Biased agonism and biased desensitisation at the 5-HT_{2A} receptor, exhibited in ARF-dependent PLD activation compared to PLC responses

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The 5-HT_{2A} receptor (5-HT_{2A}R) is an important target of the newer atypical antipsychotic agents, which act as antagonists of its $G_{q/11}$-coupled phospholipase C (PLC) activation, and is also thought to mediate the psychotropic effects of certain 5-HT_{2A}R agonists. We have shown that the 5-HT_{2A}R additionally causes activation of phospholipase D (PLD) through a novel signalling complex in which both ARF and PLD associate with distinct sites in the receptor's carboxy-terminal tail (Barclay *et al.*, 2011). Here we asked whether different 5-HT_{2A}R agonists might display differential activation of the ARF-coupled PLD pathway compared to the $G_{q/11}$-coupled PLC pathway.

Experiments were carried out mainly in COS7 cells, transfected with the 5-HT_{2A}R and other constructs, and involved whole cell radioenzymatic assays for PLC and PLD activation, co-immunoprecipitation (Co-IP) and GST-fusion protein interaction assays.

Transfection of wild type or negative mutant PLD1 increased or decreased 5-HT_{2A}R-mediated PLD responses to 5-HT respectively, while PLD2 constructs were without effect, and none affected PLC responses. Corresponding selective association of PLD1 with the 5-HT_{2A}R was seen in Co-IP and GST-fusion protein experiments. Confirming the in vivo relevance of this pathway, the 5-HT_{2A}R agonist (R)-DOI elicited PLD activation in rat frontal cortex minislices (150 x 150 □m) that was largely prevented by the highly selective 5-HT_{2A}R antagonist M100907 or by the PLD1 inhibitor VU0155069 but not by the PLD2 inhibitor BML 280. Similar to previous reports, we found that (R)-DOI and (R)-lisuride both acted as potent agonists of 5-HT_{2A}R-mediated PLC activation with pEC_{50}s of 7.7 ± 0.1 and 8.4 ± 0.1, respectively (mean ± s.e.m., n=4). However, we revealed a striking disparity in their potency for PLD activation (corresponding values of 7.9 ± 0.1 and < 4.70). Investigation of the time-course of responses revealed that (R)-lisuride-induced PLD activation (like the response to 5-HT) rapidly and completely desensitised within 2 min, whereas the (R)-DOI-induced response continued at a reduced but maintained rate for at least a further 15 min with no sign of abating. The agonists both showed slow and partial desensitisation of PLC responses.

These observations suggest that certain agonists acting at the 5-HT_{2A} R can display not only biased agonism for ARF-dependent PLD activation compared to conventional $G_{q/11}$-dependent PLC activation, but also biased desensitisation of the two outputs.

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