Electrophysiological Effects of Tamoxifen: Mechanism of Protection Against Reperfusion Arrhythmias in Isolated Rat Hearts

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Abstract: Reperfusion arrhythmias are currently attributed to ionic imbalance and oxidative stress. Tamoxifen is a potent antioxidant that also modulates some ionic transport pathways. In this work, we tried to correlate the electrophysiological effects of 1, 2, and 5 μM of tamoxifen with the incidence and severity of arrhythmias appearing on reperfusion after 10 minutes of coronary occlusion in isolated hearts from female rats. All tamoxifen concentrations inhibited the action potential shortening observed in the control hearts during late ischemia (6–10 minutes), whereas 2 and 5 μM also reduced the resting membrane potential depolarization. The incidence of sustained ventricular tachycardia and/or ventricular fibrillation on reperfusion decreased from 10 of 12 (control group) to 5 of 10 (1 μM, $P = 0.1718$), 4 of 12 (2 μM, $P = 0.0361$), and 2 of 10 (5 μM, $P = 0.0083$). The possible role of chloride currents activated by cell swelling in these effects was explored in hearts submitted to a 10-minute hypotonic challenge, where tamoxifen (5 μM) blocked the action potential shortening and the late resting membrane potential depolarization produced by hypotonicity, mimicking its action in late ischemia. Tamoxifen produced a similar increase of the total antioxidant capacity of myocardial samples at all the concentration tested. In conclusion, our data strongly suggest that the antiarrhythmic action of this agent is mediated by its electrophysiological effect derived from modulation of chloride currents activated by cell swelling.

Key Words: tamoxifen, reperfusion arrhythmias, isolated rat hearts, hypotonic challenge, oxidative stress

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INTRODUCTION

Reperfusion of ischemic myocardium induces cardiac injury manifested by arrhythmias, contractile stunning, microvascular damage, and accelerated death of the most severely harmed cells.[1] Alterations of the cellular electrical activity due to ionic imbalance and oxidative stress during early reperfusion play a key role in the generation of potentially lethal ventricular arrhythmias.[2,3] Their incidence and severity can be modified by several procedures, among others, those that reduce the oxidative stress, modulate calcium overload, or influence the action potential duration.[2,4–6]

Tamoxifen is a selective estrogen receptor modulator commonly used to treat breast cancer.[7] In addition to this primary effect, a secondary analysis of data from clinical trials performed on women chronically exposed to it revealed that this agent had a cardioprotective action.[7,8] Antiarrhythmic effects were also reported in ovariectomized rats, where the incidence and severity of ventricular arrhythmias related to ischemia and reperfusion were reduced.[9] This cardioprotection was attributed to a concomitant increase in the plasmatic antioxidant properties because tamoxifen protects cell membranes against oxidative damage.[9,10] Tamoxifen also prolongs the QT interval in humans,[11] effect attributed to its ability to block various ionic currents extensively characterized from patch clamp measurements.[12–14] However, to our knowledge, the electrophysiological mediators of the antiarrhythmic action of tamoxifen in a whole heart model of ischemia–reperfusion remain to be elucidated.

In this work, we examined the effects of clinically relevant concentrations of tamoxifen (from 0.2 to 6 μM) on the surface electrogram (ECG) and the cellular membrane potential in isolated rat hearts from female rats subjected to an ischemia–reperfusion protocol.[15,16] In addition, because of its capability to block chloride channels activated by ischemic cell swelling,[17–19] we evaluated the tamoxifen effects on the electrophysiological changes induced by hypotonicity to test the assumption that this mechanism could mediate its effects on the cellular electrical activity in ischemic myocardium. We also measured the total antioxidant capacity (TAC) in myocardial samples excised at the end of the experimental protocol.

METHODS

Langendorff-Perfused Rat Hearts

Female Sprague–Dawley rats weighing 280–380 g were used. They were housed in metal cages with food and water at

libitum and exposed to a cycle of light and darkness (12 hours each). Only rats in the diestrus stage of the estrous cycle were employed. The animals were killed by cervical dislocation after intraperitoneal administration of 60 mg/kg ketamine and 5 mg/kg xilacine, according to the protocols accepted by institutional guidelines (Committee on Ethics of Animal Experimentation, Faculty of Medical Sciences—National University of Cuyo), in agreement with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health. The hearts were rapidly removed and placed in oxygenated, 4°C Krebs–Henseleit buffer to remove extracardiac tissue. Within 4 minutes, they were mounted on a Langendorff setup and perfused with a modified Krebs–Henseleit solution containing (mM): 121 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.2 $NaHPO_4$, 25 $NaHCO_3$, and 11 glucose. When equilibrated with 5% CO_2 in O_2 at 36 ± 0.5°C and the pH was 7.4 ± 0.05.

In the experiments designed to determine the electrophysiological effects of hypotonicity, we used 2 protocols designated as A and B. In A, we perfused modified Krebs–Henseleit (described above) in the control and the recovery periods. Hypotonicity was induced using the solution that contained (mM): 70 NaCl, 5 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.2 $NaHPO_4$, 25 $NaHCO_3$, and 11 glucose during the test period (Hypo). In B, we used for the corresponding control and recovery periods a solution with 70 mM of NaCl but isotonic by adding sucrose to keep the osmolarity at 290 mOsm (IsoSuc) and Hypo for the hypotonic stress. See protocols A and B below.

Evaluation of Electrophysiological Variables and Arrhythmias

The membrane potential from subepicardial left ventricular cells was recorded with flexibly mounted glass microelectrodes. When filled with 3 M of KCl, the tip resistance was between 10 and 15 MΩ. The surface ECG obtained from 2 epicardial electrodes placed as in the conventional standard II lead was synchronously displayed with the membrane potential. The recording equipment consisted of a custom-made microelectrode amplifier, a Tektronix 565 oscilloscope, and a C4 Grass camera. Both signals were photographed from the screen of the oscilloscope. The following measurements were performed: resting potential (RP), action potential amplitude (APA), and action potential duration determined from the onset of the fast rising phase of the upstroke to 90% of repolarization (APD_{90}). The heart rate (HR) and the reperfusion arrhythmias were analyzed from the ECG and the membrane potential recordings.

Arrhythmias were classified according to the Lambeth conventions.[20] Sustained ventricular tachycardia and/or ventricular fibrillation were computed together as severe arrhythmias. Nonsustained ventricular tachycardia (NSVT) was defined as an episode of more than 4 consecutive premature ventricular beats persisting less than 30 seconds. None of the hearts included in this study presented overt tachyarrhythmias before reperfusion.

Ischemia–Reperfusion Protocol

Four groups of experiments were conducted: control (no drug, n = 12) and 3 other ones with perfusates containing 1, 2, or 5 μM of tamoxifen, here designed as TX1 (n = 10), TX2 (n = 12), and TX5 (n = 10), respectively. Tamoxifen was purchased from Sigma-Aldrich (St Louis, MO) and was present from the beginning of the experiment. The protocol consisted of a 20-minutes equilibration period followed by recording of control data during 15 minutes preischemia (PI). Regional ischemia was then induced by tightening a ligature around the anterior descending coronary artery with a 6/0 silk thread. It was maintained for 10 minutes. This procedure was performed only once in each experiment in control and in tamoxifen-exposed hearts. To analyze the time course of the electrophysiological effects of ischemia and reperfusion, we divided the data in 4 periods: PI, early ischemia (EI, from 1 to 5 minutes), late ischemia (LI, from 6 to 10 minutes), and reperfusion (REP, 10 minutes). The coronary flow was measured throughout the experiment. It was used as an index of adequate perfusion and as a criterion to assess the efficiency of coronary ligation. A reduction of at least 25% during occlusion is currently considered satisfactory.[21]

Hypotonic Stress

We applied 2 protocols designed as A and B, which only differed in the composition of the isotonic media used before and after exposure to hypotonicity: Krebs–Henseleit in A and isosucrose in B as described above. This strategy was justified by the need of assessing independently the effects of hypotonicity from those resulting from the low NaCl concentration. The experimental periods: control isotonic, hypotonicity, and isotonic recovery lasted 10 minutes each. Similar to the analysis made for the electrophysiological effects of ischemia, the data from the hypotonic period were divided into an early and a late phase. Two groups of 12 hearts were included in each protocol: control (n = 6) and exposure to 5 μM of tamoxifen (n = 6).

Determination of the TAC in Heart Tissue

The TAC was measured in myocardial samples taken from the region corresponding to the territory served by the anterior descending coronary artery at the end of the ischemia reperfusion protocols. They were weithed (100 mg), dried with filter paper, transferred to Eppendorf tubes containing phosphate-buffered saline, pH 7.4, and stored at −75°C until processing. These procedures lasted about 5 minutes. The technique described by Re et al[22] was used replacing trolox with ascorbic acid. The preformed radical $ABTS^{\bullet+}$, monocation of 2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) generated by oxidation of ABTS with potassium persulfate, was reduced with hydrogen-donating antioxidants. Ventricular homogenates (100 mg/mL) were compared using ascorbic acid (1 mM) as reference of TAC. All samples were read at 600 nm with a UV-visible Spectrometer model Helios Gamma, Helios Delta (Unicam Instruments) after 18 minutes of incubation at 37°C. The results are expressed in ascorbate equivalent per liter.

Statistical Analysis

Data are expressed as means ± standard error of mean. The results were analyzed with 2-way analysis of variance followed by the Bonferroni posttest. The incidence and severity of arrhythmias were tested with contingency tables using

the Fisher exact test. The statistical significance level was set at $P < 0.05$.

RESULTS

Tamoxifen did not influence the coronary flow in any of the experimental periods (Table 1). A mean decrease of 60% followed coronary ligation in all hearts, without significant differences between them. This reduction was far beyond the 25% accepted as the threshold for the induction of ischemia, suggesting that an area larger than 50% of the left ventricle had been affected. This view has been previously validated by us and others.[20,23] On reperfusion, the flow increased significantly, but it remained below preischemic levels.

Qualitative Analysis of the Effects of Tamoxifen on the Electrophysiological Response to the Ischemia–Reperfusion Protocol

Figure 1 illustrates typical recordings from synchronous measurements of the membrane potential and the surface ECG in a control heart and in the presence of 1 and 5 μM of tamoxifen. The records obtained less than 2 μM are not shown, but the corresponding data seem in the quantitative analysis as described below (Fig. 2). Coronary ligation produced an early decrease in RP that reached a steady state within 3 minutes in all groups. Concomitantly, the APA and the size of the overshoot diminished. Under these conditions, the onset of the fast upstroke was preceded by a slowly rising electrotonic foot whose amplitude increased with time and became more prominent toward the end of the coronary occlusion. This may be attributed to a reduction in the steady-state availability of the sodium channels subsequent to the fall in the RP in the ischemic region. These initial effects did not vary significantly throughout the rest of the ischemic period in the control and the TX1 hearts. Whereas a partial repolarization was observed in LI in the TX5 group (Fig. 1), which led to an increase in the APA and the reappearance of the overshoot. The final phase of repolarization slowed down in EI in all groups. It altered the action potential configuration but did not increase significantly the action potential duration (Fig. 2). Reperfusion triggered early and sustained ventricular arrhythmias in the controls. An example of ventricular fibrillation is shown in Figure 1. The incidence and severity of arrhythmias was markedly decreased by TX. An episode of transient ventricular tachycardia in the TX1 group is illustrated. Only few of the preparations exposed to 2 and 5 μM of tamoxifen exhibited bursts of NSVT (see below). The ECG and the pattern of the intracellular recordings recovered their preischemic characteristics almost immediately after the restitution of the coronary flow in all the hearts that did not exhibit sustained arrhythmias.

Quantitative Analysis of the Electrophysiological Variables

Tamoxifen did not affect the beating rate in any group or experimental period during the ischemia–reperfusion protocol (Table 1). The coronary occlusion induced an almost immediate and significant decrease in rate in all the experiments. This was a constant observation and can be attributed to an endogenous release of acetylcholine.[24] This bradycardia persisted throughout reperfusion in the hearts that maintained sinus rhythm, with or without tamoxifen. Figure 2 illustrates the data derived from the quantitative analysis of the left ventricular membrane potential measurements in these experiments. Tamoxifen did not alter the AP characteristics in PI. This finding differs from the data obtained in myocytes isolated from the rat right ventricle where tamoxifen alters the action potential configuration and inhibits Na^+ and K^+ currents.[13] This discrepancy may be attributed to differences in the cell type and the experimental conditions.

The initial effects of coronary occlusion were a fall of the resting membrane potential and a concomitant decrease of the APA, in addition to a nonsignificant but comparable increase in action potential duration in all groups. However, the magnitude and further evolution of these initial effects varied with time and the tamoxifen concentration. Figure 1 shows that control hearts and those exposed to 1 μM of tamoxifen exhibited an initial depolarization of 18 and 17 mV, respectively, without further changes throughout ischemia. In contrast, the initial fall in RP in the TX2 and TX5 groups underwent a partial but significant recovery in LI that resulted in a higher APA and the reappearance of the overshoot less than 5 μM of tamoxifen (Figs. 1, 2B). On reperfusion, the RP returned to preischemic levels in the hearts that did not developed sustained arrhythmias. The time course of the early drop in the membrane resting depolarization mimicked that of the extracellular K^+ accumulation, as often reported,[25] but the partial repolarization observed with 2 and 5 μM of tamoxifen in LI was an unexpected finding. In none of our previous studies on reflow arrhythmias have we observed such an effect.[6,23,26] During coronary occlusion, the

TABLE 1. Effects of Tamoxifen on Coronary Flow and HR During Control Perfusion (PI), Ischemia, and REP

		Coronary Flow (mL/min)			HR (beats/min)		
Group	n	PI	Ischemia	REP	PI	Ischemia	REP (n)
C	12	12.4 ± 1.4	4.9 ± 1.4†	7.4 ± 2.1*	278.0 ± 12.4	225.0 ± 9.1*	206.8 ± 21.1 (2)*
TX1	10	11.2 ± 1.1	5.6 ± 1.6†	7.3 ± 1.1*	277.4 ± 13.1	212.8 ± 12.3*	227.6 ± 12.1 (5)*
TX2	12	12.3 ± 1.2	4.5 ± 0.9†	7.2 ± 1.6*	302.0 ± 9.2	245.7 ± 8.9*	248.2 ± 14.6 (8)*
TX5	10	12.5 ± 1.6	4.8 ± 0.6†	7.4 ± 1.4*	263.1 ± 15.6	191.3 ± 16.6*	183.3 ± 12.8 (8)*

Data are means ± standard error of mean. The number of experiments is listed next to the group identification under n, whereas (n) to the right of REP under HR is the number of hearts that did not develop sustained ventricular arrhythmias on reperfusion.

*$P < 0.05$; †$P < 0.001$ in respect to PI.

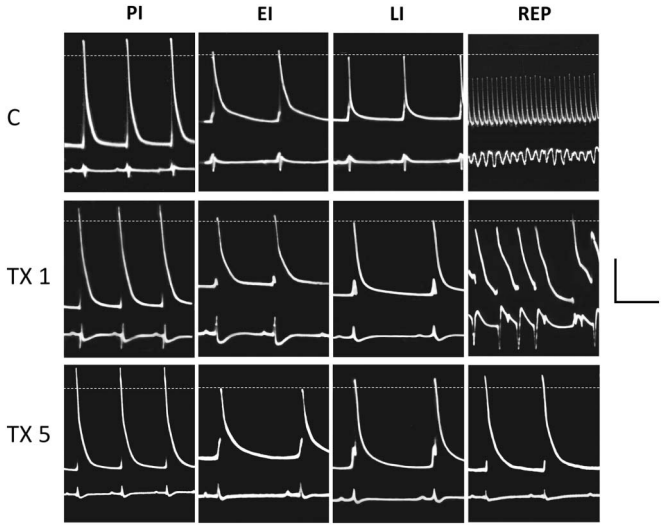

FIGURE 1. Synchronous recordings of the transmembrane potential and the surface ECG obtained at comparable times in a control heart (C) and less than 1 μM (TX1) or 5 μM (TX5) of tamoxifen. Calibration: 40 mV and 0.2 seconds. Horizontal white line, zero reference level.

action potential duration varied with a biphasic pattern in all groups: a nonsignificant early increase followed by a progressive shortening. The latter led to a 34% mean decrease of APD_{90} in the control group in LI. Although this variable evolved with a similar time course in the tamoxifen treated hearts, its decline was significantly reduced so that it reached levels comparable to those measured in PI (Fig. 2C). These findings suggest that tamoxifen counteracted a mechanism contributing to the late electrical effects of the ischemic insult.

Tamoxifen and Electrophysiological Effects of Hypotonicity

The initial pattern of electrical activity was similar in both protocols regardless of the composition of the isotonic perfusate. In contrast, the effects of the hypotonic stress varied widely between both groups (Fig. 3). In the A protocol, the RP did not vary, whereas APA fell from the beginning of hypotonicity. Tamoxifen did not antagonize this response, which was fully reversed on return to Krebs–Henseleit solution. The action potential duration markedly decreased in the second half of the hypotonic challenge. This behavior mimicked the one found with ischemia and was antagonized by tamoxifen. Hypotonicity decreased the HR. This bradycardia was insensitive to tamoxifen. In the control hearts from the B protocol, the RP decreased during the second half of the hypotonic challenge. This effect was fully reversible and antagonized by tamoxifen. The APA decreased from the beginning of hypotonicity. Tamoxifen did not prevent the initial drop of APA but attenuated its further decrease in the late period. A progressive and irreversible decrease in APD_{90} was observed throughout hypotonicity, which was fully antagonized by tamoxifen. The number of action potentials included in the analysis of the isotonic restitution after the hypotonic stress in control hearts from the B protocol was significantly reduced and could be subjected to

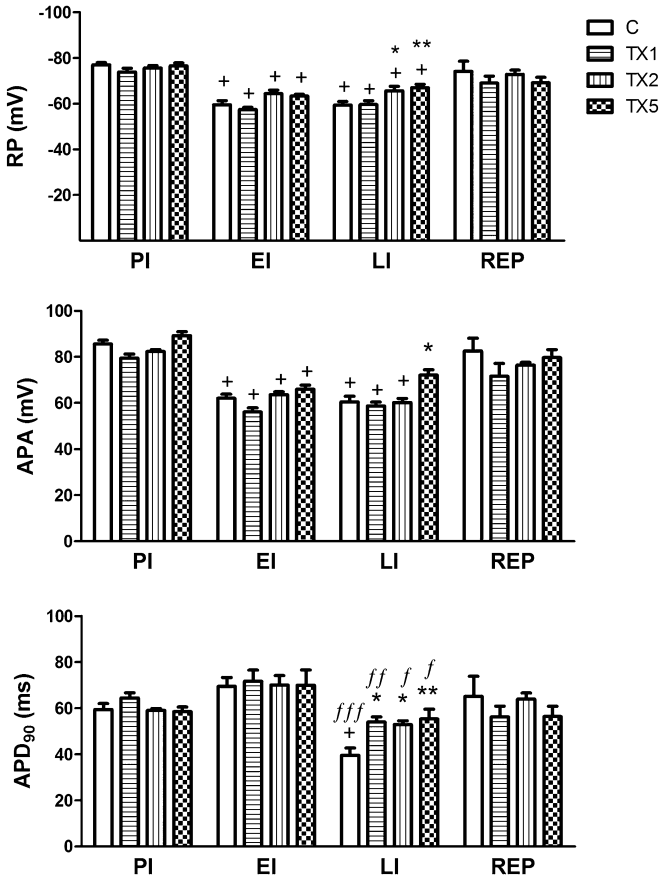

FIGURE 2. Effects of the ischemia–reperfusion protocol on the RP, the APA, and the action potential duration at 90% repolarization (APD_{90}) in control and tamoxifen-treated hearts as indicated. Data obtained before coronary occlusion (PI) during EI and LI and on reperfusion (REP). Means ± standard error of mean, n = 12 for C and TX2, 10 in the others. For reperfusion, n corresponds to the number of hearts that did not develop sustained arrhythmias: 2 in control, 5 for TX1, and 8 for both, TX2 and TX5. $*P < 0.05$; $**P < 0.01$ for tamoxifen versus control for the same period; $+P < 0.05$ in respect to PI within the same group; ƒ $P < 0.05$, ƒƒ $P < 0.01$, and ƒƒƒ $P < 0.001$ in respect to EI within the same group.

bias by the high incidence of arrhythmias during this period (see below). Hypotonicity produced a significant and persistent bradycardia in the control hearts. Tamoxifen delayed the development of the bradycardia and promoted a recovery on return to isotonicity. In addition, all the hearts in the B protocol exhibited episodes of VT and/or VF in the last half of hypotonic stress and/or on return to isotonic conditions in contrast to the tamoxifen treated group, where arrhythmic activity was only observed in 1 experiment ($P = 0.0152$).

Effects of Tamoxifen on the Incidence and Severity of Reperfusion Arrhythmias

On reperfusion, a total recovery of the action potential characteristics was observed in those hearts that maintained or reversed to sinus rhythm after an episode of NSVT. In agreement with previous data,[6,26] most of the control hearts in

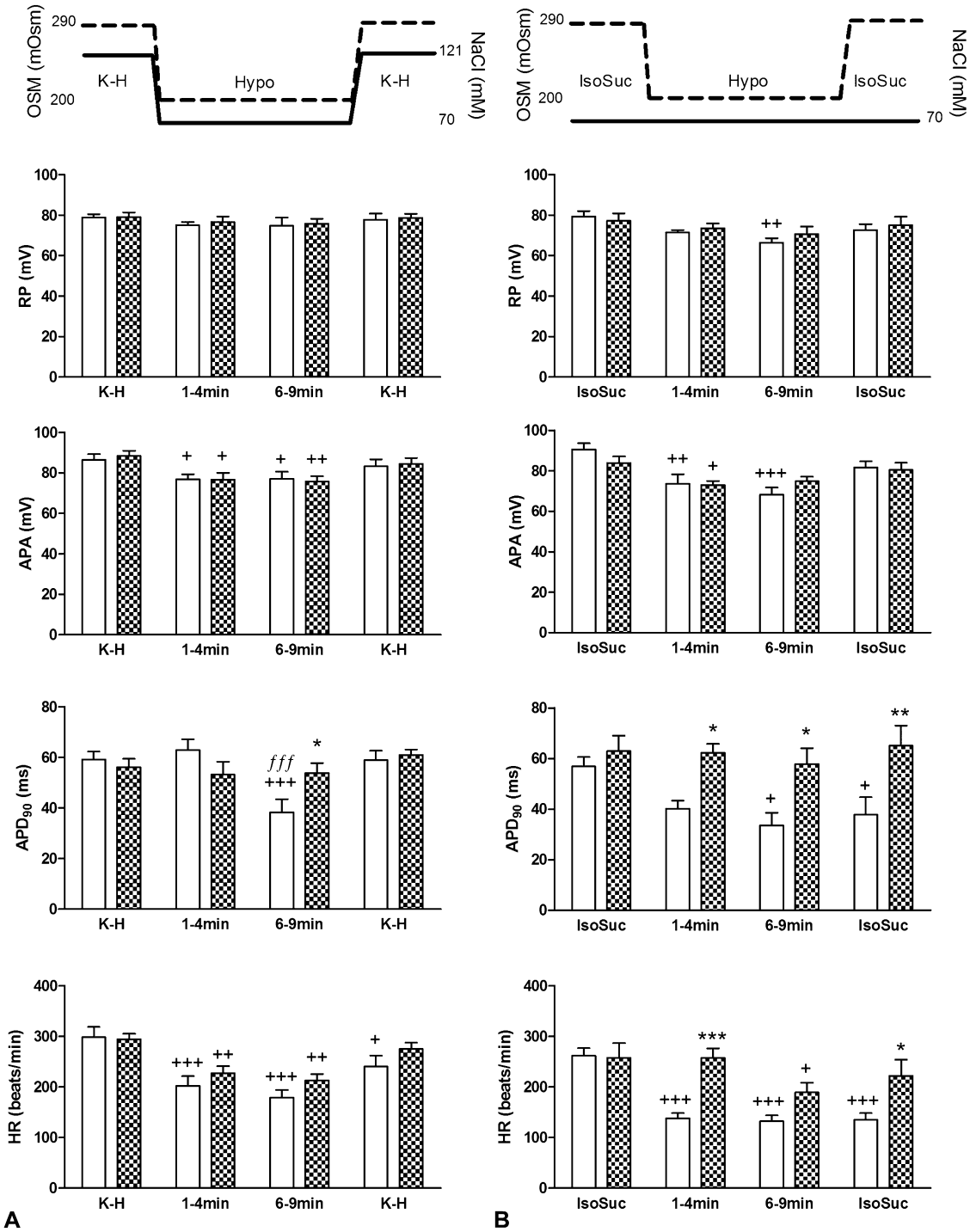

FIGURE 3. Electrophysiological effects of tamoxifen in hearts submitted to hypotonic stress. Results from early (1–4 minutes) and late (6–9 minutes) hypotonic stress are indicated by the scheme under the protocol identification (A and B). The media used before and after exposure to 10 minutes of hypotonicity (Hypo) were Krebs–Henseleit (K-H) in A and isosucrose (IsoSuc) in B. In both protocols, the empty bars correspond to the control and the chequered bars to 5 μM of tamoxifen. The results from the statistical analysis are presented as follows: (1) comparison between groups, tamoxifen versus control (*); (2) comparison within a given group in respect to the initial isotonic phase (+); or (3) versus the early phase of the hypotonic challenge (ƒ). The level of statistical significance is indicated by the number of symbols: 1 for $P < 0.05$, 2 for $P < 0.01$, and 3 for $P < 0.001$.

this study developed ventricular tachyarrhythmias early on reperfusion (Figs. 1, 4A). Their incidence was very high in the control group (83%) and decreased with tamoxifen to 50% at 1 μM, 33% at 2 μM, and 20% at 5 μM (Fig. 4A). Although at the limit of statistical significance at 1 μM, it seems that tamoxifen exhibited as well an antiarrhythmic effect, as confirmed by the plot in Figure 4B, where the number of hearts that only developed NSVT was pooled together with those that did not exhibit arrhythmias on reperfusion (no arrhythmia). This plot supports our interpretation based on the absolute number of hearts per group (Fig. 4A). Therefore, these data suggest that at concentrations compatible with its clinical use, tamoxifen exerts a dose-dependent inhibition of sustained tachyarrhythmias triggered by reperfusion after coronary occlusion.

Total Antioxidant Capacity

Figure 4C illustrates the values for the TAC expressed as ascorbate equivalent per liter in myocardial homogenates at the end of the reperfusion period. Exposure to tamoxifen induced a substantial increase in TAC in respect to the control. This effect was highly significant ($P < 0.001$) and dose independent within the concentration range used.

DISCUSSION

Our study showed that tamoxifen reduced the incidence and severity of reflow arrhythmias with a pronounced dose-dependence characteristic. The antiarrhythmic action was previously attributed entirely to its antioxidant properties by Ek et al, despite the fact that the lowest dose used by these authors did not raise the antioxidant capacity but offered the same degree of protection and the drug was administered chronically before the ischemia–reperfusion experiments.[9] Because the increase of the myocardial antioxidant capacity was the same at all the concentrations tested after an acute administration, it seems that this action provides a background level of protection to which the attenuation of the deleterious effects of ischemia on the cellular electrical activity by tamoxifen should be considered to explain its antiarrhythmic action. This acute administration scenario reduces the possibility that nuclear estrogen receptors could be involved in the effects here described and reinforce the idea that tamoxifen modulation of ionic currents underlies its antiarrhythmic effects.

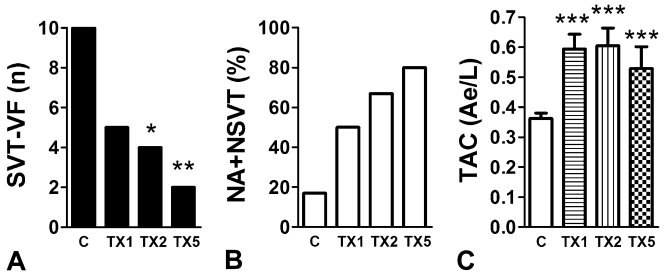

FIGURE 4. Effects of tamoxifen on reperfusion arrhythmias and the TAC. A, Number of hearts showing sustained ventricular tachycardia and/or ventricular fibrillation during reperfusion (SVT-VF). Exact Fischer test for incidence of SVT-VF in the tamoxifen groups versus control, $*P = 0.0361$ and $**P = 0.0083$ for TX2 and TX5, respectively. B, Pooled data: percentage of hearts that did not develop arrhythmias (NA) plus those that exhibited NSVT. The tendency for the dose-dependent antiarrhythmic effect was $P = 0.0222$ with the χ^2 test for trends. C, Changes in TAC with tamoxifen expressed in ascorbic equivalent per liter. Experimental groups identified under the abscissa. $***P < 0.001$ when compared with the control group by analysis of variance.

Although tamoxifen modifies several ionic currents,[12–14,27] we did not see any change in APD_{90} during the preischemic period. This could be attributed to tamoxifen simultaneous inhibition of the inward Ca^{2+} currents and the outward K^+ currents leading to a counterbalance of the mayor physiological determinants of action potential duration in the rat.[13,28] Our results are in agreement with those reported by Liu et al in isolated rabbit myocytes, despite the fact that the K^+ currents in these cells differ from dose expressed in rats.[12,13] Here, we present new information about the electrophysiological effects of tamoxifen, mainly the attenuation of the response to stressful situations, which could underlie its protective actions.

The partial recovery from the early decrease in the RP and the inhibition of the action potential shortening in LI constituted the 2 major effects of tamoxifen at 2 and 5 μM (Fig. 2). The initial RP decrease was abrupt and reflected the rapid increase in extracellular K^+ concentration that remains stable for 10–15 minutes.[25] In our experience, only a high Mg^{2+} concentration (4.8 mM) counteracts this initial decrease of the resting membrane potential, probably because of a lesser extracellular K^+ accumulation.[23] The partial restoration of RP less than 2 and 5 μM of tamoxifen in LI suggests that it was mediated by a process developing after a certain delay. The activation of chloride currents induced by cell swelling ($I_{Clswell}$) for which tamoxifen is a rather selective blocker could explain this finding.[17–19] This interpretation is based on the similarities between our results and the latency for the ischemic cell volume increase in rat hearts[29] and the onset of cell swelling in rat myocytes subjected to hypotonic stress, which is also accompanied by resting membrane potential depolarization and action potential shortening.[30] The magnitude and time course of the resting depolarization produced by hypotonic stress in our experiments (B protocol) were similar to those observed in ischemic hearts and the time course and magnitude of the APD_{90} changes during hypotonic stress with the A protocol (Figs. 2, 3). Despite the differences in their pattern, tamoxifen antagonized both responses, which supports the view that inhibition of the action potential changes induced by ischemia played a major role in its antiarrhythmic action. This agent not only blocked the APD_{90} shortening produced by hypotonic stress in both protocols but also eliminated the resting depolarization and the arrhythmias observed in the B protocol. Consequently, the ischemic action potential shortening could be attributed to $I_{Clswell}$ and I_{KATP}, both activated by swelling and blocked by tamoxifen.[17–19,27] However, I_{KATP} is not expected to influence the decrease in RP in which case, $I_{Clswell}$ would play a key role in the maintenance of a low RP in LI. It is also improbable that the protective action of tamoxifen in ischemia was mediated by a depression of myocardial contractility because this agent also antagonized the fall in the RP and the APD_{90} shortening induced by hypotonic stress during which the contractile activity was most probably enhanced by the low Na concentration in

the perfusate. In addition, our data provide the first experimental evidence linking sustained reperfusion arrhythmias with the action potential shortening induced by cell swelling.[17,18,30]

The dissociation between the early and maintained decrease in APA without concomitant changes in RP in both protocols is compatible with the low sodium concentration used. On the other hand, the late and more pronounced decrease of APA in the B protocol correlated with the resting depolarization and both were antagonized by tamoxifen, suggesting that they resulted from the activation of chloride currents. In addition, our data showed that the initial exposure to the isosucrose medium also affected electrogenesis in the sinoatrial node because the bradycardia induced by hypotonicity was irreversible and more pronounced in the B than in the A protocol and it was rather insensitive to tamoxifen. These features are consistent with the primordial role played by extracellular Na^+ in the pacemaker current I_f.[31] It is unlikely that the concomitant action potential shortening was secondary to the bradycardia because the APD_{90} from ventricular rat cells does not vary with frequencies between 140 and 380 beats per minutes.[23]

The combination of data derived from the different experimental approaches used in this work allowed us to interpret the electrophysiological changes that seem to underlie the antiarrhythmic action of tamoxifen in addition to its antioxidant properties. This agent partly restored the RP and lengthened the action potential duration of ischemic myocardium. Both effects could be attributed to a block of currents activated by cell swelling. Tamoxifen also antagonized the transient arrhythmic bursts appearing toward the end of the hypotonic challenge. The reduction of the deleterious effects of ischemia on the RP and the action potential duration seem as the main factors mediating the antiarrhythmic effect of tamoxifen through a decrease in the electrical heterogeneity within the ischemic region and in respect to the nonischemic myocardium. This would promote the maintenance of coordinated electrical activity by preventing the formation of reentry pathways, essential for the perpetuation of sustained arrhythmias triggered by delayed after depolarizations generated by Ca^{2+} overload on reperfusion.[2] Although this interpretation provides a reasonable approach to interpret the protective action of tamoxifen, it cannot reconcile the existing discrepancy between the concentration dependence of the antiarrhythmic effect and the apparent nonspecific increase in the myocardial antioxidant capacity and the insensitivity of the electrical restitution (RP and APD_{90}) to the different concentration used, despite the capability of tamoxifen to block chloride channels. It is then probable that some other targets in the chain of events responsible for the triggering of sustained arrhythmias remain to be identified. However, this possibility does not limit the eventual applicability of our finding, which showed that the reversal of the deleterious effects of ischemia on RP and APD_{90} constitutes an effective mean to prevent the occurrence of sustained tachyarrhythmias. The last argument to support this claim is that the 2 control hearts that did not developed these arrhythmias (Fig. 4B) recovered the preischemic APD_{90} values on reperfusion (Fig. 2).

CONCLUSIONS

The various mechanisms involved in the protective effects of tamoxifen illustrate once more the multifactorial nature of reperfusion arrhythmias. Our data revealed that a key element of this action was the reversal of the ischemic alterations in the cellular electrical activity, particularly the action potential duration and the resting depolarization after 5 minutes of ischemia. The combination of the data obtained from the ischemia–reperfusion protocol with those provided by the experiments with hypotonic stress led to the identification of the tamoxifen-sensitive swelling activated chloride currents as major mediators of the deleterious electrical effects of coronary ligation. Specifically, the increment of the action potential duration resulting from the blockage of these currents by tamoxifen seems to be the key element of its protective action. On the other hand, we cannot elaborate on the contribution of the increased myocardial antioxidant capacity, which may provide a rather nonspecific protection against ischemia–reperfusion injury. Nevertheless, even if tamoxifen does not counteract the processes leading to the Ca^{2+} overload on reflow, it antagonizes the sustained ventricular arrhythmias by blocking the reentry pathways essential for their perpetuation.

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