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Molecular aspects of the histamine H_3 receptor

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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

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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].

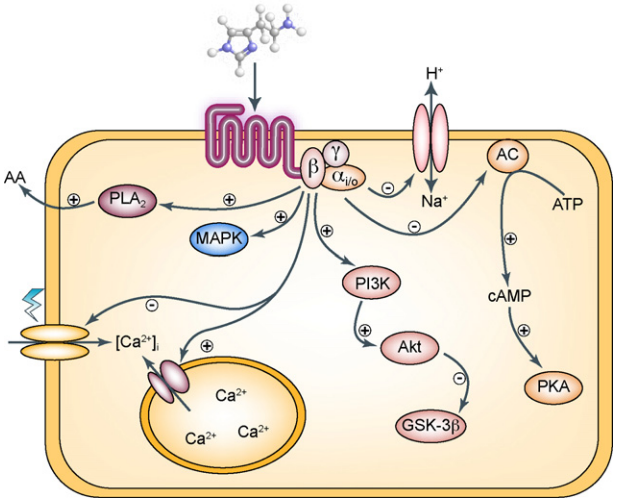

Fig. 1 – A schematic representation of the H_3R-mediated signal transduction. The H_3R has been shown to modulate several signal transduction pathways including the inhibition adenylyl cyclase (AC), mitogen-activated protein kinase (MAPK), activation of phospholipase A_2 (PLA_2), intracellular calcium 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

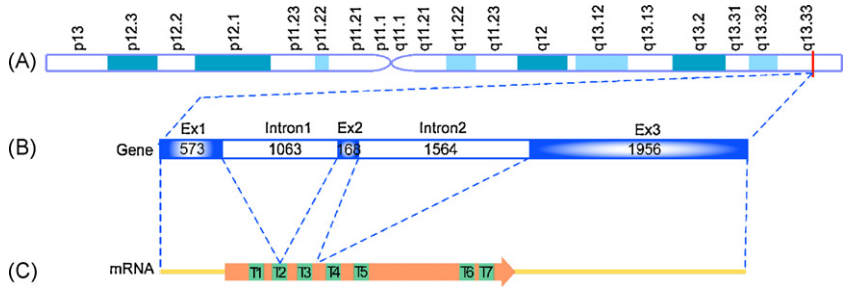

Fig. 2 – Genomic organization of the hH_3R. (A) Schematic representation of the human chromosome 20 and the location of the hH_3R gene in the q13.33 region. (B) Schematic representation of the hH_3R gene and its exons (dark blue) and introns (white boxes). (C) Schematic representation of the H_3R mRNA showing the untranslated region (in yellow), the coding regions (orange) and the transmembrane domains (green).

197–353 (hH_3R(431), hH_3R(415), hH_3R(365), hH_3R(329) and hH_3R(326) [22]). In addition to confirming the hH_3R(365) isoform, Wellendorph et al. described the cloning of four additional isoforms (hH_3R(373), hH_3R(309), hH_3R(301) and hH_3R(200) [24]). The hH_3R(373) is derived from the same splice event as the hH_3R(365), but with eight additional amino acids at the C-terminus. The alternative splicing event leading to the addition of eight amino acids was also found to occur for the hH_3R(445), leading to the hH_3R(453) isoform [28]. The hH_3R(301) and the hH_3R(309) are generated by splicing between amino acids 274 and 417, with or without the eight additional amino acids at the C-terminus. The hH_3R(200) is created by a frame shift leading to a novel stop codon. Furthermore, Tradivel-Lacombe et al. described alternative splicing event to occur between 274 and 305 leading to the hH_3R(413) isoform. Besides the scientific literature, information about H_3R isoforms can also found in the patent literature. Patent WO/2003/042359 by Merck claims twelve isoforms, including all isoforms previously published by Cogé et al. [22] and six isoforms that were not known before (hH_3R(409), hH_3R(395), hH_3R(379), hH_3R(329b), hH_3R(293), hH_3R(290) [27]). All these isoforms are formed after deletion of amino acids 7–42 and five of them are derived through a combination of already known splice events in the 197–353 region. Patents by SmithKline Beecham describe splicing events leading to the hH_3R(365) in combination with amino acid deletion the 85–98 or 393–417 region [25,26]. Besides the already known isoforms, one can envision that by combination of the known splice sites more possibilities for hH_3R isoforms exist.

Table 1 – Overview of human H_3R isoforms

H_3R(aa)	A 7-42	B 85-98	197	226	234	263	C (variable area) 274	305	315	341	353	393	417	D	Functional	First Reference
453														+8	B/F	[28]
445															B/F	[10]
431		-14													NB	[22]
415					-30										ND	[22]
413							-32								ND	[23]
409	-36														B	[27]
395	-36	-14													ND	[27]
379	-36				-30										ND	[27]
373							-80							+8	F	[24]
365							-80								B/F	[22]
351		-14					-80								ND	[25]
340							-80					-25			ND	[26]
329a				-116											B	[22]
329b	-36						-80								ND	[27]
326			-119												ND	[22]
309									-144					+8	ND	[24]
301									-144						NF	[24]
293	-36			-116											ND	[27]
290	-36		-119												ND	[27]
200	spliced at aa 170 + 30 new aa														NF	[24]
Location	N-Term	TM2	TM5				IL3				TM6	TM7		C-Term		

The H_3R isoforms are denoted by their number of amino acids. Indicated are the regions of alternative splicing and the location in the protein. H_3R isoforms were shown to have radioligand binding (B), to be functional (F), or non-functional (NF). For most isoforms neither was determined (ND). Adapted from [6].

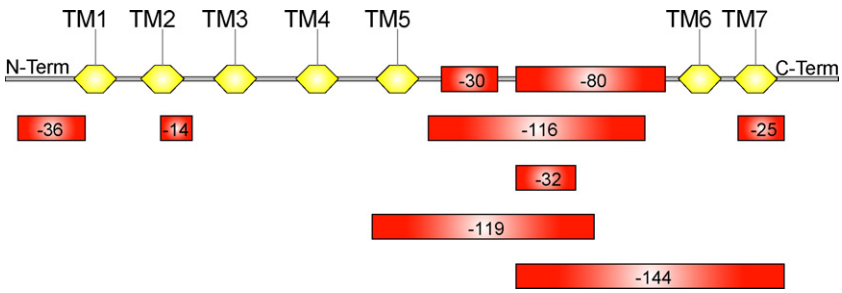

Fig. 3 – Schematic representation of the hH$_3$R protein showing the transmembrane domains (yellow) and the corresponding alternative splicing events, the numbering shows the number of deleted amino acids.

4. H$_3$R Signal transduction

4.1. *Inhibition of adenylyl cyclase*

Early experiments studying the receptor function employing pertussis toxin (PTX) using various assay systems, such as AtT-20 cells endogenously expressing the H$_3$R [17], the guinea pig atria [42] and modulation of the H$_3$R induced [^{35}S]-GTPγS binding in rat brain [43], suggested that the H$_3$R might be G$\alpha_{i/o}$-coupled [14]. Expression of the cloned H$_3$R cDNA in SK-N-MC cells confirmed the linkage of the hH$_3$R to G$\alpha_{i/o}$-proteins by showing its ability to inhibit the forskolin induced cAMP formation in a PTX sensitive manner (Fig. 1) [44]. Subsequently, the hH$_3$R was shown to couple negatively to adenylyl cyclase in variety of heterologously transfected cell lines [22,44–47] and in rat striatal slices [48]. Inhibition of adenylyl cyclase by the H$_3$R causes a decrease in intracellular cAMP and a subsequent reduction of protein kinase A (PKA) activity. PKA participates in a variety of signaling pathways leading to a range of biological responses including gene expression, synaptic plasticity, and behavior [49]. Some controversy exists on the role of cAMP in the modulation of neurotransmitter release by the presynaptic H$_3$R. H$_3$R-mediated inhibition of cholinergic neurotransmission in the guinea pig ileum and the release of norepinephrine from mouse cortex was shown to be independent of adenylyl cyclase [50–52]. However, more recent studies have shown that the H$_3$R modulates synthesis of histamine [47,53,54], as well as the exocytosis of norepinephrine both in cardiac synaptosomes, and in a transfected cell line [55] depends on the H$_3$R-mediated inhibition of cAMP levels.

Like many other GPCRs [56,57], the H$_3$R can be spontaneously active in the absence of histamine [44,45]. This constitutive activity was demonstrated by the activation of the adenylyl cyclase pathway in CHO cells, in which enhanced receptor expression of either the rat H$_3$R(445) or H$_3$R(413) was associated with an increase in the constitutive inhibition of adenylyl cyclase. Many classical H$_3$R antagonists (e.g. thioperamide, clobenpropit, ciproxyfan) were shown to reverse this constitutive inhibition of adenylyl cyclase in stably transfected CHO [45], SK-N-MC [44] and HEK293 [35] cells and, thus, are in fact H$_3$R inverse agonists. Besides agonists and inverse agonists, also neutral H$_3$R antagonists for the adenylyl cyclase pathway have been found. N-isopropylimpentamine and a propylene analogue of immepip, VUF5681, did not affect constitutive signaling, but these neutral H$_3$R antagonists competitively blocked the effect of H$_3$R agonists and H$_3$R inverse agonists [44,58]. Recently VUF5681 was used in vivo and was shown to block the effects of H$_3$R inverse agonist thioperamide on PKA-mediated synthesis of histamine in rat brain cortex, whereas it did not modulate histamine synthesis, which would be indicative for a H$_3$R agonist [53].

4.2. *Activation of phospholipase A$_2$*

Also the H$_3$R-mediated activation of phospholipase A$_2$ (PLA$_2$), leading to the release of arachidonic acid depends on the activation of G$\alpha_{i/o}$-proteins (Fig. 1). The release of arachidonic acid has been suggested to be important in the H$_3$R-mediated relaxation of the guinea pig epithelium [59]. PLA$_2$ activity is under the control of the high constitutive activity of the H$_3$R [45]. In CHO cells with moderate H$_3$R expression, proxyfan was shown to be a neutral H$_3$R antagonist in the [^{3}H]arachidonic acid release assay, but in CHO cells with high H$_3$R expression it displayed partial inverse agonism at the H$_3$R [45]. In contrast, proxyfan displayed partial H$_3$R agonism in a MAPK, [^{35}S]GTPγS and cAMP assays [60] and was therefore identified as a protean H$_3$R agonist, a ligand that depending on the system parameters, not on the receptor, displays distinct functional efficacy [61]. In general, activation of PLA$_2$ not only leads to the release of arachidonic acid, but also to the release of docosahexaenoic acid and lysophospholipids. Besides having intrinsic physiological effects, these metabolites are also substrates for the synthesis of more potent lipid mediators such as platelet activating factor, eicosanoids, and 4-hydroxynonenal. The latter is the most cytotoxic metabolite, is associated with the apoptotic type of neural cell death and markedly increased in neurological diseases like ischemia, Alzheimer's disease and Parkinson's disease [62].

4.3. *Modulation of the MAPK pathway*

Besides H$_3$R-mediated signaling through G$\alpha_{i/o}$-proteins (Fig. 1), Gβγ-subunits are known to activate specific signal transduction pathways such as the MAPK pathway [63,64]. MAPKs are known to have pronounced effects on cellular growth, differentiation and survival as well as to be important in neuronal plasticity and memory processes [65,66]. Activation of the rat H$_3$R was shown to lead to phosphorylation of MAPK in COS-7 cells heterologously expressing the rat H$_3$R. However, the level of phosphorylation varies for the different isoforms

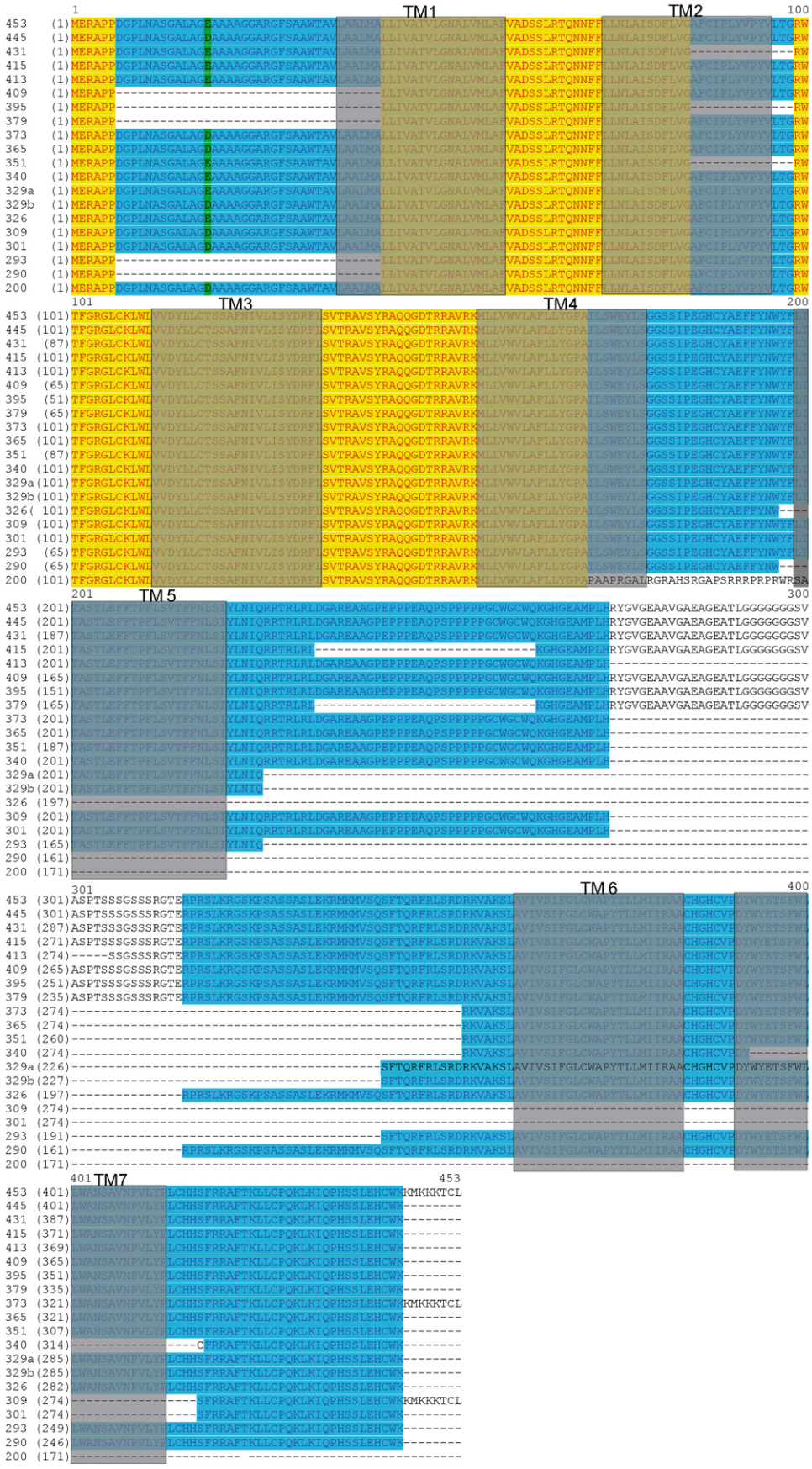

Fig. 4 – Alignment of the currently know hH_3R isoforms. The overlapping regions are in blue, partly overlapping regions yellow and the transmembrane domains are shaded black.

[29]. In contrast to the cAMP response, the H_3R was shown to exhibit little constitutive activation of the MAPK pathway [60]. Whether this MAPK phosphorylation is solely due to Gβγ-subunits, crosstalk with growth factor receptors or the use of scaffolds like β-arrestin [67], remains to be elucidated. Alternatively, a neuron-specific cascade from cAMP/PKA to MAPK comprising the critical events of hippocampus-based long-term plasticity has been described [68], but this pathway has yet not been studied in relation to the H_3R.

4.4. *Activation of the Akt/GSK-3β axis*

Also Akt/GSK-3β kinases have been shown to be activated by the H_3R in a neuroblastoma cell line, primary cultures of cortical neurons and in striatal slices of Spraque–Dawley rats (Fig. 1) [69]. Like in the previously described pathways, the H_3R constitutively activates the Akt/GSK-3β axis, which can be reversed by the H_3R inverse agonist thioperamide. The H_3R-mediated activation Akt/GSK-3β was shown to be independent of Src/EGF receptor transactivation and MAP kinase activation, but similar to other GPCRs [70] to occur through phospho-inositol-3-kinase (PI3K) activation via the Gβγ-subunits of $G\alpha_{i/o}$ proteins. In the CNS, the Akt/GSK-3β axis plays a prominent role in brain function and has been implicated in neuronal migration, protection against neuronal apoptosis [71] and is believed to be altered in Alzheimer's disease, neurological disorders [72,73] and schizophrenia [74]. Because the high expression level of the H_3R is restricted to specific areas of the brain during development [75], one could speculate that the activation of Akt might be relevant for a H_3R-mediated neuronal migration during development of the CNS exerting its effect through the Akt/GSK-3β pathway, or the MAPK kinase pathway as described above. It has been reported before that simultaneous MAPK and Akt activation are required for cortical neuron migration [76]. There is also evidence that the H_3R plays a neuroprotective role in the CNS [77]. Moreover, H_3R mRNA is upregulated in certain brain areas after induction of ischemia [78] and kainic acid-induced seizures [79]. Upregulation of the H_3R and the subsequent constitutive signaling to the Akt/GSK-3β pathway could be the mechanism by which the H_3R exerts its endogenous neuroprotective role.

4.5. *Modulation of intracellular Ca^{2+}*

In human neuroblastoma SH-SY5Y cells it was shown that H_3R activation reduced the K^+-induced intracellular calcium mobilization (Fig. 1). This signal transduction mechanism was subsequently linked to inhibitory effect of the H_3R on the norepinephrine exocytosis in these cells as well as in cardiac synaptosomes [80]. In latter studies this effect on K^+-induced calcium mobilization was linked to the H_3R-mediated inhibition of PKA activity, leading to a decreased Ca^{2+} influx through voltage-operated Ca^{2+} channels [55]. No effects on the intracellular Ca^{2+} levels were observed upon administration of H_3R agonists before the K^+-induced calcium release. In contrast, in SK-N-MC cells the heterologous expression of the hH_3R results in a rapid, but transient $G_{i/o}$-protein dependent calcium mobilization from intracellular stores upon the administration of H_3R agonists [69]. This observation is analogous to the reported signalling of the related $G_{i/o}$-coupled H_4 receptor, which mobilizes calcium in mast cells and eosinophils [81,82]. Further research is needed to study the detailed molecular pathway of the H_3R-mediated calcium mobilization in SK-N-MC cells and determine if similar findings are evident in other cell types that endogenously express H_3Rs.

4.6. *Inhibition of Na^+/H^+ exchanger activity*

The Na^+H^+ exchanger (NHE) is essential for the restoration of intracellular physiological pH by the removal of one intracellular H^+ for one extracellular Na^+ and thereby preventing acidification during ischemia [83]. The consequential increase of interneuronal Na^+ forces the reversal of the Na^+- and Cl^--dependent norepinephrine transporter and leads to an increase in carrier-mediated norepinephrine release. Activation of the H_3R was shown to diminish neuronal NHE activity (Fig. 1) and this pathway was proposed as the mechanism by which the H_3R inhibits the excessive release of norepinephrine during protracted myocardial ischemia [84]. For that reason H_3R agonists were proposed to have therapeutic potential for myocardial ischemia, the negative modulation on norepinephrine release might prevent arrhythmias and sudden cardiac death [85].

Not much is known about the mechanism by which the H_3R inhibits NHE activity. In general, GPCRs are known to activate NHE through kinases like MAPK [86]. However, little is know about the signaling mechanisms of GPCRs that attenuate NHE activity [87], although a direct interaction of $G_{\alpha i/o}$-proteins has been suggested to be involved in the inhibition of NHE [88,89].

5. Expression of the H_3R isoforms

The initial cloning of the hH_3R gene demonstrated that the full length receptor is a $hH_3R(445)$ amino acid G-protein coupled receptor that is found almost exclusively in the brain [10]. Whether the so far described isoforms indeed play an important role will depend on their expression levels and potential differential expression. Cogé et al. [22] showed by Northern blots analysis a high signal for the $hH_3R(445)$ in thalamus, caudate nucleus, putamen and cerebellum, a lower signal in the amygdala and a faint signal for the substantia nigra, hippocampus and cerebral cortex. No signal was observed in the corpus callosum, spinal cord or in peripheral tissue. Further analysis of hH_3R isoforms was done by RT-PCR, for the $hH_3R(445)$ the results was comparable to the Northern blot analysis. The $hH_3R(415)$ and the $hH_3R(365)$ showed high level expression in the thalamus, caudate nucleus and cerebellum, whereas $hH_3R(329)$ and $hH_3R(326)$ were highly expressed in the amygdala, substantia nigra, cerebral cortex and hypothalamus [22]. As described by Wellendorph et al. [24] the $hH_3R(373/365)$ isoforms are expressed at a higher level than the $hH_3R(445)$ isoform in the stomach and the hypothalamus using a RT-PCR approach [24]. The differential expression in the hypothalamus is not consistent with the findings by Cogé et al. [22]. Clearly more work needs to be done in this area.

6. Pharmacological characteristics of H_3R isoforms

Pharmacological characterization of different hH_3R isoforms have been described in two publications (Table 1). Of the six isoforms cloned by Cogé et al. [22], three isoforms ($hH_3R(445)$, $hH_3R(431)$, $hH_3R(365)$) were expressed in CHO cells and pharmacologically characterized, with a focus on the $hH_3R(445)$ and $hH_3R(365)$. The $hH_3R(431)$, which lacks 14 amino acids at the C-terminal end of TM2, showed no [^{125}I]Iodoproxyfan radioligand binding. Whereas this deletion does not affect the key-residues in ligand binding for imidazole containing ligands (D^{114} in TM3 and E^{206} in TM5 [46]), it eliminates the structurally important proline residue characteristic for amine receptors [90,91]. The 14 amino acid deletion is thereby expected to alter the structural organization and likely affects the [^{125}I]Iodoproxyfan binding. A similar pharmacology was observed for the $hH_3R(445)$ and $hH_3R(365)$ in radioligand binding studies. However, H_3R agonists did not generate a functional response on the $hH_3R(365)$ in cAMP and Ca^{2+} assays, nor in a [^{35}S]GTPγS binding assay. In contrast to the findings by Cogé et al., Wellendorph et al. [24] have shown that the $hH_3R(365)$ isoform was functional in a R-SAT™ reporter assay and displayed higher potency for typical H_3R agonists. Higher agonist potencies have also been observed for the rat H_3R isoforms, with similar deletions in the third intracellular loop 3 [29]. The truncated isoforms $hH_3R(301)$ (lacking TM6 and 7) and $hH_3R(200)$ (lacking TM5–7) failed to show a biological response, or N^{α}-[methyl-^{3}H]-histamine radioligand binding, not surprising as these isoform lack many residues important for GPCR structure and function [92]. However these isoforms can play a role in H_3R signaling as was recently published for the non-functional rat H_3R isoforms lacking TM7, that were shown to act as dominant negatives on the expression of the functional rat H_3R isoforms [93]. The $hH_3R(373)$ isoform, which corresponds to $hH_3R(365)$ with eight additional amino acids at the C-terminus, behaves as the $hH_3R(365)$ in the R-SAT™ reporter assay, suggesting that 8 extra amino acids do not dramatically alter the pharmacology of the isoforms. The $hH_3R(409)$, $hH_3R(395)$, $hH_3R(379)$, $hH_3R(329b)$, $hH_3R(293)$ and $hH_3R(290)$ isoforms lack 36 amino acids at the N-terminus, including a N-glycosylation site. Glycosylation at GPCRs have been found to be important in the stabilization dimers [94] and for correct trafficking to the membrane [95–97], however at this moment is it not known if these 36 amino acids affect hH_3R trafficking or its pharmacology.

7. Dimerization of H_3Rs

The concept of GPCR dimerization is now well documented in literature (for detailed reviews see [98]), and such direct protein–protein interactions between different GPCRs are suggested to allow a whole vista of possibilities for subtle changes in the pharmacology of these GPCRs from their monomeric, homo-dimeric or -oligomeric entities, which were previously attributed to the existence of additional receptor subtypes. In view of the recent discovery of H_3R isoforms, which are often co-expressed, the occurrence of H_3R isoform dimerization might add another level of complexity to the H_3R pharmacology.

The first evidence for H_3R isoform dimerization comes from the use of an antibody directed against the rat $H_{3C}R$ isoform (48 amino acid deletion in I3) using both native as well as heterologously expressed rat H_3Rs [99]. Subsequent *time-resolved* Fluorescent Resonance Energy Transfer (*tr*-FRET) experiments using heterologously expressed epitope tagged rat $H_{3A}Rs$ have shown the presence of oligomeric rat $H_{3A}Rs$ at the cell surface [93]. As many of the functional H_3R isoforms differ in the length of their third intracellular loop, one could envision that this might influence the capabilities of the isoforms to form domain-swap H_3R (homo- or hetero-) dimers. In addition, sequences within the third intracellular loop may serve a scaffolding function. The potential protein-protein interaction involving various functional H_3R isoforms remains unclear.

In addition to the functional H_3R isoforms, several (presumed) non-functional H_3R isoforms have been detected. These non-functional isoforms consist either of a truncated receptor comprising only the proximal part of a full-length isoform, the amino terminal domain until the second transmembrane domain, or of a C-terminal truncated isoform that in comparison to a full length isoform lacks transmembrane 7 and have been named 6TM-H_3R isoforms [93]. For these truncated isoforms neither the binding of known H_3R radioligands nor any functional responses have been observed. The roles of these non-functional H_3R isoforms are poorly understood. Intriguingly, mRNAs coding for the truncated and assumed non-functional rat H_3R isoforms are expressed in the brain to a similar extent as the functional rat H_3R isoforms [30,93], suggesting that these truncated proteins may have yet unidentified functions. Recently, we reported on the identification of three rat 6TM-H_3R isoforms which are capable to specifically negatively influence the cell surface expression of the full length functional H_3R isoforms, and that mRNA expression of these 6TM-H_3R isoforms are modulated upon treatment with the convulsant pentylenetetrazole. These observations corroborate the potential functional importance of the otherwise non-functional truncated H_3R isoforms [93]. The 6TM-H_3R isoforms appear to affect the cell surface expression of the functional isoforms through retention of these functional isoforms within the cell. Retention occurs most likely within the endoplasmatic reticulum, probably through the formation of heterodimeric H_3Rs consisting of a mixture of functional and non-functional isoforms that lack domains that are required for appropriate interactions with accessory proteins that mediate the cell-surface targeting of the receptor complex. In evidence for this, the 6TM-rH_3R isoforms lack a F(X)6LL motif that is reported to be important for interaction with a specific ER-membrane-associated protein that regulates transport of GPCRs [100], and possess an RXR ER retention signal instead [93].

8. Concluding remarks

The cloning of the hH_3R has led to the discovery of several signal transduction pathways that are modulated by the hH_3R. Some of these signaling pathways can be linked to relevant

pathophysiologies. The hH_3R-mediated inhibition of the NHE leads to a subsequent lowering in the exocytosis of norepinephrine and thereby providing an explanation for the protective role of hH_3R agonists during myocardial ischemia. The cloning of the receptor gene resulted in the elucidation of the genomic organization of the hH_3R and the discovery of many hH_3R isoforms. To date there is limited knowledge on the regulation of the expression of the H_3R isoforms or regulation of the alternative splicing of H_3R mRNA. The pharmacology and signal transduction of these isoforms is also still largely unknown and needs further investigation. Moreover, G-protein independent signaling of the H_3R, e.g. by G-protein coupled receptor kinases or β-arrestin, has not been studied and might provide additional insight in molecular aspects of the H_3R isoforms. Surprisingly, even non-signaling isoforms have been shown to have a physiological role by influencing the expression of several functional H_3R isoforms. Moreover, little is known about the hH_3R in relation the homo/heterodimerization and the existence of the hH_3R isoforms. GPCRs are shown to form non-covalent dimers through hydrophobic interactions between helices or coiled-coil structures; deletion of certain sequences in the hH_3R protein is likely to have an effect on the formation of dimers. In view of the suggested H_3R heterogeneity in both functional and radioligand binding studies, the occurrence of H_3R isoforms and (potential) dimerization might provide some of the molecular explanations.

However, how intricate the molecular details of the hH_3R might have become, the recent progress to clinical phase studies shows the therapeutic potential of hH_3R inverse agonists and substantiates that the H_3R is a promising drug target.

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