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Molecular aspects of the histamine H_3 receptor

Gerold Bongers, Remko A. Bakker [1], *Rob Leurs* *

Leiden/Amsterdam Center for Drug Research, Department of Medicinal Chemistry, Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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ABSTRACT

The cloning of the histamine H_3 receptor (H_3R) cDNA in 1999 by Lovenberg et al. [10] allowed detailed studies of its molecular aspects and indicated that the H_3R can activate several signal transduction pathways including $G_{i/o}$-dependent inhibition of adenylyl cyclase, activation of phospholipase A_2, Akt and the mitogen activated kinase as well as the inhibition of the Na^+/H^+ exchanger and inhibition of K^+-induced Ca^{2+} mobilization. Moreover, cloning of the H_3R has led to the discovery several H_3R isoforms generated through alternative splicing of the H_3R mRNA.

The H_3R has gained the interest of many pharmaceutical companies as a potential drug target for the treatment of various important disorders like obesity, myocardial ischemia, migraine, inflammatory diseases and several CNS disorders like Alzheimer's disease, attention-deficit hyperactivity disorder and schizophrenia.

In this paper, we review various molecular aspects of the hH_3R including its signal transduction, dimerization and the occurrence of different H_3R isoforms.

1. Introduction

In the historical context of histamine's pharmacology our current knowledge on the third histamine receptor has been gathered in a very short period of time. After the discovery of histamine's biological actions in 1910 [1], the first two histamine receptors were proposed in 1966 [2] and 1972 [3], based on classical pharmacological rules of drug selectivity. Using a similar strategy it was ultimately the French research group at INSERM, led by Jean-Michel Arrang and Jean-Charles Schwartz [4], which described in 1983 for the first time an additional histamine receptor, mediating a negative feedback on the release of histamine from rat brain slices.

With the rapid expansion in the knowledge on the molecular aspects of the histamine H_3 receptor (H_3R) following cloning of the receptor cDNA, it has been recognized as a promising G-protein coupled receptor (GPCR) target in the CNS for the treatment of a variety of diseases, e.g. obesity and cognitive disorders (for detailed reviews see [5–9]). Moreover, at present we are overwhelmed with a large increase in our knowledge on the molecular aspects of H_3R. Especially in the last decade important new data have been generated, following the seminal paper of the Johnson & Johnson team lead by Tim Lovenberg on the cloning of the human H_3R (hH_3R) [10]. Despite the fact that both the histamine H_1 and H_2 receptor cDNA's sequences were known since the early nineties [11,12] and substantial efforts of various laboratories to clone the H_3R cDNA on the basis of homology with the other two histamine receptors, it lasted until 1999 to elucidate the molecular architecture of the hH_3R [10]. Following a large scale

* *Corresponding author*. Tel.: +31 20 5987600; fax: +31 20 5987610.
E-mail address: r.leurs@few.vu.nl (R. Leurs).

[1] Current address: Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

Abbreviations: H_3R, histamine H_3 receptor; hH_3R, human histamine H_3 receptor; PLA_2, phospholipase A_2; TM, transmembrane domain; GPCR, G-protein coupled receptor; NHE, Na^+/H^+-exchanger; PI3K, phospho-inositol-3-kinase; PTX, pertussis toxin; PKA, protein kinase K

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effort to clone CNS-expressed (orphan) GPCRs, Lovenberg et al. [10] identified and subsequently 'deorphanised' the hH_3R. The isolated hH_3R cDNA encoded a 445 amino acid protein with all the hallmarks of the family A, rhodopsin-like GPCR [13], and finally confirmed initial suggestions of the GPCR nature of the H_3R based on H_3R agonist-induced [^{35}S]GTPγS binding [14,15], GTP- and PTX-sensitivity of H_3R radioligand binding and/or responses [14,16,17].

With the identification of the hH_3R cDNA, histamine receptor research was boosted a great deal and enormous progress has been made in the field ever since. The new information resulted in the identification of a novel histamine receptor, H_4 [18], and also evoked strong interest of many pharmaceutical companies to develop H_4R selective ligands [6,8]. Whereas the H_3R has been considered by many companies as an interesting target even before 1999, the lack of molecular information and thus the availability of recombinant systems, made most companies hesitant to start drug discovery programs. A recent review by Hancock [19] on the large drug discovery efforts by Abbott Laboratories, nicely illustrates how the lack of the hH_3R as a screening tool resulted in an initial setback in Abbott's H_3R program. Nevertheless, their early entry in the H_3R field ensured Abbott a strong position in the present H_3R field [8,19]. With the present availability of the H_3R cDNA many major pharmaceutical companies have joined the search for selective and potent H_3R antagonists [8]. The development of H_3R ligands has recently been elaborately documented in various reviews [5,8,19–21].

The cloning of the H_3R cDNA has also led to a detailed delineation of several molecular aspects of H_3R pharmacology. With the identification of the chromosomal localization and the elucidation of the genomic H_3R sequence, it became clear that the H_3R gene contains various introns and, thus, alternative splicing might result in various H_3R isoforms. Indeed, soon after the cloning of the hH_3R cDNA, at least 20 human [22–28] and several rodent [29,30] isoforms have been identified. In this review we present an overview of the H_3R isoforms and their known signal transduction pathways for a better understanding of the mechanism of action of H_3R antagonists as potential therapeutics (Fig. 1).

2. Genomic organization of the H_3R

The hH_3R gene is located on chromosome 20 at location 20q13.33 (HRH3 GeneID: 11255) and the coding region has been suggested to consist of either three exons and two introns (GenBank accession number AL078633) [31], or four exons and three introns [22]. Alternatively, the most 3′ intron has been proposed to be a pseudo-intron as it is retained in the $hH_3R(445)$ isoform, but deleted in the $hH_3R(413)$ isoform [23]. In the coding region for the $hH_3R(445)$ exon 1 codes for transmembrane domain (TM) 1 and half of TM2, exon 2 codes for half of TM2 and TM3 and exon 3 encodes the remaining TM domains (Fig. 2). The complete coding sequence spans almost 4 kbp (nt 15421–19670). As reviewed extensively elsewhere [6,32,33], soon after the cloning of the hH_3R gene, the highly conserved H_3R genes were cloned by sequence homology from various other species, including rats [29,30,34,35] guinea-pigs [36,37], mice [38], and monkeys [39].

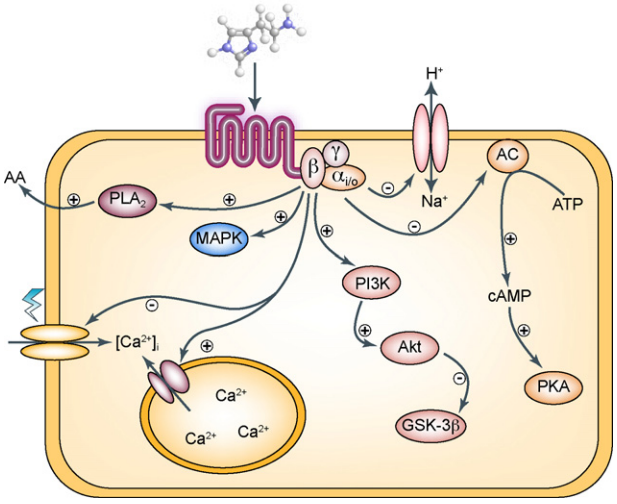

Fig. 1 – A schematic representation of the H_3R-mediated signal transduction. The H_3R has been shown to modulate several signal transduction pathways including the inhibition adenylyl cyclase (AC), mitogen-activated protein kinase (MAPK), activation of phospholipase A_2 (PLA_2), intracellular calcium mobilization, activation of the Akt/GSK-3β axis and inhibition of the Na^+/H^+ exchanger.

3. Identification of H_3R isoforms

To date at least 20 isoforms of the hH_3R are known and in addition several H_3R isoforms have been identified in rat, guinea-pig and mouse as well [22–24,28–31,37,40,41]. So far no isoforms were found for the monkey H_3R [39]. The complete spectrum of H_3R isoforms might be highly species-specific, complicating the evaluation of the various isoforms in relation to the effectiveness of H_3R ligands in vivo.

For the hH_3R, alternative splicing occurs in four different regions. In three of these regions; 7–42, 85–98 and 197–417 (following the amino acid numbering of the $hH_3R(445)$ isoform), this leads to a deletion of various amino acids. In the fourth region, alternative splicing generates isoforms that have eight additional amino acids at the C-terminus, consequently adding the amino acids KMKKKTCL to the hH_3R protein. The third region (197–417), contains several donor and acceptors sites making it a highly diverse region. Currently, nothing is known about the regulation of the splicing of the H_3R mRNA. Since alternative splicing can occur simultaneously in the different indicated regions a large variety of different H_3R isoforms can be generated (Table 1 and Figs. 3 and 4).

Alternative splicing in the first region deletes a part of the N-terminal tail and a part of TM1, whereas splicing in the second region deletes a part of the TM2. Alternative splicing in the third region between 226 and 353 generates hH_3R isoforms with a variation in the length of the third intracellular loop. Splicing in the third region, starting at amino acid 197 or ending at amino acid 417, leads to deletion of TM5 or TM6/7, respectively.

Following the cloning of the $hH_3R(445)$ by Lovenberg et al. [10], Cogé et al. described the discovery of five additional isoforms with splicing in regions the between 85–98 or