

P005 Allosteric modulation of adenosine A₁ receptors by SCH-202676
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Allosteric modulators of adenosine A₁ GPCRs are novel therapeutic agents. Recently, SCH-202676 has been shown to allosterically perturb various GPCRs. We investigated the interaction between SCH-202676 and the A₁ antagonist, [³H]DPCPX, using binding assays in CHO cells expressing the human A₁ GPCR. Radioligand dissociation assays revealed a significant slowing of the dissociation rate constant of [³H]DPCPX in the presence of 30 μM SCH-202676 ($0.13 \pm 0.01 \text{ min}^{-1}$) compared to its absence ($0.27 \pm 0.03 \text{ min}^{-1}$; n=3). Binding assays utilizing a range of SCH-202676 concentrations against a single [³H]DPCPX concentration revealed an enhanced radioligand affinity with increasing modulator concentrations. However, higher modulator concentrations caused a paradoxical decrease in [³H]DPCPX binding, in both membrane and intact cell binding assays. Additional experiments that varied the order and duration of ligand-receptor incubation had no impact on the bell-shaped binding curves, indicating that the [³H]DPCPX binding inhibition in the presence of high SCH-202676 concentrations is not due to a kinetic artefact. Our findings suggest that SCH-202676 may utilize two different binding domains on the A₁ GPCR that are likely to be accessible via the extracellular surface of the receptor.