Thioridazine Lengthens Repolarization of Cardiac Ventricular Myocytes by Blocking the Delayed Rectifier Potassium Current

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Abstract

Proarrhythmia has been observed with the antipsychotic agent thioridazine (THIO). The mechanisms underlying these effects are unknown. The objectives of this study were 1) to characterize the effects of THIO on cardiac repolarization and 2) to determine whether lengthening of the Q-T interval could be explained by blocking major K⁺-repolarizing currents. Isolated, buffer-perfused guinea pig hearts (n = 32) were stimulated at various pacing cycle lengths (150–250 ms) and exposed to THIO at concentrations ranging from 300 nM to 3 μM. THIO increased monophasic action potential duration at 90% repolarization (MAPD90) in a concentration-dependent manner from 14.9 ± 1.8 at 300 nM to 37.1 ± 3.2 ms at 3 μM. Increase in MAPD90 was also reverse frequency-dependent; THIO (300 nM) increased MAPD90 by 14.9 ± 1.8 ms at a pacing cycle length of 250 ms, but by only 7.7 ± 1.2 ms at a pacing cycle length of 150 ms. Patch-clamp experiments demonstrated that THIO decreases the time-dependent outward K⁺ current elicited by short depolarizations (250 ms; I_{K250}) in a concentration-dependent manner. Estimated IC_{50} for I_{K250} which mostly underlies I_{Ks}, was 1.25 μM. Time-dependent outward K⁺ current elicited in tsA201 cells expressing high levels of HERG protein was also decreased approximately 50% by 1.25 μM THIO. On the other hand, THIO was less potent (IC_{50} of 14 μM) to decrease time-dependent K⁺ current elicited by long pulses (5000 ms; I_{K5000}). Under the latter conditions, I_{K5000} corresponds mainly to I_{Kr}. Thus, these results demonstrate block of K⁺ currents and lengthening of cardiac repolarization by THIO in a concentration-dependent manner. This may provide an explanation of Q-T prolongation observed in some patients treated with THIO.

Thioridazine (THIO) is a phenothiazine derivative that has been used for the management of major psychotic disorders over the last 40 years. Shortly after its introduction into clinical practice, inappropriate lengthening of the Q-T interval and induction of major cardiac rhythm disturbances such as polymorphic ventricular tachycardia (torsades de pointes) were noticed (Kelly et al. 1963; Desautels et al., 1964; Huston and Bell, 1966; Schoonmaker et al., 1966; Fowler et al., 1976). Although some of these episodes occurred at high doses or overdoses of THIO, several cases of torsades de pointes and sudden death have been reported in patients receiving clinically effective doses of the drug (Kelly et al., 1963; Huston and Bell, 1966; Schoonmaker et al., 1966; Fowler et al., 1976; Kemper et al., 1983; Quieffin et al., 1991; Hulisz et al., 1994).

The study of the electrophysiological mechanism(s) responsible for the development of torsades de pointes is an area of extensive investigation. For several years, experimental studies and clinical observations have suggested that an abnormal repolarization, due either to a block of outward repolarizing potassium currents or to an increase of inward depolarizing calcium or sodium currents, could be the cause of this phenomenon (Rodén, 1991; Ben-David and Zipes, 1993). The presence of electrical intracardiac abnormalities could result in early after-depolarizations (EADs) that would...

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ABBREVIATIONS: I_{Kr}, delayed rectifier potassium current; I_{Ks}, the rapid component of I_{Kc}, I_{Kc}, the slow component of I_{Kc}; I_{K250}, time dependent outward current elicited by a depolarizing pulse of 250 ms; I_{K5000}, time-dependent outward current elicited by a depolarizing pulse of 5000 ms; I_{Ca}, the slow inward calcium current; MAPD90, monophasic action potential duration at 90% repolarization; THIO, thioridazine.
cause triggered activity and torsades de pointes (Rodent and Hoffman, 1985; Roden et al., 1986; El-Sherif, 1988; Cranefield and Aronson, 1991).

These assumptions are supported by the recent linkage of candidate genes for cardiac potassium and sodium channels with the genetically inherited forms of the long Q-T syndrome (Bennett et al., 1995; Curran et al., 1995; Schwartz et al., 1995; Wang et al., 1995; Dumas et al., 1996; Keating, 1996; Wang et al., 1996). On the other hand, etiologies for the acquired form of torsades de pointes are not as well understood. Predisposing factors to the latter type include not only slow heart rate, hypomagnesemia, or hypokalemia, but also treatment with antiarrhythmic agents, nonselective histamine H1 receptor antagonists, macrolide antimicrobials, and antifungal agents (Fish and Roden, 1989; Roden, 1991; Zimmermann et al., 1992; Ben-David and Zipes, 1993; Honig et al., 1993; Martyn et al., 1993).

Extrapolation of the currently known electrophysiological mechanisms associated with the induction of torsades de pointes to the clinical observation of THIO-induced cardiac toxicity suggests that this agent would modulate cardiac repolarization. Therefore, the goal of the present study was two-fold: 1) to investigate the action potential-lengthening effects of THIO in isolated guinea pig hearts perfused in the Langendorff mode using a monophasic action potential signal measured at 90% repolarization (MAPD90) as an index of cardiac repolarization and 2) to investigate the effects of THIO on potassium currents involved in repolarization of guinea pig ventricular myocytes using the whole-cell configuration of the patch-clamp technique. Our results demonstrate concentration and reverse frequency-dependent lengthening of MAPD90 as well as selective block of the rapid component of the delayed rectifier potassium current (I_{Ks}) by THIO.

Materials and Methods

Experiments were performed in accordance with institutional guidelines of Laval University on animal use in research. Animals were housed and maintained in compliance with the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care.

Isolated Heart Experiments

Heart Isolation and Perfusion Technique. Male Hartley guinea pigs (weight 300–350 g; Charles River Laboratories, Montreal, Quebec, Canada) were anticoagulated by injection of heparin sodium (400 IU i.p.). Thirty minutes later, animals were sacrificed by cervical dislocation, and the hearts were rapidly extirpated and immersed in cold (4°C) Krebs-Henseleit buffer containing 11.2 mM glucose, 4.7 mM KCl, 1.2 mM CaCl2, 25 mM NaHCO3, 118.5 mM NaCl, 2.5 mM MgSO4, and 1.2 mM KH2PO4. This solution was continuously gassed with 95% oxygen plus 5% carbon dioxide (pH 7.4, 37°C) and filtered through a 5.0-μm cellulose acetate membrane to remove any particulate contaminants. Each heart was cannulated and retrogradely perfused via the aorta with the Krebs-Henseleit buffer at a constant pressure equivalent to 100 cm of H2O. To permit rapid exchange of perfusion solutions, a double warming coil heart perfusion system (Harvard Apparatus, South Natick, MA) and two parallel liquid columns were used.

Electrophysiological Measurements. Hearts were electrically stimulated (programmable stimulator model 5325; Medtronic, Minneapolis, MN) at a basic cycle length of 250 ms (4 Hz) at three times threshold via two silver electrodes implanted in the epicardium of the left ventricle. A monophasic action potential catheter (Langendorff probe model 225; EP Technologies, Sunnyvale, CA) was introduced into the left ventricle through the mitral valve and securely positioned to obtain a visually adequate signal (amplitude >5 mV, stable phase 4). During the protocol, monophasic action potential signals were recorded on a computer for a duration of 3 s (digital sampling rate, 1 kHz) and stored on hard disk for analysis. Monophasic action potential duration was determined by analyzing all complete beats in the 3-s data file. These values were averaged using a routine designed specifically for this purpose and incorporated into the computer program (CVRP97 Cardiovascular Research Partner; Datton System Enr, Quebec, QC, Canada). At least 10 complexes were used for each measurement.

Protocols. Hearts were perfused during a control period of 5 min to assess stability of the monophasic action potential signal. Monophasic action potential signals were recorded at a basic length of 250 ms. Then, basic cycle length was changed to 225 ms, and the hearts were paced for 75 s before the monophasic action potential signal was recorded. The same procedure was repeated for cycle lengths of stimulation of 200, 175, and 150 ms. Thereafter, perfusion was performed with Krebs-Henseleit buffer containing THIO (300 nM, 1 μM, or 3 μM) for a period of 15 min at a basic cycle length of 250 ms. Monophasic action potential signals were recorded again at basic cycle lengths of 250, 225, 200, 175, and 150 ms. Perfusion with Krebs-Henseleit buffer containing no drug was then restarted (at 15 min postdrug) to assess reversibility of drug effects.

Statistical Analysis. Only hearts with reversal THIO effects on repolarization with buffer containing no drug were included in the analysis. Data on the magnitude of THIO effects were analyzed with Student’s paired t test. Frequency-dependent effects were compared by conditional Hotelling’s T2 test (Srivastava and Carter, 1983). All values are expressed as mean ± S.E.M. Statistical significance was set at P < .05.

Patch-Clamp Experiments

Guinea Pig Ventricular Myocyte Preparation and Solutions. Experiments were performed on single ventricular myocytes obtained from adult guinea pig hearts using an enzymatic dissociation technique. All solutions used during the cell isolation procedure were oxygenated and maintained at 37°C. The hearts were mounted on a Langendorff apparatus and retroperfused for 5 min with solution A containing 132 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl2, 10 mM HEPES, 5 mM glucose, and 1.8 mM CaCl2; pH was adjusted to 7.45 with NaOH. The hearts were then rinsed for 2 min with a calcium-free solution (solution B) containing 132 mM NaCl, 4.8 mM KCl, 1.2 mM MgCl2, 10 mM HEPES, and 5 mM glucose; pH was adjusted to 7.45 with NaOH. At the end of this period, perfusion with a low-sodium-high-potassium HEPES-buffered solution (solution C: 29 mM NaCl, 4.8 mM KCl, 128 mM potassium glutamate, 1.2 mM MgCl2, 10 mM HEPES, and 5 mM glucose; pH 7.45 with KOH) containing collagenase (final concentration, 300 U/ml; Boehringer Mannheim, Mannheim, Germany) was started and continued until the system pressure dropped to 15 mm Hg (approximately 15 min). Hearts were then perfused for 3 min with a solution (collagenase-free) made of a mixture of solution C and solution A (85:15) containing 0.3 mM CaCl2. Hearts were finally perfused with a solution made of 60% solution C and 40% solution A containing 0.75 mM CaCl2. At this point, the ventricles were cut down and minced slightly. After filtration through 200-μm nylon mesh, the dispersed cells were re-suspended in solution A and maintained at 30°C before use.

The external solution used to superfuse cells during the recording of currents contained 145 mM NaCl, 4 mM KCl, 1 mM MgCl2, 10 mM HEPES, and 5 mM glucose. Nisoldipine (0.2 μM; Bayer AG, Leverkusen, Germany) was added to eliminate the slow calcium inward current (I_{Ca}) and Ca2+ was omitted in the extracellular solution to shift I_{Kr} activation to positive potentials (Sanguinetti and Jurkiewicz, 1992). The pipette solution contained 2 mM MgCl2, 1 mM CaCl2, 11 mM EGTA, 5 mM MgATP, 5 mM K2ATP, and 10 mM

\[\text{Vol. 288}\]
HEPES. The pH was adjusted to 7.2 with KOH and the final potassium concentration was fixed at 505 mM with KCl.

THIO solutions of 300 nM and 1, 3, 10, 30, 100, and 300 μM were prepared daily by dissolving required amounts of THIO hydrochloride (Sigma, St. Louis, MO) in 100 ml of the physiological solution perfusing the cells.

Dofetilide solution of 100 nM was prepared daily by dissolving the required amount of dofetilide (Pfizer Central Research, Sandwich, Kent, United Kingdom) in dimethyl sulfoxide. A constant volume of dimethyl sulfoxide (100 μl; 0.1% v/v) was therefore added to buffer solution perfusing cells in the absence or presence of THIO.

HERG-Transfected tsA201 Cell Preparation and Solutions.

The HERG cDNA (kindly provided by Dr. Gail A. Robertson, University of Wisconsin, Madison, WI) and human CD8 receptor cDNA were used to transfect tsA201 cells after a CaCl2 precipitation protocol. Briefly, 10 μg of each construct was added to 500 μl of 250 mM CaCl2. The DNA/CaCl2 mixture was then slowly added to 500 μl of 2× HeBS (274 mM NaCl, 40 mM HEPES, 12 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). The pipette solution was composed of 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). The external Tyrode’s solution used to superfuse tsA201 cells contained 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES (pH 7). Room temperature. The culture medium was replaced just before adding the mixture to the cells. After incubating the cells overnight, anti-CD8 antibodies coupled to polystyrene beads (Dynal, Great Neck, NY) were used to identify the transfected cells.

The external Tyrode’s solution used to superfuse tsA201 cells during the recording of currents contained 137 mM NaCl, 4 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, 10 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). The pipette solution contained 130 mM KCl, 1 mM MgCl2, 5 mM EGTA, 5 mM MgATP, and 10 mM HEPES (pH 7.2 with KOH).

A THIO solution of 1.25 μM was prepared daily by dissolving the required amount of THIO hydrochloride in 100 ml of the Tyrode’s solution perfusing the cells.

Electrophysiological Measurements for Guinea Pig Ventricular Myocytes. A small aliquot of dissociated cells was placed in a 0.5-ml chamber mounted on the stage of an inverted microscope (model CK2; Olympus, Tokyo, Japan). Cells were allowed to adhere to the coverslip at the bottom of the chamber and were then superfused continuously with the external solution prewarmed at 30°C by a Peltier device (Medical System Corp., Greenvale, NY). In our experiments, complete replacement of external solution contained in the chamber was achieved within 2 to 3 min when the superfusion rate was 2 ml/min.

All currents were recorded in the whole-cell, voltage-clamp configuration of the patch-clamp technique using an Axo-patch-1D amplifier (Axon Instruments Inc., Foster City, CA). Voltage-clamp command pulses were generated by a 12-bit digital-to-analog converter (model TL-1; Axon Instruments) controlled by the PCLAMP software package (version 4.05b; Axon Instruments). Heat-polished patch-clamp pipette electrodes used (capillary glass from Radnoti Glass Technology Inc., Monrovia, CA; Starebore glass capillary tubing, 1.2 mm o.d.) had a tip resistance of 3 to 5 MΩ (when filled with the pipette solution). Series resistance was compensated 50 to 80% to improve fidelity of whole-cell voltage-clamp measurements.

Protocol for Guinea Pig Ventricular Myocytes. Rod-shaped cells with clear cross-striations, resting potential of at least −78 mV, and stable-delayed rectifier (Ikr) and inward rectifier (Iki) currents (as assessed during a baseline period of at least 4 min) were used.

Effects of THIO on the rapidly (Ik) and slowly (Iks) activating components of Ikr were studied in cells held at −40 mV (to inactivate Ih) and depolarized by pulses lasting either 250 ms (IK250) or 5000 ms (IK5000). Test potentials of depolarizing pulses varied between −20 and +50 mV for IK250 but between 0 and +50 mV for IK5000. IK was measured from the peak magnitude of tail current obtained on repolarization to −40 mV.

### Data Storage and Analysis (Guinea Pig Ventricular Myocytes)

Currents were low-pass filtered at either 2 kHz (IK250) or 100 Hz (IK5000) by a four-pole Bessel filter (−3 dB/octave). Currents were sampled at 2 kHz (IK250) and 400 Hz (IK5000) by use of a 12-bit analog-to-digital converter (TL-1 DMA; Axon Instruments) and stored on hard disk for subsequent analysis. Unless specified, data

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<td>Prolongation of MAPD90 after a 15-min THIO perfusion period at various pacing cycle lengths</td>
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**Fig. 1.** A, effects of THIO on MAPD determined in isolated, buffer-perfused guinea pig hearts, at a pacing cycle length of 250 ms. MAPD90 was measured at baseline (BAS) and during perfusion of 300 nM, 1 μM, or 3 μM THIO (n = 8 at each concentration). Asterisks indicate significant changes from baseline value (P < .05). Recordings of monophasic action potential signals during the baseline period and after 15 min of exposure to 1 μM THIO (pacing cycle length of 250 ms) are presented in B.
Electrophysiological Measurements for tsA201 Cells. Cell culture Petri dishes were directly placed on the stage of an inverted microscope (model CK2; Olympus). Cells were then superfused continuously with Tyrode’s solution at room temperature. In our experiments, complete replacement of Tyrode’s solution contained in the Petri dish was achieved within 2 to 3 min when the superfusion rate was 2 ml/min.

Data Storage and Analysis (tsA201 Cells). Currents were low-pass filtered at 2kHz (I_{K250}) by a four-pole Bessel filter (−3 dB/ octave). Currents were sampled at 2 kHz (I_{K250}) by use of a 12-bit analog-to-digital converter (TL-1 DMA; Axon Instruments) and stored on hard disk for subsequent analysis.

Results

Experiments performed in isolated guinea pig hearts (n = 8 for each concentration tested) demonstrated that THIO caused a concentration and reverse frequency-dependent increase in MAPD90. Table 1 shows that when hearts were exposed to 300 nM THIO at decremental pacing cycle lengths of 250, 225, 200, 175, and 150 ms, MAPD90 was increased by 14.9 ± 1.8, 12.9 ± 1.5, 11.3 ± 1.5, 10.1 ± 1.5, and 7.7 ± 1.2 ms, respectively. This reverse frequency-dependent effect of the drug was less apparent at 1 μM. When 3 μM THIO was used, MAPD90 could not be measured at short pacing cycle lengths, mostly because of an increase in refractoriness that was more important (relative increase) at shorter cycle lengths of stimulation. Data obtained in each heart tested for the increase in MAPD90 at a pacing cycle length of 250 ms, using THIO at 300 nM, 1 μM, and 3 μM, are shown in Fig. 1A. Typical examples of monophasic action potentials recorded at baseline and during perfusion of 1 μM THIO, at a pacing cycle length of 250 ms, are illustrated in Fig. 1B.

To understand the mechanism of the effects of THIO on cardiac repolarization, experiments were conducted in isolated cells using the patch-clamp technique. Figure 2A shows activating and tail currents of I_K elicited by a 250-ms pulse to 0 mV in a guinea pig ventricular myocyte perfused under control conditions (baseline) and in the presence of 10 μM THIO. In this cell, activating and tail currents recorded at baseline were almost eliminated by 10 μM THIO. This effect was reproducibly observed in seven myocytes tested. In addition, inhibition of I_K was assessed by exposing myocytes (n = 7 at each concentration) to 300 nM to 300 μM THIO. Estimated IC_{50}
for $I_{K_{250}}$ was 1.25 $\mu$M (Fig. 2B). Figure 2C shows activating and tail currents of $I_K$ elicited by a 250-ms pulse to +10 mV, followed by repolarization to −40 mV in a HERG-transfected tsA201 cell perfused under control conditions (baseline) and in the presence of 1.25 $\mu$M THIO (estimated $IC_{50}$ for $I_{K_{250}}$ in guinea pig ventricular myocytes). In this cell, activating and tail currents recorded at baseline were approximately half-inhibited by 1.25 $\mu$M THIO. Mean decrease in outward potassium current in four cells exposed to a similar concentration of THIO was 45%.

Figure 3 illustrates the I/V relationship of $I_{K_{250}}$ tail current measured at baseline and in myocytes exposed to 3, 10, 30, or 100 $\mu$M THIO ($n = 7$ cells/concentration). Inhibition of $I_{K_{250}}$ tail current was concentration-dependent but voltage-independent ($P < .05$; Hotelling’s $T^2$ test).

Figure 4A illustrates recordings of currents elicited by long pulses (5000 ms) at baseline and in the presence of 10 $\mu$M THIO. Activating current was elicited by a test pulse to +50 mV, while deactivating tail current was recorded after repolarization to −40 mV. A 50% reduction in both activating and tail currents was observed in this myocyte when THIO was added to dofetilide.

Almost complete inhibition of $I_{K_{5000}}$ elicited by a test pulse to +50 mV was observed by 300 $\mu$M THIO while inhibition was less than 20% at 3 $\mu$M. $IC_{50}$ determined for $I_{K_{5000}}$ was estimated at 14 $\mu$M (Fig. 4B). Figure 4C illustrates recordings of currents elicited by long pulses (5000 ms) in the presence of 100 nM dofetilide alone (a potent blocker of $I_{Kr}$ to eliminate this component) and with 14 $\mu$M THIO (estimated $IC_{50}$ for $I_{K_{5000}}$). Activating current was elicited by a test pulse to +50 mV, whereas deactivating tail current was recorded after repolarization to −40 mV. A reduction of approximately 50% in both activating and tail currents was observed in this myocyte when THIO was added to dofetilide.

Figure 5 illustrates the I/V relationship of $I_{K_{5000}}$ tail current measured at baseline and in myocytes exposed to 3, 10, 30, or 100 $\mu$M THIO ($n = 7$ cells/concentration). Inhibition of $I_{K_{5000}}$ was concentration-dependent but voltage-independent.

**Discussion**

Results obtained in this study indicate that THIO possesses direct cardiac electrophysiological effects. Exposure of isolated, buffer-perfused guinea pig hearts to THIO was as-
sociated with concentration and reverse frequency-dependent lengthening of cardiac repolarization. Patch-clamp experiments revealed selective block of IKr over IKs at low concentrations of THIO. It is believed that blocking of the components of IK by THIO gives an explanation for prolonged cardiac repolarization and potentially proarrhythmia observed in some patients treated with the drug.

Previous electrophysiological studies in animal models, as well as in patients, have demonstrated the potential of THIO to cause concentration-dependent arrhythmogenic effects. For example, in an in vivo canine model, trains of premature stimuli induced ventricular tachycardia in dogs receiving THIO at doses greater than 50 mg/kg but not at a dose of 10 mg/kg (Yoon et al., 1979). In a study involving 43 patients treated for paranoid psychosis, changes in the morphology of ECG T-wave were noticed in more than 85% of traces (82/91) when the plasma concentration of THIO was greater than 1 mM (Axelsson and Aspenstrom, 1982). In contrast, when the concentration of THIO was lower than 1 mM, no changes in T-wave were noticed in 30 of 38 ECG recordings (Axelsson and Aspenstrom, 1982).

Our results are in agreement with these previous studies (Yoon et al., 1979; Axelsson and Aspenstrom, 1982). In fact, we have demonstrated a concentration-dependent increase in MAPD90 in isolated guinea pig hearts and a concentration-dependent decrease in both IK250 and IK5000 in isolated ventricular myocytes. It was previously demonstrated that time-dependent outward current activated by short (250-ms) pulses to low depolarizing potentials (0 mV) represents mainly IKr (Sanguinetti and Jurkiewicz, 1990), which is a major constituent of outward currents involved in repolarization of human ventricular myocytes (Beuckelmann et al., 1993; Sanguinetti et al., 1995; Trudeau et al., 1995; Li et al., 1996; Spector et al., 1996). On the other hand, time-dependent outward current elicited by 5000-ms long pulses to high depolarizing potentials represents mainly IKs (Sanguinetti and Jurkiewicz, 1990). Because of the extensive binding of THIO to plasma proteins (99%), significant blocking of IKr (IC50, 1.25 mM) and/or IKs (IC50, 14 mM) by THIO should be limited to conditions resulting in high plasma levels of the drug, such as in the case of overdosage or suicide attempt (Quieffin et al., 1991; Hulisz et al., 1994). However, accumulation of THIO in some tissues such as the heart, due to its very large volume of distribution (>600 liters), may explain the occurrence of pharmacological effects (prolongation of the Q-T interval) at lower plasma concentrations (Hartigan-Go et al., 1996). Concentrations of some neuroleptics in the brain can be more than 10 times those in the blood (Sunderland and Cohen, 1987).

The relevance of IKr and/or IKs block by THIO for its therapeutic actions in the central nervous system is unknown. However, HERG genes and predicted potassium channels encoded by these genes (IKs) are not exclusively expressed in the heart. Indeed, their presence has also been shown in other excitable tissues such as the brain and the skeletal muscle. The relevance of HERG potassium channels in neu-

**Fig. 4.** A, recordings of membrane currents elicited by long pulses (IK5000) to +50 mV at baseline (BAS) and during exposure to 10 μM THIO. B, tail current amplitude of IK5000 following a test pulse to +50 mV at baseline and on exposure of myocytes to various concentrations of THIO (n = 7 cells/concentration; n = 1 at 300 μM). Percentage of inhibition of baseline current and estimated IC50 for IK5000 are also illustrated. C, bottom left illustrates recordings of membrane currents elicited by long pulses (IK5000) to +50 mV in the presence of 100 nM dofetilide alone and with 14 μM THIO in a guinea pig ventricular myocyte. Under these conditions, the IKr component was completely inhibited by dofetilide and block of IKs by THIO was close to 50% at its previously determined IC50 (14 μM).
ronal function has been further reinforced by the demonstration that the seizure locus in *Drosophila* encodes for the fly homolog of HERG (Titus et al., 1997; Wang et al., 1997).

Conduction delay even leading to conduction block occurred in some experiments performed in the course of this study with buffer-perfused isolated hearts exposed to THIO at concentrations of 3 μM and higher (data not shown). This could reflect nonselective blocking of potassium channels or blocking of sodium channels by THIO at high concentrations.

In conclusion, results obtained in this study demonstrated that THIO has direct cardiac electrophysiological effects. The drug preferentially blocks the rapid component of the delayed rectifier potassium current, *I*<sub>Kr</sub>, which may give an explanation for the observed reverse frequency-dependent prolongation of cardiac repolarization (class III effect). Some clinical attention is warranted in patients susceptible (genetically or pharmacologically) to cardiac toxicity and receiving multidrug regimens including THIO.

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**References**


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