Published in final edited form as:
Neuropharmacology. 2015 April ; 0: 103–108. doi:10.1016/j.neuropharm.2014.12.018.

Allosteric Activation of the 5-HT$_3$AB Receptor by mCPBG☆

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Abstract

The 5-HT$_3$AB receptor contains three A and two B subunits in an A-A-B-A-B order. However, serotonin function at the 5-HT$_3$AB receptor has been shown to depend solely on the A-A interface present in the homomeric receptor. Using mutations at sites on both the primary (E122) and complementary (Y146) faces of the B subunit, we demonstrate that *meta*-chlorophenyl biguanide (mCPBG), a 5-HT$_3$ selective agonist, is capable of binding to and activating the 5-HT$_3$AB receptor at all five subunit interfaces of the heteromer. Further, mCPBG is capable of allosterically modulating the activity of serotonin from these sites. While these five binding sites are similar enough that they form to a monophasic dose – response relationship, we uncover subtle differences in the heteromeric binding sites. We also find that the A-A interface appears to contribute disproportionately to the efficacy of 5-HT$_3$AB receptor activation.

Keywords

mCPBG, serotonin; 5-HT$_3$; allosteric modulation; Cys-loop receptor

1. INTRODUCTION

5-HT$_3$ receptors are excitatory ligand-gated ion channels of the Cys-loop (pentameric) receptor super-family that also includes nicotinic acetylcholine (nAChR), glycine and GABA$_A$ receptors (Lummis, 2012). Members of the super-family share a common architecture and an agonist binding site that spans the interface between two subunits. In heteromeric pentamers, some interfaces can serve as allosteric modulatory sites, being unresponsive to the native agonist but responsive to small molecule allosteric modulators. The prototypical example of this is the allosteric action of benzodiazepines at GABA$_A$ receptors.

☆This work was supported by the NIH (NS 34407)

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The 5-HT_3 family is principally composed of homomeric 5-HT_3A receptors and heteromeric receptors containing some combination of the A subunit and either B, C, D, or E subunits (Davies et al., 1999; Maricq et al., 1991; Niesler et al., 2007). The best characterized heteromer is the 5-HT_3AB receptor. In addition to contributing to the fundamental role of serotonin in the gut, the 5-HT_3AB receptor is widely expressed in the brain and has been implicated in numerous salutary and pathological processes (Krzywkowski et al., 2008; Walstab et al., 2010).

The 5-HT_3AB receptor contains three A and two B subunits (Miles et al., 2013). Fluorescence studies provide strong evidence that the most likely arrangement of the subunits is A-A-B-A-B (Miles et al., 2013; Thompson et al., 2011). Thus there are three potential types of binding sites in the 5-HT_3AB receptor: the single A-A interface also found in the homomeric receptor (**AA**), the two heteromeric binding sites with the B subunit as the primary face (**BA**), and the two heteromeric binding sites with the B subunit as the complementary face (**AB**) (Figure 1). This potential complexity might afford one signaling molecule, serotonin (5-HT), the ability to elicit diverse responses depending on the repertoire of receptors present (Jensen et al., 2008). It also opens up the possible existence of allosteric modulatory sites as are seen in $GABA_A$ receptors.

Serotonin function at the 5-HT_3AB receptor depends solely on the **AA** binding site present in the homomeric receptor (Lochner and Lummis, 2010; Michaelson et al., 2013; Thompson et al., 2011). Multiple studies have capitalized on the wealth of mutagenic data on homomeric receptors to identify crucial, non-conserved residues at both the principal and complementary faces of the B subunit that impact 5-HT function (Lochner and Lummis, 2010; Michaelson et al., 2013). Combined, these studies demonstrate that only one serotonin molecule binds to and activates the 5-HT_3AB receptor, a finding predicted by modeling of receptor kinetic parameters (Hapfelmeier et al., 2003). This makes the 5-HT_3AB receptor unusual in the Cys-loop super-family, where multiple binding sites predominate.

m-chlorophenyl biguanide (mCPBG) is a 5-HT_3 selective agonist (Kilpatrick et al., 1990). As described below, there is no detailed model for binding of mCPBG to the receptor. In the face of this relative paucity of binding information, it is not readily apparent that mCPBG should be incapable of acting at **BA** and **AB** binding sites. Indeed, a molecule has already been identified that is functional at **AB** binding sites (Thompson and Lummis, 2013; Thompson et al., 2012).

Here we demonstrate that, unlike serotonin, mCPBG is capable of binding to and activating the 5-HT_3AB receptor at all five binding sites present in the heteromer. Through mutagenesis, we identify crucial residues on the B subunit corresponding to both principal and complementary faces, establishing that full activity is dependent on **BA** and **AB** sites. Further, we show that mCPBG is capable of allosterically modulating the activity of serotonin from **BA** binding sites, demonstrating the potential for future development of 5-HT_3AB ligands with benzodiazapine-like properties.

2. METHODS

2.1 Mutagenesis and Preparation of cRNA and Oocytes

Mutant 5-HT_3A and 5-HT_3B receptor subunits, within the complete coding sequence for the human 5-HT_3A and 5-HT_3B receptor subunit (accession numbers P46098-1; NP_006019), were cloned into pGEMhe. Mutagenesis reactions were performed using the QuikChange mutagenesis kit (Stratagene), and confirmed by DNA sequencing. Harvested stage V-VI *Xenopus* oocytes were washed in four changes of Ca^{2+}-free OR2 buffer (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5), de-folliculated in 1 mg/ml collagenase for approximately 1 h, washed again in four changes of Ca^{2+}-free OR2 and transferred to ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) supplemented with 0.28 mg/ml pyruvate, 0.05 mg/ml gentamicin and 0.12 mg/ml theophylline. Oocytes were injected with 5–25 ng mRNA produced by *in vitro* transcription using the mMESSAGE MACHINE kit (Ambion, Austin, Texas, USA) from cDNA subcloned into pGEMhe as previously described (Nowak et al., 1998). Electrophysiological measurements were performed after incubation for 24–72 h post-injection at 18° C.

Forward primers used for mutagenesis are listed below (Reverse primers are the reverse complement of the sequence shown):

5-HT_3A E124Q: 5’-CGGACATTCTCAATCAGTTCGTGGATGTGGGGAAG-3’

5-HT_3A Y148A: 5’-GGCGAAGTTCAGAACGCCAAGCCCCTTCAGGTGG-3’

5-HT_3B E122Q: 5’-CGATATCATCATCAATCAGTTTGTGGACATTGAAAG-3’

5-HT_3B E122A: 5’-CGATATCATCAATGCGTTTGTGGACATTGAAAG-3’

5-HT_3B Y146A: 5’-CTGGGACCATTGAGAACGCTAAGCCCATCCAGGTGG-3’

2.2 Characterization of mutant receptors

Agonist-induced currents were recorded at 22–25° C from individual oocytes using the OpusXpress system (Molecular Devices Axon Instruments, Union City, CA). 5-HT, and *m*-chlorophenylbiguanide (mCPBG) (Sigma) were stored as 25 mM aliquots at −20° C, diluted in Ca^{2+}-free ND96 buffer (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$,, 5 mM HEPES, pH 7.5) and delivered to cells via the automated perfusion system of the OpusXpress. Glass microelectrodes were backfilled with 3 M KCl and had a resistance of ~1 MΩ. The holding potential was −60 mV. Agonist doses in Ca^{2+}-free ND96 were applied for 15 s followed by a 116 s wash with the running buffer. Dose-response data were obtained for ≥ 8 agonist concentrations on ≥ 5 cells. For 5-HT, dose concentrations ranged from 0.1 μM to 1 mM. For mCPBG, dose concentrations ranged from 0.1 μM to 50 μM. To determine EC_{50} values, concentration-response data were fitted to the four-parameter logistic equation, $I = I_{max}/[1 + (EC_{50}/[A])^{n_H}]$ where I_{max} is the maximal response plateau, [*A*] is the log concentration of agonist and *nH* is the Hill coefficient, using KaleidaGraph v3.6 software (Synergy Software, Reading, PA). Relative efficacies (ε) of *m*CPBG, are reported as $\varepsilon = I_{max\text{-}mCPBG}/I_{max\text{-}5\text{-}HT}$. Efficacy was calculated for individual cells and then averaged and reported as mean ± S.E.M. Statistics were calculated by an unpaired, two-tailed students *t* test with significance ascribed to p-values < 0.05.

3. RESULTS & DISCUSSION

3.1 5-HT and mCPBG behave differently at wild type 5-HT$_3$AB receptors

While 5-HT and mCPBG certainly bind in the same interfacial binding pocket of the receptor, the detailed binding interactions involved are not identical. The amine of 5-HT forms a cation-π interaction at W178 (loop B, Figure 3) (Beene et al., 2002), and its hydroxyl forms a hydrogen bond at E124 (loop A) (Price et al., 2008). However, a comparably detailed binding model of mCPBG has not been enumerated. While mCPBG forms no interaction at W178, it does rely upon E124 for the initiation of channel gating (Miles et al., 2012).

Serotonin activates 5-HT$_3$A receptors with a 3.4 μM EC_{50} and a relatively high Hill coefficient (n^H), indicating a high level of cooperativity (Table 1). Though it need not be the case, this measure of cooperativity has tracked with the level of ligand binding in other Cys-loop receptors (Mazzaferro et al., 2011; Tavares Xda et al., 2012). At the heteromeric AB receptor, both the EC_{50} and Hill coefficient markedly change, with a 7 fold decrease in ligand sensitivity (i.e., an increase in EC_{50}) and a diminution of cooperativity reflected in a decreased Hill coefficient from 2.8 to 1.1 (Figure 2A; Table 1). These data are consistent with the finding that 5-HT likely acts at only the single **AA** binding site in the AB receptor.

mCPBG has a similar potency to 5-HT (3.8 μM) at the homomeric receptor along with a similarly high Hill coefficient (n^H = 3.0) (Table 1). Unlike 5-HT however, both of these measures remain practically unchanged in the AB receptor (EC_{50} = 2.8 μM, n^H = 2.5), (Figure 2B; Table 1) suggesting that mCPBG may retain the ability to bind the receptor at **BA** and **AB** binding sites. Meanwhile the efficacy relative to serotonin (ε) of mCPBG surges from 0.85 to 2.75, as it transitions from a strong partial agonist to a super-agonist (Figure 2C; Table 1). This is consistent with a drop in the absolute efficacy of 5-HT due to its reduction to a single binding site in the heteromeric receptor.

3.2 The 5-HT$_3$B subunit contributes to mCPBG activation of the 5-HT$_3$AB receptor

E124 of loop A on the principal side of the 5-HT$_3$A binding site plays a critical role in mCPBG activation (Figure 3). Mutation of E124 to glutamine has been shown to selectively ablate the ability of mCPBG to activate the 5-HT$_3$A receptor, converting mCPBG to a competitive antagonist (Miles et al., 2012). However we find that the E124Q mutation in the A subunit of the heteromeric receptor, while highly deleterious, does not eliminate the ability of mCPBG to activate the 5-HT$_3$AB receptor. Relative efficacy drops sharply to 0.36 (Table 4) and the Hill coefficient falls from 3.0 to 1.0 (Table 3), suggesting the elimination of functional ligand binding sites. As the mutation disrupts **AA** and **AB** binding sites, this remaining activation suggests that mCPBG acts at **BA** binding sites.

To determine if this difference in response between the A and AB receptors was due to ligand binding at **BA** binding sites or to allosteric effects of the B subunit on the **AA** binding site, an experiment in which both agonists were applied was devised. First, an I_{max} dose of 5-HT was applied to AB receptors bearing the E124Q mutation in the A subunit. After 5-HT maximally opened the channel and saturated its binding sites, mCPBG was co-applied at its EC_{50}. Upon mCPBG addition, a second wave of receptor opening corresponding to an

additional 60 percent beyond the 5-HT I_{max} was observed (ΔI_{max} = +60 ± 9 %, n = 18) (Figure 4A). This result indicates that mCPBG binds allosterically, increasing 5-HT efficacy. When the same experiment is performed with an I_{max} concentration of mCPBG, upon co-application the current sharply declines to approximately the relative efficacy of mCPBG alone (ΔI_{max} = −72 ± 2 %, n = 20) (Figure 4B). This is consistent with mCPBG displacement of 5-HT at the **AA** binding site at higher concentrations.

3.3 mCPBG binds to all five interfaces of the 5-HT_3AB receptor

Given the ability of mCPBG to compete at the orthosteric **AA** binding site, it was presumed that allosteric binding occurs at the equivalent location on B subunit-containing **BA** and/or **AB** binding sites. To determine if the B subunit could functionally contribute to the principal, complementary or both faces of the potential binding site, mutants found to disrupt function in A subunits were introduced at the corresponding sites in the B subunit. E122Q (equivalent to E124 in the A subunit) was chosen for the principal face to probe **BA** binding sites.

Fewer crucial ligand interactions to the complementary face that could probe **AB** binding are known (Van Arnam and Dougherty, 2014). Previous studies demonstrated that Y148 on loop E of the A subunit is critical for both serotonin binding and gating of the homomeric receptor (Beene et al., 2004) (Figure 3). Mutation of Y148 to alanine also results in a loss of function for mCPBG in the homopentamer, though less than that observed for 5-HT (Tables 2 and 3). The relative efficacy of mCPBG increases over 4 fold (Table 4), suggesting that while this mutation affects mCPBG, it is more deleterious for serotonin function. 5-HT_3AB receptors bearing Y148A in the A subunits show a similar pattern of effects. However as serotonin is oblivious to mutation of the B subunit (Table 2), the comparable mutation in the B subunit should probe only whether mCPBG acts at **AB** binding sites.

5-HT_3AB receptors containing mutations that disrupt **BA** or **AB** binding sites were then assessed with both 5-HT and mCPBG. While mCPBG EC_{50} remains unchanged for both mutant receptors, relative efficacy is significantly affected. mCPBG remains a super-agonist of slightly lower efficacy, 1.94 for E122Q (**BA** disrupted) and 2.18 for Y146A (**AB** disrupted) (Figure 5 and Table 4). The lack of effect on mCPBG EC_{50} is expected if the mutations completely disrupt the binding sites that contain them while mCPBG is capable of opening the receptor, though less effectively, through the unaffected binding sites that remain. It is also possible that the apparent agonist efficacy at the receptor is decreased because the fraction of the response corresponding to the disrupted binding sites is shifted outside the observable window for mCPBG. This would not take an enormous loss of function, as mCPBG appears to be a quite effective open channel blocker at concentrations above 100 μM (Hapfelmeier et al., 2003).

The decrease in relative efficacy for the two mutations (E122Q and Y146A) indicates that the B subunit is capable of functionally contributing via both **BA** and **AB** binding sites. The similarity of the magnitude of the effect is consistent with the loss of two out of five possible binding sites in each case. As the number of remaining binding sites in either mutant

receptor would still outnumber that of serotonin (**AA** binding site only), it might be expected that mCPBG remains a super-agonist at the mutant receptors.

5-HT_3AB receptors containing both B subunit mutations (both **BA** and **AB** disrupted) show a relative efficacy that is still further decreased to 1.58. (Figure 5 and Table 4). This is significantly ($p < 0.05$) different from both single mutants, demonstrating that the two mutations are additive, as expected given their actions at different binding site types. The double mutant still has a significantly higher relative efficacy for mCPBG than is seen at the homomeric receptor ($\varepsilon = 0.85$), where mCPBG also binds only **AA** sites. This suggests either that function is not completely disrupted at the **BA** or **AB** binding sites or that the presence of the B subunit allosterically improves the relative efficacy of mCPBG activation through the **AA** binding site. In either case, the disruption of all four B subunit-containing binding sites (**BA** and **AB**) is nearly an order of magnitude less deleterious than the disruption of both **AA** and **AB** binding sites. This suggests that the five binding sites do not contribute equally to mCPBG efficacy and that the **AA** binding site has a special importance.

3.4 Evidence for differences in heteromeric binding site organization

To test whether the mutations are, in fact, completely disrupting function at the binding sites that contain them, heteromeric receptors in which the critical glutamate in every subunit (E124 in the A subunits and E122 in the B subunits) is mutated to glutamine were probed (disrupting all five binding sites). Unexpectedly, this quintuple mutant receptor is still responsive to mCPBG, with a relative efficacy only slightly worse than that of receptors with the mutation in the A subunit alone (disrupting **AA** and **AB**) (Figure 6 and Table 4). Thus it appears that there are subtle differences in the B-containing binding sites of the heteromeric receptor.

The more drastic alanine mutation at E124 shows different effects. 5-HT_3AB receptors with E122A in the B subunits (disrupting **AA** and **AB** binding sites) again showed no change in mCPBG EC_{50} but a large decrease in relative efficacy to 1.60, slightly worse than that for E122Q ($\varepsilon = 1.94$) (Figure 6 and Tables 3 and 4). Heteromeric receptors bearing E124Q in the A subunits and E122A in the B subunits (disrupting all five binding sites) now show the expected destruction of mCPBG agonist activity ($\varepsilon < 0.01$), akin to E124Q mutation in the homomeric 5-HT_3A receptor (Figure 5 and Table 4).

It appears that the **AB** binding site also behaves slightly differently with regard to mutation of E124. It would be expected based on the homomeric receptor that E124Q would destroy both the **AA** and **AB** binding sites. If so, the addition of Y146A in the B subunits (also disrupting **AB**) should have no additional effect. This is not the case however, as relative efficacy decreases from 0.36 to 0.18, suggesting that E124Q in the A subunit is not completely destroying the **AB** binding sites (Figure 6 and Table 4).

3.5 Conclusions

Unlike serotonin, which is only capable of activating 5-HT_3AB through its **AA** interface, mCPBG is capable of binding the receptor at all five subunit interfaces. Mutation of E124

(TyrA) of loop A and Y146 of Loop E on the B subunit show that mCPBG acts on both **BA** and **AB** binding sites. Importantly, we find that mCPBG is capable of allosterically modulating the serotonin response through its binding at these additional interfaces. These findings highlight the potential for the development of allosteric modulators of heteromeric 5-HT_3 receptors, akin to the benzodiazepine actions at $GABA_A$ receptors.

ACKNOWLEDGEMENTS

We would like to thank Dr. Sarah Lummis (Cambridge) and Dr. Noah Duffy for helpful discussion.

ABBREVIATIONS

5-HT	serotonin
5-HT3	serotonin type 3 receptor
mCPBG	*meta*-chlorophenyl biguanide
nH	Hill coefficient

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HIGHLIGHTS

- Unlike 5-HT, mCPBG binds at heteromeric interfaces of the 5-HT3AB receptor.
- mCPBG is capable of allosterically modulating serotonin response.
- Heteromeric binding sites subtly differ from the homomeric site.
- The homomeric binding site contributes disproportionately to receptor activation.

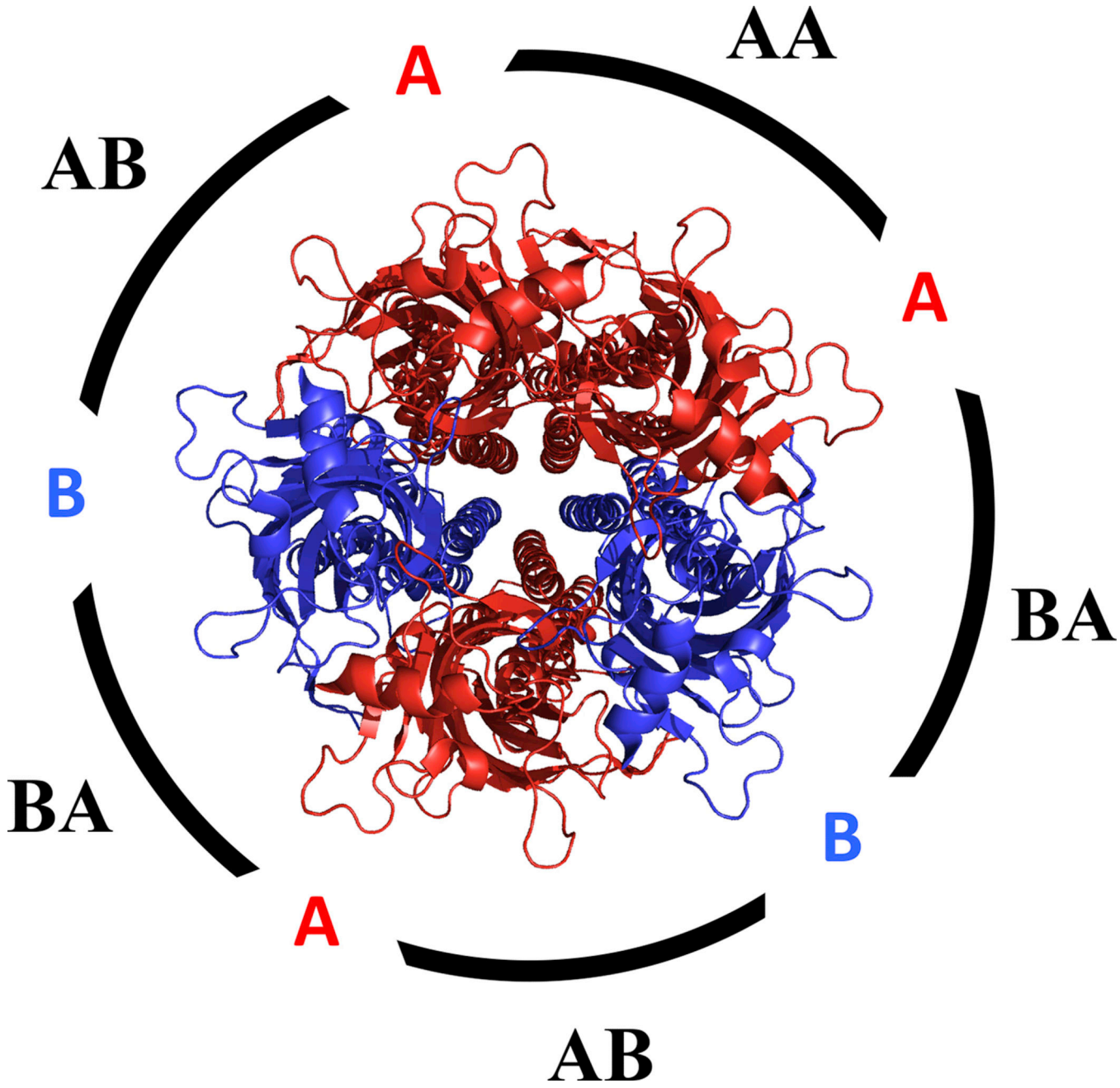

Figure 1.

Top-down view of 5-HT_3AB receptor based on GluCl crystal structure (PDB: 3RHW); interfacial agonist binding sites move in a counterclockwise direction from the principal to the complementary face. Three types of binding sites are present in the heteromeric receptor: **AA**, A subunit as both principal and complementary faces, **BA**, B subunit as principal and A subunit as complementary, and **AB**, A subunit as principal and B subunit as complementary. Serotonin binds only at **AA** interfaces, whereas mCPBG is capable of accessing all three types of binding site.

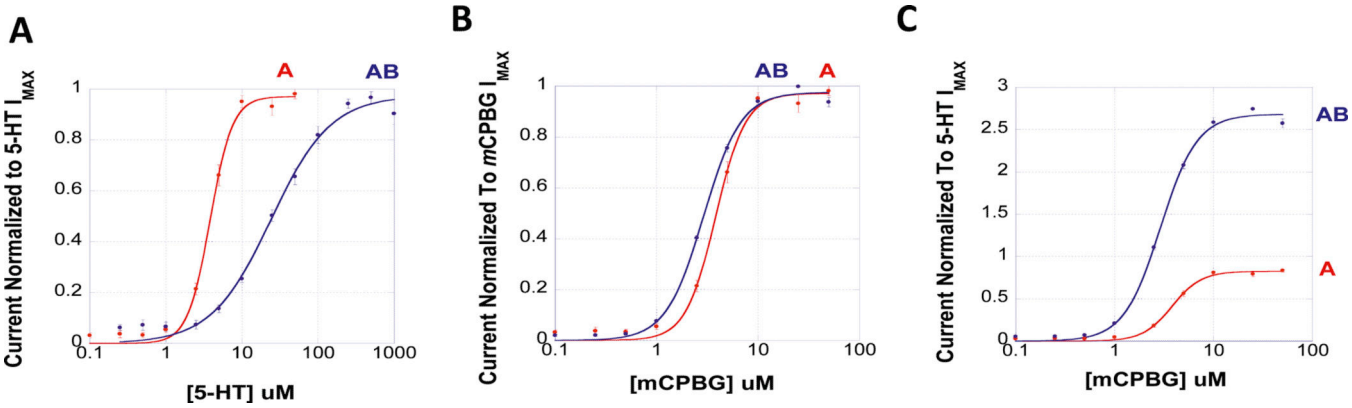

Figure 2.

5-HT and mCPBG dose-response data for wild type 5-HT_3A and 5-HT_3AB receptors. A. 5-HT dose-response data for wild type 5-HT_3A and 5-HT_3AB receptors with current normalized to the maximum elicited 5-HT current. B. mCPBG dose-response data for wild type 5-HT_3A and 5-HT_3AB receptors with current normalized to the maximum elicited mCPBG current. C. mCPBG dose-response data for wild type 5-HT_3A and 5-HT_3AB receptors with current normalized to the maximum elicited 5-HT current. 5-HT_3A data are shown in red and 5-HT_3AB data are shown in blue.

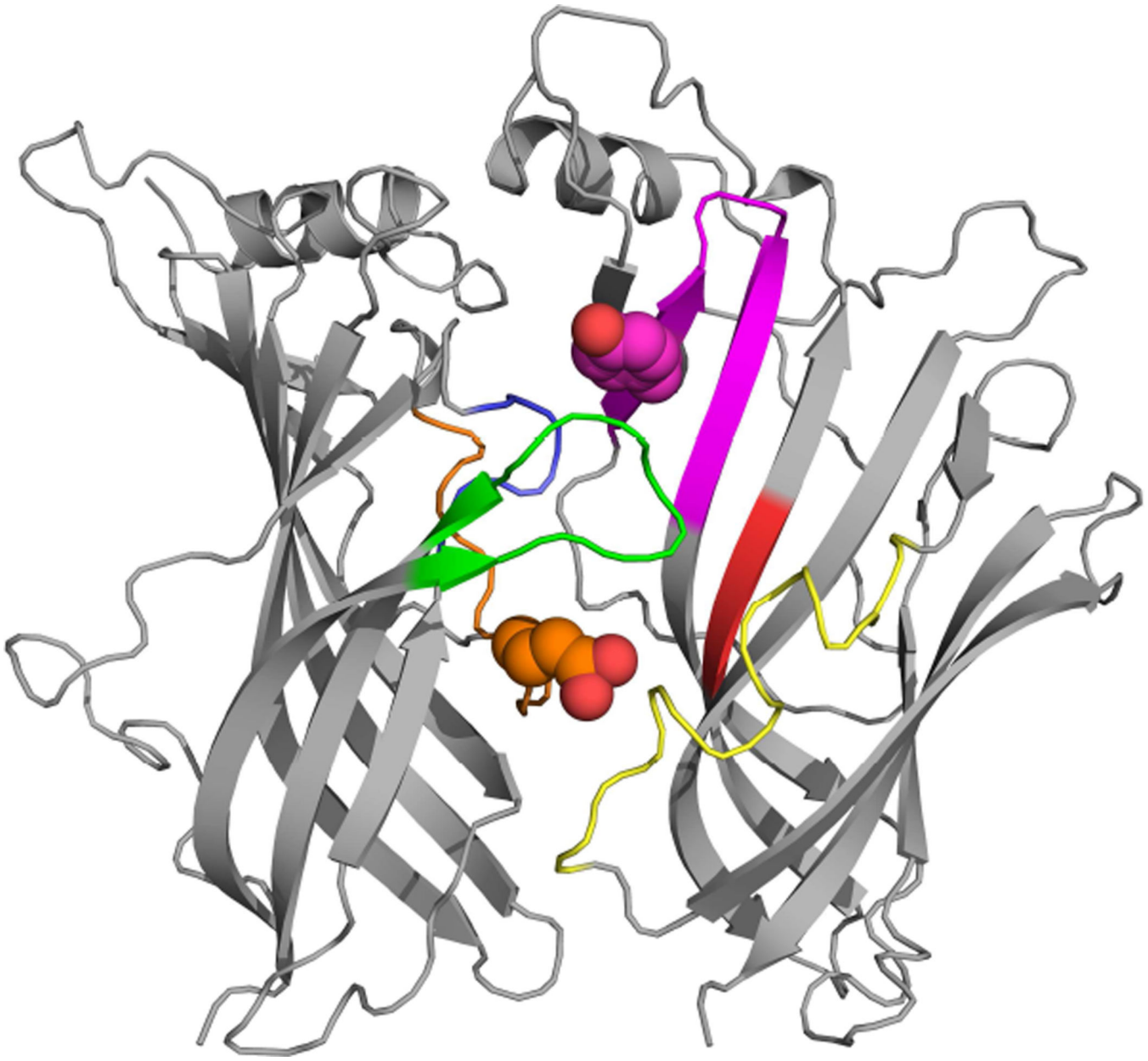

Figure 3.

Model of the 5-HT_3 receptor binding site. The principal subunit (left) contains three loops: A (orange), B (blue), and C (green). The complementary subunit (right) also contains three loops: D (red), E (purple), and F (yellow). The side chains of two conserved residues are shown: E124 of loop A and Y148 of loop E in the human 5-HT_3A subunit (E122 and Y146 in 5-HT_3B). This image has been modified from the crystal structure of acetylcholine binding protein (AChBP) from *Lymnaea stagnalis*, (PDB code 1UW6) and is for illustrative purposes only.

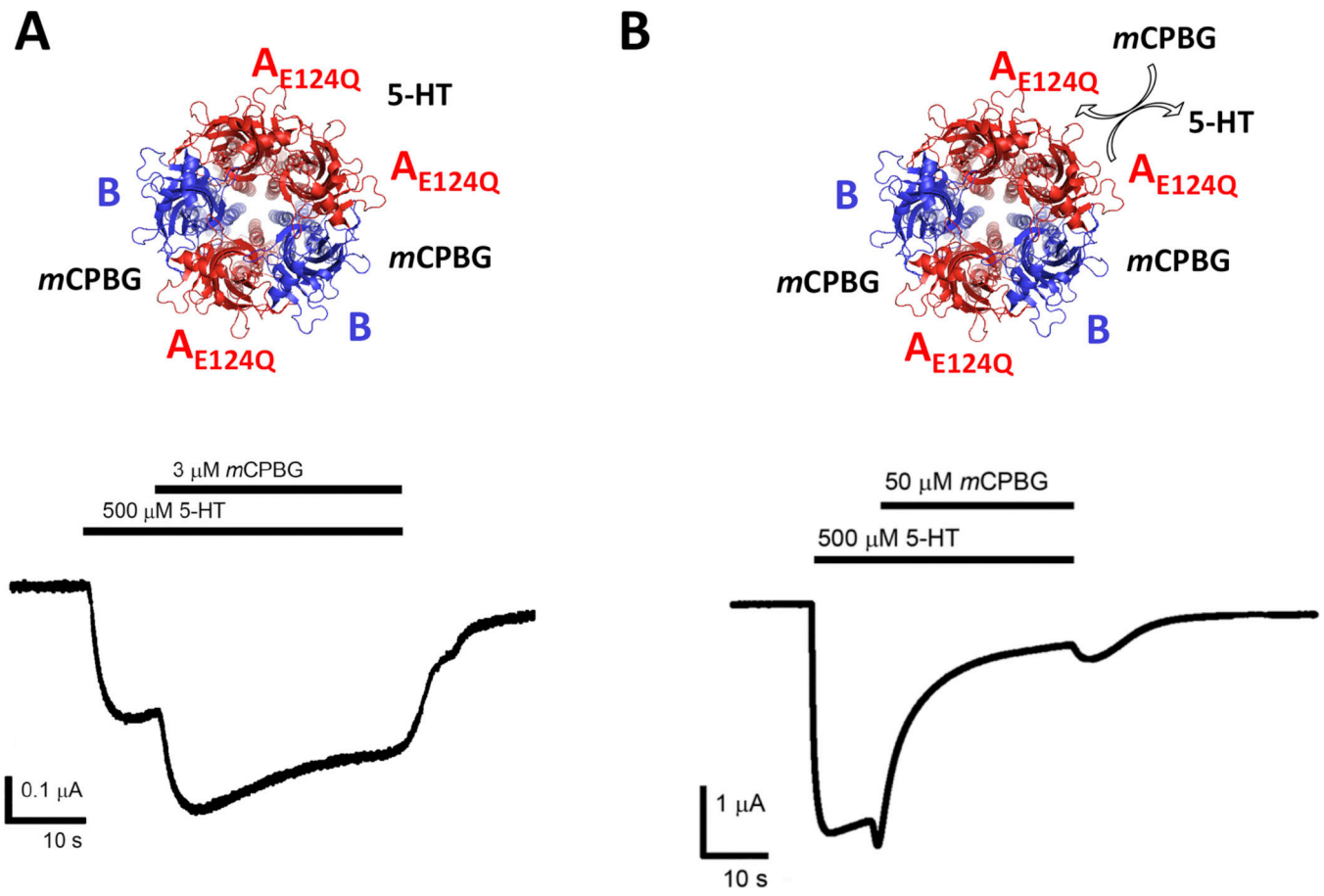

Figure 4.

Dual agonist experiment on 5-HT_3AB receptors bearing the E124Q mutation in the A subunit. A. Model of receptor binding by 5-HT and mCPBG and representative electrophysiology trace showing maximal 5-HT response followed by an additional 60 ± 9 percent (n = 18) response upon addition of EC_{50} mCPBG. B. Model of receptor ligation by 5-HT and mCPBG and representative electrophysiology trace showing maximal 5-HT response followed by a reduction in current by 72 ± 2 percent (n = 20) upon addition of saturating mCPBG, whereupon mCPBG outcompetes 5-HT for binding at the A-A interface.

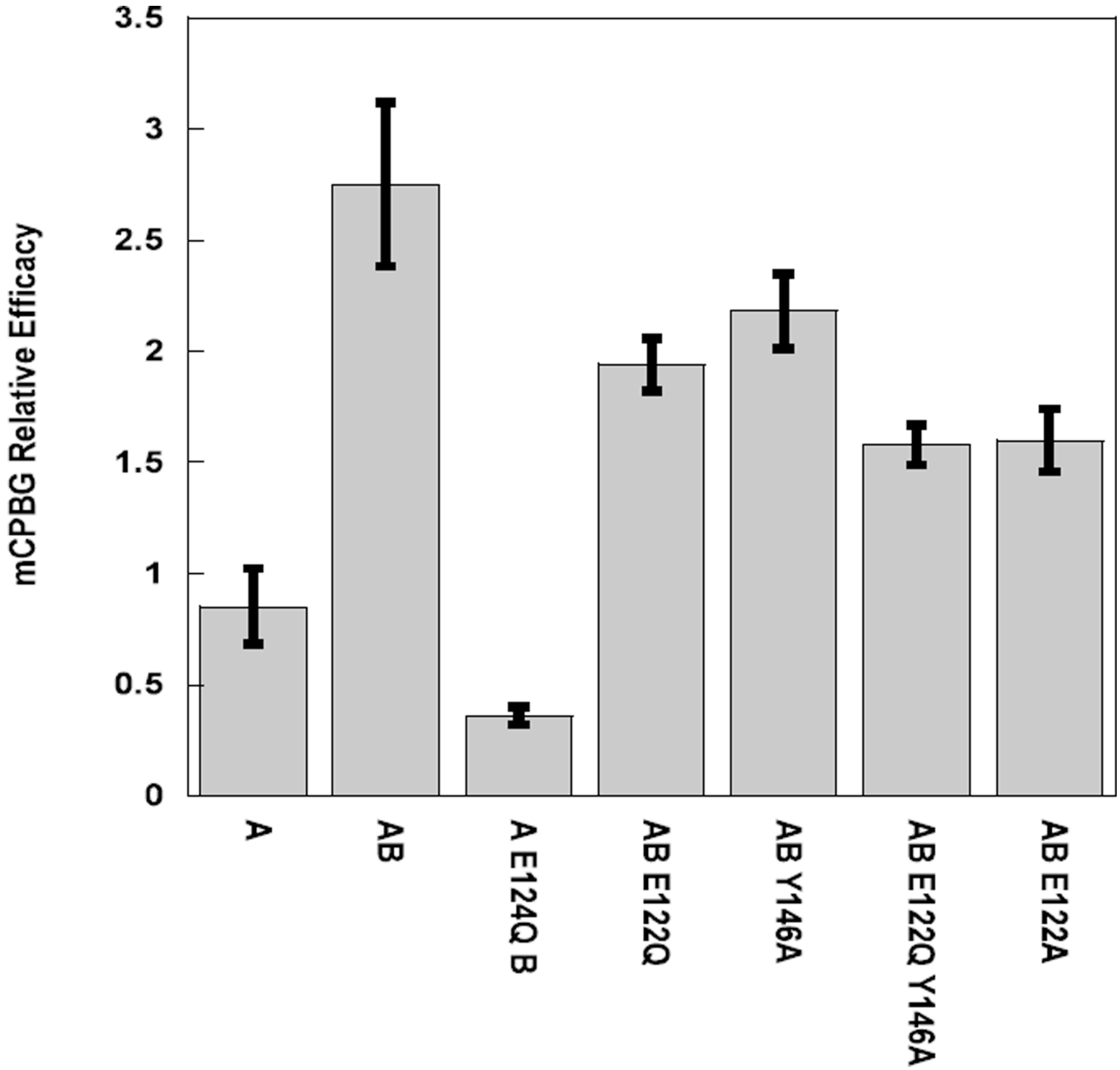

Figure 5.

Relative efficacies of mCPBG at 5-HT_3AB receptor mutants. All 5-HT_3AB receptors bearing mutations in the B subunits are significantly different ($p < 0.05$, except AB Y146A $p = 0.0501$ from wild type) than both the wild type receptor and 5-HT_3AB containing E124Q in the A subunit. 5-HT3AB bearing both E122Q and Y146A is significantly different ($p < 0.05$) from both single mutants. Values are depicted as the mean ± standard error.

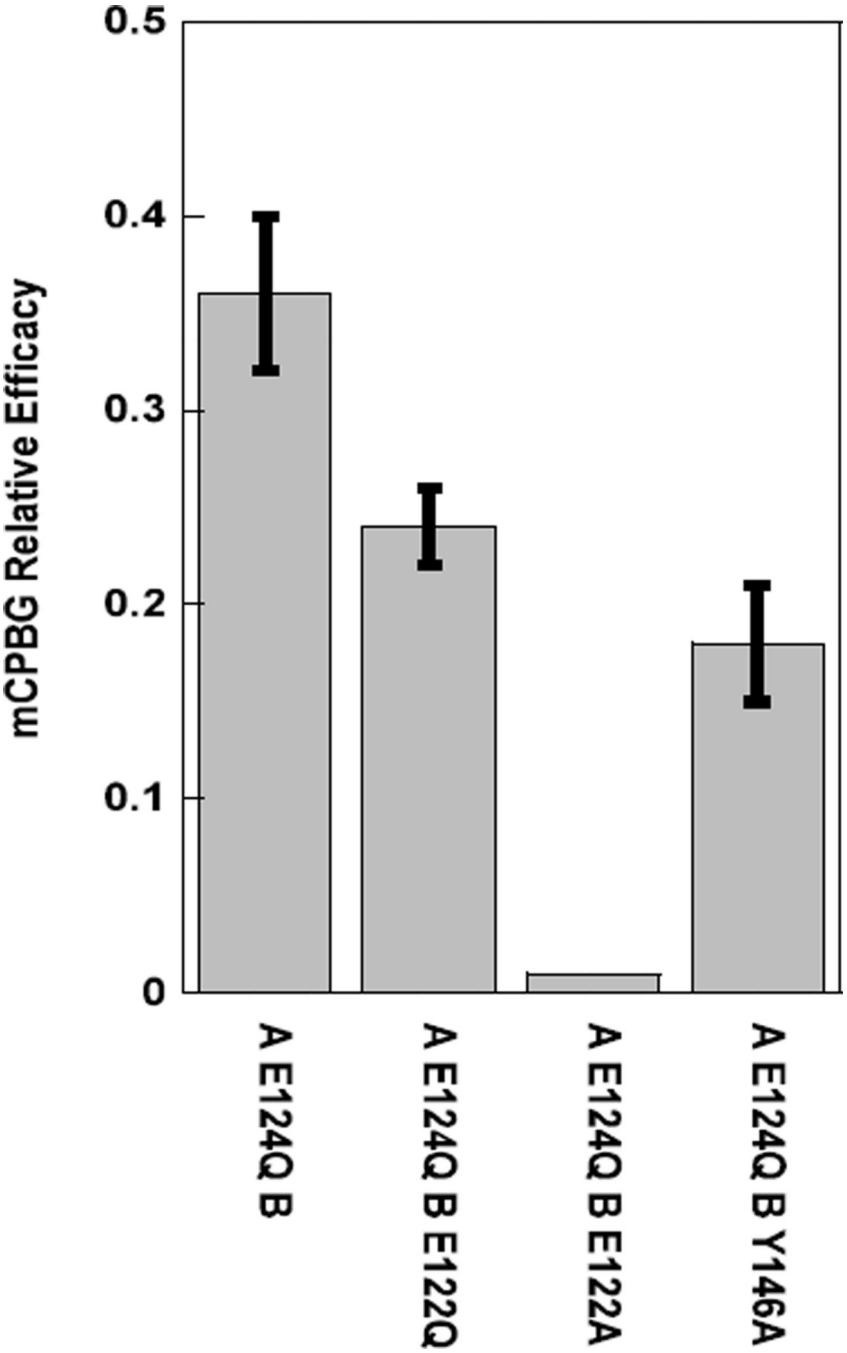

Figure 6.

Relative efficacies of mCPBG at 5-HT_3AB receptors bearing mutations in both subunits. Addition of E122Q, E122A, or Y146A mutations to the B subunits show significant ($p < 0.05$) reductions in relative efficacy. Values are depicted as the mean ± standard error.

Table 1

Functional data for wild type receptors. Hill Coefficient (n^H). Values are reported as mean ± standard error.

Receptor	Agonist	EC_{50} (μM)	n^H	# Cells	ε	# Cells
$5HT_3A$	serotonin	3.4 ± 0.1	2.8 ± 0.1	10	–	
	mCPBG	3.8 ± 0.2	3.0 ± 0.4	5	0.85 ±0.17	10
$5HT_3AB$	serotonin	24.1 ± 2.9	1.1 ± 0.1	7	–	
	mCPBG	2.8 ± 0.1	2.5 ± 0.3	7	2.75±0.19	22

Table 2

Serotonin Functional Data for 5-HT3A and 5-HT3AB Receptors. Hill Coefficient (n^H). Values are reported as mean ± standard error. Wild type (WT). No subunit supplied (−). Fold shift is reported relative to the wild type receptor of the same subunit composition.

A Subunit	B subunit	EC_{50} (μM)	Fold Shift	n^H	# Cells
WT	-	3.4 ± 0.1	-	2.8 ± 0.1	10
E124Q	-	165 ± 9	48.5	1.9 ± 0.1	6
Y148A	-	185 ± 14	54.4	2.4 ± 0.3	5
WT	WT	24.1 ± 2.9	-	1.1 ± 0.1	7
E124Q	WT	141 ± 16	5.9	1.6 ± 0.2	6
Y148A	WT	300 ± 14	12.4	1.8 ± 0.1	7
WT	E122Q	15.5 ± 1.6	0.6	1.2 ± 0.1	5
WT	E122A	16.1 ± 2.5	0.7	1.0 ± 0.1	5
WT	Y146A	14.9 ± 0.9	0.6	1.2 ± 0.1	5
WT	E122Q Y146A	8.2 ± 0.6	0.3	1.4 ± 0.1	6
E124Q	E122Q	117 ± 10	4.9	1.5 ± 0.1	5
E124Q	E122A	197 ± 3	8.2	1.9 ± 0.1	5
E124Q	Y146A	178 ± 8	7.4	1.5 ± 0.1	9

Table 3

mCPBG Functional Data for 5-HT_3 and 5-HT_3AB Receptors. Hill Coefficient (n^H), Small Response (SR) refers to I_{max} < 100nA. No Response (NR) refers to I_{max} < 30nA. Values are reported as mean ± standard error. Wild type (WT). No subunit supplied (−). Fold shift is reported relative to the wild type receptor of the same subunit composition.

A Subunit	B subunit	EC_{50} (μM)	Fold Shift	n^H	# Cells
WT	-	3.8 ± 0.2	-	3.0 ± 0.4	5
E124Q	-	NR			12
Y148A	-	24.7 ± 0.9	6.5	1.9 ± 0.1	6
WT	WT	2.8 ± 0.1	-	2.5 ± 0.3	7
E124Q	WT	3.0 ± 0.7	1.1	1.0 ± 0.2	10
Y148A	WT	11.2 ± 0.6	4	2.8 ± 0.4	7
WT	E122Q	2.3 ± 0.1	0.8	2.6 ± 0.4	5
WT	E122A	2.4 ± 0.1	0.9	2.2 ± 0.2	5
WT	Y146A	4.3 ± 0.3	1.5	2.1 ± 0.2	5
WT	E122Q Y146A	3.5 ± 0.1	1.3	2.2 ± 0.2	6
E124Q	E122Q	2.4 ± 0.4	0.9	1.2 ± 0.2	5
E124Q	E122A	SR			5
E124Q	Y146A	4.8 ± 0.7	1.7	1.5 ± 0.3	9

Table 4

5-HT_3A and 5-HT3AB Receptor Relative Efficacies of mCPBG (ε). Values are reported as mean ± standard error. Wild type (WT). No subunit supplied (−). P-values calculated by an unpaired, two-tailed student's *t* test analysis, with the mutant compared to the wild type receptor. Not determined (ND) as efficacy values were below detection limits.

A Subunit	B Subunit	ε	# Cells	P-value
WT	-	0.85 ± 0.17	10	-
E124Q	-	< 0.01	10	ND
Y148A	-	3.79 ± 0.37	7	< 0.0001
WT	WT	2.75 ± 0.19	22	-
E124Q	WT	0.36 ± 0.04	10	< 0.0001
Y148A	WT	10.6 ± 0.9	14	< 0.0001
WT	E122Q	1.94 ± 0.12	19	0.0013, < 0.0001[a]
WT	E122A	1.60 ± 0.14	11	0.0004, < 0.0001[a]
WT	Y146A	2.18 ± 0.17	13	0.0501, < 0.0001[a]
WT	E122Q Y146A	1.58 ± 0.09	13	< 0.0001, < 0.0001[a], 0.0355 [b], 0.0047 [c]
E124Q	E122Q	0.24 ± 0.02	13	< 0.0001, 0.0090 [a]
E124Q	E122A	< 0.01	8	ND
E124Q	Y146A	0.18 ± 0.03	9	< 0.0001, 0.0025[a]

[a] P-value for the mutant compared to A E124Q B wt.

[b] P-value for the mutant compared to A wt B E122Q.

[c] P-value for the mutant compared to A wt B Y146A.