Electrophysiologic Effects of Chronic Amiodarone Therapy and Hypothyroidism, Alone and in Combination, on Guinea Pig Ventricular Myocytes

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ABSTRACT

Amiodarone is a widely used antiarrhythmic drug, the mechanisms of action of which remain incompletely understood. Direct evidence suggests that the class III properties of amiodarone may be mediated by cardiac antithyroid effects. We sought to determine whether the effects of chronic amiodarone on repolarization in guinea pig hearts can be attributed to an antithyroid action by studying the changes in dofetilide-sensitive rapid (I_{Kr}) and dofetilide-resistant slow (I_{Ks}) delayed rectifier currents, inward rectifier K⁺ current (I_{K1}), and action potentials of ventricular myocytes from five groups of guinea pigs: control, hypothyroid, amiodarone-treated for 7 days, hypothyroid plus amiodarone, and vehicle (dimethyl sulfoxide) treated. I_{Kr} was reduced by amiodarone (to 61% of control, P < .05, at 50 mV) but was more strongly reduced by hypothyroidism (to 35% of control, P < .01, 50 mV). Amiodarone significantly reduced I_{Kr} and I_{Ks} (by 55 and 64% at 10 mV and −50 mV, respectively), which were unaffected by hypothyroidism. Amiodarone alone and hypothyroidism alone had similar action potential prolonging actions. Hypothyroid animals treated with amiodarone showed a combination of ionic effects (strong I_{Ks} reduction, similar to hypothyroidism alone; reduced I_{Kr} and I_{K1}, similar to amiodarone alone), along with action potential prolongation significantly greater than that caused by either intervention alone. We conclude that chronic amiodarone and hypothyroidism have different effects on ionic currents and that their combination prolongs action potential duration to a greater extent than either alone in guinea pig hearts, suggesting that the class III actions of amiodarone are not mediated by a cardiac hypothyroid state.

Amiodarone is widely believed to be the most effective antiarrhythmic drug available at present. For example, it is the only antiarrhythmic agent that has been shown to reduce arrhythmic death in patients with frequent ventricular ectopy postmyocardial infarction (Cairns et al., 1997) and appears to have superior efficacy to other antiarrhythmic drugs for sinus rhythm maintenance in patients with atrial fibrillation (Nattel, 1995). The cellular actions of amiodarone were first described by Singh and Vaughan Williams in 1970, who noted that chronic amiodarone administration increases action potential duration (APD) without affecting maximum upstroke velocity in rabbit ventricle (Singh and Vaughan Williams, 1970). They remarked on the similarity between the actions of amiodarone and hypothyroidism on the heart and showed that thyroxine reversed the effects of amiodarone, leading them to conjecture that amiodarone may exert its effects by interfering with the action of thyroid hormone on the heart.

Since the classical studies of Singh and Vaughan Williams, many investigators have evaluated possible relations between amiodarone and thyroid actions on the heart. Several investigators have noted the similarity between various cardiac effects of amiodarone and hypothyroidism on APD (Singh et al., 1970), on ventricular refractoriness (Patterson et al., 1986; Liu et al., 1996), on ventricular fibrillation threshold (Liu et al., 1996), and on ventricular myosin enzyme isoforms (Wieand et al., 1986). Hypothyroidism has been found to prevent amiodarone effects on heart rate and Q–T interval (Talajic et al., 1989) and on K⁺ currents in

ABBREVIATIONS: APD, action potential duration; APD_{90}, action potential duration to 90% repolarization; DMSO, dimethyl sulfoxide; PTU, propylthiouracil; I_{Kr}, rapid component of delayed rectifier current; I_{Ks}, slow component of delayed rectifier current; I_{K1}, inward rectifier current; I_{or}, transient outward current; R_s, series resistance.
cultured rat cardiomyocytes (Guo et al., 1997). A variety of studies suggest that amiodarone and/or its desethyl metabolite inhibit binding of triiodothyronine to its nuclear receptor and produce a variety of cardiac metabolic changes that resemble those of hypothyroidism (Latham et al., 1987; Gotzsche, 1993; Gotzsche and Orskov, 1994; Drvota et al., 1995). On the other hand, mimicking at least one of the hypothyroid actions of amiodarone, prevention of conversion of thyroxine to triiodothyronine, did not reproduce the effects of the drug on heart rate and the Q–T interval (Staubli and Studer, 1986).

Most of the work studying the relationship between the actions of amiodarone and those of hypothyroidism and the interaction between amiodarone and thyroid effects has been performed in whole-animal and standard microelectrode studies. Very little is known about the actions and interactions of chronic amiodarone therapy and hypothyroidism at the level of the ionic currents that control repolarization. The goal of the present studies was to establish the effects of chronic amiodarone administration and hypothyroidism, separately and in combination, on K⁺ currents in guinea pig ventricular myocytes. Complementary information, in terms of effects on the ECG in vivo and action potentials of isolated cells, was also obtained. In this way, we hoped to establish the extent to which the ionic effects of amiodarone can be attributed to an interaction with thyroid effects on the heart.

Materials and Methods

Experimental Groups. Adult male Hartley albino guinea pigs were assigned to one of the following groups: a control group (n = 25), a hypothyroid group (n = 14), an amiodarone-treated group (n = 9), a hypothyroid amiodarone-treated group (n = 6), and a dimethyl sulfoxide (DMSO)-treated group (n = 4). The procedures followed were in accordance with the guidelines of the Montreal Heart Institute Animal Ethics Committee and the Canadian Council on Animal Care. Animals assigned to the hypothyroid group were thyroectomized by Charles River (St. Constant, QC, Canada) after anesthesia with xylazine (5 mg/kg, i.m.; Miles Canada, Inc.) and ketamine (40 mg/kg, i.m.; Rogar/STP, Inc.). Subsequently, these guinea pigs were treated with 5-propylthiouracil (PTU; dissolved in the drinking water at a concentration of 0.05%) (Sigma Chemical Co., St. Louis, MO) and potassium perchlorate (30 mg/kg, i.p., I.P. Laboratories, New York, NY) and maintained 20 mmol/liter KCl, 110 mmol/liter potassium aspartate, 1.0 mmol/liter MgCl₂, 10 mmol/liter HEPES, 5 mmol/liter ethylene glycol (β-aminooxybutyric acid, 20 mmol/liter tauroxine, and 10 mmol/liter ethylene glycol bis(β-aminooxy) ether)-N,N,N,N’-tetraacetic acid, along with 1% albumin (pH adjusted to 7.35 with KOH).

A small aliquot of the cell-containing solution was placed in a 1-ml open perfusion chamber mounted on the stage of an inverted microscope. After a brief period to allow cell adhesion to the bottom of the chamber, the cells were perfused at 6 ml/min with a solution containing: 136 mmol/liter NaCl, 5.4 mmol/liter KCl, 2.0 mmol/liter CaCl₂, 1.0 mmol/liter MgCl₂, 0.33 mmol/liter NaH₂PO₄, 5 mmol/liter HEPES, and 10 mmol/liter glucose (pH adjusted to 7.35 with NaOH) for the recording of action potentials, inward rectifier current (Iₖ,1), and delayed rectifier current (Iₖ,2). For the recording of Iₖ,1 and Iₖ,2, Ca²⁺ current was blocked with 5 μM nifedipine (Sigma). Bath temperature was maintained at 36.2 ± 0.4°C with a thermistor-controlled heating unit for all experiments. The pipette solution contained 20 mmol/liter KCl, 110 mmol/liter potassium aspartate, 1.0 mmol/liter MgCl₂, 10 mmol/liter HEPES, 5 mmol/liter ethylene glycol bis(β-aminooxy) ether)-N,N,N,N’-tetraacetic acid, 5 mmol/liter Mg₂ATP, 0.1 mmol/liter GTP, and 5 mmol/liter phosphocreatine (pH adjusted to 7.2 with KOH) to record action potentials, Iₖ,1 and Iₖ,2.

Voltage-Clamp Technique. Only quiescent, rod-shaped cells with clear cross striations were studied. Ionic currents were recorded with the whole-cell configuration of the voltage-clamp technique. Borosilicate glass electrodes (outer diameter, 1.0 mm) with resistances from 2.5 to 6 megohms when filled with pipette solution were connected to a patch clamp amplifier (Axopatch 200A, Axon Instruments, Burlingame, CA). Data were sampled with an analog to digital converter (Digidata 1200, Axon Instruments) and stored on the hard disk of a computer for subsequent analysis. Recordings were low-pass filtered at 2 kHz.

Liquid junction potential offsets were compensated before formation of the pipette-membrane seal. After rupture of the cell membrane, pipette series resistance (Rₛ) was calculated by dividing the capacitive transient by the holding potential of Rₛ was calculated by dividing the capacitive response to a 5-mV hyperpolarizing pulse from a holding potential of −60 mV, divided by the voltage drop). The passive electrical properties of the cells included for the different experimental groups are given in Table 1. Membrane capacitance was not altered by amiodarone or DMSO treatment; however, hypothyroidism led to a statistically significant, −25% increase in cell capacitance. To control for differences in cell size, all mean current data are expressed as current densities (i.e., normalized to capacitance). Cells with significant leak current were rejected, and leakage compensation algorithms were not used.

Recorded resting membrane potentials were corrected for the junction potential. To calculate the junction potential, pipettes filled with determine the R–R, P–R, Q–R–S, and Q–T intervals. ECGs were recorded at baseline and once a week thereafter.

Cell Isolation and Solutions. Guinea pigs were sacrificed by cervical dislocation, and the hearts were quickly excised and mounted on a Langendorff apparatus. The hearts were retrogradely perfused via the aorta with oxygenated (100% O₂, pH adjusted to 7.35 with NaOH) Tyrode’s solution containing: 136 mmol/liter NaCl, 5.4 mmol/liter KCl, 2.0 mmol/liter CaCl₂, 1.0 mmol/liter MgCl₂, 0.33 mmol/liter NaH₂PO₄, 5 mmol/liter HEPES, and 10 mmol/liter glucose at 37°C. When clear of blood, the perfusate was changed to a nominally Ca²⁺-free Tyrode’s solution until contraction had ceased completely. Perfusion was then continued with the same solution containing 0.03% collagenase (Type II, Worthington Biochemical) and 1% BSA (Sigma Chemical Co.) until left ventricular tissue was softened. Small pieces of tissue were removed with forceps and mechanically dissociated by trituration. The isolated cells were kept in a storage solution containing 20 mmol/liter KCl, 10 mmol/liter KH₂PO₄, 25 mmol/liter glucose, 40 mmol/liter mannitol, 70 mmol/liter l-glutamic acid, 10 mmol/liter β-hydroxybutyric acid, 20 mmol/liter tauroxine, and 10 mmol/liter ethylene glycol bis(β-aminooxy) ether)-N,N,N,N’-tetraacetic acid, along with 1% albumin (pH adjusted to 7.35 with KOH).

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The effects of hypothyroidism and amiodarone on $I_{Ks}$ are illustrated in Fig. 3. Figure 3A shows dofetilide-sensitive $I_{Ks}$ from representative cells in the five experimental groups. Chronic amiodarone treatment and hypothyroidism were both associated with a decrease in $I_{Ks}$ step and tail currents; however, hypothyroidism led to a much stronger reduction of $I_{Ks}$ than amiodarone treatment. Amiodarone treatment in hypothyroid animals caused no additional decrease in $I_{Ks}$ amplitude compared with hypothyroidism alone. DMSO had no apparent influence on $I_{Ks}$. Mean time-dependent step current densities are shown as a function of the voltage of the test pulse in Fig. 3B and at a single reference voltage in Table 3. $I_{Ks}$ step currents had a linear current-voltage relation positive to 10 mV in all groups, but significant differences in current densities were seen among groups. Overall, amiodarone treatment (studied in eight cells) significantly reduced $I_{Ks}$ compared with control ($n = 8$), whereas DMSO had no
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and amiodarone-treated (cells (hypothyroid) compared with cells from control, hypothyroid, or a combination of the two treatments (Table 3). The current-density-voltage relation was bell shaped, with a maximum step current at 10 mV and strong inward rectification at more positive voltages. The overall shape of the current-voltage relation was not obviously different in any group, but density was significantly reduced in both amiodarone-treated groups. No significant differences in density were noted among control, hypothyroid, and DMSO-treated groups; nor were results in amiodarone-treated or hypothyroid/amiodarone-treated animals different from each other. Changes in tail densities were of the same order as those in step currents, as illustrated by the mean values following a step to 0 mV shown in Table 3.

Voltage and Time Dependence of Activation. The voltage-dependent activation of and was analyzed by normalizing dofetilide-sensitive and dofetilide-resistant tail currents at each test potential (obtained with the voltage protocols illustrated in Figs. 3 and 4) to the current at the most positive voltage. A Boltzmann function was used to fit the activation curves of and . In all groups, activation at negative voltages, with a half-activation voltage (V1/2) of −17.1 ± 3.1 mV and a slope factor (k) of 9.0 ± 1.2 mV under control conditions and no significant differences among groups. These values are similar to results reported previously in normal guinea pig ventricle (Sanguinetti and Jurkiewicz, 1990). activation at more positive potentials. For example, V1/2 under control conditions averaged 22.6 ± 2.4 mV, and k was 13.4 ± 1.1 mV. Similar to the results for activation voltage dependence was not altered in any treatment group (Table 4) and was similar to values reported previously in normal guinea pig ventricular myocytes (Sanguinetti and Jurkiewicz, 1990).

The time-dependent activation of was best fitted by a biexponential function, whereas its deactivation was best fitted by a monoexponential function. MonoeXponential relations fit both the activation and deactivation of in all groups, whereas hypothyroid cells had values 65% less than controls. was affected in the same way as steps, with a significant reduction in amiodarone-treated cells, hypothyroid cells, or a combination of the two treatments (Table 3).

Figure 4 shows original recordings of (dofetilide-sensitive current) in Fig. 4A and mean current density-voltage relations in Fig. 4B. was much smaller in cells from amiodarone-treated animals (whether or not they were hypothyroid) compared with cells from control, hypothyroid, or DMSO-treated animals (Fig. 4A). Mean data from control cells (n = 8), DMSO controls (n = 5), amiodarone (n = 8), hypothyroid (n = 9), and hypothyroid/amiodarone (n = 7) groups are shown in Fig. 4B. The current-density-voltage relation was bell shaped, with a maximum step current at 10 mV and strong inward rectification at more positive voltages. The overall shape of the current-voltage relation was not obviously different in any group, but density was significantly reduced in both amiodarone-treated groups. No significant differences in density were noted among control, hypothyroid, and DMSO-treated groups; nor were results in amiodarone-treated or hypothyroid/amiodarone-treated animals different from each other. Changes in tail densities were of the same order as those in step currents, as illustrated by the mean values following a step to 0 mV shown in Table 3.

### Table 2

<table>
<thead>
<tr>
<th>Electrocardiographic Characteristics</th>
<th>Control</th>
<th>Hypo</th>
<th>Amio</th>
<th>Hypo + Amio</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>R–R Interval (ms)</td>
<td>199 ± 4</td>
<td>294 ± 4*</td>
<td>289 ± 9*</td>
<td>319 ± 15*</td>
<td>221 ± 12</td>
</tr>
<tr>
<td>Q–T Interval (ms)</td>
<td>108 ± 2</td>
<td>163 ± 2*</td>
<td>158 ± 3*</td>
<td>173 ± 10*</td>
<td>126 ± 7</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>25</td>
<td>14</td>
<td>9</td>
<td>6</td>
<td>4</td>
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</tbody>
</table>

Hypo, hypothyroid; Amio, amiodarone-treated hypothyroid animals; DMSO, dimethylsulfosxide-treated animals.

*P < .01 versus control.

**Fig. 2.** Delayed rectifier potassium current in guinea pig ventricle. A, typical example of delayed rectifier currents recorded at 36°C with 3-s depolarizing steps to test potentials between −40 and +70 mV from a holding potential of −50 mV. Tail currents were obtained upon repolarization to −40 mV. The bath solution contained 5 μM nifedipine to block . The components of were separated pharmacologically with the use of 1 μM dofetilide, which specifically inhibits the rapid component (). B, dofetilide-resistant component () at potentials between −40 and +70 mV. These recordings were obtained after a 10-min exposure of the cell to dofetilide. C, the dofetilide-sensitive current () obtained by digital subtraction of currents in B from currents in A. For reasons of clarity, only test potentials between −30 and +30 mV are shown in C. 0 indicates zero current level.

**Fig. 5.** Cells from animals treated with amiodarone showed smaller currents than cells from the other three groups. Mean density-voltage relations for all groups (cell numbers were 13, 20, 19, 7, and 9 for control, hypothyroid, amiodarone, hypothyroid/amiodarone, and DMSO, respectively) are shown in Fig. 6A. The smaller outward currents are shown on an expanded scale in Fig. 6B. Amiodarone significantly reduced both inward and outward components of , whether in the presence or absence of hypothyroidism,
whereas hypothyroidism and DMSO did not alter $I_{K1}$ compared with control. For example, at $-100$ mV, current density for control cells averaged $-36 \pm 4$ pA/pF, compared with $-32 \pm 3$ pA/pF in DMSO-treated cells and $-34 \pm 2$ pA/pF in hypothyroid cells ($P$ was not significant for each group versus control). In contrast, the values at $-100$ mV were $-20 \pm 1$ pA/pF for amiodarone treatment alone and $-22 \pm 2$ pA/pF in hypothyroid amiodarone-treated animals. The outward component of $I_{K1}$ at voltages positive to the reversal potential of $-70$ mV was similarly affected. For example, at $-40$ mV,
control cells had an average current density of 5.3 ± 0.4 pA/pF, which was not altered by DMSO (5.9 ± 0.5 pA/pF) or by hypothyroidism (5.6 ± 0.3 pA/pF), but was substantially reduced in the amiodarone (1.7 ± 0.2 pA/pF) and hypothyroid/amiodarone (2.3 ± 0.4 pA/pF)-treated groups.

**Action Potential Characteristics.** Representative examples of action potentials of cells from each group at a stimulation frequency of 4 Hz are shown in Fig. 7A. Amiodarone treatment, hypothyroidism, and the combination of both interventions were associated with a significant prolongation of the APD. Action potential shape, resting membrane potential, and action potential amplitude were similar in all experimental groups. APD90 is plotted as a function of stimulation frequency in Fig. 7B. In cells from hypothyroid animals, APD90 was significantly longer than under control conditions for all stimulation frequencies. For example, at 4 Hz, the values were 87 ± 5 ms under control (n = 13) and 140 ± 6 ms under hypothyroid conditions (n = 15; P < .01 versus control). Amiodarone-treated cells had an APD90 that was in the range of the

![Fig. 4. Dofetilide-sensitive component (I_Kr) of the delayed rectifier potassium current. I_Kr step currents were obtained by digital subtraction of delayed rectifier current in the presence of 1 μM dofetilide from those recorded before dofetilide infusion. A, representative recordings from each group. B, I_Kr step current density-voltage relations for 8, 9, 8, 7, and 5 cells for control, hypothyroid, amiodarone, amiodarone plus hypothyroid, and DMSO groups, respectively. **P < .01 versus control; *P < .05 versus hypothyroid group.](image)

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Voltage-dependent activation of I_Ks and I_Kr</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Vh I_Ks (mV)</td>
<td>22.6 ± 2.4</td>
</tr>
<tr>
<td>k I_Ks (mV)</td>
<td>13.4 ± 1.1</td>
</tr>
<tr>
<td>Vh I_Kr (mV)</td>
<td>-17.1 ± 3.1</td>
</tr>
<tr>
<td>k I_Kr (mV)</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>Number of Cells</td>
<td>8</td>
</tr>
</tbody>
</table>

Vh, half-activation potential; k, slope factor; Hypo, hypothyroid; Amio, amiodarone-treated; Hypo + Amio, amiodarone-treated hypothyroid animals; DMSO, dimethylsulfoxide-treated animals.

<table>
<thead>
<tr>
<th>TABLE 5</th>
<th>Kinetics of I_K in various experimental groups</th>
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<tbody>
<tr>
<td></td>
<td>V (mV)</td>
</tr>
<tr>
<td>I_Ks Activation-τ1</td>
<td>50</td>
</tr>
<tr>
<td>I_Ks Activation-τ2</td>
<td>50</td>
</tr>
<tr>
<td>I_Ks Deactivation</td>
<td>-40</td>
</tr>
<tr>
<td>I_C Activation</td>
<td>0</td>
</tr>
<tr>
<td>I_C Deactivation</td>
<td>-40</td>
</tr>
</tbody>
</table>

V, voltage at which kinetics were determined; tail currents were recorded upon repolarization to -40 mV after a 3-s step to 50 mV (for I_Ks) or 0 mV (for I_Kr). Activation kinetics were analyzed during a 3-s step to voltages indicated. Numbers in parentheses indicate number of cells studied. Hypo, hypothyroid; Amio, amiodarone-treated; Hypo + Amio, amiodarone-treated hypothyroid animals; DMSO, dimethylsulfoxide-treated animals.
hypothyroid group (e.g., at 4 Hz, 149 ± 6 ms, n = 23; P < .01 versus control). Amiodarone treatment in hypothyroid animals was associated with an additional substantial AP prolongation that was most prominent at slow frequencies but remained significant at higher frequencies. At 4 Hz, APD$_{90}$ averaged 193 ± 9 ms in hypothyroid amiodarone-treated cells (n = 9; P < .001 versus control and P < .01 versus amiodarone or hypothyroidism alone). DMSO treatment had no effect on APD$_{90}$ (e.g., at 4 Hz, 102 ± 7 ms, n = 9; P was not significant, versus control).

**Discussion**

In this study, we examined the effects of chronic amiodarone therapy, hypothyroidism, and the combination of amiodarone and hypothyroidism on K$^+$ currents in guinea pig ventricular myocytes. Whereas hypothyroidism only affected $I_{Ks}$, amiodarone altered $I_{Ko}$ (albeit to a lesser extent than hypothyroidism), $I_{Kr}$, and $I_{K1}$. Combining amiodarone with hypothyroidism led to a pattern characteristic of both effects individually, with strong reductions in $I_{Ks}$ along with decreases in $I_{Ko}$ and $I_{K1}$. These observations suggest that amiodarone has actions on repolarizing current that are independent of thyroid effects, a concept supported by the additional changes in APD observed in the presence of amiodarone and hypothyroidism compared with either intervention alone.

**Comparison with Previous Studies of Actions of Amiodarone on K$^+$ Currents.** The first published voltage-clamp studies of the ionic actions of amiodarone noted reductions in “total outward current” (predominantly $I_{Ks}$) in frog atrial muscle preparations at concentrations above 10 μM (Néliat et al., 1982). Balser et al. (1991) studied the effects of acute amiodarone administration (10 μM) on guinea pig ventricular myocytes and observed a reduction in the La$^{2+}$-resistant component but no effect on the La$^{2+}$-sensitive component, compatible with principal actions on $I_{Ko}$. On the other hand, Kamiya et al. (1995) and Varró et al. (1996) observed that acute amiodarone administration decreased $I_{K}$ in rabbit ventricular myocytes, a species in which $I_{Ks}$ is the predominant component under usual experimental conditions (Colatsky et al., 1990). $I_{Iw}$ was unaffected by 10 μM amiodarone in rabbit myocytes (Kamiya et al., 1995; Varró et al., 1996). Sato et al. (1994) noted that amiodarone causes a decrease in $I_{K1}$ whole-cell current in guinea pig ventricular...
cells, along with an increase in interburst interval at the single channel level. Guo et al. (1997) showed that acute amiodarone exposure decreases the density of both $I_{to}$ and $I_{end-pulse}$ current in neonatal rat ventricular myocytes. Studies of acute amiodarone exposure are difficult to relate to the drug's ventricular repolarization-delaying effects in humans, because the latter are not apparent on acute administration and only develop during sustained therapy (Ikeda et al., 1984; Mitchell et al., 1989). Studies in experimental animals support the concept that chronic amiodarone therapy may have different effects from acute drug administration (Ohta et al., 1987; Gallagher et al., 1989; Kodama et al., 1997). It is therefore quite important to assess the ionic actions of chronic amiodarone administration. The data available in the literature are limited, have been obtained from the rabbit, and have not separated drug effects on the rapid and slow components of $I_K$. Varró et al. (1996) showed that chronic amiodarone administration (50 mg/kg daily for 3–4 weeks) reduced $I_K$ by ~50% and $I_{to}$ by ~30% without affecting $I_{K1}$. Qualitatively, similar findings have been reported by Kamiya et al. (1995). In this report, we show that chronic amiodarone administration significantly reduces the density of both $I_{Kr}$ and $I_{Ks}$, without altering their voltage dependence or kinetics. In addition, amiodarone reduces $I_{K1}$, including its outward component, potentially contributing to its repolarization-delaying action.

**Comparison with Previous Studies of Amiodarone-Thyroid Interaction.** As indicated in the Introduction, many lines of evidence point to the possibility of thyroid-mediated actions of amiodarone on cardiac repolarization; however, the findings available in the literature are insufficient to determine whether the APD-prolonging effects of amiodarone can be attributed to an inhibition of the cardiac actions of thyroid hormone. In the present study, we found qualitative differences between the changes in $K^+$ currents observed in hypothyroid compared with amiodarone-treated animals. Furthermore, guinea pigs exposed to both thyroidectomy and amiodarone therapy showed a combination of the effects of either alone and had significantly longer action potentials than animals exposed to either intervention alone. These observations suggest that chronic amiodarone therapy has effects on repolarization that are not due to inhibition of thyroid action alone and may well be due to direct inhibitory effects on cardiac $K^+$ channels, in agreement with previous studies.

**Fig. 6.** $I_{K1}$ density-voltage relations ($n = 13, 20, 19, 7,$ and $9$ cells for control, hypothyroid, amiodarone, hypothyroid plus amiodarone, and DMSO groups, respectively). A, $I_{K1}$ density-voltage relations at all test potentials studied. B, an enlargement of the inset in A to show clearly the outward currents carried by $I_{K1}$. Where error bars are absent, they fell within the symbol for mean. **$P < .01$ versus control; *$P < .01$ versus hypothyroid cells.**

**Fig. 7.** A, action potentials recorded in representative cells from each group at a stimulation frequency of 4 Hz. B, mean $APD_{90}$ for stimulation frequencies between 0.1 and 4 Hz, as recorded in 13 control cells, 15 cells from hypothyroid guinea pigs, 23 cells from amiodarone-treated guinea pigs, 9 cells from amiodarone-treated hypothyroid animals, and 9 cells from DMSO-treated guinea pigs. **$P < .01$ versus control; *$P < .01$ versus hypothyroid group; §§$P < .01$ versus amiodarone group.**
observations of the effects of acute amiodarone administration (Néliat et al., 1982; Balser et al., 1991; Sato et al., 1994; Kamiya et al., 1995; Varró et al., 1996).

We showed previously that the Q–T interval-prolonging effects of amiodarone are considerably reduced in hypothyroid guinea pigs, pointing to thyroid mediation of the class III actions of amiodarone (Talajic et al., 1989). However, even in the present study, the effect of combined amiodarone and hypothyroidism on the Q–T interval was not significantly greater than that of either intervention alone (Table 2). The discrepancy may be due to the complexity of the determinants of the Q–T interval, which include not only the duration of the action potential but also its distribution and variability. In addition, in vivo factors such as autonomic tone, heart rate, neurohormones, and interactions with anesthetic agents can affect the Q–T interval. Thus, although the lack of further significant increase in Q–T interval in hypothyroid, amiodarone-exposed animals is compatible with thyroid mediation of amiodarone action, it fails to prove the concept, and our observations of changes in K⁺ currents and APD would suggest the contrary, that not all of the effects of amiodarone on repolarization are mediated by thyroid antagonism. The possibility remains that the inhibitory actions of amiodarone on IKs are due to an inhibition of thyroid effects on the current. In fact, the observation that combined amiodarone and hypothyroidism did not reduce IKs beyond the values obtained with hypothyroidism alone is compatible with such a mechanism.

**Potential Limitations.** Any study of the effects of chronic processes like hypothyroidism and maintained amiodarone therapy must be performed in parallel and separate groups of animals, losing the statistical power of using each animal as its own control. Distortions can also occur if cells are not well distributed across hearts for each determination. We were careful to study a similar number of cells for each current measurement in each heart of each group, to avoid biasing the results. The similarity under **Results** obtained for control and DMSO groups is also reassuring in terms of the robustness of the data.

In addition to the effects on K⁺ currents studied in the present report, amiodarone has a host of other potential actions, including effects on Ca²⁺ currents (Néliat et al., 1982; Nattel et al., 1987; Nishimura et al., 1989; Valenzuela and Bennett, 1991), Na⁺ current (Mason et al., 1984; Follmer et al., 1987; Honjo et al., 1991), the Na⁺-K⁺ ATPase (Bergman et al., 1995; Gray et al., 1998), muscarinic cholinergic receptors (Watanabe et al., 1996), and β-adrenergic receptors (Venkatesh et al., 1986). We chose to concentrate on K⁺ currents in the present study because they are likely to be a major target for the class III properties of amiodarone, and we were particularly interested in determining whether these actions can be attributed to an interaction with thyroid hormone effects. Nevertheless, it is quite clear that the cardiac effects of the drug result from the interplay of a wide variety of actions of which K⁺ channel inhibition is an important, but far from the only, component.

We administered amiodarone for 1 week, based on previous studies that showed that ECG changes after 1 week of amiodarone administration to guinea pigs were the same as after 4 weeks of amiodarone (Talajic et al., 1989). We cannot exclude the possibility that additional alterations may have occurred had amiodarone administration continued for longer periods of time.

**Potential Significance.** Amiodarone is an important and widely used antiarrhythmic drug, with unique properties that are likely to result in increased use in the short term (Nademane et al., 1993; Podrid, 1995; Singh, 1995; Link et al., 1996). It is therefore important to understand its fundamental mechanisms of action. Furthermore, there is considerable interest in developing new antiarrhythmic agents that share the beneficial properties of amiodarone without some of its drawbacks, including hypothyroidism, a variety of systemic toxicities, and very slow elimination after chronic use. Two candidate compounds have already been developed (Finn et al., 1995; Manning et al., 1995a,b; Raatikainen et al., 1996). A better understanding of the mechanisms of the properties of amiodarone, particularly its important class III actions (Singh et al., 1970), is likely to be a necessary component of this effort.

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