Electrophysiological mechanisms by which hypothyroidism delays repolarization in guinea pig hearts

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1Department of Medicine and 2Research Center, Montreal Heart Institute and University of Montreal, Montreal H3C 1C8; and 3Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6

Bosch, Ralph F., Zhiguo Wang, Gui-Rong Li, and Stanley Nattel. Electrophysiological mechanisms by which hypothyroidism delays repolarization in guinea pig hearts. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H211–H220, 1999.—Thyroid hormone is known to exert important effects on cardiac repolarization, but the underlying mechanisms are poorly understood. We investigated the electrophysiological mechanisms of differences in repolarization between control guinea pigs and hypothyroid animals (thyroidectomy plus 5-propyl-2-thiouracil). Hypothyroidism significantly prolonged the rate-corrected Q-T interval in vivo and action potential duration (APD) of isolated ventricular myocytes. Whole cell voltage-clamp studies showed no change in current density or kinetics of L-type Ca2+ current, inward rectifier K+ current, or Na+ current in hypothyroid hearts. Dofetilide-resistant current (Iks) step current densities were smaller by 65%, and tail current densities were reduced by 80% in myocytes from hypothyroid animals compared with controls. The ratio of delayed rectifier step current at -50 mV to tail current at -40 mV was significantly larger in hypothyroid cells for test pulses from 60- to 4,200-ms duration, reflecting a smaller Iks. Dofetilide-sensitive current (Ikr) densities were not significantly changed. Iks half-activation voltage shifted to more positive voltages in hypothyroid (29.5 ± 2.2 vs. 21.3 ± 2.7 mV in control, P < 0.01), whereas Ikr voltage dependence was unchanged. We conclude that hypothyroidism delays repolarization in the guinea pig ventricle by decreasing Iks, a novel and potentially important mechanism for thyroid regulation of cardiac electrophysiology.

electrocardiogram; action potential; biophysics; cardiac arrhythmias; antiarrhythmic drugs; ion channels

THYROID HORMONES DISPLAY a variety of potentially important effects on cardiac electrical function. These include a positive chronotropic and inotropic effect and a shortening in repolarization (22). Hyperthyroidism is a clinically important cause of atrial fibrillation, almost certainly caused in large measure by accelerated atrial repolarization. A typical electrocardiographic feature in hypothyroid patients is a marked prolongation of the Q-T interval (32), reflecting delayed ventricular repolarization. Experimental hyperthyroidism prolongs the Q-T interval (33) as well as action potential duration (APD) measured with fine-tipped microelectrodes (11, 28).

The ionic and molecular mechanisms of thyroid hormone effects on repolarization remain poorly understood. Rubinstein and Binah (4, 23) found that, although hypothyroidism increased guinea pig ventricular APD by 31–44%, the only associated change in membrane current was a 41% reduction in L-type Ca2+ current (Ica2+) amplitude, which should, if anything, have accelerated repolarization. Hyperthyroidism has been found to decrease transient outward current (Ito) density and slow its recovery in rat ventricle (30) but does not appear to alter Ito density in rabbit atrium or ventricle at physiological temperatures (29).

The present investigation was designed to determine the ionic basis of delayed repolarization in hypothyroid guinea pigs. We sought to establish whether hypothyroidism causes changes in the density or kinetics of currents flowing during the action potential plateau (particularly K+ and Ca2+ currents) that could account for concomitant alterations in repolarization.

MATERIALS AND METHODS

Experimental groups. Adult male Hartley albino guinea pigs (500–600 g) were assigned to a control (n = 25) or a hypothyroid (n = 9) group. The procedures followed were in accordance with the guidelines of the Montreal Heart Institute and the Canadian Council on Animal Care. Animals assigned to the hypothyroid group were thyroidectomized by Charles River (St. Constant, PQ, Canada) after anesthesia with xylazine (Miles Canada, 5 mg/kg im) and ketamine (Rogar/STP, 40 mg/kg im). Subsequently, these guinea pigs were treated with 5-propyl-2-thiouracil (Sigma) for 6–8 wk (0.05% in drinking water). CaCl2 was added to the drinking water of hypothyroid animals at a concentration of 1% to avoid hypocalcemia caused by parathyroid damage. Weight and electrocardiograms (ECG) were obtained on a weekly basis. In the hypothyroid group, the first ECG changes occurred after 4 wk, and the ECG stabilized by the end of 8 wk. When ECG changes had stabilized, animals were killed by cervical dislocation, and their hearts were removed for cell isolation.

ECG recordings. Six-lead ECG recordings were obtained after sedation with acepromazine (0.1 mg/kg im) and ketamine (40 mg/kg im). The average of three measurements was used to determine the R-R, P-R, QRS, and Q-T intervals with ±2.5-ms precision. The corrected Q-T (Q-Tc) interval was calculated using Bazett’s formula (2).

Cell isolation and solutions. Left ventricular myocytes were isolated by enzymatic dissociation as previously described (6). Guinea pigs were killed by cervical dislocation, and the hearts were excised and mounted on a Langendorff apparatus. The hearts were perfused with oxygenated (100% O2, pH adjusted to 7.35 with NaOH) Tyrode solution containing (mM) 136 NaCl, 5.4 KCl, 2.0 CaCl2, 1.0 MgCl2, 0.33 NaH2PO4, 5 HEPES, and 10 glucose at 37°C. When clear of blood, the perfusate was changed to nominally Ca2+-free Tyrode solution until contraction ceased. Perfusion continued with the same solution containing 0.03% collagenase (type II, Worthington Biochemical) and 1% bovine serum albumin.
HYPOTHYROIDISM INCREASES APD BY REDUCING $I_{KS}$

Vigorously stimulated hearts were excised, and subepicardial tissue were removed and mechanically dissociated by trituration. The isolated cells were suspended at room temperature in a storage solution containing (mM) 20 KCl, 10 KH$_2$PO$_4$, 25 glucose, 40 mannitol, 70 l-glutamic acid, 10 β-hydroxybutyric acid, 20 taurine, and 10 EGTA, along with 1% albumin (pH adjusted to 7.35 with CsOH).

A small aliquot of cell-containing solution was placed in a 1-ml open perfusion chamber. After a brief period for cell adhesion to the chamber, the cells were perfused at 6 ml/min with (mM) 136 NaCl, 5.4 KCl, 2.0 CaCl$_2$, 1.0 MgCl$_2$, 0.33 NaH$_2$PO$_4$, 5 HEPES, and 10 glucose (pH adjusted to 7.35 with NaOH) for the recording of action potentials, inward rectifier current ($I_{K1}$), and delayed rectifier current ($I_{Ks}$). To record $I_{Ca}$, we used a solution containing (mM) 136 choline chloride, 5.6 CsCl, 2.0 CaCl$_2$, 1.0 MgCl$_2$, 0.33 NaH$_2$PO$_4$, 5 HEPES, and 10 glucose (pH adjusted to 7.35 with CsOH). For $I_{Na}$ recording, the solution contained (mM) 132.5 CsCl, 5.0 NaCl, 1.0 MgCl$_2$, 1.0 CaCl$_2$, 20 HEPES, and 11 glucose (pH adjusted to 7.35 with CsOH). To record $I_{K1}$ and $I_{Ks}$, $I_{Ca}$ was blocked with 5 µM nifedipine (Sigma). All experiments were performed at 36°C except for those studying fast Na$^+$ current ($I_{Na}$), for which the bath was held at 17°C with a Peltier-effect device. The pipette solution contained (mM) 20 KCl, 110 K-aspartate, 1.0 MgCl$_2$, 10 HEPES, 5 EGTA, 5 Mg$_2$ATP, 0.1 GTP, and 5 phosphocreatine (pH adjusted to 7.2 with KOH) to record action potentials, $I_{K1}$, and $I_{Ks}$. For $I_{Ca}$ recording, the pipette contained (mM) 20 CsCl, 110 Cs-aspartate, 10 HEPES, 10 EGTA, 1.0 MgCl$_2$, 5 Mg$_2$ATP, 0.1 GTP, and 5 phosphocreatine (pH adjusted to 7.2 with CsOH). To record $I_{Na}$, pipettes were filled with a solution containing (mM) 135 CsF, 5.0 NaCl, 5.0 HEPES, 10 EGTA, and 5 Mg$_2$ATP (pH adjusted to 7.2 with CsOH).

Voltage-clamp technique and action potential recording. Borosilicate glass electrodes (outer diameter 1.0 mm) with resistances of 0.8–1.2 MΩ for $I_{Na}$ recording and 2.6–6 MΩ for other experiments were connected to a patch-clamp amplifier (Axopatch 200A, Axon Instruments). The sampling frequency was 10 kHz for rapidly changing currents (such as $I_{Na}$ or $I_{Ca}$) and as low as 0.4 kHz for long recordings of slowly changing currents like $I_{KS}$.

Membrane capacitance was larger in the hypothyroid group (167 ± 6 vs. 147 ± 6 pF in control, P < 0.05), so all mean current data are expressed as current densities. Before compensation, series resistance ($R_s$) averaged 13.7 ± 1.0 and 13.2 ± 1.1 MΩ in control and hypothyroid groups, respectively, and the capacitive time constants were 2,010 ± 192 and 2,081 ± 189 µs, respectively. After compensation, $R_s$ values were 3.0 ± 0.2 and 3.3 ± 0.2 MΩ, and capacitive time constants were 424 ± 35 and 557 ± 42 µs. Cells with significant leak current were rejected.

Action potentials were recorded in current-clamp mode, beginning 5 min after membrane rupture. Stimulation with 2-ms pulses to −20 mV was applied at 0.1–4 Hz, and action potential parameters were recorded at steady state at each frequency. Recorded resting potentials were corrected for the junction potential, which averaged 11.5 mV.

Data analysis. Group data are expressed as means ± SE. Statistical comparisons between groups were made by t-test, with P < 0.05 considered statistically significant. A nonlinear least-square curve-fitting program in pCLAMP 6.0 or Sigma Plot was used for curve fitting.

RESULTS

In vivo effects of hypothyroidism. Hypothyroidism was associated with typical electrocardiographic changes, as illustrated by the representative ECG recordings in Fig. 1. The ECG recordings of control animals were similar to the baseline recordings of animals in the hypothyroid group (Table 1). At the time of euthanasia for electrophysiological study, hypothyroid animals had significantly longer R-R, Q-T, and Q-Tc intervals, but hypothyroidism did not alter P-R intervals, as illustrated by the representative ECG recordings in Fig. 1. During observation periods of 69 ± 5 days for controls and 73 ± 6 days for hypothyroid guinea pigs, the hypothyroid animals gained more weight than controls. On the day of experimental study, the average weight of hypothyroid animals was 799 ± 22 g, significantly larger than in the control group (697 ± 27 g, P < 0.05). Serum concentrations of Na$^+$, K$^+$, Ca$^{2+}$, and Cl$^-$ were unchanged in hypothyroid guinea pigs.

Action potential characteristics. In agreement with the Q-T prolongation observed in the ECG of hypothyroid guinea pigs, APD was prolonged in single ventricu-

![Fig. 1. Representative electrocardiogram (ECG) recordings in control and hypothyroid guinea pigs. Guinea pigs were sedated with acepromazine and ketamine, and ECGs were recorded at a paper speed of 200 mm/s. Decreased heart rate and marked prolongation in Q-T interval are characteristic ECG changes in hypothyroidism. II, aVL, aVF, ECG leads.](http://ajpheart.physiology.org/)

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Hypothyroidism increases APD by reducing \( I_{Ks} \)

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<th>Table 1. Electrocardiographic characteristics of control and hypothyroid guinea pigs</th>
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<td>R-R interval, ms</td>
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Results are mean ± SE; \( n = 25 \) and 9 for control and hypothyroid animals, respectively. Baseline values in hypothyroid group were obtained before exposure to propylthiouracil and within 2 days of surgical thyroidectomy. Study, values on day heart was removed for cell isolation and voltage-clamp study; Q-Tc, rate-corrected Q-T. Control animals had same initial age and weight as animals in hypothyroid group, and electrocardiogram values indicated were obtained after an observation period in control guinea pigs equivalent to that in hypothyroid animals. * \( P < 0.0001 \) vs. control.

Changes in \( I_K \) density \( I_K \) was recorded with the use of a series of 3-s depolarizing pulses (0.1 Hz) from a holding potential of \(-50 \) mV to test potentials from \(-40 \) to \(+70 \) mV, followed by a 2-s repolarizing pulse to \(-40 \) mV to record tail current \( I_{Ktail} \). Baseline measurements were performed 15 min after cell membrane rupture, and the protocol was run at least three times for each cell in 10-min intervals to detect rundown of \( I_K \). In cells with a stable \( I_K \) (<10% rundown over 20 min), 1 \( \mu M \) dofetilide was added to the bath solution to block \( I_{Kr} \). After an equilibration period of 10 min the protocol was repeated. Washout of dofetilide was obtained in five cells, and a mean reversal of 94% in drug effect was observed. Cells with rundown >10% (4% of cells) were rejected. To exclude differences between groups in the rate of early \( I_K \) rundown, the current was recorded at 5 and 15 min after membrane rupture in 14 cells for each group from 10 control and 7 hypothyroid animals. In control cells, \( I_K \) at \(+40 \) mV averaged \( 581 \pm 91 \) pA at 5 min and \( 541 \pm 93 \) pA at 15 min (7.3 ± 1.2% rundown). In hypothyroid cells, \( I_K \) averaged \( 191 \pm 20 \) pA at 5 min and \( 176 \pm 18 \) pA at 15 min (7.8 ± 0.7% rundown).

Representative \( I_K \) recordings in control and hypothyroid myocytes are illustrated in Fig. 3. Results are shown both before (Fig. 3, A and D) and after (Fig. 3, B and E) superfusion with 1 \( \mu M \) dofetilide. Dofetilide-sensitive currents (corresponding to \( I_{Kr} \), Ref. 26) obtained by digital subtraction are shown in Fig. 3, C and F. In cells isolated from hypothyroid hearts, the amplitudes of the time-dependent activating and tail cur-

\( \text{rents} \) were substantially smaller for total \( I_K \) and the slowly activating, dofetilide-resistant component \( I_{Kr} \). In contrast, the rapidly-activating, dofetilide-sensitive currents were not obviously affected by hypothyroidism.

Mean current densities are shown as a function of the voltage of the test pulse (TP) in Fig. 4. \( I_{Ks} \) step currents had a linear IV relationship in both groups, with
hypothyroid cells showing a significant decrease in step current densities at all voltages positive to 20 mV (Fig. 4A). For example, at +30 mV I_{KS} density was 1.23 ± 0.22 and 0.49 ± 0.11 pA/pF in control and hypothyroid myocytes, respectively (P < 0.05). I_{Kr} activated at more negative potentials, reached a maximum at +10 mV, and decreased thereafter. There was no statistically significant difference in I_{Kr} densities between control and hypothyroid cells, with current densities at 0 mV of 0.31 ± 0.04 and 0.30 ± 0.02 pA/pF for cells from control and hypothyroid myocytes, respectively (P = not significant (NS)).

I_{KS} tail currents also showed a smooth current-voltage relation, with current densities from cells of hypothyroid animals significantly decreased at all test potentials positive to −20 mV (Fig. 4B). Mean values for I_{KS} tails at a TP of +30 mV were 0.53 ± 0.10 pA/pF for controls and 0.10 ± 0.01 pA/pF for hypothyroid myocytes (P < 0.001). I_{Kr} tail currents approached saturation at 0 mV and had similar current densities for both groups of animals. For example, for an activating pulse to 0 mV, I_{Kr} tail current densities were 0.27 ± 0.07 pA/pF in control and 0.25 ± 0.02 pA/pF in hypothyroid myocytes (P = NS). Mean I_{Kr} step currents over the entire voltage range between −20 and +20 mV averaged 0.28 pA/pF for control cells and 0.24 pA/pF for hypothyroid cells, and corresponding values for tail currents were 0.24 and 0.22 pA/pF, respectively.

Kinetics and voltage dependence of I_{K} activation. The reduction in the amplitude of whole cell I_{KS} could involve a change in activation kinetics or a shift in the voltage dependence of activation. The time course of activation of the dofetilide-insensitive step current was best fit with a biexponential function. Hypothyroidism did not alter the kinetic properties of dofetilide-insensitive I_{KS}. For example, at a test potential of +60 mV, I_{KS} had a fast time constant (τ_{fast}) averaging 222 ± 22 ms for controls (n = 5) and 248 ± 28 ms for hypothyroid myocytes at 1- and 4-Hz stimulation frequency.

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<th>Control</th>
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<tr>
<td>V_{m}, mV</td>
<td>−83.4 ± 0.3</td>
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<td>AP amplitude, mV</td>
<td>128.6 ± 1.3</td>
<td>128.7 ± 1.1</td>
<td>128.4 ± 1.2</td>
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<td>APD_{20}, ms</td>
<td>42 ± 4</td>
<td>92 ± 4*</td>
<td>33 ± 3</td>
<td>56 ± 3*</td>
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<td>APD_{50}, ms</td>
<td>87 ± 7</td>
<td>172 ± 8*</td>
<td>71 ± 5</td>
<td>117 ± 5*</td>
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<td>APD_{90}, ms</td>
<td>103 ± 7</td>
<td>195 ± 10*</td>
<td>87 ± 5</td>
<td>140 ± 6*</td>
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Values are means ± SE; n = 13 and 15 for control and hypothyroid myocytes, respectively. V_{m}, resting membrane potential; AP, action potential; APD_{20}, APD_{50}, APD_{90}, AP duration to 20, 50 and 90% repolarization, respectively. *P < 0.0001 vs. control.

Table 2. Action potential parameters of control and hypothyroid ventricular myocytes at 1- and 4-Hz stimulation frequency.

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Hypothyroid cells (n = 5, P = NS). The corresponding values for the slow time constant (τslow) of IKs were 1,830 ± 214 and 1,439 ± 247 ms (P = NS). IKr activation kinetics were similarly unaffected by hypothyroidism. At a test potential of +10 mV, τfast of IKr was 68 ± 17 ms in controls (n = 5) and 81 ± 17 ms in hypothyroid myocytes (n = 6, P = NS), whereas τslow values were 1,785 ± 232 and 1,587 ± 253 ms for control and hypothyroid groups, respectively (P = NS).

An analysis of the voltage-dependent activation of IKr and IKs (Fig. 5) was performed by normalizing tail currents in each cell at each test potential to the current at the most positive voltage. A Boltzmann function was used to fit the activation curves of IKr and IKs. Under control conditions, IKs half-activation voltage (Vh) was 21.3 ± 2.7 mV with a slope factor (k) of 13.4 ± 1.1 mV, values equivalent to those previously reported.
in guinea pig ventricle (26). In hypothyroid animals, \( V_h \) of \( I_{K_s} \) shifted to more positive values and averaged 29.5 ± 2.2 mV (P < 0.01 vs. control); \( k \) was unchanged at 13.5 ± 1 mV (P = NS vs. control). \( I_{Kr} \) activation voltage dependence was not affected by hypothyroidism; \( V_h \) was −17.1 ± 3.1 mV in controls and −17.5 ± 3.3 mV in hypothyroid myocytes, and \( k \) averaged 9.0 ± 1.2 and 7.5 ± 1.4 mV, respectively.

Envelope of tails test. To assess the composition of \( I_K \) under control and hypothyroid conditions, we applied an envelope of tails analysis. \( I_K \) step currents (\( I_{K_{step}} \)) were elicited by depolarization from −60 to +50 mV with test pulses ranging from 60 to 4,200 ms, and tail currents were recorded on repolarization to −40 mV. Mean values for the ratio \( I_{Ktail} / I_{Kstep} \) are shown in Fig. 6. Before blockade of \( I_{Kr} \) (Fig. 6A), the envelope of tails test was not satisfied in either the control or the hypothyroid group, indicating that \( I_K \) results from the activation of more than one component. In hypothyroid myocytes, \( I_{Ktail} / I_{Kstep} \) was significantly larger at all intervals than in control cells, reflecting the larger contribution of \( I_{Kr} \) to total \( I_K \) because of the much smaller \( I_{Ks} \). After dofetilide was added to the superfusate (Fig. 6B), the envelope of tails test was satisfied. Furthermore, \( I_{Ktail} / I_{Kstep} \) was no longer different for hypothyroid compared with euthyroid cells, reflecting the fact that the same single component (\( I_{Ks} \)) remained under each condition.

Inward rectifier \( K^+ \) current. Figure 7A shows typical examples of \( I_{K1} \) in control and hypothyroid myocytes, and Fig. 7B shows mean \( I_{K1} \) densities elicited with 200-ms pulses to test potentials between −90 and +30 mV from a holding potential of −40 mV from 12 control and 20 hypothyroid myocytes. Hypothyroidism did not alter \( I_{K1} \). The reversal potential for \( I_{K1} \) was −71.0 ± 1.4 mV in hypothyroid cells, compared with −69.6 ± 1.2 mV in controls.

\( Ca^{2+} \) current. \( I_{Ca} \) is the major inward current during the plateau and is therefore crucial in the determination of APD and refractoriness under physiological conditions (17). The \( I_{Ca} \) current-voltage relation was studied at 36°C, with 400-ms steps from a holding potential of −80 mV at a frequency of 0.1 Hz. The magnitude of \( I_{Ca} \) was measured as the difference between the peak inward current and the steady-state current at the end of the depolarizing step. \( I_{Ca} \) densities were similar in control and hypothyroid myocytes at all voltages tested, as illustrated in Fig. 8. Peak current densities occurred at +10 mV in both groups and averaged 5.1 ± 0.3 pA/pF in controls and 5.9 ± 0.5 pA/pF in hypothyroid guinea pigs (P = NS). In both groups a T-type \( Ca^{2+} \) current (\( I_{Ca,T} \)) was noted as a shoulder on the total \( Ca^{2+} \) current-voltage relation. The density of \( I_{Ca,T} \) was not significantly affected by hypothyroidism.

Figure 9, A and B, shows \( I_{Ca} \) activation and inactivation voltage dependence, respectively, in 7 control and 10 hypothyroid cells. \( V_h \) averaged −11.2 ± 0.6 mV in the controls and −11.9 ± 0.8 mV in the hypothyroid group (P = NS), and mean values for \( k \) were 7.4 ± 0.5 and 7.5 ± 0.8 mV, respectively (P = NS). Voltage-dependent inactivation was also unaffected by hypothyroidism, with a mean \( V_h \) of −40.8 ± 2.3 mV in control and −37.8 ± 1.1 mV in hypothyroid myocytes and \( k \) values of 9.8 ± 0.8 and 8.6 ± 0.5 mV, respectively (P = NS).

These results indicate that differences in \( I_{Ca} \) density and voltage dependence cannot account for the APD differences between control and hypothyroid cells but do not exclude kinetic differences in \( I_{Ca} \) that could have major effects on APD. We therefore analyzed the time dependence of \( I_{Ca} \) inactivation development and recovery. The inactivation of \( I_{Ca} \) at +10 mV was best fit by a biexponential function with time constants of 5.1 ± 0.6
and 70.8 ± 4.8 ms (n = 5) in cells from control guinea pigs. In the hypothyroid group, no alteration was observed, with values of 6.1 ± 1.3 ms for $\tau_{fast}$ and 89.7 ± 8.4 ms for $\tau_{slow}$ (n = 7, P = NS vs. control for each). The recovery of $I_{Ca}$ from inactivation was studied with a two-pulse protocol (Fig. 9C). Two identical 300-ms pulses to +10 mV (P1 and P2) were delivered from a holding potential of −80 mV every 10 s at increasing P1-P2 intervals. Recovery kinetics were analyzed on the basis of the current amplitude during P2 relative to the amplitude during P1 as a function of the P1-P2 interval. Recovery was rapid under control and hypothyroid conditions and was best fitted with a biexponential function. Mean $\tau_{fast}$ was 96.8 ± 11.4 ms in controls and 99.5 ± 14.5 ms in hypothyroid cells (n = 5 and 6 cells, respectively, P = NS), whereas $\tau_{slow}$ averaged 779 ± 75 and 1,267 ± 230 ms, respectively (P = NS).

$Na^+$ current. $I_{Na}$ is the other major inward current in cardiac myocytes and is a particularly important determinant of conduction and cellular excitability. We studied $I_{Na}$ at 17°C with 40-ms steps at 0.1 Hz to test potentials between −80 and −5 mV (with 5-mV increments) from a holding potential of −120 mV. The groups had similar current densities, voltages of peak current density ($−35$ mV), and mean peak current densities (48 ± 5 and 49 ± 4 pA/pF in 10 and 11 myocytes from control and hypothyroid animals, respectively). Inactivation kinetics of $I_{Na}$ at −35 mV were best
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ventricular repolarization. This effect was associated with large reductions in $I_k$, the primary repolarizing K⁺ current in the guinea pig, which were exclusively caused by decreases in $I_{Ks}$. No other changes in K⁺, Ca²⁺, or Na⁺ currents were seen.

Comparison of ECG and action potential changes with previous reports in the literature. The ECG changes we observed in hypothyroid guinea pigs (a decrease in heart rate and a prolonged Q-T interval) are typical of clinical hypothyroidism (32) and are quantitatively similar to those described previously in guinea pigs rendered hypothyroid by $^{131}$I (33). At 4 Hz, which corresponds to the physiological heart rate in guinea pigs, APD at 90% repolarization was 61% longer in hypothyroid guinea pigs than under control conditions, whereas at slower frequencies the prolongation was more pronounced. As in previous standard microelectrode studies, all phases of repolarization were prolonged to a similar extent (11, 28).

Comparison with previous studies of ionic current changes in altered thyroid state. The voltage-dependent properties of $I_{Ks}$ and $I_k$ in myocytes from control guinea pigs were similar to those reported previously for this species (26) and comparable to delayed rectifier current in cells from human atria (37) and ventricles (16). Under hypothyroid conditions, $V_n$ of $I_{Ks}$ was shifted by 8.2 mV to more positive voltages, leading to a decrease in current amplitude at a given voltage. However, this relatively small shift is not sufficient to account for the total reduction of $I_{Ks}$, because maximal $I_{Ks}$ conductance (obtained by dividing the current amplitude by the driving force for K⁺) is decreased. For example at +50 mV, maximum conductance was 3.21 µS in control cells and 1.07 µS in cells from hypothyroid guinea pigs. Our results regarding changes in $I_k$ in hypothyroid guinea pigs differ from those of two previous publications by Binah et al. (4) and Rubinstein and Binah (23), in which no difference in $I_k$ was noted between control and hypothyroid guinea pigs. The discrepancies between our results and those of Binah and co-workers may be caused by a variety of technical factors such as experimental temperature, isolation technique, and method of $I_k$ measurement. Temperature can have a marked effect on $I_k$ currents and their response to interventions (10, 35), and $I_k$ is particularly sensitive to isolation technique (39). The analysis of current density is important in studies of hypothyroidism, because as we found and others have reported previously (19, 30), hypothyroidism can alter cell size.

$I_{K1}$ contributes to the late phase of repolarization at near-diastolic potentials. Hypothyroidism did not influence $I_{K1}$ in our study. To our knowledge, there are no published studies of the effects of hypothyroidism on $I_{K1}$. Shimoni and Banno (29) did not detect differences in $I_{K1}$ between hyperthyroid and euthyroid rabbits.

A previous study by Rubinstein and Binah (23) reported a decrease in $I_{Ca}$ amplitude in hypothyroid guinea pigs, whereas we found $I_{Ca}$ to be unchanged by hypothyroidism. A variety of technical differences may explain the discrepancy. Kosinski et al. (15) found no change in L-type Ca²⁺ channel concentration with

**DISCUSSION**

In the present study, we have demonstrated that hypothyroidism leads to important delays in guinea pig.
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Potential mechanism of hypothyroid effect on \( I_{Ks} \).
Reduced thyroid hormone activity could decrease \( I_{Ks} \) density by several mechanisms. Thyroid hormone is known to control cardiac gene expression (31). The channel that carries \( I_{Ks} \) appears to result from the coassembly of KvLQT1 and minK proteins (1, 24, 38). We reported preliminary findings suggesting that KvLQT1 mRNA concentrations were reduced in hypothyroid animals (5); however, because of problems with RNA stability, we have been unable to confirm or refute these initial data. Postranscriptional changes, such as differences in protein kinetics, insertion in the membrane, or regulation by neurohormones (3, 36) may also be involved in the decrease in \( I_{Ks} \).

Potential significance. Thyroid hormone is known to be an important regulator of cardiac repolarization. Our study is the first to provide a clear mechanism for the phenomenon in a mammalian heart. The importance of \( I_{Ks} \) in repolarizing guinea pig ventricular myocytes has been suggested by recent modeling work (40). The degree of prolongation in APD associated with \( I_{Ks} \) reduction in our guinea pigs (65% at 4 Hz) is of the same order as predicted by the mathematical model (between 38 and 70% increase for \( I_{Ks} \) reductions of 65–80%; Y. Rudy, personal communication), supporting the relevance of the latter and the role it suggests for \( I_{Ks} \) in repolarization. On the other hand, removing \( I_{Ks} \) from the action potential model strongly promotes the generation of EADs (40), which we did not see in the present study.

Because of the potential proarrhythmic effects associated with currently available class III drug therapy (21), there has been particular interest in the development of substances with novel ionic targets. Jurkiewicz and Sanguinetti (12) demonstrated that \( I_{Ks} \) contributes to rate-dependent action potential abbreviation and suggested that \( I_{Ks} \) may be an interesting target for new antiarrhythmic drugs. Of note, although APD was greater in hypothyroid guinea pigs at all frequencies, the difference was greatest at low frequencies, i.e., some reverse use dependence for APD prolongation was present. In hypothyroid patients, QT prolongation is a typical electrocardiographic feature (32), but torsades de pointes arrhythmias are rarely observed (22). Furthermore, hypothyroidism is known to have significant antiarrhythmic actions (22, 34). Hypothyroidism may therefore be an interesting natural paradigm for delayed repolarization with low associated risk of torsades de pointes. The extent to which these electrophysiological actions of hypothyroidism are caused by a depression in \( I_{Ks} \) without alteration in \( I_{Kr} \), as opposed to or in combination with other actions of hypothyroidism (e.g., reduced sympathetic activation (3)), remains to be determined. It should be pointed out in this context that KvLQT1 mutations (and presumably \( I_{Ks} \) dysfunction) cause a common and life-threatening form of the long-Q-T syndrome (1, 24, 38).

Potential limitations. We studied left ventricular cells from guinea pigs, which have important ionic differences from human ventricular myocytes. Transient outward current, which has been shown to be an important repolarizing current in human ventricle (20), is absent in the guinea pig. \( I_{Ks} \) was found to be markedly reduced in hypothyroid rat ventricle (30), whereas in rabbit ventricle it was unchanged (29). It is therefore possible that alterations in \( I_{Ks} \) play a role in the changes in repolarization in human ventricle under hypothyroid conditions. On the other hand, both components of \( I_{K} \) are present and likely to play a significant role in human ventricular repolarization (16). Furthermore, it is important to note that \( I_{Kf} \) inhibition reduces, rather than increasing, APD in dog ventricle (18). Other ionic mechanisms, such as exchangers and pumps (27), may also be affected by hypothyroidism and were not assessed in the present study. Thyroid hormone has particular important effects on the Na\(^+\)-K\(^+\) ATPase, which is significantly downregulated by hypothyroidism (7). Thus effects of hypothyroidism on cardiac pumps and exchangers may contribute significantly to cardiac electrophysiological properties in the presence of hypothyroidism, an issue that remains to be resolved.

\( I_{K} \) is very sensitive to isolation procedures (39), and therefore changes in isolation technique could contribute to differences in \( I_{K} \) when several groups are compared. To minimize possible effects of time-dependent changes in enzymes, isolation success, etc., animals from each group were studied concurrently in an alternating fashion. Rundown can be a problem when studying \( I_{K} \) and \( I_{Ca} \). All currents were therefore studied with serial measurements to screen for rundown. All cells with significant rundown were rejected for analysis. Mean values for \( I_{Kr} \) step and tail currents on steps to between −20 and +20 mV were slightly smaller (by 14 and 8%, \( \text{P} = \text{NS} \) for each) in hypothyroid compared with control cells. We cannot exclude the possibility of a very small change in \( I_{Kr} \) (of uncertain physiological significance) in hypothyroid hearts.

We studied action potentials from isolated cells in whole cell mode. These results may be different from those obtained with traditional voltage followers and fine-tipped microelectrodes in multicellular preparations. \( I_{Ks} \) activation kinetics were determined during 3-s pulses, which were only about twice the estimated time constant. This introduces an element of imprecision, and the time constants should not be considered as absolutely precise but rather as a quantitative tool to compare the relative activation rates in control versus hypothyroid cells.

In conclusion, we have shown that hypothyroidism in guinea pigs is associated with a pronounced delay in ventricular repolarization both in vivo and in isolated ventricular myocytes. The only ionic current change noted was an important reduction of the slow component of the delayed rectifier K\(^+\) current, of a magnitude potentially sufficient to account for the repolarization changes observed. These results point to an important role of \( I_{Ks} \) in controlling repolarization in ventricular
myocytes and in mediating the regulation of cardiac repolarization by thyroid hormone.

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