Redox state dependency of *HERG*S631C channel pharmacology: relation to C-type inactivation

Chris Ulens, Jan Tytgat*

Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Van Evenstraat 4, 3000 Leuven, Belgium

Received 10 March 2000; received in revised form 25 April 2000

Edited by Maurice Montal

Abstract The S631C mutation in human ether-à-go-go-related gene (*HERG*) channels has previously been reported to disrupt C-type inactivation and ion-selectivity when Cys-631 is in the oxidized state. In this study, we report the relation between pharmacology and C-type inactivation for *HERG*S631C channels. We demonstrate that *HERG*S631C in its reduced state is fully blocked by 1 μM astemizole, terfenadine and dofetilide, similar to wild-type *HERG* channels. In contrast, oxidized *HERG*S631C is insensitive for these blockers. Our results suggest that an interaction with *HERG* channels in the inactivated state might be a common mechanism to a variety of drugs known to block *HERG* channels with high affinity.

Key words: S631C; Human ether-à-go-go-related gene (HERG); C-type inactivation

1. Introduction

The human ether-à-go-go-related gene (*HERG*) was initially identified by Warmke and Ganetzky [1] and encodes ion channels that give rise to currents which are nearly identical to the rapidly activating delayed rectifier K^+ current, I_{Kr} [2]. Unlike channels formed only with *HERG*, complexes of *HERG* with MiRP1 resemble native cardiac I_{Kr} channels better in their gating, unitary conductance, regulation by K^+ and distinctive biphasic inhibition by the class III anti-arrhythmic E-4031 [3].

While LQT2, one of the less common forms of the inherited long QT syndrome (LQTS), has been linked to mutations in the *HERG* gene, the acquired form of LQTS can result from therapy with drugs that block *HERG* channels. This interaction with *HERG* channels is considered as the main mechanism underlying the potential cardiotoxic effects caused by a variety of drugs, including second-generation H_1 anti-histamines such as astemizole and terfenadine [4] and class III anti-arrhythmic agents such as dofetilide [5] and amiodarone [6]. LQTS is associated with syncope and sudden death caused by abnormal repolarization and the onset of a rare but life-threatening polymorphic ventricular tachycardia known as torsade de pointes.

Potent *HERG* channel blockers have previously been reported to interact preferentially with channels in either the open state (e.g. dofetilide [5]), the inactivated state (e.g. perhexilline [7]) or the closed state (e.g. ketoconazole [8]). It was previously shown that haloperidol block of *HERG*S631A, a channel mutant with greatly reduced C-type inactivation, was 4-fold weaker than for wild-type (WT) *HERG* channels [4]. A point mutation in the S5–S6 linker region, *HERG*S620T, abolished high-affinity block by dofetilide and also interfered with C-type inactivation [9]. Similar phenomena have been described for E-4031 [10] and nicotine [11].

Very recently, Fan et al. [12] described mutations of S631 to residues of different physicochemical properties and compared the resulting changes in the channel's inactivation process. The most dramatic differences were obtained when this position was occupied by a charged residue (S631K and S631E). When S631 was replaced by a cysteine, the mutant channel behaved like WT *HERG* channels when thiol groups are in the reduced state. Oxidizing thiol groups with H_2O_2 or modifying them with MTSET or MTSES disrupted C-type inactivation and K^+-selectivity, similar to the phenotype of S631K and S631E.

Since the pharmacological properties of *HERG*S631C are unknown, we investigated the effects of dofetilide (interacting preferentially with channels in the open state), astemizole and terfenadine (interacting preferentially with channels in the inactivated state) on *HERG*S631C, expressed in *Xenopus* oocytes. Currents were evoked using the two-microelectrode voltage clamp technique.

2. Materials and methods

2.1. Expression in oocytes

The *HERG*S631C mutant in its original vector, pALTER (Promega, USA), was first subcloned into pGEMHE. The *HERG*S631C clone was isolated by a double restriction digest with *Eco*RI and *Xba*I and ligated into the corresponding sites of pGEMHE. The ligation product, *HERG*S631C/pGEMHE, was linearized with *Xba*I and capped cRNAs were synthesized using the large-scale T7 mMESSAGE mMACHINE transcription kit (Ambion, USA). The in vitro synthesis of cRNA encoding WT *HERG* and isolation of *Xenopus* oocytes was as previously described [13]. Oocytes were injected with *HERG* or *HERG*S631C cRNA at a concentration of 10 ng/50 nl.

2.2. Electrophysiological recordings and analysis

Whole-cell currents from oocytes were recorded from 1 to 4 days after injection using the two-microelectrode voltage clamp technique (GeneClamp 500, Axon Instruments, USA). Resistances of voltage and current electrodes were kept as low as possible (approximately 200 kΩ) and were filled with 3 mol/l KCl. Currents were filtered at 200 Hz, using a four-pole low-pass Bessel filter. Capacitative and leak currents were not subtracted. To eliminate the effect of the voltage drop across the bath grounding electrode, the bath potential was actively controlled. All experiments were performed at room temperature (19–23°C). The oocytes were superfused with ND-96 solution (composition in mmol/l: KCl 2, NaCl 96, $MgCl_2$ 1, $CaCl_2$ 1.8, HEPES 5, pH 7.5). For incubation of oocytes, this solution was sup-

*Corresponding author. Fax: (32)-16-32 34 05.
E-mail: jan.tytgat@farm.kuleuven.ac.be

PII: S0014-5793(00)01586-6

plemented with 50 mg/l gentamicin sulfate. The pCLAMP program was used for data acquisition and data files (Axon Instruments, USA) were directly imported, analyzed and visualized with a custom made add-in for Microsoft Excel.

Current percentages were used for the calculation of concentration–response curves, using the Hill equation:

$$I = I_{max}/[1 + (IC_{50}/A)^{n_H}]$$

where I represents the current percentage, I_{max} the maximal current percentage, IC_{50} the concentration of the agonist that evokes the half-maximal response, A the concentration of agonist, and n_H the Hill coefficient. Averaged data are indicated as mean ± S.E.M. Statistical analysis of differences between groups was carried out with Student's t test and a probability of 0.05 was taken as the level of statistical significance.

2.3. Compounds

Astemizole, terfenadine and dofetilide were kind gifts from the Janssen Research Foundation (Belgium). Compounds were dissolved in DMSO, stored at 5°C and diluted in ND-96 appropriately for the experiments.

3. Results

To compare the pharmacology of WT *HERG* channels with *HERG*S631C channels in the reduced state, we first investigated the effect of astemizole on WT *HERG* channels. *HERG* cRNA was injected in *Xenopus* oocytes and currents were investigated 2 days after injection using the two-microelectrode voltage clamp technique. *HERG* currents were evoked by application of 1 s test pulses ranging from −70 to +30 mV in 10 mV increments from a holding potential of −90 mV. Tail currents were measured at a potential of −70 mV during 1 s (Fig. 1A). Increasing concentrations of astemizole were applied to oocytes repeatedly pulsed to 0 mV with intervals of 1 s from a holding potential of −90 mV (Fig. 1B). Peak tail current amplitudes evoked at −70 mV were then used to construct a concentration–response curve (Fig. 1C). We calculated that *HERG* channels are blocked by astemizole with an IC_{50} value of 0.735 ± 0.223 nM and a Hill coefficient of 0.461 ± 0.041 ($n = 4$). The inhibitory effect of the highest concentration of astemizole (1 μM) was only partially reversible upon long washout periods (> 15 min) with ND-96 (data not shown).

To investigate whether astemizole blocks *HERG*S631C channels, *HERG*S631C cRNA was expressed in oocytes and currents were investigated 2 days after injection. Currents were evoked using the same protocols as for WT *HERG* channels but with longer pulse intervals (8 s) since it has been reported that repeated pulses with high frequency to depolarized potentials result in disruption of C-type inactivation and ion-selectivity of *HERG*S631C [12]. When thiol groups of Cys-631 are in the reduced state, *HERG*S631C currents are characterized by fast inactivation during the test pulse and typical inward rectification of the tail currents [12], resulting in a phenotype similar to WT *HERG* channels (Fig. 2A). Under these conditions, we found that *HERG*S631C currents, evoked by application of pulses to 0 mV with intervals of 8 s from a holding potential of −90 mV, could be completely blocked by 1 μM astemizole, as measured on the peak tail current amplitudes (Fig. 2B).

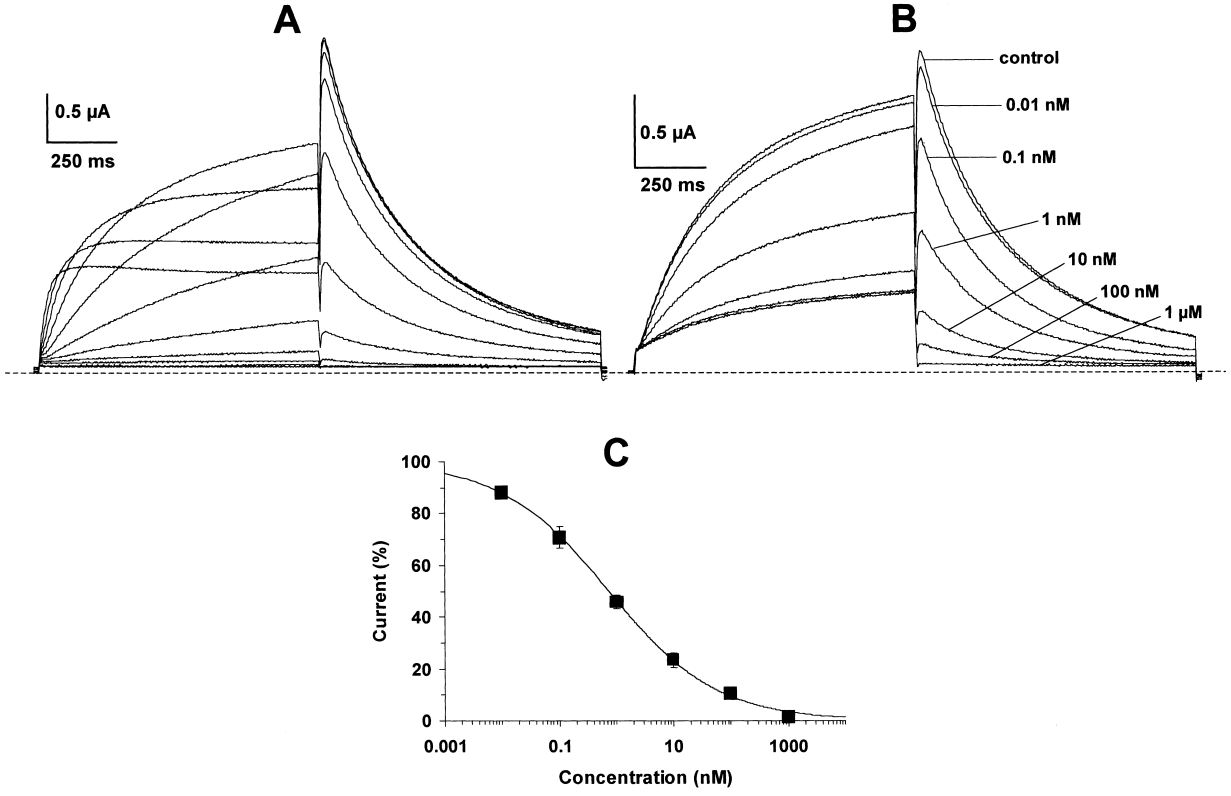

Fig. 1. A: *HERG* currents were evoked under control conditions by application of 1 s test pulses ranging from −70 to +30 mV in 10 mV increments from a holding potential of −90 mV. Tail currents were measured at a potential of −70 mV during 1 s. B: Increasing concentrations of astemizole were applied to oocytes repeatedly pulsed to 0 mV with intervals of 1 s from a holding potential of −90 mV. C: Peak tail current amplitudes evoked at −70 mV were used to construct a concentration–response curve. *HERG* currents are blocked by astemizole with an IC_{50} value of 0.735 ± 0.223 nM and a Hill coefficient of 0.461 ± 0.041 ($n = 4$).

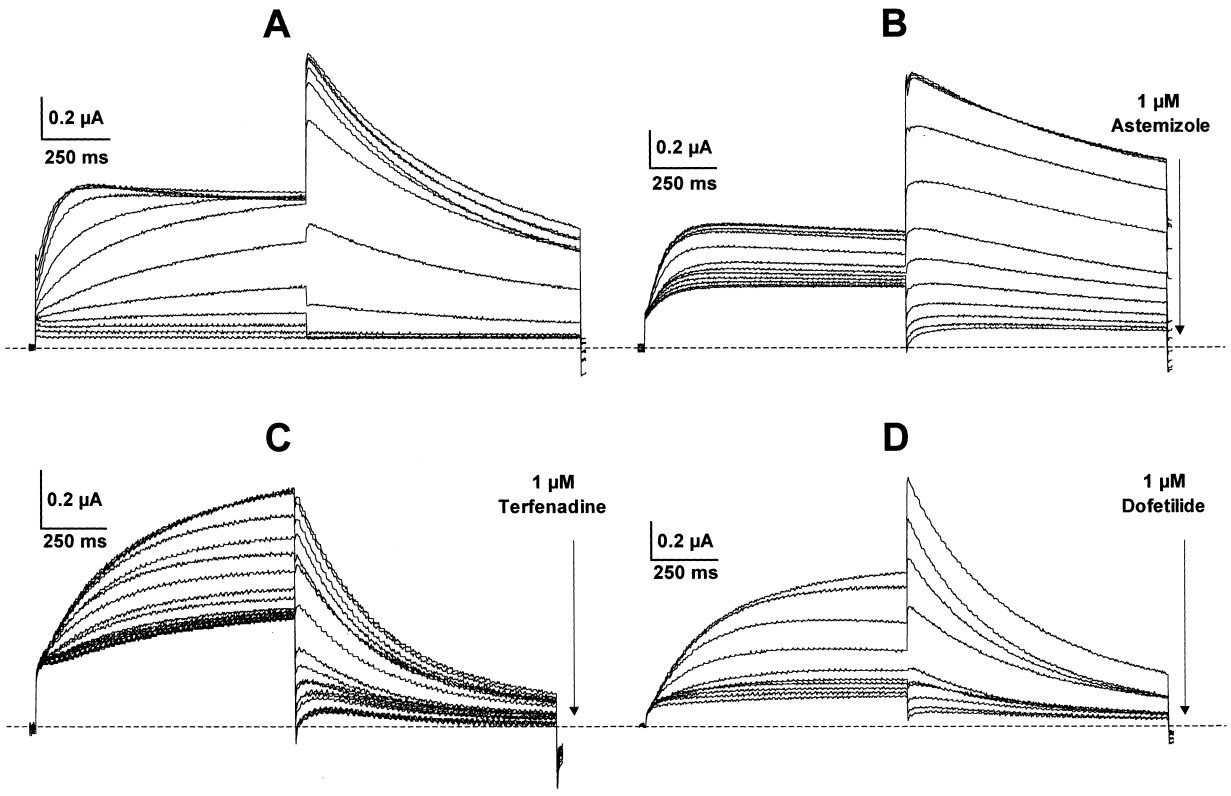

Fig. 2. A: When thiol groups of Cys-631 are in the reduced state, *HERG*S631C currents are similar to WT *HERG* currents. *HERG*S631C currents were evoked using the same protocols as for WT *HERG* currents but with pulse intervals of 8 s. When thiol groups of Cys-631 are in the reduced state, we found that *HERG*S631C currents could be completely blocked by 1 μM astemizole (B), as measured on the peak tail current amplitudes. Other potent *HERG* channel blockers affect *HERG*S631C channels in a similar manner. It was found that 1 μM of terfenadine (C) and dofetilide (D) also completely block *HERG*S631C channels ($n=4$ each). Currents in B, C and D were evoked by application of pulses to 0 mV with intervals of 8 s from a holding potential of −90 mV.

Next, we investigated whether high concentrations of other potent *HERG* channel blockers would affect *HERG*S631C channels in a similar manner. Under identical recording conditions (i.e. thiol groups of Cys-631 in the reduced state), it was found that 1 μM of terfenadine (Fig. 2C) and dofetilide (Fig. 2D) also completely block *HERG*S631C channels, as measured on peak tail current amplitudes ($n=4$ each). Again, the inhibitory effect of the highest concentration of these drugs (1 μM) was only partially reversible upon long washout periods (>15 min) with ND-96 (data not shown).

The pharmacology of *HERG*S631C channels with thiol groups of Cys-631 in the oxidized state was investigated by treating oocytes with 1% H_2O_2 for approximately 1 min. As previously demonstrated under these conditions, the *HERG*S631C phenotype is characterized by disrupted C-type inactivation and K^+-selectivity. Representative current traces shown in Fig. 3A were evoked by application of 1 s test pulses ranging from −70 to +20 mV in 10 mV increments from a holding potential of −90 mV. As a result of the disrupted ion-selectivity, current traces inwardly activated during the test pulse at potentials between −50 and −10 mV and outwardly activated at test potentials more positive than 0 mV. Tail currents inwardly deactivated at −70 mV (clearly more positive than the WT *HERG* E_{rev} of approximately −90 mV). Surprisingly, under oxidizing conditions, we found that *HERG*S631C currents, evoked by application of pulses to +20 mV with intervals of 8 s from a holding potential of −90 mV, could not be blocked by 1 μM astemizole, as measured during the test pulse (Fig. 3B). Similarly, *HERG*S631C channels with thiol groups in the oxidized state were insensitive to 1 μM terfenadine (Fig. 3C, $n=4$) and 1 μM dofetilide (Fig. 3D, $n=4$). Current percentages calculated under these conditions were 96.65 ± 4.77%, 95.92 ± 4.24% and 91.65 ± 3.50% for astemizole, terfenadine and dofetilide, respectively. To investigate whether a higher pulse frequency would affect channel block, test pulses were applied with intervals of 1 s. Under these conditions, current percentages were 90.80 ± 2.84%, 91.92 ± 2.72 and 88.72 ± 2.92 for astemizole, terfenadine and dofetilide, respectively ($n=4$ each). Statistical analysis did not reveal a significant difference.

In addition, we observed that on the same oocyte, the pharmacology of *HERG*S631C channels could be driven from sensitive to insensitive as a function of its redox state. As illustrated in Fig. 4A (peak tail current amplitudes are plotted as a function of time), *HERG*S613C with thiol groups in the reduced state could be completely blocked by 1 μM dofetilide. This inhibitory effect was partially reversible upon continuous perfusion with ND-96. *HERG*S631C currents were then investigated under oxidizing conditions (1% H_2O_2) and no inhibitory effect could be observed upon a second application of 1 μM dofetilide. Data of current percentages of *HERG*S631C channels with thiol groups in the reduced (K^+ mode, filled bars) and oxidized state (Na^+ mode, non-filled bars) are summarized in Fig. 4B for 1 μM astemizole ($n=4$), terfenadine ($n=4$) and dofetilide ($n=4$).

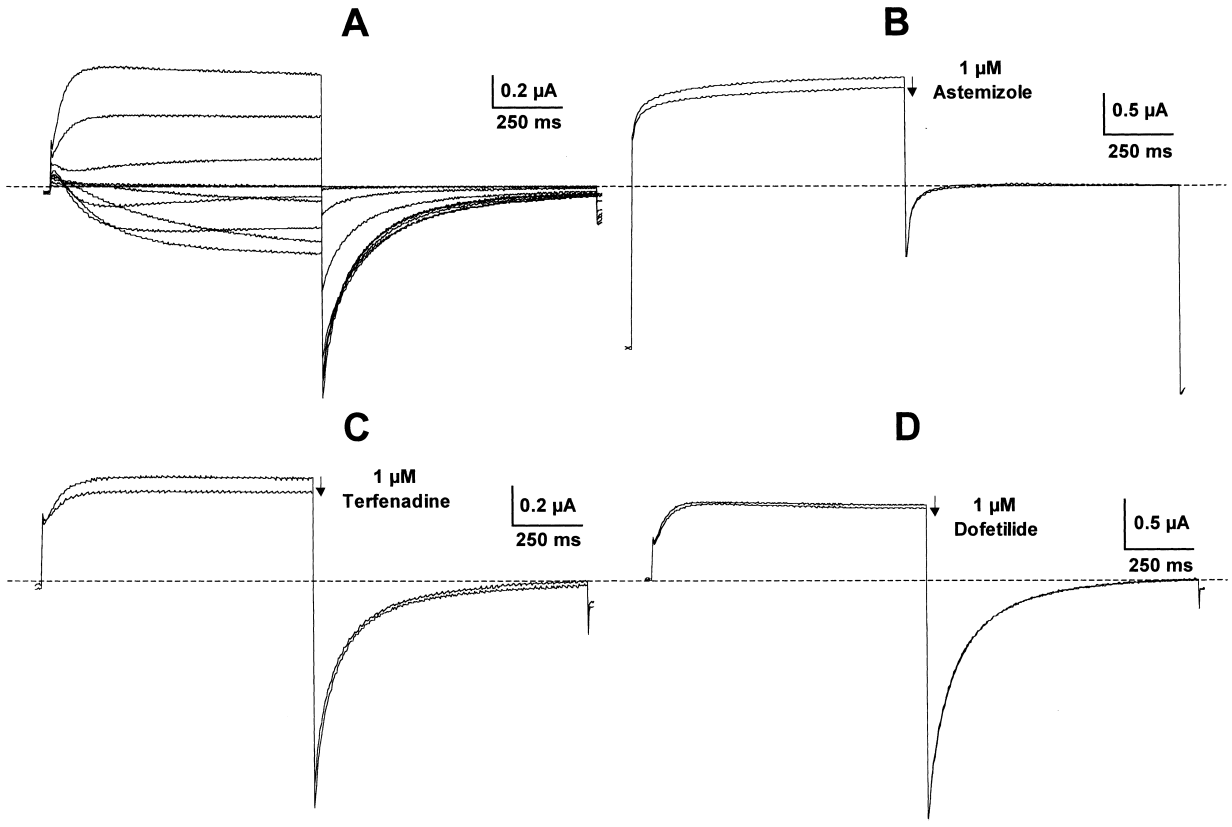

Fig. 3. When thiol groups of Cys-631 are in the oxidized state, the phenotype is characterized by disrupted C-type inactivation and K^+-selectivity. Current traces shown in A were evoked by application of 1 s test pulses ranging from −70 to +20 mV in 10 mV increments from a holding potential of −90 mV. Tail currents inwardly deactivated at −70 mV. When thiol groups of Cys-631 are in the oxidized state, we found that *HERG*S631C currents remain unaffected by application of 1 μM astemizole (B), as measured on the peak tail current amplitudes. It was found that 1 μM of terfenadine (C) and dofetilide (D) had no effect on *HERG*S631C channels either ($n=4$ each). Currents were evoked by application of pulses to 20 mV with intervals of 8 s from a holding potential of −90 mV.

4. Discussion

A preferential interaction with *HERG* channels in the open state or the inactivated state has previously been shown to underly high-affinity block of *HERG* channels. For dofetilide block, known to require channel opening for block to occur and to be modulated by inactivation gating, a point mutation in the S5–S6 linker region, *HERG*S620T, abolished high-affinity block and also interfered with C-type inactivation [9]. For haloperidol, a drug interacting preferentially with the channel in the inactivated state, a mutation in the pore region known to disrupt C-type inactivation, *HERG*S631A, reduced the high-affinity block 4-fold [4]. Very recently, Fan et al. [12] reported that most dramatic differences were obtained when S631 was replaced by a charged residue (S631K and S631E). Indeed, current–voltage relationships show that the degree of C-type inactivation is most prominent for WT *HERG* channels, retained (but to a lesser degree) for *HERG*S631A and absent for *HERG*S631K and S631E [12]. Coinciding with a dramatic loss of C-type inactivation, S631K and S631E are characterized by disrupted K^+-selectivity. Interestingly, the S631C mutant channel behaved like WT *HERG* channels when thiol groups of Cys-631 are in the reduced state. Oxidizing thiol groups with H_2O_2 or modifying them with MTSET or MTSES disrupted C-type inactivation and K^+-selectivity, similar to the phenotype of S631K and S631E [12].

In this study, we report the pharmacological properties of the *HERG*S631C mutant. For this mutant in particular, the dramatic loss of C-type inactivation can be actively controlled as a function of the redox state of Cys-631. *HERG*S631C gives rise to currents similar to WT *HERG* currents when Cys-631 is in the reduced state, whereas a phenotype similar to S631K and S631E is obtained when oocytes are treated with H_2O_2. This mutant allowed us to study the relation between pharmacology and C-type inactivation of the *HERG* channel by switching channels between two different states, one characterized by fast C-type inactivation and inward rectification of the tail currents, the other by delayed outward rectification and loss of K^+-selectivity. Voltage clamp experiments were performed on *Xenopus* oocytes injected with cRNA encoding WT *HERG* or *HERG*S631C.

Results from our study show that *HERG*S631C in its reduced state is fully blocked by 1 μM astemizole, similar to WT *HERG* channels. Saturating concentrations from terfenadine, also known to bind preferentially to *HERG* channels in the inactivated state, completely blocked *HERG*S631C channels. Similar results were obtained with 1 μM dofetilide, a drug requiring channel opening for block to occur.

In contrast, oxidized *HERG*S631C (treated with H_2O_2) is insensitive for 1 μM astemizole and terfenadine. This result suggests that indeed astemizole and terfenadine interact preferentially with the inactivated state, since *HERG*S631C becomes insensitive to saturating concentrations of these drugs when C-type inactivation of the channel is dramatically dis-

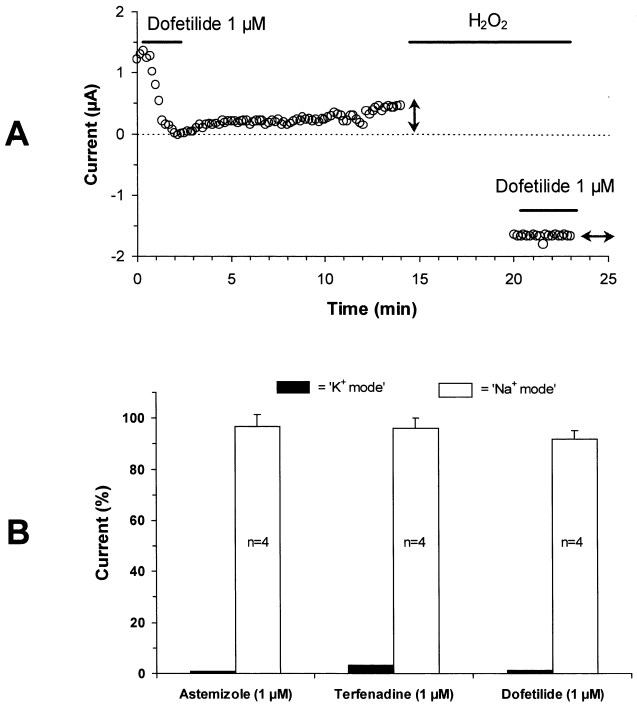

Fig. 4. A: The pharmacology of *HERG*S631C channels could be driven from sensitive to insensitive as a function of its redox state. Peak tail current amplitudes are plotted as a function of time. *HERG*S613C with thiol groups in the reduced state could be completely blocked by 1 μM dofetilide. This effect was partially reversible upon continuous perfusion with ND-96. *HERG*S631C currents were then investigated under oxidizing conditions (1% H_2O_2) and no inhibitory effect could be observed upon a second application of 1 μM dofetilide. B: Summary of current percentages of *HERG*S631C channels with thiol groups in the reduced (K^+ mode) and oxidized (Na^+ mode) state for 1 μM astemizole ($n=4$), terfenadine ($n=4$) and dofetilide ($n=4$).

rupted. Rather surprisingly, similar results were obtained with 1 μM dofetilide. Our results show that *HERG*S631C in its oxidized state becomes insensitive to 1 μM dofetilide, despite the fact that this drug has previously been shown to interact with the channel in its open state [5]. Effects which are indicative for open channel block, such as apparent inactivation during the test pulse, reduction of the peak tail current amplitude and slowing of channel deactivation, resulting in a cross-over phenomenon of the tail currents [5,14], were not observed with 1 μM dofetilide on *HERG*S631C in its oxidized state. This result illustrates the importance of the inactivated state in high-affinity block of *HERG* by astemizole and terfenadine as well as dofetilide, a drug requiring channel opening for block to occur. However, the possibility that treatment of *HERG*S631C with H_2O_2 disrupts the pharmacological binding site cannot be excluded. Additionally, we show that on the same oocyte, the pharmacology of *HERG*S631C can be driven from sensitive to insensitive as a function of its redox state. *HERG*S613C with thiol groups in the reduced state could be completely blocked by 1 μM dofetilide. This inhibitory effect was partially reversible upon perfusion with ND-96. When *HERG*S631C channels were then switched to its disrupted inactivation mode (1% H_2O_2), no inhibitory effect could be observed upon a second application of 1 μM dofetilide.

In conclusion, our results illustrate the importance of C-type inactivation in the pharmacological action of high-affinity channel blockers. Taken into account that a reduced affinity of haloperidol [4], azimilide, quinidine, RP58866 and nicotine [15] has previously been reported for *HERG*S631A, we suggest that an interaction with *HERG* channels in the inactivated state might be a common mechanism to a variety of drugs known to block *HERG* channels with high affinity.

Acknowledgements: The *HERG* clone was generously donated by Mark Keating, HHMI, University of Utah, Salt Lake City, UT, USA. The *HERG*S631C mutant was a kind gift from Gea-Ny Tseng, Virginia Commonwealth University, Richmond, VA, USA. Astemizole, terfenadine and dofetilide were gifts from the Janssen Research Foundation (Belgium).

References

[1] Warmke, J.W. and Ganetzky, B. (1994) Proc. Natl. Acad. Sci. USA 91, 3438–3442.
[2] Sanguinetti, M.C., Jiang, C., Curran, M.E. and Keating, M.T. (1995) Cell 81, 299–307.
[3] Abbott, G.W., Sesti, F., Splawski, I., Buck, M.E., Lehmann, M.H., Timothy, K.W., Keating, M.T. and Goldstein, S.A. (1999) Cell 97, 175–187.
[4] Suessbrich, H., Waldegger, S., Lang, F. and Busch, A.E. (1996) FEBS Lett. 385, 77–80.
[5] Snyders, D.J. and Chaudhary, A. (1996) Mol. Pharmacol. 49, 949–955.
[6] Kiehn, J., Thomas, D., Karle, C.A., Schols, W. and Kubler, W. (1999) Naunyn Schmiedebergs Arch. Pharmacol. 359, 212–219.
[7] Walker, B.D., Valenzuela, S.M., Singleton, C.B., Tie, H., Bursill, J.A., Wyse, K.R., Qiu, M.R., Breit, S.N. and Campbell, T.J. (1999) Br. J. Pharmacol. 127, 243–251.
[8] Dumaine, R., Roy, M.L. and Brown, A.M. (1998) J. Pharmacol. Exp. Ther. 286, 727–735.
[9] Ficker, E., Jarolimek, W., Kiehn, J., Baumann, A. and Brown, A.M. (1998) Circ. Res. 82, 386–395.
[10] Herzberg, I.M., Trudeau, M.C. and Robertson, G.A. (1998) J. Physiol. (Lond.) 511, 3–14.
[11] Wang, H.Z., Shi, H., Liao, S.J. and Wang, Z. (1999) Am. J. Physiol. 277, H1081–H1088.
[12] Fan, J.S., Jiang, M., Dun, W., McDonald, T.V. and Tseng, G.N. (1999) Biophys. J. 76, 3128–3140.
[13] Ulens, C., Daenens, P. and Tytgat, J. (1999) Cardiovasc. Res. 44, 568–578.
[14] Carmeliet, E. (1993) Circ. Res. 73, 857–868.
[15] Wang, H., Shi, H., Yang, B. and Wang, Z. (2000) Biophys. J. 78, 2026.