Ago-Allosteric Modulation of the 5-HT$_3$A Receptor via the Orthosteric Site: involvement of partial agonists

The human (h) 5-HT$_3$A receptor is a member of the cys-loop ligand-gated ion channel (LGIC) family, regarded as a prototypical model for the family. We have recently demonstrated positive allosteric modulation of this receptor by 5-chloroindole (Cl-indole; Newman et al., Br. J. Pharmacol. 169: 1228-1238, 2013).

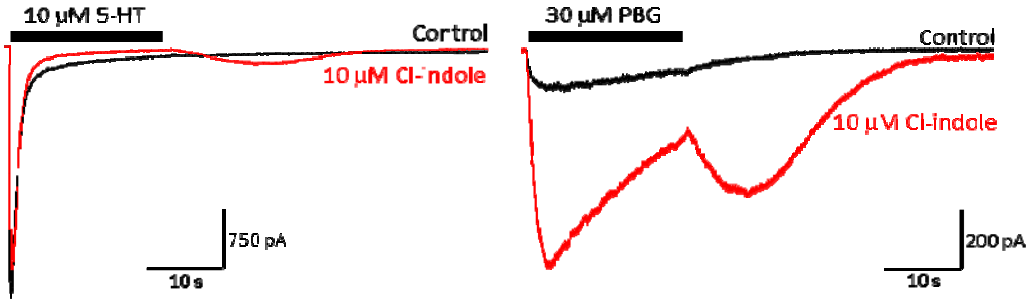
In the present study we have further explored the mechanism of action of Cl-indole at the h5-HT$_3$A receptor, with particular attention on its effect on responses evoked by partial agonists. Whole-cell patch clamp recordings were made from HEK-293 cells expressing the h5-HT$_3$A receptor. Receptor currents were evoked by a 20 second picospritz application of either 5-HT, meta-chlorophenylbiguanide (mCPBG) or phenylbiguanide (PBG). Cl-indole was bath applied via a gravity-fed perfusion system.

When using a full agonist (5-HT), Cl-indole did not potentiate 5-HT$_3$A receptor currents, whereas responses evoked by partial agonists were potentiated by Cl-indole (table 1). The kinetics of agonist-induced receptor currents were unaffected by Cl-indole (figure 1).

Table 1 – Cl-indole potentiates 5-HT$_3$A receptor responses evoked by partial agonists but not full agonists.

Agonist type	Agonist	[Agonist] (μM)	Control Current Amplitude (pA)	Cl-indole Amplitude (pA)	Paired t-test (α=0.05)
Full	5-HT	10	1635.0±483.6	1189.9±459.6	n.s
Partial	mCPBG	1	150.6±35.7	656.9±260.4	*
	PBG	30	112.4±33.8	480.8±95.6	**

Figure 1 – Cl-indole evokes tail currents after application of both full and partial agonists. In the absence of Cl-indole, current returned to baseline after removal of agonist (black trace). In constrast, removal of agonist in the presence of Cl-indole (red trace) induced a tail current with slow activation and deactivation kinetics.



We have previously demonstrated that Cl-indole induced tail currents following removal of 5-HT (Grafton *et al*., this meeting). Cl-indole induced qualitatively similar tail currents following the removal of partial agonists. We propose that Cl-indole modulates h5-HT$_3$A receptor responses by interacting with the orthosteric site, but only in the presence of an orthosteric agonist. This mechanism of action is similar regardless of whether a full or partial agonist occupies the orthosteric site, but in the presence of a partial agonist, Cl-indole is able to promote transition through the “priming” step that limits receptor activation.