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Invited review

The histamine H_4-receptor and the central and peripheral nervous system: A critical analysis of the literature

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ABSTRACT

Expression and function of histamine H_4R in central and peripheral nervous system have been a matter of controversy for more than a decade. The scientific discussion is often limited to a few publications postulating the presence of functional H_4R on neurons of the central and peripheral nervous system, but the even larger number of reports showing negative data is often neglected. In this article, we critically review the existing literature on H_4R in central and peripheral nervous system and discuss the weak points often overlooked by the community. We identified as most important problems (i) insufficient validation or quality of antibodies, (ii) missing knockout controls, (iii) uncritical interpretation of RT-PCR results instead of qPCR experiments, (iv) insufficient controls to confirm specificity of pharmacological tools, (v) uncritical reliance on results produced by a single method and (vi) uncritical reliance on results not reproduced by independent research groups. Additionally, there may be a publication as well as a citation bias favoring the awareness of positive results, but neglecting negative data. We conclude that H_4R expression on neurons of the brain is not convincingly supported by the current literature, at least as long as the positive data are not reproduced by independent research groups. Expression and function of H_4R on peripheral neurons or non-neuronal cells of the nervous system, specifically on microglia is an interesting alternative hypothesis that, however, requires further verification.

This article is part of a Special Issue entitled 'Histamine Receptors'.

1. Introduction

Histamine, a biogenic amine, which is formed by decarboxylation of the precursor amino acid L-histidine, activates four G protein-coupled receptors (GPCRs) designated H_1R, H_2R, H_3R and H_4R (Bongers et al., 2010; Walter and Stark, 2012; Seifert et al., 2013; Strasser et al., 2013). Histamine is produced by basophils and mast cells and acts at the $G\alpha_q$-coupled H_1R, causing itch, erythema and edema, the typical symptoms of immediate type (type I) allergies (Hill et al., 1997). Moreover, histamine is produced by

Abbreviations: A 943931, H_4R antagonist (4-((3*R*)-3-amino-pyrrolidin-1-yl)-6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*d*]pyrimidin-2-ylamine); ACh, acetylcholine; AKT, protein kinase B; BNP, brain natriuretic peptide; CD11b, cluster of differentiation 11b; Ch, choline; CXCR2, CXC chemokine receptor 2; DAPI, 4′,6-diamidino-2-phenylindole (dye for staining dellular nuclei); Di-8-ANEPPS, voltage-sensitive dye of the group of aminonaphthylethenylpyridinium dyes; DRG, dorsal root ganglion; EAE, experimental allergic encephalomyelitis; ENS, enteric nervous system; Fpr1, murine formyl peptide receptor 1; GPCR, G protein-coupled receptor; H_1R, H_2R, H_3R, H_4R, histamine receptor subtypes; HR, heart rate; HTMT, H_1R agonist (6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl)heptanecarboxamide); IL-1β, IL-4, IL-6, IL-10, IL-17, interleukins 1β, 4, 6, 10 and 17; JNJ 10191584, H_4R antagonist (1-[(5-chloro-1*H*-benzimidazol-2-yl)carbonyl]-4-methylpiperazine; JNJ-39758979, H_4R antagonist ((R)-4-(3-amino-pyrrolidin-1-yl)-6-isopropyl-pyrimidin-2-ylamine); JNJ 7777120, H_4R antagonist (1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine); IFN-γ, interferon-γ; JNK, a mitogen-activated protein kinase; LPS, lipopolysaccharide; MAP, mean arterial pressure; MAPK, mitogen-activated protein kinase; MH, 4-methylhistamine (H_2R/H_4R agonist); N9, N9 mouse microglia cell line (retroviral-immortalized); NE, norepinephrine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells (transcription factor); p38, a mitogen-activated protein kinase; PC-12, rat pheochromocytoma cell line; PKA, PKG, cAMP- and cGMP-activated protein kinases; A and G, qPCR, quantitative PCR; RAMH, R-α-methylhistamine; RAW 264.7, murine macrophage-like cell line; RBE4, rat brain endothelial cell line; ROS, reactive oxygen species; RT-PCR, reverse-transcription polymerase chain reaction; Sf9, *Spodoptera frugiperda* 9 insect cell line; [^{35}S]GTPγS, [^{35}S]guanosine 5-[γ-thio]triphosphate; Th1, Th2, Th17, T-helper cell subsets; TNF-α, tumor necrosis factor-α; T_R, regulatory T cells; TRPV1, transient receptor potential V1 (ion channel); VUF 6002, H_4R antagonist (= JNJ 10191584); VUF 8430, H_4R agonist (2-[(aminoiminomethyl)amino]ethyl carbamimidothioic acid ester).

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gastric enterochromaffine-like cells and stimulates gastric acid secretion via the $G\alpha_s$-coupled H_2R (Shin et al., 2008). H_2R is also responsible for the cardiac positive inotropic effect of histamine (Hill et al., 1997). Both H_1R and H_2R modulate Th1/Th2 T cell polarization (Jutel et al., 2009).

Importantly, histamine also acts as a neurotransmitter and is produced by neurons of the tuberomamillary nucleus, which sends neuronal projections to various brain regions including cerebral cortex, hippocampus or brain stem (Haas et al., 2008; Schneider et al., 2014a). Neuronal histamine activates postsynaptic H_1R and H_2R and regulates a multitude of behaviors and metabolic functions, e.g. food intake, energy consumption, respiration, susceptibility to seizures, locomotor activity, cognition, pain perception, circadian rhythm, sleep and wakefulness, arousal or emotional states (Schneider et al., 2014a). The release of neuronal histamine is controlled by a negative feedback, which is mediated by the presynaptic $G\alpha_{i/o}$-coupled H_3R (Haas et al., 2008; Schneider et al., 2014b). A growing number of reports also suggest postsynaptic functions of H_3R (Ellenbroek and Ghiabi, 2014).

The physiological role of the $G\alpha_{i/o}$-coupled histamine H_4R, which was cloned by several independent research groups more than a decade ago (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001a,b; Morse et al., 2001; Nguyen et al., 2001; Zhu et al., 2001; O'Reilly et al., 2002), has not yet been fully elucidated. On the one hand, the immunological function of H_4R is well established. H_4R is expressed on immune cells, specifically on mast cells, eosinophils and dendritic cells, where it induces calcium mobilization and chemotaxis (Neumann et al., 2014; Seifert et al., 2013). Moreover, H_4R on dendritic cells modulates Th1/Th2 balance (Neumann et al., 2014). On the other hand, only very little is known about expression and function of H_4R in the central nervous system. Since the discovery of H_4R, its functional presence in the peripheral and central nervous system has been controversially discussed (Schneider et al., 2015).

In this review, we critically discuss the available evidence on expression and function of H_4R in the nervous system from various perspectives. In the text, we differentiate between central and peripheral nervous system. In the tables, however, we use a bottom-up approach, starting with expression data on the mRNA (Table 1)- and protein level (Table 2) and ending with functional data in complex environments like cells and tissues (Table 3) or living experimental animals (Table 4).

2. H_4R expression and function in the central nervous system

2.1. Neuronal cells of the brain

Histamine H_4R and H_3R show a homology of ~40% (Oda et al., 2000; Morse et al., 2001). Both receptors couple to $G\alpha_{i/o}$ proteins and exhibit high constitutive activity (Schneider et al., 2009; Rouleau et al., 2002; Schnell et al., 2010). This similarity between H_3R and H_4R raises the question if there may also be an overlap of expression and function in the central nervous system. In fact, during the past years numerous publications have claimed the existence of H_4R on neurons, and based on such reports speculations have been made regarding potential interaction partners and functions of H_4R in the brain (Moya-Garcia et al., 2011). However, before final conclusions can be drawn, it is required to critically review the literature about H_4R expression in the brain to prevent uncritical interpretation of the available data.

The hypothesis that H_4R might be expressed in the brain was already addressed very early by RT-PCR and qPCR experiments with whole brain samples, mostly by using commercially available cDNA/mRNA libraries. It should be noted that these experiments, although they are discussed in the "neuronal cells" section of this review, do not distinguish between neuronal and non-neuronal sources of mRNA. As shown in Table 1, the majority of research groups failed to detect H_4R mRNA by RT-PCR or qPCR in whole-brain samples (Oda et al., 2000; Nakamura et al., 2000; Nguyen et al., 2001; Liu et al., 2001a, 2001b; Oda et al., 2002; O'Reilly et al., 2002; Lippert et al., 2004; Nakayama et al., 2004), suggesting absence of H_4R in the brain. In fact, brain tissue was even used as a negative control for H_4R expression in RT-PCR studies (Lippert et al., 2004). Another report uses brain tissue only as a positive control for H_3R, while bone marrow was used for H_4R (Nakayama et al., 2004). Only Liu et al. (2001a) found some H_4R mRNA in whole brain samples using an RNA protection assay.

It might be argued that H_4R mRNA is only found in specific brain regions and is too highly diluted in whole brain samples. In fact, four publications propose region-specific occurrence of H_4R mRNA (Cogé et al., 2001; Zhu et al., 2001; Strakhova et al., 2009; Shan et al., 2012). For example, H_4R mRNA was detected by RT-PCR in numerous human brain regions including amygdala, cerebellum, hippocampus and thalamus (Cogé et al., 2001; Strakhova et al., 2009). The presence of hippocampal H_4R mRNA was confirmed in mice by *in situ* hybridization (Zhu et al., 2001). Shan et al. (2012) detected low H_4R mRNA levels in human substantia nigra, putamen and caudate nucleus.

However, these data are contradictory. Specifically, H_4R mRNA expression in rat and human cortex as reported by Strakhova et al. (2009) disagrees with the negative data published by other groups (northern blot: Morse et al., 2001; RT-PCR: Cogé et al., 2001; Liu et al., 2001a). Moreover, Strakhova et al. (2009) suggests that in some brain areas (e.g. cerebellum and cortex of human and rat) H_4R mRNA is more abundant than in spleen. This, however, contradicts the majority of reports that find higher H_4R mRNA in spleen as compared to brain (Cogé et al., 2001; Oda et al., 2000; Nakamura et al., 2000; Morse et al., 2001; Liu et al., 2001b; Oda et al., 2002; O'Reilly et al., 2002; Oda et al., 2005; Zhu et al., 2001). Another problem is that the positive H_4R mRNA data reported by Cogé et al. (2001) and by Strakhova et al. (2009) were generated by conventional RT-PCR with 30 (Cogé et al., 2001) or even 40 (Strakhova et al., 2009) PCR cycles. This implies that positive signals could have been generated by over-amplification of H_4R mRNA traces that are physiologically irrelevant. Only two reports describing H_4R expression in the brain make use of the more accurate qPCR technique (Zhu et al., 2001; Shan et al., 2012). In both cases, however, qPCR revealed only relatively low levels of H_4R mRNA.

Finally, as already discussed recently (Schneider et al., 2015), positive mRNA data are not sufficient to postulate the existence of a protein. It is well-known that mRNA and protein expression do not necessarily correlate with each other (Greenbaum et al., 2003). The presence of mRNA without the corresponding protein has been shown e.g. for the α_{1D}-adrenoceptor (Yang et al., 1997). Another example is CXCR2 chemokine receptor mRNA, which could be isolated from HL-60 cells (Murphy and Tiffany, 1991), but the receptor was not detectable in Ca^{2+} assays (Klinker et al., 1996). Thus, it is crucial to confirm positive mRNA data by detection of the corresponding protein. False-negative mRNA data, however, may result, when the wrong brain regions are analyzed. In contrast to functionally active receptor proteins that are expressed in the nerve endings throughout the brain, mRNA is mostly localized in the perikarya of the neurons. Thus, if H_4R expression is expected on monoaminergic or histaminergic neurons, the search for corresponding mRNA has to occur in brainstem and hypothalamus.

Until now, there is only one report that demonstrates the presence of histamine H_4R protein in brain tissue samples (Connelly et al., 2009) and another publication about H_4R protein expression in the spinal cord of rats (Strakhova et al., 2009) and mice (Lethbridge and Chazot, 2010). Connelly et al. (2009) detected

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