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Feature Article

Effects of Antipsychotic Drugs on I_{to}, I_{Na}, I_{sus}, I_{K1}, and hERG: QT Prolongation, Structure Activity Relationship, and Network Analysis

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Purpose. To evaluate *in vitro* and computationally model the effects of selected antipsychotic drugs on several ionic currents that contribute to changes in the action potential in cardiac tissue.
Methods. Fourteen antipsychotic drugs or metabolites were examined to determine whether QT interval prolongation could be accounted for by an effect on one or more myocardial ion channels [I_{to}, I_{Na}, I_{sus}, I_{K1}, and human ether-a-go-go related gene (hERG)]. Using the patch clamp technique, drug effects on these human cardiac currents were tested.
Results. All molecules had little inhibitory effect on ion channels (blocking at concentrations >5 μM) other than hERG. A significant correlation was observed between the estimated hERG blockade and the increase in corrected QT for five of the antipsychotics. Molecular modeling identified hydrophobic features related to the interaction with hERG and correctly rank-ordered the test set molecules olanzapine and its metabolites. A network analysis of ligand and protein interactions around hERG using MetaCore™ (GeneGo Inc., St. Joseph, MI, USA) was used to visualize antipsychotics with affinity for this channel and their interactions with other proteins in this database.
Conclusion. The antipsychotics do not inhibit the ion channels I_{to}, I_{Na}, I_{sus}, I_{K1} to any appreciable extent; however, blockade of hERG is a likely mechanism for the prolongation of the QT interval.

KEY WORDS: antipsychotics; cardiac ion channels; hERG; QT.

INTRODUCTION

Many different classes of drugs have been temporally associated with cardiac arrhythmia and sudden death. During drug therapy, alterations in the electrocardiogram (ECG) suggest changes in normal patterns seen prior to initiation of therapy or in unmedicated individuals. Several classes of drugs have been shown to prolong the QT interval of the ECG, which reflects a slowing of repolarization of the ventricular myocardium (1). Although the mechanism has not been definitively established, excessive prolongation of the QT interval can lead to the potentially life-threatening ventricular tachyarrhythmia, torsade de pointes. Several ionic currents (sodium, calcium, and potassium) contribute to changes in the action potential in ventricular myocardium, with each ion being involved at specific points in the action potential (Fig. 1). Therefore, one likely mechanism by which drugs can alter repolarization and prolong the QT interval is blockade of one or more cardiac ion channels.

In cardiac tissue, a reduction in flow through any of several potassium channels is associated with QT interval prolongation (2). Evidence for changes in potassium conductance causing prolongation of the QT interval are worth reiterating here as they come from a number of sources. The most common channel linked to drug-induced QT interval prolongation in humans is the one responsible for the rapid component of the delayed rectifier potassium current (I_{Kr}). The ionic basis of the action potential therefore represents basic physiological evidence, i.e., the identification of human ether-a-go-go-related gene (hERG) in 1995 (3) and that the I_{Kr} is a co-assembly of hERG A-subunits encoded by the *KCNH2* gene and MiRP1 B-subunits encoded by the *KCNE* gene. hERG, is believed to encode the protein that underlies the delayed rectifier potassium current I_{Kr}, one of the components driving ventricular repolarization and influencing the QT interval in human myocardium (3,4) (though there is some debate about this). Mutations in hERG have

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ABBREVIATIONS: hERG, human ether-a-go-go related gene; I_{K1}, the inwardly rectifying potassium current; I_{Na}, sodium current; I_{sus}, sustained potassium current; I_{to}, transient outward potassium current.

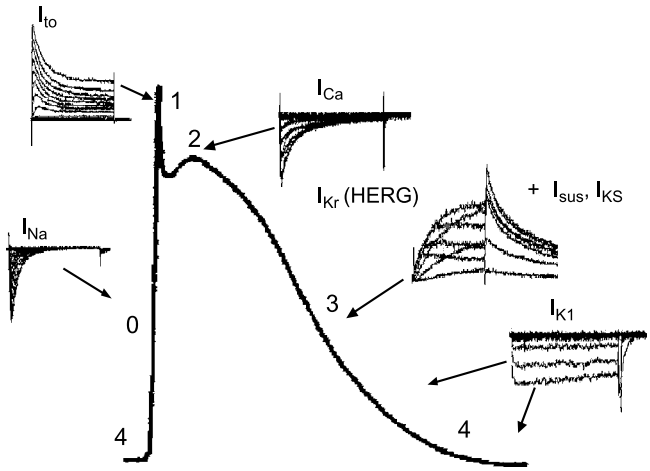

Fig. 1. Illustration of a recording of a human cardiac action potential and the currents that underlie each of its phases.

also been found to lead to one form of hereditary long QT syndrome, LTQ2 (5,6). Many drugs associated with QT interval prolongation have also been found to block hERG (7). The transgenic mouse model in which the transient outward potassium current (I_{to}) has been dramatically reduced or abolished has a markedly longer QT interval than wild-type animals (2). Similarly, genetically altered mice in which the sustained potassium current (I_{sus}) has been reduced also show prolongation of the QT interval (8).

The data collected from these studies suggest that congenital defects, genetic manipulations, or drug-induced changes in potassium conductance can prolong the QT interval and possibly lead to ventricular arrhythmias. The testing of drugs for QT effects both in clinical and nonclinical settings has resulted in the publication of several draft guidances including those from the CPMP (Committee of Proprietary Medicinal Products/986/96), ICH S7B (http://www.fda.gov/cder/guidance/5533dft.pdf), and ICH E14 (http://www.emea.eu.int/pdfs/human/ich/000204en.pdf), which are available to assist the understanding of this field.

The use of a number of antipsychotic agents has been associated with QT interval prolongation (9,10). The purpose of this current study was to characterize the human cardiac ion channel blocking profile of various antipsychotics, including clozapine, haloperidol, olanzapine, pimozide, risperidone, sertindole, thioridazine, ziprasidone, and selected antipsychotic metabolites. These drugs were examined to determine whether they blocked the current primarily responsible for depolarization of the action potential through the heart (sodium current or I_{Na}), the repolarizing potassium currents (transient outward potassium current, I_{to}), sustained potassium current (I_{sus}) or hERG, or the inwardly rectifying potassium current (I_{K1}) that is in large part responsible for the resting potential of myocardial cells. Previously, the metabolites of several of the drugs were examined to determine their inhibition of hERG alone (11). This published study also derived a general computational 3D pharmacophore model for hERG with 15 diverse molecules from the literature, which was tested with a larger set of 22 diverse molecules (including the antipsychotics included in this study) (11). We have now used the 11 antipsychotics to produce a separate pharmacophore model that was tested with olanzapine and two metabolites. This combined *in vitro* and computational study therefore provides further evidence that the blockade of potassium channels, and hERG in particular, is the primary mechanism by which certain antipsychotic drugs prolong the QT interval. In addition, the most potent hERG inhibitors share multiple common hydrophobic features as obtained with the computational 3D pharmacophore.

METHODS

Isolation of Cardiac Myocytes

Human tissue was obtained in accordance with the Tulane University School of Medicine institutional guidelines. Myocytes were isolated from specimens of human right atrial appendage obtained during surgery from hearts of eight patients (52–68 years) undergoing cardiopulmonary bypass. All atrial specimens were described as grossly normal at the time of excision, and all patients had normal P waves on the ECG. The cell isolation procedure has been described in detail (12).

Transfection and Cell Culture

Human embryonic kidney (HEK) 293 cells were stably transfected with the lipofectamine (Invitrogen, Carlsbad, CA, USA) method with the hERG cDNA. Cells were maintained in minimum essential medium with Earle's salts supplemented with nonessential amino acids, sodium pyruvate, penicillin, streptomycin, and fetal bovine serum.

Solutions

All drugs were supplied by Lilly Research Laboratories (Indianapolis, IN, USA). Drugs were dissolved in either dimethyl sulfoxide (DMSO) or deionized water to make 10-μM stock solutions that were stored at −20°C. Thioridazine stock and experimental solutions were protected from the light. Dilutions of stock solutions were made immediately before the experiment to create the desired concentrations. The "standard" external solution (solution bathing the cell) used for recording potassium currents (I_{to}, I_{sus}, I_{K1}, hERG) had an ionic composition of (in millimolars): 137 sodium chloride, 4 potassium chloride, 1.8 calcium chloride, 1.2 magnesium chloride, 11 dextrose, 10 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), adjusted to a pH of 7.4 with sodium hydroxide. The "standard" internal (pipette) potassium solution had an ionic composition of (in millimolars): 130 potassium chloride, 1 magnesium chloride, 10 sodium ATP, 5 ethyleneglycotetraacetic acid (EGTA), 5 HEPES, adjusted to a pH of 7.2 with KOH. Experiments were performed at 22 ± 1°C (I_{to}, I_{sus}, and I_{K1} recordings) or 37 ± 1°C (hERG recordings). When recording currents from human myocytes, 200 μM cadmium chloride was added to the external solution to block the L-type calcium current.

For study of the sodium current in human myocytes, cells were bathed in an "external" solution that consisted of (in millimolars): 115 trimethylamine chloride, 10 sodium

chloride, 5 cesium chloride, 1.8 calcium chloride, 1.2 magnesium chloride, 10 HEPES, 11 dextrose, adjusted to a pH of 7.4 with trimethylamine hydroxide. The chemical composition of the internal sodium solution was (in millimolars): 115 cesium fluoride, 20 cesium chloride, 10 sodium fluoride, 10 HEPES, 5 EGTA, adjusted to a pH of 7.2 with cesium hydroxide. Sodium current experiments were performed at 16 ± 0.5°C.

Data Acquisition and Analysis

Acceptable atrial myocytes were rod-shaped and lacked any visible surface membrane blebs. Currents were measured using the whole-cell variant of the patch clamp method (13). Pipette tip resistance was approximately 1.0–2.0 MΩ when filled with internal potassium solutions and 0.5–1.0 MΩ when filled with internal sodium solutions. Analog capacitance compensation and 40–60% series resistance compensation were used to yield voltage drops across uncompensated series resistance of less than 3 mV. Bath temperature was measured by a thermistor placed near the cell under study and was maintained by a thermoelectric device (model no. 806-7243-01, Cambion/Midland Ross, Cambridge, MA, USA). An Axopatch 1-B amplifier (Axon Instruments, Foster City, CA, USA) was used for whole-cell voltage clamping. Creation of voltage clamp pulses and data acquisition were controlled by an IBM PC using pClamp software (Axon Instruments).

Potassium currents recorded from human atrial myocytes were elicited by a pulse to +60 mV (800 ms) from a holding potential of −60 mV for I_{to} and I_{sus} and by a pulse to −100 mV for I_{K1}. I_{Na} recorded from human atrial myocytes was elicited by a 40-ms pulse to −20 mV from a holding potential of −140 mV. Pacing rate was 0.1 Hz for all currents.

After rupture of the cell membrane, thereby entering whole-cell mode, current amplitude and kinetics were allowed to stabilize (3–7 min) before experiments were begun. hERG currents recorded from HEK-293 cells stably transfected with hERG cDNA were elicited by a voltage pulse to +10 mV (500 ms) from a holding potential of −75 mV, and hERG tail currents were measured upon repolarization to −40 mV. Drug effects on tail current amplitude were measured after a steady-state level of blockade had been achieved as described previously (11).

Data are presented as mean ± standard error of the mean (SEM). They are given as percentage reduction in current amplitude calculated as current reduction after a steady-state drug effect had been reached relative to current amplitude before drug was introduced (control). Log-linear plots were created of the mean percentage blockade ± SEM at the concentrations that were tested. A nonlinear curve-fitting best-fit routine was utilized to fit a three-parameter Hill equation to the results using MicroCal Origin, version 6.0 software (MicroCal Software, Inc., Northampton, MA, USA). The equation is of the form:

$$y = V_{max}\frac{x^n}{k^n + x^n},$$

where x = concentration in micromolars; y = percentage blockade; and V_{max}, k, and n are unconstrained variables (except $V_{max} > 0$). The strength of the relationship between % hERG blockade and the corrected QT (QTc) increase was assessed using the Spearman correlation coefficient.

Inhibitor Pharmacophore Modeling for Antipsychotics

The computational molecular modeling studies were carried out using a Silicon Graphics Octane workstation. Briefly, models were constructed using Catalyst™ version 4.5 (Molecular Simulations, San Diego, CA, USA) as described previously with default settings (11). Catalyst models were constructed with 11 IC_{50} values for hERG inhibition by 9-hydroxyrisperidone, clozapine, clozapine-*N*-oxide, haloperidol, mesoridazine, *N*-desmethylclozapine, pimozide, risperidone, sertindole, thioridazine, and ziprasidone (Table I and Fig. 2). Catalyst automatically uses a log transformation on these data. The number of conformers generated using the best functionality of the program for each inhibitor was limited to a maximum number of 255, with an energy range of 20 kcal/mol. Hydrophobic, ring aromatic, hydrogen bond donors, hydrogen bond acceptors, and positive ionizable features were selected for possible inclusion. Ten hypotheses were generated using

Table I. Human Cardiac Ion Channel Blocking Potency of Antipsychotic Agents

Drug	hERG IC_{50} (μM)[a]	I_{Na} IC_{50} (μM)	I_{to} IC_{50} (μM)	I_{sus} IC_{50} (μM)	I_{K1} IC_{50} (μM)
Clozapine	0.320	28.2	51.7	>100	>100
Clozapine-*N*-oxide	133	Not performed	Not performed	Not performed	Not performed
N-Desmethylclozapine	4.5	Not performed	Not performed	Not performed	Not performed
Haloperidol	0.0268	19.3	54	25	>100
Olanzapine	0.231	>100	>100	>100	>>100
2-Hydroxyolanzapine	11.6	Not performed	Not performed	Not performed	Not performed
Desmethylolanzapine	14.2	Not performed	Not performed	Not performed	Not performed
Pimozide	0.0546	11.6	>100	>100	>100
Risperidone	0.148	60	>100	>100	>100
9-Hydroxyrisperidone	1.3	Not performed	Not performed	Not performed	Not performed
Sertindole	0.0147	>10	>10	>10	>10
Thioridazine	0.033	7.3	29.6	>100	>100
Mesoridazine	0.320	Not performed	Not performed	Not performed	Not performed
Ziprasidone	0.125	>10	>10	>10	>10

[a]hERG data previously published (11).

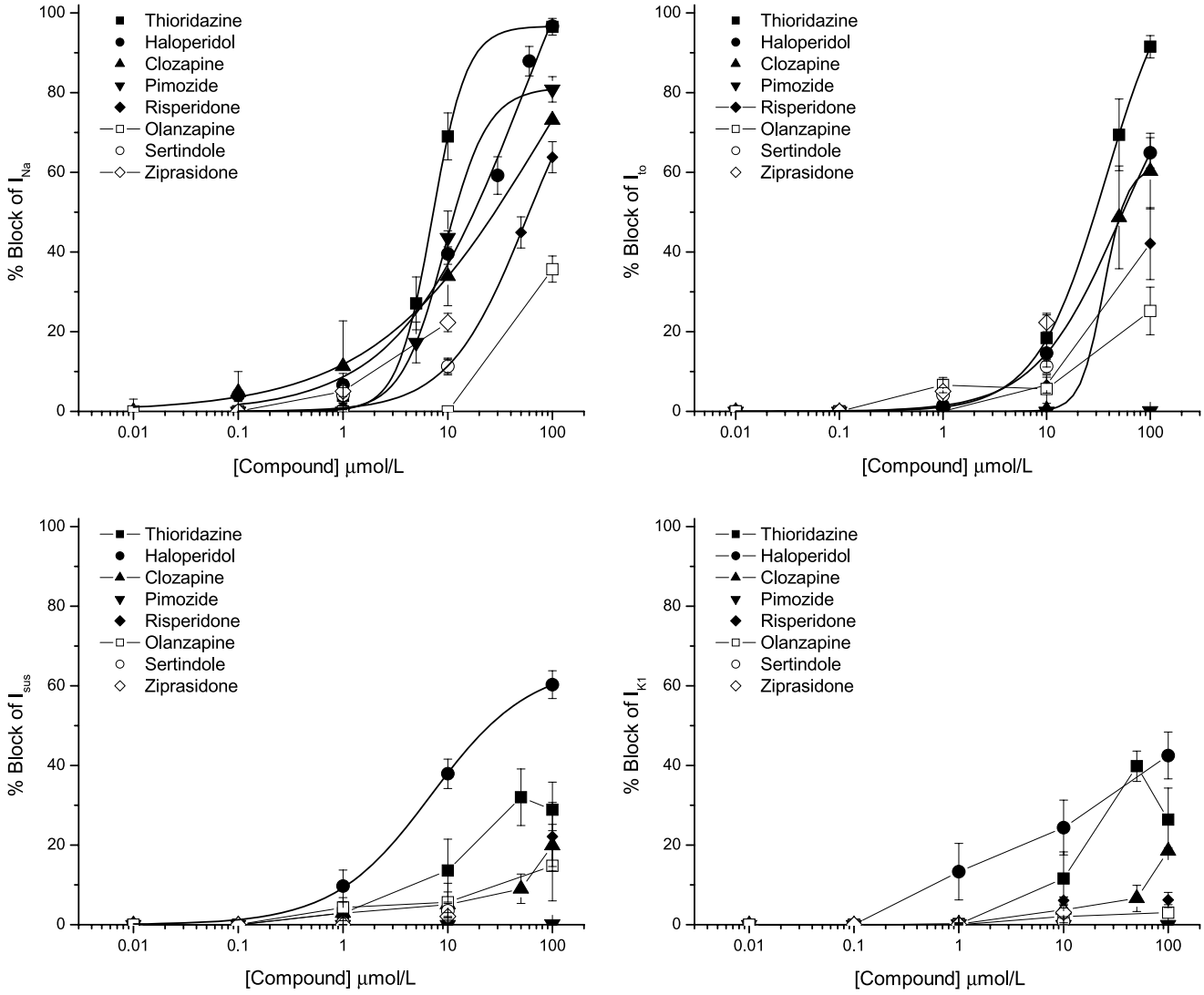

Fig. 2. Dose – response curves for drug blockade of I_{to}, I_{Na}, I_{sus}, and I_{K1}. When possible, data were fitted with an equation of the form given in "Methods." Experiments were performed at 22 ± 1°C for I_{to}, I_{sus}, and I_{K1} or 16 ± 0.5°C for I_{Na}. Symbols are mean ± SEM.

these conformers for each of the molecules and the IC_{50} values. After assessing all ten hypotheses generated, the lowest energy cost hypothesis was considered the best because this hypothesis possessed features representative of all the hypotheses. The reliability of the structure activity correlation between the predicted and observed activity values was estimated by means of an r^2 value. Olanzapine, 2-hydroxyolanzapine, and desmethylolanzapine were evaluated to generate a predicted IC_{50} value by fitting these molecules to the resultant pharmacophore. The limited number of molecules and activity range for the other cardiac channels precluded the calculation of meaningful pharmacophores.

Network Building Method

MetaCore™ (GeneGo Inc., St. Joseph, MI, USA) is an interactive database derived from manually curated literature publications on proteins and small molecules of biological relevance in humans and uses an Oracle-based (version 9.2.0.4 Standard Edition, Oracle, Redwood Shores, CA, USA) architecture. The software runs on an Intel-based 32-bit server running RedHat Linux Enterprise 3 AS (RedHat, Raleigh, NC, USA), and the web server runs Apache 1.3.x/mod_perl (http://perl.apache.org/start/index.html). Software on the server side is written in Perl, whereas the client side requires HTML/JavaScript and the Macromedia Flash Player Plug-in (Macromedia Inc., San Francisco, CA, USA). The hERG protein interactions were initially visualized in MetaCore, which generates an interaction network around the protein and finds the clusters of objects directly connected. Each connection represents a direct, experimentally confirmed, physical interaction between the objects stored in the Oracle database, described recently for this and a related tool (14,15). The manual expand algorithm, which starts with a root node as specified by the user and builds subnetworks around this object consisting of nearest neighbors, was used. In this case, the network was expanded as far as three levels. Each connection represents a direct, experimentally confirmed, physical interaction between the objects. In this case, the network was filtered to remove drugs not considered in

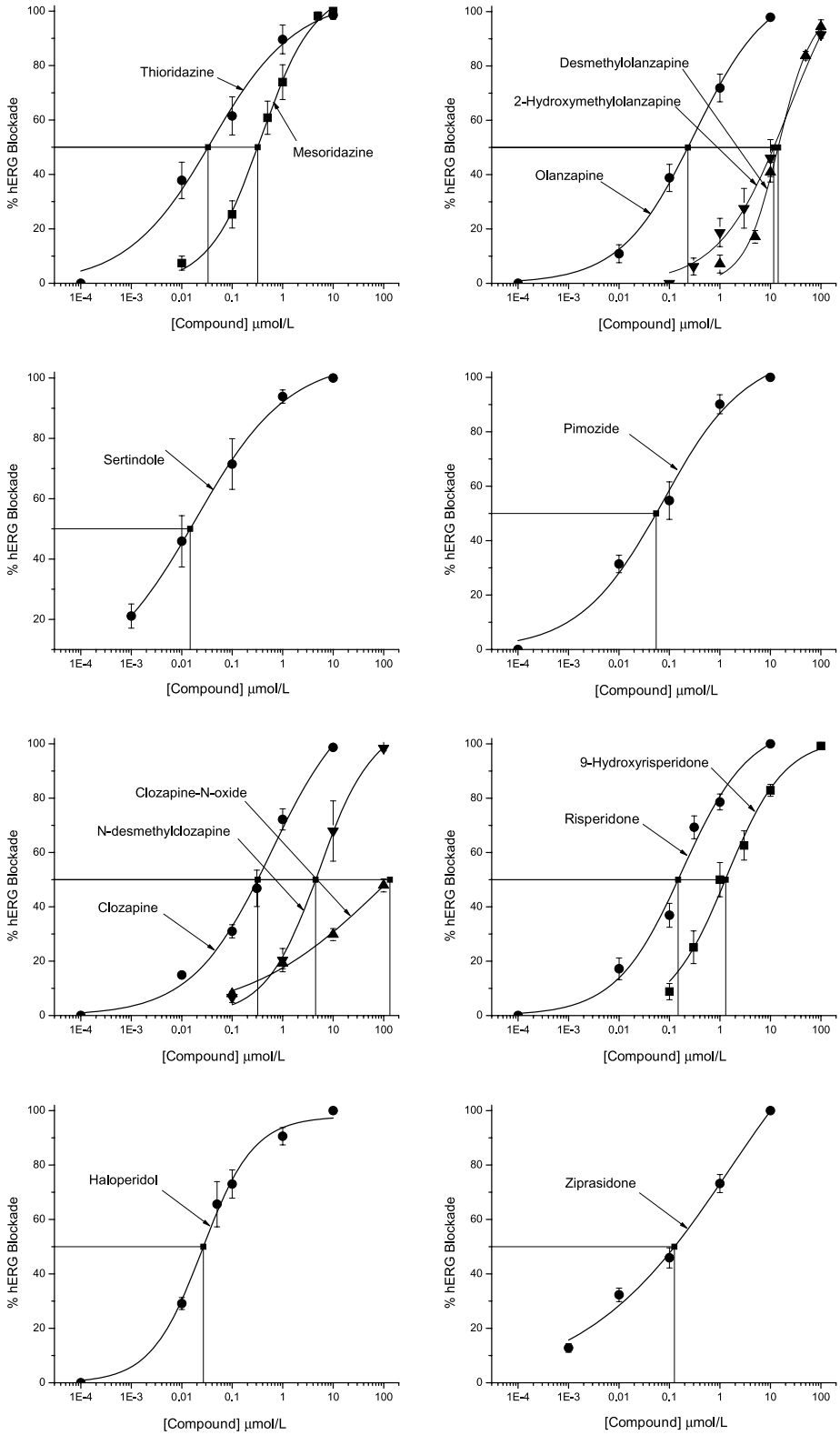

Fig. 3. Dose–response curves for blockade of hERG. Experiments were performed at 37 ± 1°C. Symbols are mean ± SEM.

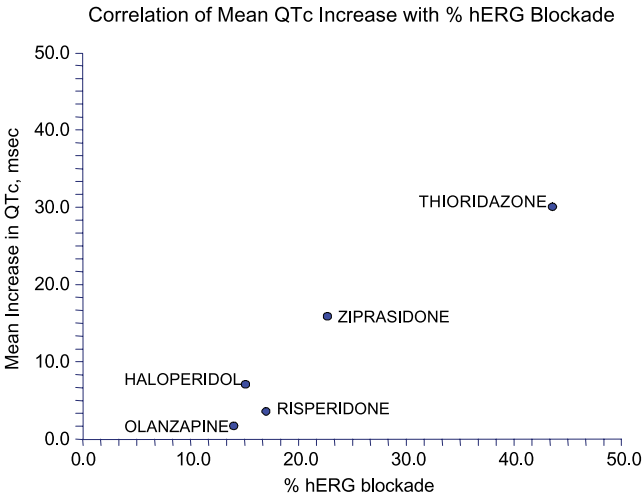

Fig. 4. Correlation of mean QTc increase with estimated percent hERG blockade at steady state C_{max} for haloperidol, olanzapine, risperidone, thioridazine, and ziprasidone.

this study and their immediate interactions (e.g., cytochrome P450s). This algorithm provides a means to look up one or more proteins of interest and identify regulatory cascades that lead to or from the gene(s) of interest.

RESULTS

The antipsychotic drugs clozapine, haloperidol, olanzapine, pimozide, risperidone, sertindole, thioridazine, and ziprasidone were tested to define their effects on ion currents that play a crucial role in depolarization and repolarization of the human myocardium (Fig. 1). All the tested antipsychotic agents had little effect on the I_{Na}, I_{to}, I_{sus}, or I_{K1} recorded from isolated human atrial myocytes with the estimated IC_{50} values for blockade greater than 5 μM with very little range of activity (Fig. 2, Table I). Because significant blockade of hERG was observed with several of the parent antipsychotics, metabolites of clozapine (clozapine-*N*-oxide and *N*-desmethylclozapine), olanzapine (desmethylolanzapine, 2-hydroxyolanzapine), risperidone (9-hydroxyrisperidone), and thioridazine (mesoridazine) were tested for blockade of hERG. The effects of these parent drugs and metabolites on the repolarizing potassium currents from hERG cDNA expressed in HEK-293 cells are shown in Fig. 3. All parent drugs and metabolites examined were found to block hERG current amplitude in a concentration-dependent manner, with thioridazine and sertindole having the greatest effect and clozapine and olanzapine having the least effect (Fig. 3). Best-fit analysis of mean concentration–response data yielded IC_{50} values ranging from 14 (sertindole) to 320 nM (clozapine) (Table I, Fig. 3). In general, the metabolites were approximately 10-fold less potent in blocking hERG than the parent drug (Table I, Fig. 3). A significantly positive association was observed between the estimated hERG blockade [based on the hERG assay results reported here and previously (11)] that would be observed with the steady-state maximum concentration (C_{max}) plasma concentrations and the increase in QTc in the Harrigan *et al.* study (16,17), as assessed by the Spearman correlation coefficient (p = 0.042, Fig. 4).

Molecular modeling was performed to gain qualitative insight into the structural feature(s) important for antipsychotic blockade of cardiac channels and, in particular, hERG. A hERG pharmacophore generated with 11 antipsychotics (IC_{50} range 0.0147–133 μM) produced a hypothesis consisting of three hydrophobic features and one ring aromatic feature (Fig. 5) with a correlation of observed and predicted IC_{50} data of r^2 = 0.77. We were unable to generate models with the other ion channel data due to the narrow range of activity. Two of the most potent hERG inhibitors in the training set, thioridazine and sertindole, fitted all of the pharmacophore features well, whereas the test set molecule olanzapine fitted the three hydrophobic features well but failed to fit the ring aromatic feature, indicative of an order of magnitude lower activity than thioridazine and sertindole (Fig. 5). The IC_{50} values for olanzapine and metabolites were correctly rank-ordered using this model, i.e., olanzapine (predicted 0.24 μM and observed 0.23 μM), 2-hydroxyolanzapine (predicted 1.2 μM and observed 11.6 μM), and desmethylolanzapine (predicted 20 μM and observed 14.2 μM).

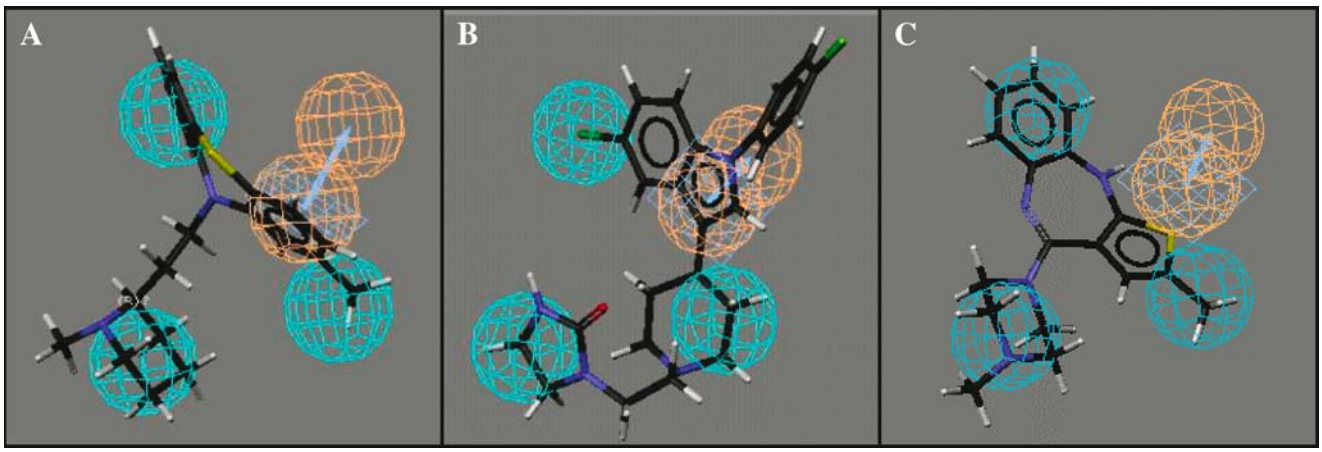

Fig. 5. Thioridazine (A), sertindole (B), and olanzapine (C) fitted to the hERG inhibitor pharmacophore derived from 11 antipsychotics. The model consisted of three hydrophobic features (cyan) and a ring aromatic feature with vector in the direction of the plane of the aromatic ring (orange). Molecules mapping to more pharmacophore features close to their centroids would be expected to possess a higher affinity for the ion channel.

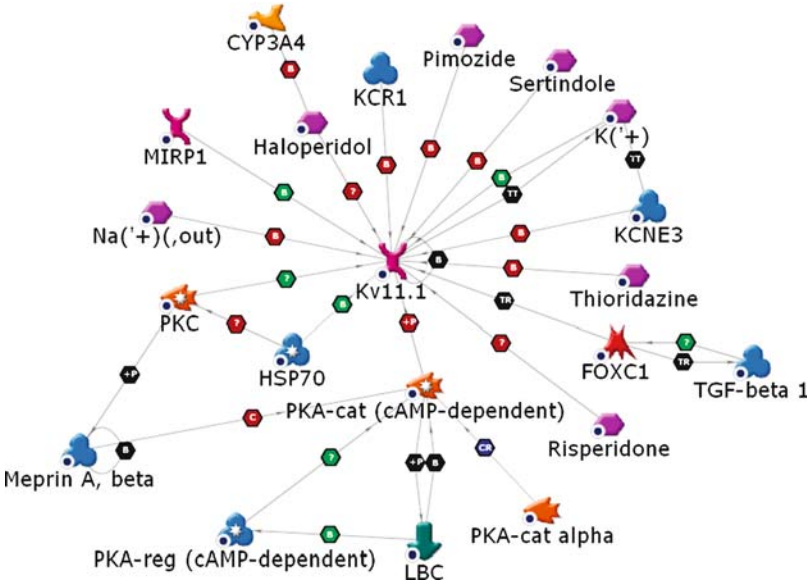

Fig. 6. A manual expand network generated with MetaCore™ (GeneGo Inc.) around the hERG channel (Kv11.1, centre pink symbol), showing the interaction of some of the antipsychotic ligands (purple hexagons) enzymes (yellow arrows), other ligands (purple hexagons), transfactors (red shapes), and other proteins (blue shapes) as well as their interactions. Small colored hexagons on the network edges between nodes represent information on the type of interaction, e.g., binding, transcriptional regulation (green = positive effect, red = negative effect, black = unspecified effect). The network represents a small fraction of the ligands known to bind hERG and illustrates the haloperidol connected to CYP3A4, which could be important for drug–drug interactions. The regulation of hERG by transcriptional factors and association with other proteins is also evident from this network.

A network analysis of the manually annotated hERG data from the literature (Fig. 6) focused on some of the antipsychotics from this study and indicates that haloperidol binds to hERG as well as CYP3A4, which also has a high affinity for hydrophobic molecules. Additionally, other proteins connected to hERG on the network are visible, representing the current extent of literature data annotated in this database. When other known hERG ligands in the database are visualized (hidden here for clarity), these were found to be overlapped with many more of these enzymes such as CYP2D6, CYP1A2, as well as transporters like P-glycoprotein (data not shown).

DISCUSSION

Several cases of QT interval prolongation and severe ventricular arrhythmias have been temporally associated with certain members of the antipsychotic class of drugs (9,10). Although the mechanism is not definitively known, a change in myocardial repolarization due to antipsychotic blockade of one or more cardiac potassium channels is the most likely explanation. There have been numerous studies that have assessed one or more antipsychotics and their effects on the hERG channel in different expression systems (7). This current study is, to our knowledge, the first to characterize the blocking effects of multiple antipsychotics against multiple human cardiac ion channels (I_{to}, I_{Na}, I_{sus}, I_{K1}, and hERG) that play important clinically significant roles in both cardiac depolarization and repolarization.

The I_{to}, I_{sus}, I_{K1}, and I_{Na} recorded from human cardiac myocytes were relatively insensitive to the agents tested in this study (Table I, Fig. 2). Although the drugs could bind to these cells differently when compared to the HEK-293 cells

Table II. Correlation Between Increase in QT_c Interval and hERG Blockade

Compound	Dose (mg/day)[a]	Mean C_{max}[a] (ng/ml)	Mean free plasma C_{max} (nM)[b]	Mean ↑ QTc (ms)[c]	% hERG blockade
Haloperidol	15	16.3	3.5	7.1	15.1
Olanzapine	20	55.1	12.3	1.7	14.0
Risperidone	16	58.7	14.3	3.6	17.0
Thioridazine	300	765	20.6	30.1	43.6
Ziprasidone	160	171	4.1	15.9	22.7

[a] Harrigan *et al.* 2004 (16,17).
[b] Calculated as mean C_{max} multiplied by proportion of drug reported as unbound [haloperidol, 8% (45); olanzapine, 7% (46); risperidone, 10% (46); thioridazine, 1% (28,47,48); ziprasidone, 1% (46)], divided by the molecular weight multiplied by 10^{-3}.
[c] QTc interval is corrected QT interval; mean changes in QTc were calculated by the baseline correction method (16,17).

expressing hERG, studies with guinea pig have indicated that determining the myocardium to plasma concentration of antipsychotics is a good indicator of arrhythmia (18). All of the drugs tested were found to block hERG current in a concentration-dependent manner with affinities in the nanomolar concentration range (Table II, Fig. 3). Blockade of I_{to}, I_{sus}, I_{K1}, and I_{Na} currents did not occur over a therapeutic concentration range, and therefore, it is unlikely that alterations of any of these currents play a role in the incidence of arrhythmias observed with the use of these agents. Therefore, blockade of hERG current is the most likely mechanism for the QT interval prolongation and arrhythmias temporally associated with the use of some antipsychotic drugs.

Although all of the drugs tested blocked hERG at some concentration, it is important to keep the concentrations associated with hERG blockade in the context of relevant plasma concentrations. Using data from a recent clinical study by Harrigan *et al.* (check 16,17), plasma concentrations of haloperidol, olanzapine, risperidone, thioridazine, and ziprasidone, as well as changes in the QTc interval, were evaluated for a potential correlation. Because the Harrigan *et al.* clinical study did not include clozapine, pimozide, or sertindole, clinical ECG data and hERG data for these drugs could not be correlated. As indicated in Table II, a reduction in hERG current amplitude, varying from approximately 14.0% to approximately 43.6%, was observed in the present study for haloperidol, olanzapine, risperidone, thioridazine, and ziprasidone based on steady-state C_{max} plasma concentration results in the Harrigan *et al.* study (16,17). In the Harrigan *et al.* study, treatment with the five antipsychotics was temporally associated with a measurable change in the QTc interval. However, the extent of these QTc interval increases may vary from relatively modest increases during treatment with olanzapine, risperidone, and haloperidol (1.7, 3.6, and 7.1 ms, respectively) to more pronounced increases for ziprasidone and thioridazine (15.9 and 30.1 ms, respectively) (16,17). Furthermore, increases in QTc of ≤5 ms are frequently observed with placebo and may represent random error due to limitations on measurement precision; only increases ≥10 ms are considered to be more likely reflective of actual drug effect (19–21). As noted by others, QT prolongation does not equal torsade de pointes and nonprolongation of QT does not mean the drug is safe (22). As assessed using the Spearman correlation coefficient, a significantly positive association (Fig. 4) was observed between the estimated hERG blockade (based on the hERG assay results reported here) that would be observed with the steady-state C_{max} plasma concentrations and the increase in QTc in the Harrigan *et al.* study (16,17). These correlation-related observations, combined with the observation that changes in QTc of <10 ms might be due to measurement imprecision or random variance noted above, further support the hypothesis that hERG blockade of ≥20% is required to result in clinically meaningful, drug-induced increases in the QTc interval (23).

Although blockade of repolarizing currents, notably the current mediated by hERG, can lead to delayed repolarization and a prolongation of the QT interval, the clinical consequences of these effects can be offset if a drug that blocks hERG also blocks depolarizing currents mediated by the sodium channel I_{Na} and/or calcium channel I_{Ca}. Blockade of these channels shortens the cardiac action potential. The concept of compensatory blockade of I_{Na} and I_{Ca} applies to metabolites as well as it does to parent drugs. As an example of this phenomenon, verapamil (24,25), as well as other drugs (26), is an hERG blocker, but it also blocks I_{Ca} at pharmacologically relevant concentrations and is not generally associated with high frequencies of occurrence of torsade de pointes. All the antipsychotics were tested in this study for I_{Na} activity without showing any potent blocking activity at pharmacologically relevant concentrations. Calcium effects could explain differences between the clinical effects predicted by the hERG assay results and the actual clinical incidence of torsade de pointes associated with a given drug. Additionally, if any drug were to contribute to maintenance of activation of the I_{Na} and/or I_{Ca} channels, this effect would prolong depolarization and augment hERG blockade with an increased risk of torsade de pointes. Such an effect is not occurring with the antipsychotics tested here due to the weak blockade of I_{Na} via the sodium channel. In addition, other modulators of clinical risk include the α-adrenoreceptor activity of drugs, as potent inhibitors of both this receptor and hERG have few, if any, reports of torsade de pointes (27). It is important to note that neither the calcium channel nor α-adrenoreceptor activity was evaluated in this study, which may represent a limitation of this study.

In addition to the effects of the parent drugs, metabolites might also contribute to QT interval prolongation. One of the active metabolites of thioridazine, mesoridazine, produces approximately a 13% reduction in hERG current amplitude at free concentrations [approximately 33 nM, Table I (11)] achieved after therapeutic doses of thioridazine (28). The combination of the hERG blocking actions of both thioridazine and mesoridazine might contribute to the relatively large prolongation of the QTc interval observed with thioridazine usage. In contrast, the primary metabolites of clozapine (clozapine-*N*-oxide and *N*-desmethylclozapine), olanzapine (2-hydroxyolanzapine and desmethylolanzapine), and risperidone (9-hydroxyrisperidone) are significantly less potent than the parent drug and are unlikely to reach concentrations that might contribute to clinically significant QT interval prolongation. The effect of the parent compounds and metabolites will also depend on the protein binding and unbound plasma concentration. In this study, we did not assess metabolites with assays other than hERG, as the parent compounds all had low activity and it is assumed that metabolites would be less potent inhibitors.

Besides the potential contribution of metabolites, several other factors might contribute to additional hERG blockade and thereby predispose patients to prolongation of the QT interval and ventricular arrhythmia in association with antipsychotic. These include elevated plasma concentrations of antipsychotics during overdose (29) or during administration by intravenous infusion (30). Drug–drug interactions may also precipitate the development of ventricular arrhythmia, such as those drugs interfering with metabolism antipsychotics that may elevate plasma concentrations (31). However, ECG abnormalities and torsade de pointes can also develop at therapeutic plasma concentrations (10). Patients suffering from psychosis are often prescribed multiple drugs during therapy, and concomitant administration of one or more drugs, in addition to an antipsychotic, that prolong the QT

interval should be avoided (32). Other factors contributing to the risk of drug-induced arrhythmia are hypokalemia (32,33), hypomagnesemia (34), elevated catecholamines, and underlying central nervous system or cardiac pathology (32). Subtle structural differences in ion channels due to genetic polymorphisms might also contribute to individual differences in QT interval changes in response to specific drugs. Based upon the preclinical data presented here and the available clinical data, it is advisable that potential risk factors should be taken into consideration prior to prescription of antipsychotics, and in some cases, careful electrocardiographic monitoring of the QT interval should be considered.

In the future, if we are to develop noncardiac drugs that avoid hERG, we must understand the structural requirements for binding this channel in the absence of a crystal structure of the protein. Previous studies have therefore generated quantitative structure activity relationship (QSAR) or pharmacophore models for hERG (35) to aid in understanding the features within molecules that are important for hERG binding. In the current study, we generated a pharmacophore that was tested with olanzapine and two metabolites. This pharmacophore illustrates the importance of both hydrophobic features as well as a ring aromatic feature (an additional hydrophobic feature likely to be involved in possible pi–pi interaction/stacking) that may interact with multiple amino acids site on this potassium channel. This pharmacophore partially confirms the work of Mitcheson *et al.* (36) who suggested the importance of electrostatic interactions between pi electrons on terfenadine, MK-499, and cisapride, and the hydrogen atoms of the aromatic rings in amino acid residues Y652/F656 of hERG are important for high-affinity binding. Piperidine containing antipsychotics sertindole and thioridazine potently inhibits hERG and fits all four of the pharmacophore features, illustrating that this is a key feature for activity, whereas weaker hERG inhibitors such as the piperazine containing olanzapine fail to fit all four features (Fig. 5). Furthermore, metabolites of clozapine, olanzapine, risperidone, and thioridazine also fail to fit all four features. The pharmacophore correctly rank-orders olanzapine and its metabolites, although this is a small test set and we would stress the qualitative nature of the pharmacophore. The current pharmacophore is different from the previously described hERG pharmacophore (11) as it lacks a positive ionizable feature, similar to another pharmacophore derived for a set of $5HT_{2A}$ inhibitors (37). This could represent a limitation of the pharmacophore modeling technique or ultimately the narrow structural diversity of the training set used. As Catalyst uses a subtractive phase, removing pharmacophore features common to active and inactive compounds, this could result in the absence of the positive ionizable feature (38). The p*K*a values of piperazine (9.8 and 5.6) and piperidine (11.1) rings differ, and therefore, this relationship with activity is missed by the pharmacophore. As described above, the most potent molecules tend to be piperidines; however, without a more detailed SAR to investigate the role of the second nitrogen in the piperazine ring, we can only speculate that p*K*a likely influences binding to hERG. It is also possible that the addition of a second nitrogen atom in the ring increases hydrophilicity and lowers affinity in this way. Many antipsychotics that possess an aromatic ring, as well as other hydrophobic groups, may be able to interact more strongly with the hERG channel binding site(s) than those that are missing some of these key features. This model represents further evidence for there being multiple overlapping pharmacophores that can be tolerated by hERG, indicative of a degree of channel flexibility. This should be considered alongside other studies describing a nonaromatic binding site within hERG (39) as well as extracellular binding sites capable of accepting scorpion toxins BeKm-1, BmTx3, and CnErg1 possessing hydrophobic surface areas (40–42). Due to the limited range (<2 log units) of IC_{50} values for I_{to}, I_{Na}, I_{sus}, and I_{K1}, it was not possible to generate meaningful pharmacophores for these cardiac channels. However, data available elsewhere for molecules binding to I_{Na} (44) may allow the construction of such models in the future.

A new computational method to visualize published interactions between ligands and proteins using a manually annotated database has previously been used to assess complex nuclear hormone–ligand interactions (15). A network analysis of the manually annotated hERG published data in MetaCore focused on some of the antipsychotics in this study (Fig. 6) and indicated the further interaction of haloperidol with CYP3A4. A larger analysis of all hERG ligands in the database indicates that there is overlap in binding with numerous CYPs (CYP3A4, CYP2D6 and CYP1A2) and P-glycoprotein (data not shown). All of these proteins have pharmacophores with numerous hydrophobic features (35,37,43). This suggests that the potential for drug–drug interactions across multiple proteins leading to hERG blockade may be important and can be investigated using such computational network approaches.

CONCLUSION

Antipsychotics do not inhibit the ion channels I_{to}, I_{Na}, I_{sus}, I_{K1} to any appreciable extent; however, blockade of hERG is a likely mechanism for the prolongation of the QT interval and the incidence of arrhythmias with certain antipsychotic agents. Correlation of available clinical data for QT interval prolongation with the degree of hERG blockade (measured under physiological conditions) suggests that marked QT interval prolongation may be predicted if unbound drug and metabolites in plasma reach concentrations that produce a 20% or greater blockade of hERG current amplitude. Differences in the arrhythmogenic potential of antipsychotics based on hERG blockade could result from differences in the magnitude of blockade, expected concentrations of free drug, and differences in concentrations of cardioactive metabolites. Although many other modeling studies have indicated the importance of a positive ionizable feature, the pharmacophore for hERG inhibition by antipsychotics suggests that limiting the number and orientation of hydrophobes will impact affinity for this channel. The network analysis of molecules binding hERG provides information on where potential drug–drug interactions that influence arrhythmogenic potential could occur.

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