FEBS Letters 417 (1997) 28–32 FEBS 19397

A mechanism for the proarrhythmic effects of cisapride (Propulsid): high affinity blockade of the human cardiac potassium channel HERG

David Rampe[a,*], Mary-Louise Roy[b], Adrienne Dennis[b], Arthur M. Brown[b]

[a] *Hoechst Marion Roussel, Inc., 2110 E. Galbraith Road, Cincinnati, OH 45215, USA*
[b] *The RammelKamp Center for Research, MetroHealth Medical Center, Case Western Reserve University, School of Medicine, Cleveland, OH, USA*

Received 12 September 1997

Abstract **Cisapride (Propulsid) is a gastrointestinal prokinetic agent commonly used to treat nocturnal heartburn as well as a variety of other gastrointestinal disorders. The use of cisapride has been associated with acquired long QT syndrome and ventricular arrhythmias such as torsades de pointes which produces sudden cardiac death. These cardiotoxic effects can be due to blockade of one or more types of K^+ channel currents in the human heart. For this reason we compared the effects of cisapride on two cloned human cardiac K^+ channels, Kv1.5 and the *human ether-a-go-go*-related gene (HERG) stably transfected into mammalian cells. Using patch clamp electrophysiology, we found that cisapride was a potent inhibitor of HERG displaying an IC_{50} value of 44.5 nmol/l when tail currents at −40 mV were measured following a 2 s test depolarization to +20 mV. When HERG currents were measured at the end of prolonged (20 s) depolarizing steps to +20 mV, the apparent affinity of cisapride was increased and measured 6.70 nmol/l. The main effect of cisapride was to enhance the rate of HERG current decay thereby reducing current at the end of the voltage clamp pulse. Furthermore, the potency of cisapride for the HERG channel was similar to that observed for the class III antiarrhythmic agent dofetilide (IC_{50} = 15.3 nmol/l) and the nonsedating antihistamine terfenadine (IC_{50} = 56.0 nmol/l). In contrast to its effects on HERG, cisapride inhibited Kv1.5 channel currents weakly displaying an IC_{50} value of 21.2 μmol/l**

gene lead to the type 2 hereditary form of long QT syndrome (LQT2) [3,4]. Furthermore, blockade of I_{Kr} is believed to cause acquired long QT syndrome associated with class III antiarrhythmic agents such as dofetilide [5].

Cisapride (Propulsid) is a gastrointestinal prokinetic agent which is widely prescribed for the treatment of gastroesophageal reflux disease (GERD) [6]. In 1996 Propulsid ranked as the 73rd most dispensed drug in the United States with over 5.4 million prescriptions filled [7]. However, a number of reports have now appeared linking cisapride use with QT prolongation and development of ventricular arrhythmias such as torsades de pointes [8–12]. This cardiotoxicity has been associated with high doses of cisapride [10], or with the concomitant use of imidazole antifungals (e.g. ketoconazole) or macrolide antibiotics (e.g. erythromycin) [11,12]. These drugs inhibit the cytochrome P-450 3A4 enzyme through which cisapride is metabolized thereby leading to higher blood levels of cisapride [11]. These reports have prompted the US Food and Drug Administration to issue a boxed warning on cisapride packaging for potential adverse cardiac events. This situation bears striking resemblance to that previously reported for the nonsedating antihistamine terfenadine (Seldane) whose use, under similar circumstances, may be associated with a prolon-

est amount of *HERG* message were then tested for HERG current. Cell line 13 (L-*HERG* 13) produced the largest HERG current and was used in all further experiments.

2.2. Electrophysiology

Cells used for electrophysiological recordings were seeded on glass coverslips 24–48 h before use. HERG currents were recorded using the whole-cell patch clamp configuration while Kv1.5 currents were recorded from cell-free inside-out membrane patches [16]. Electrodes (1–4 MΩ resistance) were fashioned from TW150F glass capillary tubes (World Precision Instruments, New Haven, CT). For whole-cell recordings electrodes were filled with the following solution (mmol/l): potassium aspartate, 120; KCl, 20; Na_2ATP, 4.0; HEPES, 5.0; $MgCl_2$, 1.0; pH 7.2 with KOH. This served as the external solution for the inside-out patch experiments. The external solution for whole-cell recordings contained (mmol/l): NaCl, 130; KCl, 5.0; sodium acetate, 2.8; $MgCl_2$, 1.0; HEPES, 10; glucose, 10; $CaCl_2$, 1.0; pH 7.4 with NaOH. Currents were recorded at room temperature using an Axopatch 1-B amplifier (Axon Instruments, Burlingame, CA) and were conditioned by a 4-pole low-pass filter with a cutoff frequency of 500 or 200 Hz. Currents were stored and analyzed using a Compaq Deskpro computer and the pCLAMP suite of software (Axon Instruments). In most cases linear leakage and capacity currents were corrected on-line using the P/4 subtraction method. In some cases passive linear leak was recorded during 100 ms depolarizations between −75 and −50 mV and least-squares fit of the data was used for passive leak correction. The IC_{50} values for all compounds were obtained by nonlinear least-squares fits of the data (GraphPAD Software, San Diego, CA).

2.3. Chemicals

Cisapride was obtained from Research Diagnostics, Inc. (Flanders, NJ). Dofetilide was obtained from Pfizer Central Research (Sandwich, Kent, England). Terfenadine was synthesized at Hoechst Marion Roussel, Inc. (Cincinnati, OH). All other compounds were obtained from Sigma Chemical Co. (St. Louis, MO).

3. Results

Fig. 1 shows the effects of cisapride on HERG channel currents. In untransfected L cells no HERG-like K^+ currents were detectable upon depolarization of the cells to +20 mV followed by a return potential of −40 mV (Fig. 1A). In cells stably transformed with the cDNA encoding HERG, slowly rising currents were apparent upon depolarization of the cell to +20 mV. The relationship between the current at the end of the pulse and membrane potential showed the characteristic bell-shaped relationship reflecting inward rectification at more positive potentials. Upon returning the cell to a potential of −40 mV, a large slowly deactivating tail current characteristic of HERG [3,14] was observed (Fig. 1B). The tail current was potently blocked by cisapride resulting in an IC_{50} value of 4.45×10^{-8} mol/l (Fig. 1B and C). Cisapride had no detectable effects on HERG tail current kinetics. When cells were returned to a potential of −100 mV, inward HERG tail currents decayed with a time constant of 61.9 ± 10.1 ms ($n = 5$). This value was unaltered in the presence of 1×10^{-7} mol/l cisapride and measured 59.0 ± 5.0 ms ($P > 0.05$, paired t-test). With this pulse protocol, the amplitude of the tail currents was reduced by 67 ± 3%.

The waveform of the current at +20 mV in Fig. 1B suggests that the blocking effects of cisapride were incomplete. To explore this phenomenon further, we examined the effects of cisapride on HERG currents induced by prolonged 20 s depolarizations to +20 mV. The extracellular K^+ was increased to 20 mmol/l to enhance current amplitude. Under these conditions current in the absence of drug showed little decay (Fig. 2A). Cisapride dramatically enhanced the rate of HERG current decay while having no apparent effect on the initial phase of current activation (Fig. 2A). Single exponential fit of the data yielded time constants for current decay ranging from 11.65 ± 1.00 s at 10 nmol/l to 0.85 ± 0.08 s at 300 nmol/l (Fig. 2B). Finally, the IC_{50} value for cisapride block of HERG under these conditions measured 6.70 nmol/l (Fig. 2C).

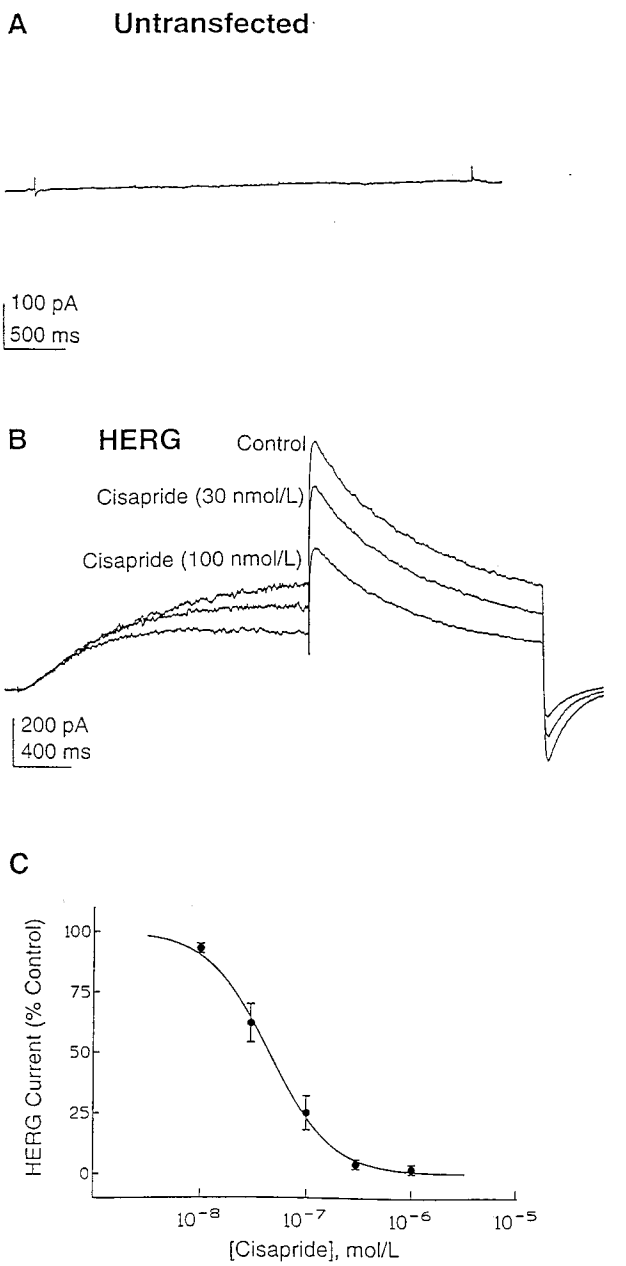

Fig. 1. Effects of cisapride on HERG. A: Untransfected L cells display no significant K^+ channel currents. Whole-cell current was elicited by a 2 s depolarizing pulse to +20 mV from a holding potential of −80 mV. The cell was then returned to −40 mV for 1.6 s. B: L cells stably transformed with the cDNA encoding HERG display slowly rising currents at +20 mV followed by large, slowly deactivating outward tail currents at the return potential of −40 mV. These tail currents were potently blocked by cisapride as shown. C: Dose-response relationships for cisapride blockade of HERG. Peak tail currents at −40 mV following the test pulse to +20 mV were used to construct the HERG dose-response curve. The IC_{50} value and Hill slope measured 44.5 nmol/l and −1.4, respectively. Error bars denote S.E.M. ($n = 5$–6).

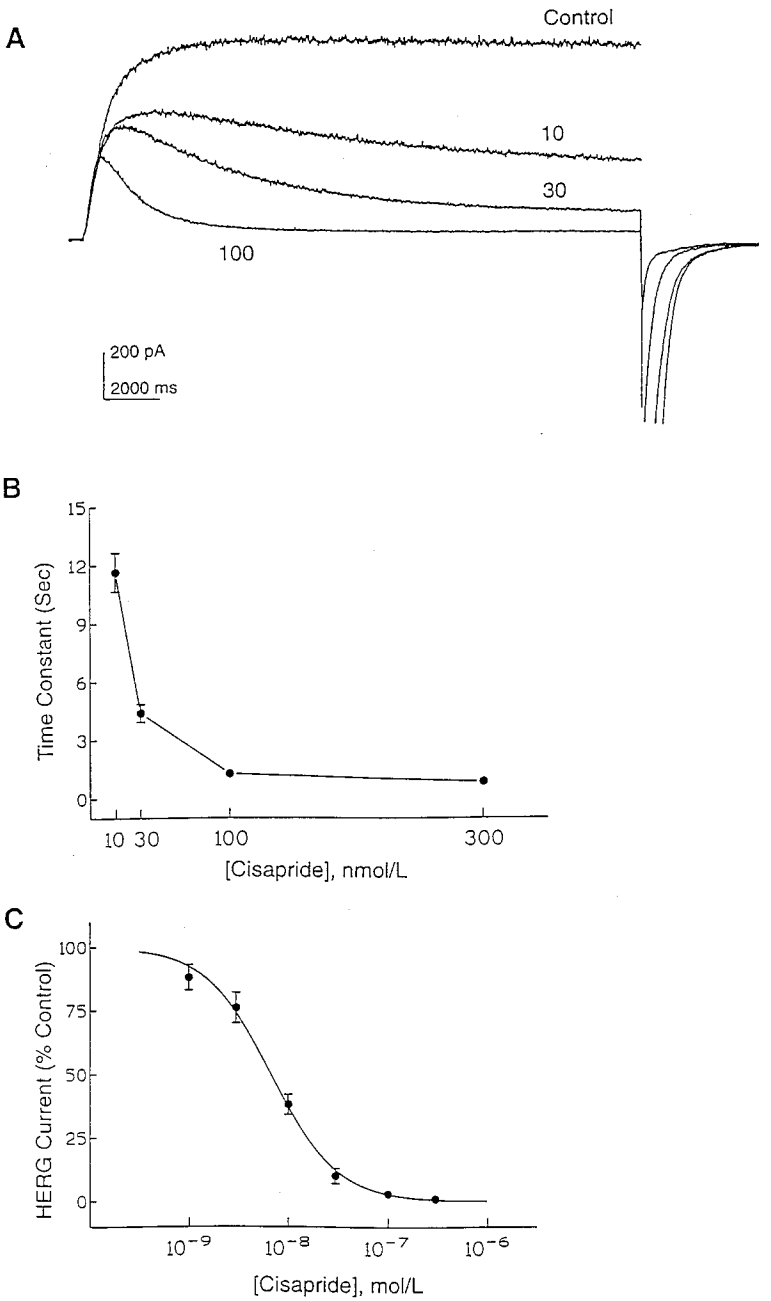

Fig. 2. Effects of cisapride on HERG during prolonged depolarization. A: HERG channel currents were elicited by a 20 s depolarization to +20 mV from a holding potential of −80 mV in the presence of 20 mM extracellular K^+. A dose-dependent increase in the rate of current decay in the presence of 10, 30 and 100 nmol/l cisapride is shown. B: Drug-induced acceleration in current decay was fitted to a single exponential and plotted as a function of cisapride concentration. Error bars represent S.E.M. ($n=6$–7). C: Dose-response relationship for cisapride blockade of HERG. Currents were sampled at the end of the 20 s depolarizing pulses to construct the dose-response relationship. The IC_{50} value and Hill slope measured 6.70 nmol/l and −1.3, respectively. Bars represent S.E.M. ($n=4$–7).

Table 1 compares the ability of dofetilide, terfenadine and cisapride to inhibit HERG. Dofetilide, a class III antiarrhythmic drug with high potency and specificity for I_{Kr} [5], inhibited HERG current with an IC_{50} value of 1.53×10^{-8} mol/l. The nonsedating antihistamine terfenadine also potently blocked *HERG* current yielding an IC_{50} value of 5.60×10^{-8} mol/l. These values are similar to those observed for the inhibition of HERG by cisapride under identical experimental conditions.

Table 1
Effects of various drugs on HERG channel currents[a]

Drug	IC_{50} value (± standard error)
Cisapride	44.5 (± 10.6) nmol/l
Dofetilide	15.3 (± 2.5) nmol/l
Terfenadine	56.0 (± 10.9) nmol/l

[a]Dose-response relationships were obtained as indicated in Fig. 1.

Fig. 3 shows the effects of cisapride on another human cardiac K^+ channel Kv1.5. As was the case for HERG, cisapride inhibited Kv1.5 channel current mainly by accelerating the rate of current decay during a depolarizing pulse (Fig. 3A). However, the IC_{50} value for cisapride block of Kv1.5

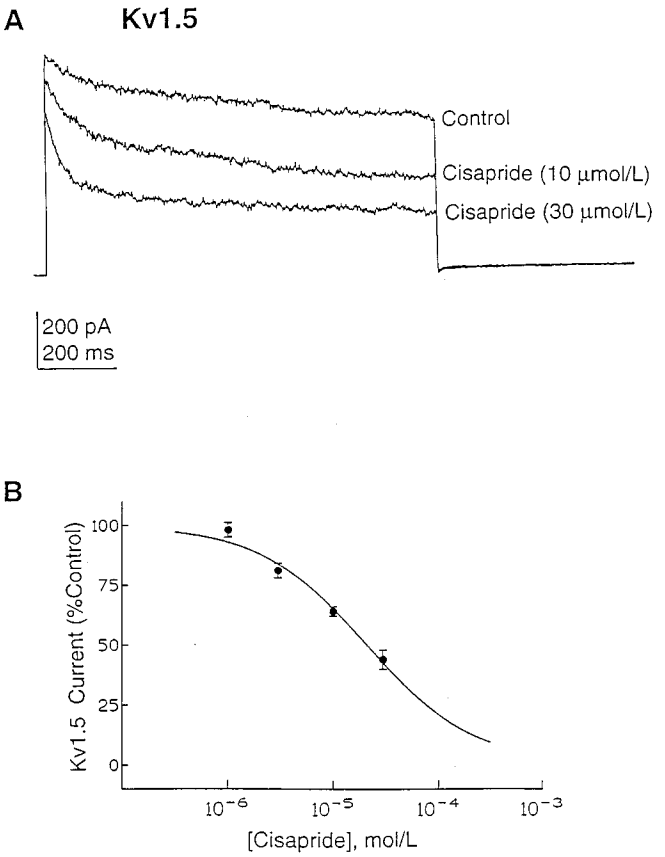

Fig. 3. Effects of cisapride on Kv1.5. A: Kv1.5 current from an inside-out membrane patch was elicited by a 1 s test depolarization to +50 mV from a holding potential of −80 mV. The effects of 10 and 30 μmol/l are shown. B: Dose-response relationship for cisapride blockade of Kv1.5. The last 50 ms of the depolarizing pulses to +50 mV were averaged to construct the dose-response curve. Concentrations of greater than 30 μmol/l could not be achieved due to solubility limitations of the drug. The IC_{50} value and Hill slope measured 21.2 μmol/l and −0.84, respectively. Bars indicate S.E.M. ($n=4$–7).

was approximately 1000-fold higher than observed for inhibition of HERG measuring 21.2 μmol/l (Fig. 3B).

4. Discussion

The present study demonstrates cisapride to be a potent antagonist of the HERG cardiac K^+ channel. The inhibitory effects on HERG were most potent when the drug was allowed to equilibrate with the channel during very prolonged depolarizations. Under these conditions cisapride enhanced the rate of current decay in a dose-dependent fashion with little effect on the initial time course of channel activation. These results suggest that cisapride blocks an activated state of HERG, possibly the open state. A similar mechanism may also explain cisapride's inhibition of Kv1.5, although much higher concentrations are necessary to produce inhibition of this cardiac K^+ channel. Indeed, the IC_{50} value of 6.70 nmol/l obtained during the prolonged voltage steps shown in Fig. 2 make cisapride one of the most potent antagonists of HERG yet described. The concentrations of cisapride needed to block HERG in the present study are likely to be encountered under certain clinical settings. Following therapeutic doses of cisapride (10–20 mg), peak plasma concentrations of the drug can average 150–300 nmol/l [6,17]. In the presence of ketoconazole or other agents which inhibit the cytochrome P450 3A4 enzyme system, levels of cisapride can be sharply higher [11,12]. Even when the extensive plasma protein binding of cisapride is considered (97–98%), concentrations of free drug can still reach levels similar to those reported here for blockade of HERG in vitro. Furthermore, blockade of HERG currents by cisapride was similar in potency to dofetilide and terfenadine, drugs which have been reported to produce acquired long QT syndrome in clinical settings [5,13].

To date, the mechanism whereby cisapride produces its cardiotoxic effects has not been determined. Cisapride is a partial agonist at the serotonin 5-HT_4 receptor and this mechanism has been proposed to underlie the drug's proarrhythmic activity [9]. Alternatively, a procainamide-like effect on the atrioventricular node has also been postulated [8]. A study in rabbit Purkinje fibers has shown a class III antiarrhythmic effect of the drug which occurs at doses of 30 nmol/l and higher [18]. Most recently cisapride has been shown to inhibit I_{Kr} in rabbit cardiomyocytes with an IC_{50} value of 9 nmol/l [19]. The gene product responsible for I_{Kr} in rabbit is unknown but I_{Kr} is probably produced by a variant of the ether-a-go-go-related gene ERG [20]. The data presented here are the first to detail the interactions of cisapride with human cardiac K^+ channels and strongly suggest that the molecular mechanism that underlies the proarrhythmic effects of cisapride is high affinity blockade of HERG. These findings also support to the hypothesis that blockade of HERG may play an important role in the development of many cases of

acquired long QT syndrome. Future studies will likely uncover more drugs which share both proarrhythmic activity and clinically relevant blockade of HERG.

References

[1] Ben-David, J. and Zipes, D.P. (1993) Lancet 341, 1578–1582.
[2] Fedida, D., Wible, B., Wang, Z., Fermini, B., Faust, F., Nattel, S. and Brown, A.M. (1993) Circ. Res. 73, 210–216.
[3] Sanguinetti, M.C., Jiang, C., Curran, M.E. and Keating, M.T. (1995) Cell 81, 299–307.
[4] Curran, M.E., Splawski, I., Timothy, K.W., Vincent, G.M., Green, E.D. and Keating, M.T. (1995) Cell 80, 795–803.
[5] Colatsky, T.J. and Argentieri, T.M. (1994) Drug Dev. Res. 33, 235–249.
[6] Wiseman, L.R. and Faulds, D. (1994) Drugs 47, 116–152.
[7] Zoeller, J. (1997) Am. Drug. 214, 32–37.
[8] Olsson, S. and Edwards, I.R. (1992) Br. Med. J. 305, 748–749.
[9] Bran, S., Murray, W.A., Hirsch, I.B. and Palmer, J.P. (1995) Arch. Intern. Med. 155, 765–768.
[10] Pettignano, R., Chambliss, C.R., Darsey, E., Heard, M. and Clark, R. (1996) Crit. Care Med. 24, 1268–1271.
[11] Ahmad, S.R. and Wolfe, S.M. (1995) Lancet 345, 508.
[12] Wysowski, D.K. and Bacsanyi, J. (1996) New Engl. J. Med. 335, 290–291.
[13] Honig, P.K., Wortham, D.C., Zamani, K., Conner, D.P., Mullin, J.C. and Cantilena, L.R. (1993) J. Am. Med. Assoc. 269, 1513–1518.
[14] Rampe, D., Wible, B., Brown, A.M. and Dage, R.C. (1993) Mol. Pharmacol. 44, 1240–1245.
[15] Roy, M.-L., Dumaine, R. and Brown, A.M. (1996) Circulation 94, 817–823.
[16] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pflüg. Arch. Eur. J. Physiol. 391, 85–100.
[17] Hofmeyr, G.J. and Sonnendecker, E.W.W. (1986) Eur. J. Clin. Pharmacol. 30, 735–736.
[18] Puisieux, F.L., Adamantidis, M.M., Dumotier, B.A. and Dupuis, B.A. (1996) Br. J. Pharmacol. 117, 1377–1379.
[19] Carlsson, L., Amos, G.J., Andersson, B., Drews, L., Duker, G. and Wadstedt, G. (1997) J. Pharm. Exp. Ther. 282, 220–227.
[20] Wymore, R.S., Gintant, G.A., Wymore, R.T., Dixon, J.E., McKinnon, D. and Cohen, I.S. (1997) Circ. Res. 80, 261–268.