

P007 Human H₃ histamine receptor isoforms can form hetero-oligomers: a biochemical study

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The H₃ Histamine receptor subtype is expressed almost exclusively in the CNS where it acts as both an autoreceptor regulating the release of histamine itself, and a heteroreceptor modulating the release of many other important neurotransmitters, including dopamine, noradrenaline, GABA, serotonin and acetylcholine. Pharmacological heterogeneity in H₃ receptors within and across species has long been recognised. The hH₃ receptor is subject to alternative splicing yielding a number of variants, which display distinct but overlapping mRNA expression profiles. We have previously provided evidence for homooligomerisation of native rodent H₃ receptors, and the major hH₃ receptor isoforms expressed in HEK 293 cells. We have developed the first panel of selective anti-H₃ receptor antibodies, one of which is the first human H₃ 445 isoform specific antibody. We have utilised this latter antibody to investigate whether hH₃ receptor isoforms can form heterooligomers. FLAG H₃ 445 and FLAG-polyHIS H₃ 329 isoforms were coexpressed in HEK 293 cells using a lipofectamine protocol, and subjected to Nickel affinity chromatography. The purified fraction were probed by anti-FLAG and anti hH₃ 445-specific antibodies, and a major species was detected by both antibodies (M_r 74,000), a size consistent with a H₃ 445/H₃ 329 heterooligomer. We are currently confirming this first evidence for H₃ receptor heterooligomerisation both pharmacologically and by using FRET.
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