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Allosteric Modulation of the Human 5-HT$_3$A Receptor by 5-(trifluoromethyl)-indole

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The 5-HT$_3$ receptor belongs to the cys-loop ligand gated ion channel superfamily of receptors. The allosteric binding site of this receptor is a potential target for the treatment of irritable bowel syndrome. In a companion study we have identified a series of halogenated indole derivatives as potential allosteric modulators of the 5-HT$_3$A receptor (Grafton et al., this meeting). One of these molecules, 5-(trifluoromethyl)-indole (5-TFMI) has been examined in more detail. The aim of this study was to investigate the effects of this molecule on the binding of full and partial orthosteric agonists and antagonists.

[^{3}H]-Granisetron binding experiments were conducted using HEK293 cells expressing stably the human 5-HT$_3$A receptor, with 5-HT (10^{-10}-10^{-4} M), ondansetron (10^{-12}-10^{-6} M), quipazine (10^{-12}-10^{-6} M) and (S)-zacopride (10^{-13}-3x10^{-7} M) competing for the radioligand in the absence and presence of 5-TFMI (3 and 10 μM). See Newman et al., Brit. J. Pharmacol. 169: 1228-1238, 2013 for detailed methodology.

5-TFMI (concentrations up to 10 μM) did not enhance 5-HT-induced calcium release in fluorescence-based assays (data not shown), suggesting a lack of binding of 5-TFMI at the orthosteric site at these concentrations. 5-TFMI failed to impact the ability of ondansetron, a selective 5-HT$_3$ receptor antagonist to compete for [^{3}H]-granisetron (p=0.1). In contrast, the presence of 5-TFMI (3 μM and 10 μM) significantly increased the apparent affinities of 5-HT and quipazine (at 10 μM only) – the latter a 5-HT$_3$ receptor partial agonist – for the [^{3}H]-granisetron-labelled 5-HT$_3$A receptor (Table 1). A slight leftward shift in (S)-zacopride competition curve was noticeable but not significant. 10μM 5-TFMI also significantly increased the Hill coefficients for the 5-HT and quipazine competition curves, suggesting modified cooperativity.

	pEC$_{50}$	**Hill coefficient**
5-HT	6.07 ± 0.07	2.38 ± 0.22
5-HT + 3μM 5-TFMI	6.55 ± 0.16 *	1.40 ± 0.21
5HT + 10μM 5-TFMI	6.72 ± 0.14 **	1.06 ± 0.27*
Quipazine	8.26 ± 0.07	2.11 ± 0.24
Quipazine + 3μM 5-TFMI	8.61 ± 0.09	1.68 ± 0.07
Quipazine + 10μM 5-TFMI	9.19 ± 0.23 **	1.06 ± 0.19 **
S-Zacopride	9.24 ± 0.26	1.03 ± 0.12
S-Zacopride + 10μM 5-TFMI	9.62 ± 0.35	1.01 ± 0.15

Table 1. Ability of 5-TFMI to alter the interaction of agonists and partial agonists with the [^{3}H]-granisetron-labelled 5-HT_3A receptor. Data represent mean ± SEM, n = 4-6 independent experiments. * $p < 0.05$. ** $p<0.01$, Mann-Whitney U test

The data show 5-TFMI is able to impact agonist affinity for the radiolabelled 5-HT_3 receptor at concentrations that do not interact with the orthosteric site of the receptor. 5-TFMI could prove a useful tool to help locate the molecular site of the allosteric binding site within the 5-HT_3 receptor complex, which will aid rational drug design of suitable allosteric modulators of the 5-HT_3 receptor with therapeutic potential.