Identification of the antiarrhythmic drugs amiodarone and lorcainide as potent H3 histamine receptor inverse agonists

Andria L. Del Tredici1,2, Jian-Nong Ma1, Fabrice Piu1,3, and Ethan S. Burstein1,#

1ACADIA Pharmaceuticals Inc., 11085 Torreyana Road, Suite 100, San Diego CA 92121

2Current address: Millennium Laboratories, 16981 Via Tazon, San Diego, CA 92127

3Current address: Otonomy Inc., 6275 Nancy Ridge Drive, Suite 100, San Diego, CA 92121

#corresponding author
Running title: Antiarrhythmic drugs as H3 receptor inverse agonists

To whom correspondence should be addressed:

Ethan S. Burstein

Address: ACADIA Pharmaceuticals Inc., 11085 Torreyana Road, Suite 100, San Diego CA 92121

Email: eburstein@acadia-pharm.com

Phone: 858-320-8623

Fax: 858-558-2872

Text pages: 13

Tables: 5

Figures: 5

References: 45

Abstract: 166

Introduction: 492

Discussion: 1201

Abbreviations: GPCR (G-protein coupled receptor), R-SAT™, Receptor Selection and Amplification Technology

Recommended section assignment: Drug Discovery and Translational Medicine
ABSTRACT

Using molecular pharmacology to re-profile older drugs discovered before the advent of recombinant technologies is a fruitful method to elucidate mechanisms of drug action, expand understanding of structure-activity relationships between drugs and receptors, and in some cases, repurpose approved drugs. The H3 histamine receptor is a G-protein coupled receptor (GPCR) primarily expressed in the central nervous system where among many things it modulates cognitive processes, nociception, feeding and drinking behavior and sleep/wakefulness. In binding assays and functional screens of the H3 histamine receptor, the antiarrhythmic drugs Lorcanide and Amiodarone were identified as potent, selective antagonists/inverse agonists of human and rat H3 histamine receptors, with relatively little or no activity at over 20 other monoamine GPCRs including H1, H2, and H4 receptors. Potent antagonism of H3 receptors was unique to Amiodarone and Lorcanide out of 20 antiarrhythmic drugs tested, representing six pharmacological classes. These results expand the pharmacophore of H3 histamine receptor antagonist/inverse agonists and may explain, in part, the effects of Lorcanide on sleep in humans.
INTRODUCTION

The existence of a third histamine receptor subtype was first proposed in 1983 based on the observation that histamine could inhibit its own release from neurons in cortical brain slices with a pharmacology that deviated from known H1 and H2 receptor pharmacology (Arrang et al., 1983). Subsequent discovery of H3-selective ligands (Arrang et al. 1987), followed by the cloning of the H3 receptor (Lovenberg et al., 1999) has greatly facilitated research into the physiological functions of this receptor.

The H3R couples to Gαi G-proteins to inhibit cyclic AMP production, and is expressed primarily in the central nervous system (Lovenberg et al., 1999) where it acts as an autoreceptor, controlling histamine release as well as the release of other neurotransmitters including acetylcholine, dopamine, gamma amino butyric acid, 5-hydroxytryptamine and peptides (Haas et al., 2008). The H3R undergoes extensive splicing to produce a number of distinct isoforms (Bakker, 2004), which have distinct pharmacological properties (Coge et al., 2001; Wellendorph et al, 2002). The H3R displays a high degree of constitutive activity in vivo and this tonic activity is likely an important feature contributing to its control of neurotransmitter release (Morisset et al., 2000). For example, reduction in the tonic activity of H3 autoreceptors increases histamine release that stimulates postsynaptic H1 histamine receptors, leading to increased wakefulness. Due to its role in modulating the activities of many important neurotransmitter systems, ligands that modulate H3R activity affect central nervous system control of cognitive processing, nociception, feeding and drinking behavior and sleep/wakefulness. H3 histamine receptor antagonists/inverse agonists are being tested clinically as therapeutic agents to treat narcolepsy, cognitive impairment in Alzheimer’s disease, schizophrenia, and obesity, among others (Passani and Blandina, 2011).
Lorcainide and Amiodarone are two structurally, and pharmacologically distinct antiarrhythmic drugs. Lorcainide is a Class 1C antiarrhythmic that works by blocking open fast acting voltage-gated sodium channels (subtype Nav1.5), slowing the upstroke of ventricular myocyte action potential (Amery et al, 1981). Lorcainide is used to treat patients with premature ventricular contractions and Wolff-Parkinson-White syndrome (Samánek et al., 1987). Disturbed sleep is a prominent side-effect of Lorcainide usage (see Eiriksson and Brogden, 1984]. Amiodarone is a Class III antiarrhythmic that prolongs the repolarization phase of the cardiac action potential by blocking potassium channels (Rosenbaum et al., 1983). Although Amiodarone is an effective antiarrhythmic agent, it has side effects on a number of organ systems, limiting its use (Santangeli et al., 2012).

To better understand the molecular basis for the clinical actions of drugs, we have been systematically screening and profiling collections of clinically used compounds for activity in functional assays using heterologously expressed GPCRs. Using a cellular proliferation assay called R-SAT™ (Burstein et al., 2006), we identified lorcainide and amiodarone as potent H3 histamine receptor antagonists; activity that was unique to these two agents among a diverse collection of antiarrhythmic drugs tested. Further profiling revealed both agents display inverse agonist activity at certain H3 receptor isoforms, as well as surprising selectivity for H3 receptors over other GPCRs.
MATERIALS AND METHODS

Materials: NIH-3T3 cells (CRL 1658) and human embryonic kidney 293T (HEK-293T, CRL 11268) cells were purchased from American Tissue Culture Collection. O-nitrophenyl-β-D-galactopyranoside and nonidet P-40 were from Sigma. Tissue culture media used was Dulbecco’s modified Eagles medium (DMEM) (Gibco-BRL) 96-well tissue culture dishes were from Falcon. Hanks balanced salt solution without magnesium chloride, magnesium sulfate, and calcium chloride, Trypsin-EDTA were all from Gibco-BRL.

Drugs: All compounds were solubilized as 10 mM stock solutions in DMSO, and subsequently diluted into working solutions with the appropriate buffer. In no case was the DMSO concentration greater than 0.5% at the top concentration of any working solution, a DMSO concentration that we have found does not affect assay performance. Working dilutions were made in the appropriate assay buffers. All compounds were obtained from Tocris (Bristol, United Kingdom) or Sigma/RBI (St. Louis, MO).

Cell culture: NIH-3T3 cells were incubated at 37 °C in a humidified atmosphere (5% CO2) in DMEM supplemented with 4500-mg/l glucose, 4 nM L-glutamine, 50 U/ml penicillin G, 50 U/ml streptomycin (PSG, HyClone from Fisher Scientific Logan, UT) and 10% calf serum. HEK-293T cells were incubated at 37°C in a humidified atmosphere (5% CO2) in Dulbecco’s modified Eagles tissue culture medium with the same supplements used for NIH 3T3 cells except plus 10% Fetal calf serum was used instead of calf serum.

Constructs: The human D2 (short form), 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT2C(vgv), 5-HT7, alpha1A, alpha1B, alpha2A, alpha2B, alpha2C, M1, M2, M3, M4, M5, H1, and H3 (isoforms 1, 2 and 4) receptors have been described previously (Burstein et al, 2011; Wellendorph et al, 2002). The human H2 and H4 receptors and the rat
ortholog of isoform 1 of the human H3 receptor were cloned by PCR. All clones were subcloned into the pSI vector (Promega Corp., Madison, WI) and sequence verified before use.

Cellular Proliferation Assays: Receptor Selection and Amplification Technology (R-SAT™) assays were performed as described (Burstein et al., 2011). The data for human and rat H3 receptors, and human H2 and H4 receptors were generated as follows: Cells were plated one day before transfection using 7x10^3 cells in 0.1 ml of media per well of a 96-well plate. Cells were transiently transfected with 1 to 10 ng/well of receptor DNA, and 30 ng/well pSI-β-galactosidase (Promega, Madison, WI) per well of a 96-well plate using Polyfect (Qiagen, Valencia, CA) according to the manufacturer’s instructions. In addition, “helper” DNAs encoding accessory proteins were transfected including 20 ng/well ras/rap1B(AA), 2 ng/well adenyl cyclase 2, 4 ng/well of Gqi5 (for H3 and H4 receptors only), 0.8 ng/well of Grk2 and 6 ng/well RGS1 (Burstein et al, 2006; 2011). One day after transfection media was changed and cells were combined with ligands in DMEM supplemented with 25% Ultraculture synthetic supplement (Cambrex, Walkersville, MD) instead of calf serum to a final volume of 200 ul/well. After five days in culture β-galactosidase activity was measured and responses quantified on a plate-reader (Bio-Tek EL 310 or Molecular Devices). The data for 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, 5-HT1F, 5-HT2A, 5-HT2B, 5-HT2C(vgy), 5-HT7, alpha1A, alpha1B, alpha2A, alpha2B, alpha2C, M1, M2, M3, M4, M5 and H1 receptors was generated using a similar method using cells grown in larger volumes (632 cm^2 cell factory flasks, Nalgene Nunc International, Rochester, NY), transfected, and frozen at -135°C until use as described previously (Burstein et al., 2011).

Cyclic AMP Inhibition Assays: Cyclic AMP Inhibition assays were conducted using human H3 cAMP Hunter™ kits (DiscoveRx, Fremont, CA) according to the manufacturer’s
instructions using 96 well plates. For agonist/inverse agonist assays, forskolin (20 µM final concentration) was added and the plates incubated for another 30 min at 37°C. For functional antagonist assays, R-α-methyl histamine (3 nM, the EC₈₀ concentration) was included with the forskolin. The plates were read on a TopCount NXT (PerkinElmer).

**Membrane preparations:** Membranes were made as previously reported (Burstein et al., 2011) with the following modifications: HEK-293T cells were seeded at 13.5×10⁶ cells per 15 cm dish and were transfected 24 hrs later by mixing 11 ug of DNA in 900 ul DMEM, adding 33 ul FuGENE (Roche Applied Science, Indianapolis, IN) dropwise, incubating the mixture for 15 minutes at room temperature, and adding it to the plate. Cells were not centrifuged following cell scraping, but were collected directly into the ice-cold nitrogen cavitation chamber.

**Radioligand binding assays:** Membranes were prepared as described above. For binding assays, membranes expressing H3 (5 ug/well) or H4 (10 ug/well) were incubated with 0.5 nM [³H]-R-α-methyl histamine ([³H]-RAMH) for H3 or 10 nM [³H]-Histamine for H4 for 1 h at room temperature in binding buffer 1 (50 mM Tris, 10 mM MgCl₂, 1 mM EDTA, 0.1% BSA, pH 7.4). Membranes expressing M1 (5 µg/well), M2 (5 µg/well), M3 (8 µg/well), M4 (8 µg/well), and M5 (4 µg/well), were incubated with 0.4 nM [³H]-N-methylscopolamine (0.5 nM for M5) for 2 h at room temperature in binding buffer 2 (25 mM sodium phosphate, 5 mM MgCl₂, 0.1% BSA, pH 7.4). Binding reactions were terminated by filtration through UniFilter-96 GF/B filters (from Perkin-Elmer, presoaked with 0.1% polyethylenimine for 1 h) with a Brandel 96-well harvester. Filters were washed with ice-cold wash buffer (25 mM HEPES, 250 mM NaCl, 1 mM CaCl₂, 5 mM MgCl₂, pH 7.4; 500 ml/plate) and then allowed to air-dry for overnight. 50 µl MacrosScint-20 scintillation cocktail was added to each well, the plates were sealed, and then counted 3 h later on a TopCount NXT (PerkinElmer).
Data analysis: Agonist curves from R-SAT™, cyclic AMP, and radioligand binding experiments were fitted to a sigmoidal dose-response function: $Y = B + \frac{(T-B)}{1+10^{(\text{LogEC50-LogX)}*H}}$ where $Y$ is the response, $B$ is the baseline, $T$ is the top or maximum response, $H$ is the slope of the curve (Hill Slope), and $X$ is the concentration of ligand. For R-SAT™ and cyclic AMP assays $H$ was set to 1. Calculation of $K_i$ values in the functional antagonist assays was performed using the Cheng-Prusoff method (Cheng and Prusoff, 1973). All data analysis was performed using GraphPad Prism version 4.0 (San Diego, CA).
RESULTS

We have developed a high-throughput cellular proliferation assay that is compatible with most G-protein coupled receptors and that detects constitutive receptor activity with high sensitivity (R-SAT™; Receptor Selection and Amplification Technology, Burstein et al., 2006). To identify novel small molecule ligands, R-SAT™ was used to screen the human H3 histamine receptor against a collection of more than 200,000 compounds. The compound library included products of combinatorial chemical synthesis, medicinal chemistry synthesis, and more than 2000 clinically used drugs. A large number of novel antagonists and inverse agonists at the H3 receptor were identified which will not be described here.

Among the “hits” identified were two antiarrhythmic drugs, the Class 1C sodium channel blocker lorcainide, and the Class 3 potassium ion channel blocker amiodarone (Figure 1). Full concentration-response experiments run with H3 receptors demonstrated that both compounds were potent inverse agonists at human histamine receptors (Figure 2, Table 1). The inverse agonist activities of both compounds were most apparent at isoform 2, and less so at the other isoforms tested. The high constitutive activity of isoform 2 of the human H3 receptor is consistent with a previous report (Bongers et al., 2007). Interestingly, the H3 receptor antagonist clobenpropit displayed partial agonist activity, especially at isoforms 2 and 4.

Full concentration-response experiments run with H3 receptors in the presence of histamine demonstrated that amiodarone and lorcainide were potent functional antagonists at H3 histamine receptors (Figure 3). Compared to H3 receptor reference compounds, both lorcainide and amiodarone were more potent than thioperamide and less potent than clobenpropit or iodophenpropit (Table 1). Clobenpropit only partially suppressed histamine-induced activation of isoform 2, consistent with its partial agonist activity at that isoform. The potencies of
amiodarone and lorcainide were similar at isoform 1 of the human H3 receptor and its rat ortholog. In contrast, thioperamide and clobenpropit were approximately 10-fold more potent at rat H3 receptors, consistent with previous reports (Ligneau et al., 2000; Yao et al., 2003; Stark et al., 2001).

H3 receptors couple to Ga1 G-proteins and inhibit production of cyclic AMP (Lovenburg et al., 1999). Therefore to confirm results obtained using R-SAT™, cyclic AMP assays were run. Both amiodarone and lorcainide were each able to block histamine-induced cyclic AMP inhibition more potently than thioperamide (Figure 4A, Table 2). Similar to the R-SAT™ results, both lorcainide and amiodarone displayed inverse agonist activity, apparent as increases in cyclic AMP levels above baseline (Figure 4B, Table 2). Clobenpropit also had inverse agonist activity in cyclic AMP assays, despite showing partial agonist activity in the cellular proliferation assays.

Recently amiodarone was reported to bind allosterically to muscarinic acetylcholine receptors (Stahl et al., 2010; 2011). We therefore tested lorcainide and amiodarone in radioligand binding assays to H3 and H4 histamine receptors, and all five subtypes of muscarinic acetylcholine receptors. Both lorcainide and amiodarone were able to fully displace [3H]-NAMH from both human and rat H3 receptors (Figure 5). In contrast, at H4 receptors, only amiodarone could completely displace the radioligand, and only at much higher concentrations. Similarly, neither drug was able to fully displace [3H]-NMS from any of the muscarinic receptor subtypes. Amiodarone and lorcainide were slightly more potent at human H3 receptors in the binding assays, whereas the reference antagonists, especially thioperamide, were more potent at rat H3 receptors (Table 3).
We profiled amiodarone and lorcainide in R-SAT™ at a collection of 23 other monoaminergic GPCRs, including the other histamine receptor subtypes, the five muscarinic receptor subtypes, the D2 dopamine receptor, five alpha adrenergic receptor subtypes and nine serotonergic receptor subtypes (Table 4). The profiles were conducted at each receptor alone (agonist/inverse agonist mode), and in the presence of the relevant reference agonist for each receptor (antagonist mode). Weak agonist activity of amiodarone at H2 histamine receptors, and weak antagonist activity of amiodarone at M2 muscarinic and 5-HT2B serotonin receptors and lorcainide at M2 muscarinic receptors was observed. None of these interactions at other GPCRs for either amiodarone or lorcainide were comparable in terms of potency to their interactions with H3 histamine receptors.

The potent interactions with H3 receptors were unique to amiodarone and lorcainide among the antiarrhythmic drugs we tested. There were no significant interactions with H3 receptors seen for 18 other compounds spanning the six major pharmacological classes of antiarrhythmic drugs (Table 5). Recently dronedarone was developed to be a safer alternative to amiodarone (Adlan and Lip, 2013), but despite its structural similarity to amiodarone, had little to no affinity for H3 receptors (Table 5).
DISCUSSION

We have used a high-throughput functional screen to identify the antiarrhythmic drugs lorcainide and amiodarone as potent H3 histamine receptor inverse agonists. Using two different functional assays and radioligand binding assays, we showed that lorcainide and amiodarone could block histamine and R-α methyl histamine induced functional responses, reduce basal or constitutive activity of H3 receptors, and displace [3H]-N-α-methyl histamine binding to H3 histamine receptors. In contrast, none of the 17 other antiarrhythmic drugs tested showed any appreciable activity at H3 receptors. The antagonist/inverse agonist actions of lorcainide and amiodarone at H3 receptors were more potent than the reference H3/H4 antagonist thioperamide, and selective for H3 receptors, with little or no activity at 23 other GPCRs tested. Amiodarone has previously been shown to directly activate G-proteins (Hagelüken et al., 1995), but at micromolar concentrations, much higher than the antagonist potencies described herein. The potencies of amiodarone and lorcainide were similar or slightly higher at isoform 1 of the human H3 receptor compared to the rat ortholog of isoform 1 in both functional assays and radioligand binding assays, in contrast to the reference ligands thioperamide, iodophenpropit and clobenpropit which were each approximately 3 to 10-fold more potent at rat H3 receptors. The inverse agonist activities of amiodarone and lorcainide were most apparent at isoform 2, which has an 80 amino acid deletion in intracellular loop 3 (i3 loop), and had the greatest level of constitutive activity of the isoforms tested herein. The inverse agonist activities of amiodarone and lorcainide were least apparent at isoform 4, which in addition to the 80 amino acid i3 loop deletion, also has a much shorter, alternatively spliced c-terminus compared to isoform 1 and isoform 2 (see Wellendorph et al., 2002).
Compared to their potencies at their ‘targets’, the potencies of amiodarone and lorcanide at H3 histamine receptors are remarkably high. For example, amiodarone binds to human ether-a-go-go related protein (hERG) channels with an affinity of 220 nM (Waldhauser et al., 2008), displaces $[^3]$H-nitrendipine binding to guinea pig cerebral cortex membranes with an IC$_{50}$ of 97 nM (Nokin et al., 1986), inhibits fast inward Na$^+$ currents in human atrial cells at 3 to 30 µM concentrations (Lalevée et al., 2003), and inhibits L-type Ca$^{2+}$ channels expressed in Xenopus oocytes at 10 µM concentrations (Ding et al., 2001). Similarly, 200 µM lorcanide displaces approximately 50% of $[^3]$H-ouabain binding to the Na$^+$-K$^+$ ATPase in guinea pig heart microsomes (Abdulrahman et al., 1999). In contrast, we observed potencies ranging from 100 nM to as low as 3 nM for amiodarone and lorcanide at H3 histamine receptors across the various functional and binding assays examined herein. While making direct comparisons between the H3 receptor data and the aforementioned experiments with various ion channels may be difficult due to differing experimental conditions, it is clear that the potencies of amiodarone and lorcanide at H3 receptors are least as high, if not higher, than their potencies at their therapeutic targets.

As therapeutic agents, lorcanide and amiodarone are both used to treat cardiac arrhythmias, but their specific therapeutic uses, mechanisms of action, and attendant side effects are quite different. Amiodarone is a Class III antiarrhythmic that prolongs the repolarization phase of the cardiac action potential by blocking potassium channels. Amiodarone has also been called a ‘broad spectrum’ antiarrhythmic drug with actions that are similar to those of antiarrhythmic classes Ia, II, and IV including beta blocker-like effects, and effects on sodium channels (Punnam et al, 2010). The beta-blocker effects may be due to downregulation of beta adrenergic receptors, and not a consequence of direct receptor-ligand interaction (Drvota et al.,
1999), although low affinity (micromolar) interactions with beta receptors have been reported (Schnabel et al., 1999). We observed no significant interactions with beta adrenergic receptors (unpublished observations). Amiodarone causes a number of side effects, particularly on thyroid function due to its chemical similarity to thyroxine, possibly by antagonizing T3 binding to thyroid hormone receptor (Drvota et al, 1995). In addition, amiodarone causes significant peripheral organ toxicity, including liver toxicity, corneal deposits, pulmonary fibrosis and peripheral neuropathies, but no significant central nervous system (CNS) side effects (Latini et al., 1984; Santangeli et al., 2012). The peripheral side effects and lack of CNS side effects are consistent with the known tissue distribution of amiodarone to liver, lung, heart and adipose tissue but limited uptake into brain (Riva et al., 1982).

Lorcainide (Lorcainide hydrochloride) is a Class 1c antiarrhythmic agent that works by blocking open fast acting voltage-gated sodium channels (subtype Nav1.5) (Amery et al., 1981; Eiriksson and Brogden, 1984). Lorcainide is used to help restore normal heart rhythm and conduction in patients with premature ventricular contractions, ventricular tachycardia and Wolff-Parkinson-White syndrome (Amery et al., 1981; Eiriksson and Brogden, 1984; Winkle et al., 1984). Compared to amiodarone, lorcainide distributes better to the brain (Klotz et al., 1980) and lorcainide usage is associated with an increased prevalence of central nervous system effects, especially disturbed sleep (see Eiriksson and Brogden, 1984). Interestingly, insomnia is a reported side effect of H3 antagonists in humans (Herring et al., 2012; Othman et al., 2013), and H3 antagonists are in development as treatments for narcolepsy (Inocente et al., 2012). Possibly, the H3 antagonist activity of Lorcainide contributes to its effects on sleep in humans.

Pharmacophores for H3 antagonists have been studied extensively (Singh and Jadhav, 2013; Lebois et al., 2011; Berlin et al., 2011). Structurally, it is possible to rationalize the H3
antagonist/inverse agonist activities of lorcainide, and amiodarone. The classic H3 ligands, both agonists (e.g. N-α-methylhistamine and R-α-methylhistamine) and antagonists (e.g. thioperamide and clobenpropit), contain an imidazole functionality (see Figure 1). However new pharmacophores for H3 antagonists in which the imidazole is replaced by other basic groups are now common. Typically, the basic groups are cyclic amines (e.g. piperidines or pyrrolidines). Certain structural fragments such as alkylamino-alkoxyphenyl motifs (most frequently dialkylamino-propanoxyphenyl) are found in numerous series (see Figure 1 for examples). The diethylamino-ethoxy-diiodophenyl fragment of amiodarone is consistent with this theme, with the ethoxyphenyl spacing a novel variation compared to many of the recently described H3 antagonists. The more linear nature of amiodarone is also reminiscent of both classic and recent examples of H3 antagonists. The diodo substitutions in amiodarone are unique, and not seen in any other H3 antagonists however interestingly, the marine natural product and H3 antagonist aplysamine contains dibromo substitutions in analogous positions (see Figure 1). Similarly, the isopropyl-aminopiperidine moiety in lorcainide fits the pattern found in many recent examples of H3 antagonists.

It is interesting to speculate whether or not the H3 receptor antagonist activities of lorcainide or amiodarone contribute to their antiarrhythmic properties. Functional H3 histamine receptors are expressed in sympathetic nerve endings in human atria where they have been shown to reduce norepinephrine (NE) release, such as occurs during ischemia/reperfusion injury (Imamura et al., 1996). H3 knockout mice show enhanced norepinephrine release in an ischemic heart model and as a consequence, increased severity of cardiac arrhythmia (Koyama et al., 2003). These data suggest a cardioprotective role for H3 receptor agonists rather than antagonists, and so the H3 antagonist activities of lorcainide and amiodarone probably do not
contribute to their therapeutic effects. This is consistent with the observation that no other antiarrhythmic drug tested had H3 receptor antagonist activity.
Acknowledgements: We thank Uli Hacksell for critical reading of the manuscript.

Authorship Contributions:

Participated in research design: Del Tredici, Piu, Burstein.

Conducted experiments: Del Tredici, Ma.

Performed data analysis: Del Tredici, Ma, Burstein.

Wrote the manuscript: Burstein.
REFERENCES


Cheng Y, Prusoff WH. (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol. 22:3099-3108.


Drvota V, Carlsson B, Häggblad J, Sylvén C. (1995) Amiodarone is a dose-dependent noncompetitive and competitive inhibitor of T3 binding to thyroid hormone receptor subtype beta 1,
whereas disopyramide, lignocaine, propafenone, metoprolol, dl-sotalol, and verapamil have no inhibitory effect. *J Cardiovasc Pharmacol*. **26**:222-226.


Figure Legends

Figure 1. Structures of amiodarone, lorcainclide, and other H3 antagonists. The other H3 antagonists shown have been discussed (see Singh et al., 2013; Lebois et al., 2011; Berlin et al., 2011).

Figure 2. Profile of H3 receptor ligands in cellular proliferation assays. Cellular proliferation assays (R-SAT™) were run as described in the methods using A) human H3 receptor isoform 1; B) human H3 receptor isoform 2; C) human H3 receptor isoform 4; and D) rat H3 receptors together with the indicated concentrations of R-α-methyl histamine (RAMH, circles); histamine (open circles), clobenpropit (diamonds), amiodarone (triangles) and lorcainclide (inverted triangles). Responses are normalized to the response of histamine with zero denoting the basal response in the absence of added ligands.

Figure 3. Functional antagonism of H3 receptors in cellular proliferation assays. Cellular proliferation assays (R-SAT™) were run as described in the methods using A) human H3 receptor isoform 1 and 500 nM histamine; B) human H3 receptor isoform 2 and 300 nM histamine; and C) rat H3 receptors and 500 nM histamine together with the indicated concentrations of thioperamide (open circles), clobenpropit (open diamonds), amiodarone (triangles) and lorcainclide (inverted triangles). Responses are normalized to the response of thioperamide.
Figure 4. Profile of H3 receptor ligands in cyclic AMP assays. Cyclic AMP inhibition assays were run as described in the methods using human H3 receptor isoform 1 with A) the indicated concentrations of thioperamide (open circles), amiodarone (triangles) and lorcanide (inverted triangles) together with fixed concentrations (3 nM) of R-α-methyl histamine (RAMH) or B) with the indicated concentrations of R-α-methyl histamine (RAMH, circles), clobenpropit (open diamonds), amiodarone (triangles) and lorcanide (inverted triangles) alone. Responses are normalized to the response of histamine with zero and the dashed line denoting the basal response in the absence of added ligands.

Figure 5. Binding of Amiodarone and Lorcanide to H3, H4 and muscarinic receptors. Radioligand binding studies were conducted as described in the methods using A) human H3 receptors (isoform 1) and [3H]N-α-methyl histamine, B) rat H3 receptors (ortholog to isoform 1) and [3H]N-α-methyl histamine; C) human H4 receptors and [3H]histamine, D) human M1 muscarinic receptors and [3H]N-methyl scopolamine, E) human M3 muscarinic receptors and [3H]N-methyl scopolamine, and F) human M5 muscarinic receptors and [3H]N-methyl scopolamine together with the indicated concentrations of amiodarone (triangles) and lorcanide (inverted triangles). Reference ligands (open circles) were A – B) histamine, C) thioperamide, and D – F) atropine. Responses were normalized to the reference ligands in each case.
Tables

**Table 1. Profile of H3 receptor ligands in cellular proliferation assays.** Cellular proliferation assays were run with human H3 receptors (isoform 1, 2 or 4) or rat H3 receptors (ortholog to isoform 1) as described in the methods using the indicated ligand alone (Agonist/Inverse Agonist), or in the presence of fixed concentrations of histamine (Antagonist). Agonist/Inverse agonist potency is reported as the negative logarithm of the EC50 (pEC50) ± SEM. Agonist/Inverse agonist efficacy is normalized to the efficacy of histamine. Antagonist potency is reported as the negative logarithm of the Ki (pKi) ± SEM. Antagonist inhibition is normalized to the inhibition of thioperamide.
Table 2. Profile of H3 receptor ligands in cAMP assays. Cyclic AMP assays were run as described in the methods. Agonist/inverse agonist assays were performed using the indicated ligand alone. Antagonist assays were performed in the presence of fixed concentrations (3 nM) of R-α-methyl histamine (RAMH). Agonist/Inverse agonist potency is reported as the negative logarithm of the EC50 (pEC50) ± SEM. Agonist/Inverse agonist efficacy is normalized to the efficacy of RAMH. Antagonist potency is reported as the negative logarithm of the Ki (pKi) ± SEM. Antagonist inhibition is normalized to the inhibition of thioperamide.
Table 3. Radioligand binding activity of amiodarone and lorcaïnide at histamine and muscarinic receptors. Shown are the average values for two or more independent experiments. Data were fitted using Prizm as described in the methods. Antagonist potency is reported as the negative logarithm of the IC50 (pIC50). The pIC50 values for the reference ligands were 6.8 for thioperamide at H4 receptors, and 8.0, 8.2, 7.9, 8.0 and 8.6 at M1-M5 receptors, respectively. The human H3 receptor was isoform 1 and the rat H3 receptor was the ortholog to isoform 1 of the human H3 receptor.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Human H3</th>
<th>Rat H3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>7.5 ± 0.2</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>Lorcaïnide</td>
<td>7.0 ± 0.2</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>Histamine</td>
<td>8.0 ± 0.2</td>
<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>Clobenpropit</td>
<td>8.8 ± 0.2</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td>Iodophenpropit</td>
<td>8.6 ± 0.0</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td>Thioperamide</td>
<td>6.5 ± 0.2</td>
<td>7.4 ± 0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Amiodarone</th>
<th>Lorcaïnide</th>
</tr>
</thead>
<tbody>
<tr>
<td>H4</td>
<td>5.4 ± 0.2</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>M1</td>
<td>&lt; 5.0</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>M2</td>
<td>&lt; 5.0</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>M3</td>
<td>&lt; 5.0</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>M4</td>
<td>&lt; 5.0</td>
<td>&lt; 5.0</td>
</tr>
<tr>
<td>M5</td>
<td>&lt; 5.0</td>
<td>&lt; 5.0</td>
</tr>
</tbody>
</table>
4. Profile of amiodarone and lorcainide at 23 other GPCRs in cellular proliferation assays. Cellular proliferation assays were run using amiodarone, lorcainide, and the indicated reference agonists and antagonists to obtain pEC\textsubscript{50} and Eff\% values, and Inh\% values, respectively. The antagonist assays evaluated the abilities of amiodarone, lorcainide or the relevant reference agonist to block responses to fixed concentrations (approximately the EC\textsubscript{80} concentration) of the relevant reference agonist potency is reported as the negative logarithm of the EC\textsubscript{50} (pEC\textsubscript{50}). Agonist efficacy is normalized to the efficac
avored reference agonists. Antagonist potency is reported as the negative logarithm of the Ki (pKi). Antagonist inhibited to the inhibition of the indicated reference antagonists. ND denotes not done.
Table 5. Profile of other antiarrhythmic drugs at human H3 histamine receptors. Cellular proliferation assays were run as described in the methods using the indicated ligand in the presence of fixed concentrations of histamine at human H3 histamine receptor isoform 1. Antagonist percent inhibition is normalized to the percent inhibition of thioperamide, and represents the percent inhibition at 10 µM, the highest concentration tested. (–) indicates the inhibition constant could not be estimated. *data generated in radioligand binding assays with H3 histamine receptor isoform 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Class</th>
<th>Mechanism</th>
<th>pKi</th>
<th>%inh</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disopyramide</td>
<td>IA</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Procainamide</td>
<td>IA</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>52</td>
</tr>
<tr>
<td>Quinidine</td>
<td>IA</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>63</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>IB</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>IB</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Mexiletine</td>
<td>IB</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Propafenone</td>
<td>IC</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Flecainide</td>
<td>IC</td>
<td>Na⁺ blocker</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Atenolol</td>
<td>II</td>
<td>Beta blocker</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>Propranolol</td>
<td>II</td>
<td>Beta blocker</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Acebutolol</td>
<td>II</td>
<td>Beta blocker</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>II</td>
<td>Beta blocker</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Nadolol</td>
<td>II</td>
<td>Beta blocker</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Sotalol</td>
<td>II/III</td>
<td>Beta / K⁺ blocker</td>
<td>-</td>
<td>27</td>
</tr>
<tr>
<td>Dronedarone</td>
<td>III</td>
<td>K⁺ blocker / others</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Verapamil</td>
<td>IV</td>
<td>Ca²⁺ blocker</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>IV</td>
<td>Ca²⁺ blocker</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>IV</td>
<td>Ca²⁺ blocker</td>
<td>-</td>
<td>17</td>
</tr>
</tbody>
</table>
Figure 1

amiodarone
lorcainide
aplysamine
RWJ-20085
JNJ-7737782
clobenpropit
JNJ-520752
#52 in Lebois et al., 2011
thioperamide
Figure 2

A. Isoform 1

B. Isoform 2

C. Isoform 4

D. Rat H3

- **RAMH**
- **Histamine**
- **Clobenpropit**
- **Amiodarone**
- **Lorcainide**
Figure 4