Molecular Interaction of Droperidol with Human Ether-a-go-go-related Gene Channels

Prolongation of Action Potential Duration without Inducing Early Afterdepolarization

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Background: The cardiac safety of droperidol given at antiemetic doses is a matter of debate. Although droperidol potently inhibits human ether-a-go-go-related gene (HERG) channels, the molecular mode of this interaction is unknown. The role of amino acid residues typically mediating high-affinity block of HERG channels is unclear. It is furthermore unresolved whether droperidol at antemetic concentrations induces action potential prolongation and arrhythmogenic early afterdepolarizations in cardiac myocytes.

Methods: Molecular mechanisms of HERG current inhibition by droperidol were established using two-electrode voltage clamp recordings of Xenopus laevis oocytes expressing wild-type and mutant channels. The mutants T623A, S624A, V625A, Y652A, and F656A were generated by site-directed mutagenesis. The effect of droperidol on action potentials was investigated in cardiac myocytes isolated from guinea pig hearts using the patch clamp technique.

Results: Droperidol inhibited currents through HERG wild-type channels with a concentration of half-maximal inhibition of 0.6–0.9 μM. Droperidol shifted the channel activation and the steady state inactivation toward negative potentials while channel deactivation was not affected. Current inhibition increased with membrane potential and with increasing duration of current activation. Inhibition of HERG channels was similarly reduced by all mutations. Droperidol at concentrations between 5 and 100 nM prolonged whereas concentrations greater than 300 nM shortened action potentials. Early afterdepolarizations were not observed.

Conclusions: Droperidol is a high-affinity blocker of HERG channels. Amino acid residues typically involved in high-affinity block mediate droperidol effects. Patch clamp results and computational modeling allow the hypothesis that interaction with calcium currents may explain why droperidol at antiemetic concentrations prolongs the action potential without inducing early afterdepolarizations.

DROPERIDOL is a highly potent butyrophenone that has been commonly used for more than 30 yr in the therapy of postoperative nausea and vomiting. High doses of droperidol (0.25 mg/kg) given for the induction and maintenance of anesthesia have been reported to prolong the QT interval in perioperative patients. After several cases of excessive QT-prolongation leading to fatal arrhythmias associated with the use of droperidol at high doses, the US Food and Drug Administration released a “black box” warning in 2001.†† As a consequence, the clinical use of droperidol in postoperative nausea and vomiting therapy has decreased dramatically. However, not a single case of cardiac arrhythmia has been reported after application of droperidol at antiemetic doses (0.625–1.25 mg). Furthermore, droperidol given at antiemetic doses does not seem to be associated with a clinically significant prolongation of the QT interval. Also, the cardiotoxic potential of droperidol at antiemetic doses has not been addressed in controlled clinical trials. The safety of droperidol in postoperative nausea and vomiting therapy, therefore, remains a matter of debate.

Prolongation of the QT interval reflects an increase of action potential duration (APD) in ventricular cardiomyocytes. Contradicting results have been obtained in animal studies regarding the effects of droperidol on APD and its potential to induce early afterdepolarizations (EADs). Some authors demonstrated action potential (AP) shortening, whereas others reported AP prolongation or no effect on AP. Drug-induced prolongation of cellular APD and induction of torsades de pointes ventricular arrhythmia is often caused by high-affinity block of the delayed rectifying K⁺ current Ikr. The channel underlying Ikr is encoded by the human ether-a-go-go-related gene (HERG). High-affinity block of HERG channels is transmitted by two aromatic amino acid residues in the S6 helix (Tyr652 and Phe656) and by several residues located at the base of the pore helix (Thr623, Ser624, Val625). Based on the concentration of half-maximal inhibition (IC₅₀) value for inhibition of HERG channels, droperidol may be regarded as a high-affinity blocker. Currently, however, it is unknown whether the amino acid residues typically mediating high-affinity block are involved in the interaction of droperidol with HERG channels. Furthermore, it
is unknown whether droperidol interacts with these channels in a way mechanistically similar to high-affinity blockers such as dofetilide and E-4031.19,21

Therefore, despite the “black box” warning of the US Food and Drug Administration on the cardiac safety of droperidol, several issues of the cardiotoxic profile of droperidol remain unresolved. The molecular mode of interaction of the drug with HERG channels has not been defined. Furthermore, it is unclear whether droperidol at antiemetic concentrations induces AP prolongation and early afterdepolarizations in ventricular cardiomyocytes. The aim of our study, therefore, was to characterize the molecular mode of interaction of droperidol with HERG channels. In addition, it was intended to suggest a possible explanation why droperidol may not induce EADs despite its interaction with HERG channels.

Materials and Methods

All animal experiments were conducted in accordance with institutional guidelines and approved by local authorities (Ministry of Science and Health, Hamburg, Germany).

Solutions and Chemicals

Ringer’s solution contained 75 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 5 mM Na-pyruvate, and 5 mM HEPES, titrated to pH 7.5 with NaOH. Two-electrode voltage clamp experiments were performed using different extracellular solutions: HERG and HERG mutants were investigated using NaCl-79 solution, which contained 79.5 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES, pH adjusted to 7.5 with NaOH. NaCl-95 solution used for measurement of Iᵦ₅, contained 95 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, and 10 mM HEPES, titrated to pH 7.40 with tris-(hydroxymethyl)-aminomethane. Cardioplegic solution contained 15 mM NaCl, 9 mM KCl, 4 mM MgCl₂, 0.35 mM NaH₂PO₄, 0.015 mM CaCl₂, 10 mM glucose, and 238 mM mannitol, titrated to pH 7.40 with NaOH. For patch clamp experiments, myocytes were bathed in modified Tyrode solution containing 138 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 0.33 mM NaH₂PO₄, 2 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, titrated to pH 7.30 with NaOH. The pipette solution contained 120 mM K-glutamate, 10 mM KCl, 2 mM MgCl₂, 10 mM EGTA, 10 mM HEPES, and 2 mM Na₂-ATP, titrated to pH 7.20 with KOH. Droperidol (Sigma, Deisenhofen, Germany) was dissolved in dimethyl sulfoxide. The maximal concentration of dimethyl sulfoxide in the respective extracellular solution was 1%, whereas the concentration used for kinetic analysis was less than 0.1%. At these concentrations, dimethyl sulfoxide did not by itself inhibit currents (data not shown), and its effect on HERG channel gating is negligible.22

Molecular Biology, cRNA Preparation

The HERG mutants T623A, S624A, V625A, Y652A, and F656A were created by site directed mutagenesis.23 All constructs were cloned in the pGEM expression vector for complementary RNA (cRNA) synthesis. The cRNA was synthesized in vitro with the mMESSAGE mMACHINE Kit (Ambion, Austin, TX) according to the manufacturer’s protocol. The cRNA was purified with a phenol/chloroform extraction. The integrity of the cRNA was analyzed in a denaturing gel. The concentration was determined with the Ribogreen method (Ribogreen RNA Quantification Reagent; Molecular Probes, Eugene, OR). For preparation of oocytes, female Xenopus laevis frogs (n = 37) were anesthetized with tricaine solution, and ovarian lobes were surgically removed. Oocytes were isolated by enzymatic digestion using collagenase A (Roche, Mannheim, Germany; Serva, Heidelberg, Germany) for 2–4 h. Enzymatic digestion was stopped by incubation in Ringer’s or NaCl-95 solution. Defolliculated oocytes (stages V and VI) were injected with cRNA of HERG, HERG mutants, or KCNQ1/KCNE1 (in a ratio of 1:1).

Two-electrode Voltage Clamp

Whole cell currents were measured 2–7 days after cRNA injection with the two-electrode voltage clamp technique using an Oocyte-Clamp OC-725C amplifier (Warner Instrument Corporation, Hamden, CT) or a TurboTec 05 amplifier (npi electronic, Tamm, Germany), both controlled by Pulse software (HEKA Elektronik, Lambrecht, Germany). Sharp electrodes were pulled from borosilicate glass capillary tube (World Precision Instruments, Sarasota, FL or Clark Electromedical Instruments, Reading, United Kingdom) and filled with 3 M KCl and 3% agar. Whole cell currents were measured at room temperature (21°–24°C) while oocytes were superfused at a constant rate.

Isolation of Guinea Pig Ventricular Myocytes and Patch Clamp Experiments

For isolation of cardiomyocytes, male Dunkin-Hartley guinea pigs (n = 12; 350–450 g) were deeply anesthetized by thiopental-Na⁺ (200 mg/kg body weight), and hearts were excised and quickly placed into cold (4°C) cardioplegic solution. Myocytes of the center part of the left ventricular free wall were enzymatically isolated as previously described using a Langendorff apparatus.24,25 Single myocytes were stored at rool temperature in Ca²⁺ free modified Tyrode solution. Only single rod-shaped cells with clear cross-striations and no spontaneous contractions were used for experiments within 12 h after isolation. The ruptured-patch whole cell configuration was used as previously described using an EPC-9 amplifier controlled by the Pulse software. Membrane voltages were recorded in the zero current clamp mode.
Pipette potentials were corrected online for liquid junction potentials. APs were elicited at 0.2 Hz by depolarizing current injections of 5 or 10 ms in duration. All experiments were performed at room temperature (21°C–24°C) while cells were superfused at a constant rate.

Data Analysis

Data were analyzed using PulseFit software (HEKA Elektronik), Igor (WaveMetrics, Lake Oswego, OR), and KaleidaGraph software (Synergy Software, Reading, PA). The normalized tail currents during the activation protocol were fitted by a modified Boltzmann equation: 

\[ I = I_{\text{max}} / [1 + \exp((V_{0.5} - V_m)/k)], \]

where \( V_{0.5} \) is the membrane potential of half-maximal activation, \( V_m \) is the membrane potential, and \( k \) is the slope factor. For the analysis of the steady state inactivation, maximal current amplitudes during the tail were corrected for the closing during the hyperpolarizing step at very negative potentials as described previously. The corrected curves were fitted by a Boltzmann function. The inhibition of currents by droperidol was quantified by the reduction of the maximal current during the test pulse. The fractional block \( f \) was calculated by the following formula:

\[ f = 1 - (I_{\text{max, drug}}/I_{\text{max, control}}). \]

Concentration–response curves were fitted by a Hill equation: 

\[ f = 1/[1 + (c/IC_50)^h], \]

where \( c \) is the concentration of droperidol and \( h \) is the Hill coefficient. The impact of droperidol on the AP at 90% repolarization (APD\(_{90}\)) was quantified by the following formula: 

\[ y = (\text{APD}_{90, \text{drug}}/(\text{APD}_{90, \text{control}} + \text{APD}_{90, \text{washout}}))/2, \]

where \( \text{APD}_{90, \text{control}} \) is the \( \text{APD}_{90} \) before application, \( \text{APD}_{90, \text{drug}} \) is the \( \text{APD}_{90} \) in the presence of droperidol, and \( \text{APD}_{90, \text{washout}} \) is the \( \text{APD}_{90} \) after maximal possible washout of the drug.

Statistical Analysis

All data are given as mean ± SD; \( n \) values indicate the number of experiments. When only two groups were compared, statistical significance was calculated by the Student \( t \) test, otherwise by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test using Excel (Microsoft, Redmond, WA) or PRISM (GraphPad Software Inc., San Diego, CA). Where appropriate, paired \( t \) tests were performed. Statistical significance was defined as \( P < 0.05 \).

Results

Effects of Droperidol on HERG and KCNQ1/KCNE1 Currents

The effect of droperidol on HERG and KCNQ1/KCNE1 currents was studied in oocytes injected with corresponding cRNA. Throughout all experiments, the holding potential was −80 mV, and repetitive pulses were applied to ensure steady state conditions.

Different pulse protocols were employed to assess the effect of droperidol on HERG currents. The ramp protocol was used to elicit a HERG current similar to that during a normal AP. Oocytes were depolarized to +60 mV and repolarized to −80 mV within 1 s, and the maximal current \( I_{\text{max}} \) was determined. Using square pulses, the cells were depolarized to +0 mV for 2 s, and tail currents were then recorded at −40 mV (\( I_{\text{tail}} \) −40) or at −120 mV (\( I_{\text{tail}} \) −120). Figure 1A shows representative current traces elicited by the different pulse protocols under control conditions and after application of 1 μM droperidol. A strong suppression of HERG currents could be detected with each pulse protocol. The concentration dependent fractional inhibition of \( I_{\text{max}} \) in \( I_{\text{tail}} \) −40 and \( I_{\text{tail}} \) −120 were described by Hill equations (fig. 1B and table 1). The IC\(_50\) was in the range of 0.6–0.9 μM. The inhibition at a concentration of 1 μM droperidol did not significantly differ between the applied pulse protocols (statistically not significant; ANOVA). Therefore, this concentration was chosen to further analyze the effect of droperidol on channel gating properties. The Hill coefficients of all Hill equations were comparable.

To investigate the effect of droperidol on KCNQ1/KCNQ1 currents, oocytes were depolarized to +60 mV for a duration of 5 s (\( I_{\text{test}} \)). Tail currents were recorded at −60 mV (\( I_{\text{tail}} \) −60). Figure 1C shows representative current traces under control conditions and after application of 10 μM droperidol. Whereas 1 μM droperidol did not influence the current amplitude of \( I_{\text{test}} \) (\( n = 4 \)), 10 μM droperidol reduced \( I_{\text{test}} \) in two of five experiments by 3% and 5%. \( I_{\text{tail}} \) −60 was not affected by either concentration.

Effects of Droperidol on HERG Channel Gating

HERG Activation. To analyze the influence of droperidol on the activation of HERG currents, the pulse protocol shown in figure 2A was used. Depolarizing steps between −70 mV and +80 mV activated time-dependent outward currents. The amplitude of the outward currents at the end of the depolarizing pulse (\( I_{\text{test}} \)) increased with more positive voltage steps and reached a maximum value at approximately 0 mV. Depolarization to more positive values caused a current decrease. To visualize the current inhibition by droperidol, \( I_{\text{test}} \) was normalized to the maximum control current and plotted against the activation potential under control conditions and in the presence of 1 μM droperidol (fig. 2B). Whereas droperidol strongly inhibited the amplitude of
no influence on the voltage-dependence of activation was detected.

After the depolarizing step, the tail current \( I_{\text{tail}} \) was elicited by a repolarization to \(-40\) mV. The amplitude of \( I_{\text{tail}} \) increased with depolarizing steps from \(-70\) to \(+20\) mV and reached a current plateau upon further depolarization (fig. 2C). \( I_{\text{tail}} \) was normalized to the maximum \( I_{\text{tail}} \) of each cell and plotted against the depolarizing membrane potential. The resulting current-voltage curves (fig. 2C) were described by Boltzmann equations (see table 2 for details). Droperidol significantly shifted the membrane potential of half-maximal activation \( (V_{0.5}) \) to-ward negative potentials \((P < 0.001)\) and decreased the slope factor \((P < 0.001)\). The inhibition of \( I_{\text{tail}} \) by droperidol significantly increased with depolarization of the membrane potential \((P < 0.001, \text{ANOVA}, n = 8; \text{fig. 2D})\).

**HERG Inactivation.** The influence of droperidol on the steady state inactivation was assessed by a three-step protocol (fig. 3A): The membrane was depolarized for 2 s to \(+40\) mV, then pulses from \(-120\) to \(+60\) mV in steps of \(10\) mV were applied for \(50\) ms, and finally the membrane potential was kept at \(-40\) mV for 1 s. Figure 3A shows representative current traces under control conditions and after application of \(1\) \(\mu\)M droperidol. The magnitude of the resulting tail current at \(+40\) mV was

\[
l_{\text{test}}, \text{ no influence on the voltage-dependence of activation was detected.}
\]

of each cell and plotted against the depolarizing membrane potential. The resulting current-voltage curves (fig. 2C) were described by Boltzmann equations (see table 2 for details). Droperidol significantly shifted the membrane potential of half-maximal activation \( (V_{0.5}) \) toward negative potentials \((P < 0.001)\) and decreased the slope factor \((P < 0.001)\). The inhibition of \( I_{\text{tail}} \) by droperidol significantly increased with depolarization of the membrane potential \((P < 0.001, \text{ANOVA}, n = 8; \text{fig. 2D})\).

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\[
l_{\text{test}}, \text{ no influence on the voltage-dependence of activation was detected.}
\]
normalized to the maximum tail current and was plotted against the depolarizing voltage steps (fig. 3B). The parameters of the corresponding Boltzmann functions are given in table 2. Whereas droperidol caused a significant left shift of the inactivation midpoint \( (P < 0.001) \), the slope factor was not influenced. The fractional inhibition of \( I_{\text{tail}} \) significantly depended on the membrane potential \( (P < 0.001, \text{ANOVA, } n = 7) \); fig. 3C). The inactivation time constants were analyzed using a three-step pulse protocol (data not shown): depolarization to 0 mV (2 s) followed by a repolarization to \(-80\) mV for 50 ms and recording of the tail currents between \(-80\) and \(+40\) mV. The time constants of the monoexponential fits of the current decay under control conditions were similar to the ones found after application of \( 1 \mu M \) droperidol \( (\text{droperidol, } 18.0 \pm 5.7 \text{ ms; control, } 20.1 \pm 6.2 \text{ ms at a pipette potential of } 0 \text{ mV; } n = 7) \).

**HERG Deactivation.** The influence of droperidol on HERG deactivation was investigated using the following pulse protocol: depolarization to \(+60\) mV (2 s) followed by repolarization steps to \(-120\) to \(-50\) mV (2 s). The current decay was best described by exponential functions with one or two time constants, respectively. There was no significant difference between the time constants under control conditions and in the presence of \( 1 \mu M \) droperidol \( (n = 8 \text{ oocytes investigated; ANOVA: statistically not significant; data not shown}) \).

**Time Dependence of Droperidol Inhibition.** The time dependence of HERG block was investigated with an “envelope of tails” protocol (fig. 4). The pulse protocol as well as representative current traces are depicted in figure 4A. After a depolarization to \(+40\) mV for a duration \( (\Delta t) \) of \(100-700\) ms, tail currents were recorded at \(-40\) mV. Fractional inhibition of the tail currents was calculated for each depolarization (fig. 4B). The time-dependent increase of inhibition followed a monoexponential function with a time constant of \(186 \pm 36\) ms.

### Table 2. Influence of Droperidol on HERG Channel Kinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1 ( \mu M ) Droperidol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation ( V_{0.5}, \text{mV} )</td>
<td>(-7.4 \pm 1.8)</td>
<td>(-11.9 \pm 2.0^*)</td>
</tr>
<tr>
<td>Slope factor, mV</td>
<td>(8.8 \pm 0.5)</td>
<td>(7.4 \pm 1.1^*)</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Steady state inactivation ( V_{0.5}, \text{mV} )</td>
<td>(-38.7 \pm 7.8)</td>
<td>(-45.3 \pm 10.9^*)</td>
</tr>
<tr>
<td>Slope factor, mV</td>
<td>(-17.8 \pm 0.9)</td>
<td>(-18.8 \pm 1.0)</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Membrane potential of half-maximal activation/steady state inactivation \( (V_{0.5}) \) and slope factor are parameters of the corresponding Boltzmann equations. Data are given as mean \( \pm SD \).

\(^* P < 0.001 \) droperidol vs. control values.

HERG = human \( ether-a-go-go \)-related gene; n = number of cells investigated.

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Fig. 2. Activation of human \( ether-a-go-go \)–related gene (HERG) currents and the influence of \( 1 \mu M \) droperidol. (A) Representative current traces under control conditions and in the presence of \( 1 \mu M \) droperidol evoked by the depicted pulse protocol. (B) Voltage dependence of \( I_{\text{test}} \) activation and the influence of \( 1 \mu M \) droperidol. \( I_{\text{test}} \) was normalized to the maximal current amplitude under control conditions. (C) To visualize the effect of droperidol on the voltage-dependent activation of tail current amplitude, \( I_{\text{tail}} \) was normalized to the maximal tail current and fitted by Boltzmann functions (parameters are shown in table 2). Droperidol significantly shifted the voltage of half-maximal activation \( V_{0.5} \) toward more negative voltages and decreased the slope factor \( (P < 0.001) \). (D) Inhibition of the tail current significantly depended on the membrane potential \( (P < 0.001, \text{analysis of variance}) \). For each parameter, eight cells were investigated.
Frequency Dependence of Droperidol Inhibition.
The frequency dependence of HERG current inhibition by droperidol was investigated after the drug was allowed to wash in for 10 min at $-80$ mV (fig. 4C). HERG currents were activated by repetitive current pulses (depolarization to $+20$ mV of 300 ms duration, followed by a subsequent repolarization to $-40$ mV for 300 ms) at a basic cycle length of 1 s ($n = 5$), 2 s ($n = 4$), 4 s ($n = 4$), or 10 s ($n = 4$). After exposure to 1 $\mu$M droperidol, application of the pulse train at the different basic cycle lengths decreased tail current amplitude by 47 ± 5, 43 ± 10, 46 ± 12, or 44 ± 5% at a basic cycle length of 1, 2, 4, or 10 s, respectively. The time course of the inhibition was described by monoeponential functions with time constants of 1.2 ± 0.4, 1.9 ± 0.3, 4.3 ± 0.3, or 7.9 ± 1.3 s at a basic cycle length of 1, 2, 4, or 10 s, respectively. Although the development of block was faster at higher stimulation frequencies ($P < 0.001$, ANOVA), the extent of steady state inhibition did not depend on the stimulation frequency.

Interaction Sites of Droperidol
The inhibitions of mutated HERG constructs (Y652A, F656A, T623A, S624A, and V625A) by droperidol were investigated after an incubation time of 10 min at $-80$ mV. Trains of 100 depolarizing pulses, as shown in the inset, were applied at a basic cycle length of 1 s ($\Delta t = 100$ ms; $n = 5$), 2 s ($\square$, $n = 4$), 4 s ($\bullet$, $n = 4$), or 10 s ($\bigcirc$, $n = 4$). The resulting mean relative tail current amplitudes of the first 60 s are plotted versus time. The onset of inhibition significantly depended on the basic cycle length ($P < 0.001$, analysis of variance), whereas the steady state block was not dependent on the basic cycle length.
established to identify the molecular sites of interaction with HERG channels. The mutants S624A and Y652A show similar gating behavior as wild-type HERG channels. Inhibition by 1 μM droperidol was assessed using the three pulse protocols shown in figure 1A. The mutants T623A, V625A, and F656A gave rise to only small currents in response to either the ramp protocol or the protocol eliciting tail currents at −40 mV. Therefore, these mutants were investigated by assessing the inhibition of tail currents at −120 mV. Representative current recordings of wild-type HERG and mutant channels under control conditions and in the presence of 1 μM droperidol are shown in figure 5A. The residual tail currents at −120 mV are depicted in figure 5B. Inhibition of all mutants by 1 μM droperidol was significantly smaller than inhibition of wild-type channels (see also table 3).

**Influence of Droperidol on APD of Guinea Pig Cardiac Myocytes**

Figures 6A and B show representative APs recorded from isolated guinea pig left ventricular myocytes under control condition and in the presence of droperidol after an incubation time of 3 min. APs were elicited at 0.2 Hz by short depolarizing currents under steady state conditions. Figure 6C visualizes the influence of droperidol on APD90.

![Table 3. Inhibition of HERG Mutant Channels by 1 μM Droperidol](image)

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Imax</th>
<th>Itail −40</th>
<th>Itail −120</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>wt</td>
<td>51.4</td>
<td>54.4</td>
<td>44.1</td>
<td>10</td>
</tr>
<tr>
<td>T623A</td>
<td>6.7</td>
<td>6.3</td>
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</tr>
<tr>
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<td>5.6</td>
<td>7.4</td>
<td>6</td>
</tr>
<tr>
<td>V625A</td>
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<td>—</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>wt</th>
<th>T623A</th>
<th>S624A</th>
<th>V625A</th>
<th>Y652A</th>
<th>F656A</th>
</tr>
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<tbody>
<tr>
<td>Imax</td>
<td>51.4%</td>
<td>6.7%</td>
<td>5.6%</td>
<td>—</td>
<td>5.6%</td>
<td>—</td>
</tr>
<tr>
<td>Itail −40</td>
<td>54.4%</td>
<td>6.3%</td>
<td>5.6%</td>
<td>—</td>
<td>7.4%</td>
<td>—</td>
</tr>
<tr>
<td>Itail −120</td>
<td>44.1%</td>
<td>3.4%</td>
<td>7.4%</td>
<td>1.0%</td>
<td>1.0%</td>
<td>—</td>
</tr>
</tbody>
</table>

I<sub>max</sub>, I<sub>tail −40</sub>, and I<sub>tail −120</sub> are fractional inhibition of currents evoked by corresponding pulse protocols (figure 1A) by 1 μM droperidol. Data are given as mean ± SD.

* P < 0.001 mutant vs. human ether-a-go-go-related gene (HERG) wild type (wt).

n = number of cells investigated.
n

served at a concentration of 100 nM. At a concentration
manner with a maximal prolongation of 17
droperidol increased APD in a concentration-dependent
even higher concentrations of up to 10


Discussion
In the current study, the molecular mechanisms under-
lying inhibition of HERG channels by droperidol were
characterized. The role in drug channel interaction of
several amino acid residues located in the S6 domain
and within the channel pore were established. Furthermore,
possible consequences of this interaction for the excit-
ability of isolated ventricular cardiomyocytes were elu-
cidated.

Droperidol blocked HERG currents in Xenopus oo-
cytes with an IC50 value between 0.6 and 0.9 μM. This
value is well within the range of that reported for other
high-affinity blockers such as dofetilide and E-4031 (0.4
and 0.6 μM, respectively).19 The difference between the
value reported in the current study and the value re-
ported by Drolet et al.12 (approximately 0.03 μM in
HEK293 cells) is most likely caused by differences in the
expression systems.30 Droperidol did not significantly
influence KCNQ1/KCNEL currents, which confirms that
droperidol specifically interacts with IKr but not with IKs.
Whereas droperidol potently inhibited the amplitude of
HERG currents, it had only small effects on channel
gating. Channel activation and steady state inactivation
were slightly shifted toward negative potentials,
whereas deactivation was not influenced. Current inhib-
ition by droperidol significantly increased with the
membrane potential and with increasing duration of
current activation. Furthermore, the development of
block depended on the stimulation frequency. These
results indicate that the inhibition by droperidol may be
state dependent, with a preference for open channels.
Therefore, droperidol acts similarly to other high-affinity
blockers of HERG channels.19,21

Several residues located in the S6 domain (Tyr652,
Phe656) and within the channel pore (Thr623, Ser624,
Val625) are important for the interaction of high-affinity
blockers with HERG channels.16,17,19,32 In the current
study, these amino acid residues were identified to also
mediate inhibition of HERG channels by droperidol.
However, these mutations are known to alter channel
gating behavior.16,20 Whereas S624A slows and V625A
abolishes channel inactivation, the other mutants accel-
erate inactivation. Moreover, all mutations shift the volt-
age dependence of activation and/or inactivation. Al-
though it cannot be excluded that the reduced inhibition
by droperidol is related to changes in channel gating,
this seems less likely. The reduction in inhibition is
similar for all mutations despite the changes in gating
being qualitatively different. The role of inactivation in
HERG channel block is controversial: Abolishing C-type
inactivation by introducing the double mutant
HERGG628C/S631C reduces inhibition of HERG channel by
blockers such as dofetilide33 and E-4031.34 However,
other mutations affecting HERG inactivation yield incon-
sistent results regarding the role of inactivation for the
inhibition by high-affinity blockers.35,36 Our results dem-
onstrate that inhibition of HERG channels by droperidol
is reduced to a similar extend by a mutation that accel-
irates inactivation (T623A) and by a mutation that abol-
ishes inactivation (V625A). Reduced droperidol affinity
of noninactivating HERG mutant channels may thus re-
sult from inactivation gating-associated reorientation of
residues in the S6 domain that comprise the droperidol
binding site rather than resulting from a direct effect of
the inactivation. Taken together, our findings may, there-
fore, suggest that inactivation is neither a sufficient nor a
necessary prerequisite for high-affinity block of HERG
channels by droperidol.

Consistent with its potency to inhibit IKr, droperidol
prolonged the AP in guinea pig cardiomyocytes. At
concentrations between 5 and 100 nM, droperidol in-
creased APD in a concentration-dependent manner with
a maximal increase of approximately 20% at 100 nM.
Further increases of the droperidol concentration of 300
nM or greater, however, attenuated the AP prolongation
and reversed the effect at even higher concentrations.
This dual concentration-dependent effect on APD of
ventricular cardiomyocytes may explain why some au-
tors found AP shortening,8,9 whereas others found AP
prolongation11,12 or no effect on APD.10 The potency of
droperidol to prolong APD and to induce EADs is a
matter of debate.8-12 Droperidol at antiemetic concen-
trations is estimated to reach free plasma concentrations

| Table 4. Influence of Droperidol on Action Potential Characteristics |
|-------------|-------------|-----------------|-----------|
| Vm, mV       | Overshoot, mV | APD90% of Control | n         |
| Control      | −89 ± 2      | 46.5 ± 4.8       | —         | 45        |
| Droperidol   |              |                  |           |
| 5 nM         | −91 ± 3      | 40.6 ± 2.6       | 104 ± 4*  | 5         |
| 10 nM        | −88 ± 4      | 41.5 ± 3.9       | 109 ± 1†  | 4         |
| 30 nM        | −88 ± 6      | 45.5 ± 1.6       | 112 ± 5‡  | 7         |
| 100 nM       | −90 ± 4      | 46.5 ± 4.9       | 117 ± 5‡  | 9         |
| 300 nM       | −92 ± 4      | 44.1 ± 1.3       | 102 ± 4   | 6         |
| 1 μM         | −86 ± 2      | 45.2 ± 1.9       | 91 ± 3‡  | 7         |
| 10 μM        | −89 ± 5      | 43.0 ± 4.5       | 79 ± 10†  | 7         |

APD90, and table 4 summarizes AP details. Droperidol
influenced neither the resting membrane potential nor
the AP overshoot. At low concentrations (5–100 nM),
droperidol increased APD in a concentration-dependent
manner with a maximal prolongation of 17 ± 5% ob-
served at a concentration of 100 nM. At a concentration
of 300 nM, the AP prolongation was abolished, whereas
even higher concentrations of up to 10 μM shortened
APD compared with control.
INTERACTION OF DROPERIDOL WITH HERG CHANNELS

Fig. 7. Computational modeling of cardiac action potentials of epicardial, midmyocardial, and endocardial myocytes using the Luo-Rudy dynamic model. (A) Simulation of ventricular action potentials under standard conditions (control) and with 50% inhibition of $I_{CaL}$. Action potential prolongation was larger in endocardial (20 ms or approximately 9%) than in epicardial myocytes (13 ms or approximately 7%). In midmyocardial cells, repolarization did not occur within one stimulation interval, and early afterdepolarizations developed. (B) Simulation of control action potentials and with 50% inhibition of $I_{Kr}$ and 40% inhibition of $I_{CaL}$. While the action potential duration in midmyocardial cells was prolonged by 28 ms (approximately 10%), such a combined channel block abolished the prolongation of the action potential duration in endocardial and epicardial cells. No early afterdepolarizations developed. All simulations were performed with a basic cycle length of 1,000 ms for 500 cycles at body temperature.

of approximately 30 nM.³⁷–³⁹ At this concentration, $I_{Kr}$ would be inhibited by a maximum of 50%.¹² Assuming that droperidol at these plasma concentrations exclusively inhibits HERG currents, computational modeling based on the Luo-Rudy dynamic model would predict APD prolongation and induction of EADs (fig. 7A). However, EADs were not observed in our experiments with ventricular cardiomyocytes. Furthermore, EADs and severe ventricular arrhythmias have not been reported during the antiemetic application of droperidol.²,⁴ Our results may, therefore, suggest that despite being a high-affinity blocker of HERG channels, additional pharmacologic effects of droperidol may counterbalance these proarrhythmic effects.

It is well known that several HERG blockers exhibit additional effects on $Ca^{2+}$ channels.⁴⁰–⁴³ Because $I_{CaL}$ is involved in the development of EADs,⁴⁴–⁴⁸ blockade of this current has repeatedly been suggested to diminish the proarrhythmic effects induced by inhibition of HERG channels.³²,⁴⁹,⁵⁰ The calcium channel antagonist verapamil also is a highly potent inhibitor of $I_{Kr}$ and specifically interacts with HERG channels.⁴⁰ Despite its specific effects on HERG channels, verapamil abolishes EADs and does not induce torsades de pointes ventricular arrhythmia.⁴¹–⁴⁵,⁴⁷,⁵¹–⁵⁴ Although direct effects of droperidol on $I_{CaL}$ are unknown, indirect evidence suggests that droperidol interacts with $I_{CaL}$.⁸,⁹,¹¹,⁵⁵ Droperidol has recently been reported to decrease the potassium chloride–induced increase of $Ca^{2+}$ concentration in myocytes, indicative of an interaction with $I_{CaL}$.⁵⁵ Inhibition of $I_{CaL}$ would explain the AP shortening by droperidol reported in the current study and previous studies.⁸,⁹ Therefore, we simulated 50% $I_{Kr}$ block with different degrees of $I_{CaL}$ block. Computational modeling indicates that inhibition of $I_{CaL}$ by 40% would prevent the occurrence of EADs (fig. 7B). It may, therefore, be hypothesized that concomitant inhibition of $I_{Kr}$ and $I_{CaL}$ prevents induction of EADs by droperidol in cardiomyocytes. Such a dual mechanism has been established for verapamil and also for the neuroleptic agent risperidone.⁴³,⁵⁰ Furthermore, this dual effect may offer an explanation why severe ventricular cardiac arrhythmia have not been reported after application of droperidol at antiemetic doses.²,⁴ In conclusion, this study provides evidence that droperidol is a high-affinity blocker of HERG channels causing only minor alterations of channel gating. The residues Thr623, Ser624, Val625, Tyr652, and Phe656 are important for the drug effect. Despite this molecular mode of interaction, droperidol does not induce EADs in ventricular cardiomyocytes. Computational modeling allows us to hypothesize that interaction with other depolarizing currents such as $I_{CaL}$ may explain why droperidol at antiemetic concentrations prolongs the APD without inducing EADs. This hypothesis warrants further investigation.

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