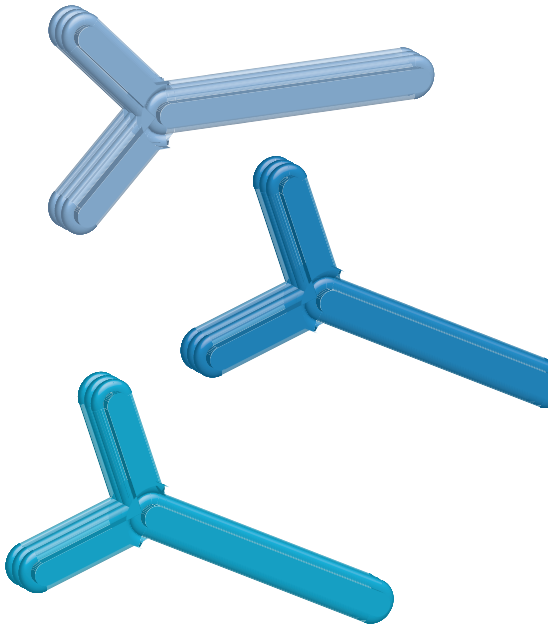
TECHNOLOGY

Specificity concerns with antibodies for receptor mapping



Immunohistochemical mapping — in which a particular protein in a biological sample is identified by its interaction with antibodies that have been developed to specifically bind to it — is widely used to investigate the distribution and localization of proteins, as well as to draw inferences on their potential as drug targets. A group of seven papers published in *Naunyn Schmiedeberg's Archives of Pharmacology* now raises concerns about the specificity of multiple antibodies from commercial and academic sources that are used for mapping a wide range of receptors of therapeutic interest, including adrenergic, muscarinic and dopaminergic receptors.

In many cases, antibodies for mapping proteins, such as G protein-coupled receptors, are raised against synthetic peptide antigens that correspond to fragments of the protein. The specificity of the antibody is typically confirmed by the absence of the band thought to correspond to the protein in western blots when the antibody probe is pre-blocked by the synthetic peptide. However, a key concern is that these small peptides might not be able to replicate the secondary and tertiary structures that are unique to the protein of interest, leading to erroneous detection of the protein.

One rigorous negative control to alleviate this concern would be to monitor the bands in western blots obtained using antibodies thought to be specific for a particular protein in wild-type mice and mice genetically modified to lack the protein: the appropriate band should be present in the wild type, and absent in the knockout. Four of the recent papers applied this strategy to various receptors, with concerning results: nearly all of the antibodies tested failed to meet the criterion for specificity, with the same pattern of bands in studies of both wild-type and knockout mice. The three other papers applied alternative techniques, but again indicated that the antibodies tested lacked the specificity intended.

Overall, these papers indicate that caution is needed when performing and interpreting experiments using the various antibodies tested. Although such concerns have been raised for particular antibodies in the past, the breadth of evidence in these recent papers suggests that rigorous validation of antibodies should be emphasized more strongly to address these concerns.

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ORIGINAL RESEARCH PAPERS Jositsch, G. *et al.* Suitability of muscarinic acetylcholine receptor antibodies for immunohistochemistry evaluated on tissue sections of receptor gene-deficient mice. *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 389–395 (2009) | Pradidarcheep, W. *et al.* Lack of specificity of commercially available antisera against muscarinergic and adrenergic receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 397–402 (2009) | Hamdani, N. & van der Velden, J. Lack of specificity of antibodies directed against human beta-adrenergic receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 403–407 (2009) | Jensen, B. C., Swigart, P. M. & Simpson, P. C. Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are nonspecific. *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 409–412 (2009) | Bodei, S. *et al.* Should we be cautious on the use of commercially available antibodies to dopamine receptors? *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 413–415 (2009) | Lu, X. & Bartfai, T. Analyzing the validity of GalR1 and GalR2 antibodies using knockout mice. *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 417–420 (2009) | Everaerts, W. *et al.* Where is TRPV1 expressed in the bladder, do we see the real channel? *Naunyn Schmiedebergs Arch. Pharmacol.* **379**, 421–425 (2009)