Direct Myocardial Depressant Effect of Methylmethacrylate Monomer

Mechanical and Electrophysiologic Actions in vitro

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Background: The present study explored the mechanism of direct myocardial depression by methylmethacrylate monomer (MMA).

Methods: Isometric contraction of isolated guinea pig right ventricular papillary muscle was measured in modified normal and 26 mM K⁺ Tyrode solutions at various stimulation rates. Normal and slow action potentials were evaluated by conventional microelectrode technique. MMA effects on various aspects of sarcoplasmic reticulum function were evaluated by its effect on rapid-cooling contractures, restated-state contraction in rat papillary muscle in modified normal Tyrode solution, and in guinea pig papillary muscle under low Na⁺ (25 mM) Tyrode solution. Whole cell patch clamp techniques were applied to measure the inward Ca²⁺ currents (IₐCa).

Results: MMA (0.5, 1.5, and 4.7 mM) caused concentration-dependent depression of peak force and maximal rate of force development by approximately 70%, 50%, and 20% of baseline from rested state to 3 Hz stimulation rates, respectively. Depression of peak force and maximal rate of force development by MMA was dependent on stimulation frequency, with less depression at higher stimulation rates. In low Na⁺ Tyrode solution, 1.5 mM MMA depressed peak force of rat and guinea pig myocardium by 20–30%. In 26 mM K⁺ Tyrode solution, 0.5 and 1.5 mM MMA caused selective and marked concentration-dependent depression of late force development (0.5 mM: approximately 60% of baseline, 1.5 mM: approximately 30% of baseline) with no alteration in early force development. MMA (1.5 mM) depressed rapid-cooling contracture to 53 ± 10% of baseline, accompanied by approximately 63% prolongation of time to peak contracture. In patch clamp studies, MMA reduced IₐCa in a concentration-dependent manner. In isolated vascular smooth muscle in rabbit, 5 and in rat uterine preparation, 14 the exact mechanisms that contribute to direct myocardial depression have not yet been clearly defined. The present study was undertaken to elucidate the mechanism of action of MMA on direct myocardial depression using a variety of inotropic and electrophysiologic interventions.

Materials and Methods

Effects on Contractility

Experiment with Modified Normal Tyrode Solution. Following a procedure approved by the Yonsei University College of Medicine Animal Research Committee, the heart was removed from female guinea pigs (400–450 g) or Sprague–Dawley rats (400–450 g) after inhalation anesthesia with halothane 2.5–4 vol%. The right ventricular papillary muscles were excised, mounted horizontally in a tissue bath, and superfused (8 ml/min) at 37°C with modified normal Tyrode solution (mM: 143 Na⁺, 5 K⁺, 2 Ca²⁺, 127 Cl⁻, 1.2 MgSO₄, 25 HCO₃⁻, 11 glucose, 0.10 EDTA). EDTA was used to chelate trace contaminant heavy metals. Solution was circulated through the bath from unsealed reservoirs through which 95% O₂ and 5% CO₂ was bubbled (flow rate: 500 ml/min) maintaining pH at 7.4 ± 0.5. The tendinous end of the papillary muscle was attached to a Grass FT03 force transducer (GRASS Instruments, Quincy, MA) while the other end of the muscle was pinned to the bottom of the tissue bath. Muscle length was adjusted to the lowest resting force at which maximum twitch force was obtained. The muscles were stimulated at 0.5 Hz for 60 min for stabilization. The muscles were field-stimulated by a GRASS S44 stimulator (GRASS Instruments) at an intensity of approximately 120% of exposure to MMA, transient systemic hypotension can occur in anesthetized patients within seconds or minutes1,2 and, occasionally, can progress to cardiovascular collapse, cardiac arrest, and death.3,4 Several mechanisms have been proposed as causes of this hemodynamic effect, including direct vasodilatation,5 direct myocardial depression,6,7 pulmonary embolization of bone marrow fat,8 air,9 platelet aggregates,10 and complement activation with release of anaphylatoxins.11

Direct myocardial depressant action of MMA has been proposed and demonstrated in humans12 and in vivo7 and in vitro6,13 animal studies. Although indirect evidence exists for inhibition of Ca²⁺ entry as a cause of contractile depression in isolated rat and rabbit atrium,6 in isolated vascular smooth muscle in rabbit,9 and in rat uterine preparation,14 the exact mechanisms that contribute to direct myocardial depression have not yet been clearly defined. The present study was undertaken to elucidate the mechanism of action of MMA on direct myocardial depression using a variety of inotropic and electrophysiologic interventions.

METHYL METHACRYLATE monomer (MMA), an aromatic volatile liquid, is one component of acrylic bone cements and has been employed in orthopedic surgical procedures to anchor prosthetic devices to bone.
the lowest level that elicited a response. After a 15-min rest, a rested-state (RS) contraction was elicited followed by stimulation rates of 0.1, 0.5, 1, 2, and 3 Hz sequentially. At each stimulation rate except RS, duration of stimulation was preset and kept constant during the experiment. Following baseline measurements, muscles were exposed to each calculated concentration of MMA (3, 10, and 30 mM) for 15 min successively before the recording of responses. A 15 min application of MMA was sufficient to produce a stable and consistent effect in pilot experiments. Concentrations were referenced by an in vitro vascular smooth muscle study and determined by preliminary experiments on contractile force in modified normal Tyrode solution. Recovery responses were measured after washout for 20 min. Effects on contractile function are presented as peak force (PF) and maximal rate of force development (dF/dt-max).

After each experiment, the muscle cross-sectional area was estimated from muscle length, weight, and density (1.04 g/ml), assuming a cylindrical form. Mean cross-sectional areas were 0.83 ± 0.32 mm² (n = 32), ranged from 0.24 to 1.46 mm², and 0.55 ± 0.38 mm² (n = 8), ranged from 0.14 to 1.22 mm², in guinea pigs and rats, respectively. Thirteen muscles were used for action potential (AP) experiments and 21 myocytes were used for electrophysiologic measurement.

To evaluate MMA effects on Ca²⁺ release by rapid depolarization from the sarcoplasmic reticulum (SR), rat papillary muscles were used. Following baseline measurements at RS, muscles were exposed to 10 mM MMA for 15 min (unstimulated), followed by muscle stimulation at 0.1, 1, and 2 Hz stimulation rates, yielding a stable contraction. The duration of stimulation at each stimulation rate was preset and kept during the experiment. As in the control setting, rapid cooling was applied immediately after cessation of stimulation. The isometric force and bath temperature were continuously recorded during the experiments, and recovery responses were observed following washout for 20 min.

**Experiment with Low Na⁺ (25 mM) Tyrode Solution.** Experiments using guinea pig papillary muscles with low Na⁺ (25 mM) Tyrode solution (mM: 25 Na⁺, 5K⁺, 9Cl⁻, 2 Ca²⁺, 1.2 MgSO₄, 25 HCO₃⁻, 11 glucose, 0.1 EDTA, 234 sucrose) were performed to confirm actions in rat force studies and to rule out the species-dependent differences in SR Ca²⁺ release. Because Ca²⁺ extrusion via the Na-Ca exchange is markedly reduced by low extracellular Na⁺ concentration, Ca²⁺ is accumulated in SR during rest and causes enhanced contraction at RS and low-stimulation rates. The contraction in low Na⁺ Tyrode solution does not require a large inward Ca²⁺ current (I_{Ca}), as evidenced by the fact that its inhibition by nifedipine in this preparation does not alter the contractile force. After obtaining baseline measurements at RS after 15 min rest, muscles were exposed to 10 mM MMA for 15 min. Recovery responses were measured following washout for 20 min.

**Experiment with 26 mM K⁺ Tyrode Solution.** Guinea pig papillary muscles were used to observe the effect on biphasic contraction and slow AP, which can be observed in 26 mM K⁺ Tyrode solution (mM: 122 Na⁺, 26K⁺, 121 Cl⁻, 2 Ca²⁺, 1.2 MgSO₄, 25 HCO₃⁻, 11 glucose, 0.1 EDTA), which causes partial depolarization to −40 to −45 mV and inactivates fast sodium channels. Concurrent application of 0.1 μM isoproterenol results in an increase in open probability of Ca²⁺ channels via the β-adrenergic pathway to permit the measurement of slow APs. Under these conditions, contractility, inward Ca²⁺ current, and Ca²⁺ uptake into the SR are enhanced. The pattern of force after rest and up to 0.5–1 Hz stimulation rate consistently demonstrated a distinct early and late phase of force development (dF/dt-max and dF/dt-max, respectively). While the early peak of the biphasic contraction elicited under a variety of conditions in various species is activated by Ca²⁺ available for release. The late PF is inhibited by inhibitors of Ca²⁺ entry such as nifedipine and Mn²⁺. In contrast, ryanodine decreases the initial force development by putting the Ca²⁺ channel in a low conductance state and decreasing SR Ca²⁺ available for release. Increasing concentrations of MMA (3 and 10 mM) were applied sequentially in contractile force experiments. Recovery responses were measured following washout for 20 min.

**Electrophysiologic Studies**

**Papillary Muscles.** Papillary muscle membrane potential was monitored by conventional 3 m KCl-filled glass microelectrodes (10–20 MΩ) attached to a VF-1 preamplifier (World Precision Instruments, Sarasota, FL). Membrane potential and its maximal rate of rise during

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the AP (dV/dt-max) were monitored on a digital storage oscilloscope. Because higher stimulation rates (2 and 3 Hz) frequently caused dislodgement of impaled micro-electrodes, only results from an impalement maintained throughout the whole sequence at 0.25 Hz stimulation rate were tabulated. Stimulation intensity was maintained at approximately 120% of the lowest intensity necessary to elicit a response and then adjusted to maintain approximately the same latency (stimulus-AP interval, usually 5–10 ms). AP amplitude, dV/dt-max, and AP duration at 30, 50, and 90% repolarization (APD<sub>30</sub>, APD<sub>50</sub>, and APD<sub>90</sub>, respectively) were measured at 0.25 Hz stimulation rate in modified normal Tyrode solution and in 26 mM K⁺ Tyrode solution with 0.1 mM isoproterenol.

**Isolated Myocyte Studies.** Whole-cell voltage clamp measurements were applied to isolated myocytes to examine the effect of MMA on Ca²⁺ current. Guinea pig ventricular myocytes were isolated enzymatically as previously described. Guinea pigs (~300 g) were anesthetized with 50 mg/kg sodium pentobarbital according to the guidelines of the University of Virginia Animal Care and Use Committee. The heart was quickly excised, perfused by a Langendorff system with modified normal Tyrode solution for 10 min, followed by perfusion for 5 min with nominally Ca²⁺-free Tyrode solution that caused cessation of the contractions. The Ca²⁺-free buffer was then supplemented with 25 mM CaCl₂, 0.5 mg/ml albumin, and 0.5 mg/ml type II collagenase (Worthington Biochemical, Freehold, NJ) and continuously recirculated. After 20 min, ventricles were excised, minced with scissors into the enzyme solution, and agitated by slow bubbling of the solution with 95% O₂ and 5% CO₂ for 30 min. The resulting slurry was filtered through 200-μm nylon meshes and centrifuged for 2 min at 80g. Cells were then washed twice in Krebs-Henseleit buffer with 200 mM CaCl₂, resuspended in the buffer with 1 mCi CaCl₂, and stored in a 95% air and 5% CO₂ incubator set at 37°C.

The external bathing solution contained (in mM): 140 Na<sup>+</sup>, 5 K<sup>+</sup>, 2 Ca<sup>2+</sup>, 1 Mg<sup>2+</sup>, 151 Cl<sup>−</sup>, 10 HEPES, adjusted to pH 7.4 with 1 N NaOH. For measurement of inward Ca<sup>2+</sup> current (I<sub>Ca</sub>), the patch pipette solution contained (in mM): 120 Cs<sup>+</sup>, 20 tetraethylammonium, 1 Ca<sup>2+</sup>, 140 Cl<sup>−</sup>, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N,N-tetra-acetic acid, 10 HEPES, 5 Mg<sub>2</sub>-adenosine triphosphate, adjusted to pH 7.3 with 1 N HCl. Standard whole-cell voltage clamp methods were employed, using the Axopatch 200 (Axon instruments, Foster city, CA) patch clamp amplifier. One whole-cell recording was achieved, the bathing solution was exchanged to (in mM): 125 Cs<sup>+</sup>, 20 tetraethylammonium, 2 Ca<sup>2+</sup>, 1 Mg<sup>2+</sup>, 151 Cl<sup>−</sup>, 10 HEPES, adjusted to pH 7.4 with 1 mM CsOH. Patch electrodes were prepared from borosilicate glass model KIMAX-51 (American Scientific, Charlotte, NC) heat-polished with a micro-forge, giving resistances of 2 and 3 MΩ when filled with internal solution. Myocyte experiments were conducted at room temperature (20–22°C). Data acquisition was performed using a pCLAMP system version 6.0.3 (Axon Instruments) coupled with an IBM-compatible, 486-based microcomputer. Voltage-dependent Ca²⁺ current (I<sub>Ca</sub> L) was evoked by step depolarizations from −30 mV to 70 mV from a holding potential of −40 mV.

**Drugs**

Liquid MMA (2-methylpropenoic acid methyl ester, molecular weight 100.13) was purchased from Sigma (St. Louis, MO), as were all other chemicals unless otherwise noted. For the papillary muscle experiments, an appropriate aliquot of liquid MMA was added to the volume of the perfusate to achieve the final calculated concentrations of 3, 10, and 30 μM. At 37°C, gas chromatographic measurement yielded measured concentrations of 0.5 ± 0.04 (n = 4), 1.5 ± 0.1 (n = 4), and 4.7 ± 0.1 (n = 4) μM MMA in modified normal Tyrode solution when equilibrated for 15 min with each, successive concentration of MMA (3, 10, and 30 μM). The chromatographic method employed N₂ as a carrier gas, a flame-ionization detector, and a fused silica capillary column (HP-FFAP, 60 m × 0.33 mm inner diameter, film thickness 0.2 μm) maintained isothermal at 40°C. N-hexene (chromatography grade) was used for liquid-liquid extraction. Calculation was based on a standard curve prepared each time.

For isolated myocyte studies, an appropriate aliquot of liquid MMA was added to the volume of the perfusate to achieve the final calculated concentrations of 0.5, 0.75, 1.0, and 2.0 μM.

**Statistical Analysis**

Because there are important differences in baseline values from one muscle to another, inotropic responses to each concentration of MMA were expressed as a percentage of baseline values. In papillary muscle and isolated myocyte experiments, repeated measures of analysis of variance followed by Student-Newman-Keuls test were applied to test for significant differences among the stimulation rates and among the anesthetic concentrations. All values are expressed as mean ± SD. A P value less than 0.05 was considered significant.

**Results**

**Effects on Contractility**

**Experiment with Modified Normal Tyrode Solution.** MMA (0.5, 1.5, and 4.7 μM measured) caused concentration-dependent depression of PF and dF/dt-max of approximately 70, 50, and 20% of baseline from RS to 3 Hz stimulation rates, respectively. At all concentrations, the effect of MMA on PF and dF/dt-max seemed...
to be significantly less at 3 Hz when compared to the other stimulation rates from RS to 2 Hz (figs. 1A and B). The contractile force was recovered to approximately 50% of baseline following washout for 20 min (data not shown). To verify the stimulation frequency-dependent effect of MMA, we examined six muscles where 10 mM MMA (calculated concentration) was only applied, which showed significantly less depression at 2 and 3 Hz stimulation rates (figs. 1C and D). In recovery following a 20-min washout, more recovery was observed at 2 and 3 Hz stimulation rates. To verify the volatile characteristic of MMA, we examined one muscle where spontaneous recovery of contractile force to approximately 80% of baseline under 0.5 Hz stimulation rate following 45 min application of 10 mM MMA (calculated concentration) was observed (baseline: 0.29 mN/mm², 10 mM: 0.11 mN/mm², recovery: 0.24 mN/mm²). The baseline values of PF and dF/dt-max in modified normal Tyrode solution from RS to 3 Hz stimulation rates are shown in table 1.

Rat papillary muscles exhibit negative force-frequency relationship, which may be related to hypoxia. Because of potential for hypoxic alteration, especially at higher stimulation rates, the effect of 1.5 mM MMA were only examined at RS. Depression of PF to 70 ± 17% of baseline at RS was shown (6.44 ± 0.46 vs. 4.61 ± 0.47 mN/mm², n = 8, P < 0.05). Recovery was complete following a 20-min washout (6.44 ± 0.46 vs. 5.53 ± 0.63 mN/mm², NS).

Rapid Cooling Contracture. MMA (1.5 mM) depressed RCC following a 2-Hz stimulation rate to 53 ± 10% of baseline (3.45 ± 2.69 vs. 1.92 ± 1.76 mN/mm², n = 7, P < 0.05) with prolongation of time-to-peak contracture (163 ± 40% of baseline, 13.46 ± 2.72 vs. 21.69 ± 5.94 s, P < 0.05). Almost complete recovery to baseline level (86 ± 13% of baseline, 3.45 ± 2.69 vs.
Table 1. Baseline Values of Main Mechanical Parameters in Modified Normal Tyrode Solution

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rested State</th>
<th>0.1 Hz</th>
<th>0.5 Hz</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>3 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Force</td>
<td>0.34</td>
<td>0.68</td>
<td>0.68</td>
<td>0.74</td>
<td>2.16</td>
<td>3.86</td>
</tr>
<tr>
<td>mN/mm²</td>
<td>0.13</td>
<td>0.29</td>
<td>0.29</td>
<td>0.32</td>
<td>0.91</td>
<td>1.39</td>
</tr>
<tr>
<td>dF/dt-max</td>
<td>3.27</td>
<td>6.40</td>
<td>6.13</td>
<td>7.98</td>
<td>30.24</td>
<td>57.23</td>
</tr>
<tr>
<td>mN·s⁻¹·mm⁻²</td>
<td>1.18</td>
<td>2.81</td>
<td>2.46</td>
<td>3.41</td>
<td>12.49</td>
<td>20.50</td>
</tr>
</tbody>
</table>

Results are mean ± SD (n = 8). dF/dt-max = maximum rate of force development.

3.15 ± 3.00 mN/mm² (NS) was shown in RCC amplitude following a 20-min washout with returning of time-to-peak contracture to baseline level (13.46 ± 2.72 vs. 15.26 ± 2.35 s, NS) (fig. 2).

Experiment with Low Na⁺ (25 mM) Tyrode Solution. MMA (1.5 mM) depressed RS contraction to 83 ± 17% of baseline (2.05 ± 1.51 vs. 1.70 ± 1.20 mN/mm², n = 6, P < 0.05). Complete recovery was shown following a 20-min washout.

Experiment with 26 mM K⁺ Tyrode Solution. MMA (0.5 and 1.5 mM) caused selective and marked concentration-dependent depression of late force development (0.5 mM: ~60% of baseline, 1.5 mM: ~30% of baseline) with no alteration of early force development (figs. 3A-C and 4A). Complete recovery was shown following a 20-min washout (data not shown). The baseline values of PF, dF/dt-max, and dF/dt-max are shown in table 2.

**Electrophysiologic Effect**

**Papillary Muscles.** In normal APs, MMA (1.5 and 4.7 mM) altered neither the amplitude nor dV/dt-max at 0.25 Hz stimulation rate, while APD₅₀ and APD₉₀ were significantly reduced at 4.7 mM MMA (APD₅₀: 88 ± 10% of baseline, APD₉₀: 90 ± 8% of baseline) (fig. 3). No change in the resting membrane potential, which ranged from −85 mV to −89 mV, was observed at either concentration of MMA. In slow APs, 1.5 and 4.7 mM MMA did not change either AP amplitude or dV/dt-max at 0.25 Hz stimulation rate as similar as in modified normal Tyrode solution. AP durations were significantly reduced by 4.7 mM MMA (APD₅₀: 84 ± 2% of baseline, APD₉₀: 87 ± 1% of baseline, P < 0.05) while modest reduction of AP duration by 1.5 mM MMA was shown (fig. 3, fig. 4B). No changes in resting membrane potential, which ranged from −52 mV to −54 mV, were observed at either concentration of MMA.

**Isolated Myocyte Studies.** Application of 1 mM MMA resulted in reduction of peak current of Ica, L to 51 ± 9% of baseline (538 ± 296 pA vs. 274 ± 161 pA, n = 5, P < 0.05) (fig. 5A). As shown in figure 5B, 0.5, 0.75, 1, and 2 mM MMA decreased Ica, L 91 ± 15%, 82 ± 13%, 51 ± 9%, and 36 ± 13% of baseline, respectively, in a concentration-dependent fashion. The resulting curve was fitted by the following formula: I MMAX/I MAX = Kd/C/(MMAX)N + Kd/C. The I MMAX and I MAX are Ica at baseline and at each MMA concentration, and Kd is an MMA concentration, which decreases I MAX to the 50% of baseline level. N is the slope factor, Kd = 1.33 mM, and the n = 1.93.

**Discussion**

MMA is an aromatic volatile liquid (boiling point, 100–101°C) with a density similar to water (0.94 g/ml). The present study shows that MMA depresses the contractile force in a concentration-dependent manner with somewhat less depression at higher stimulation rates compared to low stimulation rates. The myocardial depressant action of MMA has been reported in isolated ventricular tissues, in intact animals, and in clinical settings. Baran et al. in their study with isolated rat and rabbit atria demonstrated approximately 45 and 28% depression of contractile force, respectively, by 10 mM MMA. In a rat heart-lung preparation, Kashimoto et al. reported that 1000 μg/ml (10 mM) MMA caused depression of cardiac output approximately 50–40% of baseline. Considering the similar depression of contractile force in our result, the used, calculated concentration of the previous studies may be close to the measured concentration by gas chromatography since the low solubility and loss to the atmosphere resulted in lower measured concentrations than calculated from the amount of drug.

In a muscle, we observed spontaneous recovery (ap-
approximately 80% of a baseline value) at 45 min following 10 mM MMA administration, indicating the volatile character of MMA. In the present study, the measured concentrations of the superfusate by gas chromatography definitely support the evidence of volatility. Blood clearance of MMA has been reported to be rapid and pulmonary excretion is considered the main mechanism of elimination. Gentil et al. in their measurement of methylmethacrylate plasma concentrations during total hip arthroplasty reported that the half-life of MMA was 3.0 ± 0.7 min and 55 ± 8% of MMA was cleared during the transpulmonary passage.

An interesting observation is that MMA caused less depression at higher stimulation rates, as shown in figures IC and D, which has been interpreted as less disturbance of Ca\(^{2+}\) release from the SR than sarcolemmal Ca\(^{2+}\) influx. The SR of the isolated rat ventricle does not become depleted of Ca\(^{2+}\) during rest so that RS contraction in rat papillary muscle reflects force development mediated by Ca\(^{2+}\) released from the SR. Unlike rat myocardium, Ca\(^{2+}\) is depleted progressively during rest in guinea pig myocardium. However, Ca\(^{2+}\) is accumulated in guinea pig SR during rest when extracellular Na\(^+\) concentration is reduced, an effect that has been used to assess the effect on SR Ca\(^{2+}\) release by depolarization in guinea pig myocardium. The minimal effect of MMA on RS force development in the present rat experiment is consistent with a modest effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release. To rule out the species-different effect on SR Ca\(^{2+}\) release.
rat experiment, also indicating a modest effect of MMA on SR Ca\(^{2+}\) release.

Our findings in partially depolarized \(\beta\)-adrenergically stimulated myocardium showed marked concentration-dependent depression of late force development (0.5 mM: approximately 60% of baseline, 1.5 mM: approximately 30% of baseline) with no alteration of early force development. As depression of the late contraction is mediated in part by depression of the transsarcolemmal Ca\(^{2+}\) influx, marked concentration-dependent depression of late force development by MMA may be caused by depression of the transsarcolemmal Ca\(^{2+}\) influx or by the reduction of late SR release of Ca\(^{2+}\), which represents transient accumulation into the SR of the entering Ca\(^{2+}\). During prolonged rest, isolated guinea pig ventricular muscle becomes depleted of its intracellular store of Ca\(^{2+}\) so that RS contraction and those at low stimulation rates are dependent on the transsarcolemmal influx of Ca\(^{2+}\) for activation.\(^{16}\) Depression of RS contraction in our results is consistent with reduction of Ca\(^{2+}\) entry or reduction of late SR release of Ca\(^{2+}\). Considering the modest effect on SR Ca\(^{2+}\) release, therefore, depression of the late force development in 26 mM K\(^+\) Tyrode solution and RS contraction in modified normal Tyrode solution is likely to be mediated by depression of the inward Ca\(^{2+}\) current.

The RCC provides an index of the availability of activator Ca\(^{2+}\) available for release from the SR\(^{15}\) and the Ca\(^{2+}\) release pathway from the SR activated by rapid cooling does not appear to be the same Ca\(^{2+}\) release channel by depolarization. Although the pool for Ca\(^{2+}\) that generates RCCs and electrically evoked contractions appears to be the same,\(^{21}\) Feher and Rebeyka\(^{27}\) suggested that electrically evoked contractions and rapid-cooling induced RCCs might rely on ryanodine-sensitive and -insensitive pathways, respectively, for Ca\(^{2+}\) release from the SR. In the present study, 1.5 mM MMA depressed the RCC to 53 ± 10% of baseline and prolonged time to peak contracture to 163 ± 40% of baseline. The most likely explanation for the depression of the RCC is the inhibition of rapid cooling-induced Ca\(^{2+}\) release pathway rather than an alteration of SR Ca\(^{2+}\) uptake.

In our microelectrode experiments in papillary muscles, we could not observe any alteration of slow AP dV/dt-max. As dV/dt-max of AP actually measures net current, simultaneous decrease of inward and outward currents may have counteracting effect. In our result, the failure to detect the decrease of the slow AP dV/dt-max with 1.5 and 4.7 mM MMA do not eliminate possible depression of Ca\(^{2+}\) currents if coincident depression of K\(^+\) conductance was occurring. Therefore, although K\(^+\) currents primarily determine AP duration, changes in Ca\(^{2+}\) conductance may also alter the duration of the ventricular AP. The whole cell voltage-clamp studies were carried out to directly demonstrate the effect on Ca\(^{2+}\) entry, which revealed a concentration-dependent

### Table 2. Baseline Values of Main Mechanical Parameters in 26 mm K\(^+\) Tyrode Solution with 0.1 \(\mu\)M Isoproterenol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rested State</th>
<th>0.1 Hz</th>
<th>0.5 Hz</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>3 Hz</th>
</tr>
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<tbody>
<tr>
<td>Peak Force (mN/s/mm(^2))</td>
<td>1.84</td>
<td>1.92</td>
<td>2.00</td>
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<td>1.76</td>
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<td>mN/s/mm(^2)</td>
<td>1.93</td>
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<td>2.07</td>
<td>1.90</td>
<td>1.67</td>
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<td>dF(_E)/dt-max</td>
<td>5.46</td>
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<td>6.42</td>
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<tr>
<td>dF(_E)/dt-max</td>
<td>16.44</td>
<td>16.78</td>
<td>18.48</td>
<td>13.78</td>
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<tr>
<td>mN/s(^{-1}) mm(^{-2})</td>
<td>17.40</td>
<td>17.83</td>
<td>19.39</td>
<td>16.34</td>
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</tbody>
</table>

Results are mean ± SD (n = 6).

dF\(_E)/dt-max = maximum rate of early force development; dF\(_L)/dt-max = maximum rate of late force development.

### Table 3. Effects of MMA on Normal and Slow Action Potential Characteristics at 0.25 Hz Stimulation Rate

<table>
<thead>
<tr>
<th>Condition</th>
<th>Amplitude (mV)</th>
<th>dV/dt-max (V/s)</th>
<th>APD(_{50}) (ms)</th>
<th>APD(_{90}) (ms)</th>
<th>APD(_{30}) (ms)</th>
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</thead>
<tbody>
<tr>
<td><strong>Normal AP (n = 8)</strong></td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>130 ± 12</td>
<td>126 ± 43</td>
<td>178 ± 31</td>
<td>217 ± 33</td>
<td>131 ± 34</td>
</tr>
<tr>
<td>1.5 mM MMA</td>
<td>129 ± 9</td>
<td>129 ± 49</td>
<td>168 ± 29</td>
<td>206 ± 28</td>
<td>118 ± 31</td>
</tr>
<tr>
<td>4.7 mM MMA</td>
<td>133 ± 13</td>
<td>116 ± 40</td>
<td>152 ± 15*</td>
<td>190 ± 17*</td>
<td>118 ± 24*</td>
</tr>
<tr>
<td>Recovery</td>
<td>135 ± 13</td>
<td>134 ± 53</td>
<td>188 ± 30*</td>
<td>224 ± 31</td>
<td>141 ± 28</td>
</tr>
<tr>
<td><strong>Slow AP (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>96 ± 12</td>
<td>13 ± 5</td>
<td>196 ± 25</td>
<td>230 ± 27</td>
<td>166 ± 13</td>
</tr>
<tr>
<td>1.5 mM MMA</td>
<td>93 ± 10</td>
<td>13 ± 6</td>
<td>180 ± 17*</td>
<td>218 ± 24</td>
<td>158 ± 20</td>
</tr>
<tr>
<td>4.7 mM MMA</td>
<td>89 ± 5</td>
<td>10 ± 13</td>
<td>156 ± 13*†</td>
<td>191 ± 12*†</td>
<td>136 ± 11*†</td>
</tr>
<tr>
<td>Recovery</td>
<td>94 ± 7</td>
<td>14 ± 6</td>
<td>181 ± 36</td>
<td>208 ± 38*</td>
<td>138 ± 30*</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

* Differences from baseline (\(P < 0.05\)). † Differences from 1.5 mM MMA (\(P < 0.05\)).

AP = action potential; APD\(_{50}\), APD\(_{90}\), and APD\(_{30}\) = duration of AP at 50, 90, and 30% repolarization, respectively; dV/dt-max = the maximum rate of depolarization of the AP; MMA = methymethacrylate monomer.
The depression of $I_{Ca}$ by MMA in the present voltage clamp studies shows a concentration dependence similar to the depression of contractile forces of guinea pig papillary muscle in modified normal Tyrode solution. The $I_{Ca}$, 1.33 mM, in the dose-response curve of $I_{Ca}$ may explain the similar depression of contractile force, approximately 50% of baseline, by 1.5 mM MMA (by gas chromatography) in modified normal Tyrode solution. Since the patch clamp studies typically employed a shorter exposure interval to the perfusate, the perfusate was not bubbled, and the bath temperature was maintained at room temperature (20–22°C), there would have been less MMA lost by volatilization and the concentration in solution may have been more accurate.

MMA is a volatile compound with short half-life, and to assess blood concentrations accurately is known to be difficult. Frequently, MMA concentrations were undetectable. Wide ranges in MMA blood concentrations (0–590 μM) have been reported in patients undergoing hip replacement. Concentrations of 0–26 μM were present in pulmonary artery samples taken 3 min after insertion of cement in 13 patients with total hip replacement; 2.4–151 μM were present in central venous samples taken 5 min after insertion of the cement into the acetabulum and femur in 8 patients; 0.5–318.9 μM were present in peripheral venous samples taken 30 s after implantation of the acrylic cement into the femur in 20 patients; 0.2–590 μM were present in pulmonary artery, radial artery, and superior vena cava samples in 15 total hip replacement patients; and 1–123 μM were present in pulmonary artery and radial artery samples obtained 2 min after insertion of cement into the acetabulum and femur in 11 patients. In contrast to these relatively low blood concentrations, Pahuja et al. in their patients undergoing total hip replacement reported an extremely high concentration (20 mM) of MMA in one of five patients, while the other four patients’ concentrations ranged between 16 and 110 μM. Based on these common clinical blood concentrations, the level of applied concentrations, over 3 mM (0.5 mM by gas chromatography), in this study is higher. Thus, the direct contribution of MMA itself to cardiac depression may be less than the other factors, which cause hypotension during MMA implantation.

In summary, MMA depresses myocardial contractility directly in a concentration-dependent manner, which may be at least caused in part by depression of Ca$^{2+}$ influx through cardiac membrane. The rapid initial release of Ca$^{2+}$ from the SR by depolarization seems to be modestly inhibited, and a certain release pathway, specifically induced by rapid cooling, appears to be depressed. Considering the common clinical concentrations, the direct myocardial depressant effect of MMA may contribute less than the other factors that cause hypotension after acrylic bone cement implantation during orthopedic surgical procedures.

\[ \text{Fig. 5. (A) Effect of 1 mM methylmethacrylate (MMA) on voltage-sensitive Ca$^{2+}$ current ($I_{Ca}$) evoked from isolated guinea pig ventricular myocytes at room temperature. L-type } I_{Ca} (I_{Ca,k}) \text{ triggered by a voltage step to 0 mV from a holding potential of } -40 \text{ mV was obtained. (B) Concentration-dependent depressant effect of MMA on } I_{Ca}. \text{ Current amplitude of the } I_{Ca} \text{ measured in the presence of MMA is normalized to the respective baseline amplitude in the absence of MMA, and thus normalized current amplitudes of the } I_{Ca} \text{ are plotted as a function of MMA concentration (n = 5 at each concentration except 0.1 mM [n = 1]). The apparent dissociation constant and slope factor were 1.33 mM and 1.93, respectively.} \]

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References


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