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An investigation of allosteric modulation of the human 5-HT$_3$A receptor using 5-halo-indoles

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The human 5-HT$_3$A receptor is a member of the cys-loop ligand-gated ion channel family. Various molecules had been identified as positive allosteric modulators of this receptor, including alcohols, volatile anaesthetics and most recently 5-chloroindole. In the present study we have used binding assays, fluorescence indicators and patch clamp techniques to explore the allosteric modulation of human 5-HT$_3$A receptor stably expressed in HEK293 cells. Affinity (Ki) and Hill slope values arising from various 5-HT$_3$ receptor agonists (DDP 733, (s)-zacopride and quipazine) and an antagonist (ondansetron) were consistent with the pharmacology of the human 5-HT$_3$ receptor. Neither 5-chloroindole nor 5-iodoindole competed with [^{3}H]-granisetron for the 5-HT$_3$A receptor orthosteric site (concentrations up to 100 µM). Furthermore neither of these halogenated indoles (10-30 µM) altered the affinity of ondansetron for the receptor. In contrast, 5-chloroindole provoked a concentration dependent increase in the affinity of the partial agonist quipazine for the 5-HT$_3$A receptor from 19 ± 3 nM to 11 ± 1 nM and 6 ± 1 nM at 10 µM and 30 µM, respectively. DDP 733 displayed an affinity for the 5-HT$_3$A receptor of 2.3 ± 0.5 nM and 5-chloroindole increased the affinity to 2.2 ± 0.04 (10 µM 5-chloroindole), 1.6 ± 0.05 (30 µM 5-chloroindole), and 1.6 ± 0.05 (100 µM 5-chloroindole). Similarly 5-iodoindole provoked an increase in the affinity of quipazine from 23 ± 1 nM to 8 ± 1 nM and 3 ± 0.2 nM at 10 µM and 30 µM, respectively. 5-Iodoindole also increased the affinity of DDP773 for the receptor from 3.9 ± 0.1 nM to 1.9 ± 0.4 nM (10 µM 5-iodoindole) and 1.5 ± 0.2 nM (30 µM 5-iodoindole). Finally, 10 and 30 µM 5-iodoindole provoked an increase in affinity of (S)-zacopride from 0.98 ± 0.11 nM to 0.61 ± 0.07 nM and 0.64 ± 0.07 nM, respectively. Determination of functional responses (increase in intracellular calcium assayed by fluorescence dye on Flex Station and current recorded by whole cell patch clamp) demonstrated the ability of 5-chloroindole and 5-iodoindole (10-30 µM) to potentiate responses to the partial agonists.

In this study we have extended our previous observations of the usefulness of 5-chloroindole (and now 5-iodoindole) as a pharmacological tool for exploring the allosteric modulation of the 5-HT$_3$A receptor. Further investigations with chimeric receptors to map the binding site(s) for these halogenated indoles will allow rational design of drugs that may become useful therapeutic agents via modulation of 5-HT$_3$ receptor function.