

# Electrophysiologic Effects of Chronic Amiodarone Therapy and Hypothyroidism, Alone and in Combination, on Guinea Pig Ventricular Myocytes<sup>1</sup>

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## ABSTRACT

Amiodarone is a widely used antiarrhythmic drug, the mechanisms of action of which remain incompletely understood. Indirect 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.  $I_{Ks}$  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  $I_{K1}$  (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 (Wiegand 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

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**ABBREVIATIONS:** APD, action potential duration; APD<sub>90</sub>, 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;  $I_{to}$ , 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 (Stäubli 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 thyroidectomized 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) for 6 to 8 weeks. In addition,  $CaCl_2$  was added to the drinking water of hypothyroid animals at a concentration of 1% to avoid the possibility of hypocalcemia due to parathyroid damage at the time of thyroidectomy. Control animals were followed in the same way and for the same period of time as hypothyroid animals. In the animals of the amiodarone group, the drug was administered i.p. at a daily dose of 80 mg/kg body weight (dissolved in DMSO) over 7 days. Animals assigned to the hypothyroid amiodarone-treated group were subjected to both thyroidectomy/PTU treatment and amiodarone injections, the latter begun when hypothyroid effects reached steady state (8 weeks) and continued for 7 days. DMSO-treated animals were injected i.p. daily for 7 days with DMSO, the vehicle for amiodarone administration, in the same quantities as used for amiodarone administration. Weight and ECGs were obtained on a weekly basis. At the end of the observation period, animals were sacrificed by cervical dislocation, and their hearts were removed for cell isolation.

**ECG Recordings.** Six-lead electrocardiographic recordings (leads I, II, III, aVL, aVR, and aVF) were obtained (MT 95000, Astro-Med Inc.; platinum subdermal needle electrodes) after sedation with acepromazine (0.1 mg/kg, i.m.; Ayerst Laboratories, New York, NY) and ketamine (40 mg/kg, i.m.; Rogar/STP, Inc.). A paper speed of 200 mm/s was used to achieve a measurement accuracy of  $\pm 2.5$  ms. The average of three successive measurements was used to

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%  $O_2$ , pH adjusted to 7.35 with NaOH) Tyrode's solution containing: 136 mmol/liter NaCl, 5.4 mmol/liter KCl, 2.0 mmol/liter  $CaCl_2$ , 1.0 mmol/liter  $MgCl_2$ , 0.33 mmol/liter  $NaH_2PO_4$ , 5 mmol/liter HEPES, and 10 mmol/liter glucose at 37°C. When clear of blood, the perfusate was changed to a nominally  $Ca^{2+}$ -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  $KH_2PO_4$ , 25 mmol/liter glucose, 40 mmol/liter mannitol, 70 mmol/liter L-glutamic acid, 10 mmol/liter  $\beta$ -hydroxybutyric acid, 20 mmol/liter taurine, and 10 mmol/liter ethylene glycol bis( $\beta$ -aminoethyl 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  $CaCl_2$ , 1.0 mmol/liter  $MgCl_2$ , 0.33 mmol/liter  $NaH_2PO_4$ , 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 ( $I_{K1}$ ), and delayed rectifier current ( $I_K$ ). For the recording of  $I_{K1}$  and  $I_K$ ,  $Ca^{2+}$  current was blocked with 5  $\mu$ M nifedipine (Sigma). Bath temperature was maintained at  $36.2 \pm 0.4^\circ C$  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  $MgCl_2$ , 10 mmol/liter HEPES, 5 mmol/liter ethylene glycol bis( $\beta$ -aminoethyl ether)-*N,N,N,N'*-tetraacetic acid, 5 mmol/liter  $Mg_2ATP$ , 0.1 mmol/liter GTP, and 5 mmol/liter phosphocreatine (pH adjusted to 7.2 with KOH) to record action potentials,  $I_{K1}$  and  $I_K$ .

**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 megaohms 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 ( $R_s$ ) was electrically compensated to minimize the capacitive surge on the current recording and the voltage drop across  $R_s$ .  $R_s$  was calculated by dividing the capacitive time constant, obtained by fitting the decay of the capacitive transient, by the calculated membrane capacitance (the time integral of 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

TABLE 1  
Passive electrical properties of cells from different experimental groups

	Control	Hypo	Amio	Hypo + Amio	DMSO
Cell Capacitance (pF)	142.2 ± 6.6	174.8 ± 8.6 <sup>a</sup>	140.4 ± 5.4	181.2 ± 11.5 <sup>a</sup>	157.0 ± 13.6
$R_S$ before Compensation (MΩ)	13.3 ± 1.0	13.6 ± 1.3	16.3 ± 0.8	14.1 ± 1.3	12.7 ± 1.2
$\tau$ before Compensation ( $\mu$ s)	2160 ± 194	2368 ± 246	2487 ± 141	2520 ± 344	2113 ± 255
$R_S$ after Compensation (MΩ)	3.0 ± 0.3	3.7 ± 0.3	2.6 ± 0.2	3.2 ± 0.3	2.9 ± 0.4
$\tau$ after Compensation ( $\mu$ s)	423 ± 37	609 ± 52	468 ± 43	519 ± 49	412 ± 30

$R_S$ , pipette series resistance;  $\tau$ , capacitive time constant. Hypo, hypothyroid; Amio, amiodarone-treated hypothyroid animals; DMSO, dimethylsulfoxide-treated animals.  
<sup>a</sup>  $P < .01$  versus control.

pipette solution were immersed into a solution that was identical with the pipette solution and subsequently into the bath solution used for action potential recording. The potential difference recorded was the junction potential of the pipette. The average of 10 pipettes ( $11.5 \pm 0.2$  mV) was subtracted from the measured resting membrane potential.

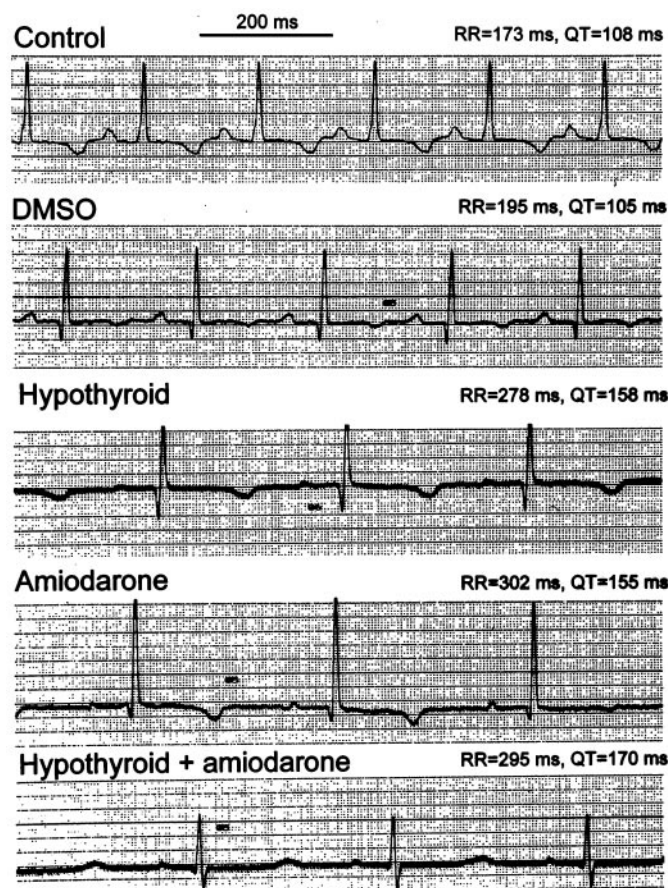
**Data Analysis.** Group data are expressed as mean ± S.E.M. Statistical comparisons between the different experimental groups were obtained with ANOVA. Differences with a two-tailed  $P < 0.05$  were considered statistically significant. Comparisons between multiple group means were performed with a Bonferroni-corrected  $t$  test for all pairwise group comparisons. A nonlinear least-square curve-fitting program (CLAMPFIT in pCLAMP 6.0 or Sigma Plot) was used to perform curve-fitting procedures.

## Results

**Effects on ECG.** In hypothyroid animals, the first electrocardiographic changes were noted after 4 weeks of PTU exposure, and the ECG stabilized by the end of 8 weeks. We have shown previously that amiodarone effects on the ECG of the guinea pig reach steady state after 1 week of amiodarone therapy (Talajic et al., 1989). Examples of typical ECGs of one animal/group at the end of the study period in each group are shown in Fig. 1. Animals in the amiodarone, hypothyroid, and amiodarone-hypothyroid group had significantly increased R–R and Q–T intervals compared with controls, with no change in P–R or R–R intervals. DMSO itself had no effect on the ECG. Table 2 shows mean R–R and Q–T intervals at the time of study for each group of animals.

**Effects on Delayed Rectifier Current ( $I_{K_r}$ ).**  $I_{K_r}$  was recorded with the use 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. Initial measurements were performed 15 min after cell-membrane rupture, and the protocol was run at least three times in each cell in 10-min intervals to detect rundown of  $I_{K_r}$ . In cells with a stable  $I_{K_r}$  ( $<10\%$  rundown over 20 min),  $1 \mu$ M dofetilide was added to the bath to block  $I_{K_r}$ , and after 10 min, the protocol was repeated.  $I_{K_r}$  was evaluated on the basis of dofetilide-sensitive  $I_{K_r}$ , and  $I_{K_s}$  was determined from the dofetilide-resistant component (Lei and Brown, 1996; Salata et al., 1996). Washout of dofetilide was obtained in seven cells, and a mean reversal of 92.4% in drug effect was observed. Cells with rundown of  $>10\%$  (6.3% of cells) were rejected. Examples of currents recorded before and after dofetilide, as well as dofetilide-sensitive currents obtained by digital subtraction, are shown in Fig. 2, A, B, and C, respectively.

The effects of hypothyroidism and amiodarone on  $I_{K_s}$  are illustrated in Fig. 3. Figure 3A shows dofetilide-resistant  $I_{K_s}$  from representative cells in the five experimental groups. Chronic amiodarone treatment and hypothyroidism were



**Fig. 1.** Electrocardiographic recordings from guinea pigs of the five different experimental groups. ECGs were obtained at a paper speed of 200 mm/s 1 day before the patch-clamp experiments were performed. The figure shows a representative example of lead II for each experimental group. Individual R–R and Q–T intervals are also given for each recording. Please note that DMSO treatment did not alter heart rate or Q–T intervals. In contrast, hypothyroidism, amiodarone treatment, and the combination of both substantially prolonged the R–R and the Q–T interval.

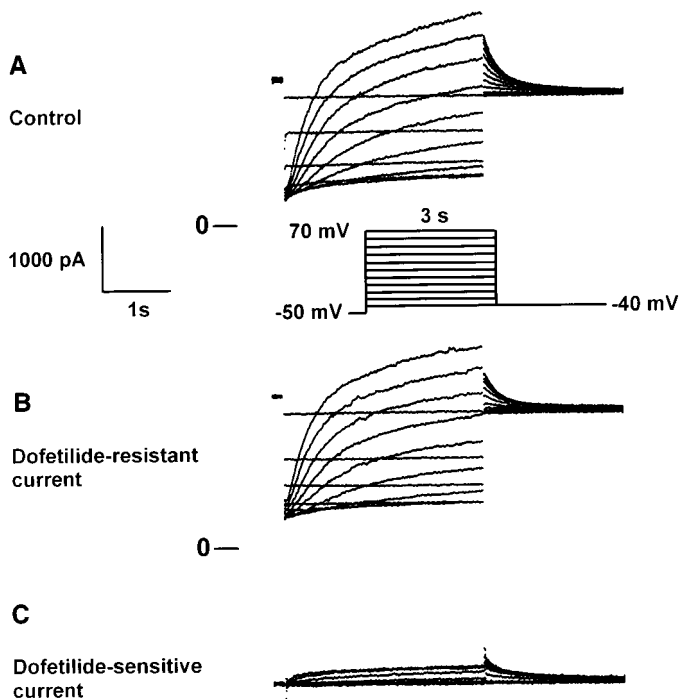
both associated with a decrease in  $I_{K_s}$  step and tail currents; however, hypothyroidism led to a much stronger reduction of  $I_{K_s}$  than amiodarone treatment. Amiodarone treatment in hypothyroid animals caused no additional decrease in  $I_{K_s}$  amplitude compared with hypothyroidism alone. DMSO had no apparent influence on  $I_{K_s}$ . 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.  $I_{K_s}$  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  $I_{K_s}$  compared with control ( $n = 8$ ), whereas DMSO had no

TABLE 2  
Electrocardiographic characteristics

	Control	Hypo	Amio	Hypo + Amio	DMSO
R-R Interval (ms)	199 ± 4	294 ± 4 <sup>a</sup>	289 ± 9 <sup>a</sup>	319 ± 15 <sup>a</sup>	221 ± 12
Q-T Interval (ms)	108 ± 2	163 ± 2 <sup>a</sup>	158 ± 3 <sup>a</sup>	173 ± 10 <sup>a</sup>	126 ± 7
Number of Animals	25	14	9	6	4

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

<sup>a</sup>  $P < .01$  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  $\mu$ M nifedipine to block  $I_{Ca}$ . The components of  $I_K$  were separated pharmacologically with the use of 1  $\mu$ M dofenilide, which specifically inhibits the rapid component  $I_{Kr}$  of  $I_K$ . B, dofenilide-resistant component ( $I_{Kr}$ ) at potentials between -40 and +70 mV. These recordings were obtained after a 10-min exposure of the cell to dofenilide. C, the dofenilide-sensitive current ( $I_{Ks}$ ), 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.

effect ( $n = 5$ ). Cells from hypothyroid animals ( $n = 9$ ) and from hypothyroid/amiodarone-treated animals ( $n = 7$ ) had  $I_{Ks}$  that was significantly smaller than both control ( $P < .01$ ) and amiodarone-treated ( $P < .05$  at voltages positive to +50 mV) cells. For example, at +50 mV,  $I_{Ks}$  density was reduced by ~41% in cells from amiodarone-treated animals compared with control animals, whereas hypothyroid cells had  $I_{Ks}$  values 65% less than controls.  $I_{Ks}$  tail current densities were 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).

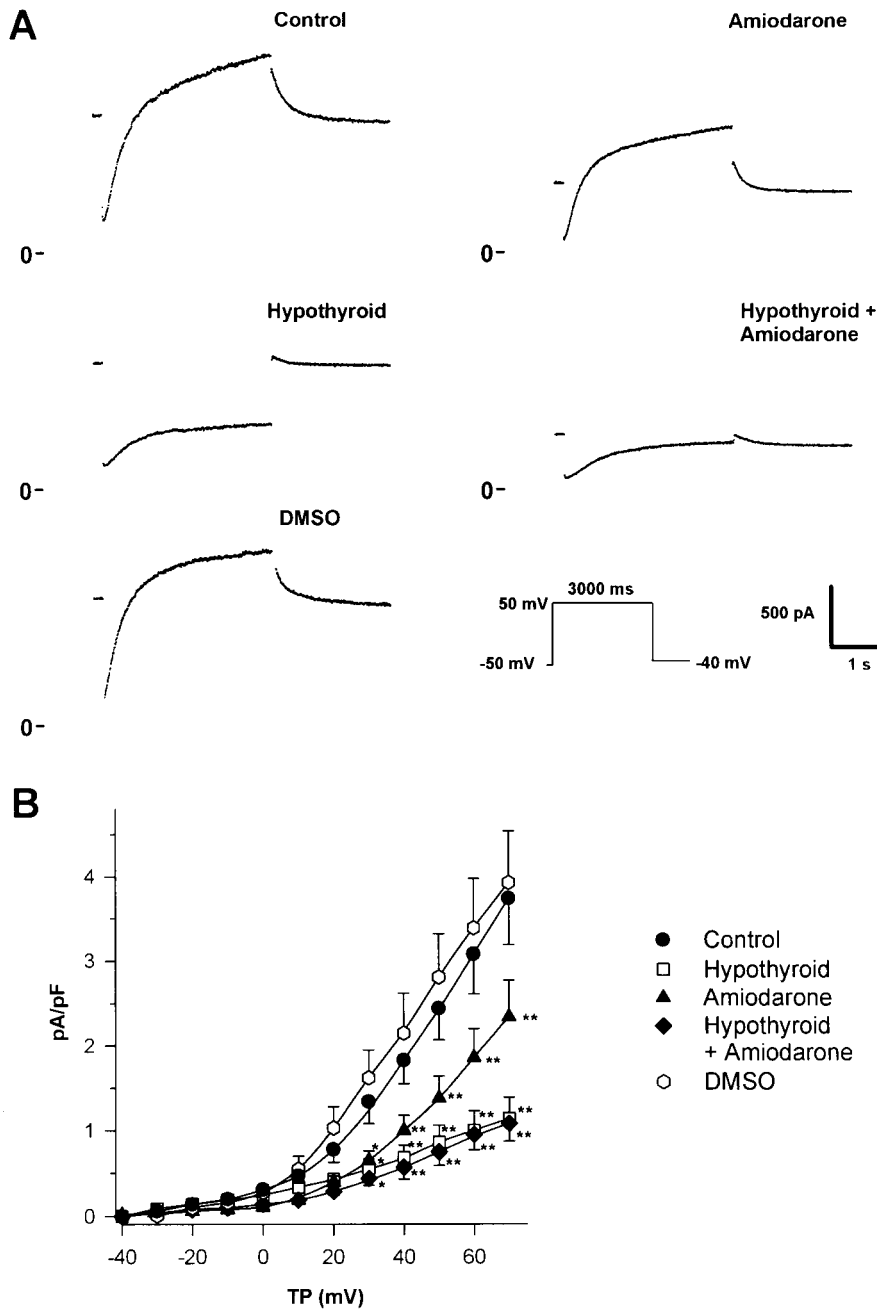
Figure 4 shows original recordings of  $I_{Kr}$  (dofenilide-sensitive current) in Fig. 4A and mean current density-voltage relations in Fig. 4B.  $I_{Kr}$  was much smaller in cells from amiodarone-treated animals (whether or not they were hypothyroid) compared with cells from control, hypothyroid, 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  $I_{Kr}$  step 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  $I_{Kr}$ -voltage relation was not obviously different in any group, but  $I_{Kr}$  density was significantly reduced in both amiodarone-treated groups. No significant differences in  $I_{Kr}$  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  $I_{Kr}$  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  $I_K$  Activation.** The voltage-dependent activation of  $I_{Kr}$  and  $I_{Ks}$  was analyzed by normalizing dofenilide-sensitive and dofenilide-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  $I_{Kr}$  and  $I_{Ks}$ . In all groups,  $I_{Kr}$  activated at negative voltages, with a half-activation voltage ( $V_h$ ) of  $-17.1 \pm 3.1$  mV and a slope factor ( $k$ ) of  $9.0 \pm 1.2$  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).  $I_{Ks}$  activated at more positive potentials. For example,  $V_h$  under control conditions averaged  $22.6 \pm 2.4$  mV, and  $k$  was  $13.4 \pm 1.1$  mV. Similar to the results for  $I_{Kr}$ , the  $I_{Ks}$  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  $I_{Ks}$  was best fitted by a biexponential relation, whereas its deactivation was best fitted by a monoexponential function. Monoexponential relations fit both the activation and deactivation of  $I_{Kr}$ . Table 5 provides the best-fit activation and deactivation time constants of  $I_{Ks}$  (at 50 and -40 mV, respectively) and corresponding results for  $I_{Ks}$  (at 0 and -40 mV). None of the interventions studied significantly altered the kinetics of  $I_{Kr}$  or  $I_{Ks}$ .

**Inward Rectifier  $K^+$  Current.** Representative recordings of  $I_{K1}$  from various experimental groups are shown in Fig. 5. Cells from animals treated with amiodarone showed smaller currents than cells from the other three groups. Mean  $I_{K1}$  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  $I_{K1}$ , whether in the presence or absence of hypothyroidism,



**Fig. 3.** Dofetilide-resistant component ( $I_{Ks}$ ) of the delayed rectifier potassium current. A, representative recordings obtained with the protocol shown at 36°C, in the presence of 1  $\mu$ M dofetilide to block  $I_{Kr}$ . B, mean  $I_{Ks}$  density-voltage relations recorded during 3-s voltage steps from -50 mV to the test potentials (TP) indicated. The results shown are step current densities for 8, 9, 8, 7, and 5 cells for control, hypothyroid, amiodarone, amiodarone plus hypothyroid, and DMSO groups, respectively. \*, \*\* $P < .05, 0.01$  versus control, respectively.

**TABLE 3**  
 $I_K$  current densities

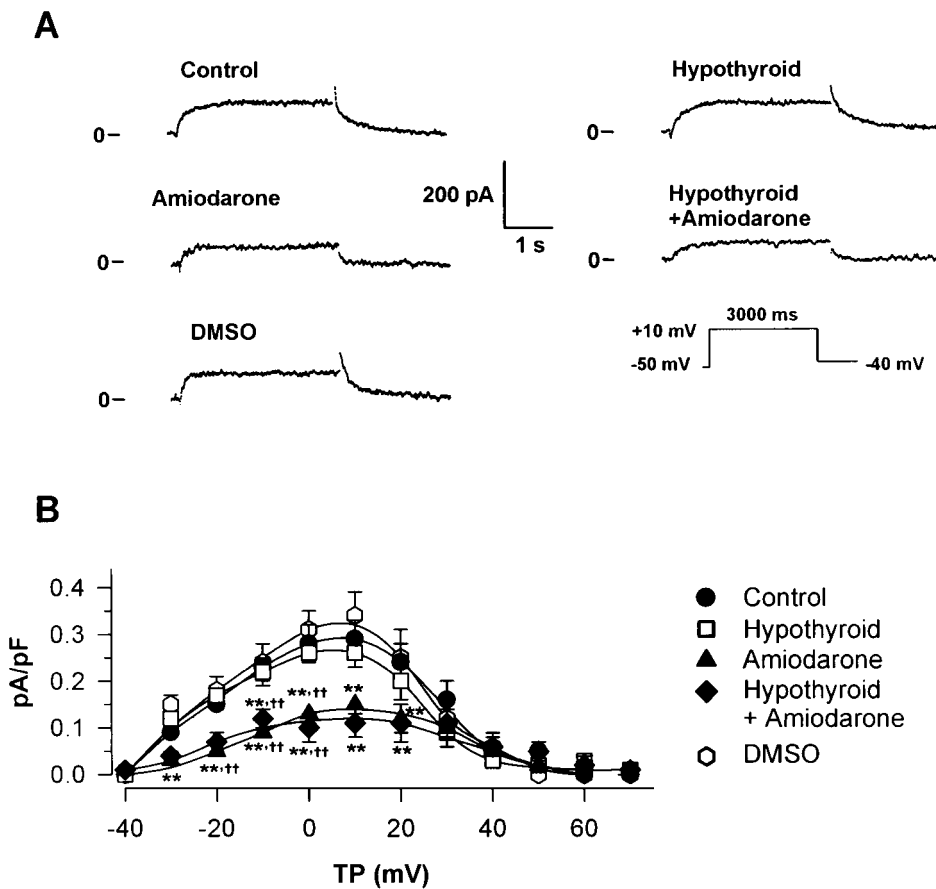
	TP (mV)	Control	Hypo	Amio	Hypo + Amio	DMSO
$I_{Ks}$ Step (pA/pF)	+50	2.44 ± 0.37	0.86 ± 0.20 <sup>b</sup>	1.38 ± 0.26 <sup>a,d</sup>	0.95 ± 0.18 <sup>b</sup>	2.81 ± 0.51
$I_{Ks}$ Tail (pA/pF)	+50	0.72 ± 0.13	0.15 ± 0.02 <sup>c</sup>	0.38 ± 0.06 <sup>a,d</sup>	0.20 ± 0.04 <sup>b</sup>	0.79 ± 0.18
$I_{Kr}$ Step (pA/pF)	0	0.28 ± 0.04	0.26 ± 0.02	0.12 ± 0.02 <sup>c,e</sup>	0.10 ± 0.03 <sup>c,e</sup>	0.31 ± 0.04
$I_{Kr}$ Tail (pA/pF)	0	0.28 ± 0.07	0.24 ± 0.02	0.08 ± 0.01 <sup>a,d</sup>	0.09 ± 0.03 <sup>a,d</sup>	0.31 ± 0.08
Number of Cells		8	9	8	7	5

TP, test potential; tail currents were recorded upon repolarization to -40 mV from TP indicated; Hypo, hypothyroid; Amio, amiodarone-treated; Hypo + Amio, amiodarone-treated hypothyroid animals; DMSO, dimethylsulfoxide-treated animals.

<sup>a</sup>  $P < .05$ , <sup>b</sup>  $P < .01$ , <sup>c</sup>  $P < .001$  versus control; <sup>d</sup>  $P < .05$ , <sup>e</sup>  $P < .001$  for Amio or Hypo + Amio versus Hypo.

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,



**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  $1 \mu\text{M}$  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;  $^{++}P < .01$  versus hypothyroid group.

TABLE 4

Voltage-dependent activation of  $I_{Kr}$  and  $I_{Ks}$ 

	Control	Hypo	Amio	Hypo + Amio	DMSO
$V_h I_{Ks}$ (mV)	$22.6 \pm 2.4$	$29.5 \pm 2.2$	$29.7 \pm 1.4$	$25.9 \pm 1.9$	$24.8 \pm 2.9$
$k I_{Ks}$ (mV)	$13.4 \pm 1.1$	$13.5 \pm 1.0$	$10.5 \pm 1.2$	$11.6 \pm 0.7$	$12.7 \pm 1.3$
$V_h I_{Kr}$ (mV)	$-17.1 \pm 3.1$	$-17.5 \pm 3.3$	$-14.2 \pm 2.0$	$-18.9 \pm 3.1$	$-16.6 \pm 3.2$
$k I_{Kr}$ (mV)	$9.0 \pm 1.2$	$7.5 \pm 1.4$	$9.8 \pm 2.0$	$6.9 \pm 2.8$	$9.4 \pm 1.9$
Number of Cells	8	10	9	7	5

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

TABLE 5

Kinetics of  $I_K$  in various experimental groups

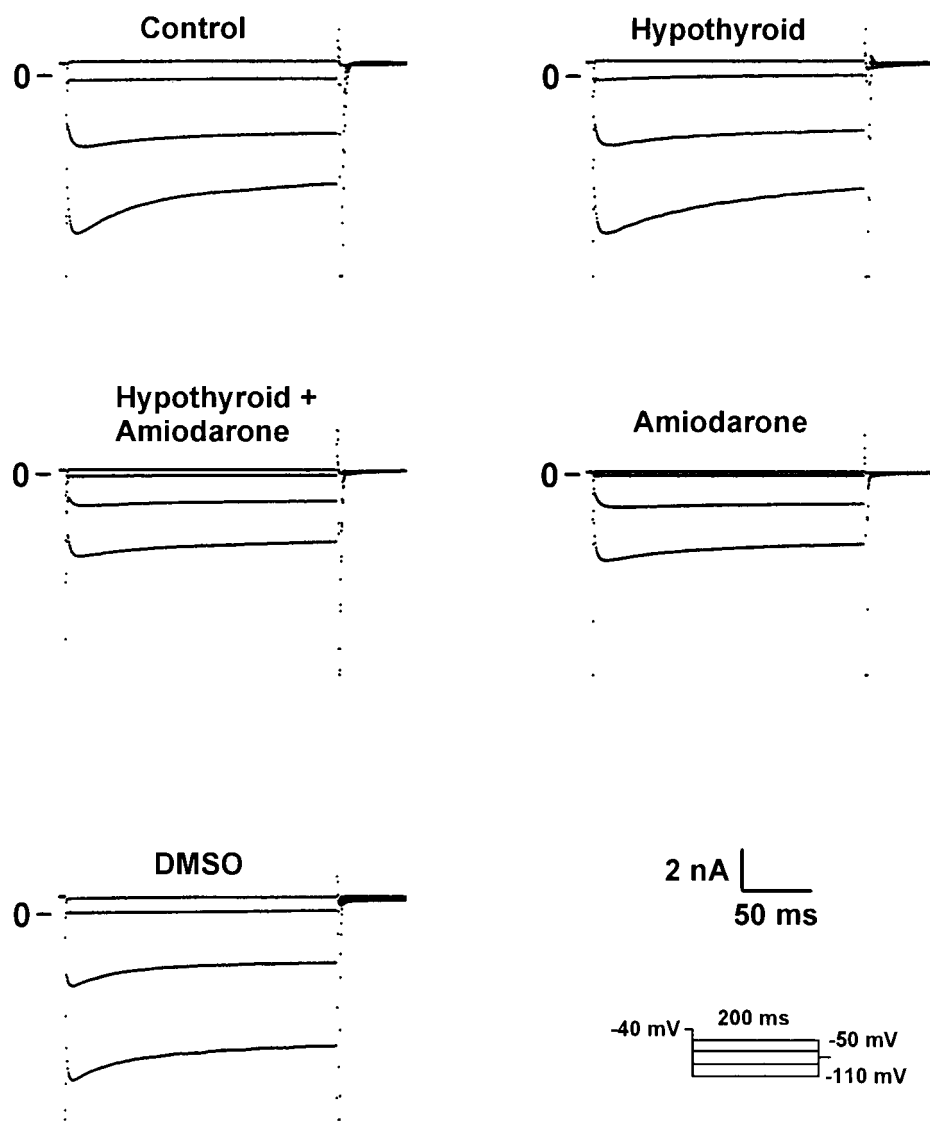
	V (mV)	Control	Hypo	Amio	Hypo + Amio	DMSO
$I_{Ks}$ Activation- $\tau_1$	50	$202 \pm 24$ (14)	$180 \pm 23$ (9)	$169 \pm 18$ (8)	$185 \pm 21$ (6)	$207 \pm 27$ (6)
$I_{Ks}$ Activation- $\tau_2$	50	$1808 \pm 101$ (14)	$1828 \pm 168$ (9)	$1639 \pm 158$ (8)	$1622 \pm 267$ (6)	$1736 \pm 196$ (6)
$I_{Ks}$ Deactivation	-40	$380 \pm 45$ (7)	$374 \pm 39$ (7)	$372 \pm 36$ (7)	$418 \pm 52$ (6)	$386 \pm 49$ (5)
$I_{Kr}$ Activation	0	$129 \pm 20$ (9)	$118 \pm 12$ (8)	$143 \pm 16$ (11)	$163 \pm 28$ (6)	$151 \pm 23$ (5)
$I_{Kr}$ Deactivation	-40	$118 \pm 18$ (8)	$151 \pm 21$ (8)	$137 \pm 25$ (7)	$147 \pm 23$ (6)	$140 \pm 27$ (5)

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.

control cells had an average current density of  $5.3 \pm 0.4$  pA/pF, which was not altered by DMSO ( $5.9 \pm 0.5$  pA/pF) or by hypothyroidism ( $5.6 \pm 0.3$  pA/pF), but was substantially reduced in the amiodarone ( $1.7 \pm 0.2$  pA/pF) and hypothyroid/amiodarone ( $2.3 \pm 0.4$  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. APD to 90% repolarization (APD<sub>90</sub>) is plotted as a function of stimulation frequency in Fig. 7B. In cells from hypothyroid animals, APD<sub>90</sub> was significantly longer than under control conditions for all stimulation frequencies. For example, at 4 Hz, the values were  $87 \pm 5$  ms under control ( $n = 13$ ) and  $140 \pm 6$  ms under hypothyroid conditions ( $n = 15$ ;  $P < .01$  versus control). Amiodarone-treated cells had an APD<sub>90</sub> that was in the range of the



**Fig. 5.** Amiodarone treatment but not hypothyroidism decreases  $I_{K1}$ . Inward rectifier currents recorded from representative cells in each group at 36°C. Test pulses were 200-ms steps at 0.5 Hz to test potentials shown. 0 indicates zero current level.

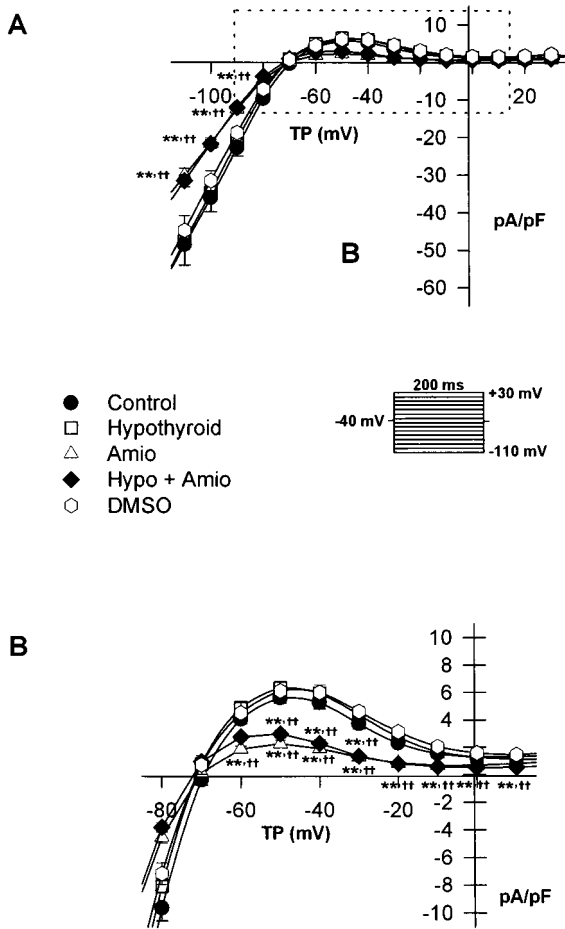
hypothyroid group (e.g., at 4 Hz,  $149 \pm 6$  ms,  $n = 23$ ;  $P < .01$  versus control). Amiodarone treatment in hypothyroid animals was associated with an additional substantial AP prolongation that was most prominent at slow frequencies but remained significant at higher frequencies. At 4 Hz,  $APD_{90}$  averaged  $193 \pm 9$  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,  $102 \pm 7$  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  $I_{Ks}$  (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  $I_{Kr}$  and  $I_{K1}$ . These observations suggest that amiodarone has actions on repolarizing current that are inde-

pendent 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  $I_K$ ) in frog atrial muscle preparations at concentrations above  $10 \mu M$  (Nélat et al., 1982). Balsler et al. (1991) studied the effects of acute amiodarone administration ( $10 \mu M$ ) on guinea pig ventricular myocytes and observed a reduction in the  $La^{2+}$ -resistant component but no effect on the  $La^{3+}$ -sensitive component, compatible with principal actions on  $I_{Ks}$ . 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  $I_{Kr}$  is the predominant component under usual experimental conditions (Colatsky et al., 1990).  $I_{to}$  was unaffected by  $10 \mu M$  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



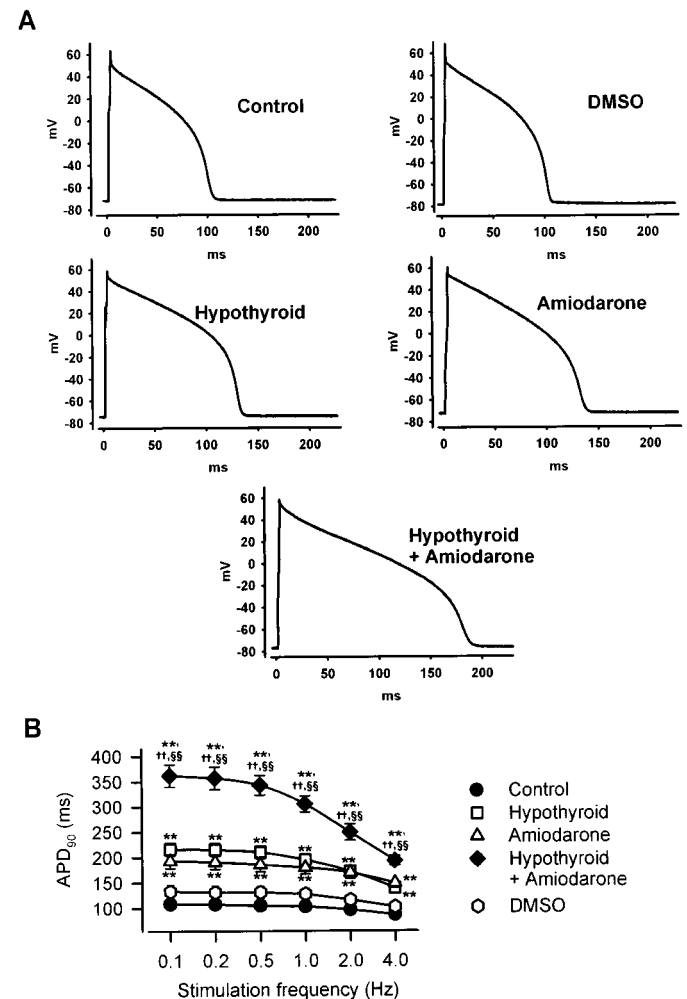
**Fig. 6.**  $I_{K1}$  density-voltage relations ( $n = 13, 20, 19, 7,$  and  $9$  cells for control, hypothyroid, amiodarone, hypothyroid plus amiodarone, and DMSO groups, respectively). A,  $I_{K1}$  density-voltage relations at all test potentials studied. B, an enlargement of the inset in A to show 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.

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 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



**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;  $SSP < .01$  versus amiodarone group.



observations of the effects of acute amiodarone administration (Nélat 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  $I_{Ks}$  are due to an inhibition of thyroid effects on the current. In fact, the observation that combined amiodarone and hypothyroidism did not reduce  $I_{Ks}$  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  $Ca^{2+}$  current (Nélat 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  $\beta$ -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 (Nademanee 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 (Finance 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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