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Comparison of the chronic and acute effects of amiodarone on the calcium and potassium currents in rabbit isolated cardiac myocytes

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1 The acute and chronic effects of amiodarone were studied on the transmembrane ionic currents in rabbit single ventricular myocytes at 35°C by applying the whole-cell configuration of the patch-clamp technique.

2 Acute exposure to 1 and 5 μM amiodarone significantly reduced the amplitude ($-53.9\pm3.9\%$, $n=5$ and $-64.0\pm2.0\%$, $n=3$, $P<0.01$), but chronic amiodarone treatment (i.p. 50 mg kg^{-1} day^{-1} for 3-4 weeks) changed neither the amplitude nor the kinetics of the inward calcium current.

3 Both acute superfusion with amiodarone (1 and 5 μM) and chronic amiodarone treatment significantly decreased the amplitude of the delayed rectifier outward potassium current (I_K).

4 Acute application of amiodarone (1 and 5 μM) did not alter but chronic amiodarone treatment moderately depressed the transient outward current (I_{to}).

5 Neither the acute (1 and 5 μM) nor the chronic amiodarone treatment changed the magnitude of the inward rectifier potassium current (I_{k1}).

6 It is concluded that acute amiodarone application and chronic amiodarone treatment alter transmembrane ionic currents of ventricular myocytes differently. This may explain, at least in part, the marked differences in the cardiac electrophysiological effects observed after acute and chronic amiodarone treatment in patients.

Keywords: Amiodarone; I_{Ca}; I_K; I_{to}; I_{k1}; chronic treatment; rabbit ventricular myocytes

Introduction

Earlier the CAST (1989) and recently the SWORD (1994) studies provided evidence that some class I/C antiarrhythmic drugs and (-)-sotalol, a 'pure' class III drug, significantly increased postinfarction mortality. These failures in the long term antiarrhythmic therapy shifted interest more toward amiodarone, a drug which, in contrast with flecainide and (-)-sotalol, seems able to reduce postinfarction mortality in ongoing trials (Pfisterer *et al.*, 1992; The CASCADE investigators, 1993).

Amiordarone, a widely used old antiarrhythmic drug, was recognized first to lengthen cardiac action potential duration after chronic treatment for 6 weeks in rabbits by Singh & Vaughan Williams in 1970 and since it has been termed a class III antiarrhythmic agent (Vaughan & Williams, 1975). Later however it became evident that amiodarone has multiple actions, including use-dependent block of the maximal rate of depolarization (Mason *et al.*, 1984; Varro *et al.*, 1985) and inward sodium current (Follmer *et al.*, 1987); as well as the slow response action potential (Nattel *et al.*, 1987) and inward calcium current (Nishimura *et al.*, 1989; Valenzuela & Bennett, 1991). Amiodarone also blocks α- and β-adrenoceptors in a noncompetitive manner (Polster & Broekhuysen, 1976). It must be emphasized that the cardiac eletrophysiological effects of amiodarone differ markedly after acute and chronic administration (Wellens *et al.*, 1984; Kadoya *et al.*, 1985; Varro *et al.*, 1988). This latter fact suggests that the ionic mechanisms which underlie its marked effects on cardiac electrical activity can be understood only if both acute and chronic effects are analyzed in detail.

The influence of amiodarone on various transmembrane ionic currents has recently been investigated in several laboratories. These experiments however were carried out after acute drug application (Follmer *et al.*, 1987; Nishimura *et al.*, 1989; Balser *et al.*, 1991; Carmeliet, 1993), and there are no full length reports available so far, on the possible modulation of ionic currents in the heart after long term treatment. Therefore, in this study we compared the chronic and acute effects of amiodarone on some important transmembrane ionic currents in single ventricular myocytes of the rabbit.

Methods

Preparation of myocytes

Single ventricular myocytes were obtained by enzymatic dissociation of rabbit hearts. Rabbits (1-2 kg) were killed by cervical dislocation after receiving 400 iu kg^{-1} heparin intravenously. The chest was opened and the heart was quickly removed and placed in cold (4°C) solution with the following composition (mM): NaCl 135, KCl 4.7, KH_2PO_2 1.2, $MgSO_4$ 1.2, HEPES 10, $NaHCO_3$ 4.4, glucose 10, $CaCl_2$ 1.8, (pH 7.2). The heart was mounted on a modified, 60 cm high Langendorff column and perfused with oxygenated and prewarmed (37°C) solution mentioned above. After washing out the blood (3-5 min) the heart was perfused by pump (Cole Palmer) with nominally Ca-free solution (flow rate 24 ml min^{-1}) for 4 min followed by 12-15 min perfusion (12 ml min^{-1}) with the same solution containing 0.5 mg ml^{-1} collagenase (Sigma 1A) and 0.04 mg ml^{-1} pronase E (Sigma) with 0.1% albumin. After 5 min of the enzyme perfusion the calcium concentration was increased by 200 μM. After removing the heart from the cannula the free wall of the right ventricle was placed in enzyme-free solution containing 1.8 mM $CaCl_2$ and 1% albumin and equilibrated at 37°C for 10 min. The tissue was then cut into small fragments. After gentle agitation, the cells were separated from the chunks by filtering through nylon mesh. The

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cells were then washed three times with solution containing 1.8 mM $CaCl_2$ and stored at room temperature. One drop of cell suspension was placed in transparent recording chamber mounted on the stage of an inverted microscope (TMS Nikon Co, Tokyo, Japan) and the individual myocytes were allowed to settle at the bottom of the recording chamber for at least 10 min before superfusion. HEPES-buffered Tyrode solution was used as normal superfusate. This solution contained (mM): NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, HEPES 5.0 at pH of 7.4. Superfusion was maintained by gravity flow.

Electrical measurements

Suction pipettes with tip diameters of 1–2 μm were made from glass capillaries (Clark; UK). These pipettes had tip resistances of 2–3 MΩ when filled with the standard internal solution. This solution consisted of (mM): KCl 140, K_2ATP 5, $MgCl_2$ 4, EGTA 1 and HEPES 10 at pH 7.2. The electrode potential was adjusted to zero immediately before the pipette was attached. After establishing a high (1–5 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1–5 ms. The input resistance and cell capacitance were measured under voltage control during application of a 10 mV hyperpolarizing pulse from a holding potential of −10 mV. Measurements were carried out at 35°C with a NDB TC-1 type temperature controller (NB Datyner Co, Stony Brook, NY, U.S.A). Recordings were obtained by using an Axopatch 1D patch-clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.). Outputs from this amplifier were digitized with a 333 kHz A/D converter (Digidata 1200, Axon) under software control (Pclamp 6.0, Axon Instruments) installed on a 40 MHz IBM 386 compatible computer.

Rabbits used to study the chronic effect of amiodarone were treated either with 50 mg kg^{-1} day^{-1} amiodarone (Cordarone, Labaz) or by the solvent (10% Tween 80, 10% ethanol in distilled water; 1 ml kg^{-1}) intraperitonally for 3–4 weeks. The acute effects of amiodarone were tested in separate experiments in animals which had not been treated previously with amiodarone or with its solvent.

The results were analyzed using software programmes purchased from Axon Instruments (Pclamp 6.0). Statistical analysis was performed by Student's *t* tests for paired or unpaired data. Mean values were regarded as significantly different from each other at $P<0.05$.

Results

In all rabbits used to measure the chronic effect of amiodarone on the transmembrane ionic currents the main ECG parameters were determined before and at the end of the 3–4 week treatment. The results are shown in Table 1. Chronic amiodarone treatment significantly increased QT_c and widened QRS intervals without significantly changing the spontaneous cycle length (RR) and PQ interval. There were no significant ECG changes observed in the solvent-treated group. After taking the last ECG recordings following the 3–4 week treatment, cells were isolated from the 5 amiodarone and from the 5 solvent-treated animals. Successful current recordings were obtained in 33 cells (5 rabbits) in the amiodarone and 32 cells (5 rabbits) in the solvent-treated groups. The capacitive surface areas were 122.3±5.3 pF in the solvent (control) and 122.4±5.3 pF in the amiodarone-treated groups i.e. not different from each other. The results regarding the amplitudes of the transmembrane current in the chronic amiodarone experiments and its control are expressed in pA/pF in order to minimize further the possible errors due to individual differences in cell size.

Effect on the inward calcium current (I_{CA})

The effect of amiodarone on the L-type calcium current (I_{Ca}) was studied in experiments in which the KCl in the pipette was replaced by 120 mM CsCl and 20 mM TEA in order to block the potassium currents. I_{Ca} was elicited from the holding potential of −40 mV to eliminate fast inward sodium current (I_{Na}). Command pulses of 400 ms duration with pulse interval of 3 s were applied in the voltage range of −35 to +50 mV to elicit I_{Ca}. The amplitude of I_{Ca} was measured as the peak inward current at each voltage pulse.

In cells exposed to acute amiodarone (1 μM) superfusion for 5–10 min in untreated rabbits, significant reduction (at 0 mV −53.9±3.9%; $n=5$; $P<0.01$) of the peak inward current was observed. This effect was partially reversed upon 5–7 min washout of the drug (Figure 1). Stronger effect (at 0 mV −64.0±2.0%; $n=3$; $P<0.01$) was seen after application of a higher concentration of amiodarone (5 μM).

In 16 cells obtained from 5 chronically treated rabbits, neither the amplitude nor the inactivation and recovery kinetics of I_{Ca} were significantly different from those measure in 15 cells isolated from 5 rabbits treated with the solvent (Figure 2; Table 2).

Effect on the delayed rectifier outward potassium current (I_K)

The effect of amiodarone on the delayed rectifier outward potassium current (I_K) was measured by applying 3 s long depolarizing voltage steps from the holding potential of −40 mV. Clamping the cells back to −40 mV, outward tail currents were observed which were attributed to I_K. It is known that in rabbit ventricle I_K represents only the rapid component (I_{Kr}) of the delayed rectifier outward potassium current (Carmeliet, 1993), therefore in the subsequent text no distinction will be made between I_{Kr} and I_{Ks}. The external solution in all experiments used to measure potassium current contained 0.25 mM $CdCl_2$ in order to block completely I_{Ca}.

Acute exposure to 1 μM amiodarone superfusion for 5–10 min significantly reduced (at +30 mV −28.7±7.1%; $n=6$; $P<0.01$) the amplitude of the tail current (Figure 3). This effect was further increased by elevating the concentration to 5 μM (at 30 mV −78.7±5.0%; $n=3$; $P<0.01$).

Current recordings obtained in 16 cells from 5 rabbits treated chronically with amiodarone showed reduced amplitude of I_K. This was significantly different from that measured in 15 cells from the solvent-treated 5 animals (Figure 4). There

Table 1 Effect of chronic amiodarone treatment on the ECG parameters in conscious rabbits

Parameters	*Control*	*Solvent*	*Control*	*Amiodarone*
RR (ms)	260±10	240±10	255±17	270±11
PQ (ms)	55.0±2.2	61.0±3.3	58.7±1.2	60.0±0.05
QRS (ms)	27.0±1.2	28.0±1.2	27.5±1.4	33.7±1.2 $P<0.05$
QT_c (ms)[a]	142±2	139±3	134±2	167±9 $P<0.05$

[a] QT_c was calculated by using the equation given by Carlsson *et al.* (1993): $\{QT_c=QT-0.175(RR-300)\}$. Mean±s.e., $n=5$.

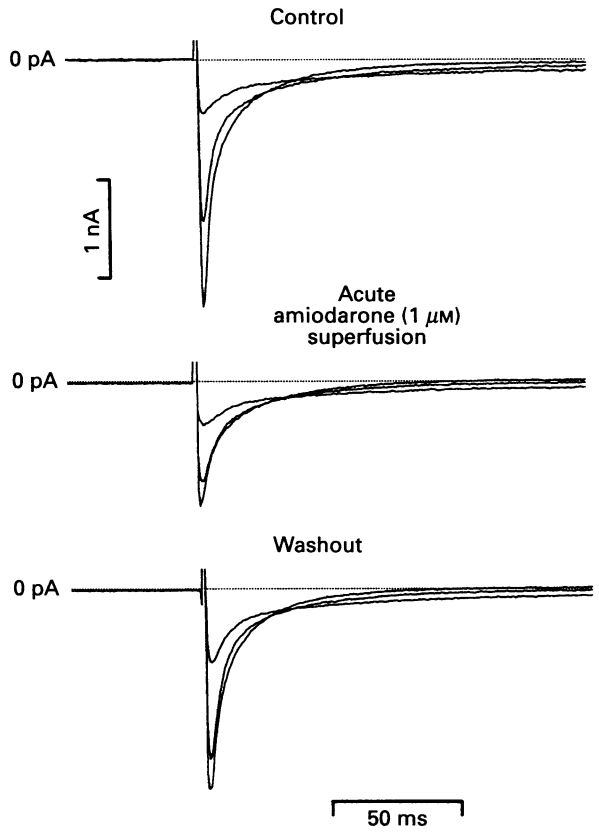

Figure 1 Effect of 1 μM amiodarone superfusion on the inward calcium current (I_{Ca}) in rabbit ventricular myocytes. The holding potential was −40 mV and currents were recorded at −20, −10 and 0 mV. Pulse frequency: 0.3 Hz. (Result of a representative experiment).

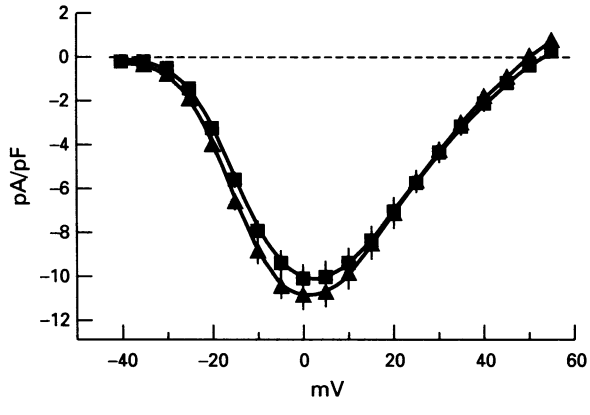

Figure 2 Lack of effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3–4 weeks) on the peak inward calcium current (I_{Ca}) in rabbit ventricular myocytes. Holding potential: −40 mV. Pulse frequency: 0.3 Hz. (■) Control, 5 animals, 15 cells; (▲) amiodarone, 5 animals, 16 cells.

Table 2 Lack of effect of chronic amiodarone treatment on the inactivation and recovery kinetics of the inward calcium current (I_{Ca})

Parameters	*Solvent* (5 animals 15 cells)	*Aminodarone* (5 animals 16 cells)
Inactivation		
τ_{fast} (ms)	7.13 ± 0.27	7.08 ± 0.16
AMP (pA/pF)	6.6 ± 0.5	6.6 ± 0.4
τ_{slow} (ms)	41.09 ± 1.40	43.38 ± 1.61
AMP (pA/pF)	2.6 ± 0.2	2.8 ± 0.2
Recovery from inactivation		
τ (ms)[a]	139.49	132.13

[a] τ was calculated from pooled data (5–5 animals 14–16 cells). Holding potential = −40 mV; Command potential = 0 mV. Means ± s.e.

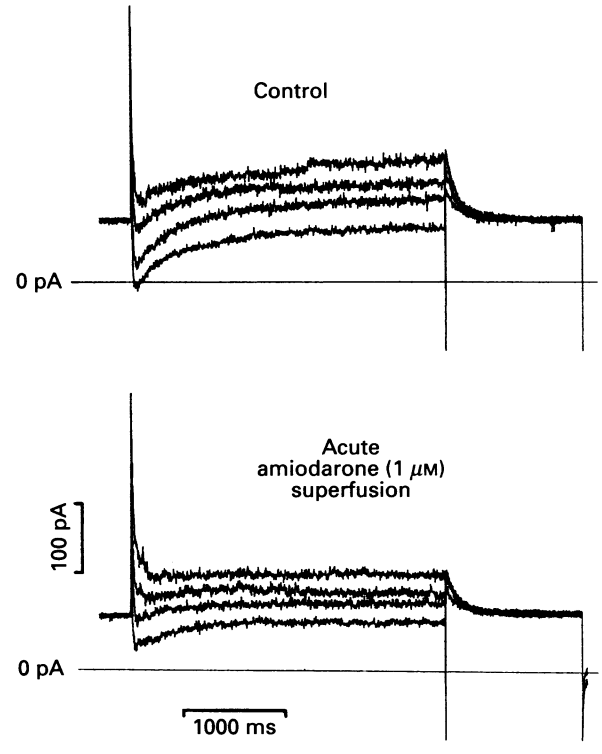

Figure 3 Effect of 1 μM amiodarone superfusion on the delayed rectifier outward potassium current (I_K) in rabbit ventricular myocytes. The holding potential was −40 mV and test potentials of 3 s durations were applied to 0, +10, +20 and +30 mV. Then voltage was returned to −40 mV and outward tail currents were recorded. Pulse frequency: 0.2 Hz. (Result of a representative experiment). (●) Control, 5 animals, 15 cells; (▲) amiodarone, 5 animals, 16 cells.

were however no significant differences in deactivation kinetics of I_K between the two groups (from +30 mV 104.5 ± 7.7 ms; 15 cells; from 5 control rabbits vs. 98.3 ± 12.0 ms; 15 cells; from 5 amiodarone-treated rabbits).

Effect on the transient outward current (I_{to})

The effect of amiodarone on the transient outward current (I_{to}) was studied by applying 400 ms long depolarizing pulses from the holding potential of −90 mV. The amplitude of I_{to} was measured as the difference between the peak outward and the steady state current measured at the end voltage pulses. This approach has some limitations. Since it is difficult to separate adequately I_K and some other outward currents like I_{Kp} from I_{to} these currents most likely 'contaminated' measurements of I_{to}, but the amplitudes of the latter currents are small relative to those of I_{to}. Although both activation and inactivation of I_{Na} is faster than that of I_{to}, it is theoretically possible that changes of I_{Na} may influence the measurements of I_{to}. With less negative holding potential, I_{Na} could have been inactivated but in this case the amplitude of I_{to} would have been smaller due to partial inactivation of I_{to}. Because continuous application of

TTX throughout the measurements to eliminate I_{Na} would have greatly increased the cost of the study, we tested the effect of 50 μM TTX on I_{to} in 5 separate experiments. Application of TTX did not significantly alter the amplitude of I_{to} in the voltage-range of -10 to $+60$ (not shown) suggesting that the possible influence of I_{Na} is negligable.

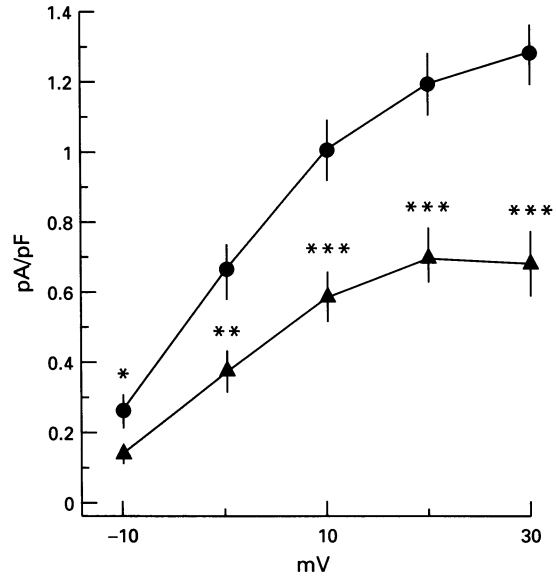

Figure 4 Effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3–4 weeks) on the peak outward tail current (I_K) amplitude in rabbit ventricular myocytes. Tail current was measured at -40 mV holding potential after applying 3 s long depolarizing pulses to $-10-+30$ mV. (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

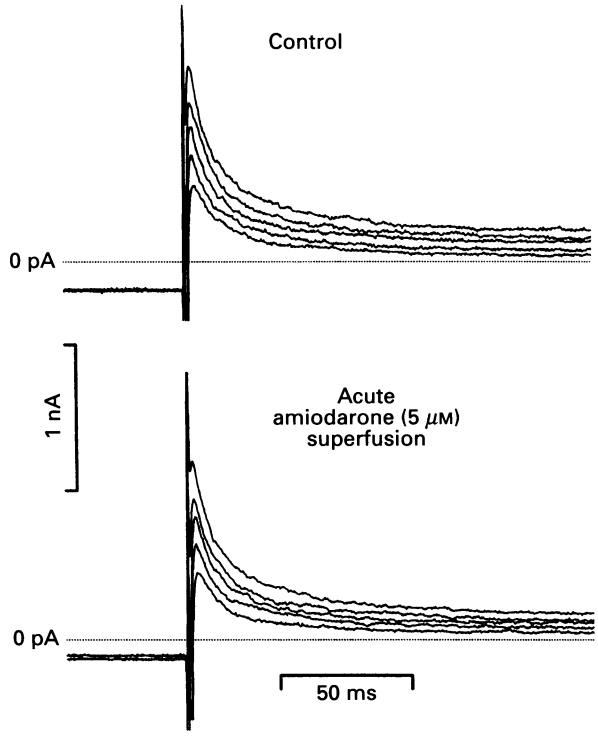

Figure 5 Lack of effect of 5 μM amiodarone superfusion on the transient outward current (I_{to}) in rabbit ventricular myocyte. The holding potential was set at -90 mV and currents were recorded at $+10$, $+20$, $+30$, $+40$ and $+50$ mV. Pulse frequency: 0.3 Hz. (Result of a representative experiment).

As the results of a representative experiment indicate (Figure 5), acute exposure to a relatively high concentration of amiodarone (5 μM) did not change the amplitude and the inactivation kinetics of I_{to}. Similar results were obtained in 4 other cells. I_{to} measured in myocytes obtained from chronically treated animals (5 rabbits; 15 cells) showed moderate but significant reduction of the current amplitude without changing its inactivation (Figure 6, Table 3) as compared with that of the control (solvent) group.

Effect on the inward rectifier potassium current (I_{k1})

The effect of amiodarone on the inward rectifier potassium current (I_{k1}) was studied by measuring the steady state current level at the end of 400 ms long voltage pulses in the range of -120 to 0 mV. Neither the chronic amiodarone treatment shown in Figure 7 (5-5 rabbits; 17-17 cells) nor the acute perfusion with a relatively high amiodarone concentration

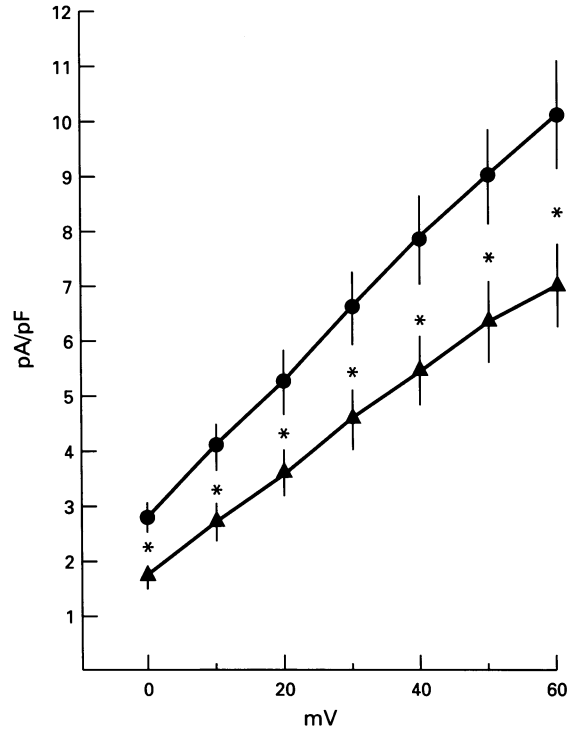

Figure 6 Effect of chronic amiodarone treatment (50 mg kg^{-1} day^{-1} for 3–4 weeks) on the peak transient outward current (I_{to}) in rabbit ventricular myocytes. Holding potential: -90 mV. Pulse frequency: 0.3 Hz. (*$P<0.05$). (●) Control, 5 animals, 15 cells; (▲) amiodorane, 5 animals, 15 cells.

Table 3 Lack of effect of the chronic amiodarone treatment on the inactivation kinetics of the transient outward current (I_{to})

Parameters	*Solvent* (5 animals 16 cells)	*Aminodarone* (5 animals 17 cells)
Inactivation		
τ_{fast} (ms)	8.13 ± 0.31	8.23 ± 0.48
AMP (pA/pF)	6.6 ± 1.0	4.4 ± 0.5
τ_{slow} (ms)	55.7 ± 5.45	51.59 ± 6.29
AMP (pA/pF)	2.7 ± 0.2	2.2 ± 0.3

Holding potential = –90 mV; command potential = + 50 mV; pulse cycle length 3 s. Mean ± s.e.

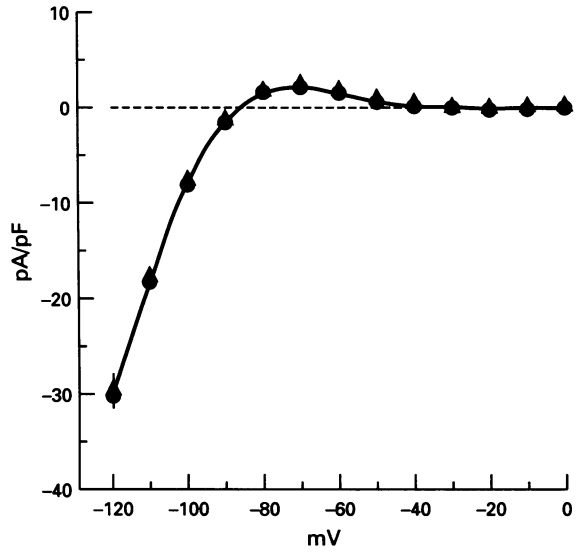

Figure 7 Lack of effect of chronic amiodorane treatment ($50\,mg\,kg^{-1}\,day^{-1}$ for 3–4 weeks) on the steady-state current-voltage relation (inward rectifier potassium current, I_{k1}) in the voltage range of −120–0 mV in rabbit ventricular myocytes. Holding potential: −90 mV. Pulse duration: 400 ms. Pulse frequency: 0.3 Hz. (●) Control, 5 animals, 17 cells; (▲) amiodarone, 5 animals, 17 cells.

(5 μM, $n=5$; not shown) influenced significantly the steady state current voltage relation, suggesting the lack of effect of amiodarone on the inward rectifier potassium current.

Discussion

In this study we compared the effects of chronic administration and acute application of amiodarone on some important transmembrane ionic currents in rabbit ventricle. Our main findings are: (A) acute but not chronic amiodarone application decreased I_{Ca}; (B) I_K was depressed after both acute exposure to and chronic administration of amiodarone; (C) I_{to} was moderately decreased after chronic amiodarone treatment and not changed after acute amiodarone superfusion; (D) I_{k1} was not influenced by amiodarone regardless of whether it was applied as chronic treatment or administered directly to the tissue bath.

The acute effects of amiodarone on the various transmembrane ionic channels including I_{Na}, I_{Ca}, I_K and I_{k1} have been investigated previously by others (Follmer *et al.*, 1987; Nishimura *et al.*, 1989; Balser *et al.*, 1991; Sato *et al.*, 1994). In these whole cell voltage clamp studies the drug concentrations were similar to those used in our experiments; this dose-range is also very close to the reported therapeutic plasma amiodarone levels (Plomp *et al.*, 1984; Debbas *et al.*, 1984; Ikeda *et al.*, 1984). Effective amiodarone concentrations used earlier by several laboratories in multicellular preparations appeared to be higher. It is not clear why amiodarone is more effective in single cells than in multicellular preparations or in *in vivo* experiments. One may speculate that this relates to the high degree of lipid solubility and the rather special pharmacokinetics of amiodarone (Plomp *et al.*, 1984; Debbas *et al.*, 1984).

It is well established that acute superfusion of amiodarone inhibits I_{Na} (Follmer *et al.*, 1987), I_{Ca} (Nishimura *et al.*, 1989; Valenzuela & Bennett, 1991) and I_K (Balser *et al.*, 1991; Carmeliet, 1993; 1994) in guinea-pigs, rabbit and cat single myocytes. The acute effects of amiodarone on I_{k1} and I_{to} are however not well defined. It seems that a high concentration of amiodarone (20 μM) can inhibit I_{k1} (Sato *et al.*, 1994). Only preliminary data are available regarding the acute effect of I_{to} (Toyama *et al.*, 1994) and very little is known about the chronic influence of amiodarone on the transmembrane ionic currents (Toyama *et al.*, 1994). These preliminary data agree with our results regarding potassium currents but partly differ from the calcium current measurements, since in contrast to our data diminished I_{Ca} was noted after chronic amiodarone treatment by Toyama *et al.* (1994).

It is known that the acute and chronic electrophysiological effects of amiodarone substantially differ from each other either based on data from *in vivo* investigation (Wellens *et al.*, 1984) and *in vitro* measurements (Kadoya *et al.*, 1985; Varro *et al.*, 1988) settings. This suggests that there must also be differences in the drug-evoked changes on the underlying transmembrane ionic currents between acute and chronic amiodarone applications. After acute amiodarone administration the dominant ECG change is an increase of PQ interval (Wellens *et al.*, 1984). QT_c lengthening was usually observed only after chronic amiodarone treatment (Debbas *et al.*, 1984; Wellens *et al.*, 1984; Kodama *et al.*, 1992). Acute amiodarone exposure in the tissue bath either does not change (Ikeda *et al.*, 1984; Kadoya *et al.*, 1985; Varro *et al.*, 1988) or slightly increases (Northover, 1984; Mason *et al.*, 1984; Yabek *et al.*, 1986) or decreases (Varro *et al.*, 1988) the duration of the action potential (APD) in various cardiac preparations. Chronic amiodarone treatment always markedly increases APD in ventricular muscle fibres (Singh & Vaughan Williams, 1970; Kato *et al.*, 1988) and does not (Papp, 1978; Varro *et al.*, 1988) or only moderately lengthens (Gallagher *et al.*, 1989) APD in Purkinje fibres. Considering the results of the present and previous studies it seems reasonable to assume that since acute amiodarone inhibits both inward and outward currents, the net effect of the drug on APD would depend on the relative contribution of these currents to the repolarization in a particular tissue type (Toyama *et al.*, 1994). Therefore APD lengthening, shortening or no change may follow acute application of amiodarone in various cardiac fibres. After chronic treatment, however, the inhibitory effect of the drug on I_K and I_{to} prevail, which results in lengthening of repolarization in ventricular muscle.

The mechanism of the amiodarone-evoked inhibition of the transmembrane ionic currents is not fully elucidated. The acute effects on I_{Na}, I_{Ca} and I_K can be best explained by direct state dependent interactions of the drug with the sodium (Mason *et al.*, 1984; Follmer *et al.*, 1987), calcium (Nattel *et al.*, 1987; Nishimura *et al.*, 1989) and potassium (Balser *et al.*, 1991; Carmeliet, 1993) channels. The chronic effect of amiodarone seems to be more complex. The effect of long-term amiodarone treatment on thyroid function was recognised as early as 1970 by Singh & Vaughan Williams (1970). Later it was demonstrated the repolarization lengthening effect of chronic amiodarone treatment was attenuated by experimental hypothyroidism (Talajic *et al.*, 1989), suggesting that at least some of the chronic effects of amiodarone are mediated via thyroid action. In support of this idea it was found that amiodarone blocked the nuclear thyroid receptors in the heart and liver (Latham *et al.*, 1987.).

In summary, we conclude that acute application of amiodarone in the tissue bath and chronic amiodarone treatment alter transmembrane ionic currents differently. This may help to understand the marked differences in the amiodarone-induced electrophysiological changes observed after acute and long term treatments with the drug in patients.

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