Histamine is not only a crucial cytokine in the periphery but also an important neurotransmitter and neuromodulator in the brain. It is known to act on metabotropic H1-H4 receptors, but the existence of directly histamine-gated chloride channels in mammals has been suspected for many years. However, the molecular basis of such mammalian channels remained elusive whereas in invertebrates, genes for histamine-gated channels have been already identified.

In this report we demonstrate that histamine can directly open vertebrate ion channels and identified β2 subunits of GABA(A) receptors as potential candidates for histamine-gated channels. In Xenopus oocytes expressing homomultimeric β2 channels, histamine evoked currents with an EC50 of 212 µM (β2) and 174 µM (β3), whereas GABA is only a very weak partial agonist. We tested several known agonists and antagonists for the histamine binding site of H1-H4 receptors and described for β2 channels a unique pharmacological profile distinct from either of these receptors.

In heteromultimeric channels composed of α1β2 or α1β2γ2 subunits, we found that histamine is a modulator of the GABA response rather than an agonist, as it potentiates GABA-evoked currents in a γ2 subunit controlled manner. Despite the vast number of synthetic modulators of GABA(A) receptors widely used in medicine, which act on several distinct sites, only few endogenous modulators have yet been identified. We show here for the first time that histamine modulates heteromultimeric GABA(A) receptors and may thus represent an endogenous ligand for an allosteric site.
neurotransmitter in the CNS. Most of the rapid inhibitory neurotransmission in the CNS is mediated by the GABA type A receptors (14). GABA(A) receptors are heteropentameric proteins constructed of various subunits (α, β, γ, δ, θ, ε and π). The most prominent native receptors are heteromers of α, β and γ subunits (14) but at least in recombinant systems, functional homomultimeric receptors composed of β or γ subunits alone exist (15-19). Such homomultimeric ion channels differ in many aspects from the conventional, heteromultimeric GABA(A) receptors and were therefore candidates for receptors with unexpected new properties.

**Experimental Procedures**

GABA(A) cDNAs and RNAs- Rat α1 and β1 cDNAs were cloned by PCR based methods using standard molecular biology procedures. Rat β2 cDNA was kindly provided by R. Rupprecht (Munich, Germany). Mouse γ2L and human β3 cDNA was obtained from RZPD (Berlin, Germany). All cDNAs were subcloned into pSGEM (courtesy of M. Hollmann, Bochum, Germany) or pCDNA3 (Invitrogen, Karlsruhe, Germany) for HEK293 cell expression.

Expression of receptor cRNA in Xenopus oocytes- cRNAs were synthesized using the AmpliCap T7 high yield message maker Kit (Epicentre, Madison, WI, U.S.A.), according to the manufacturer’s protocol, with PacI linearized pSGEM-plasmids as templates. Xenopus laevis oocytes were prepared by standard methods. After 24 h stage V–VI oocytes were injected with cRNA (typically 5-25 ng per oocyte), incubated at 16 °C in Barth’s solution and tested for functional expression of GABA(A) receptors by two-electrode voltage-clamp recording after 3–7 days. Agonists and antagonists were diluted to the concentrations indicated with Frog-Ringer’s solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl2, 10 mM HEPES, pH 7.2). Recording was done with a two-electrode voltage-clamp amplifier (TURBO TEC-03, npi, Tamm, Germany) and pCLAMP software (Axon Instruments, Union City, CA, U.S.A.) with typical membrane potential of -40 to -60 mV. The pH of all Ringer’s solutions containing histamine was adjusted to pH 7.2 if necessary.

The quality of the water used for solution preparation can be critical for the amount of contamination by traces of Zn^{2+} or Cu^{2+}. Previously it was shown that in native cerebral Purkinje neurons, co-application of histidine enhances GABA-evoked currents by complexing trace amounts of copper ions (20). To exclude that the observed histamine effects depend on the complexation of such divalent cation contaminations, potentiation and dose-response curves experiments were performed in Ringer’s solution prepared with ultra-pure water (‘‘AMPUWA” water, Fresenius, Bad Homburg, Germany) as suggested (20).

In experiment targeting the effect of histamine on heteromultimeric receptors, it was vital to ensure the absence of any contaminating population of homomultimeric channels composed of β subunits when expressing α1β2γ2 receptors as such could mimic a histamine effect. In preliminary studies with oocytes injected with a 1:1:1 ratio of α1β2γ2 subunits, in some experiments small currents were directly evocable by 1 mM histamine, obviously caused by a small, contaminating population of homomultimeric β2 channels. Therefore, we injected an excess of α1 and γ2 over β2 subunit RNA (ratio α1:10, β2: 1, γ2:2). In these oocytes, 1 mM histamine itself evoked no detectable currents, (<< 1% of the maximum GABA evoked current) proving the absence of a contaminating population of homomultimeric β2-subunits. Same is valid for receptors composed of α1β2 subunits, where the α subunit was also injected in a tenfold excess over β. To ensure the incorporation of the γ2L subunit, oocytes were screened with 10 µM Zn^{2+} in the presence of GABA. While αβ subunit combinations are highly sensitive for an inhibition by Zn^{2+}, the αβγ isoforms are insensitive.

**Statistics**- For electrophysiological measurements, statistical analysis and curve fitting was done by Hill equation using SigmaPlot V8.0 (Systat Software, San Jose, CA). All mean values are ± SEM.

**Patch-clamp experiments**- HEK293 cells were maintained under standard conditions in a minimum essential medium supplemented with 10% foetal bovine serum, 100 units/ml penicillin and streptomycin, and 2 mM L-glutamine. Semiconfluent cells were transfected in 35-mm dishes (Becton...
Results

Homomultimeric β channels - We investigated the action of histamine on homomultimeric GABA(A) receptors. Complementary RNAs (cRNA) from β2-3 subunits were expressed in Xenopus laevis oocytes. 2-6 days after injection, histamine- and GABA-elicited currents were recorded in the voltage clamp configuration. We used the known β channel agonist pentobarbital (Pb) (15;16;18) as a positive control and found that 3 mM histamine could also directly open homomultimeric β3 channels with an average current amplitude of 536 nA (±95, n=15). Compared to saturating concentrations of Pb, histamine evoked currents were smaller, reaching 67% (±6%, n=8, p=0.008) of the Pb response (Fig. 1A). 3 mM histamine also opened homomultimeric β3 channels, although with lower average amplitudes (132 nA (±21, n=7), data not shown). Dose-response curves show that homomultimeric receptors composed of β2 or β3 were similarly sensitive to histamine, with an EC50 of 212 μM ± 29 μM (n = 4) and an EC50 of 174 μM ± 14 μM (n = 11), respectively (Fig. 1B,C). It has been reported that homomultimeric β channels are activated by GABA (15-18). Our experiments expressing β2 or β3 confirmed the effect of GABA but at high, saturating GABA concentrations (3 mM) the amplitudes were only 10% of the histamine-activated currents (β3: I_{GABA} (3 mM) / I_{histamine} (10 mM) = 0.11 ± 0.012 (n = 10)). GABA is a weak partial agonist compared to histamine (Fig. 1A).

Homomultimeric channels composed of GABA(A) β subunits are rather histamine-gated than GABA-gated channels. Histamine evoked a current reversing typically at -23 mV under standard ion conditions in oocytes expressing β3 (Fig. 1D), which is consistent with the previously reported chloride selectivity of β channels (15).

The histamine effect is independent on the oocyte expression system. In whole cell-patch clamp experiments on Human Embryonic Kidney 293 (HEK293) cells expressing β3 channels (Fig. 1E), 1 mM histamine evoked currents with an average amplitude of 203 pA (±31, n=8) which was typically 60% of the pentobarbital response (21). The EC50 of histamine with 386 μM (±24, n=6) was in the same range as in Xenopus oocytes (Fig. 1F).

Pharmacology of the histamine response - The currents evoked by 1 mM histamine expressed in Xenopus laevis oocytes expressing β3 were completely blocked by 10 μM PTX but 100 μM of the GABA-antagonists bicuculline or gabazine were ineffective (data not shown). Histamine analogs like histidine and tele-methylhistamine were also found to be agonists for β3 channels (Fig. 2A, supplementary Fig. 1,2), although with lower potencies (EC50 1.14 mM ± 0.15 μM (n = 5) and (EC50 1.1 mM ± 0.13 μM (n = 3), respectively) demonstrating that metabolic precursors and metabolites could also be active agonists. For the histamine-binding sites of metabotropic H1-H4 receptors specific agonists and antagonists are known (22). We investigated whether the histamine binding site of GABA(A) β subunits match one of those pharmacological profiles. In oocytes expressing β3 channels, current evoked by 300 μM histamine was effectively blocked by the H3/4 antagonist thioperamide. Higher concentrations of thioperamide additionally blocked a fraction of spontaneously open channels as indicated by the apparent ‘outward’ current; it thus behaves like an ‘inverse agonist’ (Fig. 2B). The IC50 was 7.2±0.7 µM (n=5) in the absence of histamine (Fig. 2D). In the presence of 300 μM histamine, the IC50 for thioperamide was 32±3.8 μM (n=5) determined by a 4 parameter Hill fit of the complete blocking curve, but it would be in the range of 10 μM, if one would relate it only to the histamine evoked current. Therefore, the found value is only a rough estimate, as the population of open channels interferes with the analysis. Next, we tested if thioperamide and...
histamine might compete for the same binding site and found that at 10 mM histamine, thioperamide was a far less effective blocker; a clear indication for a competitive mechanism (Fig. 2C). The H2 antagonist famotidine (IC\textsubscript{50} = 154±21 \mu M (n = 5)) and the H1/2 agonist HTMT (IC\textsubscript{50} = 162±19 \mu M (n = 5)) were also found to be blockers of the histamine-evoked current as well as the population of open channels (supplementary Fig. 3).

Other ligands for metabotropic histamine receptors, such as cimetidine (H2 antagonist), pyrilamine (H1 antagonist) and dimaprit (H2 agonist), were ineffective in concentrations up to 500 \mu M (data not shown). Our data reveal that the histamine-binding site of GABA(A) β subunits has a unique pharmacology not matching any of the metabotropic receptors.

Histamine analog H2 blockers act on heteromultimeric GABA(A) receptors as reported previously (23;24). Our findings may explain the molecular basis of this observed action as we found that such molecules directly act on β subunits. But our findings imply that GABA(A) receptors are not specifically blocked by H2 antagonists alone but agonists or antagonists of other metabotropic histamine receptors act also.

**Histamine's action on α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors** - The action of histamine on the β subunits resembles that of allosteric GABA(A) modulators like propofol and barbiturates which also activate currents at homomultimeric β receptors directly (15;16;18). At heteromultimeric GABA(A) receptors, such modulators potentiate the action of GABA (25). To investigate the action of histamine on heteromultimeric receptors, we investigated recombinant α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors, which are the most abundant GABA(A) synaptic receptor type in the CNS (14). In oocytes expressing α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors, 1 mM histamine potentiated the current evoked by 10 \mu M GABA (about EC\textsubscript{50}) (Fig. 3A). The potentiation at 10 \mu M GABA was 1.5 fold on average (I(GABA + histamine)/I(GABA) = 1.52±0.43, n=12) but had a considerable variability, reaching from zero in few oocytes up to 2.4 fold. In the same set of oocytes, 1 mM histamine was virtually ineffective on the current evoked by saturating concentrations (300 \mu M) of GABA (1.01±0.06) pointing out that potentiation by histamine was significantly much more effective at submaximal GABA concentrations (p=0.0012, n=12). This fit to the observation that 1 mM histamine significantly lowered the EC\textsubscript{50} for GABA from 15.8 ± 2.1 \mu M to 11.1 ± 1.7 \mu M (p=0.0044, n = 5) (Fig. 3A, B). 1 mM histamine itself didn't evoke detectable currents, (< 1% of the maximum GABA evoked current, Fig. 3A).

The histamine potentiation of recombinant GABA(A) receptors was independent of the expression system and could also be observed at α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors expressed in HEK293 cells. Histamine showed potentiating effects similar to those in oocytes, demonstrating that the effect was not restricted to the oocyte expression system. At cells stimulated with 3 \mu M GABA, 1 mM histamine evoked an up to 2.0-fold potentiation of the GABA current (1.4-fold potentiation on average; n = 12) (Fig. 3C), but 1 mM histamine alone never evoked any detectable currents.

**Histamine's action on α\textsubscript{1}β\textsubscript{2} receptors** - It is known that the presence of a γ\textsubscript{2} subunit modulates the potentiation by allosteric modulators like benzodiazepines, that are only effective in γ subunit containing receptors (26). In the case of potentiators acting on β subunits like propofol, it was reported that the γ\textsubscript{2} subunit alters the mode of potentiation (27). To address the question if the presence of a γ\textsubscript{2} subunit alters histamine's potentiation, we compared the effect of histamine on heteromultimeric α\textsubscript{1}β\textsubscript{2} and α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors. In oocytes expressing α\textsubscript{1}β\textsubscript{2} receptors, 1 mM histamine potentiated currents evoked by GABA. Histamine potentiation was strongly dependent on the GABA concentration but in a different manner as for α\textsubscript{1}β\textsubscript{2}γ\textsubscript{2} receptors. At α\textsubscript{1}β\textsubscript{2} GABA receptors, 1 mM histamine potentiated best at saturating GABA concentrations (300 \mu M) but in the average not at submaximal concentrations of 3 \mu M GABA (Fig. 3D,E). At 300 \mu M GABA, average potentiation was 1.26 fold (±0.24, n = 14) and significantly greater (p=0.013, n=14) than at 3 \mu M GABA in the same set of oocytes (1.04±0.07, n = 14). Also in these experiments, some oocytes had GABA currents not potentiated by histamine at all. 1 mM histamine itself didn't evoke detectable currents (< 1% of the maximum GABA evoked current). The average EC\textsubscript{50} for GABA was not significantly affected by 1 mM histamine (p=0.22, n=4). These experiments demonstrate that the γ\textsubscript{2} subunit has a vital,
modulatory role in histamine potentiation of GABA(A) receptors.

Histamine potentiates GABA receptors in a dose-dependent manner. At \( \alpha_3 \beta_2 \) receptors, the EC_{50} of potentiation is 965 \( \mu \text{M} \) (\( \pm 306, n = 4 \)) (Fig. 3 F). The potentiation effect requires higher histamine concentrations as the direct action on homomultimeric \( \beta_2 \) channels.

**DISCUSSION**

Our study shows that histamine directly opens homomultimeric GABA(A) receptors that thus can function as histamine-gated channels. The existence of such histamine-gated chloride channels in mammals has been suggested for a long time (4;9). The putative histamine receptor described by Hatton and Yang (9) in the SON nucleus shares some pharmacological similarities with homomultimeric \( \beta \) channels (e.g. the PTX-sensitivity). However, there are also some pronounced differences (e.g. the affinity for histamine and the sensitivity to cimetidine), suggesting that the native receptor may need additional components. Compared to the high affinity of metabotropic histamine receptors, the EC_{50} of \( \beta \)-channels with app. 200-400 \( \mu \text{M} \) is quite low and they are not expected to be activated by typical extracellular histamine concentrations in the brain. However, at synaptic transmission, high enough concentrations could be reached in the synaptic cleft as histamine concentration in synaptic vesicles be as high as 670 \( \text{mM} \) (28). Nevertheless, our findings support the idea that GABA(A) subunits may be vital parts of potential native mammalian histamine-gated channels and could provide the histamine binding site.

Further, we demonstrate that histamine potentiates GABA responses in heteromultimeric receptors and thereby identified a new type of allosteric potentiator for GABA(A) receptors. The mode of potentiation resembles anaesthetics such as propofol: like propofol it targets \( \beta \) subunits, opens homomultimeric \( \beta \) channels directly but modulates the GABA response at heteromultimeric channels (15;16;18;27;29;30). Also the influence of the \( \gamma \) subunit is the similar. In receptors composed of \( \alpha_3 \beta_2 \gamma \) subunits, both histamine and propofol potentiate effectively at saturating GABA concentrations increasing the maximally evoked currents. In contrast, at \( \alpha_1 \beta_2 \gamma \) receptors, histamine and propofol were non effective at high concentrations but are shifting the GABA dose-response curve leftwards (30).

In our oocyte expression system, we found that at recombinant heteromultimeric receptors the strength of histamine potentiation was quite variable, an indication that potentiation might be regulated for example posttranscriptionally by a yet unknown mechanism. Such variability of receptor properties in recombinant expression systems is often observed and can have several reasons: for example, differences in expression level (31), receptor clustering (32), different amounts of \( \gamma \) subunits relative to \( \alpha \) or \( \beta \) in \( \alpha \beta \gamma \) GABA(A) receptors (33) as well as different receptor phosphorylation, just to mention a few. Interestingly, phosphorylation regulates GABA(A) receptor potentiation by neurosteroids (34).

In contrast to the variability of the histamine effect on heteromultimeric channels, exclusively all measured homomultimeric \( \beta \) channels responded to histamine and none were found that only responding to GABA or pentobarbital.

For GABA(A) receptors, about 10 different sites for allosteric modulators are known including neurosteroids, benzodiazepines, general anaesthetics and ethanol (25;35). With the exception of neurosteroids, no endogenous modulators have been identified so far. Our findings that histamine potentiates GABA action on GABA(A) receptors suggests that it is an endogenous ligand for an allosteric site located on the \( \beta \) subunits. Therefore, our results suggest an additional function for histamine *in vivo*, apart from the action on metabotropic histamine and NMDA receptors (36). All histaminergic neurons in the mammalian brain are found in the TM nucleus, and send axons to almost all parts of the CNS (10). Some of these neurons contain both GABA and histamine. In addition, histamine could diffuse out of a histaminergic synapse by a ‘spillout’ effect as described for GABAergic synapses (37) and thus may act on neighbouring synaptic or extrasynaptic GABA(A) receptors. Further, mast cells in the brain are a source for histamine. Mast cells occur in the CNS of many species and up to 50% of the brain histamine is attributable to the presence of these cells. By direct gating of channels or by affecting GABA(A) receptor currents, histamine should modulate processes in which rapid GABA-evoked currents participate (8).
REFERENCES


FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Action of histamine on homomultimeric β channels. (a-d) Homomultimeric β receptors are activated by histamine. β-expressing oocytes were voltage-clamped at potentials between -40 and -60 mV and various histamine concentrations (10 µM - 10 mM) were bath-applied. (a) Current induced by histamine, pentobarbital (Pb) and GABA in Xenopus oocytes expressing β3 receptors, 10 µM PTX blocked the population of spontaneously open channels that is typical for β channels (15-18). (b) Dose-dependency of β3 activation. (c) Histamine dose-response curves for β2−3. (d) I/V relationship of currents induced by 1 mM histamine in an individual oocyte expressing β3 receptors. (e) Whole cell currents induced by histamine, pentobarbital and GABA in HEK293 cells expressing β3 receptors (upper trace) and by histamine in increasing concentrations (lower trace). The off-response after pentobarbital application is typical for saturating concentrations. (f) Histamine dose-response curves for β3 in HEK293 cells

Fig. 2. (a) Action of histamine analogs on β3 expressing Xenopus oocytes. (b) Pharmacology of thioperamide. For each concentration of thioperamide, three consecutive measurements were done as indicated by 1-3 in (b). 1st: 300 µM histamine as control, 2nd: various concentrations of thioperamide co-applied with 300 µM histamine and 3rd: thioperamide alone. Apparent outward currents were caused by block of spontaneously open channels. Amount of spontaneous activity was determined by application of the blocker 10 µM PTX. (c) Action of thioperamide on currents evoked by 10 mM histamine (d), dose-inhibition curves for thioperamide from measurements as in (b) or (c) (filled circle: thioperamide co-applied with 300 µM histamine, circle: thioperamide alone, inverted triangle: thioperamide co-applied with 10 mM histamine)

Fig. 3. Histamine potentiation of GABA-evoked currents. Potentiation of GABA responses by histamine. (a) α1β2γ2-expressing Xenopus oocytes were voltage-clamped and various GABA concentrations with or without 1 mM histamine were bath-applied, (b) effect of 1 mM histamine on the dose-response curve for GABA in oocytes expressing α1β2γ2. Data points are the mean of five individual oocytes (filled circle: GABA, circle: GABA + 1 mM histamine), (c) potentiation of GABA responses in α1β2γ2-expressing HEK293 cells measured by whole-cell patch clamp experiments, (d) action of 1 mM histamine on GABA-evoked currents in Xenopus oocytes expressing α1β2 receptors, (e) effect of 1 mM histamine on the dose-response curve for GABA in oocytes expressing α1β2. Data points are the mean of five individual oocytes (filled circle: GABA, circle: GABA + 1 mM histamine). (f) Dose-response curve for histamine potentiation of currents evoked by 300 µM GABA in oocytes expressing α1β2. Data points are the mean of four individual oocytes.
Fig. 1
Fig. 2
Fig. 3
Histamine action on vertebrate GABA(A) receptors: direct channel gating and potentiation of GABA-responses
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