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Biochemical Pharmacology 62 (2001) 431–438

Biochemical Pharmacology

Agonist-induced functional desensitization of recombinant human 5-HT2 receptors expressed in CHO-K1 cells

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Received 11 January 2001; accepted 9 April 2001

Abstract

The desensitization characteristics of recombinant human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors (VSV and INI isoforms) stably expressed in CHO-K1 (Chinese hamster ovary) cells was investigated by calcium fluorimetry. Comparative desensitization characteristics of the agonists 5-HT, m-chlorophenylpiperazine (mCPP), and 2,5-dimethoxy-4-iodoamphetamine hydrobromide (DOI) were performed. Human 5-$HT_{2C\ (INI)}$ receptors exhibited a greater degree of desensitization to all agonists tested than edited 5-$HT_{2C\ (VSV)}$ receptors. A 2-hr exposure to 5-HT resulted in a significantly larger reduction in response upon re-exposure to 5-HT at 5-$HT_{2C\ (INI)}$ receptors, as compared to 5-$HT_{2C\ (VSV)}$ receptors (72% and 47% respectively, $P < 0.01$). Both receptor isoforms were expressed at similar densities. Human 5-HT_{2B} receptors exhibited the most dramatic degree of desensitization, with prior exposure to 5-HT reducing subsequent response to 5-HT by 80%, with an extremely rapid time-course ($t_{\frac{1}{2}} < 5$ min). The response at 5-HT_{2A} receptors was reduced by 54%. The partial agonists mCPP and DOI also elicited desensitization, generally in line with their relative efficacies at each receptor, but exhibited more rapid kinetic profiles than 5-HT. Heterologous desensitization of an endogenously expressed $G_{q/11}$-coupled purinergic receptor was also examined following preincubation of the cell lines with 10 μM 5-HT. Only stimulation of 5-$HT_{2C\ (VSV)}$ receptors resulted in a profound attenuation of subsequent ATP mediated responses. These results demonstrate differing degrees of both homologous and heterologous desensitization of 5-HT_2 receptors. Additionally, the different desensitization profiles of 5-$HT_{2C\ (INI)}$ and 5-$HT_{2C\ (VSV)}$ receptor may be due to signal transduction differences caused by RNA editing.

Keywords: Serotonin; Receptor; 5-HT_2; RNA editing; Desensitisation

1. Introduction

The actions of the neurotransmitter 5-HT are mediated by a family of receptors of which the 5HT_2 receptors represent a closely related subgroup [1]. There are three 5-HT_2 receptor subtypes, termed 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. The 5-HT_{2C} receptor has recently been demonstrated to undergo RNA editing, which is thought to reduce the efficiency of G-protein receptor coupling and hence the ability of agonists to induce a functional response [2].

Agonists active at 5-HT_2 receptors have been proposed to be of therapeutic utility for a variety of CNS disorders including anxiety, depression, and obesity [3–7]. Additionally, agonist action at 5-HT_{2A} receptors is believed to be a mechanism responsible for the effects of various hallucinogenic compounds [8,9]. However, GPCRs are known to undergo desensitization, which is a regulatory mechanism whereby prolonged receptor stimulation results in a reduction of agonist-induced responses [10,11]. Determination of the functional adaptive consequences of 5-HT_{2A} and 5-HT_{2C} receptor stimulation *in vivo* is complex, and may depend on the route of drug administration, behavioral outcome measure, and drug of interest [12–14]. For example, the available evidence suggests that there are different functional consequences of prolonged 5-HT_{2C} receptor stimulation, the hypolocomotion rapidly tolerates, whereas the hy-

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Abbreviations: 5-HT, 5-hydroxytryptamine; CHO, Chinese hamster ovary; PI, phosphoinositide; FLIPR, fluorimetric imaging plate reader; mCPP, m-chlorophenylpiperazine; DOI, 2,5-dimethoxy-4-iodoamphetamine hydrobromide, GPCR, G-protein-coupled receptor; and PCR, polymerase chain reaction.

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PII: S0006-2952(01)00677-3

pophagia and loss of body weight is maintained [12,15]. The molecular mechanisms for such differences are currently unknown. Although the 5-HT_{2B} receptor has not been studied to the same degree, chronic infusion of 5-HT in rats for 10 days has been demonstrated to result in a slight (but significant) reduction in the maximal 5-HT-induced contractile response in stomach fundus [16], a common *in vitro* measure of 5-HT_{2B} receptor function. However, a conflicting report which included detailed behavioral analysis following maintained paroxetine or BW 723C86 administration concluded that the 5-HT_{2B} receptor was resistant to agonist-induced desensitization [17].

In order to study the desensitization characteristics of all the 5-HT_2 receptors in a similar cellular environment and using the same functional measure, we expressed human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors at low density in CHO-K1 cells. Additionally, we wished to investigate the impact of RNA editing on the desensitization characteristics of the 5-HT_{2C} receptor by comparing the unedited (INI) receptor with the isoform that has been reported to be the most abundantly expressed edited variant (VSV) in the human brain [18]. Since all three receptor subtypes couple to PI hydrolysis with a subsequent release of intracellular calcium, we used a FLIPR to monitor 5-HT-induced intracellular Ca^{2+} mobilization in recombinant cell lines as previously described [19]. As RNA editing has been reported to decrease the efficiency of G-protein coupling, we extended our previous study which examined the 5-$HT_{2C\ (VSV)}$ receptor to include the unedited (5-$HT_{2C\ (INI)}$) receptor isoform. The desensitization characteristics of the receptors following acute exposure to the full agonist 5-HT and the partial agonists mCPP and DOI were compared. We also investigated any heterologous interaction between prolonged stimulation of 5-HT_2 receptors and an endogenously expressed purinergic receptor that also couples to intracellular calcium mobilization [20,21]. Although 5-HT_{2A} and 5-HT_{2C} receptors have previously been shown to be susceptible to agonist-induced desensitization *in vitro* [22–24], to our knowledge this study represents the first direct demonstration of human 5-HT_{2B} receptor desensitization and is the first study to compare the desensitization characteristics of differentially edited 5-HT_{2C} receptor variants.

2. Materials and methods

2.1. Mutagenesis of the human 5-$HT_{2C\ (VSV)}$ receptor to the 'unedited' 5-$HT_{2C\ (INI)}$ isoform

The 5-$HT_{2C\ (VSV)}$ receptor previously available [19] underwent site-specific mutation by splice overlap extension PCR, based on the method of Horton *et al*. [25], to create the unedited 5-$HT_{2C\ (INI)}$ isoform. Primers were based on the DNA sequence (Genbank accession no. M81778) and two separate PCR reactions were performed on 5-$HT_{2C\ (VSV)}$ template DNA cloned in pUC18. Primer 1 and Primer 2 amplified a 488-bp product (Primer 1—5'*GGT GAA CCT GAG GAA TGC GGT GC* 3'(Bsu 36 I site underlined) and Primer 2—5' *CGG CTA TGC TCA ATA GGA TTA CGA ATT GCT ACA TAC CGA TC* 3'). Primers 3 and 4 amplified a 259-bp product (Primer 3—5' *GAT CGG TAT GTA GCA ATT CGT AAT CCT ATT GAG CATAGC CG* 3' and Primer 4—5' *GTC AGG CAA TAC GTA ATC AC* 3' (SnaBI site underlined), using the same template and PCR conditions. PCR proceeded for 60 sec at 94°, 60 sec at 55°, and 60 sec at 72° for 30 cycles. Each reaction contained 50 pmol of each primer, 3 mM $MgSO_4$, 200 μM dNTP, 20 mM Tris pH 8.3, 66 mM KCL, 0.1% Triton, 20 ng template DNA, and 2.5 U Vent DNA polymerase (NEB) in a final volume of 50 μL.

PCR products were gel-purified, using Wizard purification resin (Promega) and combined in equimolar quantities. A second PCR was then performed on this mixture, using Primer 1 and Primer 4. The final 707-bp product was treated with polynucleotide kinase (20 U) and 0.5 mM ATP in 1 X polynucleotide kinase buffer at 37° for 30 min. Following gel purification, the final product was subcloned into pUC18. The presence of the unedited DNA sequence was confirmed by fluorescent dideoxy sequencing, and a Bsu 36 I–SnaB I fragment was removed and exchanged with the VSV-containing fragment from the original template DNA by subcloning. The final complete unedited isoform was subsequently subcloned into a cytomegalovirus enhancer promoter-based mammalian expression vector, pcDNA6 (Invitrogen) as a BamH I–Xba I fragment.

2.2. Cell culture

Recombinant CHO-K1 cell lines were routinely cultured in Dulbecco's modified Eagle's medium (Sigma) containing 10% heat-inactivated dialyzed foetal bovine serum (dFBS), 1% penicillin/streptomycin, 1% L-glutamine, and 1% non-essential amino acids. Human 5-HT_{2A}, 5-HT_{2B}, and 5-$HT_{2C\ (VSV)}$ cell lines were grown under selection with 0.8 mg/mL of G-418. A stably expressing cell line for the 5-$HT_{2C\ (INI)}$ receptor was created using the calcium phosphate method as previously described [19] and cultured under selection with 5 μg/mL of blasticidin. A clonal 5-$HT_{2C\ (INI)}$ cell line expressing at 215 fmol/mg was chosen, as this expression level most closely resembled that of the 5-$HT_{2C\ (VSV)}$ cell line which expressed at 238 fmol/mg as previously described [19].

2.3. Desensitization assays and FLIPR

Recombinant cell lines were seeded at a density of 30,000 cells/well in black-walled clear bottom 96-well plates and incubated overnight before use. The following day cells were dye-loaded by incubation with 4 μM FLUO-3 in 100 μL/well of Hanks' balanced salt solution (HBSS) (GIBCO) containing 20 mM HEPES and 2.5 mM probenecid (Sigma). Plates were dye-loaded at 37° for 60–

120 min prior to washing to remove unincorporated dye, leaving 100 μL HBSS/well. 5-HT was added to cultures (in FLIPR) in a volume of 50 μL at a rate of 70 μL/sec. Maximal fluorescence responses occurred within 10–15 sec after agonist addition.

Agonist-induced desensitization was investigated by pre-incubating the cells with 10 μM agonist in order to achieve 100% receptor occupancy (0–24 hr as indicated in the fig. legends) prior to washing, and re-exposure to either 5-HT or ATP as indicated in the fig. legends. Preincubation times of 2 hr or less were performed during the dye loading procedure. In all cases, the cells were subjected to a wash procedure for approx. 3 min prior to being placed in FLIPR. Hence, there was an effective recovery time of up to 4 min prior to the addition of 5-HT or ATP. Responses were measured as a percentage of response to 10 μM 5-HT in control wells expressing the receptor of interest.

2.4. Drugs

All compounds were obtained from Sigma-RBI. Fluo-3-AM and pluronic acid were purchased from TefLabs and Molecular Probes, respectively. Oligonucleotides were synthesised to order by OSWEL. All cell culture reagents were purchased from Sigma or GIBCO. Cell culture plastic ware was purchased from Falcon or Corning Costar.

2.5. Data analysis

Agonist dose–response curves were constructed using a four-parameter logistic equation from GraphPad Prism where $Y = \text{bottom} + (\text{Top} - \text{Bottom})/1 + 10^{(\text{LogEC}_{50} - X)\text{nH}}$. The relative efficacy was determined from the Top value (the maximum value of the Y plateau). The concentration of agonist that produced a half-maximal response is represented by the EC_{50} value. Each 96-well plate contained 4 wells dedicated to a positive control defined as 10 μM 5-HT and 4 wells as a negative control defined as assay buffer alone. For pharmacological characterization, all data were normalized to the positive control wells, which were expressed as 100% signal.

Statistical analysis was performed using one-way ANOVA in combination with Tukey's test for multiple comparisons with 95% confidence limits.

3. Results

3.1. Pharmacological characterization of agonists

We have previously investigated the potency and efficacy of 5-HT, mCPP, and DOI at the 5-HT_{2A}, 5-HT_{2B}, and 5-$HT_{2C\ (VSV)}$ receptors [19]. In the present study, we investigated the potency and efficacy of 5-HT, mCPP, and DOI at the unedited 5-$HT_{2C\ (INI)}$ receptor subtype using the same assay (measurement of Ca^{2+} mobilization in FLIPR). 5-HT, mCPP, and DOI exhibited pEC_{50} values of 8.50 ± 0.02, 7.57 ± 0.06, and 8.16 ± 0.04 respectively (mean ± SEM; N = 4). Both mCPP and DOI acted as partial agonists at the 5-$HT_{2C\ (INI)}$ receptor subtype with relative efficacies of 75 ± 3% and 60 ± 3%, respectively. In agreement with previous reports [2,18], agonists stimulated the edited 5-HT_{2C} receptor with lower potency than the unedited receptor [19].

3.2. Time-course of 5-HT-, mCPP-, and DOI-induced desensitization

Time-course desensitization experiments (Fig. 1, A) show that preincubation with 5-HT, mCPP, or DOI (10 μM) induced varying degrees of desensitization at each of the 5-HT_2 receptor subtypes. In all cases, maximal desensitization was apparent following 20–30 min preincubation. The rapidity of onset for maximal desensitization occurred in the order 5-HT_{2B} > 5-$HT_{2C\ (INI)}$ > 5-$HT_{2C\ (VSV)}$ > 5-HT_{2A}. Greatest levels of desensitization were observed for the 5-HT_{2B} receptor with maximal responses reduced to 10–20% of control levels (Fig. 1B). Comparison of maximal desensitization following 2-hr preincubation with 10 μM 5-HT at the 5-$HT_{2C\ (VSV)}$ and 5-$HT_{2C\ (INI)}$ receptors revealed that desensitized responses at the unedited 5-$HT_{2C\ (INI)}$ receptor subtype were significantly lower than the 5-$HT_{2C\ (VSV)}$ receptor (28% of control compared to 53% respectively of control) ($P < 0.01$) (Fig. 1, C and D). Additionally, the partial agonists mCPP and DOI caused a very rapid desensitization of the 5-$HT_{2C\ (VSV)}$, exceeding the level of 5-HT-induced desensitization. Interestingly, the desensitization induced by mCPP and DOI appeared to recover following more prolonged incubation times (e.g. mCPP reduced 5-HT-induced response to 26% after 5 min but this recovered to 44% of 5-HT response following a 2-hr incubation). The final degree of desensitization after 2 hr, and maintained for 24 hr, was almost identical for all 3 agonists at the edited receptor (Fig. 1C and Fig. 2C). The unedited receptor exhibited similar desensitization profiles for all 3 agonist compounds with no apparent resensitization following more prolonged (2- to 24-hr) pre-exposure to 5-HT (Fig. 1D and Fig. 2D). 5-HT_{2A} and 5-HT_{2B} receptors were generally more resistant to mCPP-induced desensitization after 24 hr with DOI and 5-HT inducing a greater reduction in subsequent 5-HT-induced responses than at 2 hr (Fig. 1D and Fig. 2D).

5-$HT_{2C\ (VSV)}$ receptors appeared to elicit a greater functional response as compared to 5-$HT_{2C\ (INI)}$ receptors (Fig. 3). Both receptors were expressed at the same density, plated into the same 96-well plate at 30,000 cells/well, and assayed side by side from the same stock solutions. Both receptors yielded similar baseline fluorescence values (e.g. 17684 and 19798 for the VSV and INI subtypes, respectively), indicating similar cell densities and dye incorporation. Hence the final 5-HT-mediated calcium signal (as measured by increase in fluorescence) was greater for the desensitized 5-$HT_{2C\ (VSV)}$ receptor than for control 5-$HT_{2C\ (INI)}$ receptors (Fig. 3).

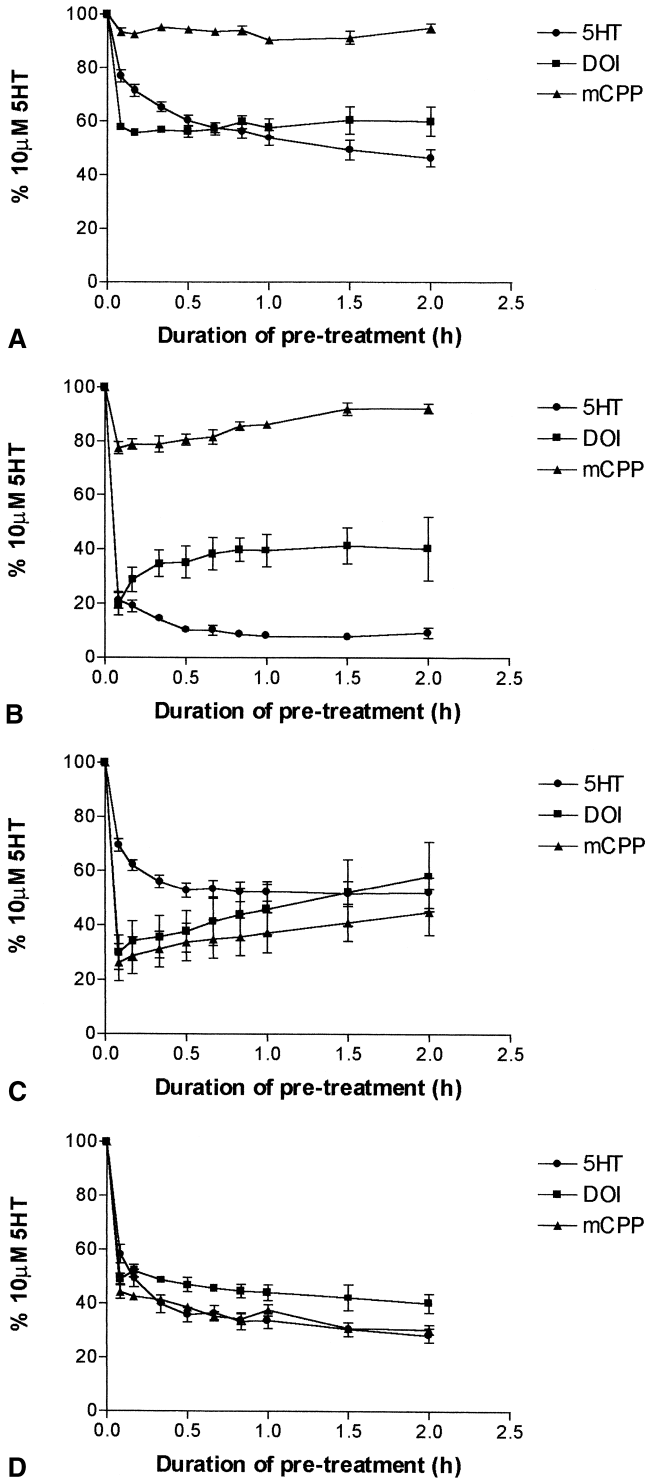

Fig. 1. Time-course of desensitization induced by full and partial agonists at cloned 5-HT_2 receptor subtypes. Cells expressing the (A) 5-HT_{2A}, (B) 5-HT_{2B}, (C) 5-$HT_{2C\ (VSV)}$, and (D) 5-$HT_{2C\ (INI)}$ receptors were dye-loaded and simultaneously preincubated with 10 μM 5-HT, DOI, or mCPP for varying amounts of time as described in Materials and Methods. Following washout cultures were then re-exposed to supramaximal (10 μM) 5-HT on FLIPR and peak responses recorded. Values are means ± SEM of 4–6 independent observations.

5-HT_{2B} receptors exhibited extremely rapid and pronounced desensitization following prior exposure to 5-HT with a $t_{\frac{1}{2}}$ of < 5 min and responses reduced to approximately 20% control levels. DOI exhibited an equally rapid desensitization, but in contrast to 5-HT some recovery was evident at longer time points (Fig. 1B and Fig. 2B). Prior exposure to mCPP induced a smaller degree of desensitization in line with a lower relative efficacy of this compound, which also appeared to recover almost to control levels following 2-hr incubation (97% of control values; Fig. 2B).

The 5-HT_{2A} receptor was most resistant to desensitization, with 5-HT and DOI eliciting a similar degree of desensitization to approx. 50–60% control values. However, the time-course for DOI-induced desensitization appeared more rapid than 5-HT itself (Fig. 1A). mCPP did not elicit a significant degree of desensitization at 5-HT_{2A} receptors up to a 2-hr exposure (Fig. 1A), in line with its low relative efficacy at the receptor. However prolonged treatment with all 3 compounds for 24 hr greatly increased the degree of desensitization upon re-stimulation with 5-HT (Fig. 2A).

3.3. Concentration-dependence of 5-HT-induced desensitization of 5-HT_2 receptor subtypes

The concentration-dependence of 5-HT-induced desensitization was examined in each cell line by preincubation with varying concentrations of 5-HT (10 nM–10 μM) for 60 min followed by agonist washout and re-exposure to 5-HT (0.01 nM–10 μM). Concentration–response curves were generated by expressing responses of pretreated cultures as a percentage of the response to 10 μM 5-HT in control cultures expressing the appropriate receptor. The results (Fig. 4, A–D) show that at all 5-HT_2 receptor subtypes tested, preincubation with increasing concentrations of 5-HT led to a decrease in the potency of 5-HT-induced Ca^{2+} mobilization and a decreased maximal response in comparison to control cultures. Maximal responses following preincubation of cultures with 10 μM 5-HT were in line with the results obtained from the time-course experiments (Fig. 1).

3.4. Heterologous desensitization of endogenously expressed purinergic receptor responses

CHO-K1 cells endogenously express a purinergic receptor that is coupled to intracellular Ca^{2+} mobilization [20, 21]. ATP acts as an agonist at this receptor with an EC_{50} ranging from 80–182 nM in the recombinant 5-HT_2 receptor cell lines (Fig. 5). Preincubation with 10 μM 5-HT for 60 min caused a differential degree of heterologous desensitization upon stimulation with ATP. 5-HT_{2A}, 5-HT_{2B}, and 5-$HT_{2C\ (INI)}$ receptor stimulation elicited small decreases in the potency of ATP, and in the case of the 5-HT_{2B} and 5-$HT_{2C\ (INI)}$ receptors, small decreases in subsequent maximal responses (12% and 16%, respectively) (Fig. 5, A, B, and D). However, preincubation of cells expressing 5-$HT_{2C\ (VSV)}$ receptors resulted in a more profound reduction of maximal

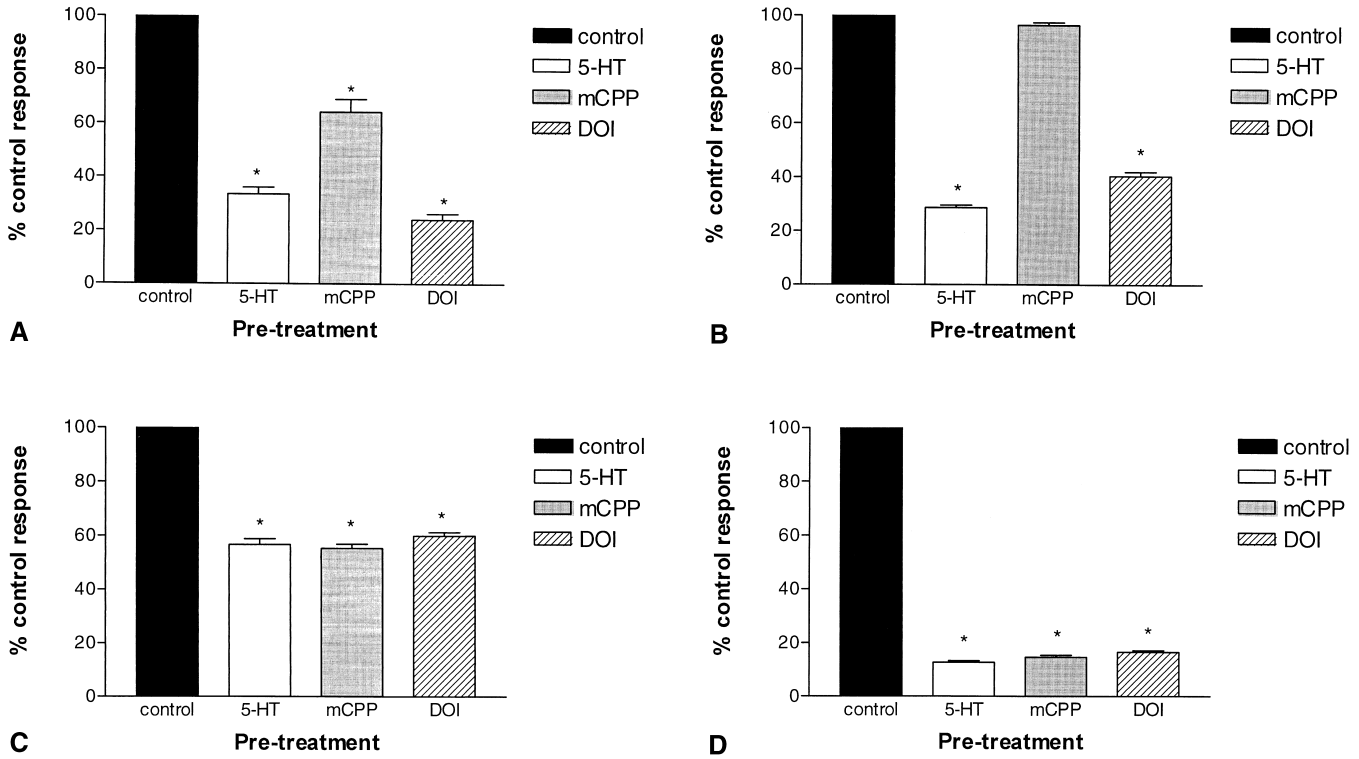

Fig. 2. Desensitization of cloned 5-HT_2 receptor subtypes following prolonged (24 hr) preincubation with full and partial agonists. Cells expressing the (A) 5-HT_{2A}, (B) 5-HT_{2B}, (C) 5-$HT_{2C\ (VSV)}$, and (D) 5-$HT_{2C\ (INI)}$ receptors were preincubated with 10 μM 5-HT, DOI, or mCPP for 24 hr. Following washout cultures were then re-exposed to supramaximal (10 μM) 5-HT on FLIPR and peak responses recorded. Data are expressed as percentage of response in control cultures. Values are means ± SEM of 3 independent observations. * Significantly different from control ($P < 0.05$).

ATP-induced response to 59% of control levels and a consequent reduction in EC_{50} from 119.2 to 496.6 nM (Fig. 5C).

4. Discussion

In an attempt to better understand the molecular regulation of 5-HT_2 receptors, we have studied the desensitization characteristics of the receptors expressed in CHO-K1 cells in order to control for cell-specific factors that might regulate receptor function. In addition, agonists were added at supramaximal concentrations (10 μM) in order to achieve 100% receptor occupancy and circumvent any issues arising as a consequence of the varying potency of 5-HT at these receptors. Hence, the finding that the 5-HT_{2B} receptor is more sensitive to agonist-mediated desensitization than either the 5-HT_{2A} or 5-HT_{2C} receptors suggests that this is a receptor-specific phenomenon. However, we note that despite the slightly higher expression level of the 5-HT_{2B} receptor [19], we have previously shown that stimulated fluorescence responses resulting from activation of this receptor are lower than responses from either 5-HT_{2A} or 5-$HT_{2C\ (VSV)}$ receptors [19]. We suggest that this phenomenon may occur as a result of reduced coupling efficiency of the 5-HT_{2B} receptor to intracellular Ca^{2+} mobilization. Additionally, it has previously been reported in a study that compared the desensitization characteristics of 5-HT_{2A} and 5-HT_{2C} receptors with respect to PI hydrolysis and arachidonic acid, the two pathways were differentially susceptible to desensitization [26]. While different agonists may have a greater propensity to preferentially stimulate one pathway than another, [27], no such information is available for 5-HT_{2B} receptors. Thus, we have chosen to monitor release of intracellular Ca^{2+} as a common end point for all three receptors, and compared each receptor to its control in order to assess the effect of prior exposure to agonists of different relative efficacies.

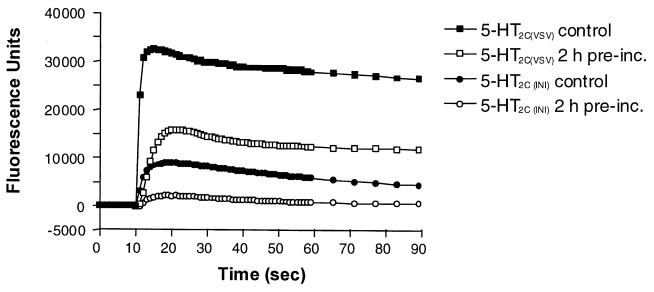

Fig. 3. Peak fluorescence responses measured using FLIPR in cultures expressing both unedited and edited isoforms of the human 5-HT_{2C} receptor. Cells expressing either the 5-$HT_{2C\ (VSV)}$ or 5-$HT_{2C\ (INI)}$ receptor subtype were exposed to 10 μM 5-HT with or without 2-hr preincubation with 10 μM 5-HT. Values shown are the absolute fluorescence levels taken from a representative experiment.

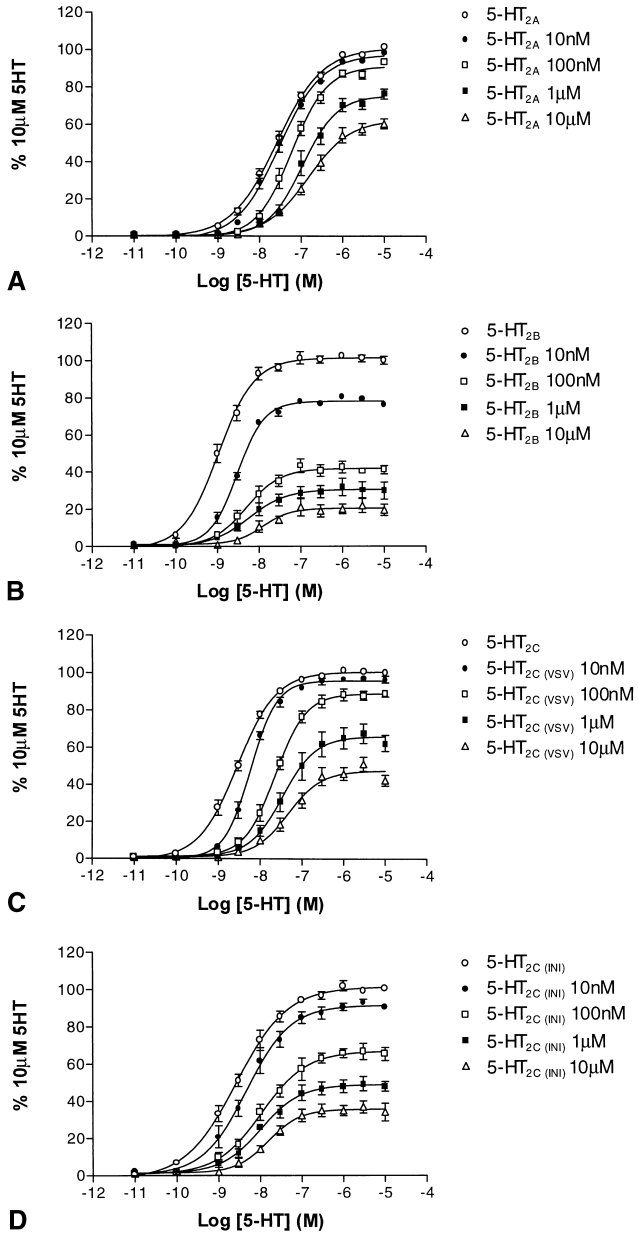

Fig. 4. Concentration-dependence of 5-HT-induced desensitization of 5-HT_2 receptor subtypes. Cells expressing the (A) 5-HT_{2A}, (B) 5-HT_{2B}, (C) 5-$HT_{2C\ (VSV)}$, and (D) 5-$HT_{2C\ (INI)}$ receptors were preincubated with varying concentrations of 5-HT as shown prior to washout and measurement of 5-HT concentration-response (0.01 nM–10 μM) on FLIPR. Values are means ± SEM of 3 independent observations.

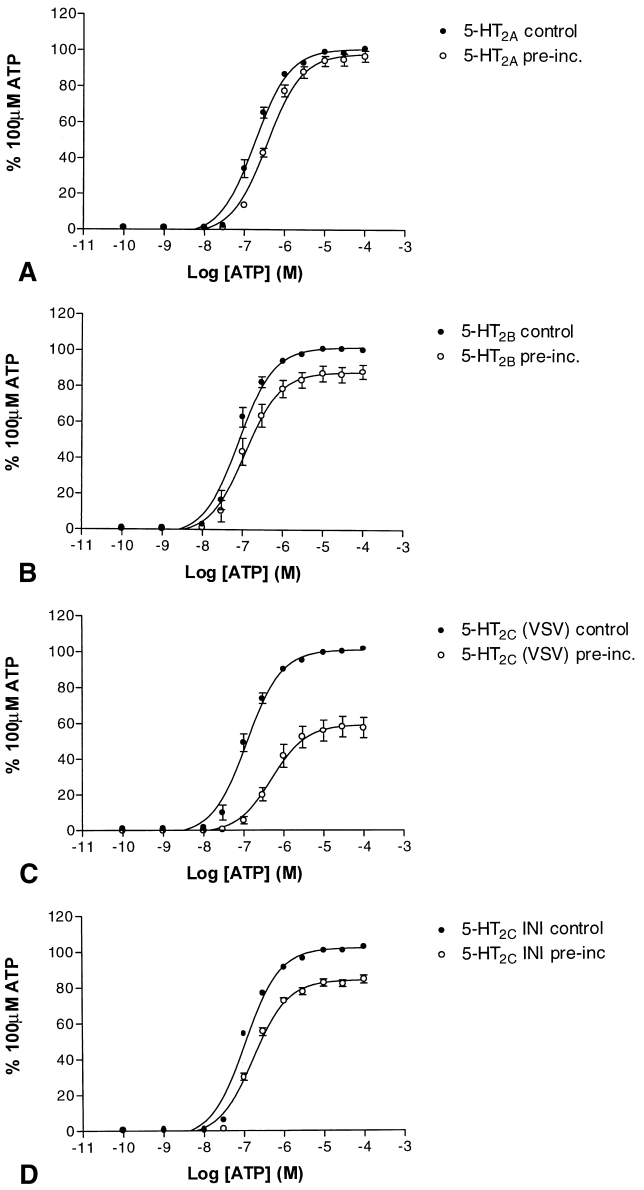

Fig. 5. Heterologous desensitization of an endogenous purinergic receptor following preincubation with 10 μM 5-HT. Heterologous desensitization was investigated by preincubating cells expressing the (A) 5-HT_{2A}, (B) 5-HT_{2B}, (C) 5-$HT_{2C\ (VSV)}$, and (D) 5-$HT_{2C\ (INI)}$ receptors with 10 μM 5-HT for 60 min. followed by washout and re-exposure to the purinergic agonist ATP (0.1 nM–100 μM). Data points were calculated from the peak response obtained at each concentration of agonist. Values are means ± SEM of 3 independent observations. Relative efficacies are calculated as percentage of the maximal response to 100 μM ATP.

Recent studies have shown that messenger RNA (mRNA) transcripts that encode for the 5-HT_{2C} receptor are subject to adenosine–inosine RNA editing at a number of sites [2,18]. This results in a number of differentially edited 5-HT_{2C} receptor variants that have reduced G-protein coupling efficiency and decreased levels of constitutive activity and altered agonist responsiveness [18,28]. In the present study, we compared the desensitization characteristics of the unedited (5-$HT_{2C\ (INI)}$) and the reported most abundant edited receptor expressed in human brain (5-$HT_{2C\ (VSV)}$). Our results show that 5-HT induces a greater level of desensitization of 5-$HT_{2C\ (INI)}$ responses compared to 5-$HT_{2C\ (VSV)}$ responses following 2- to 24-hr preincubation. After a 2-hr preincubation, both mCPP and 5-HT induced similar levels of desensitization of 5-$HT_{2C\ (INI)}$ receptors whilst the slightly lower efficacy partial agonist DOI was less effective. A similar pattern of results has been reported for the β_2-adrenoceptor where partial agonists (at concentrations resulting in high receptor occupancy) induced lower levels

of desensitization compared to the full agonist adrenaline [29]. In contrast, comparison of full and partial agonist-induced desensitization of the edited 5-$HT_{2C\ (VSV)}$ receptor subtype showed that following brief (5 min) preincubation, the partial agonists DOI and mCPP triggered a greater level of desensitization than the fall agonist 5-HT. Following more prolonged preincubation (2–24 hr), the desensitization elicited by DOI and mCPP exhibited a slow recovery towards similar levels to 5-HT. Thus, our results show that editing of the 5-HT_{2C} receptor results in an enhancement of initial desensitization in response to preincubation with partial agonists and an increased propensity for resensitization following more prolonged exposure. At present, the mechanisms accounting for the differential agonist- and receptor-specific time-course of desensitization of the 5-$HT_{2C\ (INI)}$ and 5-$HT_{2C\ (VSV)}$ receptors are unknown. However, the greater degree of desensitization of the 5-$HT_{2C\ (INI)}$ receptor is in contrast to fact that the edited receptor studied encodes a casein kinase II (CKII) phosphorylation site that is not present in the 5-$HT_{2C\ (INI)}$ receptor. Our results also show that the absolute fluorescence increases measured in both control and desensitized cultures are much greater for the 5-$HT_{2C\ (VSV)}$ receptor than for the unedited 5-$HT_{2C\ (INI)}$ receptor (Fig. 3). It has been suggested that the unedited 5-$HT_{2C\ (INI)}$ receptor may exhibit a greater degree of constitutive activity as a consequence of improved G-protein coupling efficiency in relation to the edited 5-$HT_{2C\ (VSV)}$ receptor [2]. Hence, a possible explanation for the reduced calcium responses observed for the 5-$HT_{2C\ (INI)}$ receptor would be if increased levels of constitutive activity resulted in a smaller stimulated increase in PI hydrolysis. However, it is also possible that the different 5-HT_{2C} receptor isoforms couple with differing efficiencies to different signaling pathways that this study would not have detected [27].

Of all the 5-HT_2 receptor subtypes tested, the 5-HT_{2A} receptor is generally the least sensitive to 5-HT-induced desensitization, exhibiting slower rapidity of onset and lower maximal levels of desensitization. Acute (0–2 hr) pretreatment of 5-HT_{2A} receptor-expressing cells with mCPP, however, did not trigger desensitization of subsequent responses to 5-HT. This is consistent with the fact that mCPP acts as a low-efficacy partial agonist at this receptor [19]. However, longer preincubation of 5-HT_{2A}-expressing cells with mCPP for 24 hr did result in a significant inhibition (~25%) of subsequent responses to a supramaximal concentration of 5-HT (Fig. 2A), suggesting that low-efficacy partial agonists may require longer preincubation times to trigger desensitization. However, 5-HT_{2A} and 5-HT_{2C} receptors have previously been reported to undergo a paradoxical down-regulation in response to antagonists as well as agonist treatments [30–33]. Pretreatment of 5-HT_{2A} receptor-expressing cells with the higher efficacy (~60%) partial agonist DOI gave rise to similar levels of desensitization following either 2- or 24-hr preincubation. Interestingly, despite the fact that the 5-HT_{2A} receptor displayed the slowest desensitization kinetics in response to 5-HT pretreatment, DOI-induced desensitization was equally rapid ($t_{\frac{1}{2}} < 5$ min) at all four receptor subtypes with peak-levels of desensitization occurring after only 5 min of preincubation (Fig. 2, A–D).

5-HT_{2B} receptors were the most sensitive to desensitization. Following 5-min preincubation, 5-HT and the partial agonist DOI induced a similar degree of desensitization. 5-HT-induced desensitization of 5-HT_{2B} receptor responses exhibited a slight recovery following more prolonged (24 hr) pre-exposure in contrast to DOI, which exhibited a partial resensitization within 20–30 min. To our knowledge, this study is the first to report direct agonist-induced desensitization of the human 5-HT_{2B} receptor, and the compound-specific profiles may in part help explain the conflicting reports in the literature following prolonged *in vivo* administration [16,17].

We also studied the effect of 5-HT preincubation on the level of ATP-induced calcium mobilization via activation of endogenously expressed $G_{q/11}$-coupled purinergic receptors [20,21]. Both 5-HT_{2A} and 5-HT_{2B} receptors caused a small degree of heterologous desensitization, in contrast to that elicited by 5-HT_{2C} receptor-expressing cells (Fig. 5). These data suggest that desensitization of 5-HT_{2A} and 5-HT_{2B} receptors expressed in CHO-K1 cells may involve post-translational modification of the receptors themselves and not of any of the components of the signal transduction cascade leading to the release of intracellular Ca^{2+}. The observation that prior stimulation of particularly the edited subtype of 5-HT_{2C} receptors elicited greater reductions in subsequent ATP stimulated responses suggests that there is a divergence in the intracellular signaling cascades of 5-HT_2 receptors. The greater degree of heterologous desensitization from stimulation of 5-$HT_{2C\ (VSV)}$ receptors implies that RNA editing may alter the second messenger pathways of 5-HT_{2C} receptors. The potential physiological relevance of such a finding is difficult to infer, and may depend on whether RNA editing of the receptor is altered following drug treatment. Such differential effects on heterologous desensitization between 5-HT_{2A} and 5-HT_{2C} receptors is in agreement with a previous study that demonstrated a similar disparity on the ability of stimulation of each receptor to reduce subsequent agonist stimulation of an endogenous 5-HT_{1B}-like receptor in CHO-K1 cells [34]. These contrasting results between homologous and heterologous desensitization paradigm for the 5-HT_{2B} receptor and even between 5-HT_{2C} receptor isoforms suggest that additional and diverging signal transduction mechanisms are operating.

In conclusion, this study reports that the human 5-HT_2 receptors are differentially susceptible to agonist-mediated desensitization when expressed in a common cellular environment. Additionally, the different kinetics obtained between different agonists and the contrasting results obtained between heterologous and homologous desensitization suggest that the receptors (and 5-HT_{2C} receptor isoforms) are differentially regulated, and differentially affect other receptor systems. The long-term consequences of prolonged

5-HT_2-receptor stimulation are therefore difficult to predict and may extend beyond the serotonergic system per se.

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