

GENERATION AND USE OF ANTIBODIES TO STUDY HISTAMINE H₃ AND H₄ RECEPTORS

Paul L Chazot and Fiona C Shenton

*Centre for Integrative Neuroscience
Durham University
Durham
DH1 3LE, UK*

paul.chazot@dur.ac.uk

Our group has been interested for a number of years in the structure, heterogeneity and function of the two newest members of the histamine receptor family, namely the H₃ and H₄ receptors. Both receptor subtypes undergo species-dependent alternative splicing to yield a wide range of isoforms, the relevance of which is still unclear.

Antibodies have been widely used in biochemistry as selective probes to study the structure and function of important biological macromolecules. The utility of antibodies as research tools lie in their unique structure. The antibody molecule consists of two distinct domains: the Fab or “business” end of the molecule, which binds to the respective antigen with its exquisite specificity and the Fc region, which can be readily labelled with a wide range of tags. Neurotransmitter receptors, such as histamine H₃ and H₄ receptors, are expressed at very low concentrations in the mammalian brain, ie. fmol receptors/mg protein, and it is difficult to purify sufficient material for the generation of useful antibodies. The availability of cDNAs has revolutionised the generation of anti-receptor antibodies. Our group is interested in generating multi-use specific antibodies, and to achieve this goal, we use the primary sequence information and select a suitable short oligopeptide to generate amino-acid sequence-specific anti-receptor antibodies⁶. This is vitally important because of the close sequence similarities between individual GPCRs, histamine receptor subtypes and respective H₃ and H₄ receptor splice isoforms.

The development of anti-H₃ and H₄ receptor antibodies have proved invaluable in providing new information relating to the precise localisation of the H₃ and H₄ receptor polypeptides in the human and rodent brain (and parts of the periphery) and providing evidence for both rodent and human H₃R and H₄R isoforms and homo- and hetero-oligomerisation¹⁻⁴. For example, in collaboration with Professor Lindsay Hough and Dr Frank Rice’s groups (Albany, USA), we have provided the first clear evidence, by investigating the distribution of H₃R immunofluorescence in the skin, dorsal root ganglia, and spinal cords of rats, wild type mice, and H₃R knockout (H₃KO) mice, that the H₃R is expressed on small-caliber periarterial fibers of the deep dermis, but not on small-caliber fibers of the epidermis or superficial dermis. In contrast to previously published suggestions, no evidence for the existence of H₃R-containing C fibers was obtained. This work establishes that H₃Rs are located on deep dermal, A δ peptidergic, periarterial fibers, and that

these fibers are likely to play a key role in mechanical nociception and inflammation³.

The key goals of our recent work are to further develop antibodies specific to the different hH₃R isoforms, and use them to delineate the structure and distribution of native hH₃ receptor homo- and hetero-oligomers. We have developed the first human isoform specific antibody, which is an extremely powerful tool. This work is vital to our basic understanding of the structure and function of the H₃R, and moreover the rationale development of H₃R-directed medicines. We are at the stage of entering clinical development of H₃ receptor antagonists, with the preclinical data showing great promise. Clear heterogeneity of action in animal models is becoming a feature of the preclinical data, and is thus an important issue to address⁵. We know little about potential human H₃ receptor heterogeneity. Recently, we have used our unique anti-H₄ receptor probe to demonstrate, for the first time that the H₄R is expressed in the human brain, as well as many immune cells. We have also provided the first evidence that the H₄ receptor forms an oligomer *in vitro* and *in vivo*, and our collaborator Rob Leurs's group has shown that this subtype undergoes alternative splicing in man, and can regulate the full length receptor as a dominant negative isoform¹.

It is vitally important that we understand the implications of H₃ and H₄ receptor isomerisation and oligomerisation, especially in humans, to fully realise the therapeutic potential of newly developed therapeutic candidates⁵.

References

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