Histamine and \( H_3 \) Receptor in Alcohol-Related Behaviors

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ABSTRACT

Data from rat models for alcohol preference and histidine decarboxylase knockout (HDC KO) mice suggest that brain histamine regulates alcohol-related behaviors. Histamine levels are higher in alcohol-preferring than in alcohol-nonpreferring rat brains, and expression of histamine \( H_3 \) receptor (\( H_3R \)) is different in key areas for addictive behavior. \( H_3R \) inverse agonists decrease alcohol responding in one alcohol-preferring rat line. Conditioned place preference induced by alcohol is stronger in HDC KO mice than in control mice. The HDC KO mice display a weaker stimulatory response to acute alcohol than the wild-type (WT) mice. In male inbred C57BL/6 mice the \( H_3R \) antagonist ciproxifan inhibits ethanol-evoked stimulation of locomotor activity. Ciproxifan also potentiates the ethanol reward, but does not alone result in the development of place preference.

At least in one rat model developed to study alcohol sensitivity high histamine levels are characteristic of the alcohol-insensitive rat line, and lowering brain histamine with a HDC inhibitor increases alcohol sensitivity in the tilting plane test. However, the motor skills of HDC KO mice do not seem to differ from those of the WT mice. Current evidence suggests that the histaminergic system is involved in the regulation of place preference behavior triggered by alcohol, possibly through an interaction with the mesolimbic dopamine system. Histamine may also interact with dopamine in the regulation of the corticostriato-pallido-thalamo-cortical motor pathway and cerebellar mechanisms, which may be important in different motor behaviors beyond alcohol-induced motor disturbances. \( H_3R \) ligands may have significant effects on alcohol addiction.

Rat Models of Alcohol-Related Behaviors

Several inbred and outbred rat lines with different alcohol preferences have been developed and reported (for a review, see Sinclair et al., 1989; Sommer et al., 2006). These include the high alcohol-preferring (HAP) and low alcohol-preferring (LAP) rats (Kitanaka et al., 2004), inbred alcohol-preferring and nonpreferring rats (McBride et al., 2010), and outbred alko alcohol (AA) and alko nonalcohol (ANA) rats (Sinclair et al., 1989; Sommer et al., 2006). A summary of rat and mouse models used in alcohol research is shown in Table 1.

The AA and ANA rat lines were among the first lines produced using a bidirectional selection method (Eriksson, 1968) for alcohol preference, and these rats have now been maintained beyond the 100th generation (Sommer et al., 2006). The AA rats have higher levels of dopamine in several brain regions, including striatum and limbic forebrain, than the ANA rats (Ahtee and Eriksson, 1975), and tyrosine hydroxylase activity is also 42% higher in AA than ANA rats (Pispa et al., 1986). The levels of noradrenaline are also higher in AA rats than in ANA rats in the cortex and limbic areas (Ahtee and Eriksson, 1975). Noradrenaline turnover also appears higher in AA rats, because the levels of 3-methoxy-4-hydroxy-phenylglycol are higher in AA rats (Sommer et al., 2006). However, there is no difference in the alcohol-induced release of dopamine between the lines, so the sensitivity to ethanol is not the major difference between the lines (Sommer et al., 2006). Serotonin levels are higher in AA rats than in ANA rats in all brain regions studied (Ahtee and...
Eriksson, 1972), but metabolite levels are normal. Thus, there are differences in major catechol and indolamine neurotransmitter systems between the rat lines. Histamine levels in AA rats are 20 to 170% higher in different brain regions in AA than in ANA rat brain (Lintunen et al., 2001) (Table 2). It is noteworthy that in almost all brain areas histamine levels in Sprague-Dawley (SD) rats are between the values seen in AA and ANA rats, suggesting correlation of histamine levels to alcohol preference. Levels of tele-methylhistamine are generally 30 to 70% higher in AA (Lintunen et al., 2001).

<table>
<thead>
<tr>
<th>Animal Strain or Line</th>
<th>Description</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprague-Dawley</td>
<td>Outbred line</td>
<td>General multipurpose model, albino</td>
</tr>
<tr>
<td>Wistar</td>
<td>Outbred line</td>
<td>General multipurpose model, albino</td>
</tr>
<tr>
<td>AA and ANA Lines</td>
<td>Lines selectively bred for high and low alcohol preference and consumption</td>
<td>Breeding program started in Finland in 1960s; derived from Wistar and Sprague-Dawley background; Lewis and Brown Norwegian genotype was introduced later</td>
</tr>
<tr>
<td>P and NP Lines</td>
<td>Lines selectively bred for high and low alcohol preference and consumption</td>
<td>Breeding was initiated in 1970s in the United States; Wistar background, inbred</td>
</tr>
<tr>
<td>sP and sNP Lines</td>
<td>Lines selectively bred for high and low alcohol preference and consumption</td>
<td>Breeding started in 1981 in Sardinia; Wistar background</td>
</tr>
<tr>
<td>AT and ANT Rats</td>
<td>Rats with genetic tolerance or hypersensitivity towards alcohol-induced ataxia</td>
<td>Breeding started in 1970s in Finland; several background lines</td>
</tr>
<tr>
<td>C57BL/6J Mice</td>
<td>Inbred strain, J refers to the substrain from The Jackson Laboratory (Bar Harbor, ME)</td>
<td>Multipurpose model; used in ethanol drinking studies because of the high ethanol consumption; EthOH-induced CPP and low stimulation</td>
</tr>
<tr>
<td>DBA/2J Mice</td>
<td>Inbred strain from The Jackson Laboratory</td>
<td>Multipurpose model; used in place preference studies; do not drink ethanol voluntarily</td>
</tr>
<tr>
<td>129/Sv Mice</td>
<td>Inbred strain, typically the background strain when generating knockout mice</td>
<td>Moderate to low responses to alcohol, high alcohol CPP, low spontaneous activity</td>
</tr>
<tr>
<td>HAP/LAP Mice</td>
<td>High/low alcohol consumption</td>
<td>Derived from the HS/igb eight-way inbred strain cross, United States</td>
</tr>
<tr>
<td>HDID-1, HDID-2 Mice</td>
<td>High drinking in the dark</td>
<td>Derived from the HS/igb eight-way inbred strain cross, United States</td>
</tr>
</tbody>
</table>

Histamine levels in AA rats are 20 to 170% higher in different brain regions in AA than in ANA rat brain (Lintunen et al., 2001) (Table 2). It is noteworthy that in almost all brain areas histamine levels in Sprague-Dawley (SD) rats are between the values seen in AA and ANA rats, suggesting correlation of histamine levels to alcohol preference. Levels of tele-methylhistamine are generally 30 to 70% higher in AA (Lintunen et al., 2001).
than ANA rats, with pons as the only exception where no difference is seen (Lintunen et al., 2001). Thus, there is a distinct difference in histamine turnover between the rat lines. Significantly higher histamine and tele-methylhistamine concentrations were found in several brain regions relevant for the alcohol preference, including the frontal cortex, striatum, septum, hypothalamus, and hippocampus. Immunohistochemical analysis showed that this increased histamine resided in nerve fibers rather than mast cells. Higher histamine-immunoreactive fiber densities in AA rats were found in medial and lateral septum, nucleus accumbens, and medial preoptic nucleus (Lintunen et al., 2001) (Fig. 1). Generally, histamine levels in different rat strains are variable because of the different numbers of mast cells found predominantly in the thalamus and median eminence. In agreement with this concept, histamine levels in the thalamus of SD rats were significantly higher than in either AA or ANA rats, whereas the levels in hypothalamus, septum, and hippocampus were approximately twice as high in AA as in ANA or SD rat brain (Lintunen et al., 2001). Histamine H3 receptor (H3R) regulates histamine synthesis and release (Arrang et al., 1983), and in rat brain the different isoforms of this receptor are also differentially expressed (Drutel et al., 2001). Because of its function, possible changes in H3R expression and/or receptor radioligand binding may be caused by a primary difference in the histamine. On the other hand, primary changes in receptor regulation may lie behind changes in histamine turnover. In AA rats, a distinct statistically significant difference to ANA rats in H3R radioligand binding was found in the motor cortex, nucleus accumbens, and CA1 area of the hippocampus, all areas which may be of relevance for the behavioral phenotype. H3R radioligand binding was lower in AA rats than in ANA rats in only these areas, whereas there was no difference in lateral septum or tuberomammillary nucleus (Lintunen et al., 2001). These differences suggest no direct correlation between histamine levels and H3R radioligand binding. Thus, the observed differences in receptor binding are not merely caused by higher histamine levels and release in these areas, but may depend on other factors that are potentially important for the behavioral differences. It is noteworthy that histamine H1 receptor (H1R) expression was generally significantly lower or showed a tendency to lower values in AA rats in all brain areas (Lintunen et al., 2001). This can be interpreted as a downregulation caused by high histamine level and release. Behaviorally, the H1R antagonist mepyramine did not affect ethanol self-administration in AA rats, whereas two H3R inverse agonists, thioperamide and clobenpropit, significantly and in a dose-dependent manner reduced self-administration of ethanol (Lintunen et al., 2001). These differences in histaminergic system function have not been tested similarly in the other rat line models of high/low alcohol consumption. However, the histamine-stimulated phosphoinositide hydrolysis in the cerebral cortex is significantly lower in HAP than in LAP rats (Takekura et al., 2003). Although the histamine levels were not significantly different in that study, the mean of histamine concentration in the cortex was more than twice as high as in LAP rats, and the lack of significance may be caused by the low number of experimental animals (n = 3) (Kitanaka et al., 2004). The μ-opioid receptor antagonist n-Phe-Cys-Tyr-d-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP) injected into the amygdala and the δ-opioid receptor antagonist naltrindole administered into the nucleus accumbens or basolateral amygdala also decrease ethanol responding in a two-lever operant system in both AA and Wistar rats (Hyttia, 1993; Hyttia and Kiianmaa, 2001). AA rats also show lower spontaneous release of β-endorphin in the hypothalamus (Nylander et al., 1994; de Waele et al., 1994) and lower levels of proenkephalin peptides in the nucleus accumbens and prodynorphin peptides in the ventral tegmental area than ANA rats. Differences in pro-opiomelanocortin expression have been reported in both AA and ANA rats (Gianoulakis et al., 1992) and HAP/LAP rats (Kinoshita et al., 2004), although in somewhat different brain regions, which suggests that the same mediators are at least common phenotypic signs of the selectively bred animals.

Rat lines have also been developed for studying sensitivity to a moderate dose (2 g/kg) of alcohol (Rusi et al., 1977), and

Fig. 1. Histamine-immunoreactive nerve fibers in ANA rat brain regions (a, c, e, and g) and AA rat brain regions (b, d, f, and h) show distinct differences in the medial septum (a and b), lateral septum (c and d), nucleus accumbens (e and f), and medial preoptic nucleus. 3V, third ventricle; Aca, commissural anterior; acb, nucleus accumbens; LSV, lateral septum, ventral part; LV, lateral ventricle; MPO, medial preoptic nucleus; Pe, periventricular nucleus. Scale bar: 100 μm. Reproduced with permission from Lintunen et al. (2001).
in these alcohol-sensitive (alcohol nontolerant) and alcohol-insensitive (alcohol tolerant) rats distinct differences have been observed in histamine levels, H₃R mRNA expression, H₃R radioligand binding, and H₄R agonist-induced G protein activation (Lintunen et al., 2002). The alcohol-sensitive rats have significantly reduced histamine levels in the frontal cortex, septum, hypothalamus, and hippocampus and increased H₃R mRNA expression in several brain regions. In these rats, brain histamine may be causally linked to the differences in alcohol sensitivity. Administration of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase (HDC), induces a decline in both brain histamine levels and motor coordination as measured on a tilting plane in alcohol-insensitive (alcohol tolerant) rats, which have high brain histamine levels (Lintunen et al., 2002). Both histamine- and immepep-evoked guanosine 5′-O-3-[35S]thio(triphosphate) binding is higher in alcohol-sensitive (alcohol nontolerant) rats in the primary motor cortex, insula, and caudate putamen (Lintunen et al., 2002). These regions are important in motor disorders such as Tourette's syndrome, where functional imaging has revealed abnormalities in a network involving the prefrontal cortex, insula, caudate, and premotor and primary motor cortex (Stern et al., 2000). Tourette's syndrome is also characterized by increased dopamine release after amphetamine challenge in both striatal (Singer et al., 2002) and extrastriatal (Steeves et al., 2010) sites. It is possible that histamine/dopamine interactions are widely important in several motor disorders, because in both Parkinson's disease and experimental rat models the H₃R expression and radioligand binding are altered in substantia nigra and caudate putamen (Ryu et al., 1994; Anichtchik et al., 2000a,b, 2001). Thus, interactions of histamine and dopamine probably span a wide range of disorders ranging from motor system to addiction.

The alcohol-induced motor incoordination may also depend on cerebellar mechanisms. Indeed, the diazepam-insensitive binding of the benzodiazepine [3H]ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-1,4-benzodiazepine-3-carboxylate ([3H]Ro15–4513) is lacking in the granule cells of the ANA rats, a phenomenon that is caused by a point mutation in the α6 subunit of the GABAₐ receptor (Korpi et al., 1993). This mutation renders the α subunit benzodiazepine-sensitive (Korpi et al., 1993), suggesting that the altered GABAergic signaling may contribute to the behavior of the ANA rats. The cerebellum receives a direct hypothalamicocerebellar input from the histaminergic tuberomammillary nucleus neurons in both rodents and humans (Panula et al., 1989, 1993), and fibers pass through the granule cell layer and Purkinje cell layer perpendicularly, to then turn 90° to make contact with Purkinje cell dendrites (Panula et al., 1993). Thus, both extensive systems (basal ganglia and cerebellum) that control the upper motor neuron functions are modulated by the histaminergic system in a manner that may contribute to alcohol-dependent motor incoordination.

**Mouse Strain Properties and Differences, Methods Used to Study, Pros and Cons**

Alcohol has strong behavioral effects in mice, and most studied are the reinforcing and rewarding effects and the stimulation of locomotor activity. In addition, the motor-impairing effect of alcohol is well known and can be studied in mouse behavioral models. The reinforcing and rewarding properties of alcohol are studied by using self-administration paradigms or conditioned place preference (CPP). Even though both of these methods are thought to measure reinforcement it is possible that they do not measure exactly the same aspects of alcohol reinforcement and the brain areas involved can be different (Green and Grahame, 2008). Thus, a combination of the two methods is advisable. Self-administration of alcohol has been largely done by using a two-bottle choice test where mice have a chance to choose between water or alcohol solution. However, most mouse strains do not drink alcohol initially at concentrations that are pharmacologically relevant (Sanchis-Segura and Spanagel, 2006). Thus, procedures to train mice to drink have been developed, such as ascending concentrations of alcohol and addition of a sweet flavor agent that can progressively fade out or not. The recently described drinking in the dark method (Rhodes et al., 2005) offers a faster option for screening the effects of drugs on alcohol drinking. The drinking in the dark method is based on the high voluntary alcohol consumption of C57BL/6J mice during their active period of day and has received a lot of interest among alcohol researchers as a model for binge drinking.

CPP is a form of Pavlovian conditioning where environmental cues become associated with alcohol administration (Tzschentke, 2007). CPP is routinely used to measure the reinforcing and rewarding or aversive effects of drugs. The use of CPP in alcohol studies requires careful planning of the study design because the alcohol dose, the length of the conditioning period, and the use of a biased versus an unbiased protocol might lead to unexpected or misleading results (Cunningham et al., 2006). The mice of the DBA/2J strain show strong alcohol CPP and are commonly used, whereas C57BL/6J mice do not develop alcohol-evoked CPP (Cunningham and Noble, 1992) or it is rather weak (Gremel et al., 2006). Examples of different mouse strains used in alcohol studies are shown in Table 1. Modifications of CPP and operant alcohol self-administration paradigms are used when other addictive features beyond reinforcement, such as alcohol seeking and relapse, are the focus of research.

The stimulatory effect of alcohol can be detected by measuring the horizontal locomotor activity in response to alcohol administration. Typical to alcohol stimulation is that it occurs only with fairly low doses and is rather short-lived. Sensitivity of different mice strains toward the stimulatory effect of alcohol vary to a great extent, the DBA/2 and FVB strains being most stimulated by alcohol and 129/Sv and C57BL/6 strains showing moderate to complete loss of activation by alcohol (Crabbe et al., 1994, 2005). In repeated alcohol administration some strains, but not all, develop sensitization to the stimulatory effect of alcohol, a phenomenon that is commonly seen with psychostimulants such as amphetamine and cocaine. Locomotor stimulation and the development of psychomotor sensitization have been suggested to predict the addictive property of a drug (Wise and Bozarth, 1987; Robinson and Berridge, 1993, respectively). However, these theories have been widely discussed (Sanchis-Segura and Spanagel, 2006), and many studies, including ours (Nuutinen et al., 2010), do not support this view. Acute sedative effects of alcohol can be examined with various behavioral tests of which the rotating rod test is the most widely used. However, this test has been criticized for its lack
of sensitivity (Stanley et al., 2005). Different mouse strains also differ in their sensitivity for ethanol in the rotarod task (Rustay et al., 2003). A more sensitive task for studying alcohol-induced ataxia and motor incoordination in mice is the balance beam walking test. However, finding the right combination of alcohol and size of the beam that shows marked, but not too strong, impairment by alcohol can be rather challenging, and it is important to measure not only time to cross the beam but more importantly how many foot slips the mouse makes while walking on the beam. In addition, sedation can be studied by using a grid or a dovel test or ataxia can be observer-rated. Very high doses of alcohol (e.g., 4 g/kg) induce the loss of the righting reflex, which can be also a marker for alcohol sedation. However, because of the high dose, loss of the righting reflex test measures different aspects of sedation than the other motor function tests mentioned above.

Histamine in Mouse Models of Addiction

Histamine has been suggested to have an inhibitory role in reward and reinforcement. This is supported by the findings that cocaine-induced CPP is attenuated by increasing brain histamine levels with L-histidine, whereas inhibition of histamine synthesis by α-fluoromethylhistamine potentiates CPP by cocaine (Suzuki et al., 1996). In line with this, the CPP induced by ethanol (Nuutinen et al., 2010) (Fig. 2) and morphine (Gong et al., 2010) is stronger in HDC KO mice. However, no difference for cocaine reward was found in the CPP model between HDC KO and WT mice (Brabant et al., 2009). A CPP study using thioperamide/aspirate activates (Suzuki et al., 1996), diphenhydramine induces cocaine CPP with a cocaine dose that is not reinforcing alone (Nguyen et al., 2010) in mice. However, this effect probably is caused by the unspecific effect of diphenhydramine on dopamine transporter (Tanda et al., 2008). It is noteworthy that histamine receptor triple knockout mice (H1, H2, H3 KO) do not differ from wild-type animals in place preference induced by methamphetamine (Okuda et al., 2009).

Studies using histamine receptor ligands have resulted in variable findings concerning the behavioral effects of abused drugs. Similar to what was shown in rats with first-generation H3R antagonists (Suzuki et al., 1996), diphenhydramine induces cocaine CPP with a cocaine dose that is not reinforcing alone (Kamei et al., 2003) even though ebastine does not affect dopamine uptake (Fujisaki et al., 2002). Also histamine H2 receptor antagonists can enhance reward in mice as was shown in the study by Suzuki et al. (1995) with zolantidine in combination with morphine.

Studies concerning the role of H2R in the effects of drugs of abuse are contradictory, because methamphetamine (Clapham and Kilpatrick, 1994; Morisset et al., 2002; Fox et al., 2005) and alcohol stimulation are decreased by the H2R antagonist but cocaine-induced hyperactivity is potentiated by H2R inactivation (Brabant et al., 2006, 2009). One reason for the different results can be the ability of thioperamide to inhibit cocaine metabolism and increase plasma concentrations of cocaine (Brabant et al., 2009). A CPP study using thioperamide showed that H2R inactivation resulted in cocaine reward with a low dose of cocaine that is not rewarding alone (Brabant et al., 2006). In agreement, we showed that ethanol-induced CPP was stronger in response to ciproxifan (H2R antagonist/inverse agonist) pretreatment (Nuutinen et al., 2010). In contrast, H2R KO mice were not different from the wild-type mice in the methamphetamine-induced reward.

Although the CPP responses differ for ethanol and cocaine, the HDC KO mice show decreased locomotor stimulation in response to ethanol (Nuutinen et al., 2010) and cocaine (Brabant et al., 2007). These findings suggest that histamine is needed for the acute stimulation by drugs of abuse, whereas the reward and reinforcement might be inhibited by neuronal histamine. Histamine’s inhibitory role in drug reward is further supported by the stronger methamphetamine-induced CPP in H1R KO mice (Takino et al., 2009). It is noteworthy that histamine receptor triple knockout mice (H1, H2, H3 KO) do not differ from wild-type animals in place preference induced by methamphetamine (Okuda et al., 2009).

Fig. 2. Lack of histamine synthesis leads to alterations in responses to alcohol. A, male HDC KO mice are not stimulated by acute alcohol (1.5 g/kg i.p.). Data represent cumulative distance moved within 30 min. *** p < 0.001 from the baseline of WT control mice. B, alcohol-induced reward is stronger in HDC KO mice than in WT control animals. An unbiased, counterbalanced CPP paradigm was used where each animal received four 5-min conditioning trials with alcohol and saline on alternating days. Grid + stands for the conditioning subgroup where a metal grid floor was paired with alcohol (2 g/kg i.p.) administration. In the Grid – group plastic floor material was paired with alcohol injection. Place preference was indexed by comparing the Grid + and Grid – groups. *** p < 0.001 from the corresponding Grid + group. Two-way analysis of variance yielded a significant conditioning subgroup × genotype interaction, indicating that the alcohol preference in HDC KO mice was stronger than in control animals.
although they were less stimulated by methamphetamine than the control animals (Okuda et al., 2009). Altogether, these findings suggest a role for histamine receptors, especially for H₃Rs in the regulation of behavioral effects of drugs of abuse. The H₃R has a multifunctional role that includes the control of not only histamine release but also the release of several other neurotransmitters and the recently described interaction of postsynaptic H₃Rs with dopamine receptors (Ferrada et al., 2008, 2009). Thus, more studies are needed to clarify the actual role of histamine in addictive behaviors and the degree the effects seen with the H₃R ligands are caused by modulation of dopamine neurotransmission and signaling. In this respect, and in chemical nature and pharmacological properties, the different H₃R ligands (Table 3) have significantly different properties.

The studies described above have used either the CPP paradigm or measured activation of locomotion by drugs of abuse. Thus, it would be important to get data from self-administration paradigms to better understand the role of histamine in addiction. Also it is worth noting that an acute hyperactive response is not a direct measure of reinforcement and reward drug. Indeed, findings from our laboratory using HDC KO mice (Nuutinen et al., 2010) and in DARPP-32 (dopamine cAMP-regulated phosphoprotein of 32,000 kDa), the key dopamine signaling molecule, knockout mice (Ris-inger et al., 2001) suggest that alcohol CPP and stimulatory response are dissociated. Thus a combination of many behavioral methods examining different aspect of reinforcement and other addictive behaviors are needed.

Are the Models Relevant for Human Alcoholism?

The basic structure and organization of the histaminergic system with all neurons in the posterior hypothalamic tuberomamillary nucleus in human brain are very similar to that of all other studied mammals (Airaksinen et al., 1991) (Fig. 3). Very little is known about the functions of brain histamine in human alcoholics and addicts. However, in one study on the postmortem brains of type 1 alcoholics (later onset, often females, low degree of association with violence) and type 2 alcoholics (early onset, often males, high degree of association with violence) histamine levels were significantly higher in cortical gray matter of type 1 alcoholics than in normal control brains (Alakärppä et al., 2002). The levels of the first metabolite, tele-methylhistamine, were significantly increased in type 2 alcoholics, indicating increased histamine release and turnover (Alakärppä et al., 2002). This may mean that histamine synthesis and/or metabolism are primarily altered in alcoholics, or that the possibly associated liver pathology lies behind the abnormal findings. Indeed, histamine concentration is increased 4-fold in the caudate-putamen and significantly in the cortical regions of patients with hepatic encephalopathy, and tele-methylhistamine levels are

Table 3

Examples of brain-penetrating H₃R ligands

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Mechanism of Action</th>
<th>Human pKᵢ</th>
<th>Rat pKᵢ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imetit</td>
<td>Agonist</td>
<td>pKᵢ = 9.2–9.7</td>
<td>pKᵢ = 9.4</td>
<td>Ireland-Denny et al., 2001; Schnell et al., 2010</td>
</tr>
<tr>
<td>Immepip</td>
<td>Partial agonist</td>
<td>pKᵢ = 9.6</td>
<td>pKᵢ = 9.0</td>
<td>Ireland-Denny et al., 2001</td>
</tr>
<tr>
<td>R-α-methylhistamine</td>
<td>Agonist</td>
<td>pKᵢ = 8.9–9.2</td>
<td>pKᵢ = 8.6–8.7</td>
<td>Ireland-Denny et al., 2001; Schnell et al., 2010</td>
</tr>
<tr>
<td>Antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Imidazole-based</td>
<td></td>
<td></td>
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<tr>
<td>Ciproxifan</td>
<td>Inverse agonist</td>
<td>pKᵢ = 7.0</td>
<td>pKᵢ = 8.9–9.2</td>
<td>Ireland-Denny et al., 2001; Schnell et al., 2010</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>Inverse agonist</td>
<td>pKᵢ = 7.1–7.3</td>
<td>pKᵢ = 7.9–8.1</td>
<td>Ireland-Denny et al., 2001; Schnell et al., 2010</td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>Inverse agonist</td>
<td>pKᵢ = 9.1–9.3</td>
<td>pKᵢ = 9.1–9.4</td>
<td>Ireland-Denny et al., 2001; Schnell et al., 2010</td>
</tr>
<tr>
<td>Nonimidazole compounds</td>
<td></td>
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<tr>
<td>Pitolisant (BP2.649)</td>
<td>Inverse agonist</td>
<td>IC₅₀ = 5.3 nM</td>
<td>Kᵢ = 17 nM</td>
<td>Ligneau et al., 2007</td>
</tr>
<tr>
<td>ABT-239</td>
<td>Inverse agonist</td>
<td>pKᵢ = 9.4</td>
<td>pKᵢ = 8.9</td>
<td>Cowart et al., 2004; Esbenshade et al., 2005</td>
</tr>
<tr>
<td>JNJ-10181457</td>
<td>Neutral antagonist</td>
<td>pKᵢ = 8.1</td>
<td>pKᵢ = 8.2</td>
<td>Bonaventure et al., 2007</td>
</tr>
<tr>
<td>VUF-5681</td>
<td>Neutral antagonist</td>
<td>pKᵢ = 8.4</td>
<td>pKᵢ = 8.4</td>
<td>Kitbunnadaj et al., 2003</td>
</tr>
</tbody>
</table>

pKᵢ = −logKᵢ, the greater the pKᵢ value → the higher the affinity toward H₃Rs.

BP2.649, 1-[3-[4-(4-chlorophenyl)propoxy]propyl]piperidine hydrochloride; ABT-239, 4-[2-(2-hydroxyethyl)-5-methylpyrrolo[2,3-b]pyridin-1-yl]benzonitrile; JNJ-10181457, 4-[3-(4-piperidin-1-ylbut-1-ynyl)benzyl]morpholine; VUF-5681, 4-[3-(1H-imidazol-4-yl)propyl]piperidine dihydrobromide.
also increased, suggesting increased histamine turnover, whereas H\(_2\)R radioligand binding is decreased (Lozeva et al., 2003). A concomitant increase in densities (\(B_{\text{max}}\)) of H\(_3\)R in the frontal cortex has also been reported in patients with hepatic encephalopathy (Lozeva et al., 2001). Similar increases in brain histamine have been reported in portocaval shunted rats (Fogel et al., 2002). Taken together, changes in brain histamine, H\(_3\)R, and H\(_2\)R are found in patients with hepatic failure, and these changes coincide with sleep disturbances, abnormal circadian rhythm, and other neuropsychiatric disturbances. These findings, although they suggest that histaminergic drugs may be useful in treatment of alcohol-related disorders associated with hepatic failure, do not imply that histamine alone would be necessary or essential. Alterations in brain circuits containing multiple transmitters relevant for those in alcohol addiction, including the hypothalamo-cortical or hypothalamo-striatal/accumbal pathways may be important.

**Authorship Contributions**

**Participated in research design:** Panula and Nuutinen.

**Conducted experiments:** Panula and Nuutinen.

**Performed data analysis:** Panula and Nuutinen.

**Wrote or contributed to the writing of the manuscript:** Panula and Nuutinen.

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