British Journal of Pharmacology (1997) 120, 968–974

The inhibitory effect of the antipsychotic drug haloperidol on HERG potassium channels expressed in *Xenopus* oocytes

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1 The antipsychotic drug haloperidol can induce a marked QT prolongation and polymorphic ventricular arrhythmias. In this study, we expressed several cloned cardiac K^+ channels, including the human ether-a-go-go related gene (HERG) channels, in *Xenopus* oocytes and tested them for their haloperidol sensitivity.

2 Haloperidol had only little effects on the delayed rectifier channels Kv1.1, Kv1.2, Kv1.5 and I_{sK}, the A-type channel Kv1.4 and the inward rectifier channel Kir2.1 (inhibition <6% at 3 μM haloperidol).

3 In contrast, haloperidol blocked HERG channels potently with an IC_{50} value of approximately 1 μM. Reduced haloperidol, the primary metabolite of haloperidol, produced a block with an IC_{50} value of 2.6 μM.

4 Haloperidol block was use- and voltage-dependent, suggesting that it binds preferentially to either open or inactivated HERG channels. As haloperidol increased the degree and rate of HERG inactivation, binding to inactivated HERG channels is suggested.

5 The channel mutant HERG S631A has been shown to exhibit greatly reduced C-type inactivation which occurs only at potentials greater than 0 mV. Haloperidol block of HERG S631A at 0 mV was four fold weaker than for HERG wild-type channels. Haloperidol affinity for HERG S631A was increased four fold at +40 mV compared to 0 mV.

6 In summary, the data suggest that HERG channel blockade is involved in the arrhythmogenic side effects of haloperidol. The mechanism of haloperidol block involves binding to inactivated HERG channels.

Keywords: Haloperidol; human ether-a-go-go related gene (HERG); K^+ channel; arrhythmia, torsades de pointes

Introduction

Torsades de pointes is a life-threatening form of polymorphic ventricular tachycardia with a characteristic ECG morphology where the points of the QRS complexes appear to twist around the isoelectric line (Gallagher, 1985). This arrhythmia typically occurs in the setting of a prolonged QT interval (Stratmann & Kennedy, 1987), reflecting delayed myocardial repolarization and prolonged action potential duration. Prolongation of the QT interval may be congenital or may result from electrolyte abnormalities, dietary deficiencies, toxins or exposure to a number of medications (Jackman *et al*., 1988) such as class IA and class III antiarrhythmic drugs (Zipes, 1987), phychotropic agents, vasodilators or the non-sedating histamine receptor antagonists, terfenadine and astemizole (Simons *et al*., 1988; Monohan *et al*., 1990).

Haloperidol, first synthesized by Janssen Laboratories in 1958, is a widely used potent butyrophenone antipsychotic drug (DiMascio, 1972). To date, several cases of QT prolongation and torsades de pointes have been found with the oral use of haloperidol (Fayer, 1986; Henderson *et al*., 1991) and recently also after parenteral administration (Metzger & Friedman, 1993; Hunt & Stern, 1995). The prolongation of the QT interval seen during haloperidol cardiotoxicity suggests that delayed repolarization may play a role in the arrhythmogenic potential of this medication. To determine the cellular electrophysiological basis for the arrhythmogenic effects of this drug, we investigated the action of haloperidol on various cardiac potassium channels (K^+ channels). Special attention was paid to HERG channels, which are likely to represent the K^+ conductance I_{Kr} in cardiac myocytes (Sanguinetti *et al*., 1995). I_{Kr} contributes significantly to the action potential repolarizing currents, as inhibition of I_{Kr} prolongs action potential duration (Sanguinetti *et al*., 1991). HERG channels expressed in *Xenopus* oocytes show identical biophysical properties as the cardiac K^+ conductance I_{Kr} (Sanguinetti *et al*., 1991; 1995). Furthermore, HERG mutations are associated with inherited long QT-2 syndrome suggesting a likely cellular mechanism for torsades de pointes (Curran *et al*., 1995). This provides a mechanistic link between some forms of inherited and drug-induced prolongation of the QT interval.

Methods

Handling and injection of *Xenopus* oocytes and synthesis of cRNA have been described previously in detail (Busch *et al*., 1996). The two-microelectrode voltage-clamp configuration was used to record currents from *Xenopus laevis* oocytes. In several sets of experiments, oocytes were individually injected with cRNA encoding for the HERG K^+ channels (Warmke & Ganetzky, 1994), HERG S631A (Schönherr & Heinemann, 1996), human I_{sK} (Murai *et al*., 1989), rat Kv1.1 (Stühmer *et al*., 1988), rat Kv1.2 (McKinnon, 1989), rat Kv1.4 (Stühmer *et al*., 1989), human Kv1.5 (Kamb *et al*., 1989) or rat Kir2.1 (Fakler *et al*., 1994). Recordings were performed at 22°C with a Geneclamp amplifier (Axon Instruments, Foster City, U.S.A.) and MacLab D/A converter and software for data acquisition and analysis (AD Instruments, Castle Hill, Australia). To estimate deactivation kinetics (τ_{deact}) of HERG

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channels, a single exponential function was fitted to the tail currents at −85 mV after depolarizations to −15 mV. The control solution contained (mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5 (titrated with NaOH to pH 7.4). The microelectrodes were filled with 3 M KCl solution and had resistances between 0.5 to 0.9 MΩ. Haloperidol and its metabolite, reduced haloperidol, were purchased from Sigma. Both were prepared as stock solutions in dimethylsulphoxide (DMSO). Data are presented as arithmetic means ±s.e.mean and *n* represents the number of experiments performed. Relationships between concentration and blocking effect were calculated with the Hill equation. Student's *t* test was used to test for statistical significance, which was assumed to be obtained for $P<0.05$.

Results

Injection of oocytes with the cRNAs encoding for Kv1.1, Kv1.2, Kv1.4, Kv1.5, I_{sK} and Kir2.1 channels resulted in the induction of K^+ currents as previously described (Stühmer *et al.*, 1988; 1989; McKinnon, 1989; Kamb *et al.*, 1989; Murai *et al.*, 1989; Fakler *et al.*, 1994). Kv1.1, Kv1.2 and Kv1.5, which may underly the cardiac ultrarapid potassium current I_{Kur} (Paulmichl *et al.*, 1991; Fedida *et al.*, 1993; Tamkun *et al.*, 1994), exerted fast-activating, non-inactivating outward currents. Kv1.4 channels, which may underly the cardiac transient outward current I_{to} (Po *et al.*, 1993), showed both fast activation and inactivation. The I_{sK} channel which, does not fully activate even after 15 s of depolarizing stimulus, resembles the slow delayed rectifier I_{Ks}, described in guinea-pig and human cardiac myocytes (Busch *et al.*, 1994; Wang *et al.*, 1994). The inward rectifier Kir2.1 may resemble a component of the cardiac conductance, I_{K1}, which is primarily responsible for the maintenance of the resting membrane potential. Haloperidol (at 3 μM) had only weak or no blocking effect on the above channels (Figure 1). The blockade was $3.4\pm0.9\%$ ($n=4$) for Kv1.1 at 0 mV, $5.1\pm1.2\%$ ($n=4$) for Kv1.4 at 0 mV, $1.4\pm0.7\%$ ($n=4$) for Kv1.5 at 40 mV and $5.5\pm1.0\%$ ($n=4$)

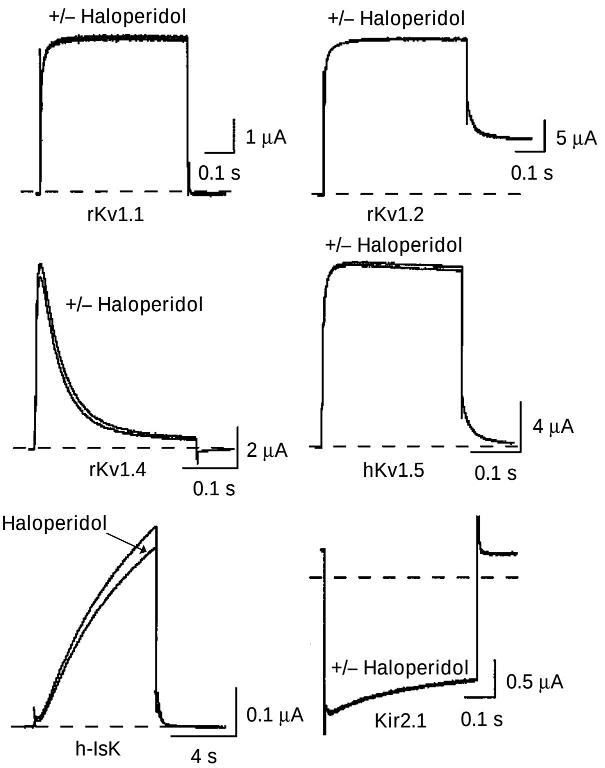

Figure 1 Effects of 3 μM haloperidol on rat Kv1.1, rat Kv1.2, rat Kv1.4, human Kv1.5, human I_{SK} and rat Kir2.1 channels expressed in *Xenopus* oocytes. Outward currents through Kv1.1 and Kv1.4 channels were evoked with 0.5 s and 0.3 s depolarizing pulses, respectively, to 0 mV every 3 s. K^+ outward currents through Kv1.2 and Kv1.5 were evoked every 3 s with 0.3 s depolarizing pulses to 0 mV and 40 mV, respectively; tail currents were analysed at −30 or −80 mV. The holding potential for all Kv channels was −80 mV and the currents were filtered at 1 kHz. I_{SK} was evoked with 15 s voltage steps to −10 mV from a holding potential of −80 mV (filtered at 10 Hz). Kir2.1 currents were evoked with 0.5 s polarizing pulses to −120 mV from a holding potential of −40 mV (filtered at 1 kHz). The amplitudes of the recorded currents were measured at the end of the test voltage steps; currents through inactivating Kv1.4 channels were measured at their maximum.

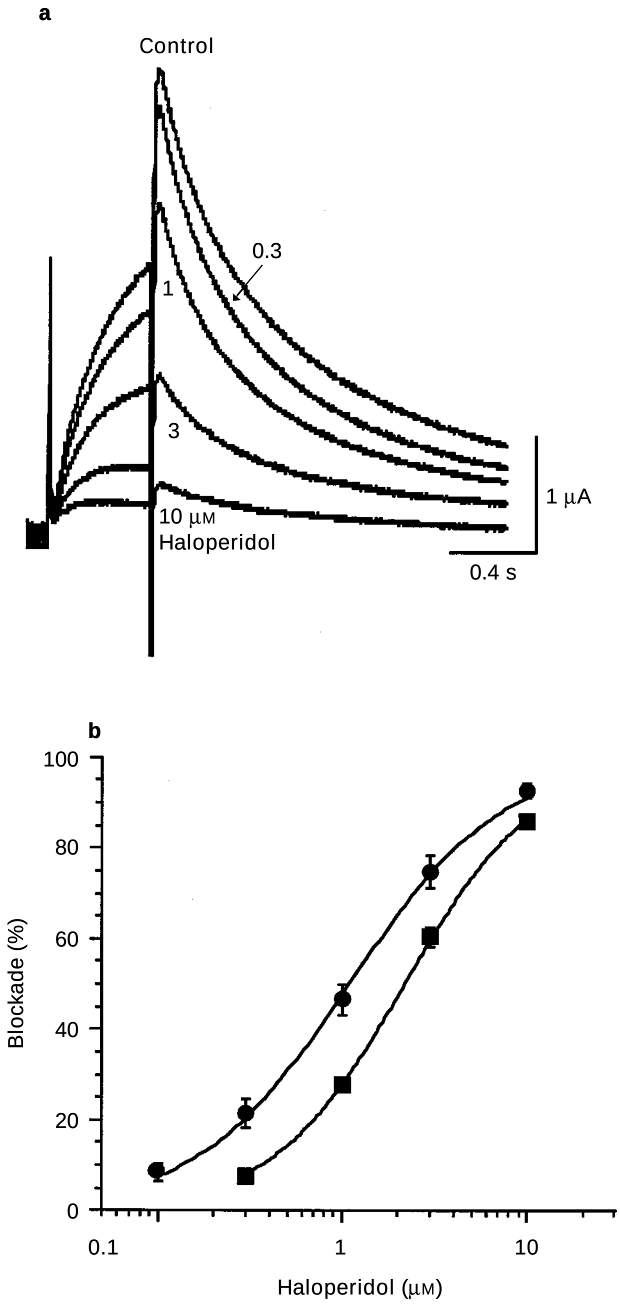

Figure 2 Concentration-dependent blockade of HERG channels by haloperidol. (a) HERG currents were evoked with 0.5 s depolarizing pulses to 0 mV from a holding potential of −80 mV every 3 s. Tail currents were recorded at −70 mV (filtered at 0.5 kHz). (b) The relationship between concentration and blockade by haloperidol of HERG channel tail currents after depolarizations to 0 (■) and +40 (●) mV. The Hill equation was fitted to the data with 100% blockade as a fixed maximal effect. Data are given as arithmetic means and vertical lines show s.e.mean.

for I_{sK} channels at −10 mV. There was no effect of 3 μM haloperidol on either Kv1.2 ($n=4$) at 0 mV or on Kir2.1 ($n=4$) at −120 mV. Based on the entire concentration-effect relationship, haloperidol blocked I_{sK} channels with an IC_{50} value of 22.6±1.9 μM ($n=4$).

Injection of oocytes with cRNA encoding for HERG channels resulted in the induction of a K^+ conductance with previously described activation and rectification properties (Sanguinetti *et al.*, 1995). Channels were activated by depolarization, but because of their more rapid C-type inactivation compared to their activation, outward currents at 0 mV were relatively small. However, the tail currents, obtained during repolarizing steps to −70 mV were large as a result of a fast relief from inactivation combined with slow deactivation. Haloperidol blocked both the relatively small outward currents during 0.5 s depolarizing pulses to 0 mV and the large tail outward current at −70 mV (holding potential was −80 mV; interval of pulses was 3 s). Figure 2a shows original current traces illustrating the concentration-dependent blockade of HERG channels by haloperidol. Analysis of the blockade of the tail currents with the Hill equation resulted in an IC_{50} value of 2.2±0.1 μM and a Hill coefficient of 1.2 ($n=5$). Depolarizations to 40 mV at the same frequency increased the apparent affinity for haloperidol (IC_{50}: 1.1±0.2 μM; Hill coefficient: 1.1; $n=5$; Figure 2b). Under the same conditions, the primary hydroxymetabolite of haloperidol (reduced haloperidol) produced a half-maximal block at 2.6±0.5 μM (Hill coefficient: 1.1; $n=5$; data not shown). The effects of haloperidol and reduced haloperidol on HERG channels were completely reversible upon washout (washout period >5 min).

To study the voltage-dependence of block more extensively, the effects of haloperidol on HERG current-voltage relationship and deactivation kinetics were analysed in more detail. Three second voltage steps from −45 to 30 mV (increment 15 mV, Figure 3a) were performed and the tail currents measured and it was found that the voltage required to half-maximally activate HERG channels was shifted from −22.5±2.8 mV under control conditions ($n=5$) to −30.1±2.0 mV after 1 μM haloperidol (Figure 3b; $n=5$). As can be seen in Figure 3 the conductance of HERG channels decreased at depolarized potentials (>0 mV) in the presence of haloperidol as a result of the increased block. Please note in Figure 3 that the outward currents at potentials >−15 mV decreased as a result of an increased C-type inactivation, whereas the tail currents were increased at more depolarized potentials. This is a consequence of both an increased activation at positive potentials and a rapid relief of the inactivation at very negative potentials.

Blockade of HERG channels by haloperidol was strongly use-dependent. To analyse this use-dependence, HERG channels were activated by 0.5 s depolarizing steps to 40 mV at

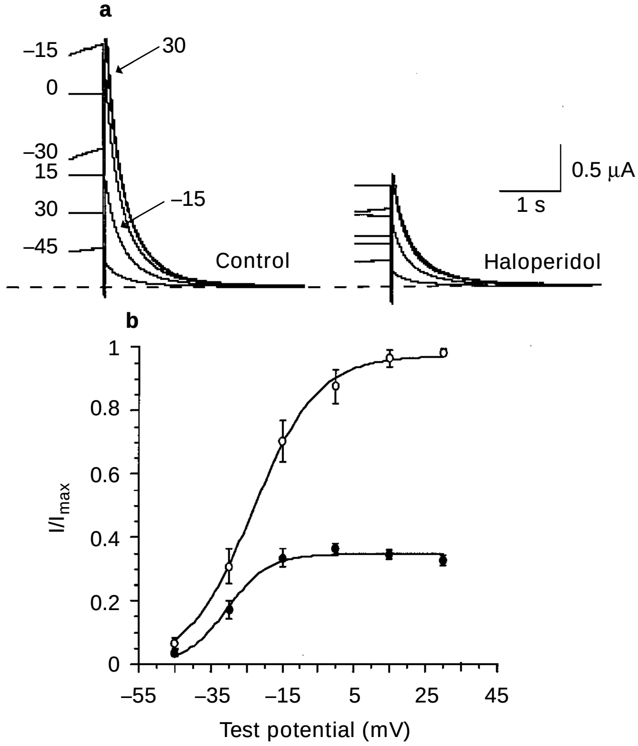

Figure 3 (a) Current traces showing the voltage-dependence of HERG channel activation and its blockade by haloperidol. Currents were evoked by 3 s depolarizing pulses from −45 mV to +30 mV (increment 15 mV), from a holding potential of −80 mV every 5 s. Only the final 0.5 s of the currents at the test potentials are shown. Please note that the outward currents at the test potentials decrease at potentials >−15 mV whereas activation increases with further depolarization as indicated for the tail currents at −15 and +30 mV. (b) Analysis of the effect of 1 μM haloperidol (●, $V_{0.5}$: −30.1±2.0 mV) on HERG channel current-voltage relationship. Tail current amplitudes were normalized relative to the maximum value obtained under control conditions (○, $V_{0.5}$: −22.5±2.8 mV). $V_{0.5}$ indicates the calculated voltage needed to evoke half-maximal HERG channel currents. Data are given as arithmetic means; vertical lines show s.e.mean.

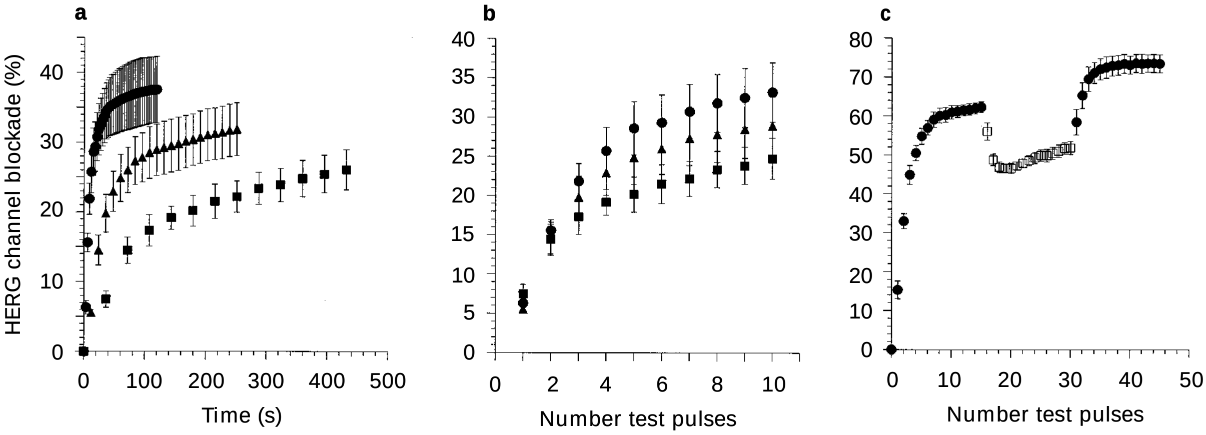

Figure 4 (a) Use-dependent HERG channel blockade by 1 μM haloperidol. Tail currents were recorded at −85 mV after a 0.5 s depolarizing pre-pulse to +40 mV from a holding potential of −80 mV every 3 s (●), 12 s (▲) and 36 s (■), respectively. Before the first depolarizing pulse (time=0) was administered the oocytes were superfused for 30 s with the drug-containing solution to warrant complete exchange of the bath. (b) The HERG channel blockade data from (a) were plotted against the number of test pulses. (c) Steady-state HERG channel blockade by 1 μM haloperidol after 15 test pulses at 3 s intervals (●). Increasing the depolarization intervals to 36 s in the presence of haloperidol (□) resulted in a partial relief, and changing back to 3 s intervals increased HERG channel blockade again. Data are given as arithmetic means; vertical lines show s.e.mean.

intervals of 3 s, 12 s or 36 s in the presence of 1 μM haloperidol ($n=4$). Figure 4a illustrates that the time course in which HERG channel blockade occurs is dramatically dependent upon the activation frequency, but not on the time of drug superfusion. HERG-blockade by haloperidol occurred much faster at higher activation frequencies. In Figure 4b the HERG blockade data from Figure 4a are plotted as function of the number of test pulses at different intervals. After the same number of test pulses the block of 1 μM haloperidol was somewhat weaker at a low activation frequency than at higher activation frequencies, indicating favoured unbinding of the drug at low frequencies. In additional experiments, steady-state HERG channel blockade by haloperidol was initially obtained with depolarizations at 3 s intervals. Subsequent increase of the depolarization interval to 36 s resulted in a partial relief of HERG channel blockade (see Figure 4c).

The use- and voltage-dependence of haloperidol-mediated HERG blockade suggests that the drug binds to the open or inactivated state of HERG channels. One feature of open-channel blockers is their partial unbinding from the open channel during deactivation, thereby increasing the apparent deactivation time constant, τ_{deact} (Yang *et al*., 1995). However, haloperidol (1 μM) did not alter the rate of HERG deactivation at -85 mV; τ_{deact} was 305 ± 9 ms under control and 312 ± 18 ms at 1 μM haloperidol.

Figure 5a shows a series of tail currents under control conditions and with 1 μM haloperidol. HERG channels were activated with 20 ms, 40 ms, 80 ms, 160 ms, 320 ms and 640 ms depolarizing steps to 40 mV at a pulse interval of 3 s. The traces in Figure 5a show that with depolarizing steps $\geqslant$ 160 ms peak tail currents reached steady-state, i.e. HERG channels were maximally activated. Although HERG channel activation was maximal after 160 ms depolarizations, HERG blockade continued to increase with longer durations of the test pulses from 36.0 $\pm$ 2.3% at 160 ms to 57.4 $\pm$ 2.2% at 640 ms (see Figure 5b; $n=4$). Therefore, the fraction of open channels does not exclusively determine the degree of blockade. This indicates either that haloperidol block of open channels occurs at a very slow rate or that haloperidol binds to inactivated channels.

To test the effects of haloperidol on HERG inactivation kinetics, the onset of this inactivation process was revealed by a modified voltage-clamp pulse protocol. After HERG channel activation with long depolarizing pulses (>15 s), the membrane was hyperpolarized for 20 ms. This brief hyperpolarization was sufficient for recovery from inactivation, but too short to cause significant deactivation. After this short hyperpolarization, the depolarization-mediated initial outward

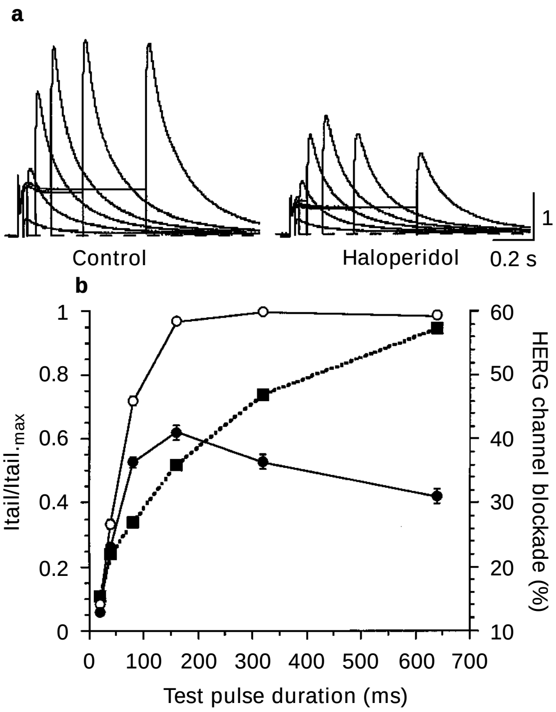

Figure 5 (a) Envelope of tail test for HERG channels under control conditions and at 1 μM haloperidol. HERG channels were activated with 20 ms, 40 ms, 80 ms, 160 ms, 320 ms and 640 ms depolarizing steps to 40 mV at a pulse interval of 3 s. Currents were measured after steady-state block had been achieved (see Figure 4a). (b) The relative peak tail currents under control conditions (○), at 1 μM haloperidol (●) and the HERG channel blockade (■) are plotted against the test pulse duration. Data are given as arithmetic means; vertical lines show s.e.mean.

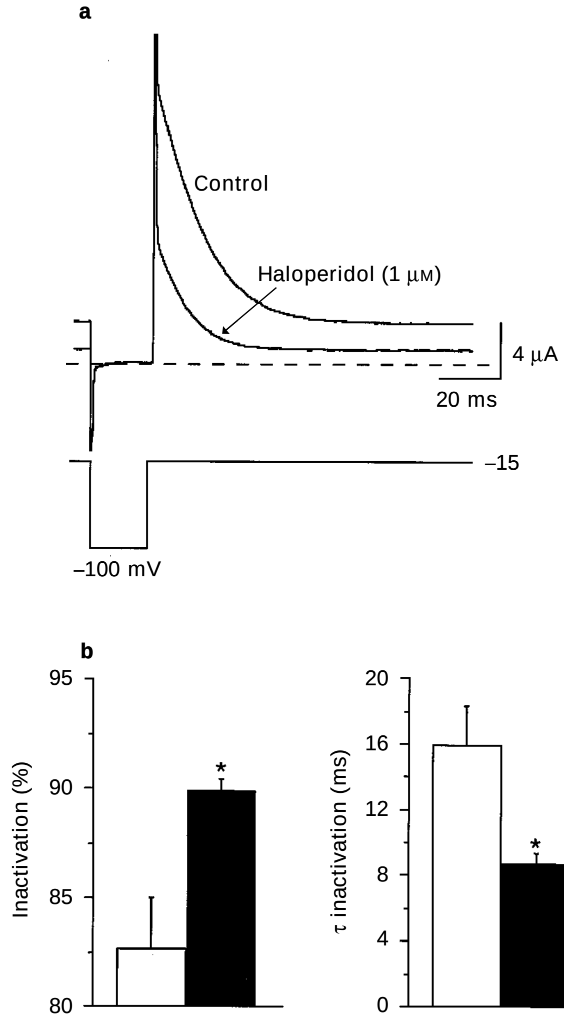

Figure 6 Effects of haloperidol on HERG inactivation. (a) The onset of this inactivation process was revealed by a modified voltage-clamp pulse protocol illustrated below the current trace. After a long depolarizing pulse (>15 s) to -15 mV to activate the HERG channels maximally, the membrane was hyperpolarized to -100 mV for 20 ms for a rapid relief of inactivation. The instantanous outward current observed after depolarization back to -15 mV reflects the activated HERG channel population at -15 mV. This instantanous current inactivated again to the outward currents recorded before the hyperpolarizing voltage step. Please note that the tail currents reflect inactivating but not deactivating HERG channels. The dashed line indicates 0 current. (b) The percentage of inactivation of HERG channels was calculated from the currents measured immediately after the hyperpolarizing pulse and the steady-state current at -15 mV under control conditions (□) and 1 μM haloperidol (■). Capacitive currents were subtracted. Inactivation time constants were obtained by fitting the declining currents at -15 mV with a single exponential function. Data are given as arithmetic means; vertical lines show s.e.mean.

current reflects approximately the activated HERG channel population which then again inactivates rather quickly to the current level before the short hyperpolarization (Smith *et al.*, 1996; Schönherr & Heinemann, 1996). Therefore the tail currents shown in Figure 6a reflect HERG channel inactivation but not deactivation. The inactivation time course at −15 mV could be fitted by a single exponential function revealing a time constant (τ_{inact}) of 15.9 ± 2.4 ms ($n=5$). At 1 μM haloperidol, τ_{inact} decreased to 8.7 ± 0.6 ms ($n=5$). Furthermore, the extent of inactivation increased significantly from 82.7 ± 2.4% under control conditions to 89.9 ± 0.6% ($n=5$) in 1 μM haloperidol (Figure 6b). These data demonstrate that haloperidol affects HERG inactivation.

Recently, Schönherr & Heinemann (1966) presented data on a HERG mutant (HERG S631A) with a greatly reduced C-type inactivation. Because we hypothesized that haloperidol affects inactivation of HERG channels, this less-inactivating channel mutant was tested for its haloperidol sensitivity. HERG S631A channels showed indeed a reduced affinity for haloperidol (Figure 7a). Analysis of the data with the Hill equation revealed IC_{50} values of 8.3 ± 1.7 μM ($n=4$) and 1.9±0.2 μM ($n=4$) at depolarizing pulses (0.5 s) to 0 and 40 mV, respectively (Figure 7b).

Subsequently, we compared the inactivation properties from HERG wild-type and mutant channels by the same method as described for the determination of τ_{inact} and increased the holding potentials from −45 to 45 mV in increments of 15 mV. Figure 8a illustrates that the HERG S631A channels inactivated at potentials ⩾ 0 mV whereas wild-type channels were already greatly inactivated at −45 mV (Figure 8b; $n=5$). At 45 mV the inactivation was 37.3 ± 2.5% and 96.4 ± 0.4% for HERG S631A and HERG channels, respectively.

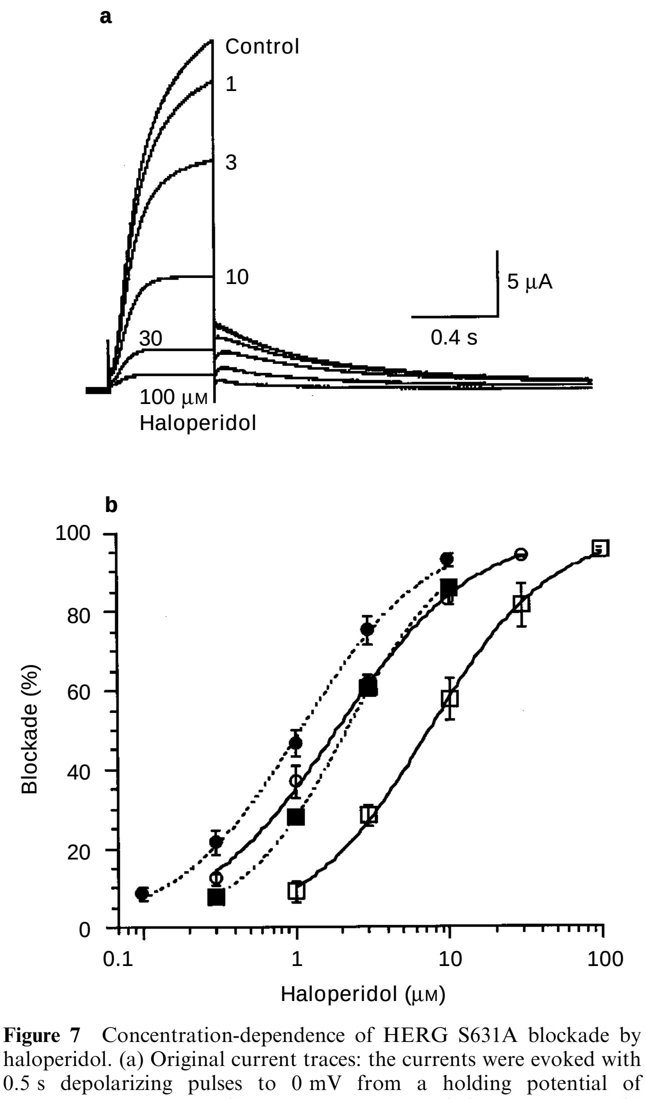

Figure 7 Concentration-dependence of HERG S631A blockade by haloperidol. (a) Original current traces: the currents were evoked with 0.5 s depolarizing pulses to 0 mV from a holding potential of −80 mV every 3 s. Tail currents were recorded at −70 mV. (b) Relationship between concentration and blockade by haloperidol of HERG S631A obtained for currents evoked with 0.5 s depolarizing pulses every 3 s to 0 mV (□) or to 40 mV (○), respectively. For comparison concentration-dependent blockade by haloperidol of HERG wild-type channel tail currents after depolarizations to 0 (■) and +40 (●) mV are shown. The data were fitted with a Hill equation with 100% blockade as a fixed maximum. Data are given as arithmetic means; vertical lines show s.e.mean.

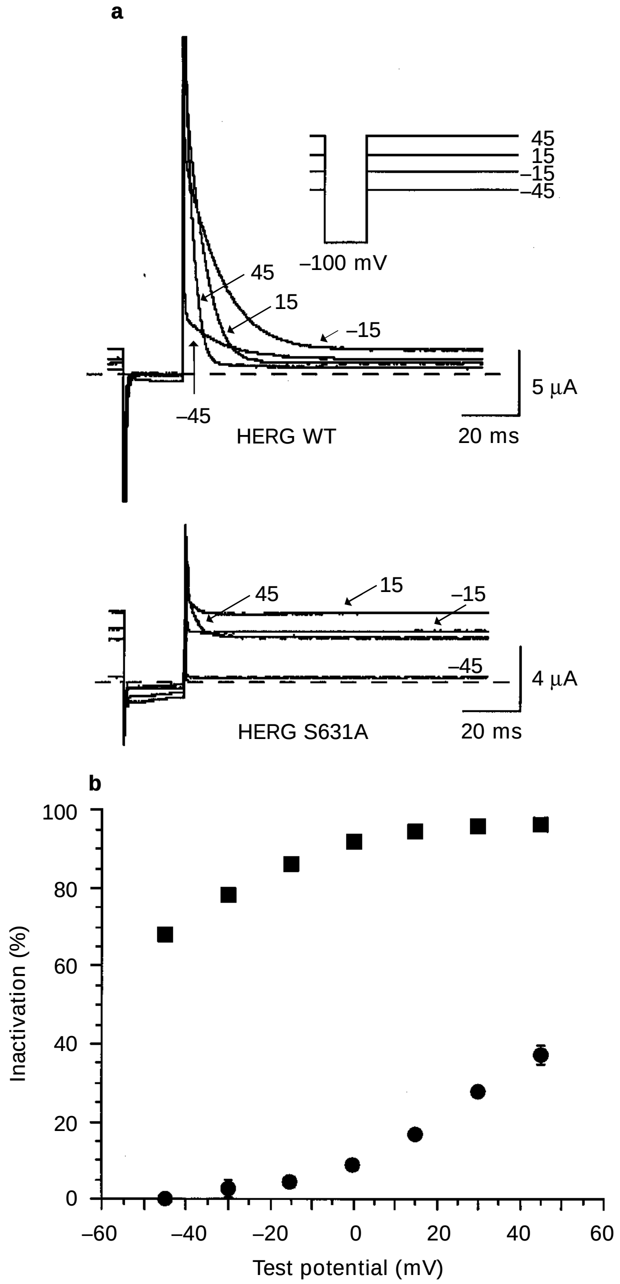

Figure 8 Inactivation properties of HERG wild-type (■) and HERG S631A (●) channels. (a) The holding potential was altered from −45 mV to +45 mV (increment 15 mV) and 20 ms steps to −100 mV were applied to cause a relief from inactivation. The traces at −30, 0 and +30 mV were omitted for reasons of clarity. (b) Fractional inactivation of HERG channels was calculated from the currents measured immediately after the hyperpolarizing interpulse and the steady-state current at the holding potential. Data are given as arithmetic means; vertical lines show s.e.mean.

Finally, we analysed HERG S631A blockade by haloperidol for its voltage-dependence. We tested the steady-state effects of haloperidol on HERG S631A channel currents during 3 s depolarizing pulses to −30 mV up to +45 mV (increment 15 mV; interval 3 s) and plotted the blockade against the voltage (Figure 9b). Haloperidol 1 μM blocked HERG S631A channels by 12.4 ± 1.8% (n=5) on depolarizing to −30 mV and by 48.9 ± 3.1% (n=5) at +45 mV (Figure 9a,b). The increased block of HERG S631A with 1 μM haloperidol, therefore, coincided with an increase in HERG S631A inactivation.

Discussion

Several cases of severe ventricular arrhythmias associated with haloperidol therapy have been documented (Henderson *et al.*, 1991; Metzger & Friedman, 1993; Hunt & Stern, 1995). How haloperidol might influence heart excitability is unknown, but the QT prolongation in the ECG indicates that blocking effects of haloperidol on repolarizing potassium currents might be involved (Hunt & Stern, 1995). This study was therefore designed to identify the putative cardiac targets for the action of haloperidol and to identify its mechanism of action. For this purpose we tested distinct K^+ channels, previously shown to be expressed in the heart, for their haloperidol sensitivity.

In the present study haloperidol and its reduced hydroxymetabolite blocked HERG channels much more potently than the other K^+ channels examined, suggesting that HERG channel blockade may underly the cardiotoxicity of haloperidol. Two further points support this hypothesis: HERG blockade occurred at concentrations similar to the plasma concentrations of patients with haloperidol-induced QT prolongation (Ereshefsky *et al.*, 1984; Metzger & Friedman, 1993). Furthermore, genetic defects of HERG or HERG blockade by class III antiarrhythmics are known to prolong the QT interval (Curran *et al.*, 1995; Sanguinetti *et al.*, 1995). Like haloperidol, the non-sedating histamine receptor antagonist terfenadine is a specific HERG channel blocker (Suessbrich *et al.*, 1996) which causes prolongation of the QT interval in some patients (Simons *et al.*, 1988; Monahan *et al.*, 1990). Interestingly, terfenadine and haloperidol share similarities in their chemical structure.

The use- and voltage-dependence of haloperidol block suggests that drug binding occurs to the open or inactivated state of HERG channels. Our results suggest that open-channel binding and unbinding of haloperidol occurs at a very slow rate for two main reasons: (1) the envelope of tails test demonstrated an increase in HERG channel blockade with increasing duration of depolarizing steps even after steady state activation had been reached, and (2) the rate of HERG deactivation was not affected by haloperidol, excluding fast unbinding before channel closing (Yang *et al.*, 1995). Although we cannot exclude slow binding of haloperidol to open channels, we provide evidence that haloperidol binds to inactivated HERG channels. Thus, haloperidol increased both the rate and extent of HERG inactivation. Finally, the HERG channel mutant S613A, which shows markedly reduced inactivation, was significantly less potently blocked by haloperidol. In contrast to HERG wild-type channels, HERG S631A inactivation was only apparent at potentials more positive than 0 mV. The increased inactivation of HERG S631A channels at such potentials corresponded to an increased haloperidol affinity, supporting the hypothesis that the inactivated state of the channel favours haloperidol binding. However, these data cannot exclude voltage-dependent binding of haloperidol (which is positively charged at physiological pH), within the electrical field of the membrane, to open channels. Indeed, the decay of HERG S631A outward currents (obvious in Figure 9a) suggests partial open-channel blockade of the HERG channel mutant.

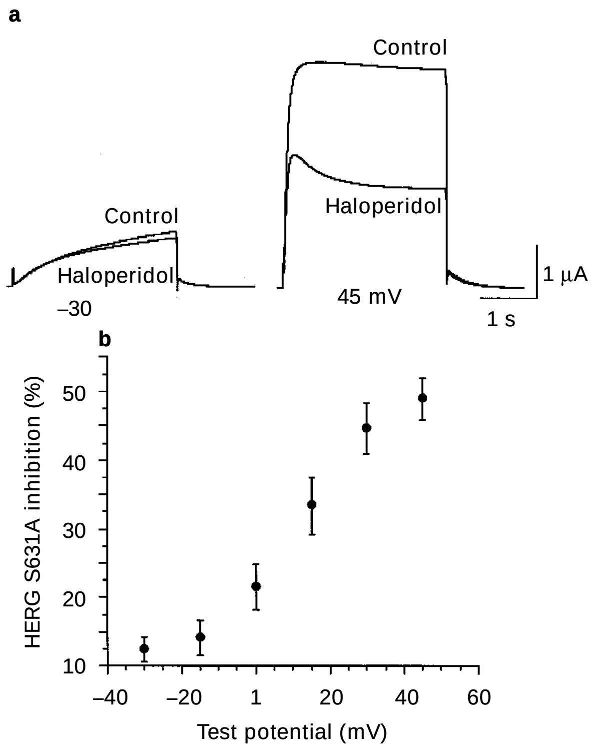

Figure 9 Voltage-dependence of the block by haloperidol of HERG S631A channels. (a) Currents were evoked with 3 s depolarizing pulses to −30 mV and +45 mV, respectively, from a holding potential of −80 mV every 5 s. (b) HERG S631A channel block caused by 1 μM haloperidol was plotted against the test potential. Data are given as arithmetic means; vertical lines show s.e.mean.

In summary, the present study suggests that the arrhythmogenic properties of haloperidol can be attributed to its blockade of cardiac HERG channels. The mechanism of haloperidol block seems to involve a preferential binding to inactivated HERG channels.

A.E.B. is a Heisenberg Fellow. The work was supported by a grant from the Deutsche Forschungsgemeinschaft (Bu 704/3-1 to AEB). The authors are indebted to Drs R. Swanson, J.P. Ruppersberg and M. Keating for providing the I_{sK}-, Kir2.1- and HERG clones and to Dr G.L. Busch for critical reading of the manuscript. We thank B. Noll and E. Sailer for the preparation and handling of oocytes and RNA synthesis.

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(Received October 2, 1996
Revised November 26, 1996
Accepted November 27, 1996)