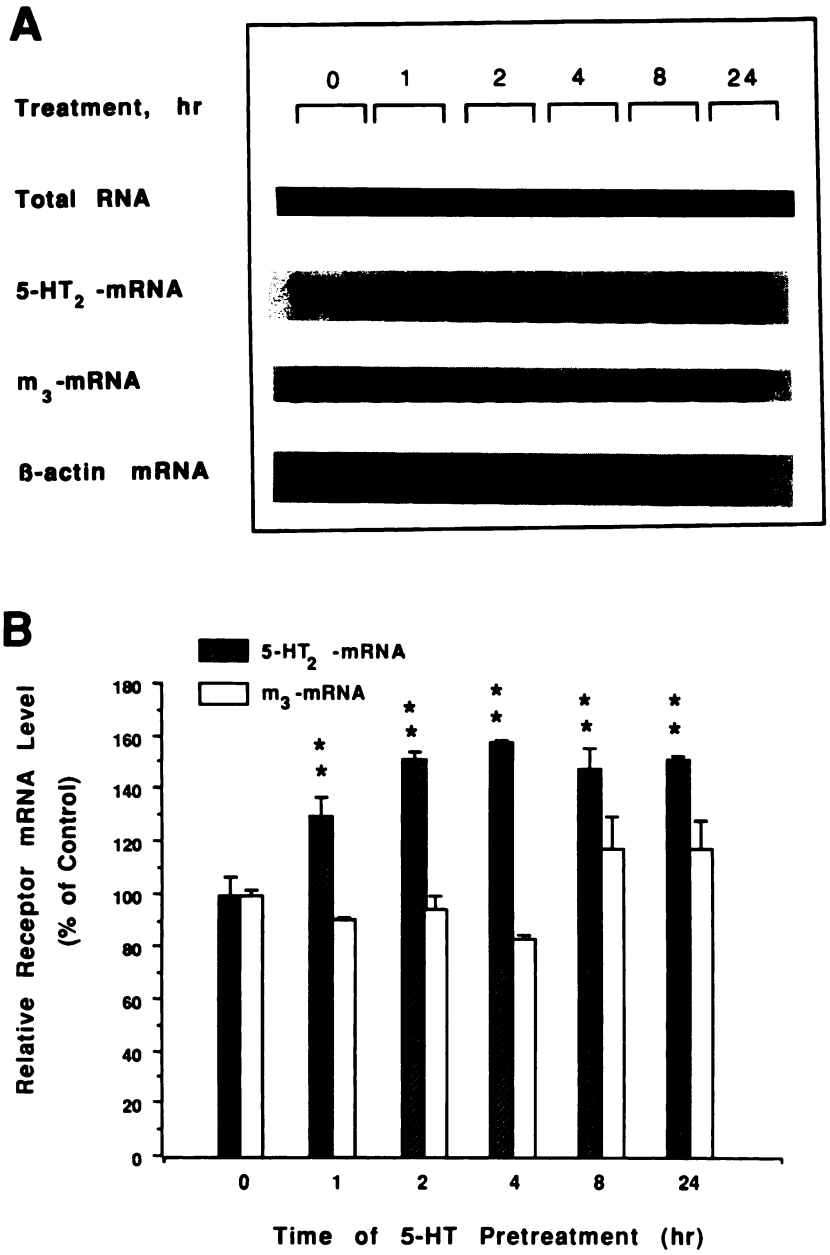

Fig. 6. Time course of changes of 5-HT$_2$ receptor mRNA after treatment with 10 μM 5-HT. Cerebellar granule cells after 8 days in culture were exposed to 10 μM 5-HT for indicated periods of time, by sequential addition of 5-HT. A, Autoradiographs of blots hybridized to probes for 5-HT$_2$ receptor, m$_3$-mAChR, and β-actin mRNAs. B, Quantification by a βetagen βetascope of autoradiograms. Levels of 5-HT$_2$ receptor mRNA have been normalized to total cellular RNA at each time point. The data represent the mean ± standard deviation of duplicate samples. The experiment has been repeated three times with virtually identical results. **, $p < 0.05$, compared with the time 0 control.

DOI pretreatment induced a delayed (>1 hr) increase in the B_{max} of [^{3}H]ketanserin binding to 5-HT$_2$ receptors in membranes or intact cells. Northern blot hybridization demonstrated that the increased density of 5-HT$_2$ receptor binding sites was paralled by an increased level of 5-HT$_2$ receptor mRNA. Neither total RNA nor the level of mRNA for m$_3$-mAChR or β-actin was significantly changed by 5-HT or DOI treatment. One may argue that the up-regulation of 5-HT$_2$ receptors by 5-HT is secondary to an activation of other receptor subtypes, such as 5-HT$_{1A}$ and 5-HT$_{1C}$, in granule cells. However, the observation that a similar effect was observed using the putative 5-HT$_2$ receptor agonist DOI argues against this possibility. Taken together, these data suggest that persistent 5-HT$_2$ receptor activation enhances the expression of this receptor, probably by transcriptional and/or post-transcriptional regulation. The temporal association between the increase in [^{3}H]ketanserin binding and the up-regulation of 5-HT$_2$ receptor mRNA also suggests that the level of 5-HT$_2$ receptor mRNA dictates the density of receptor binding sites. In this context, the observation by Roth *et al.* (25) that 5-HT$_2$ receptor protein and its mRNA were increased in parallel in the rat brain during development also suggests a prominent role for 5-HT$_2$ receptor mRNA level in the expression of this 5-HT receptor subtype.

The up-regulation of 5-HT$_2$ receptor binding sites and mRNA contrasts sharply with the down-regulation of mAChR observed in cerebellar granule cells pretreated with the mAChR agonist carbachol (26). In those studies, we found that carbachol pretreatment induces a homologous desensitization and subsequent loss of mAChR binding sites and differential down-regulation of m$_2$- and m$_3$-mAChR mRNA. The molecular mechanisms underlying the distinct regulation of 5-HT$_2$ and mAChR mRNA are unclear. In this study, we found that the concentration of 5-HT that elicits an increase in 5-HT$_2$ binding sites and their mRNA lies within the concentration range of 5-HT that stimulates PI hydrolysis. However, the role of inositol trisphosphate and/or diacylglycerol in stimulating 5-HT$_2$ receptor expression remains to be defined. In smooth muscle cells 5-

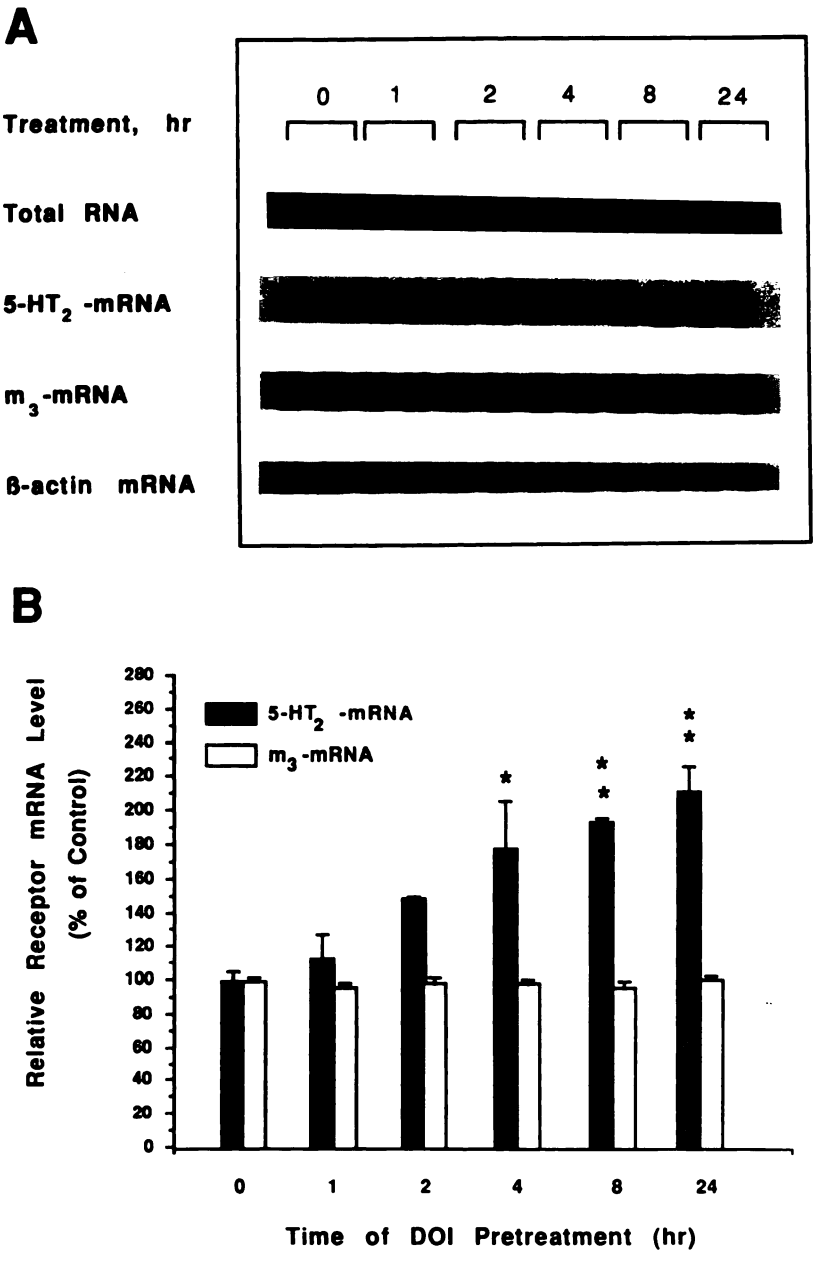

Fig. 7. Time course of changes of 5-HT_2 receptor mRNA after treatment with 10 μM DOI. Cerebellar granule cells were exposed to 10 μM DOI for indicated time periods. A, Autoradiographs of blots hybridized to probes for the 5-HT_2 receptor, m_3-mAChR, and β-actin mRNAs. B, Quantification by βetagen blot analyzer of the autoradiograms. Levels of 5-HT_2 receptor mRNA have been normalized to total cellular RNA at each time point. The data represent the mean ± standard deviation of duplicate samples. The experiment has been repeated three times with identical results. *, $p < 0.05$; **, $p < 0.01$, compared with the time 0 control.

HT_2 receptors have been suggested to be coupled directly or indirectly to calcium channels (2, 27–29) and prostacyclin production (30, 31). It is unknown whether these effectors are coupled to 5-HT_2 receptors in cerebellar granule cells and, thereby, responsible for the enhanced expression of this 5-HT receptor subtype. Recently, it was reported by Bailey *et al.* (32) that, with repeated or prolonged stimulation of *Aplysia* neurons, 5-HT acts like a growth factor to elicit changes in the surface and internal membrane structure by initiating rapid protein synthesis. Consistent with this notion, we found that the 5-HT-induced up-regulation of 5-HT_2 receptor mRNA in cerebellar granule cells is blocked by cycloheximide, a protein synthesis inhibitor (data not shown).

After treatment of cells with 5-HT or DOI for 4 or 24 hr, both the B_{max} and K_d values for [^{3}H]ketanserin binding to 5-HT_2 receptors were increased. These changes were not found in cells treated with either agonist for only 1 hr, suggesting that the decrease in the affinity is not due to residual agonist bound to the receptor. Rather, it reflects a 5-HT-induced modification of the binding characteristics of 5-HT_2 receptors. Because desensitization induced by 5-HT or DOI pretreatment was evident even at 30 min after treatment, it is unlikely that this event is the result of a loss of receptor affinity for 5-HT to elicit PI turnover. Consistent with this interpretation is the observation that the desensitization of the 5-HT_2 receptor-mediated PI response in granule cells is associated with an attenuation of the maximal extent of inositol phosphate production, with no apparent change in the EC_{50} value of 5-HT (16). Thus, it seems to be more likely that 5-HT-induced desensitization results from a rapid uncoupling of 5-HT_2 receptors from their corresponding G protein-phospholipase C complex. Paradoxical up-regulation has also been observed in the rat brain nicotinic receptor subtype after chronic agonist treatment (33). It seems possible that, in their studies and ours, a robust compensatory increase in receptor expression occurs after desensitization of the effector response, resulting in a net

increase in the receptor density. Recently, delayed functional supersensitivity of 5-HT$_2$ receptor-mediated head-twitch behavior was observed in mice acutely or chronically treated with DOI (34), a paradoxical phenomenon consistent with our report.

The inhibition of 5-HT- and DOI-induced PI response by PTX pretreatment suggests that the G protein involved is subject to regulation by ADP-ribosylation. This PTX sensitivity is similar to 5-HT$_2$ receptor-mediated PI hydrolysis in hamster lung fibroblasts (35) but differs from the PI responses mediated by m$_3$-mAChRs in cerebellar granule cells (15) and 5-HT$_2$ receptors in P11 cells (36); in the latter systems, PTX fails to affect the agonist-induced responses. It is interesting to note that in P11 cells exposure to 5-HT results in a decrease in the density of 5-HT$_2$ receptors, which follows the onset of homologous desensitization (17). In our studies, the simultaneous presence of PTX with 5-HT during pretreatment abolishes the up-regulation of 5-HT$_2$ receptor binding sites (data not shown). It remains to be explored whether the disparity in receptor regulation reflects a cell type-specific phenomenon or is the result of differences in the nature of the corresponding G proteins and the coupling mechanisms of the receptors.

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