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Binding Properties of Dipropyltryptamine at the Human 5-HT1a Receptor

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Serotonin · Hallucinogens · cAMP · γ-S-GTP

Abstract
Dipropyltryptamine (DPT) is a synthetic indolealkylamine first characterized in the 1960s. Largely forgotten since the discovery of multiple serotonin receptor subtypes, some of the properties of DPT at the cloned human 5-HT1a receptor are described here. When [^{3}H]8-OH-DPAT is bound to the receptor, DPT inhibits the interaction with an IC_{50} of 0.1 μmol/l. This interaction is shown to be competitive when double-reciprocal plots of the DPT/agonist interaction are analyzed. DPT's effects in the signal transduction system are complex. While DPT alone (0.1–1,000 μmol/l) activates G_i when both cAMP and γ-S-GTP incorporation are measured, in the presence of 5-HT (0.1–10 μmol/l), DPT blocks the agonist effect. In combination, the findings suggest that DPT is a moderate affinity partial agonist at the human 5-HT1a receptor. These results provide evidence that DPT has potential as a versatile experimental tool at 5-HT1a receptors.

Introduction

Relationship of N,N-Dipropyltryptamine (DPT) to N,N-Dimethyltryptamine (DMT)

DPT was first synthesized in the 1950s. Unlike its chemical relative DMT, which occurs naturally in plants (e.g., *Psychotria* spp., *Phalaris* spp.), current understanding indicates that DPT only occurs as a synthetic compound [1, 2]. Although DPT was studied briefly in the 1960–1970s, it has been largely forgotten since the identification of multiple serotonin (5-hydroxytryptamine; 5-HT) receptor (R) subtypes [3, 4] following the molecular biology revolution. DPT has been virtually unknown in the scientific literature since the mid-1970s when its use as an adjunct to psychotherapy faded. Although this early clinical experiment with DPT yielded mixed results, it was nevertheless obvious that the drug had substantial pharmacological activity [5]. DMT, often considered to be a prototypical psychedelic compound, is a Schedule I Drug under the Controlled Substances Act. While DPT is not explicitly so recognized, it might, nevertheless, be construed as such under the loose categorizations of the Controlled Substance Analogue Enforcement Act [6].

The much better known DMT is a potent hallucinogen [7–11], and its biochemical pharmacology has been at least partially characterized. DMT is structurally related to serotonin and the serotonin-like hallucinogens LSD, psilocin, psilocybin, and bufotenin [12]. Many of the

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pharmacological properties of these serotonin analogues, including hallucination, can be explained by interactions with 5-HTR. The structural relationship of DMT and DPT, coupled with the known activity of DMT, suggests considerable opportunity for exploration of the biological potential of DPT in these serotonergic receptor systems [13].

Attention was directed at DPT in the current investigation due to an expectation that it, or structural analogues, might represent possible candidates for migraine treatment. Field investigations among the Machiguenga tribe in the Peruvian Amazon indicated that they use eye drops from the leaves of *Psychotria* spp. (known to contain DMT) in the successful treatment of migraine headaches [14, 15]. DMT is known to display agonistic activity at 5-HT1aRs, and antagonistic activity at 5-HT2aRs [11], precisely the same qualities suggested as pertinent to acute symptomatic and prophylactic treatment of migraine, respectively [14, 16–18].

Subjective reports of DPT experiences are available in published form [1, 19]. These seem to indicate a psychedelic (entheogenic) threshold of 1 mg/kg. Thus, DPT is hallucinogenic, but is a much less potent hallucinogen than DMT and especially LSD. This difference suggests that serotonergic properties of DPT, potentially useful in treatment of migraine, could possibly be obtained at doses in which hallucinogenic potential is low. Exploration of the biochemical pharmacology of DPT and comparison of its properties with DMT may shed light on possible structural alterations [20] that could be exploited in drug development [21]. Even if DPT's pharmacology proves to be less than useful in an applied sense, greater understanding of its receptor activity could provide a valuable experimental tool. With the recent placement of 5-methoxy-N,N-diisopropyltryptamine (foxy-methoxy) into Schedule I [22] and the substantial lack of fundamental pharmacological studies for many of these active substances, it would seem an appropriate time to promote studies of the psychoactive tryptamines.

Biochemical Pharmacology of DPT

The studies reported here represent the initial characterization of DPT at cloned human (H) 5-HT1aR [23–26]. H5-HT1aR [28] is a seven transmembrane domain (7TMD), G-protein-coupled (GPC) receptor [29–31], and has been linked to biomedical conditions such as depression, anxiety, and migraine headache. Since H5-HT1aR is negatively coupled to the adenylyl cyclase signal transduction system via G_i in the Chinese hamster ovary (CHO) cells used here [32], functional attributes of ligands that bind to the receptor can be monitored by quantification of intracellular cyclic AMP (cAMP).

Additionally, since H5-HT1aR is a GPCR, receptor activity can be followed by GTP incorporation techniques. Recently, we have reported studies with H5-HT1aR using peptide probes derived from the intracellular loop 2 and 3 regions of the receptor. These peptide studies are designed to further understand the interfacial biology between H5-HT1aR and G_i [24, 25]. Coupled with findings from other laboratories, we have developed a working model of the receptor/G protein interface [33]. The findings about DPT interactions at H5-HT1aR's ligand-binding site and implications from these findings, in combination with a large body of structure-activity observations at 5-HT1aR [34, 35], should increase the likelihood of refinement of a comprehensive model of the receptor [36]. Results reported here suggest that DPT is a high-affinity partial agonist at H5-HT1aR. These conclusions have implications for use of DPT or structural analogues at the ligand-binding site of H5-HT1aR [21]. A preliminary report of these findings has appeared [37], including brief observations about DPT's activity at another 7TMD/GPC serotonin receptor [27, 42], the rat 5-HT2aR.

Materials and Methods

Cell Culture

CHO cells expressing the H5-HT1aR [38–39] were cultured in Ham's F-12 medium fortified with 10% fetal calf serum and 200 μg/ml geneticin. Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells were subcultured or assayed upon confluency (5–8 days). Cloned H5-HT1aR was kindly provided by Dr. John Raymond (Medical University of South Carolina).

Receptor Preparation

Cells were harvested by trypsinization and centrifuged at low speed in ice-cold medium. The pellet was re-suspended in ice-cold Earle's balanced salt solution followed by centrifugation. Cells were re-suspended in 10 ml of ice-cold binding buffer (50 mmol/l Tris, 4 mmol/l $CaCl_2$, 10 μmol/l pargyline, pH 7.4), homogenized with Teflon-glass, and centrifuged at 450,000 *g* at 4°C. To produce a crude membrane preparation, the pellet was re-suspended in 30 ml of ice-cold binding buffer, and homogenized, first with Teflon-glass and then with a Polytron (setting 4) for 5 s. The receptor preparation was stored on ice and assayed within the next 1.5 h.

Assay of Receptor Activity

Binding of the agonist [^{3}H]8-OH-DPAT ([^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin) to H5-HT1aR followed well-characterized in vitro protocols [25, 40]. Radioligands were purchased from New England Nuclear (Boston, Mass., USA). 1-ml reaction mixtures, in triplicate, were incubated for 30 min in a 30°C shaker bath. Composition of the 1-ml reaction mixture was: 700 μl of receptor prep-

aration; 100 μl of either binding buffer (for total binding) or 10 μmol/l 5-HT (final concentration for non-specific binding), 100 μl of the tritiated agent (final concentration of 0.5 nmol/l [^{3}H]8-OH-DPAT), and 100 μl of diluted DPT or binding buffer in the case of controls. In the case of experiments that varied in both DPT concentration and [^{3}H]8-OH-DPAT concentration (fig. 3), the following radioligand concentrations were used: 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/l. To achieve double-reciprocal plots of the results, the concentrations were represented as the following numbers respectively: 5.0, 2.5, 1.67, 1.25, and 1.0 (see x-axis, fig. 3).

Reactions were stopped by addition of 4 ml of ice-cold 50 mmol/l Tris buffer, pH 7.4, and subsequent vacuum filtration on glass fiber filters (Whatman GF/B). Filters were rinsed twice in 5 ml of ice-cold Tris buffer, dried, and counted in 5 ml of Ecoscint (National Diagnostics) liquid scintillation fluid in a Beckman LS 6500 instrument. Homogenates were assayed for protein to maintain a nominal value of 50 μg protein per filter over weekly assays [41]. Total and non-specific binding tubes were run in triplicate.

cAMP Assay

CHO cells were cultured to confluency in 12- or 24-well plates. Medium was aspirated and the cells were rinsed twice in warm serum-free F-12 medium. Cells were then incubated for 20 min at 37°C in 0.5 ml of serum-free F-12 medium containing 100 μmol/l isobutylmethylxanthine and the following substances (final concentrations) alone or in combination (see fig. 4): 30 μmol/l forskolin (for all treatments); 1–10 μmol/l 5-HT; 0.1–1 μmol/l DPT, and 0.1 μmol/l 8-OH-DPAT. Reactions were stopped by aspiration of medium and addition of 0.5 ml of 100 mmol/l HCl. After 10 min, well contents were removed and centrifuged at 4,000 rpm. Supernatants were diluted in 100 mmol/l HCl, and cAMP was quantified [24] directly in a microplate format by colorimetric enzyme immunoassay with a kit from Assay Designs (Ann Arbor, Mich., USA). Triplicate independent samples at a minimum were assayed in quadruplicate to increase precision.

γ-S-GTP Assay

H5-HT1aR membranes from transfected CHO cells were incubated with 5-HT (0.1 μmol/l) and/or DPT (0.1–1,000 μmol/l; see fig. 5), and the following incubation mixture: 20 mmol/l Hepes buffer, pH 7.4, 5 mmol/l $MgCl_2$, 1 mmol/l EDTA, 1 mmol/l DTT, 100 mmol/l NaCl, 100 μmol/l GDP, 10 μmol/l pargyline, 0.2 mmol/l ascorbate, and 0.1 nmol/l [^{35}S]γ-S-GTP [43]. Mixtures were incubated for 30 min at 30°C, and were terminated by dilution in cold buffer. The mixture was filtered on GF/C filters, rinsed twice in buffer, followed by drying and liquid scintillation counting. Negative control (basal incorporation) was the above mixture minus DPT or 5-HT. Non-specific binding was determined in the presence of cold γ-S-GTP (10 μmol/l). Positive control was H5-HT1aR membranes in the same incubation mixture plus 5-HT. All values reported in figure 5 are for specific binding (total – non-specific) of triplicates.

Synthesis, Storage and Dilution of DPT; Data Analysis

DPT was synthesized as outlined in figure 1, and stored in the dark at 4°C. Various spectral and physical analyses of DPT are available on request. DPT is very soluble in water and dilutions were made with distilled water. Final dilution was in the binding buffer of the assay. All statistics (means, standard errors of the mean (SEM), and Pearson correlation coefficients) and graphical procedures (including drug-receptor binding analysis) in the study were conducted with PSI-Plot (Version 7) software (Poly Software International), or on a Hewlett-Packard Graphing Calculator, HP48.

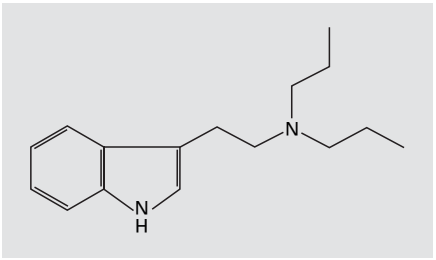

Fig. 1. Structure and synthesis of dipropyltryptamine (DPT). Tryptamine (1.6 g; 10 mmol) and diisopropylethylamine (1.04 ml; 60 mmol) were dissolved in diethyl ether (60 ml) at 0°C. 1-Iodopropane (1.02 g; 0.6 ml; 60 mmol) was added dropwise with stirring over 1 h. The reaction stirred 16 h at room temperature, was filtered to remove salts, and evaporated to an oil. The crude product, which was contaminated with N-propyltryptamine and some unreacted tryptamine, was chromatographed on silica to afford the product, N,N-di-n-propyltryptamine in 46% yield. The purified product had elemental and spectral characteristics consistent with literature values.

Results

Displacement of [^{3}H]8-OH-DPAT from H5-HT1aR

DPT was synthesized at the University of Montana (fig. 1) for the purpose of characterizing some of its binding and signal transduction characteristics at the H5-HT1aR. The first series of experiments were designed to determine the affinity of DPT at H5-HT1aR present in membranes isolated from cultured CHO cells. Figure 2 shows that DPT produced concentration-dependent displacement of the specific agonist [^{3}H]8-OH-DPAT (0.5 nmol/l) from the H5-HT1aR ligand-binding site. The displacement of [^{3}H]8-OH-DPAT at H5-HT1aR indicates that DPT has moderately high affinity with an apparent IC_{50} of about 0.1 μmol/l.

In figure 3, two different concentrations of DPT (40 and 200 nmol/l) were compared with a control lacking DPT against various concentrations of [^{3}H]8-OH-DPAT (0.2, 0.4, 0.6, 0.8, and 1.0 nmol/l) bound to H5-HT1aR. In this adaptation of the Lineweaver-Burke plot of enzyme kinetics, both agonist [^{3}H]8-OH-DPAT concentrations (x-axis) and agonist-receptor complex concentrations (y-axis) are inverted. The resulting straight lines allow estimation of the nature of DPT/agonist interactions.

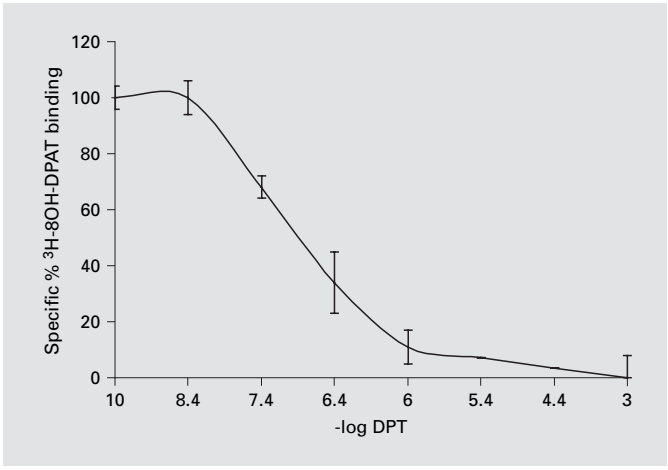

Fig. 2. Inhibition of [^{3}H]8-OH-DPAT binding by dipropyltryptamine (DPT) in membrane preparations expressing the human 5-HT1a receptor (H5-HT1aR). DPT concentrations are –log mol/l. Results are mean ± SEM with n's of 2–5. More detailed experimental conditions of cell culture, membrane preparation, and drug-receptor binding are outlined in Methods.

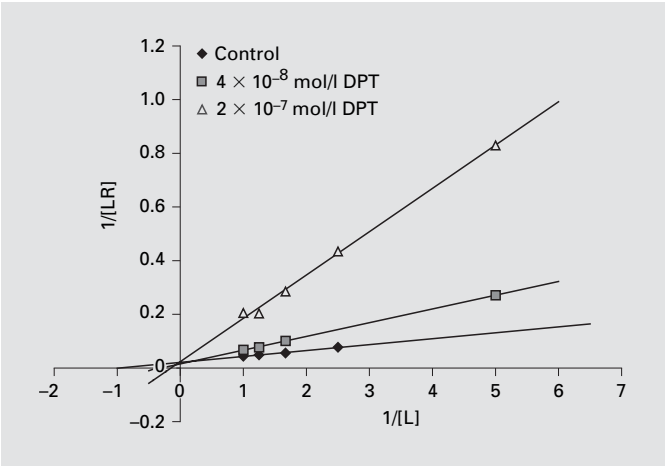

Fig. 3. Concentration dependency of [^{3}H]8-OH-DPAT binding in the presence of dipropyltryptamine (DPT) in membrane preparations of the human 5-HT1aR (H5-HT1aR). L is the concentration of [^{3}H]8-OH-DPAT. On the x-axis, 1 = 1 nmol/l; 5 = 0.2 nmol/l, etc. On the y-axis, LR is the concentration of the [^{3}H]8-OH-DPAT-H5-HT1aR complex. All points are triplicates of specific binding with minimum n's of 2. Detailed experimental conditions are outlined in Methods. Correlation coefficients exceed 0.99 for control (lowest line) and 4 × 10^{-8} mol/l DPT (middle line); correlation coefficient for the upper line (2 × 10^{-7} *M* DPT) is 0.89.

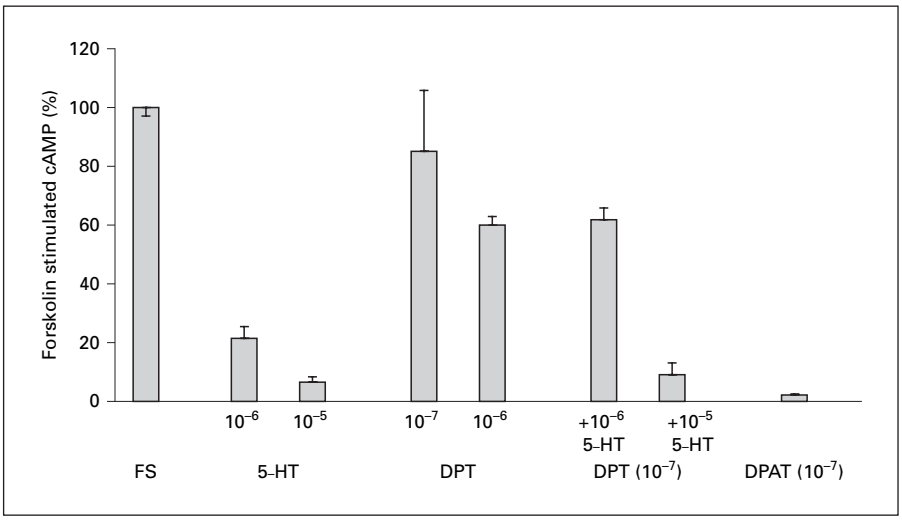

Fig. 4. Effects of serotonin (5-HT), 8-OH-DPAT (DPAT) and dipropyltryptamine (DPT) on forskolin-stimulated cyclic AMP (cAMP) formation in whole cell preparations of the human 5-HT1a receptor (H5-HT1aR). All conditions contain forskolin (FSK) at 30 μmol/l final concentration. Results are expressed as percentage of FSK control as mean ± SEM with n's of 3 (DPAT), 7 (5-HT)–22 (FSK), with intermediate values for all other conditions. Final molar concentrations for all conditions are listed below the bars. Additional experimental conditions are described in Methods.

Interpretation of the experiments analyzed here is consistent with DPT inhibiting agonist binding competitively at H5-HT1aR's ligand-binding site, as the lines intersect on the y-axis. Thus, the two concentrations of DPT yield increases of [^{3}H]8-OH-DPAT's apparent K_d on the negative x-axis.

cAMP Determination

DPT's ability to bind at the H5-HT1aR raised the question of its potential to trigger the signal transduction system. We had preliminary data that suggested antagonistic effects of DPT in this negatively coupled system. Thus, determination of intracellular cAMP concentra-

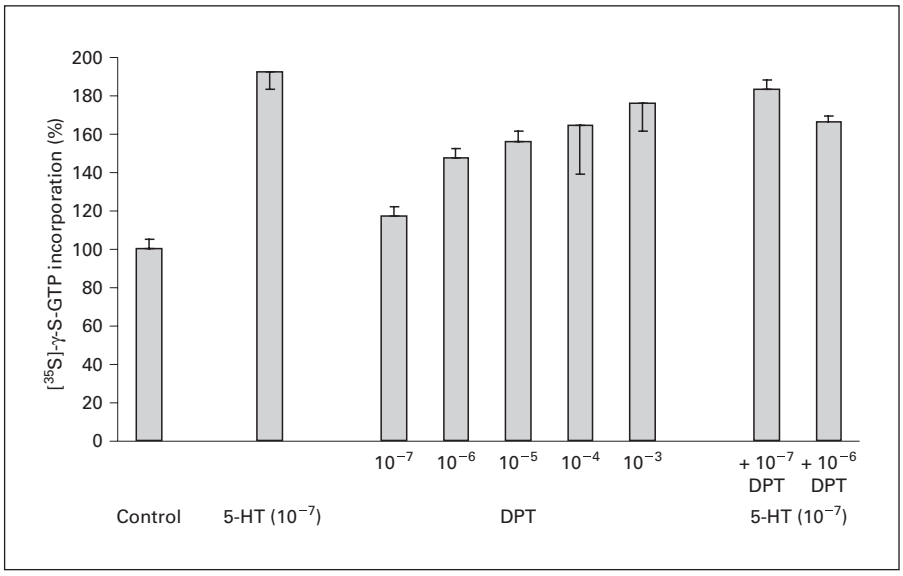

Fig. 5. Effects of serotonin (5-HT) and dipropyltryptamine (DPT) on incorporation of [^{35}S]γ-S-GTP into membrane preparations of the human 5-HT1a receptor (H5-HT1aR)-G protein complex. Control represents incorporation in the basal setting (buffer). 5-HT concentration is 0.1 μmol/l in all cases. Molar DPT concentrations are listed below the bars. Results are expressed relative to basal incorporation as mean ± SEM with n's of 4 (1 mmol/l DPT)–16 (control). Additional experimental conditions are described in Methods.

tions following DPT binding tested the hypothesis that DPT is an antagonist. Figure 4 demonstrates that DPT reduced forskolin-stimulated cAMP concentrations. While at 0.1 μmol/l DPT the effect is not significant, at 1 μmol/l it clearly is. This action is consistent with agonistic rather than antagonistic effects. The full agonist serotonin dramatically stimulated the receptor, reducing cAMP levels in a concentration-dependent manner. Additionally, for comparison to the binding experiments, 0.1 μmol/l 8-OH-DPAT (DPAT), a full agonist, decreased forskolin-stimulated cAMP by more than 95%. In combination with 5-HT, 0.1 μmol/l DPT diminished the effects observed with 5-HT alone. This result could be consistent with the conclusion that DPT is an antagonist. However, any agent, agonist or antagonist that is competing with a higher affinity full agonist, could potentially reduce the full agonist's effects. Such combinations of agents would produce a complicated set of effects, dependent on the relative concentrations and affinities of the two agents. Thus, it is not possible to completely test the hypothesis with these results. With another different but related measure of the signal transduction system, we directly tested the intrinsic activity of DPT.

γ-S-GTP Incorporation

Figure 5 summarizes another approach to testing DPT's capacity to trigger signal transduction at H5-HT1aR. As anticipated, 5-HT (0.1 μmol/l) by itself incorporated 92% more than the basal (buffer) incorporation of [^{35}S]γ-S-GTP. Additionally, DPT showed significant concentration-dependent incorporation of [^{35}S]γ-S-GTP. From baseline at 0.1 μmol/l, DPT increased incorporation to a maximum of 76% above control at 1 mmol/l. When 5-HT (0.1 μmol/l) and DPT (0.1 or 1.0 μmol/l) were administered together, the effect of 5-HT alone was reduced by DPT. Together, DPT and 5-HT were not able to exceed the effect of 5-HT alone or to even equal the 5-HT effect alone. This would indicate that DPT is acting as an antagonist in the presence of the full agonist serotonin, an effect consistent with that of a partial agonist. By raising the DPT concentration to 1 mmol/l when administered alone, the incorporation effect (76%) is almost as large as that produced by 5-HT alone (92%) at a 10,000-fold lower concentration (0.1 μmol/l). While the near full efficacy of DPT at 1 mmol/l compared to 5-HT at 0.1 μmol/l could suggest that DPT is a full agonist with much lower affinity than 5-HT, the overall impression left by these observations is that DPT is a partial agonist.

Discussion

DPT represents an interesting example of a drug that has seen therapeutic and recreational uses while undergoing few pre-clinical experimental analyses. While many closely related analogues of DPT have been carefully investigated with respect to medicinal chemistry and in vitro and in vivo pharmacology, DPT itself has been on the sidelines.

The results described in figures 2 and 3 provide strong evidence that DPT binds competitively to the H5-HT1aR ligand-binding site. With an IC_{50} of 0.1 μmol/l as measured by displacement of the high-affinity (subnanomolar K_d) agonist [^{3}H]8-OH-DPAT, DPT's affinity is moderate. This conclusion is in line with previously published results for DMT at the 5-HT1aR [9, 11], where the IC_{50} was in the range of 0.1–0.2 μmol/l. Since [^{3}H]8-OH-DPAT is a highly characterized, widely accepted specific ligand at the 5-HT1aR ligand-binding pocket, the results appearing in figure 3 provide compelling data that DPT and [^{3}H]8-OH-DPAT compete with each other for this site. We have conducted repeated Hill analyses at 5-HT1aR with [^{3}H]8-OH-DPAT [42] and now with DPT (data not shown); these results consistently demonstrate non-cooperative, unitary site binding, and addition of DPT to the mix changes nothing about this conclusion.

With the additional bulkiness of DPT's alkyl substituents, we originally hypothesized that DPT would be antagonistic. Upon testing, it appears that this assumption is not correct, and we now believe that the data shown in figures 4 and 5 suggest that DPT is a partial agonist. Since H5-HT1aR is negatively coupled to AC via G_i in CHO, agonists will activate G_i. In the experimental paradigm used here, forskolin-stimulated cAMP levels should be reduced in the presence of agonist, as shown by 5-HT at two different concentrations. 1 μmol/l DPT, a concentration that should produce significant effect as judged by the binding data shown in figures 2 and 3, also produced lower cAMP levels, an effect consistent with agonistic behavior. The combination of 5-HT and DPT produced an effect lower than that of 5-HT alone. Together these observations are consistent with the hypothesis that DPT is a partial agonist; however, the results are not conclusive.

To more completely test the agonistic effects of DPT, we conducted concentration-effect experiments at the G protein level. An agonist such as 5-HT should be able to increase incorporation of [^{35}S]γ-S-GTP into G_i. The results presented in figure 5 demonstrate this effect at 0.1 μmol/l serotonin. At a 10,000-fold concentration range of DPT (from 0.1 μmol/l to 1 mmol/l), graded increases in [^{35}S]γ-S-GTP incorporation were observed. A lower-affinity full agonist should be able to achieve the effectiveness of a higher-affinity full agonist, albeit at higher concentration. Comparison of DPT at 1 mmol/l with 5-HT at 0.1 μmol/l almost allows this conclusion, but not quite, as DPT produced a 76% incorporation increase compared to 92% for 5-HT. Judging from the binding data of DPT (fig. 2, 3), 1 mmol/l is a very high, saturating concentration.

With DPT's binding affinity in the submicromolar range (IC 50 of 0.1 μmol/l), binding theory would imply that a full agonistic effect should be achievable at 1,000-fold higher concentration (0.1 mmol/l). However, even at 1 mmol/l, DPT still is not able to produce the agonistic effect of 5-HT, and as in the cAMP experiments, the higher magnitude effect of 5-HT alone was never achieved when 5-HT was in the presence of DPT. Thus, even though DPT comes close to fulfilling the requirements for a full agonist, it falls short. Interpretation of the binding data and two different measures of efficacy suggest that DPT is best classified as a moderate affinity partial agonist at the H5-HT1aR.

Published studies with DMT in rat brain [11] established the full agonist properties of the drug using both cAMP determinations and GTP inhibition studies. If results with DMT in rat 5-HT1aR and our findings with DPT in H5-HT1aR can be compared, the two drugs have similar affinities for both receptors; both drugs also show agonistic properties at the receptors. However, while DMT is a full agonist in comparison to the full agonist [^{3}H]8-OH-DPAT, DPT fails to achieve the full efficacy of the agonist, 5-HT, even at a very high concentration (1 mmol/l). Thus, it would appear that the concentration and magnitude effect characteristics of DPT, while similar to those of DMT, better fit the definition of partial agonist at H5-HT1aR. It would be interesting to conduct thermodynamic studies with DMT and DPT to analyze potential enthalpic and/or entropic differences in binding parameters that may lead to the transition from full to partial agonist properties at 5-HT1aR.

In preliminary results at the rat 5-HT2aR [37] we have previously shown that DPT is a low-affinity agonist or partial agonist (IC_{50} of about 4 μmol/l). The results provide a provocative contrast with the findings of Deliganis et al. [11] with DMT at rat 5-HT2aR. That is, while DMT is an antagonist, DPT appears to be an agonist. These differences cannot be explained on a species basis since both used rat receptors; however, the DMT work used rat cerebral cortex while our DPT studies utilized cloned receptor. 5-HT1aR and 5-HT2aR are known to have very different binding determinants, and the structural changes from DMT to DPT, reducing agonism at 5-HT1aR, as we have observed in this study, may well lead to the opposite transition at 5-HT2aR [44]. Additional binding, signal transduction, and thermodynamic studies with DMT and DPT at 5-HT2aR would be instructive, especially with regard to potentially explaining the in vivo differences between DMT and DPT in humans.

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