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Commentary

Histamine-gated ion channels in mammals?

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ABSTRACT

There is ample pharmacological and physiological evidence for yet unidentified histamine receptors in mammalian brain that are linked to a Cl^- conductance. In invertebrates, two histamine-gated chloride channels (HisCl α1 and α2) are already well known. HisCl channels are members of the Cys-loop receptor superfamily of ligand-gated ion channels and are closely related to the mammalian $GABA_A$ and glycine receptors (GlyR). Indeed, they share particularly strong homology within the ligand binding and ion channel domains. Here we discuss the possibility that mammalian HisCl channels might exist among the known $GABA_A$ or GlyR subunits. Studies published to date support this hypothesis, including evidence for direct histamine gating of $GABA_A$ β homomers, histamine potentiation of $GABA_A$ $\alpha\beta$ and $\alpha\beta\gamma$ heteromeric receptors, and $GABA_A$ receptor blockade by some antihistamines. We explore what is known about the binding-site structure, function and pharmacology of invertebrate HisCl channels and other histamine binding sites to support and inform a broader search for HisCl channels among the mammalian $GABA_A$ and GlyR subunits. The discovery and identification of HisCl-like channels in mammals would not only enhance understanding of inhibitory signaling and histamine function in the mammalian brain, but also provide new avenues for development of therapeutic compounds targeting this novel histamine site. This commentary is therefore intended to foster consideration of a novel and potentially important target of histamine and histaminergic drugs in the CNS.

1. Introduction

Histamine is an important neuromodulator in the nervous systems of a wide range of organisms. In the mammalian CNS, histaminergic fibers emanating from hypothalamic tuberomamillary neurons project widely throughout the brain and spinal cord to regulate excitability and contribute to the control of cognition, appetite, arousal, and other brain functions. The reader is referred for additional information to several thorough reviews [1,2]. The highest densities of histaminergic fibers are seen in hypothalamus, particularly in the supraoptic, periventricular, medial preoptic, and superchiasmatic nuclei, and also in the septal nuclei, the supramamillary nucleus and the diagonal band. Strong histaminergic innervation is also found in the mesencephalic trigeminal nucleus and in mesencephalic reticular areas that give rise to the ascending reticular activating system. Other notable targets of moderate to dense histaminergic innervation include the major aminergic cell groups in the ventral tegmentum and substantia nigra (dopaminergic), the locus coeruleus (noradrenergic) and the raphe nuclei (serotonergic). Although the focus of the present work is on histamine's function in the CNS, it should be mentioned that histamine is also an important physiological and pathological mediator outside of the brain, including gastric secretion, inflammation, allergy and immunomodulation.

1.1. Histamine receptors in mammals

Histamine is known to act through four metabotropic (G protein-coupled) receptors (GPCRs, H_1–H_4); three of these are widely distributed within the brain and are important in brain histaminergic transmission [2]. H_1 receptor activation, linked via G_q, increases intracellular calcium levels, promotes arousal, and is important in maintaining circadian rhythms and cognitive function. H_2 receptors, linked to G_s, promote cyclic AMP synthesis, and are thought to contribute to memory consolidation. H_3 receptors, linked though $G_{i/o}$, function as both autoreceptors and heteroreceptors. The latter are capable of regulating the activity of a wide variety of other transmitters. H_3 antagonists are in clinical development for the possible treatment of dementia, obesity or attention deficit hyperactivity disorder [3]. H_4 receptors exist in brain [4,5] and share high homology and signaling characteristics with H_3 receptors [6], but are most important in immune and inflammatory signaling [7]. Selective agonists and antagonists are known for all of the metabotropic histamine receptors.

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1.2. Evidence of additional histamine receptors

Several observations point to the existence of additional receptors or ion channels that recognize and respond to histamine in the mammalian brain. Nearly half a century ago, it was determined that iontophoretic application of histamine to cortical neurons has inhibitory effects that are mimicked by the histamine metabolite tele-methylhistamine, which is virtually inactive on metabotropic histamine receptors [8]. Long-chain congeners of histamine also have behavioral effects following CNS administration that cannot be accounted for by actions on known histamine receptors [9]. The H_2 agonist dimaprit, which is an antagonist of invertebrate histamine-gated chloride channels (see below), produces neurotoxic lesions in rat brain that are not blocked by H_2 antagonists nor mimicked by other H_2 agonists [10]. Likewise, some H_2 antagonists (e.g. cimetidine) that closely resemble histamine's structure produce excitation and seizures, but these effects are not mimicked by other H_2 antagonists [11]. In addition, intraventricular injection of cimetidine evokes antinociceptive responses that are not mediated by H_2 receptors but are mimicked by congeners lacking H_2 receptor properties [12].

1.3. Histamine-activated chloride channels in mammals

Evidence suggests there are two types of histamine-activated chloride channels expressed in mammalian gut [13] and brain [14–16], neither of which has yet been identified. The first type appears to be activated indirectly by H_2 receptor activation of cAMP signaling. In cultured small intestinal AH-type myenteric neurons from guinea pig, for example [13], exogenous application of 1 μM histamine induced a slow, stable increase in chloride conductance that was blocked by the H_2 antagonist, cimetidine, and could be mimicked by the H_2 agonist, dimaprit, or by membrane-permeable cAMP analogs. Similarly, picodrop applications of histamine inhibited GABAergic neurons of the thalamic perigeniculate nucleus in brain slices from ferret [16]. The inhibition resulted from a slow, stable increase in chloride conductance that was blocked by cimetidine and famotidine (H_2 antagonists) but not by diphenhydramine (H_1) thioperamide ($H_{3/4}$) or picrotoxin ($GABA_A$). These indirectly activated chloride channels are not the focus of our commentary.

The second type is very different and appears to reflect a chloride channel that is gated by histamine directly. The most compelling evidence for these comes from studies using rat hypothalamic slices to examine synaptic responses rather than exogenous application of histamine [14,15]. Hatton and Yang examined the fast IPSPs evoked by tuberomamillary stimulation that occur specifically in the population of oxytocin neurons of the supraoptic nucleus and not in neighboring vasopressin neurons. They discovered a histamine-activated chloride conductance that was effectively blocked by H_2 receptor antagonists, cimetidine and famotidine at 1–2 μM. The IPSP was also blocked by 20 μM picrotoxin, but not by 10 μM bicuculline or 50 μM strychnine. G-protein mediation was ruled out because the IPSP was not blocked by pertussis toxin, GDP-βs or Rp-cAMPs, had fast kinetics, and could follow stimulation frequencies up to 100 Hz. The authors were led to conclude the existence of a histamine-gated chloride channel underlying the IPSPs evoked by tuberomamillary stimulation. To date, these channels remain unidentified. Our focus will be on the possible molecular identity of such elusive histamine-gated channels in the mammalian brain.

1.4. Histamine-gated chloride channels (HisCl) in invertebrates

Histamine is known to signal directly through chloride channels (HisCl) in invertebrates ranging from nematodes to arthropods. The HisCl channels are expressed in both neurons and glia and are important sensory components in several invertebrate nervous systems (e.g. *Drosophila* phototransduction and arousal, olfactory, thermal and mechanosensation; [17–19]. The reader is referred to more comprehensive reviews for further information on the subject of histamine signaling in invertebrate nervous systems [20–23]. Of note, invertebrates express a variety of related ligand-gated chloride channels that are activated by biogenic amines, but for which there are, as yet, no known mammalian counterparts [24–29].

Two cDNAs coding for HisCl channels were cloned from *Drosophila* and characterized by four groups simultaneously [17,30–32]. Alternate names were given by these groups, but here we adopt Gisselmann's nomenclature of HisCl α1 (a.k.a. HCLA, HA-CL I, $HisCl_2$) and HisCl α2 (a.k.a. HCLB, HA-CL II, $HisCl_1$). Both subunits made homomeric chloride channels when expressed in *Xenopus* oocytes or *Drosopohila* S2 cells that could be activated by micromolar concentrations of histamine. Functional histamine EC_{50} estimates for HisCl α1 ranged from 14 to 166 μM and for HisCl α2 ranged from 4 to 11 μM, but with all reports consistently finding the α2 subunit to be more sensitive [19,32,33]. The HisCl channels were not activated by other neurotransmitters (up to 1 mM) including GABA, glycine, glutamate, acetylcholine, dopamine, serorotonin, epinephrine, norepinephrine or ATP. They were also insensitive to the GABA/glycine receptor antagonists picrotoxin, bicuculline and strychnine, but were blocked by the nicotinic receptor antagonist, *d*-tubocurarine ($IC_{50} \sim 5$ μM) and less potently by dimaprit and certain histamine receptor antagonists, including cimetidine, pyrilamine and α-methyl-histamine [30,33]. The pharmacological profiles of native HisCl in lobster, crayfish, honeybee and flies are generally similar to the recombinant *Drosophila* HisCl channels. They are activated by histamine ($EC_{50} \sim 25$ μM) and blocked by *d*-tubocurarine and various histamine receptor antagonists, including pyrilamine and diphenhydramine (H_1), tiotidine, cimetidine and ranitidine (H_2) [17,19,34,35]. Notably, heteromeric HisCl receptors formed by the combined expression of α1 and α2 subunits were apparently more primed to open than the homomeric receptors. They generated spontaneous currents in the absence of agonist, were ~3-fold more sensitive than the homomers to histamine ($EC_{50} \sim 1$–3 μM) and could also be activated by GABA, albeit weakly ($EC_{50} \geq 1$ mM) [19,32,33].

2. Mammalian HisCl homologs

The search to identify mammalian HisCls might reasonably focus on the closest known mammalian homologs of these invertebrate HisCl α1 and α2 channels that are already known to exist. Screening these against published sequences from the rat genome reveals the greatest homology with particular subunits of GABA- and glycine-gated chloride channels, which are members of the inhibitory class of the Cys-loop family of ligand-gated ion channels. Structurally, the HisCl, $GABA_A$ and GlyR subunits are all predicted 4 transmembrane proteins of approximately 420–500 amino acids in length that most likely form pentameric ion channels in various combinations [36–39].

Fig. 1 depicts the relatedness of invertebrate HisCl and major rat $GABA_A$ and GlyR subunits based on sequence identities. In pairwise comparisons, overall sequence identities between HisCl α1 and α2 subtypes are 41%, whereas overall identities between the full-length HisCl subunits and $GABA_A$ or GlyR subunits are 18–29%. Not surprisingly, there is little or no homology in the signal peptide sequence, the distal N-terminus, or the M3–M4 intracellular loop. These regions are extremely variable across the entire family of mammalian $GABA_A$ and GlyR subunits. The HisCl, $GABA_A$ and GlyR subunits have considerably greater homology in the pre-M1 domain, which includes the Cys-loop signature sequence (C-13-C,

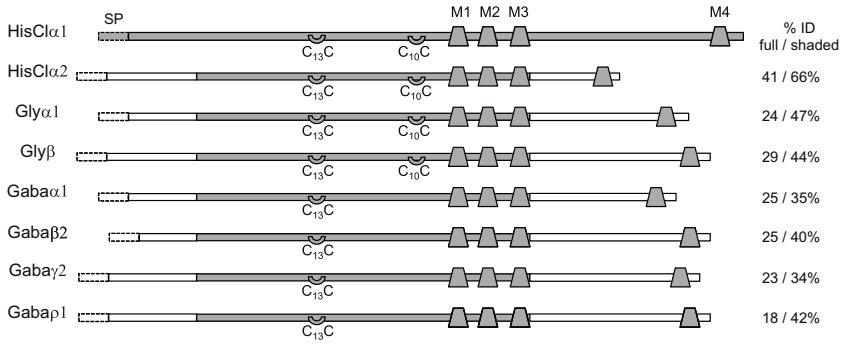

Fig. 1. Linear structure comparison of invertebrate HisCls and mammalian homologs. Positions of the signal peptide (SP), cysteine-loops ($C_{13}C$, $C_{10}C$) and M1–M4 transmembrane domains are shown. Regions having the greatest homology to HisCl α1 are shaded, and percent sequence identities with HisCl α1 are given at right for the full-length and shaded regions.

a pair of cysteine residues separated by 13 amino acids) and the agonist binding site, and in the transmembrane domains (the channel pore). Thus the elements that are most important for ligand binding and receptor function are also the best conserved.

Based on sequence identities in the homologous region, HisCl subunits show the strongest similarities to mammalian GlyR α1–3 and β subunits (44–50%), followed by $GABA_A$ ρ1–3 (42–45%), $GABA_A$ β1–3 subunits (40–44%), and the various $GABA_A$ α, γ, δ subunits (33–39%). Other mammalian Cys-loop receptor subunits had lesser homology. Additionally, the HisCl and GlyR subunits all contain a secondary Cys-loop (C-10-C) immediately preceding the M1 transmembrane domain that is not present in $GABA_A$ subunits; structurally the secondary Cys-loop resides in loop C on the (+) side of the agonist binding domain (discussed below), however its disulfide state and functional significance have not been established.

To date, there have been no studies published examining the histaminergic pharmacology of mammalian GlyRs. Likewise, the published literature includes very few reports examining the histaminergic pharmacology of $GABA_A$ receptor function [11,40,41]. Notably however, these few reports uniformly confirm that histamine-related compounds can inhibit or activate these channels, or potentiate GABA's effects on the most commonly expressed $GABA_A$ receptor subtypes.

2.1. Glycine receptors and histamine

The GlyR subfamily in mammals includes only four subunits (α1–3, β), which make pentameric glycine-gated Cl^- channels [36,37,39]. Native glycine-gated channels are usually heteromers composed of αβ subunits [39]. The α subunits can form functional homomeric channels in recombinant systems, whereas the β subunit alone does not make a glycine-gated channel [42]. Gly α and β subunits predominate in spinal cord and medulla, where they are therapeutic targets for non-opioid analgesics [43]. Gly α subunits are also expressed in midbrain, hypothalamus, thalamus, and sparsely elsewhere in brain with α1 being most widespread [44,45], but α2/3 being relatively more abundant in hypothalamus [46]. Curiously, the β subunit is expressed at high levels throughout the brain including many areas apparently devoid of Gly α subunits [45]. It remains unclear what role, if any, the Gly β subunits might serve when expressed alone since they have no other reported functions aside from co-assembly with Gly α subunits. Gly β has not been shown to self-assemble, traffic to plasma membrane or respond to other agonists in neurons, and biochemical analyses indicate that it does not self-assemble or traffic in recombinant systems [47].

It has been proposed that the mammalian GlyRs evolved from the invertebrate glutamate-gated chloride channels (GluCl) [24,26]. Phylogenetic analysis suggests that both derived from a remote common ancestor and that specialization of the GluCl and GlyRs occurred after the divergence of invertebrates and vertebrates such that GluCl are unique to invertebrates while GlyRs are unique to vertebrates. Alternatively, the same case could be made for GlyRs having evolved from HisCl channels. Indeed, the relatively strong homology and the absence of glycine-gated channels in invertebrates led us to wonder if mammalian GlyRs might retain some sensitivity to histamine that could explain the synaptic responses observed in supraoptic neurons [14,15]. Because the hypothesis that histamine or histamine-related drugs might act on GlyRs had not been investigated in published studies, we made recordings from recombinant GlyR subunits expressed in HEK293 cells to test for histamine-gated channels. In these experiments however, we could not find evidence for activation of rat Gly α1, β, or α1β receptors by histamine up to 3 mM concentration. On the contrary, 1 mM histamine inhibited ~50% of the currents evoked by 50 μM glycine at both Gly α1 and α1β receptors. We take this as evidence to suggest that some remnant of a histamine binding site is present in GlyRs but direct histamine gating is not. It remains to be determined whether this site might be relevant to histaminergic drug actions.

2.2. $GABA_A$ receptors and histamine

The $GABA_A$ subfamily in mammals includes a heterogeneity of α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1–3 subunits, which combine to make pentameric GABA-gated Cl^- channels [38]. Of these, α, β, γ2 and δ are considered the major subunits, having by far the greatest expression, while γ1, γ3, ρ1–3, ε, θ, and π are considered minor subunits with very limited expression and uncertain function. Native $GABA_A$ receptors are usually composed of αβ or αβγ subunits that are expressed throughout the CNS [38,48]. These are the principal targets of benzodiazepines, barbiturates, anesthetics, and other neuroactive drugs used to treat anxiety, insomnia and epilepsy [48]. The α, β and γ subunits have also been shown to form functional homomeric channels in recombinant systems [49–54]. However, the significance of $GABA_A$ homomers is unclear, and native receptors are generally thought to include all of the co-expressed αβγ subunits. Of the relatively less common subunits, several are noteworthy later in this commentary. Of these, the δ subunit is primarily expressed in cerebellum and thalamus [55], ε and θ subunits are expressed in hypothalamus and in monoaminergic neurons [56,57], and the ρ subunits are expressed primarily in retina and the visual pathways [58–60]. The ρ subunits are sometimes referred to as $GABA_C$ receptors because they form homopentameric receptors and may not co-assemble with other $GABA_A$ subunits. The π subunit is expressed only in peripheral tissues and has not been detected in brain. As discussed below,

there is some uncertainty regarding the scope of co-assembly and function of $GABA_A$ subunits in both recombinant and native systems because interpretations have historically relied heavily on responses to GABA.

In regards to histamine actions at $GABA_A$ receptors, a report by Saras et al. [40] provides support for the idea that a $GABA_A$ receptor of some type, specifically involving the β subunits, are a target for histamine action and may be the elusive mammalian HisCl channel. Having recently cloned the *Drosophila* HisCl subunits and finding that heteromeric α1α2 channels could be activated by GABA [30,33], they turned attention to the reverse possibility that histamine might act on mammalian $GABA_A$ receptors. Indeed, this was the case. They expressed recombinant $GABA_A$ β subunits alone as β homomers or in combination with α and γ subunits as αβ or αβγ heteromers. Remarkably, the β homomers were found to harbor a previously unknown binding site for histamine, which was fully capable of opening the channel to gate a chloride current with an EC_{50} of 174 (β2) and 212 μM (β3). The pharmacology of the β channels was unusual. Besides histamine, they could also be activated by the histamine precursor, L-histidine, or the histamine metabolite, tele-methyl-histamine with somewhat lower potencies ($EC_{50} \sim 1$ mM). They were also blocked by various classes of histamine GPCR antagonists, including famotidine (H_2), histamine trifluoromethyl toluidine ($H_{1/2}$), and thioperamide ($H_{3/4}$), but not by cimetidine. Notably, the β channels were nearly insensitive to GABA, which was a very weak partial agonist at 3 mM concentration, and they were not blocked by 100 μM bicuculline or gabazine, but were blocked by 10 μM picrotoxin. Also, like some invertebrate HisCl channels noted above, the β homomers showed spontaneous gating in the absence of agonist that was also blocked by picrotoxin or thioperamide. In αβ or αβγ heteromers, histamine by itself was insufficient to open the channels directly whereas it potentiated GABA-evoked currents. The EC_{50} for histamine potentiation of α1β2γ2 heteromeric receptors was ~5-fold higher (965 μM) than the EC_{50} for activation of β2 homomers (174 μM), suggesting that the binding sites for histamine may differ as a function of $GABA_A$ receptor subunit composition (see Fig. 2). Nonetheless, the key finding of Saras et al. [40] was that histamine may be capable of acting on mammalian $GABA_A$ receptors in essence like an endogenous barbiturate, complete with both modulatory and direct gating behaviors that had previously gone unknown for decades.

The results of Saras et al. raise three intriguing questions. First, do $GABA_A$ β homomers or another configuration of $GABA_A$ (or GlyR) subunits make endogenous HisCl channels in mammalian brain? Second, if so, do they account for the synaptic histamine-gated chloride channels in supraoptic oxytocin neurons? [14,15] And third, what are the implications of His/GABA signal integration at the receptor level and the potential for development of novel therapeutics targeting the histamine site on the receptors to modulate inhibitory signaling in the CNS? To begin to answer these questions, it is important to consider how histamine and GABA bind and activate the receptor channels, and then to compare what is known about the pharmacology of the histamine-IPSPs in oxytocin neurons with HisCl and GABA receptors.

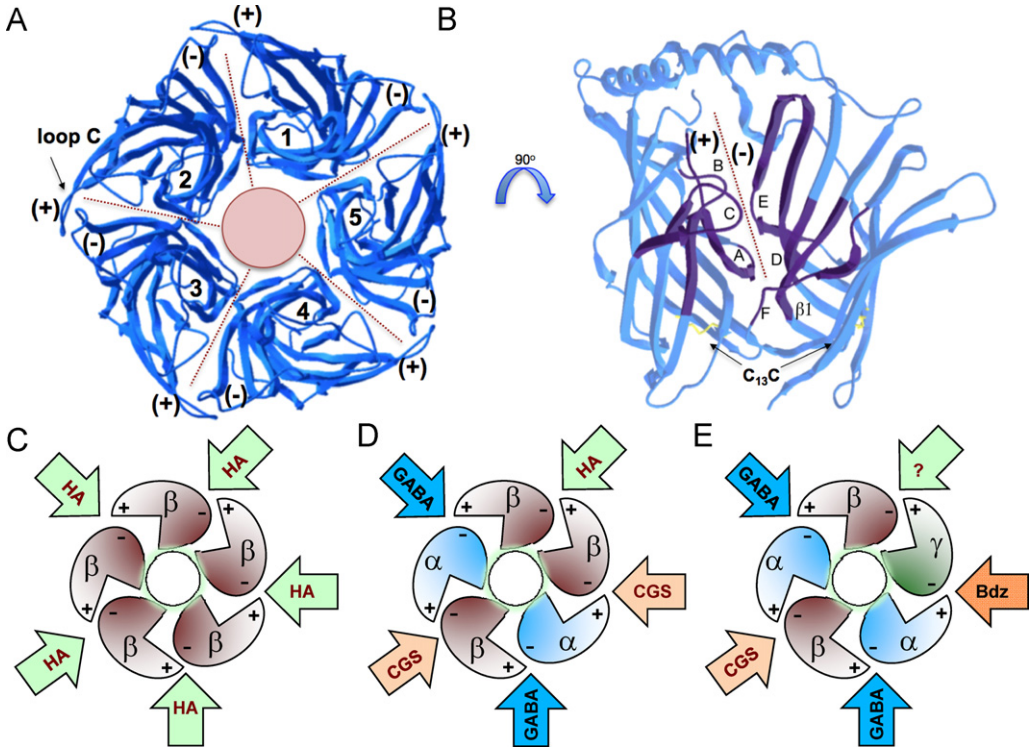

Fig. 2. Pentameric structures illustrating the variability of the subunit interface domains as a function of subunit composition. (A) Top-down view of the pentameric structure of AChBP is shown (PDB ID: 2BYN), which has proven to be a good predictive model of other Cys-loop receptors. Five subunits are arranged symmetrically around a central pore and the agonist binding site resides at the interface that is formed by (+) and (−) sides of the partner subunits. (B) Side view of the (+)/(−) interface between two adjacent subunits. The (+) side includes loops A, B and C, where loop C wraps the outside edge of the solvent–accessible interface. The (−) side includes loops D, E, F, and the β1 sheet that precedes loop A in the linear structure. (C) $GABA_A$ β homomers have five identical binding domains at the β(+)/β(−) interface. These are not activated by GABA but are activated by histamine (HA). (D) Co-assembled αβ heteromers are activated by GABA binding to two β(+)/α(−) interface domains. CGS9895 potentiates GABA currents by binding to two additional α(+)/β(−) interface domains. Histamine also potentiates GABA currents, presumably acting at the β(+)/β(−) interface as in β homomers. (E) Co-assembled αβγ heteromers are activated by GABA binding to two β(+)/α(−) interfaces. Benzodiazepines (Bdz) potentiate GABA currents by binding to the α(+)/γ(−) interface. CGS9895 potentiates GABA currents by binding to the α(+)/β(−) interface. Histamine acts less potently here, probably because its preferred site at the β(+)/β(−) interface is not present and a secondary site at the γ(+)/β(−) or α(+)/β(−) interface is less accommodating to histamine binding.

3. The Cys-loop agonist binding site

Assuming histamine opens HisCl channels in the same manner as GABA, glycine, ACh and other agonists act at related Cys-loop receptors, the molecular determinants of agonist binding and activity should reside at the interface between adjacent subunits in the pentameric complex. This is the most likely case, and their concentration-response curves having Hill slopes between 1.6 and 2.7 suggest at least 2 or in some cases at least 3 binding sites are required for histamine activation [19]. The locations of the critical determinants of agonist binding and activity have been inferred from structural and mutational analyses. While experimentally determined structures for the HisCl, $GABA_A$ and glycine receptors have yet to be solved, the current understanding of the architecture of Cys-loop receptors generally, and the ligand binding sites of these receptors specifically, is derived from site-directed mutagenesis studies and informed by homology models based on the structures of related receptors. The templates for structural interpretations include the EM structure at 4 Å resolution of the muscle type nAChR from the *Torpedo* electric ray [61] and X-ray structures at higher resolution of soluble acetylcholine binding proteins (AChBP) from snails that have the architecture of the extracellular domain of Cys-loop receptors [62–64].

In homology models of the mammalian $GABA_A$ and GlyRs, and presumably in HisCl channels as well, the agonist binding site is composed of a series of β sheet and loop segments (shown in Fig. 2A and B) that are discontinuous in the linear protein sequence but adjacent in the folded structure. Loops A, B and C are on one side of the subunit interface, called the (+) side or principal face. Loops D and E, and minor loops F and β1 are on the complementary side of the interface, also called the (−) side [65]. This structure is preserved in both homomeric and heteromeric configurations of individual subunits, with the difference being that all 5 of the (+)/(−) interface domains would be identical in the case of homomers whereas many configurations are possible in heteromers (Fig. 2C–E).

3.1. Agonist binding residues

Within the binding loops, three aromatic residues were identified early after cloning of the $GABA_A$ receptor subunits that were proven to be critical determinants of GABA potency in heteromeric αβγ receptors. The first was phenylalanine (F64) in the $GABA_A$ α subunit, which was identified as the site for photoaffinity labeling by [^{3}H]-muscimol [66] and where a conservative mutation F64L reduced GABA potency (increased EC_{50}) by more than 200 fold [67]. Equivalent mutations in β or γ subunits did not have similar effects. Two others were tyrosine residues (Y157, Y205) in the β subunit, where conservative mutations Y157F or Y205F each reduced GABA potency by more than 50 fold whereas equivalent mutations in α or γ subunits were ineffective [68]. An additional aromatic residue (β Y97) was subsequently shown to make a cation–π interaction with the agonist [69]. Together, these four key residues define an "aromatic box" for agonist binding at the interface between α and β subunits that is strongly conserved across the Cys-loop family of receptors. A few nearby mutations had similar effects, most notably to β T160 and T202, but not in α or γ subunits [68]. Structurally, the α F64 residue is in (−) loop D, whereas the β Y97, Y157, and Y205 residues are in (+) loops A, B and C, respectively. Other residues with lesser effects on agonist potency, perhaps indirectly, have been found throughout the loops. In GlyRs, which make functional homomeric receptors, agonist binding involves some but not all of these same residues which are located at the α(+)/α(−) interface [39,70,71]. These residues are indicated by asterisks in Fig. 3.

3.2. Subunit combinations and interfaces

Most GABA receptors in mammalian CNS have a stoichiometry of 2α, 2β, and 1γ [38], and the subunits are most likely arranged in the counter-clockwise order β–α–β–α–γ [72]. Such arrangement has been deduced over many years and particularly aided by the

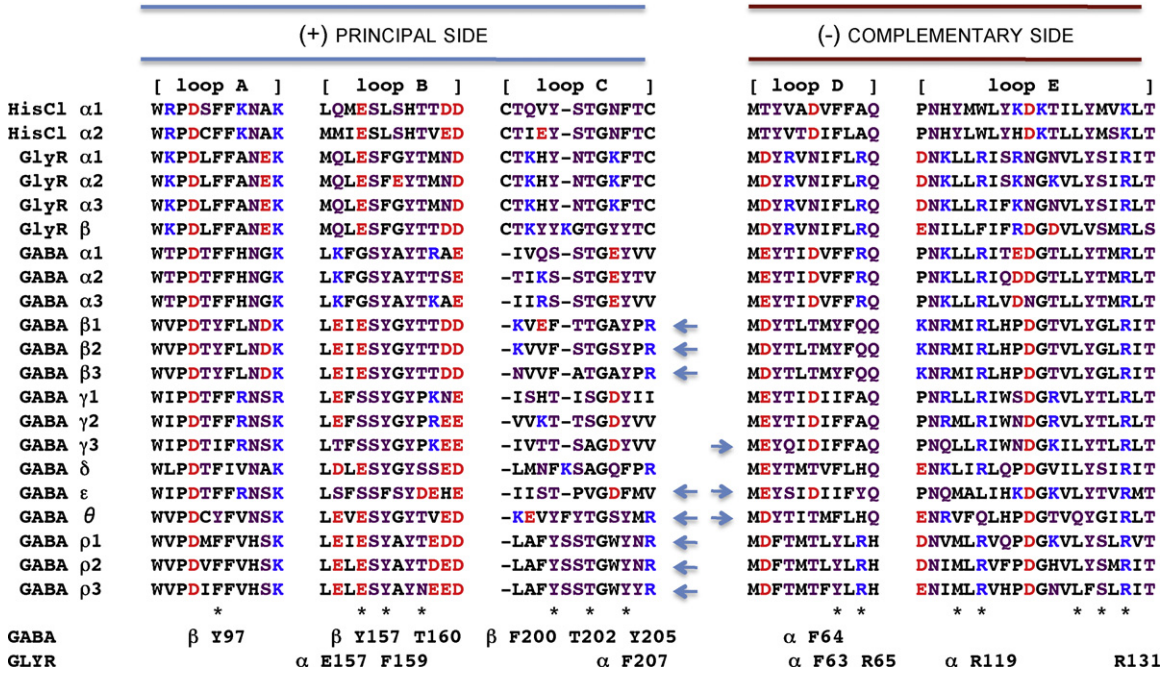

Fig. 3. Amino acid sequence alignments within the (+) and (−) binding loops of HisCl and mammalian chloride channels. HisCl sequences are from *Drosophila melanogaster*. $GABA_A$ and GlyR subunits are from rat. To facilitate comparisons with HisCl and the expected features of a histamine binding site, acidic residues are shown in red, basic residues in blue, and other residues with side chain oxygen atoms in purple. Asterisks below the sequence alignments indicate residues that have been identified previously by others as critical determinants of ligand binding (see text). Residue numbers are given below from either $GABA_A$ or GlyRs. Arrows identify interface domains of interest on the (+) and (−) sides based on conservation with HisCl subunits and particular features expected to facilitate histamine recognition.

use of concatenated subunits to impose a particular order by linking the N- and C-termini of adjacent subunits together [73,74]. Thus it has been determined that $GABA_A$ receptor function requires at least 2 copies each of the α and β subunits so that GABA and other competitive ligands can bind at 2-sites composed of the β(+)/α(−) interfaces [38,75]. This leaves the remaining 3 homologous interface domains unoccupied. The identities of the particular α(1–6) and β(1–3) subunits determine the potency of activation by GABA, among other things. The identity of the fifth subunit (β, γ, δ, ε, θ or otherwise) determines two unique non-GABA interfaces that may or may not have endogenous ligands, but could likely modulate GABA function and might provide unique targets for the development of novel therapeutics.

The fact that $GABA_A$ receptors have 5 homologous but imperfectly conserved subunit interfaces, 3 of which do not bind GABA, no doubt contributes to their complex pharmacology. Benzodiazepines, for example, potentiate GABA currents allosterically by binding to the α(+)/γ(−) interface [38,48]. One might speculate that other ligands could likewise bind to the α(+)/β(−) or β(+)/β(−) interface in αβ heteromers, or to the γ(+)/β(−) interface in αβγ heteromers, or the various (+)/(−) interface configurations introduced by other less common subunits (e.g., δ, ε, θ). In fact, the first such compound (CGS9895) was only recently proven to bind at the α(+)/β(−) interface and, not surprisingly, it was shown to potentiate GABA currents in a manner similar to benzodiazepines [76]. It is highly likely that histamine works in much the same way by binding at least to the β(+)/β(−) interface, as required to activate β homomers, if not at other interface configurations as well.

3.3. What makes a histamine binding site?

Clearly GABA β homomers make a histamine binding site, albeit one with >100 μM EC_{50}. This indicates that histamine can bind reasonably well to the β(+)/β(−) interface even if this might not be the ideal configuration. Therefore, we should like to know if other $GABA_A$ subunit configurations might make a better binding site for histamine. Unfortunately, the structures of invertebrate HisCl channels have not been solved and no homology models have yet been produced. In lieu of these, we might hope to draw inferences regarding the expected nature and composition of a histamine binding site from other histamine-binding proteins. Histamine itself consists of an imidazole ring and an amino group connected by two carbon atoms. Both the ring and the amino group are basic centers. At physiological pH, the amino group is positively charged, however the imidazole ring is neutral and the remaining proton is carried by either of the two nitrogen atoms [77]. Several histamine-binding proteins from the lipocalin family of proteins in ticks have been resolved with histamine bound at 1.2–1.4 Å resolution [78,79]. In these structures, the ligand binding pocket is lined by negatively charged side groups and very few positive charges, ideally suited to attract and accommodate a basic ligand, and by hydrophobic side chains that make van der Waals contact with the ligand. The ammonium group of histamine makes a cation–π interaction with a tyrosine residue, similar to ligands binding the Cys-loop receptors [65,69,71], and also forms hydrogen bonds or ionic interactions with the carbonyl group of a serine residue or the carboxylate groups of aspartate and glutamate residues. The imidazole ring of histamine forms a hydrogen bond with tyrosine, and in some cases makes additional hydrogen bonds involving serine, aspartate or glutamate residues and a water molecule.

3.4. Predicting histamine interactions with other subunits

One would expect to find similar characteristic features in other histamine binding sites, including at least one prominent aromatic residue, a strong bias for acidic over basic residues facing into the binding pocket, and a relative abundance of side chain oxygen atoms that could interact with the basic centers of histamine. In fact, these same core features of histamine recognition appear to be preserved in the histamine GPCRs and related enzymes [80–82]. Therefore, if we judge the HisCl, $GABA_A$ and GlyR subunits on the basis of acidic, basic, and aromatic substitutions in the (+) and (−) binding loops, we might at least begin to decipher why histamine is able to bind at the β(+)/β(−) interface and predict how various other subunits or subunit combinations might impact the favorability of histamine binding at those (+)/(−) interfaces. Or at least we can begin to identify potential clashes. Too much speculation, however, regarding the identities of specific histamine binding residues would be premature at this time in the absence of significantly more structural and mutational analyses.

Fig. 3 shows a partial sequence alignment of HisCl, $GABA_A$ and GlyR subunits within the (+) and (−) binding site loops, with residues color coded for ease of analysis. Comparing across all subunits, the four major aromatic GABA- or glycine-binding residues discussed above stand out as particularly worth mentioning, as all but one are conserved in the HisCl subunits. First is the aromatic residue in the center of loop A (Y97 in $GABA_A$ β), which is a phenylalanine in both HisCl subunits. Second is the aromatic residue in the center of loop B (Y157 in $GABA_A$ β or F159 in GlyR α), which is replaced by leucine in both HisCl subunits. While the aromatic residue in this position is a critical determinant of agonist binding in $GABA_A$ and GlyRs, it is apparently not so in the HisCl receptors. Third is the aromatic residue at the end of loop C (Y205 in $GABA_A$ β or F207 in GlyR α), which is phenylalanine in the HisCl subunits. The other aromatic residue in the center of loop C (F200 in $GABA_A$ β) is conserved in HisCl and GlyRs but only in some $GABA_A$ subunits. Fourth is the conserved aromatic residue in loop D (F64 in $GABA_A$ α or F63 in GlyR α), which is also phenylalanine in the HisCl subunits. With respect to charged residues, we note that the basic arginine near the end of loop D (labeled R65 in Fig. 3) is present in $GABA_A$ α, ρ and all of the GlyR subunits. The positive charge here introduces a potential clash with histamine that is not found in the HisCl channels or other $GABA_A$ subunits. Likewise, the $GABA_A$ α subunits have basic substitutions in (+) loops B and C and (−) loops D and E, as do the GlyR subunits in (+) loop C and (−) loops D and E. More negative charges and fewer positive charge substitutions are observed in the $GABA_A$ β, ε, θ and ρ subunits, possibly making an interaction with a basic ligand like histamine more favorable at these sites. Overall, the seemingly most compatible interface domains for histamine binding are indicated in Fig. 3 by arrows on the (+) or (−) sides of the interface.

3.5. $GABA_A$ β as a HisCl candidate

A more detailed examination of the pharmacology of histamine-activated chloride currents in oxytocin neurons is warranted to assess their identity with $GABA_A$ β homomers. Sadly little is known about the pharmacology of the native channels in these neurons, except that they were entirely blocked by picrotoxin (20 μM), cimetidine (2 μM) or famotidine (2 μM) but insensitive to block by bicuculline (10 μM) or strychnine (50 μM) [14,15]. By comparison, the *Drosophila* HisCl channels were also insensitive to block by bicuculline (100 μM) and strychnine (10 μM), but they were, in contrast, at least 10-fold less sensitive to block by picrotoxin (≥500 μM) or cimetidine (>20 μM) [30,32,33]. There are no data concerning famotidine inhibition of HisCls. Regarding the pharmacology of recombinant rat $GABA_A$ β homomers, these channels were completely blocked by picrotoxin (10 μM), but only weakly inhibited by famotidine (≥150 μM) and entirely insensitive to cimetidine up to 500 μM [40].

So there would appear to be some pronounced differences between the supraoptic chloride channels and recombinant

histamine-gated channels, with the greatest discrepancy being their sensitivity to cimetidine, but also to picrotoxin and famotidine. Clearly more information regarding the pharmacology of native histamine-gated channels expressed in supraoptic neurons is needed. However, what is known makes it highly unlikely that the supraoptic channels are homomeric GABA β receptors. Beyond their insensitivity to cimetidine, their apparent affinity for histamine in the 150 μM range would put them at the lower range of agonist potencies at Cys-loop receptors, lower in fact than the most direct parallel of HisCl at the invertebrate photoreceptor synapse ($EC_{50} \sim 25$ μM) [19].

While the data indicate that many of the critical elements for histamine binding and gating are preserved in $GABA_A$ β subunits, it would be far easier to argue in favor of the physiological relevance of histamine-gated channels that are activated below 50 μM histamine concentrations. Even these would require higher levels of histamine release compared to H_1–H_4 receptors, for example as might occur at direct synaptic contacts. But classical histaminergic synapses may be relatively uncommon in mammals compared to signaling by volume transmission [1,2]. Additionally, although GABA β homomers are produced in recombinant systems, they are not commonly believed to exist in natural systems, whereas αβγ configurations have been demonstrated conclusively and there is high probability that αβ heteromers exist as well [38]. A final additional challenge for endogenous $GABA_A$ β homomers is that they are very potently inhibited by trace Zn^{2+} ions ($IC_{50} \sim 150$ nM) at concentrations ~50-times lower than resting levels of Zn^{2+} estimated in the extracellular environment [53,83].

3.6. Are other subunits involved?

As discussed above, neither GlyRs nor $GABA_A$ β homomers are likely to make native HisCl channels in mammalian brain. Nevertheless, the histamine gating of $GABA_A$ β proves there is sufficient conservation in at least some of the $GABA_A$ subunits to produce a histamine binding site with reasonable affinity. The β homomers would therefore provide a useful template for mutagenesis and homology modeling to map the ligand binding residues. Moreover, the properties of this site might be somewhat more favorable for synaptic activation in other $GABA_A$ receptors having different subunit compositions. For example, one recent study of recombinant αβγ heteromers demonstrated that the α4, α5 and β3 subunits conferred more robust histamine potentiation of GABA-evoked currents than other α or β subunits [41]. Further studies are needed to determine more broadly which $GABA_A$ subunits can combine with the β-subunits to make viable, histamine-gated channels, even in the absence of α subunits.

A complementary approach to identify potential HisCl channels among the $GABA_A$ subunits is to determine which subunits are expressed in the histaminergic cells themselves and in the targets of histamine signaling. Because histaminergic neurons project widely throughout the brain and spinal cord, our focus is limited to areas receiving dense histamine innervation. Several studies have produced a detailed mapping of the distribution of various $GABA_A$ α, β, γ and δ subunits in rat brain by in situ hybridization and immunohistochemistry [84,85].

In the hypothalamus generally, these reports found the most abundant subunits were α2 and β3. Combined with dual-labeling studies of magnocellular oxytocin and vasopressin neurons [86,87] they suggest that the predominant subtypes in the preoptic, supraoptic and paraventricular regions would include α2 > α1, β3 > β2, γ1 and γ2 subunits, but with α1 being far less abundant in paraventricular neurons. Identified histaminergic neurons in the tuberomamillary nucleus have been examined by single-cell RT-PCR [88]. These cells expressed the $GABA_A$ α1, α2 and α5 subunits, usually all together. The β3 subunit was expressed in almost all cells, whereas relatively few contained β1 or β2. About half of the cells examined expressed γ1 and/or γ2 mRNA, while the other half had no γ subunits. The $GABA_A$ ε subunit was also expressed in ~20% of identified histaminergic cells and always in combination with β3 and multiple α subunits. These results were consistent with the previous mapping studies, except that those studies had not examined the distribution of $GABA_A$ ε subunits.

Two particularly intriguing candidates in the search for mammalian HisCl channels are the $GABA_A$ ε and θ subunits, or heteromeric receptors containing these subunits. Compared to the classical αβ or αβγ receptors, $GABA_A$ receptors containing the ε or θ subunits are somewhat more obscure because the ε and θ subunits themselves have rather more limited expression and distribution in the mammalian CNS and very few studies have been done to characterize their functional or pharmacological properties. In the context of this commentary however, these subunits are of interest not only from analysis of the binding site residues, but also because they are predominantly expressed in monoaminergic neurons and in hypothalamus [56,57,89] most notably in a subset of histaminergic tuberomamillary neurons [88] and in nearly all oxytocin neurons in the supraoptic and paraventricular nuclei [90]. Indeed the striking correspondence of expression of the $GABA_A$ ε, and θ subunits has led others to suggest they may be co-regulated along with α3 as part of a gene cluster on the X-chromosome [91]. Recombinant studies indicate that ε subunits can self-assemble as viable surface receptors like the β subunits or they can replace either the β or γ in combination with an α subunit [92–94]. But only αβε combinations produced functional GABA currents. The θ subunit required co-assembly with α to make viable surface receptors, and only made a functional GABA receptor in combination with other α, β and γ subunits [89]. Thus, there is evidence that both the $GABA_A$ ε and θ subunits can assemble and traffic in complexes that are not responsive to GABA and for which there are no known agonists. Very few heteromeric combinations have been attempted to date as these subunits await further characterization.

Finally, we should consider the $GABA_A$ ρ1–3 subunits, which are often referenced as $GABA_C$ [38,95,96]. Examination of their agonist binding site residues in Fig. 3 reveals good correspondence with HisCl subunits, particularly on the (+) side of the interface, and like HisCl they are relatively insensitive to bicuculline inhibition. However, their CNS distribution and functional properties seem incompatible with a role in histamine signaling. The ρ subunits are mostly confined to retina and the visual pathways, also in dorsal root ganglia, but only sparsely elsewhere [58–60]. Recombinant studies indicate that each of the ρ subunits can self-assemble to make viable GABA-activated receptors. In fact, GABA is considerably more potent at homomeric ρ receptors than at other $GABA_A$ subtypes, so it seems unlikely that homomeric ρ receptors might also respond to a basic ligand like histamine. However, no published studies have addressed this question. Moreover, it is also doubtful that ρ-containing heteromers exist in brain, as co-expression studies with $GABA_A$ α, β, γ, or Gly β subunits have failed to detect any changes in the functional properties of ρ receptors that would be expected if they co-assembled [95].

4. Summary and implications for mammalian HisCl receptors

Available evidence suggests that mammalian HisCl channels are likely to be produced by certain combinations of $GABA_A$ or GlyR subunits. Studies published in support of this hypothesis include evidence for histamine-gated Cl^- channels in hypothalamic neurons [14,15], direct histamine gating of $GABA_A$ β homomers [40], histamine potentiation of $GABA_A$ αβ and αβγ heteromeric receptors [40,41], and $GABA_A$ receptor block by some antihistamines [11]. Yet the elusive mammalian HisCl channel remains to be identified and more fully characterized.

The present analysis highlights the implication that unconventional combinations of $GABA_A$ subunits can make unique receptors. In this context, it is important to remark that both the interpretation of which subunits can co-assemble and the interpretation that they assemble in a fixed stoichiometric arrangement are largely based on functional responses to GABA. In this case, any combinations or arrangements of subunits that lack the β(+)/α(−) interface would be scored as non-functional even if they might produce viable surface receptors for other ligands. The curious case of histamine activation of GABA β homomers proves this point, as these receptors by necessity must exist as a symmetrical arrangement of subunits having 5 identical β(+)/β(−) interface domains. They have no GABA site. Whether such receptors exist in native systems is another question altogether. But it would be prudent to consider the possibility that certain other non-GABA binding configurations might also be functional and might have physiological relevance in mammals, as do their homologs in invertebrates. We acknowledge that thinking about GABA receptors as anything else might seem like a strange notion indeed, and one that some might consider to be controversial. However, we prefer to remain open minded on the matter. After all, if other conformations of $GABA_A$ subunits were viable but activated by different ligands, would they still be "GABA receptors?"

Regarding the identity of the HisCl channels in hypothalamic oxytocin neurons or elsewhere in mammalian CNS, the jury is still out. While $GABA_A$ β homomers are the strongest lead thus far, no native or recombinant channel has yet been reported that exhibits such potent block by cimetidine and famotidine in addition to histamine gating. The present overview suggests a few attractive candidates for further study, but such analysis would clearly benefit from more refined structural homology models and mutational comparisons of HisCl channels and $GABA_A$ β subunits. Still, we suggest the most attractive candidates going forward would include various $GABA_A$ β heteromeric configurations, especially involving the β3 subunit, as well as combinations involving the $GABA_A$ ε or θ subunits. These subunits show particularly high homology with HisCl in the agonist binding domains and high expression in histaminergic neurons and prominent targets of histamine signaling. There is also a need to develop ligands for the histamine binding sites of recombinant receptors, as such ligands could be used to identify mammalian HisCl channels in situ and determine their contributions to histamine signaling. The most promising lead compounds that have been identified thus far would appear to be cimetidine, which inhibits the TM-evoked IPSP in vivo [15], and thioperamide, which inhibits the $GABA_A$ β receptors [40]. One would expect related compounds could be developed that act allosterically to enhance or inhibit GABA-evoked currents by binding to the histamine site, thereby exhibiting therapeutic properties similar to drugs acting at the benzodiazepine site.

These findings should support a broader search for HisCl channels among the mammalian $GABA_A$ and GlyR subunits, which would not only enhance understanding of inhibitory signaling and histamine function in the mammalian brain, but also provide new avenues for development of therapeutic compounds targeting this novel histamine site.

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