ABSTRACT

The mechanisms of the inotropic effect of mitoxantrone (MTO), a synthetic dihydroxyanthracenedione derivative with antineoplastic activity, were investigated in guinea pig ventricular myocytes using whole-cell patch-clamp methods combined with fura-2 fluorescence and cell-edge tracking techniques. In right ventricular papillary muscles, 30 μM MTO increased isometric force of contraction as well as action potential duration (APD) in a time-dependent manner. The force of contraction was increased approximately 3-fold within 4 h. This positive inotropic effect was accompanied by a prolongation of time to peak force and relaxation time. In current-clamped single myocytes treated with 30 μM MTO for 30 min, an increase of cell shortening by 77% and a prolongation of APD by 19% was observed. Peak amplitude of the intracellular Ca2+ transients was also increased by 10%. The contribution of APD prolongation to the enhancement of cell shortening induced by MTO was assessed by clamping control myocytes with action potentials of various duration. Prolongation of ADP 90 (ADP measured at 90% of repolarization) by 24% led to an increase of cell shortening by 13%. When the cells were clamped by an action potential with constant APD, MTO still caused an increase of cell shortening by 59% within 30 min. No increase of the peak intracellular Ca2+ transients, however, was observed under this condition. We conclude that both the APD prolongation and a direct interaction with the contractile proteins contributed to the positive inotropic effect of MTO.

Mitoxantrone (MTO) is a synthetic dihydroxyanthracenedione derivative (Fig. 1) that possesses antineoplastic activity both in animal and in vitro test systems. Structurally similar to anthracyclines, MTO was developed as a replacement for doxorubicin to circumvent the cardiotoxic effects of anthracyclines. Clinically, MTO, applied either as a single agent or as a component of combination treatment regimens, has shown therapeutic efficacy in the treatment of a wide range of solid tumors such as breast cancer and hematological malignancies (Faulds et al., 1991). MTO has demonstrated considerably better tolerability than anthracycline drugs, but contrary to what was initially thought, acute and delayed adverse cardiac effects have been described in MTO recipients, particularly in those previously exposed to anthracyclines or presenting cardiovascular diseases (Faulds et al., 1991; Wiseman and Spencer, 1997). Described cardiac effects include decreases in left ventricular ejection fraction, congestive heart failure, and, in some cases, dysrhythmias. Comparative studies of doxorubicin and MTO in chronic cardiotoxicity animal models have shown remarkable qualitative similarities between both compounds, although ultrastructural changes induced by MTO were generally less severe when compared with doxorubicin. Changes in the cardiac myocytes of MTO-treated animals consisted of dilatation of the sarcoplasmic reticulum (SR), moderate to marked myofibrillar loss, and focal mitochondrial swelling (Alderton et al., 1992; Herman et al., 1997). Distension of the t-tubular system of the SR was also the earliest morphological change observed in myocardial biopsies from patients receiving anthracyclines (Unverferth et al., 1983). There is compelling evidence that, in both the acute and chronic cardiotoxicity, anthracyclines alter the function and the density of cardiac ryanodine receptors that constitute the Ca2+ release channel of junctional SR (Pessah et al., 1992; Dodd et al., 1993; Boucek et al., 1997). When the acute effects of doxorubicin were investigated in functionally intact guinea pig cardiomyocytes, a decreased Ca2+-induced Ca2+ release from cardiac SR was observed (Wang and Korth, 1995). This effect was comparable to that of ryanodine, which at submicromolar concentrations locks the Ca2+ release channel of the SR in an open state, facilitating spontaneous SR Ca2+ release. Several lines of evidence indicate that MTO also interferes with SR Ca2+ release channels. In skeletal muscle, MTO is a potent stimulator of Ca2+ release from SR membrane vesicles (Abramson et al., 1988), whereas the open probability of sheep cardiac Ca2+ release channels incorpor-
rated in bilayer lipid membranes was variably influenced (Holmberg and Williams, 1990). Little intrinsic activity toward activation of Ca\(^{2+}\) release channels was found with MTO in rat cardiac SR preparations (Kim et al., 1994). When assessed in the presence of doxorubicin, however, MTO behaved like a competitive antagonist of the doxorubicin-modified channel. Despite deprivation of the ability of the SR to retain Ca\(^{2+}\), doxorubicin produced a slowly developing positive inotropic effect, which in part may have been due to the pronounced prolongation of action potential duration (APD) (Wang and Korth, 1995). Recently, MTO was also shown to prolong APD in guinea pig cardiomyocytes by inhibition of the inward and the delayed rectifier K\(^+\) channels (Wang et al., 1999). Taken together, MTO and doxorubicin seem to share some important cardiac effects, which are possibly due to the common anthraquinone moiety of both drugs.

Because information about acute effects on cardiac contractility is still lacking, we investigated in this study the influence of MTO on excitation-contraction coupling in single cardiomyocytes and in multicellular preparations. We show that MTO produces a positive inotropic effect that involves mechanisms dependent on and independent of an increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)).

**Materials and Methods**

**Chemicals.** MTO hydrochloride (batch 331110) was kindly provided by Lederle GmbH (Münster, Germany) and was dissolved in distilled water to give a 30 mM stock solution. Appropriate concentrations of this stock solution were added to the bath solution just before use to achieve final concentrations. Tetrodotoxin (TTX) and amphotericin B were obtained from Sigma (Deisenhofen, Germany). Fura-2 acetoxymethylester and ionicomycin were obtained from Calbiochem (La Jolla, CA).

**Multicellular Preparations.** Guinea pigs of either sex weighing 250 to 350 g were anesthetized with halothane and subsequently sacrificed by cervical dislocation. Right ventricular papillary muscles (diameter, 0.5–0.8 mm) were rapidly excised from the isolated heart and minced in a two-chambered organ bath with internal circulation of the bath solution (volume, 50 ml). The bath solution was constantly gassed and kept in circulation by 5% CO\(_2\) in O\(_2\); the pH was 7.4. After perfusion, the heart was minced and homogenized, and the resulting homogenate was used by means of amphotericin B. The compound was dissolved in 1% BSA and kept for use at room temperature under a continuous flow of O\(_2\). The homogenate was centrifuged at 37,000 × g for 3 min and the supernatant solution containing 1% BSA and bicarbonate was transferred to a 37°C water bath. A drop of cell suspension was added to Tyrode’s solution in the recording chamber (volume, 0.5 ml) mounted on an inverted microscope (Axiovert 10; Carl Zeiss, Jena, Germany). The Tyrode’s solution contained 138 mM NaCl, 1.2 mM MgSO\(_4\), 2 mM CaCl\(_2\), 5 mM KCl, 10 mM glucose, 5 mM HEPES; the pH was 7.4. After the cells had attached to the bottom, the bath was perfused at a flow rate of 4 ml/min with prewarmed Tyrode’s solution gassed continuously with O\(_2\). The temperature in the bath (34–35°C) was continuously monitored.

**Measurement of Contractility and Action Potential.** The papillary muscles were stimulated at their base through two puncate platinum electrodes with square-wave pulses of 2 ms in duration and an intensity slightly above threshold. To suppress the stimulation-evoked release of endogenous catecholamines, all experiments were performed in the presence of 20 nM TTX, which blocks Na\(^+\) channels of nerve cells (the exocytotic release of endogenous catecholamines depends on the depolarization of sympathetic nerve endings in the heart) but has almost no effects on the electrical and mechanical properties of myocardial cells. The force of contraction was measured isometrically by means of an inductive force transducer (Q-11, 10p; Hottinger Baldwin Meßtechnik, Germany) connected to an oscilloscope and a digital audio tape recorder (DAT-recorder DTR-1202; Bio-Logic, Claix, France). The resting force was kept constant at 4 mN throughout the experiment. An equilibration period of at least 1 h at a stimulation frequency of 1 Hz preceded each experiment. Subsequently, the frequency of stimulation was lowered to 0.5 Hz, and the drug intervention was started as soon as the force of contraction had reached a steady state. The following parameters of the isometric contraction were evaluated: peak force of contraction, time to peak force, and relaxation time (measured at 90% of relaxation).

Transmembrane electrical activity was recorded with conventional glass microelectrodes that were filled with 3 M KCl and that had tip resistances of 10 to 20 MΩ. Transmembrane potentials were measured by means of an electrometer amplifier (model 773; World Precision Instruments, New Haven, CT), stored on a DAT-recorder (DTR-1202; Bio-Logic), and evaluated subsequently by a computer. The maximum rate of rise of the action potential (V\(_{max}\)) was obtained by an electronic differentiator with linear differentiation in the range 0 to 1000 V/s. Only experiments with microelectrode impalements lasting throughout the entire experimental period were accepted for evaluation.

**Single-Cell Isolation.** Isolated myocytes were prepared from ventricles of adult guinea pigs by enzymatic dissociation according to Powell et al. (1980) with small modifications. Briefly, the heart was perfused retrogradely at 37°C and at a constant rate of 10 ml/min with the following solutions: 5 min with a nominally Ca\(^{2+}\)-free Joklik solution (Joklik-MEM; Biochrom, Berlin, Germany) supplemented with NaHCO\(_3\), and then 5 to 10 min with the same solution to which 50 μM CaCl\(_2\), collagenase (Worthington type II, 25 mg/50 ml; Biochrom), protease (type XIV, 10 mg/50 ml; Sigma), and 0.1% BSA (fraction V, Sigma) had been added. All solutions were gassed with 5% CO\(_2\) in O\(_2\); the pH was 7.4. After perfusion, the heart was minced and incubated for 5 min in fresh enzyme solution. Then the cells were disaggregated by gentle mechanical agitation. After filtration through a nylon mesh, the cells were centrifuged at 37°C for 3 min and then resuspended in modified Krebs-Henseleit solution containing 1% BSA and kept for use at room temperature under a continuous stream of 5% CO\(_2\) in O\(_2\).

**Whole-Cell Patch-Clamp.** A cell of suspension was added to Tyrode’s solution in the recording chamber (volume, 0.5 ml) mounted on an inverted microscope (Axiovert 10; Carl Zeiss, Jena, Germany). The Tyrode’s solution contained 138 mM NaCl, 1.2 mM MgSO\(_4\), 2 mM CaCl\(_2\), 5 mM KCl, 10 mM glucose, 5 mM HEPES; the pH was 7.4. After the cells had attached to the bottom, the bath was perfused at a flow rate of 4 ml/min with prewarmed Tyrode’s solution gassed continuously with O\(_2\). The temperature in the bath (34–35°C) was continuously monitored. Myocytes were stimulated at 0.5 Hz by either current clamp or action potential clamp (AP clamp) in the whole-cell patch-clamp configuration (Hamill et al., 1981). For AP clamp, action potentials were recorded from a typical cell in the current-clamp mode and stored in a computer. These action potentials served as voltage commands to clamp other cells and to elicit contractions or Ca\(^{2+}\) transients. Patch electrodes were fabri cated from borosilicate glass capillaries (World Precision Instruments) and filled with a filtered solution containing 125 mM KCl, 5 mM MgSO\(_4\), 5 mM NaCl, 5 mM K\(_2\)ATP, 5 mM HEPES, adjusted to pH 7.3 by adding KOH. The resistance of the electrodes ranged from 1.5 to 3 MΩ. In some experiments, perforated patch-clamp technique was used by means of amphotericin B. The compound was dissolved in electrode-filling solution to reach a final concentration of 300 μg/ml. The whole-cell clamp was achieved by the use of a patch clamp amplifier (EPC7; List Medical Electronics, Darmstadt, Germany) connected via a 16-bit analog/digital interface to a pentium IBM clone computer. The cell capacitance and series resistance were compensated. Sampling rate for action potentials under current
clamp was 2 kHz. Data acquisition and analysis was performed with an ISO-3 multitasking patch-clamp program (MFK, Niedernhausen, Germany).

**Cell Shortening.** Cell length was monitored using a stable light source (Gossen-Konstanter, Erlangen, Germany) to form a bright field image of the cell, which was projected onto a photodiode array (Laser 2000, Welfling, Germany) with a 4-ms scan rate, and changes in cell length during contraction were quantified via edge tracking. The signal was then transmitted to a computer for on-line analysis. Peak shortening, time to peak shortening, relaxation time, and shortening duration (measured at 80% of relaxation) were evaluated.

**Ca\(^{2+}\) Transients.** Myocytes were added to the recording chamber mounted on an inverted microscope adapted for epifluorescence measurement. Single cells were loaded for 5 to 10 min via the patch electrode filled with 30 μM the Ca\(^{2+}\)-sensitive dye fura-2 pentasodium salt dissolved in the electrode filling solution. The dye was alternately (200 Hz) excited at 340 and 380 nm wavelengths of light generated by a Deltascan illumination system (Photon Technology International, Brunswick, NJ). Emission fluorescence at 510 nm was generated by a Deltascan illumination system (Photon Technology International, Brunswick, NJ). Emission fluorescence at 510 nm was detected with a photon-counting photomultiplier tube. Autofluorescence of control myocytes did not vary during the experiment.

The signal was then transmitted to a computer for on-line analysis. Peak shortening, time to peak force and relaxation time were determined by superimposing the original recordings from a typical experiment. Maximal force was expressed as a percentage of the predrug control value after 1 h (control), and cell shortening was expressed as a percentage of the maximal cell shortening induced by MTO in guinea pig papillary muscle. Cell length was monitored using a stable light source (Gossen-Konstanter, Erlangen, Germany) to form a bright field image of the cell, which was projected onto a photodiode array (Laser 2000, Welfling, Germany) with a 4-ms scan rate, and changes in cell length during contraction were quantified via edge tracking. The signal was then transmitted to a computer for on-line analysis. Peak shortening, time to peak shortening, relaxation time, and shortening duration (measured at 80% of relaxation) were evaluated.

**Statistics.** Where appropriate, results are presented as means ± S.E. Significance tests were performed using Student's *t* test for paired observations. Differences between means were regarded statistically significant at *P* < .05.

**Results**

**Inotropic Effects and APD in Papillary Muscles.** The anthracenedione derivative MTO produced a concentration-dependent positive inotropic effect in isometrically contracting guinea pig papillary muscles (Fig. 2). The positive inotropic effect developed slowly over several hours (Fig. 2A). With 30 μM MTO, the increase in force of contraction became visible after a latency of 30 min and was significant compared with the predrug control value after 1 h (*P* < .05). After 4 h, force had increased approximately 3-fold from 1.4 ± 0.2 (control) to 4.1 ± 0.5 mN (n = 8; Fig. 2C). When 10 μM MTO was applied to the bath solution, the positive inotropic effect became significant only after 3 h, and after 4 h, force of contraction had increased by 58% from 1.2 ± 0.1 (control) to 1.9 ± 0.3 mN (n = 5; Fig. 2C). Similar positive inotropic effects were observed when the muscles had been pretreated for 30 min with 10 μM TTX (data not shown). Experiments carried out in the absence of MTO showed a continuous decline in the force of contraction over time. After 4 h, force had decreased to 81.2 ± 5.5% of the control level (n = 6). The MTO-induced increase in force of contraction was accompanied by a marked prolongation of contraction duration. As shown in Fig. 2, A and D, this effect was due to a prolongation of time to peak force and of relaxation time. Both time parameters were significantly prolonged after 1 h of incubation with 30 μM MTO (*P* < .05). In eight papillary muscles, time to peak force and relaxation time increased within 4 h from 136 ± 5 and 119 ± 6 ms (control values) to 178 ± 5 (by 31%) and 175 ± 9 ms (by 47%), respectively. When papillary muscles were exposed to 30 μM MTO for more than 4 h, some preparations developed slowly rising contractures that usually terminated the positive inotropic effect.

As shown in Fig. 2B and substantiated in four other papillary muscles, 30 μM MTO produced a time-dependent prolongation of APD at all levels of repolarization. Three hours after the application of MTO, APD measured at 90% repolarization (APD\(_{90}\)) had increased from 223 ± 14 (control) to 295 ± 18 ms, i.e., by 32% (n = 5; *P* < .05). MTO had no significant effect on resting membrane potential, V\(_{\text{max}}\) and action potential amplitude. The values before (n = 5) and 3 h after the addition of 30 μM MTO were respectively −84 ± 1 and −85 ± 2 mV for the resting potential, 214 ± 19 and 203 ± 16 V/s for V\(_{\text{max}}\) and 120 ± 4 and 118 ± 3 mV for action potential amplitude.

**Cell Shortening and APD in Single Cells.** When the effects of MTO on cell shortening and action potential were investigated in isolated ventricular myocytes that had been current-clamped at 0.5 Hz, results similar to those obtained in papillary muscles were observed. Figure 3A shows superimposed original recordings from a typical experiment; superfusion of a cell with 30 μM MTO produced a time-dependent prolongation of APD accompanied by an enhancement and prolongation of cell shortening. APD\(_{90}\) increased from 221 ms (control) to 260 and 391 ms, whereas cell shortening increased from 9.2 μm (control) to 15.8 and 20.5 μm after 30 and 50 min, respectively. Simultaneously, time to peak shortening and relaxation time were prolonged from 128 and 124 ms (control) to 135 and 151 ms at 30 min and to 140 and 170 ms after 50 min. In nine cells in which the shortening could be followed continuously over 30 min, MTO induced a 77%
increase of cell shortening from 6.0 ± 0.6 (control) to 10.6 ± 1.1 μm (Fig. 3B). Shortening duration was prolonged by 24% from 263 ± 25 to 325 ± 29 ms (Fig. 3C). APD90 of these cells was prolonged by 19% from 254 ± 16 to 301 ± 19 ms. Shortening of control cells that were not exposed to MTO decreased slightly but significantly within 30 min from 7.2 ± 1.0 to 6.8 ± 0.9 μm (n = 7; Fig. 3B), whereas shortening duration was not affected (Fig. 3C).

To investigate the influence of APD on cell shortening, myocytes were AP-clamped at various APDs. Figure 4A (upper traces) shows two superimposed action potentials that were obtained from a cell before (trace a) and 1 h after superfusion with 30 μM MTO (trace b). Recorded action potentials served then as voltage commands to clamp another cell to elicit corresponding contractions (Fig. 4A, lower traces). It can be seen that a prolongation of APD90 from 258 (trace a) to 433 ms (trace b) was accompanied by an enhancement of cell shortening from 3.9 (trace a) to 6.1 μm (trace b). Shortening of the first contraction after APD prolongation was not changed but increased during the following contractions. Time to peak shortening was slightly decreased from 164 to 159 ms by APD prolongation, whereas relaxation followed a more complex time course. Relaxation developed an inflection so that a rapid relaxation phase that dominated approximately 75% of total cell shortening was followed by a tail that was markedly slowed by the prolonged APD. Figure 4B shows the relation between the percentage of increase of APD90 and the respective change in peak amplitude of cell shortening. The control values for APD90 and peak amplitude of cell shortening were 258 ms and 8.5 ± 1.4 μm, respectively.

To investigate whether MTO enhanced cell shortening independent of APD prolongation, ventricular myocytes were AP-clamped with an action potential of constant duration. In the experiment shown in Fig. 5A, a cell was AP-clamped at 0.5 Hz with an APD90 of 258 ms. Application of 30 μM MTO to the superfusing solution resulted in an increase in cell shortening from 6.4 μm (control) to 7.8 and 9.6 μm after 20 and 30 min, respectively. This increase was accompanied by a prolongation of time to peak shortening and of relaxation time from 158 and 108 ms (control) to 162 and 132 ms after 20 min and to 184 and 156 ms after 30 min, respectively. A summary of the results is shown in Fig. 5, B and C.

![Fig. 3. Effects of MTO on cell shortening and APD in current-clamped guinea pig ventricular myocytes. Stimulation frequency, 0.5 Hz. A, superimposed original recordings showing the time-dependent increase of APD (upper traces) and cell shortening (lower traces) in the presence of 30 μM MTO. Amphotericin B (300 μg/ml) was included in the electrode solution to permeabilize the membrane patch. The action potential and cell shortening were measured simultaneously in the same cell. B and C, summary of the changes in peak amplitude of cell shortening (B) and shortening duration (C) within 30 min either in the absence (Drug-free; n = 7) or in the presence of 30 μM MTO (n = 9). *P < .05; ***P < .001 versus the respective control.](image)

![Fig. 4. Increase of cell shortening induced by prolongation of APD in guinea pig ventricular myocytes clamped with action potentials of various duration. The action potentials had been recorded before from a myocyte at different times during its exposure to 30 μM MTO. These action potentials served then as voltage commands for other cells to trigger contraction at 0.5 Hz. A, superimposed original recordings showing two different commanding action potentials (upper traces) and the respective cell shortening (lower traces). The APD90 of the action potentials were 258 (a) and 433 ms (b). B, relation between percentage of increase of APD90 and the respective change in peak amplitude of cell shortening. The control values for APD90 and peak amplitude of cell shortening were 258 ms and 8.5 ± 1.4 μm, respectively.](image)
micromolar MTO enhanced the shortening of six cells by 59% from 7.1 ± 1.2 to 11.3 ± 1.2 μm and prolonged shortening duration by 22% from 252 ± 6 to 307 ± 12 ms after 30 min. In five control cells that were not exposed to MTO, cell shortening decreased significantly within 30 min by 10% from 7.2 ± 0.5 to 6.5 ± 0.3 μm (Fig. 5B), whereas shortening duration did not change.

APD and Ca2⁺ Transients. To determine the influence of APD and of MTO on intracellular Ca2⁺ transients, myocytes were AP-clamped with action potentials of various duration. The original recordings of Fig. 6A show action potentials (upper traces) that had been recorded before at different times from a myocyte exposed to 30 μM MTO. The recorded action potentials served then as voltage commands for another cell to elicit Ca2⁺ transients (lower traces). The recorded action potentials served then as voltage commands for another cell to elicit Ca2⁺ transients (lower traces). It can be seen that the stepwise prolongation of APD is accompanied by an increase in the peak of the respective Ca2⁺ transient. As in the contraction experiments, three to four action potentials were necessary to obtain a steady-state increase in the Ca2⁺ transient. Figure 6B summarizes the results obtained in six cells: prolongation of APD₉₀ by 24, 48, and 68% increased the peak of the Ca2⁺ transient by 11.3 ± 1.6, 21.3 ± 2.0, and 26.0 ± 2.6%, respectively. When a myocyte was current-clamped at 0.5 Hz and superfused with 30 μM MTO for 30 min, APD₉₀ increased by 26% from 258 ms and 670 ± 52 nM, respectively.

274 to 345 ms, and the simultaneously evoked Ca2⁺ transient rose by 13% from 656 to 741 nM (Fig. 7A). The summary of the results from six cells demonstrates a significant increase in peak [Ca2⁺]i, by 10% from 746 ± 97 to 822 ± 104 nM (Fig. 7A). In contrast, when a myocyte was AP-clamped with constant APD (APD₉₀ = 258 ms), 30 μM MTO produced within 30 min a small decline of peak [Ca2⁺]i from 788 to 734 nM (Fig. 7B). The combined data obtained from five cells showed a significant decrease in Ca2⁺ transients by 7.2% from 713 ± 52 to 662 ± 58 nM (Fig. 7C). In control cells that were not treated with MTO, Ca2⁺ transients decreased slightly within 30 min from 701 ± 67 to 657 ± 69 nM (by 6.3%, n = 5, P < .05) under current clamp and from 669 ± 93 to 631 ± 89 nM (by 5.7%, n = 4, P < .05) under AP clamp.

Discussion

The results reported in this article show that in guinea pig papillary muscles and in individual myocytes the anthracene-based antineoplastic agent MTO produced a gradual increase in force of contraction and cell shortening that was only in part due to an increase in the amplitude of the Ca2⁺ transients. The positive inotropic effect of MTO was accompanied by a progressive prolongation of APD, implying that both effects could be related to each other. The mechanism by which MTO prolonged APD has been recently elucidated and involves depression of
depolarizing steps has been described in cardiac myocytes of several species (Bridge et al., 1988; Bers and Bridge, 1989; Bers et al., 1990). It was proposed that the voltage-independent relaxation is probably due to rapid Ca\textsuperscript{2+} sequestration by the SR that increases with the cytosolic [Ca\textsuperscript{2+}]), whereas the voltage-dependent relaxation reflects the ceasing of the Ca\textsuperscript{2+} influx and the enhancement of Ca\textsuperscript{2+} extrusion via Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange. In comparison with control cells that were clamped with action potentials of various duration, myocytes exposed to MTO responded with a stronger shortening at a comparable APD accompanied by a prolongation of time to peak shortening and of relaxation time (Fig. 3). Evidence that the MTO-induced increase in cell shortening was in part independent of APD prolongation evolved from the finding that MTO enhanced the shortening of myocytes that were AP-clamped with a constant APD. In these cells, a significant prolongation of contraction duration was also observed. Mechanisms by which MTO could have increased force of contraction beyond the level of APD prolongation are an enhancement of the transmembrane Ca\textsuperscript{2+} current via voltage-gated L-type Ca\textsuperscript{2+} channels either by cyclic AMP accumulation or by direct modulation of channel gating and elevation of intracellular Na\textsuperscript{+} activity either by inhibiting Na\textsuperscript{+}-K\textsuperscript{+} pump or by increasing transmembrane Na\textsuperscript{+} influx (Varro and Papp, 1995). Drugs that increase intracellular cyclic AMP levels, such as \(\beta\)-adrenoceptor agonists or inhibitors of phosphodiesterase, enhance Ca\textsuperscript{2+} influx via L-type Ca\textsuperscript{2+} channels and stimulate the activity of the SR Ca\textsuperscript{2+} pump that becomes functionally effective as a shortening of relaxation time (Raffaeli et al., 1989; Brixius et al., 1997). The finding that MTO prolonged relaxation time and had no influence on L-type Ca\textsuperscript{2+} current (Wang et al., 1999) excludes cyclic AMP-dependent pathways and a direct channel modulation. In addition, the positive inotropic effect of cardioactive steroids that is due to inhibition of Na\textsuperscript{+}-K\textsuperscript{+} pump is not accompanied by a prolongation of contraction (Reiter, 1972). Prolongation of relaxation time accompanies the positive inotropic effect of many drugs and toxins that increase transmembrane Na\textsuperscript{+} influx (Honer-jäger, 1982; Buggisch et al., 1985). In contrast to the Na\textsuperscript{+} channel modulators, inotropic effects as well as APD prolongation of MTO were not influenced by the Na\textsuperscript{+} channel blocker TTX (this study and Wang et al., 1999). Recently, the anthracycline derivative doxorubicin, another highly effective antineoplastic agent, was demonstrated to produce a slowly developing positive inotropic effect in guinea pig papillary muscles (Wang and Korth, 1995). MTO and doxorubicin are anthraquione-based compounds, and there are similarities between both compounds, such as positive inotropic effect and APD prolongation, but also striking differences in their cardiac actions. Despite its strong positive inotropic effect, doxorubicin markedly reduced contraction velocity and prolonged the time to peak force. This effect resembles the action of ryanodine in cardiac cells (Lewartowski et al., 1990) and is due to an increase of the open probability of Ca\textsuperscript{2+} release channels in the SR (Nagasaki and Fleischer, 1989; Holmberg and Williams, 1990; Wang and Korth, 1995). Reports on the action of MTO on SR Ca\textsuperscript{2+} channels are conflicting (Abramson et al., 1988; Holmberg and Williams, 1990; Kim et al., 1994), but our experiment does not support a doxorubicin- or ryanodine-like action of MTO on SR function. The possibility, however, that the moderate prolongation of time to peak shortening is the manifestation of a weak effect of MTO on SR Ca\textsuperscript{2+} release channels cannot be completely ruled out. In any case, a ryanodine-like mechanism

Fig. 7. Effects of MTO on intracellular Ca\textsuperscript{2+} transients in guinea pig ventricular myocytes under current clamp or AP clamp. The Ca\textsuperscript{2+} transients were measured by loading the cells with 30 \(\mu\)M fura-2 pentasodium salt through the patch pipette. A, superimposed original recordings showing simultaneous increase of APD (upper traces) and Ca\textsuperscript{2+} transients (lower traces) 30 min after superfusing a current-clamped myocyte with 30 \(\mu\)M MTO. The stimulation frequency was 0.5 Hz. B, original recordings showing the effect of 30 \(\mu\)M MTO (30 min) on Ca\textsuperscript{2+} transients (lower traces) in a cell clamped with a constant APD (upper trace) at 0.5 Hz. The commanding action potential (APD\textsubscript{90} = 258 ms) had been recorded before from a different myocyte. C, summary of the effects of 30 \(\mu\)M MTO (30 min) on peak Ca\textsuperscript{2+} transients under either current clamp (n = 6) or constant AP clamp (n = 5). ** P < .01 versus the respective control.
should rather impair SR Ca\(^{2+}\) load and hence contraction. In a recent study using rat skinned cardiac fibers, doxorubicin and other anticholines were shown to increase tension by direct interaction with the force-generating filaments (Bottone et al., 1997). In contrast, no direct effect of doxorubicin on the Ca\(^{2+}\) sensitivity of the myofilaments could be demonstrated in membrane-permeabilized cardiac fibers of rabbit (Boucek et al., 1997). During the last decade, drugs have been discovered that increase force of contraction by an increase in the Ca\(^{2+}\) sensitivity of the myofilaments proteins rather than by elevation of [Ca\(^{2+}\)]. Many of these Ca\(^{2+}\)-sensitizing drugs are characterized by a positive inotropic effect associated with an increase in time course of contraction and a reduction in the amplitude of the Ca\(^{2+}\) transient (Blinks and Endoh, 1986; Lee and Allen, 1991; White et al., 1993). When MTO was applied to fura-2-loaded and current-clamped myocytes, a small but significant rise (10%) of the peak Ca\(^{2+}\) transient was observed after 30 min. This rise in [Ca\(^{2+}\)] must have been due to APD prolongation because MTO-naive myocytes, when AP-clamped with the same prolonged APD that was recorded before in a current-clamped cell, showed a similar extent of increase of peak Ca\(^{2+}\) transients. However, when MTO was applied to cells that were AP-clamped with a constant APD, a decrease instead of an increase in contraction force seems to be irrele-

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