0026-895X/00/061152-06$3.00/0
MOLECULAR PHARMACOLOGY

MOL 57:1152–1157, 2000 /13072/824864

Doxorubicin Impairs Crossbridge Turnover Kinetics in Skinned Cardiac Trabeculae after Acute and Chronic Treatment

EVERT L. DE BEER, ANTONIO E. BOTTONE, JOLANDA VAN DER VELDEN, and EMILE E. VOEST

Department of Medical Physiology and Sports Medicine, Utrecht University, Utrecht (E.L.d.B., A.E.B.); Laboratory for Physiology, Institute for Cardiovascular Research, Free University, Amsterdam (J.v.d.V.); and Department of Internal Medicine, University Hospital Utrecht, Utrecht (E.E.V.), The Netherlands

Received November 4, 1999; accepted February 1, 2000

This paper is available online at http://www.molpharm.org

ABSTRACT

Crossbridge dynamics underlying the acute and chronic inotropic effects of doxorubicin (Dox) were studied by application of releasing length steps (amplitude, 0.5–10%) to skinned cardiac trabeculae. Acute incubation of trabeculae with 20 μM Dox for 30 min resulted in a decrease of the velocity of unloaded shortening (V_0, from 9.3 $\pm$ 1.1 to 7.7 $\pm$ 0.7 μm/s, $P < .05$) and in an increase of the rate of force redevelopment (τ_r, from 56 $\pm$ 4 to 65 $\pm$ 3 ms, $P < .05$) in response to step amplitudes ranging from 5 to 10%. In contrast, chronic Dox treatment in rats (2 mg/kg/week for 4 weeks) significantly impaired trabecular crossbridge dynamics after step releases of 0.5%. This was reflected by an increase of all time constants describing tension recovery: τ_1, from 10 $\pm$ 1 to 14 $\pm$ 1 ms; τ_2, from 65 $\pm$ 6 to 82 $\pm$ 6 ms; τ_3, from 92 $\pm$ 7 to 293 $\pm$ 67 ms; $P < .05$. In addition, V_0 was decreased (from 8.6 $\pm$ 0.6 to 6.8 $\pm$ 0.3 μm/s, $P < .05$) and τ_r was increased (from 67 $\pm$ 4 to 89 $\pm$ 3 ms; $P < .05$) in the slack-test. We found that chronic Dox treatment resulted in a shift from the "high ATPase" α-myosin heavy chain (MHC) isoform toward the "low-ATPase" β-MHC isoform in the ventricles (control: α-MHC 79 $\pm$ 2% and β-MHC 21 $\pm$ 2%; Dox-treated: α-MHC 53 $\pm$ 2% and β-MHC 47 $\pm$ 2%; $P < .05$). The present results show that acute Dox incubation affects the detachment rate of crossbridges, which leads to a delayed relaxation and an arrest of crossbridges in strongly bound states. In contrast, chronic Dox treatment leads to an impairment of both the attachment and detachment rates in the crossbridge cycle, which may be explained by an altered MHC isoform composition in ventricular myocardium. Interfering with Dox-induced alterations of crossbridge kinetics may provide a new strategy to prevent Dox-associated cardiotoxicity.

The clinical use of doxorubicin (Dox) in the treatment of a wide variety of malignancies is limited by the risk of developing cardiomyopathies beyond a cumulative dose of 550 mg/m^2 (Doroshow, 1991). The precise mechanism of this cardiotoxicity is still incompletely understood, but several hypotheses have been proposed (for review, see Singal et al., 1987).

In previous experiments, we studied the inotropic effect of Dox on skinned cardiac preparations after both acute drug incubation and after chronic treatment of rats with Dox. In these studies, we used cardiac preparations in which both inner and outer membranes were permeabilized, leaving only the contractile machinery intact. We reported a strong and direct positive inotropic action of Dox on the actin-myosin contractile system after acute administration in both skeletal (De Beer et al., 1992) and cardiac muscle (Bottone et al., 1997). In contrast, the maximal tension of trabeculae was decreased after chronic treatment of rats with Dox (Bottone et al., 1998). Based on our previous findings, we suggested that these inotropic actions of Dox are best explained by a direct interaction of Dox with the actin-myosin crossbridge system.

Crossbridges cycle repetitively through force-generating and non–force-generating states during tension development (for review, see Cooke, 1997). Since the early discoveries of Huxley and Simmons (1971) and Julian et al. (1974), complex mechanical and biochemical schemes characterized by a large number of intermediate crossbridge states have been made. Based on schemes of Lymn and Taylor (1971) and Eisenberg and Hill (1985), three ensembles of crossbridges can be discriminated within the crossbridge cycle: detached, pre–power-stroke, and force-generating crossbridges. The action of crossbridges is controlled by intracellular Ca^{2+} through the regulatory proteins troponin and tropomyosin, located on the actin filaments. An increase of the Ca^{2+}-activated tension generally results from an increase in the number of actomyosin interactions, whereas relaxation occurs when crossbridges accumulate in non–force-producing states. Crossbridge turnover kinetics may be studied by application of rapid length perturbations in Ca^{2+}-activated preparations and measuring the subsequent tension re-

This research was supported by Grant 93.074 from the Netherlands Heart Foundation.

ABBREVIATIONS: Dox, doxorubicin; MHC, myosin heavy chain.

sponse. The amplitude of the step release determines whether using relatively small steps changes the orientation of the attached crossbridges alone or applying larger steps slackens all crossbridges. The adaptation of the preparation to its new steady-state length contains information about the kinetics of transitions within the crossbridge cycle.

The results of the present study show that crossbridge kinetics in skinned cardiac trabeculae are affected by both acute and chronic Dox exposure. We provide evidence that acute Dox exposure results in an attenuation of the detachment rate of crossbridges, which results in an arrest of crossbridges in the strongly bound states and a higher isometric tension level. In contrast, chronic Dox treatment results in a decrease of both the attachment and detachment rates of crossbridges resulting in a lower isometric tension response. The chronic effects may be explained by the simultaneously found alterations in isomyosin composition in the ventricles of Dox-treated rats.

Materials and Methods

Animals and Cardiac Preparations. Male Wistar rats were used in all experiments. Animals were given water and standard chow ad libitum, and were kept on a 12-h light/dark cycle. The University Experimental Animal Committee approved all experiments.

For measurement of the acute effect of Dox, cardiac preparations were obtained from rats with a body weight of 250 to 350 g at the moment of sacrifice. To study the effect of long-term Dox administration, rats with a starting weight of 310 to 330 g were i.v.-administered Dox at a dose of 2 mg/kg body weight once a week for 4 weeks (total cumulative dose, 8 mg/kg). The animals were used in experiments 4 weeks after the last infusion with Dox. Previous experiments showed that this experimental protocol provides a reproducible model to study Dox-related cardiotoxicity (Bottone et al., 1998).

At the designated time, rats were anesthetized with Nembutal (60 mg per kg body weight, i.p.). After tracheotomy, the thorax was opened and the aorta was cannulated. The heart was rapidly removed and connected to a Langendorff perfusion system, and retrogradely perfused with a solution composed of 130 mM NaCl, 4.7 mM KCl, 0.42 mM Na_2HPO_4, 20.2 mM $NaHCO_3$, 10.1 mM glucose, 1.0 mM $MgCl_2$, and 2.0 mM $CaCl_2$. This solution was continuously gassed with a mixture of 95% O_2 and 5% CO_2 to maintain pH at 7.4 at 30°C. 2,3-Butanedione monoxime (15 mM) was added to the perfusion solution to prevent contraction and muscle damage during dissection (Mulieri et al., 1989). Free running trabeculae, ranging 50 to 150 μm in diameter and 1 to 2 mm in length, were dissected carefully from the right ventricle wall and skinned by exposure to Triton X-100 (1% v/v) for 30 min. Triton X-100 renders both the sarcolemma and inner membrane structures permeable for small ions and molecules. The skinning solution was removed by washing with relaxation solution. The contents of the various solutions have been described elsewhere (Bottone et al., 1997).

Myosin Heavy Chain Isoform Analysis. After isolation of trabeculae, right and left ventricles of both Dox-treated rats and control rats were frozen in liquid nitrogen and stored at −80°C. The freeze-dried samples were dissolved in a buffer containing 62.5 mM Tris, pH 6.8, 15 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 0.5 mM leupeptin, 1% (w/v) SDS, 0.01% (w/v) bromphenol blue, and 15% (v/v) glycerol. Gel electrophoresis was performed as described previously (Van der Velden et al., 1998), using an acrylamide to bis-acrylamide ratio of 200:1 in the separating gel (12% total acrylamide; pH 9.3) and of 20:1 in the stacking gel (3.5% acrylamide; pH 6.8). A Protean II xi cell was used for electrophoresis (Bio-Rad, Hercules, CA). Samples (1 μg) were run at constant current (24 mA) for 5 h (approximately 1800 V-h). Silver staining of the gels was performed as described by Giulian et al. (1983). Laser scanning densitometry was performed to identify differences in myosin isoform composition. Bands corresponding to contractile proteins were identified by Western immunoblotting using specific antibodies against rat α-myosin heavy chain (α-MHC) and β-MHC: monoclonal antibody 249-SA4 equals anti-α-MHC and monoclonal antibody 169–1-D5 equals anti-β-MHC in the rat. The production of these antibodies has been described previously (De Groot et al., 1989).

Measuring Device. The displacement generating system was based on that described by De Winkel et al. (1993) and consisted of a coil moving in a permanent magnet, making step length changes up to 200 μm (10% of muscle length) completed within 1 ms. The displacement was detected by a photosensitive element behind a small vane that is part of the mover. Light of a stable light source is projected continuously on the photosensitive element and movement of the vane leads to a change in the output voltage of the photosensitive element. The output of the photosensitive element is filtered, amplified, and fed back to the coil to fix the end position of the mover. The noise of the length displacement was 65 nm peak to peak. Isometric tension of the muscle preparations was measured with a Sensonor AE 801 force transducer (Sensonor, Horten, Norway). All tension signals and displacement signals were digitized by a computer with an AD-card (Keithley DAS 1602) for further analysis.

The preparation was mounted between the force transducer and a support connected to the coil of the displacement system with a cyanoacrylate glue (Sicomet 77; Henkel, Düsseldorf, Germany). The glue was colored with congo red to check the interface of the glue and the functional preparation to obtain a precise measure for preparation length. Trabeculae were stabilized for 15 min in relaxation solution before start of the experiments. Sarcomere length was measured by means of laser diffraction and was adjusted to 2.15 μm at the start of the experiments. Only preparations of which the sarcomere length remained constant throughout the experiments were used. In a series of test experiments, we followed the change in muscle length and the change in sarcomere length simultaneously during step releases. These experiments showed that the relative change in sarcomere length was equal to the change in muscle length, which was accomplished by means of the stiff fixation of the muscle ends by the cyanoacrylate glue (De Winkel et al., 1995). Tension was calculated as force divided by cross-sectional area. The latter was calculated from the muscle dimensions by assuming an ellipsoid shape. Experiments were performed at 22°C and at pH 7.0.

Quick Release Experiment. The adaptation of the mechanical transient to the sudden length change reflects processes such as conformational changes within a crossbridge while attached and the attachment or detachment of cycling crossbridges (Huxley and Simmons, 1971). In the present protocol, we investigated the force adaptation to sudden length changes with an amplitude of 0.5% of the muscle length.

The passive Young's modulus (i.e., the stiffness normalized for the length and the diameter in relaxed preparations) was determined at the start of each experimental protocol. The Young's modulus is defined as $\Delta T/(\Delta l/l)$, where ΔT is the amplitude of the tension response, and $\Delta l/l$ is the relative change in length. Next, the preparation was incubated in activation solution at pCa 4.0. At steady-state maximal contraction, tension transients resulting from quick releases were recorded. The resulting tension transients were fitted with a sum of three exponential functions, yielding the rate constants τ_1, τ_2, and τ_3, together with two extreme tension values, T_1 and T_2, reflecting the adaptation of the preparation to its new length.

In the acute experiments, two groups were formed: preparations that were allowed to stabilize for 30 min in control relaxation solution, and preparations that were incubated for 30 min in relaxation solution containing 20 μM Dox. We compared the effect of 30-min Dox incubation with the effect of 30-min stabilization in control solution. After the Dox incubation, tension responses were recorded in both the relaxed and the activated state. Control experiments consisted of tension recordings in control solutions.

Slack-Test. The velocity of unloaded shortening was determined by the slack-test (Edman, 1979). Trabeculae were activated with Ca^{2+} and allowed to develop a steady-state tension level. Protocols were performed at two activation levels: pCa 6.0, resulting in a submaximal contraction, and pCa 4.0, resulting in a maximal contraction. At steady-state contraction, the preparation was slackened by imposing a series of six quick releases of increasing magnitude on the preparation. The step size of these quick releases was calibrated in the relaxed preparation and ranged from 5 to 10% of the length of the functional preparation. After each step release, the muscle was returned to its initial length using a slow ramp restretch (500-ms duration) starting 500 ms after the step release. Each step release was initiated from the same isometric tension level. Adoption of this protocol allows for the collection within 60 s of a complete slack-test containing six releases during a single contraction. The time required to take up the slack (i.e., the duration of the unloaded shortening) was measured as the interval between the beginning of the length step and the onset of tension redevelopment. The onset of tension redevelopment was calculated from a linear regression fit of the force data during the initial phase of force redevelopment and its intersection with the force baseline. The force baseline was determined by a linear regression fit of the force data during unloaded velocity of sarcomere shortening of the largest release step (i.e., 10% of the fiber length); this baseline was then applied to all releases. The magnitude of the release step was plotted as a function of the duration of unloaded shortening. The slope of this relation, which was calculated by linear regression, corresponds to the velocity of unloaded shortening (V_0). The subsequent redevelopment of force after the shortening of the preparation under zero load was fit by a monoexponential function of the form Force $= a \times (1 - e^{-t/\tau r})$, where a is the redeveloped steady force, t is the time, and τ_r is the time constant describing tension recovery.

Statistical Analysis. Data values are given as mean ± S.E.M. for n observations. In our statistical analysis, we included data on just one preparation in each protocol of each individual animal. Subsequent preparations showed similar results. An analysis of variance (ANOVA) was used to compare differences between control preparations and Dox-treated preparations. Differences with $P < .05$ were considered to be significant.

Results

Animals and Preparations. All rats that received Dox in the chronic study ($n = 20$) started to lose weight during the treatment period. Six rats died during the post-treatment period because of Dox-related complications. Their mean starting weight amounted 314 ± 4 g ($n = 14$). At the time of sacrifice (4 weeks after treatment), the mean body weight was 238 ± 9 g and the mean heart weight 0.93 ± 0.03 g. In earlier experiments, we showed that loss of body weight per se does not affect the contractile properties of isolated trabeculae (Bottone et al., 1998). The dimensions of the right ventricular trabeculae were not significantly affected by the treatment with Dox. The overall mean diameter was 131 ± 7 μm for all preparations used in the acute experiments ($n = 30$) and 142 ± 10 μm for all preparations of rats that were treated with Dox in the chronic study ($n = 14$).

Differences in MHC Isoform Composition. A typical silver-stained polyacrylamide gel of the electrophoretically separated proteins is shown in Fig. 1A. The fast α-MHC and slow β-MHC could be separated (B), and subsequent laser densitometric scans (C) revealed the presence of two corresponding peaks. Chronic Dox treatment in rats significantly increased the ratio of β-MHC over α-MHC in ventricular tissue compared with control animals, as can be seen in D.

Doxorubicin Affects Maximal Ca^{2+}-Activated Tension. Incubating trabeculae with relaxation solution containing 20 μM Dox for 30 min resulted in an increase of the maximal Ca^{2+}-activated tension compared with 30-min stabilization in control relaxation solution: 93 ± 7 kN/m^2 ($n = 15$) and 75 ± 5 kN/m^2 ($n = 15$), respectively ($P < .05$). In trabeculae obtained from rats of the chronic study, the maximal tension of Dox-treated trabeculae was significantly decreased compared with the maximal tension of control trabeculae: 62 ± 4 kN/m^2 ($n = 14$) and 84 ± 4 kN/m^2 ($n = 30$), respectively ($P < .05$).

Effect on Transition Times between Crossbridge States. In preparations that were maximally activated (at pCa 4.0), the tension transients after step releases of 0.5% were fitted with a sum of three exponential functions, yielding the rate constants τ_1, τ_2, and τ_3, together with two extreme tension values, T_1 and T_2 (Fig. 2). Dynamic experiments in relaxed preparations showed that the passive stiffness, quantified by the Young's modulus, was not significantly altered upon acute or chronic Dox exposure. The control value for Young's modulus amounted 320 ± 50 kN/m^2 ($n = 14$), which is the same as values reported by others in both skeletal and cardiac muscle preparations (Jung et al., 1988; De Winkel et al., 1995). This implies that the formation of connective tissue upon chronic Dox treatment is negligible, which agrees with observations in earlier experiments (Bottone et al., 1998). Table 1 summarizes the results of Dox treatment on the three time constants in the quick release protocol. Acute Dox incubation resulted in a slight increase of τ_2 compared with preparations that were allowed to stabilize for 30 min in control relaxation solution (from 47 ± 7 to 59 ± 6 ms; P = N.S.). Chronic treatment of rats with Dox resulted in a significant increase of all three time constants describing the tension recovery compared with control preparations (τ_1, from 10 ± 1 to 14 ± 1 ms; τ_2, from 65 ± 6 to 82 ± 6 ms; τ_3, from 92 ± 7 to 293 ± 67 ms; $P < .05$). The ratio T_1/T_0 and T_2/T_0 upon releasing steps of varying amplitude remained unchanged upon both acute and chronic Dox treatment, indicating that the functionality of crossbridges remains unaffected upon Dox treatment (data not shown).

Doxorubicin Affects Slack-Test Parameters. Figure 3 shows a typical recording of a complete slack-test obtained in a maximally activated trabecula (A) and superimposed traces of one slack-test protocol (B) with step amplitudes ranging from 5 to 10%. The duration of unloaded shortening was shown to be linearly proportional to the amplitude of the quick release step, and the velocity of unloaded shortening (V_0) can be determined by the slope of this relation. Table 1 summarizes the results of the slack-test. In the acute study, V_0 of trabeculae was significantly decreased upon 30 min exposure to Dox at pCa 4.0 compared with control specimens (from 9.3 ± 1.1 to 7.7 ± 0.7 μm/s; $P < .05$). V_0 of trabeculae of chronically Dox-treated rats was significantly decreased compared with control values at both pCa 4.0 (from 8.6 ± 0.6 to 6.8 ± 0.3 μm/s; $P < .05$) and at pCa 6.0 (from 10.9 ± 1.0 to 6.7 ± 0.6 μm/s; $P < .05$). In addition to V_0, we calculated the time constant of the force redevelopment (τ_r) at all step releases ranging from 5 to 10%. We found that τ_r was dependent of the amplitude of the step and decreased with increasing step amplitudes (B). The average τ_r at a 10% length step was taken as standard and was calculated at both pCa 4.0 and pCa 6.0 (see Table 1). The τ_r was independent of the level

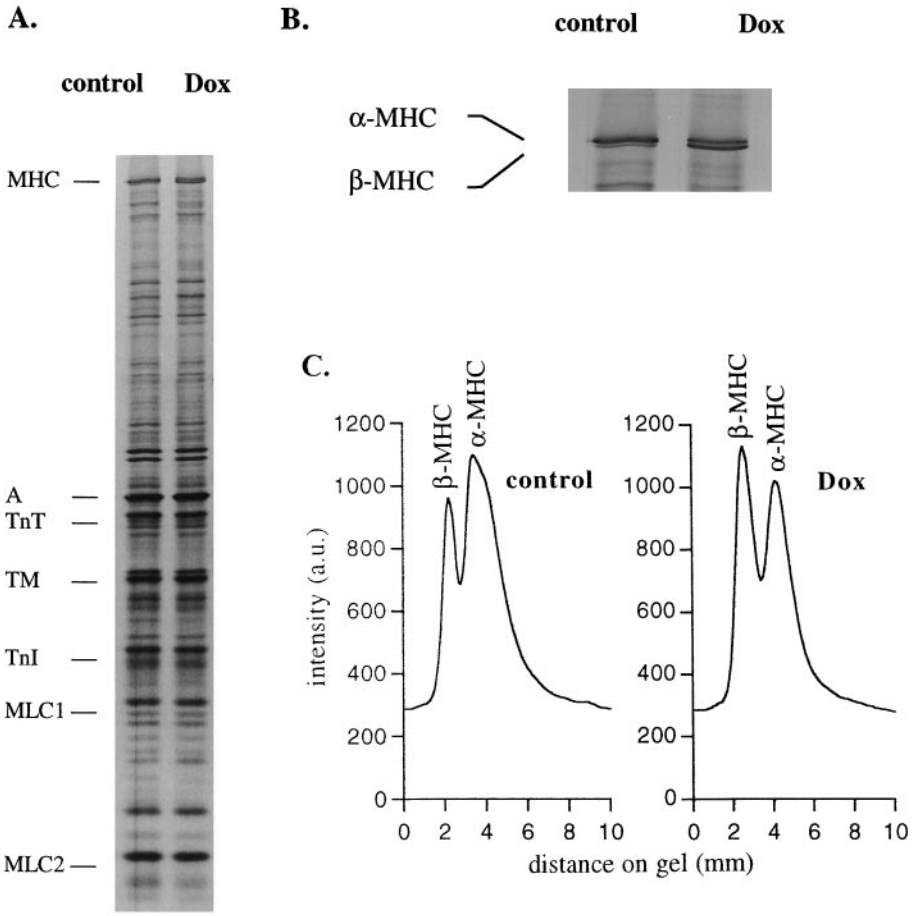
of Ca^{2+} activation at a given step release amplitude. At maximal Ca^{2+} activating levels, acute Dox incubation resulted in a significant increase of τ_r compared with preparations that were stabilized for 30 min in control relaxation solution (from 56 ± 4 to 65 ± 3 ms; $P < .05$). In Dox-treated rats, τ_r was significantly higher compared with control values in both maximally and partially activated preparations: 89 ± 3 and 67 ± 4 ms, respectively, at pCa 4.0; 106 ± 11 and 63 ± 8 ms, respectively, at pCa 6.0; $P < .05$.

Discussion

This study demonstrates that both acute and chronic Dox treatment impair crossbridge kinetics in skinned cardiac trabeculae, although the underlying kinetics is different. Acute Dox incubation resulted in a lower rate of crossbridge cycling due to a delayed uncoupling of attached crossbridges. This leads to arrest crossbridges in strongly bound states, which explains the increased isometric tension upon acute Dox exposure (Bottone et al., 1998). Chronic Dox treatment in rats resulted in an overall lower rate of crossbridge cycling in skinned cardiac trabeculae. Both coupling and decoupling processes in the crossbridge cycle were impaired upon chronic Dox treatment, leading to a decreased isometric tension level (Bottone et al., 1998). The impaired contraction cycle upon chronic Dox treatment coincided with a shift in the ventricular MHC isoform composition toward the "low ATPase" β-MHC isoform.

The concentration of Dox used in the acute experiments was set at 20 μM, the upper limit of the peak plasma concentration. We (Bottone et al., 1997) have shown that the acute effect of Dox is dose-dependent. Because we want to investigate the underlying molecular mechanism, we used the high concentration. In this respect, it is important to note that the tissue clearance time is 7 days. This implies that Dox is trapped in the tissue [e.g., bound to cardiolipin (Goormaghtigh and Ruysschaert, 1984)].

The time course of tension redevelopment mainly reflects the reapproach of the isometric steady-state distribution of crossbridges between the force-generating states and the non–force-generating states (Brenner, 1988). Acute incubation of trabeculae with Dox resulted in an increase of τ_r together with a decrease of V_0 in the slack-test, indicating that time constants during the crossbridge cycle were al-



D.	control	Dox
α-MHC, %	79 ± 2	53 ± 2[a]
β-MHC, %	21 ± 2	47 ± 2[a]

Fig. 1. Typical photograph of silver-stained SDS-polyacrylamide gel of ventricular contractile proteins (A), a closer view of the MHC composition (B), and the corresponding laser densitometric scan (C). D summarizes the relative distribution of the α- and β-MHC isoforms in ventricular tissue. Data are means ± S.E. (n = 5). [a] $P < .05$.

tered. Because acute Dox incubation significantly increases the maximal Ca^{2+} activated tension in trabeculae, the results of the slack-test point toward an increase of the population of strongly bound crossbridges. An accumulation of crossbridges in the strongly bound states can be caused either by an increased rate of crossbridge attachment or by a decreased rate of crossbridge detachment. The former is unlikely, because the decrease of V_0 and the increase of τ_r reflect a slower rate of crossbridge cycling. Therefore, we conclude that a decreased rate of crossbridge detachment underlies the lower crossbridge cycling rate upon acute Dox incubation, which explains the higher isometric tension level of trabeculae upon acute Dox exposure. The observation that the time constant describing the net detachment of crossbridges (τ_2) in the quick-release protocol is slightly increased, supports this interpretation. A slower relaxation was also observed when skinned cardiac trabeculae were relaxed after a maximal contraction in the presence of Dox compared with control experiments (data not shown). Our results are in accordance with observations of a slower relaxation of cardiac preparations upon Dox treatment (Boucek et al., 1987; Asayama et al., 1992). The precise mechanism for the acute Dox effect remains unclear but may involve binding of Dox to cardiac actin filaments, thereby hindering crossbridge cycling (Lewis et al., 1982).

The chronic effects of Dox treatment were subsequently studied. Both the results of the quick release protocol and the slack-test showed that chronic Dox treatment in rats results in a significant decrease of the crossbridge cycling rate in isolated skinned trabeculae. Assuming three ensembles of crossbridge states [detached, prepower-stroke (weakly bound), and force-generating crossbridges (strongly bound)], we conclude that: 1) conformational changes in the strongly bound crossbridges are impaired as measured by τ_1; 2) the net uncoupling of crossbridges resulting in a decrease of the isometric tension is impaired, as reflected by τ_2; and 3) the net coupling of crossbridges resulting in a slow increase in isometric tension is impaired, as measured by τ_3. Results of the slack-test strengthen these observations. V_0 was significantly decreased and τ_r was significantly increased upon chronic Dox indicating that the turnover rate of cycling crossbridges was significantly decreased. In addition, chronic Dox treatment resulted in a significant decrease of the maximal Ca^{2+} activated isometric tension level. The passive stiffness of trabeculae after chronic remained unaffected in the present study. In an earlier study we showed that the decrease of the maximal tension could not be explained by cell loss, myofibrillar loss, or the formation of connective tissue (Bottone et al., 1998). In addition, evidence was provided that the impairment of the isometric tension response is drug-related and cannot be ascribed to the loss of body weight associated with Dox treatment. Taken together, we conclude that chronic Dox treatment significantly increases transition times between ensembles of crossbridges, which results in an overall lower rate of crossbridge cycling.

To elucidate the mechanism behind the impaired crossbridge kinetics upon chronic Dox treatment, we focused on changes in the MHC isoform composition in ventricular tissue. The rat heart expresses two types of MHCs, the high ATPase α-MHC and the low ATPase β-MHC. The contractile velocity of the heart correlates with the relative amount of each MHC (Ebrecht et al., 1982; Holubarsh et al., 1985). Chronic Dox treatment induced a shift toward a β-MHC composition in the ventricles, in agreement with the findings

TABLE 1

Maximal Ca^{2+}-activated tension T_{max} (kN/m^2) of skinned trabeculae (A), time constants describing tension recovery after a quick release step of 0.5% in maximally Ca^{2+}-activated preparations (B), and unloaded shortening velocity V_0 (μm/s) and rate of force redevelopment (τ_r, ms) after the slack at a length step of 10% (C). Control values were obtained after stabilization for 30 min in control relaxation solution. The effect of Dox was measured after 30-min incubation in relaxation solution containing 20 μM Dox. Initial values before stabilization were: τ_1, 10 ± 1; τ_2, 65 ± 6; and τ_3, 92 ± 7, n = 14. Initial values of V_0 before stabilization were 8.6 ± 0.6 (pCa 4.0) and 10.9 ± 1.0 (pCa 6.0), n = 15; initial values of τ_r before stabilization were 67 ± 4 (pCa 4.0) and 63 ± 8 (pCa 6.0), n = 13. The effect of chronic Dox treatment was measured 4 weeks after the last Dox infusion.

		pCa	Control	Dox Acute	Dox Chronic
A	T_{max}	4.0	75 ± 5	93 ± 7*	62 ± 4*
B	τ_1	4.0	10 ± 1	11 ± 1	14 ± 1*
	τ_2	4.0	47 ± 7	59 ± 6	82 ± 6*
	τ_3	4.0	82 ± 8	89 ± 14	293 ± 67*
C	V_0	4.0	9.3 ± 1.1	7.7 ± 0.7*	6.8 ± 0.3*
		6.0	9.6 ± 1.7	8.8 ± 0.7	6.7 ± 0.6*
	τ_r	4.0	56 ± 4	65 ± 3*	89 ± 3*
		6.0	61 ± 7	61 ± 5	106 ± 11*

* $P < .05$ (ANOVA).

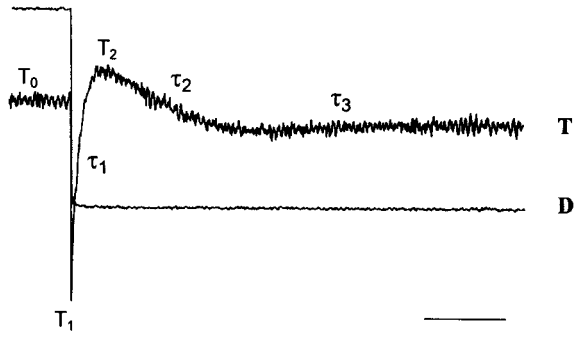


Fig. 2. Typical tension transient (T) of a cardiac trabecula resulting from a step release of 0.5% of the muscle length at pCa 4.0. The tension transient was fitted with a sum of three exponential functions, yielding the rate constants τ_1, τ_2, and τ_3, together with two extreme tension values, T_1 and T_2. T_0 is the maximal isometric tension level. D is the displacement signal. Calibration, 100 ms.

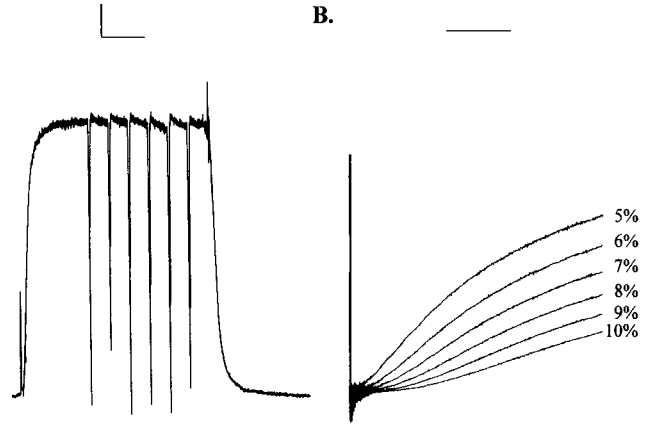


Fig. 3. Typical recording a of slack-test protocol obtained during maximal contraction in a trabecula. A, tension traces resulting from step releases with amplitudes ranging from 5to 10%, respectively. B, superimposed tension traces at a higher magnification, showing the point at which tension redevelops after each step release. Calibration: A, horizontal 15 s, vertical 10 kN/m^2; B, horizontal 20 ms.

of other studies (Cappelli et al., 1989). Because of the clear correlation between the decreased contractile performance and the relative increase of the expression of β-MHC, the contractile alterations in the present study may be explained by a Dox-induced change in myosin isoform composition in ventricular myocardium.

In summary, evidence is provided that acute Dox exposure leads to a shift in the population of cycling crossbridges toward the strongly bound states. The decreased rate of relaxation upon acute Dox exposure leads to the higher isometric tension response. In contrast, chronic treatment with Dox results in an overall lower crossbridge cycling rate accompanied by a higher relative expression of the β-MHC isoform in ventricular muscle. Both the attachment and detachment rate of crossbridges are impaired, which accounts for the overall lower isometric tension response. Our present results raise the possibility that preventing perturbations in crossbridge kinetics may be a novel approach treat anthracycline-induced cardiomyopathy. The clinical relevance of our findings is supported by our preliminary experiments in rats that show that the drug dexrazoxane suppresses both the transition of α-HMC to the β-isoform and the Dox-induced changes in crossbridge kinetics (Bottone, 1999).

References

Asayama J, Yamahara Y, Tatsumi T, Miyazaki H, Inoue M, Omori I, Inoue D and Nakagawa M (1992) Acute effects of doxorubicin on skinned cardiac muscle fibers of guinea pigs. *Cardiovasc Res* **26:**371–375.

Bottone AE (1999) Doxorubicin and cardiac contractile function. Febodruk, Enschede. Ph.D. Thesis, Utrecht University. ISBN:90–393-1589–2.

Bottone AE, de Beer EL and Voest EE (1997) Anthracyclines enhance tension development in cardiac muscle by direct interaction with the contractile system. *J Mol Cell Cardiol* **29:**1001–1008.

Bottone AE, Voest EE and de Beer EL (1998) Impairment of the actin-myosin interaction in permeabilized cardiac trabeculae after chronic doxorubicin treatment. *Clin Cancer Res* **4:**1031–1037.

Boucek RJ Jr, Olson DE, Brenner EM, Ogunbumni M, Inui M and Fleischer S (1987) The major metabolite of doxorubicin is a potent inhibitor of membrane-associated ion pumps: A correlative study of cardiac muscle with isolated membrane fractions. *J Biol Chem* **262:**15851–15856.

Brenner B (1988) Effect of Ca^{2+} on cross-bridge turnover kinetics in skinned single rabbit psoas fibers: Implications for regulation of muscle contraction. *Proc Natl Acad Sci USA* **85:**3265–3269.

Cappelli V, Moggio R, Monti E, Paracchini L, Piccinini F and Reggiani C (1989) Reduction of myofibrillar ATPase activity and isomyosin shift in delayed doxorubicin cardiotoxicity. *J Mol Cell Cardiol* **21:**93–101.

Cooke R (1997) Actomyosin interaction in striated muscle. *Physiol Rev* **77:**671–697.

de Beer EL, Finkle H, Voest EE, Van Heijst BGV and Schiereck P (1992) Doxorubicin interacts directly with skinned single skeletal muscle fibers. *Eur J Pharmacol* **214:**97–100.

De Groot IJM, Lamers WH and Moorman AFM (1989) Isomyosin expression patterns during rat heart morphogenesis: An immunohistochemical study. *Anat Rec* **224:** 365–373.

De Winkel ME, Blangé T and Treijtel BW (1993) The complex Young's modulus of skeletal muscle fibre segments in the high frequency range determined from tension transients. *J Muscle Res Cell Motil* **14:**302–310.

De Winkel ME, Blangé T and Treijtel BW (1995) Viscoelastic properties of cross bridges in cardiac muscle. *Am J Physiol* **268:**H987–H998.

Doroshow JH (1991) Doxorubicin-induced cardiac toxicity. *N Engl J Med* **324:**843–845.

Ebrecht G, Rupp H and Jacob R (1982) Alterations of mechanical parameters in chemically skinned preparations of rat myocardium as a function of isoenzyme pattern of myosin. *Basic Res Cardiol* **77:**220–234.

Edman KAP (1979) The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibers. *J Physiol (Lond)* **291:**143–159.

Eisenberg E and Hill TL (1985) Muscle contraction and free energy transduction in biological systems. *Science (Wash DC)* **227:**999–1006.

Giulian GG, Moss RL and Greaser ML (1983) Improved methodology for analysis and quantitation of proteins on one-dimensional silver-stained slab gels. *Anal Biochem* **129:**277–287.

Goormaghtigh E and Ruysschaert JM (1984) Anthracycline glycoside-membrane interactions. *Biochim Biophys Acta* **779:**271–288.

Holubarsch H, Goulette RP, Litten RZ, Martin BJ, Mulieri LA and Alpert NR (1985) The economy of isometric force development, myosin isoenzyme pattern and myofibrillar ATPase activity in normal and hypothyroid rat myocardium. *Circ Res* **56:**78–86.

Huxley AF and Simmons RM (1971) Proposed mechanism of force generation in striated muscle. *Nature (Lond)* **233:**533–538.

Julian FJ, Sollins KR and Sollins MR (1974) A model for the transient and steady-state mechanical behaviour of contracting muscle. *Biophys J* **14:**546–562.

Jung DWG, Blangé T, De Graaf H and Treijtel BW (1988) Elastic properties of relaxed, activated and rigor muscle fibres measured with microsecond time resolution. *Biophys J* **54:**897–908.

Lewis W, Kleinerman J and Puszkin S (1982) Interaction of adriamycin in vitro with cardiac myofibrillar proteins. *Circ Res* **50:**547–553.

Lymn RW and Taylor EW (1971) Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry* **10:**4617–4626.

Mulieri LA, Hasenfuss G, Ittleman F, Blanchard EM and Alpert NR (1989) Protection of human left ventricular myocardium from cutting injury with 2,3 butanedione monoxime. *Circ Res* **65:**1441–1444.

Singal PK, Deally CMR and Weinberg LE (1987) Subcellular effects of adriamycin in the heart: A concise review. *J Mol Cell Cardiol* **19:**817–828.

Van der Velden J, Klein LJ, Van der Bijl M, Huybregts MAJM, Stooker W, Witkop J, Eijsman L, Visser CA, Visser FC and Stienen GJM (1998) Force production in mechanically isolated cardiac myocytes from human ventricular muscle tissue. *Cardiovasc Res* **38:**414–423.

Send reprint requests to: Dr. E. L. de Beer, Department of Medical Physiology and Sports Medicine, Utrecht University, P.O. Box 80043, 3508 TA Utrecht, the Netherlands. E-mail: e.l.debeer@med.uu.nl