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Receptor regulatory properties evident in the molecular similarity of serotonin receptor ligands and purine nucleotides

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Abstract

Previous computational studies have explored the relative molecular similarity inherent in the ligands of neurotransmitter-regulated cell receptors and purine nucleotides. This study presents the results of an investigation of the major serotonin (5-HT) receptor classes, using molecular superimposition and fitting data. Ligands for $5HT_{1B/C/D}$ and $5HT_{4/7}$ receptors identified pharmacophores in the adenine ring of ATP. 5-HT_2 and 5-HT_3 receptor ligands identified pharmacophores in the guanosine nucleotide and cyclic nucleotide, respectively. The described molecular similarity is consistent with the cyclic nucleotide responses observed during signal transduction events initiated by 5-HT, and the reported similarity between ligands of the 5-HT_{1B} and 5-HT_{1D}, 5-HT_{1A} and 5-HT_7, and 5-HT_4 and 5-HT_3 receptors. The results are discussed in terms of current pharmacophoric models and signal transduction events involving interaction between G-protein receptors and catalytic sites.

Introduction

The classification of the serotonin (5-HT) receptor family into at least 14 subtypes is based on gene structure, amino acid sequence homology and signalling cascades (Hoyer et al 1994). A distinct distribution pattern of autoreceptors has been described on serotonin neurones (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}), whereas other subtypes function as heteroreceptors (5-$HT_{1B,D}$, 5-$HT_{2A,C}$, 5-HT_3, 5-HT_4) regulating the release of neurotransmitters that include noradrenaline, dopamine and GABA (Barnes & Sharp 1999). The characteristic 7-unit transmembrane protein structure of G-protein receptors is shared by six of the major 5-HT classes. 5-HT_3 operates a ligand-gated cation channel, characterized by fast depolarizing responses (Hannon & Hoyer 2002). The 5-HT_2 receptor subtype is distinctive in coupling to phosphatidylinositol hydrolysis (Hannon & Hoyer 2002). All other receptor subtypes couple to adenylate cyclase, 5-HT_1 negatively so (Barnes & Sharp 1999).

In common with other neurotransmitters, signal transduction events instigated by 5-HT are associated with concentration-dependent changes in the nucleotides of adenine and guanine (Raymond et al 2001). Changes in adenylate cyclase and phospholipase C activities require the participation of a guanine nucleotide binding protein (Sanders-Bush & Canton 2000). The two subfamilies of membrane guanylate cyclases, consisting of single membrane-spanning modular proteins, are responsive to either hormone activation of a cell surface receptor or Ca^{2+} signalling (Sharma 2002).

Synergy between agonists from different receptor classes, long evident in functional tests on intact cells, may be considered in terms of cross-talk between G-proteins, receptor oligomerization, or interaction at the level of receptor binding (Devlin & Christopoulos 2002). In the aforementioned study, Devlin and Christopoulos describe the interaction between 5-HT and a guanine nucleotide on cannabinoid agonist binding in rat cerebellar membranes. This type of interaction is central to the rationale for the present study: commonality in the structures of ligands belonging to the different small molecular weight neurotransmitter/vasoactive amine receptor classes, based on

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adenine and guanine nucleotide structure (Williams et al 2002). Investigation of the structures of receptor ligands should improve our understanding of receptor cross-talk and signal transduction mechanisms. Here, we explore the relative molecular similarity contained in the ligand structures of the major serotonin receptor subtypes and the purine nucleotides.

Materials and Methods

Representative 5-HT receptor agonist and antagonist structures were selected, if available, for the receptor subtypes under investigation. The Nemesis program (Oxford Molecular version 2.1) was used to carry out charge calculations, conformational analysis and superimposition of the molecular structures. Purine nucleotide structures (ATP, GTP) are from the program library file. The molecular structures of cyclic GMP (cGMP), 5-HT and selective 5-HT agonists and antagonists are built from structures in the program fragment file. 5-HT_1: LY301317, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), WAY100635, GR127935, SB258719 (Gaster & King 1998); sumatriptan (Hibert et al 1990), PNU109291 (Slassi 2002), RU24969 (O'Neill & Parameswaran 1997), methyl-2-(1-napthyloxy)-ethylamine (Ishmaiel et al 1997); LY344370, BRL54443 (Gaster & King 1998); 5-HT_2: MDL100907 (Gaster & King 1998); BW723C86, ORG37684, SB242084, RO60175 (Gaster & King 1998), 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane (DOB), 9-(aminomethyl)-9-10-dihydroanthracene (AMDA), cyproheptadine (Westkaemper & Glennon 2002); 5-HT_3: quipazine (Oh et al 2001), MDL72222, MD-354 (Dukat et al 2001); 5-HT_4: UCM21195, metoclopramide, cisapride (Lopez-Rhodriguez et al 2002), SB20470 (Gaster & King 1998); 5-HT_7: SB269970a, (4,5-dihydroimidazol-2-yl)-biphenylamine (Hagan et al 2000).

Nitrogen atoms in agonists and antagonists corresponding to the protonated nitrogen atom on 5-HT are designated as Nsp^{3+}. Data from conformational analysis of the ligands investigated were obtained by rotating about the molecular bonds given in Table 1. For 5-HT, torsion angle labels relate to the $5HT_{1A}$ structure in Figure 1. All structures are minimum energy conformers, although it is considered that thermal energy will allow all conformers within 3 kcal of the minimum energy conformation to be valid. The low energy conformers of the purine nucleotides, ATP and GTP, have been used previously (Williams et al 2002).

Molecules are fitted to the nucleotide structures using three points of contact that incorporate the protonated nitrogen atom and other atoms showing similarity of type, atomic distance and partial charge. The quality of fit is expressed by the root mean square (rms) value in the tables containing the fitting data. With respect to data for the agonists and antagonists given in the figures and tables, atom N2 is equivalent to atom N6, as the designation depends on whether the fit relates to the guanine (N2) or adenine (N6) purine rings.

Table 1 Conformational data

Molecule	Figure reference	Torsion angles (°)
ATP	2c	O6C7C8O9 −59, O9C10N9C6 −38
GTP	4b	O6C7C8O9 −68, O9C9N1C5 −47
5-HT_{1A}	1	C10, C11C1C2 −98, C11C1C2N6 −52
RU24969	2c	C2C3C4C5 153
Methyl-2-(1-napthyloxy)-ethylamine	2d	C3C2O5C7 175, C2O5C7C8 177, O5C7C8N6 −32, C7C8N6C9 −179
S-PNU109291	2e	C9C10N7C8 −64, C7N6C6C5 161, N6C6C5C4 −48, C6C5C4O1 67
Sumatriptan	2f	C5N6C3C1 −144, N6C3C1C7 −55, C3C1C7C2 −88, C10C9C8S1 117, C9C8S1N7 −180, C8S1N7C6 −103
LY344370	3a	C7C3C4C5 −102, C1C6N9C8 −19, C6N9C8C9 180, N9C8C9C10 171
BRL54443	3b	C9C6C4C5 62
S-UCM21195	3c	C12C11C10N9 2, C11C10N9C8 −179, C10N9C8C7 101, N9C8C7C1 −175
R/*R*-cisapride	3d	C1C9C2N8 164, C9C2N8C7 156, C2N8C7C1 −66, C6N6C10C11 68, N6C10C11C12 51, C10C11C12O8 −64, C11C12O8C13 −172, C12O8C13C14 149
R-SB269970	3e	C9C8S1N8 99, C8S1N8C7 89, S1N8C7C5 109, N8C7C5C10 −62 C7C5C10N6 62, C5C10N6C11 −170
Dihydroimidazole-biphenylamine	3f	C6C7N5C4 −98, C7N5C4N6 −9
R-MDL100907	4a	C8C9C12C13 −94, C9C12C13C3 −65, C7N2C10C11 −159, N2C10C11C12 41, C10C11C12C13 71
S-BW723C86	4b	C2C5C6C7 −137, C5C6C7N2 36, C3C4O7C8 163, C4O7C8C9 −60, O7C8C9S1 113
S-ORG37684	4c	C4C5O9C3 94, C5O9C3C11 −69
Quipazine	4d	N5C2C1C3 −112
S-MDL72222	4e	C1C6C9O2 −0, C6C9O2C2 −180, C9O2C2C3 −64
MD354	4f	C2C1N3C7 115, C1N3C7N2 3

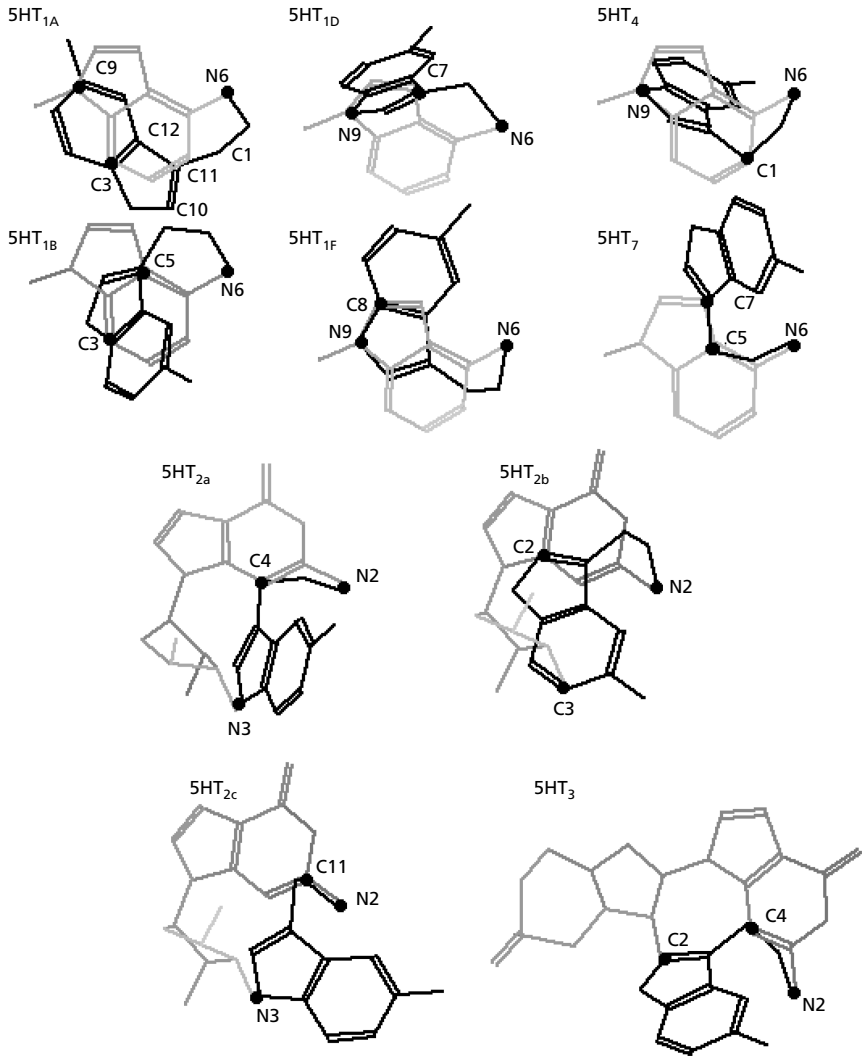

Figure 1 Fitting of 5-HT to adenine and guanine nucleotide structures.

Results

Fitting of 5-HT$_1$, 5-HT$_4$ and 5-HT$_7$ receptor ligands to ATP

For the purposes of this study, the adenine ring of ATP is defined by imidazole ring A and pyrimidine ring B. There are at least 13 different fits of the minimum energy conformer of 5-HT to atom groups in ring A inclusive of N6, with summed interatomic distances for the three fitting atomic pairs ranging from 0.17Å to 0.59Å. There are four exclusive fits of 5-HT to ring B (summed atomic distances 0.36–0.62Å). Furthermore, there are nine fits involving both A and B rings (summed atomic distances 0.33–0.67Å). These data (not given) indicate that the 5-HT conformer is of little use for identifying relevant pharmacophores within the ATP structure, without the use of subtype-specific 5-HT ligand structures. Further study of the selected conformers of 5-HT, adenine and the adenine nucleotide reveals that the atomic distances in 5-HT do not permit a fit to atoms that include N2 and a ribose ring hydroxyl group (N–O8 distance = 6.8Å). The fitting of 5-HT to ATP in this study is thus confined to the adenine ring.

The 5HT$_{1A}$ agonists 8-OH-DPAT and LY301317 are based on rigid cyclic ring structures. Both structures provide equivalent fits to the adenine ring, with N6–C3 and N6–C9 distances of 4.3Å and 4.9/5.1Å (Table 2; Figure 2). The *S*-enantiomer of 8-OH-DPAT and the 5-HT$_{1A}$ antagonist WAY100635 fit in the same manner to ATP (data not given). RU24969 and methylnapthyloxyethylamine, representative 5-HT$_{1B}$ receptor agonists, provide good fits to the pyrimidine moiety of the adenine ring. In contrast, structures of the 5-HT$_{1D}$ agonists, sumatriptan and PNU109291, relate to atoms in the imidazole moiety of the adenine ring. Dimensions of the 5-HT$_{1B}$ (2.2, 2.8, 4.2Å) and 5-HT$_{1D}$ (2.3, 2.8, 4.6Å) pharmacophores, based on the 5-HT structure, are similar. These 5-HT$_{1B/D}$ pharmacophores are also evident in the structure

Table 2 Molecular superimposition data for fitting of 5-HT_1, 5-HT_4 and 5-HT_7 receptor ligands to the adenine nucleotide

Molecule	Superimposed atomic distance (Å)			Intramolecular distance (Å)			rms value
	N6	**C3**	**C9**	**N6–X3**	**N6–X9**	**X3–X9**	
ATP				4.1	4.6	2.5	
5-HT_{1A}	0.18	0.24	0.25	4.2	4.3	2.8	0.0588
R-8-OH-DPAT	0.13	0.05	0.09	4.3	4.9	2.4	0.0108
LY301317	0.24	0.14	0.26	4.3	5.1	2.4	0.0381
	N6	**C5/O5**	**C3**	**N6–X3**	**N6–X5**	**X3–X5**	
ATP				4.1	2.4	2.4	
5-HT_{1B}	0.17	0.24	0.09	4.2	2.8	2.2	0.0156
Methyl-2-(1-napthyloxy)-ethylamine	0.11	0.16	0.13	4.3	2.4	2.4	0.0110
RU24969	0.06	0.03	0.07	4.3	2.5	2.4	0.0139
	N6	**N/C7**	**N/X9**	**N6–X7**	**N6–X9**	**X7–X9**	
ATP				3.1	4.6	2.2	
5-HT_{1D}	0.12	0.16	0.04	2.8	4.6	2.3	0.0196
Sumatriptan	0.03	0.06	0.05	3.0	4.7	2.3	0.0066
S-PNU109291	0.12	0.19	0.25	2.8	4.8	2.5	0.0440
	N6	**C6**	**N9/C9**	**N6–C6**	**N6–X9**	**C6–N9**	
ATP				4.3	4.6	1.4	
5-HT_{1F}	0.09	0.10	0.02	4.2	4.6	1.4	0.0085
BRL54443	0.08	0.12	0.18	4.3	4.9	1.4	0.0112
LY344370	0.18	0.15	0.03	4.7	4.9	1.4	0.0020
	N6	**C1**	**C9/N9**	**N6–C1**	**N6–X9**	**C1–X9**	
ATP				2.3	4.6	4.1	
5-HT_4	0.07	0.15	0.13	2.5	4.6	3.8	0.0210
UCM21195	0.10	0.14	0.08	2.5	4.7	3.8	0.0267
Cisapride	0.09	0.14	0.06	2.5	4.6	4.2	0.0056
	N6	**X5**	**C7**	**N6–X5**	**N6–X7**	**X5–X7**	
ATP				2.4	3.1	1.4	
5-HT_7	0.14	0.19	0.20	2.5	2.8	1.5	0.0355
Dihydroimidazole	0.08	0.13	0.14	2.5	2.9	1.4	0.0380
SB269970a	0.05	0.14	0.09	2.6	3.1	1.5	0.0240

of the 1B/1D antagonist GR127935 (data not given). Fits of the minimum energy conformer of 5-HT to the above pharmacophores in the adenine ring are presented in Figure 1.

The limited rotational property of the agonist BRL54443 facilitates identification of the 5-HT_{1F} pharmacophore (Figure 3). The indole nitrogen of 5-HT participates in fitting to the 5-HT_{1F} pharmacophore in the adenine ring, a feature common to the agonist LY344370. Cisapride, a 5-HT_4 receptor agonist, and the antagonist UCM21995 identify a pharmacophore of dimensions 2.5, 3.8/4.2, 4.6/4.7Å in the adenine ring. The 5-HT_4 agonist metoclopramide fits in a similar manner (data not given). In comparison with the structure of 5-HT, cisapride and UCM21995 fit with carbonyl groups positioned over the pyrrole moiety of 5-HT. The 5-HT_7 pharmacophore is identified by the agonist dihydroimidazolbiphenylamine and the antagonists SB269970 and SB258719 (data not given). There are two alternative fits of 5-HT to the 5-HT_7 pharmacophore; the one shown is most similar to fits of the aforementioned ligands. In view of the reported 5-HT_7 agonist activity of 8-OH-DPAT, the fit of this structure to the 5-HT_7 pharmacophore was tested and confirmed (N6 0.09Å, C5 0.18Å, C7 0.15Å, rms 0.0418).

Fitting of 5-HT_2 receptor ligands to GTP

In contrast to the restricted fitting of 5-HT to the purine ring of ATP, the 5-HT_2 receptor ligands relate to the nucleoside moiety of GTP. In particular, the N2–O3 distance (5.1Å), incorporating the ribose ring hydroxyl, is evident in the pharmacophores of the 5-HT_2 receptor subtypes (Table 3; Figures 1 and 4). The indole nitrogen of 5-HT participates in fitting to the 5-HT_{2A} and 5-HT_{2c} pharmacophores. The interatomic distances of fitted 5-HT_{2B} ligands show less variation than those of the other subtypes. The 5-HT receptor ligand structures of DOB, *S*-RO60175 cyproheptadine, AMDA and SB242084, fit to the 5-HT_2 pharmacophores in keeping with their known selectivity (data not given). In view of the reported stimulatory effect of 8-OH-DPAT on guanyl cyclase activity, the fitting of this structure to the guanine nucleotide was investigated and confirmed for the 5-HT_{2A} (N2 0.11Å, C3 0.15Å, O3 0.18Å) and 5-HT_{2C} (N2 0.14Å, C11 0.09Å, C3 0.11Å) pharmacophores defined in GTP.

Fitting of 5-HT_3 receptor ligands to cGMP

The potent and selective 5-HT_3 antagonist, MDL72222, and quipazine, a 5-HT_3 receptor agonist, have three common

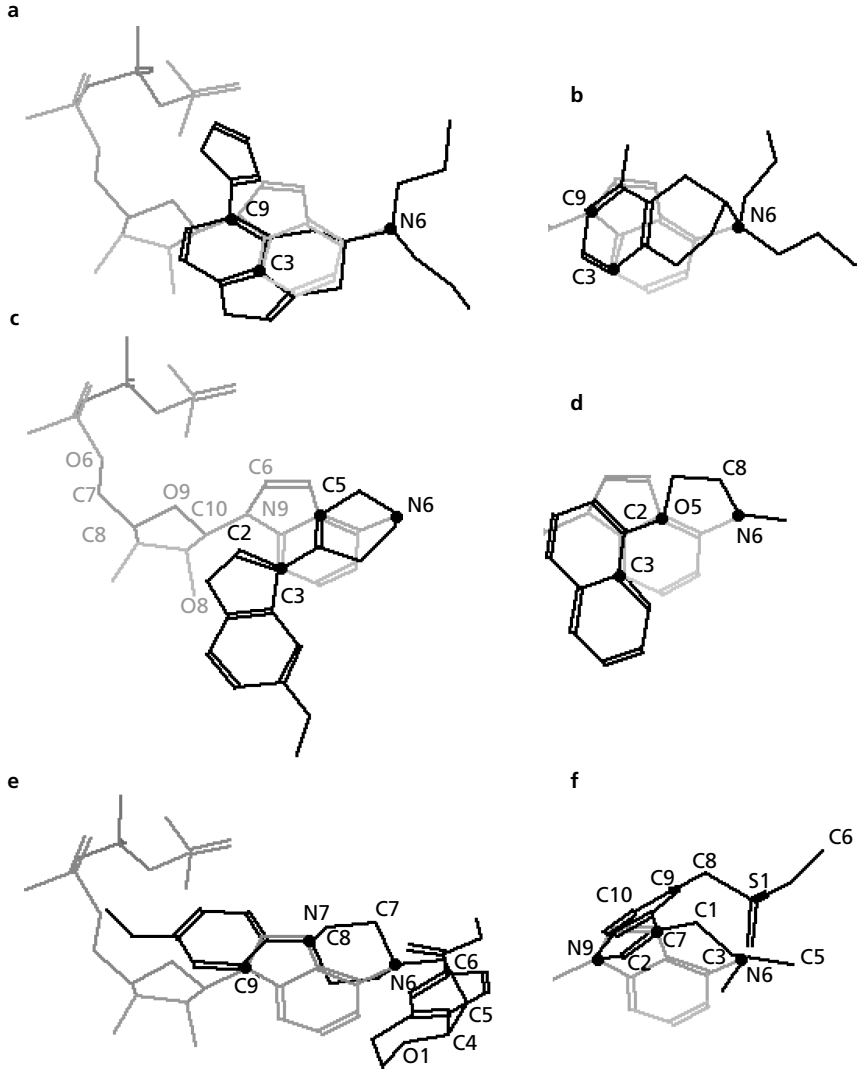

Figure 2 Fitting of 5-HT receptor ligands to the adenine ring of ATP: 5-HT_{1A} (a) LY301317, (b) *R*-8-hydroxy DPAT; 5-HT_{1B} (c) RU24969, (d) methyl-2-(1-napthyloxy)-ethylamine; 5-HT_{1D} (e) *S*-PNU109291, (f) sumatriptan.

centres of charge at nitrogen atom N2, and at distances 2.4/2.5Å and 4.1/4.2Å from atom N2. The latter distances correspond to atoms N3 and O2 in the structure of cGMP (Figure 4). 5-HT and MD354 provide very similar fits to the cGMP structure. The triangular pharmacophore described by the above 5-HT_3 ligands has the dimensions 2.5, 2.4–2.6, 3.9–4.2Å.

Discussion

The 5-HT receptor parsimonious tree provides evidence of three major divisions in evolutionary topology (5-HT_2; 5-HT_1; 5-HT_4, 5-HT_6, 5-HT_7), which correspond, respectively, to signal transduction mechanisms involving phosphatidylinositol (Gq) and adenyl cyclase (Gi and Gs) rather than to ligand specificity (Cravchik & Goldman 2000).

Hibert et al (1990) list some of the proposed minimum energy conformations of 5-HT, derived by different methodological approaches, and conclude that conformations and energy prescriptions do not promote any one conformer corresponding to the receptor bound state, as intramolecular energy may balance against intermolecular interaction forces. Receptor binding phenomena are governed by the complementarity between the 3D arrangement of the ligand atomic groups capable of interaction, and the 3D arrangement of suitable receptor residues (Cappelli et al 2002a). Though sharing the same pharmacophoric features and binding site, different modalities may exist for agonists and antagonists, such as a difference in the negatively charged amino acid selected as counter-ion (Cappelli et al 2002a, b). The current approach searches for computational evidence of pharmacophoric groups within the nucleotide structures influenced by 5-HT ligands during cell signal

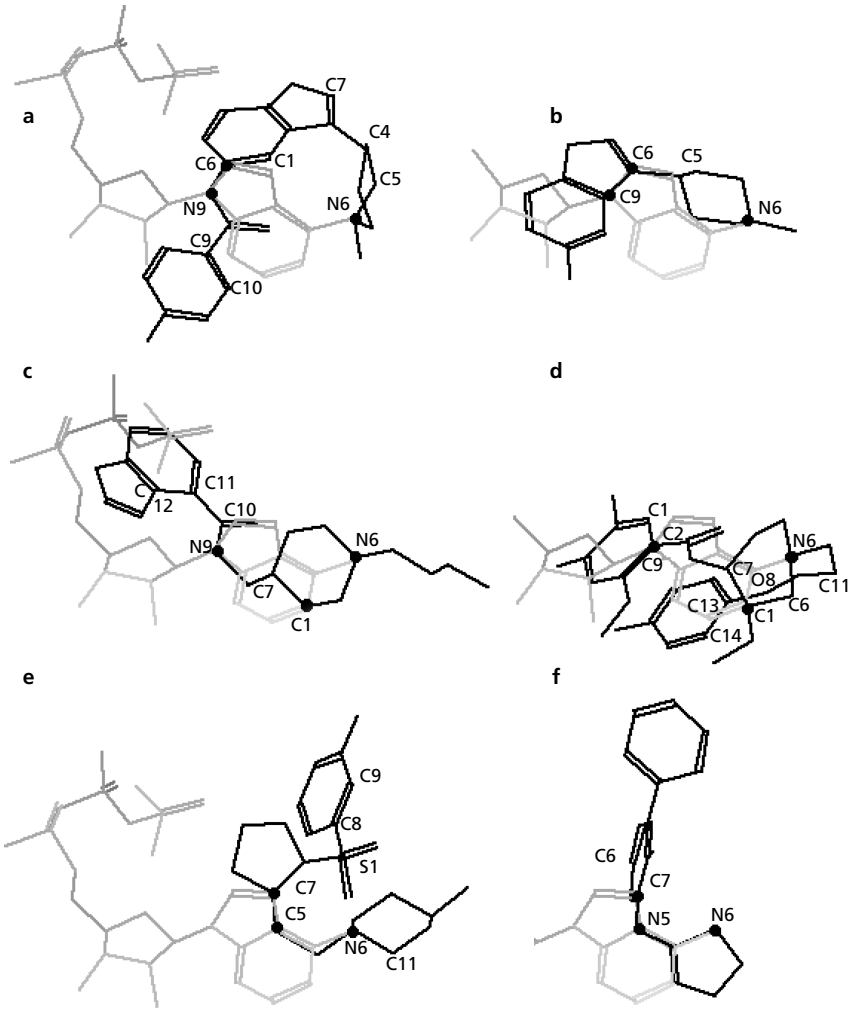

Figure 3 Fitting of 5-HT receptor ligands to the adenine ring of ATP: 5-HT_{1F} (a) LY344370, (b) BRL54443; 5-HT_4 (c) *S*-UCM21195, (d) *R*/*R*-cisapride; 5-HT_7 (e) *R*-SB269970A, (f) 4,5-dihydroimidazol-2-yl-biphenylamine.

transduction events. These biochemical events include the regulation of cAMP and cGMP levels. As mentioned above, these signal transduction mechanisms characterize the 5-HT receptor subtypes. In the present study, however, one conformation of 5-HT is able to represent the structural characteristics found in the 5-HT receptor agonists and antagonists, and the purine nucleotides.

With respect to 5-HT, the structural requirements at 1A and 1B receptor sites are known to be quite different, whereas 1B and 1D receptors are especially similar in their amino acid sequences (Slassi 2002). The affinity of 5-HT for 1B sites is almost 10-times less than at 1A sites but 100-times greater than at 5-HT_2 sites (Hibert et al 1990). The 5-HT_{1A} pharmacophore described by Hibert et al is based on the common structural feature of an aromatic ring with an almost coplanar N atom at 5.2Å (Casy 1993). In the present study, the 5.2Å distance of the 5-HT_{1A} pharmacophore equates to the distal atoms in the aromatic ring rather than to the centroid. The pharmacophores identified by 5-$HT_{1B/1D}$ ligands are similar in their dimensions. There is, however, no relationship between goodness of fit to the purine nucleotides and the documented affinity of 5-HT at 5-HT_1 and 5-HT_2 sites.

The purine nucleotide model also links 8-OH-DPAT with the 5-HT_7 and 5-HT_2 pharmacophores. This observation concurs with the known agonist or partial agonist properties of 8-OH-DPAT at the 5-HT_7 receptor, and the capacity of 8-OH-DPAT to stimulate cGMP formation (Thomas et al 1999; Regina et al 2003).

Similarity in the transmembrane amino acid sequences of 5-HT_{1A} and α_1-adrenergic receptors (45% homology) accounts for poor drug selectivity between these receptors (Trump-Kallmeyer et al 1992). This similarity has also resulted in the previous allocation of the 5-HT_{1A} pharmacophore identified in the guanosine nucleotide to the α_1-adrenergic receptor (Williams et al 1998), a finding that will require re-evaluation in the light of the present study.

The essential molecular features necessary for 5-HT_2 receptor binding, evident in the structures of LSD and DOB, have been identified from 3D rhodopsin and bacteriorhodopsin

Table 3 Molecular superimposition data for fitting of 5-HT_2 and 5-HT_3 receptor ligands to the guanine nucleotides

Molecule	Superimposed atomic distance (Å)			Intramolecular distance (Å)			rms value
	N2	**C4**	**N3/O3**	**N2–C4**	**N2–X3**	**C4–X3**	
GTP				2.3	5.1	4.1	
5-HT_{2A}	0.22	0.16	0.30	2.5	4.6	3.8	0.0304
R-MDL100907	0.08	0.20	0.14	2.4	5.1	3.7	0.0153
	N2	**C2**	**C3**	**N2–C2**	**N2–X3**	**C2–X3**	
GTP				3.6	5.1	4.5	
5-HT_{2B}	0.16	0.15	0.03	3.9	5.1	4.5	0.0082
S-BW723C86	0.13	0.10	0.03	3.8	5.2	4.5	0.0188
	N2	**C11**	**N/X3**	**N2–C11**	**N2–X3**	**C11–X3**	
GTP				1.4	5.1	4.9	
5-HT_{2C}	0.20	0.14	0.31	1.5	4.6	4.5	0.0073
S-ORG37684	0.09	0.19	0.26	1.5	4.8	4.5	0.0079
	N2	**X3**	**X2**	**N2–X3**	**N2–X2**	**X2–X3**	
cGMP				2.3	4.2	2.8	
5-HT_3	0.16	0.16	0.24	2.5	3.9	2.6	0.0321
Quipazine	0.05	0.25	0.20	2.5	4.1	2.4	0.0128
MD-354	0.13	0.20	0.29	2.5	3.9	2.4	0.0066
S-MDL72222	0.07	0.22	0.16	2.5	4.2	2.5	0.0003

structures, structural–activity relationships, and site-directed mutagenesis studies (Westkaemper & Glennon 1994). 5-HT_2 receptor models incorporate the electrostatic interaction of a protonated amine with the conserved receptor aspartate residue, and two receptor donated hydrogen bonds to relevant alkoxy, carbonyl, double bond or indole nitrogen species (Westkaemper & Glennon 1994). More recently, Westkaemper & Glennon (2002) have described the 5-HT_2 antagonistic properties of cyproheptadine and AMDA, which consists of a phenylethylamine skeleton within a tricyclic ring system. Although AMDA does not meet the requirements of existing 5-HT_2 models, this structure provides good fits to the 5-HT_2 pharmacophores identified in the guanine nucleotide.

The 5-HT_4 receptor antagonist model described by Lopez-Rodriguez et al (2002) highlights the similarity between 5-HT_3 and 5-HT_4 pharmacophores, and a difference in the acceptable substituent group volume on the basic N atom. A large substituent promotes selective binding at the 5-HT_4 receptor. The common pharmacophore contains aromatic and coplanar carbonyl groups, and a protonated N atom. The carbonyl oxygen is positioned at about 3.6Å from the centroid of the aromatic ring. The aromatic ring and carbonyl oxygen are, respectively, 8.0Å (7.5Å for 5-HT_3) and 5.4Å from the basic N atom. These pharmacophoric features, present in receptor antagonists and agonists, are more difficult to reconcile with the structure of 5-HT. In the present study, conformations of the 5-HT_4 receptor ligands are based on the structure of metoclopramide. N (basic)–O (carbonyl) distances in the 5-HT_4 agonists are 2.5Å, and 4.2Å in the antagonist UCM21195. Respective N-centroid distances are 5.8Å and 7.4Å. N-centroid and N–O (carbonyl) agonist distances presented in this study are thus shorter than equivalent distances in the antagonist structures. In relation to ATP, structures of the 5-HT_3 and 5-HT_4 antagonists are similar, with a fit at 2.5Å, and N (basic)–O (carbonyl) distances of 3.8/4.2Å.

Cappelli et al (2002a) have described the derivatization and refinement of a 5HT_3 pharmacophore based on the agonist quipazine. The pharmacophoric features of this model include a charge assisted hydrogen bond involving the protonated terminal piperazine N atom, hydrogen bonding between the heterocyclic N atom and receptor, interaction between the aromatic ring and receptor, and short range interactions in the receptor area corresponding to the c-edge of the quinoline nucleus. 5HT_3 receptor agonist models fail, however, to accommodate the shorter centroid to amine distance of 5HT_3 agonist arylguanidines (3.7–4.9Å), which most probably bind in a similar manner to arylbiguanides (Dukat et al 2001). In the present study, the fit of the arylguanidine structure to the cyclic guanosine nucleotide is most similar to that of 5-HT.

Lopez-Rodriguez et al (2000) have described a pharmacophoric model of 5-HT_7 antagonism that contains a basic N atom, a hydrophobic region at 4.8–5.8Å, an aromatic ring at 7.6–8.6Å, and a H-bond acceptor group at 7.3–8.3Å. The aforementioned molecular distances here relate to the distal atoms of the aromatic and hydrophobic rings in SB269970A, the 5-HT_7 antagonist used in this study.

In previous studies on receptors for catecholamines, the receptor ligands promoting adenyl or guanyl cyclase activity have fitted to a nucleoside ribose ring hydroxyl group (Williams et al 2002). The current results show that the fitted structures of some inhibitory 5-HT_1 agonists clearly influence the ribose-triphosphate moiety that participates in formation of the cyclic nucleotide. In contrast, stimulatory 5-HT_4 and 5-HT_7 agonists fit away from this site.

Protein kinases acting on G-protein activated phospholipase C enzymes are dependent on the cyclized form of

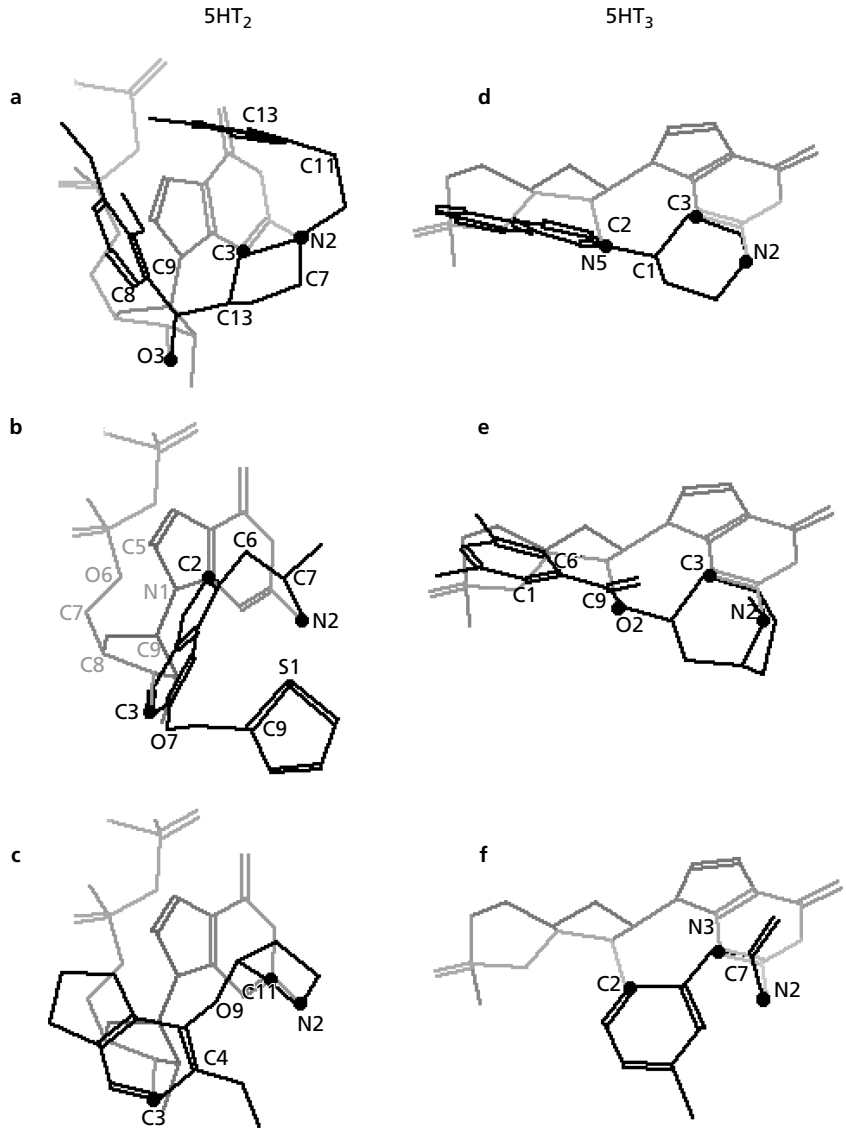

Figure 4 Fitting of 5-HT receptor ligands to guanine nucleotide and cyclic nucleotide structures: (a) 5-HT_{2A} *R*-MDL100907, (b) 5-HT_{2B} *S*-BW723C86, (c) 5-$HT2_C$ *S*-ORG37684; 5-HT_3 (d) quipazine, (e) *S*-MDL72222, (f) MD354.

the guanine nucleotide (cGMP) (Xia et al 2001). cGMP is implicated in the signal transduction mechanisms of 5-HT_2 and 5-HT_3 receptors. Tohda et al (1991, 1995) have reported on the mechanisms of cGMP formation induced by two 5-HT receptors linked to cytosolic and membrane-bound guanylate cyclases, in neuroblastoma N18TG-2 cells. Kanada et al (1993) and MacNaughton (1993) have described a 5-HT_3 receptor responsible for raising cGMP in rat and guinea-pig intestines. Stimulation of 5-$HT_{2A,B,C}$ receptors triggers cGMP production by membrane-bound guanylate cyclases in brain tissue (Kaufman et al 1995; Nebigil et al 2001; Regina et al 2003). Currently, there is no evidence for a direct link between 5-HT receptor activation and cGMP formation, as has been reported for $\alpha_{2D.A}$-adrenergic receptor activation of a Ca^{2+} modulated membrane guanylate cyclase, in pinealocytes and a rat adrenocortical carcinoma cell line (Sharma 2002).

Perspectives on the relationship between G-protein cell membrane receptors and enzyme catalytic sites are changing on the basis of evidence that the extracellular loop participates in the binding of small biogenic amines and reconstitution of the catalytic site (Gu et al 2001; Shi & Javitch 2004). Conformational changes project the two cytosolic catalytic domains of adenylate cyclase to the plasma membrane, facilitating functional activity in the intact cell (Gu et al 2001). The link between pharmacophoric events at cell receptors and subsequent signal transduction processes may be more direct than was once thought. Future investigations should focus on the substrate occupancy of the mobile catalytic domain, and the

potential for interaction between the nucleotide substrates and ligands of G-protein receptors.

Conclusion

Neurotransmitter amines and the purine nucleotides of adenine and guanine show relative molecular similarity. With respect to serotonin, the complexity of the diversity evident in receptor subtypes and signalling mechanisms may be studied at a basic level in the structures of the aforementioned nucleotides. The findings of this study show that adenine and guanine nucleotides provide a link between agonist and antagonist pharmacophoric models and their roles in signal transduction processes.

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