Initial reperfusion with 2,3 butanedione monoxime is better than hyperkalemic reperfusion after cardioplegic arrest in isolated guinea pig hearts

Abstract  Objective. Initial warm cardioplegic reperfusion is widely used to ameliorate cardiac reperfusion damage after cardioplegic arrest. However, undesired effects of the high potassium concentration of the cardioplegic perfusate may limit the beneficial effect of this treatment. Contraction uncoupling by a negative inotropic and vasodilating agent such as 2,3-butanedione monoxime (BDM) may be superior to warm cardioplegic reperfusion in reducing reperfusion damage. Thus, initial reperfusion with BDM was compared with hyperkalemic reperfusion (HKR) after global ischemia of Langendorff-perfused guinea pig hearts.

Methods. Cardiac arrest was induced in 16 hearts using hyperkalemic Krebs' solution and hearts were stored unperfused at 37 °C for 40 min. Two groups were studied: HKR, initial reperfusion with 37 °C oxygenated hyperkalemic Krebs' solution and hearts were stored unperfused at 37 °C for 40 min, and BDM, addition of 20 mM BDM to normokalemic Krebs' for 5 min. Results. BDM increased reactive coronary reflow (128±8%; all data mean±SEM of baseline) much more than HKR treatment (65±5%). O2 consumption was reduced more by HKR (28±1%) than by BDM (42±4%), but the O2 supply/consumption ratio was higher with BDM. During perfusion with normal Krebs' solution, flow stabilized at about 75% of baseline in both groups. Post-ischemic responses to adenosine, serotonin, and nitroprusside were depressed to a similar degree in both two groups. Recovery of left ventricular developed pressure was better in BDM (69±2%) than in HKR (61±3%)-treated hearts. Reperfusion dysrhythmias were markedly reduced after BDM reperfusion.

Conclusions. These data indicate that treatment in the initial 5-min reperfusion period with BDM is more effective than hyperkalemic reperfusion in reducing reperfusion damage.

Key words Warm cardioplegic reperfusion • Adenosine • 5-Hydroxy-tryptamine • Serotonin • Nitroprusside • Endothelium

Introduction

Ischemic cardioplegic arrest and cardiac reperfusion as performed for cardiac transplantation and during most cardiopulmonary bypass protocols can result in decreased myocardial contractility (stunning) [3, 10], dysrhythmias (ventricular fibrillation (VF) or tachycardia (VT)), as well as depressed coronary flow, flow reserve, and endothelial function [13, 22]. Initial reperfusion with a 37 °C oxygenated hyperkalemic solution (warm cardioplegic reperfusion) has been shown to improve post-ischemic recovery of myocardial function [3, 6], possibly by minimizing the cardiac myocyte oxygen (O2) demand, thus redirecting metabolic activity toward tissue repair processes and the replenishment of intracellular energy stores before restitu-
tion of the demand for cardiac work. In a previous study we demonstrated that hyperkalemic reperfusion (HKR) for 5 min improved left ventricular (LV) function in isolated, Langendorff perfused guinea pig hearts, but had no effect on dysrhythmias, recovery of coronary flow, and depression of the post-ischemic endothelium—dependent or—Independent flow responses [9]. In the same study we reported that when 1 mM or 5 mM adenosine was added to the HKR perfusate, initial reflow increased, and that 5 mM adenosine had a sustained effect on the recovery of coronary flow and LV systolic and diastolic functions.

2,3 Butanediol monoxide (BDM) is an effective negative inotropic and vasodilating agent, believed to decrease the sensitivity of contractile proteins to Ca++, or to directly inhibit actin-myosin cross-bridge cycling [1, 8, 15, 17, 20, 31]. 2,3 Butanediol monoxide considerably improves the myocardial O2 balance, not only by reducing myocardial O2 demand but, unlike hyperkalemia, by increasing O2 delivery [2, 28]. We, therefore, hypothesized that after hyperkalemic arrest, initial reperfusion with BDM solution might be at least as effective as initial reperfusion with high K+ solution in improving the initial myocardial O2 balance and the long-term recovery of myocardial function. The use of a normokalemic solution may allow more rapid restoration of transsarcolemmal ion gradients and the resting membrane potential, while the addition of BDM maintains depression of contractility during the initial reperfusion period. This approach might allow a quicker return to regular electric activity, i.e. to sinus rhythm, and contractile function than a hyperkalemic solution which causes membrane depolarization and intracellular Ca++ accumulation. Our aim was to compare the effectiveness of initial BDM reperfusion with initial high K+ reperfusion on the recovery of cardiac function in isolated guinea pig hearts.

Materials and methods

Following Animal Studies Committee approval, 16 albino English short-haired guinea pigs (250–350 g) were injected intraperitoneally with 20 mg of ketamine and 1000 units of heparin, and were decapitated when unresponsive to noxious stimuli. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health. Our methods have been published in detail [2, 26, 28]. After thoracotomy, the aorta was cannulated distal to the aortic valve and the venae cavae and the pulmonary artery were cut. Each heart was immediately perfused retrogradely through the aorta and excised. All hearts were perfused at a controlled perfusion pressure of 55 mmHg, measured at the aortic root. The perfusate, a modified Keb's-Ringer solution, was filtered in-line (5 μm pore size; Astrodisc) and contained (in mM): Na+, 137; K+, 4.5; Mg++, 1.2; Ca++, 2.5; Cl−, 134, HCO3-, 15.5; H2PO4-, 1.2; glucose, 11.5; pyruvate, 2; mannitol, 16; ethylene-diaminetetraacetic acid (EDTA), 0.05; and insulin, 5 units/l. Hyperkalemic Kreb's solution had an equivalent osmolarity of 300 mosmol/l but differed in Na+ (123 mM), K+ (28 mM), and Ca++ (1.25 mM). Perfusion and bath temperatures were maintained at 37 °C using a thermostatically controlled water circulator (VWR 1130)2. Both solutions were equilibrated with a gas mixture of 97% O2 and 3% CO2, which resulted in a pH of 7.4. Left ventricular pressure (LVP) was measured isovolumetrically with a transducer (DTX)3 connected to a flexible, saline-filled latex balloon inserted into the LV through a cut in the left atrium via the mitral valve. The balloon volume was adjusted to maintain an end-diastolic LVP of 6 mmHg and left ventricular developed pressure (LVDP) was calculated as LVP'systolic − LVPend-diastolic. Positive and negative peak of pressure change velocity (dP/dtmax) were obtained electronically with an analog differentiator.

Electrograms were recorded with two pairs of bipolar electrodes (plastic-coated silver, diameter 125 μm) placed on the right atrial appendage and on the right ventricle pulmonary conus. The electrode signals were amplified and displayed continuously on a digital oscilloscope ( Nicolet310) and audibly monitored. Electrogram intervals for the determination of heart rate and atrioventricular (AV) conduction time were measured on-line by digital timer systems that allowed instantaneous interval and rate analyses. Ventricular tachycardia was defined by the presence of uniform or multifractular ventricular wave forms and a faster ventricular than atrial rate. Ventricular fibrillation was defined by the presence of erratic activity in the ventricular electrogram and by the absence of pressure generation by the LV. Coronary flow was measured on-line using a ultrasonic flow probe (T.106 small animal blood flow meter)6. Coronary sinus effluent was collected by a cannula placed in the right ventricle via the pulmonary artery after ligating the venae cavae. Coronary outflow O2 tension was measured continuously on-line (Instech 203 B)7. The temperature-controlled Clark electrode was recalibrated periodically by using a bypass circuit with perfusate gassed with room air. Both inflow and outflow O2 tensions were verified off-line with a self-calibrating analyzer system (ABL-3)8. Derived parameters were calculated according to standard formulae:

\[ \text{DO}_2 = \text{inflow PO}_2 \times \text{O}_2 \text{ solubility} \times \text{coronary flow} \]

Myocardial O2 consumption:

\[ \text{MVO}_2 = (\text{inflow PO}_2 - \text{outflow PO}_2) \times \text{O}_2 \text{ solubility} \times \text{coronary flow} \]

Cardiac efficiency in utilizing consumed O2 for generating work:

\[ \text{EFF} = \frac{\text{LV systolic pressure} \times \text{heart rate}}{\text{MVO}_2} \]

All directly measured electronic signals were displayed on a high resolution, eight-channel recorder (Astro-Med Dash 8)9, tape recorded for back-up (Vetter D1)10, and stored on floppy discs by a personal computer (model 310)11, equipped with a 12-bit analog-to-digital converter sampling at 100 hz (AD 200)12. Each value is the mean of a 10 s data segment which was visually inspected to assure the absence of artifacts.

The time constant of isovolumic pressure decline (tau), which reflects the time required for LVP to fall about one-third of its value at peak -dP/dt, was calculated as described previously [24]. Peak -dP/dt was used as zero time, and pressure was recorded for 60 ms/
beat for 3–4 consecutive beats. The logarithm of LVP at time t (lnP) was plotted against t, and tau was defined as the negative reciprocal of the slope of the linear section of the curve, according to the equation lnP = -t/tau + lnP0, where P0 is LVP at time t, and P0 is LVP at peak -dP/dt. This method has been successfully applied to the Langendorff perfused guinea pig heart by using a fluid filled LV-balloon system, connected to a pressure transducer by short tubing of stiff polyethylene material [12].

The study protocol is schematically shown in Fig. 1. Following completion of the preparation and a 10-min period of stabilization, a 0.2 ml bolus of 2 mM adenosine was injected into the inflow line to determine maximal coronary reserve. Only hearts that increased flow by 100% or more were included. After 30 min of further stabilization, flow responses to 2-min infusions of 5-hydroxytryptamine (1 μM) and nitroprusside (100 μM) were assessed. Fifteen minutes after stopping these drugs, the baseline values of all measured variables were again obtained and cardioplegic arrest was induced by perfusing oxygenated hyperkalemic Krebs’ (37°C) for 5 min. The LV balloon was then deflated and the hearts remained unperfused and submerged in the same hyperkalemic Krebs’ at 37°C for 40 min. On reperfusion, the hearts were divided into two groups: HKR (n=8) was instituted using 37°C oxygenated hyperkalemic Krebs’ for 5 min. This was followed by reperfusion with regular Krebs’ for 90 min. 2,3 Butanedione monoxime monooxygenase reperfusion (n=8) was initiated with normokalemic (K⁺ 4.5 mM) Krebs’ with 20 mM BDM for 5 min before the perfusate was switched to normal Krebs’. The LV balloon was reinfated to 6 mmHg end-diastolic pressure at 15 min of reperfusion, and all hearts were bolus infused with lidocaine (0.05 ml of 1%) at 20 min to convert any hearts not already in sinus rhythm. Coronary flow was measured at baseline, during the induction of cardioplegia, during HKR or BDM reperfusion, and at 1, 2, 3, 4, 5, 15, 30 and 60 min during reperfusion with normal Krebs’.

Coronary reserve was assessed again at 30 min. The postischemic recovery of all other variables was determined after 60 min of reperfusion and the responses to 5-hydroxytryptamine (1 μM) and nitroprusside (100 μM) were again assessed.

All data are expressed as means±standard error of the mean. After testing for normal distribution of the data, statistical differences among groups were obtained by unpaired t-test. When multiple testing occurred, as for coronary flow, MVO₂ or DO₂/MVO₂, a Bonferroni correction was performed. For within-group comparisons repeated measures with two-way analysis of variance followed by Dunnett’s method for multiple comparisons was employed. The incidence of reperfusion dysrhythmias was compared by Fisher’s exact test. Differences were considered significant at P<0.05.

Results

Heart rate and cardiac rhythm

The initial heart rate in HKR and BDM hearts was 232±6 and 231±3 bpm, respectively. In HKR hearts all electromechanical activity was absent during hyperkalemic reperfusion and commenced after 1–3 min of normal Krebs’ perfusion. In contrast, five of eight hearts displayed regular electrical activity already during BDM reperfusion and all hearts of this group were beating within 1 min of normal Krebs’ reperfusion. At 5 min, only two of eight HKR, but seven of eight BDM hearts had sinus rhythm (P=0.021); the remaining hearts had either VT or VF, Fig. 2). The total duration of VT and VF during the first 15 min of regular Krebs’ perfusion was 9.4±1.5 min in HKR and 3.4±1.9 min in BDM groups (P=0.027). Lidocaine, was given in all experiments at 20 min, although only five HKR hearts and one BDM heart needed conversion at that time. At 60 min the heart rate was not different from baseline in either group.

Left ventricular systolic and diastolic function

The initial LVDP in HKR and BDM hearts was 123±6 mmHg and 125±5 mmHg, respectively. Recovery of LVDP was significantly (P=0.029) better in BDM (69±2%) than in HKR (61±2%) hearts, and +dP/dtmax also tended to return to higher values in the BDM, versus the HKR, group (Table). In contrast, LV diastolic function, as assessed by dP/dtmin and tau, was similar in the two groups (Table). In our laboratory up to 8 h of continuous perfusion only slightly attenuated LVDP by about 10–15% [28].
Coronary flow and O₂ consumption

The baseline coronary flow was 62±3 ml/min/per g dry weight in HKR and 55±2 ml/min/per g dry weight in BDM hearts. During the induction of cardioplegia before ischemia, the flow decreased by more than 20% in both groups (Fig. 3). During reperfusion in HKR hearts the flow reached an early reactive hyperemic peak (100±8%), but quickly decreased to 65±5% of baseline at the end of 5 min hyperkalemic reperfusion. On continued perfusion with regular Krebs', the flow stabilized between 75 and 80% of the pre-ischemic baseline. During BDM reperfusion, however, the initial reflow reached a peak of 150±9%, was well above baseline after 5 min of BDM reperfusion (129±8%) and remained elevated during BDM washout with regular Krebs' for over 5 min. Thirty and 60 min after the onset of reperfusion, the coronary flow remained at 77±2% of baseline and was similar in the two groups (Fig. 3).

During induction of cardioplegia before ischemia, myocardial MVO₂ decreased similarly by 79±4% in HKR and by 80±2% in BDM hearts. After cardioplegic arrest, MVO₂ was increased slightly (from 20±2% to 28±1%) during HKR but doubled during BDM reperfusion (from 21±4% to 42±4%). The early phase of reperfusion with regular Krebs' after HKR or BDM reperfusion was characterized by an earlier increase of MVO₂ in BDM hearts than in HKR hearts. While MVO₂ remained below baseline in HKR hearts until the end of the experiments, it was not statistically different from baseline in BDM hearts after 2 min and until 60 min of reperfusion (Fig. 4). The balance of O₂ consumption and supply, as indicated by the quotient of O₂ delivery and MVO₂, increased four to five fold during the induction of cardioplegia, remained elevated during initial reperfusion with either HKR or BDM, and returned to baseline values already at 15 min (Fig. 5). The myocardium's efficiency in utilizing consumed O₂ for generating work was similarly decreased in both groups at 60 min (Table).

Table 1 Recovery of functional parameters after 60 min reperfusion in percent of baseline (HR heart rate, LVDP left ventricular developed pressure, dP/dt max peak of pressure increase velocity, dP/dt min peak of pressure decrease velocity, tau time constant of relaxation, Flow coronary flow, MVO₂ myocardial O₂ consumption, DO₂/MVO₂ ratio of O₂ delivery to O₂ consumption, Efficiency efficiency of myocardial O₂ utilization for cardiac work).

<table>
<thead>
<tr>
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<th>HKR</th>
<th>BDM</th>
<th>P</th>
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<tr>
<td>HR</td>
<td>99.2±5.0</td>
<td>92.8±7.3</td>
<td>n.s.</td>
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<tr>
<td>LVDP</td>
<td>61.1±2.4</td>
<td>69.2±2.3</td>
<td>0.029</td>
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<tr>
<td>dP/dt max</td>
<td>69.5±4.6</td>
<td>76.3±4.0</td>
<td>n.s.</td>
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<tr>
<td>dP/dt min</td>
<td>58.6±3.7</td>
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<td>n.s.</td>
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<tr>
<td>tau</td>
<td>120.5±9.3</td>
<td>117.9±2.1</td>
<td>n.s.</td>
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<tr>
<td>Flow cor.</td>
<td>75.4±4.8</td>
<td>76.7±2.0</td>
<td>n.s.</td>
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<tr>
<td>MVO₂</td>
<td>80.4±4.3</td>
<td>87.7±2.9</td>
<td>n.s.</td>
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<tr>
<td>DO₂/MVO₂</td>
<td>93.1±5.4</td>
<td>88.4±6.7</td>
<td>n.s.</td>
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<tr>
<td>Efficiency</td>
<td>77.3±1.8</td>
<td>74.4±2.5</td>
<td>n.s.</td>
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Coronary reserve and vascular function

Prior to ischemia and reperfusion, coronary reserve, as assessed by adenosine bolus injection, was 100±10% in HKR and 121±10% in BDM hearts (Fig. 6). Vasodilatory responses to 5-hydroxytryptamine and nitroprusside were also comparable in the two groups. After ischemia and reperfusion, responses to all vasodilators were depressed in both groups much more than would be expected after an equivalent time of continuous perfusion [26].

Discussion

The most important findings of this study after cardioplegic arrest and reperfusion in isolated perfused guinea pig hearts are: reperfusion with BDM increases the initial O₂ delivery to demand ratio more than HKR; BDM has a greater beneficial effect than HKR on the short-term, but not on the long-term recovery of coronary flow; BDM markedly reduces reperfusion dysrhythmias compared to HKR; and BDM improves recovery of LVDP significantly more than HKR.

Initial HKR reduces O₂ demand for myocardial pump work, thus allowing O₂ to be utilized for cellular repair mechanisms and replenishment of energy stores [3, 5, 6]. By also reducing the reperfusate Ca²⁺ concentration, O₂ demand is further reduced [6]. Simultaneously the vasodilating effect of reduced Ca²⁺ may enhance O₂ delivery by attenuating the K⁺ induced vasoconstriction [28]. In our study BDM was not quite as effective as HKR in minimizing O₂ consumption during the initial reperfusion with BDM or HKR. This may be due to a different mechanism of action. Hyperkalemic reperfusion causes membrane depolarization and cardiac arrest whereas BDM does not eliminate the electrical function of the heart. Rather BDM uncouples muscle contraction from excitation by decreasing the Ca²⁺ sensitivity of contractile proteins [1, 15, 17, 31], or by directly affecting actin-myosin cross-bridge kinetics [8, 20]. In concentrations above 5–10 mM (20 mM in the present study) BDM also alters intracellular Ca²⁺ kinetics and transsarcolemmal Ca²⁺ transport [4, 11, 15, 20, 25], lowers the heart rate and increases AV conduction time [2, 17]. Thus, electrical activity restarted already during BDM reperfusion and O₂ consumption increased to 42±4% of the baseline, compared to 28±1% during HKR, which is similar to the 20±2% increase found during pre-ischemic induction of hyperkalemic cardioplegia. The increase in O₂ consumption during BDM reperfusion, however, was more than compensated for by the higher flow in these hearts so that the DO₂/MVO₂ ratio was 4.3±0.6 in BDM hearts, compared to only 3.1±0.4 in HKR hearts. This suggests that despite the higher MVO₂, O₂ supply is more than sufficient during BDM reperfusion compared with HKR. Besides improving the O₂ balance, the increased initial
Fig. 2 Effects of 5 min BDM reperfusion versus 5 min of HKR reperfusion on incidence and duration of ventricular fibrillation (VF) and ventricular tachycardia (VT) during 15 min normal Krebs' perfusion after 40 min of cardioplegic ischemic arrest (* P<0.05 vs HKR)

Fig. 3 Time course of coronary flow as a percent of baseline (pre-ischemic) flow for HKR and BDM groups. Measurements were taken at: baseline (BL), the end of 5 min perfusion with hyperkalemic Krebs' (CP), the reactive hyperemic peak flow, which occurred within 1 min of reperfusion (RH), the end of the initial 5 min reperfusion with either oxygenated, hyperkalemic Krebs' (HKR) or BDM Krebs' (IR), and at the given time points of reperfusion in min after the start of perfusion with normal Krebs'. ISCH 40 min of 37°C cardioplegic ischemia. For better identification of single data points the initial 15 min of reperfusion were expanded on the x-axis scale (* P<0.05 vs HKR; # P<0.05 vs baseline)

Fig. 4 Time course of oxygen demand (MVO2) as a percent of baseline (pre-ischemic) flow for HKR and BDM groups. Measurements were taken at: baseline (BL), the end of 5 min perfusion with hyperkalemic Krebs' (CP), the end of initial 5 min reperfusion with either oxygenated, hyperkalemic Krebs' (HKR) or BDM Krebs' (IR), and at the given time points of reperfusion in min after the start of perfusion with normal Krebs'. ISCH 40 min of 37°C cardioplegic ischemia (* P<0.05 vs HKR; # P<0.05 vs baseline)

Fig. 5 The time course of the ratio of O2 delivery (DO2) to O2 consumption (MVO2) for HKR and BDM groups. Measurements were taken at: baseline (BL), the end of 5 min perfusion with hyperkalemic Krebs' (CP), the end of initial 5 min reperfusion with either oxygenated, hyperkalemic Krebs' (HKR) or BDM Krebs', (IR), and at the given time points of reperfusion in min after the start of perfusion with normal Krebs'. ISCH 40 min of 37°C cardioplegic ischemia (* P<0.05 vs HKR; # P<0.05 vs baseline)

Fig. 6 Flow responses to three different acting coronary vasodilators before and after cardioplegic arrest and reperfusion. (C1–C8 baseline flow immediately before or after the respective vasodilator, ADE adenosine bolus (0.2 ml of 2 mM), 5-HT 5-hydroxytryptamine (1 μM), NP Na+-nitroprusside (100 μM)). After ischemia and reperfusion coronary reserve and endothelium-dependent (5-HT) and endothelium-independent (NP) flow responses were equally depressed in the two groups.
flow during BDM reperfusion may also accelerate toxic metabolite wash out and enhance the nutrient supply to the myocardium.

While potassium promotes vasoconstriction (Fig. 3) [9, 28], BDM in this and previous studies has been shown to be a potent vasodilator [2, 7, 27, 28, 30]. In endothelin preconstricted isolated canine cerebral arteries we found that the ED₅₀ for BDM induced vasodilation was about 1 mM [19]. This suggests that the 20 mM concentration used in the present study produced near maximal dilation. The mechanism of BDM-induced vascular smooth muscle relaxation may have similarities to its negative inotropic effect in myocardium. In guinea pig taenia coli smooth muscle, BDM was suggested to inhibit myosin light chain phosphorylation, to directly decrease force generation at the cross-bridge level and to inhibit intracellular Ca²⁺ translocation [20]. In isolated human uterine artery smooth muscle cells BDM was found to inhibit the depolarization-induced increase in intracellular Ca²⁺ transients [21]. In addition, the vasodilatory potency of BDM does not seem to be mediated by nitric oxide, cGMP, or prostaglandins [19]. After switching from BDM to normal Krebs' in our study, coronary flow remained elevated for only 5–15 min (Fig. 3). At 60 min, the recovery of flow, DO₂/MVO₂ and cardiac efficiency were similar in the two groups. This lack of a long-term effect of BDM suggests that the pronounced beneficial effect during early reperfusion does not necessarily result in better salvage of the coronary vasculature, while LVDP remained improved. This is also supported by the vasodilator responses after cardioplegic arrest and HKR or BDM reperfusion. Coronary reserve, as well as endothelium-dependent and -independent flow increases were present, but depressed, similarly in the two groups (Fig. 6).

In a number of protocols, BDM, when given before or during myocardial ischemia and reperfusion, has improved the recovery of myocardial function. As an additive to University of Wisconsin solution, 30 mM BDM abolished contracture and reduced ATP loss in rabbit hearts stored for 24 h [29]. In dogs, the infusion of BDM (6 ml, 10 mM) into the distal left anterior descending artery (LAD) bed during a 15-min LAD occlusion markedly reduced myocardial stunning, as assessed by measurement of the regional wall function [18]. In isolated guinea pig hearts subjected to low flow cold perfusion for 22 h and to subsequent rewarming, the addition of 10 mM BDM decreased contracture, improved systolic function and reduced arrhythmias [27, 28]. Also in guinea pig hearts, pretreatment with 5 or 10 mM BDM improved myocardial function and reduced reperfusion arrhythmias after 30-min global ischemia [2]. The mechanism of myocardial protection by BDM during ischemia and reperfusion may be related to enhanced O₂ delivery, to reduced myocardial O₂ demand [2, 30], to attenuated ischemic and reflow contracture, and thus cell-to-cell progression of necrosis [7], to its direct depressant action on the contractile apparatus [8, 20] or to a combination of all these effects.

In contrast to other studies discussed above, in the present study BDM was added only during the initial reperfusion period. Our protocol also resulted in an improved myocardial systolic function, i.e. higher LVDP and a tendency toward a higher +dP/dt max as late as 60 min after BDM reperfusion. Although the difference between the HKR and BDM groups seems minor, it was found in comparison to initial hyperkalemic reperfusion (a well established treatment of reperfusion damage) which was very effective as compared to a control group without any treatment in a previous study from our laboratory [9]. Recovery of variables of LV diastolic function, -dP/dt max and tau, was not affected by BDM in our study. In one study BDM (54 mM, 9 ml/min) was directly infused into the LAD only during the first 30 min of reperfusion following 51 min of LAD occlusion in pigs [7]. The authors observed decreased regional systolic function during early reperfusion in the presence of BDM, and reduced infarct size, reduced contraction band necrosis and increased coronary blood flow during reperfusion after BDM [7].

The lack of any beneficial effect of BDM over HKR on the diastolic function in our study may have two reasons. First, we vented the LV during ischemia and early reperfusion, thereby possibly minimizing or masking the damaging effect of reperfusion contracture. Second, we added BDM only for the initial 5 min of reperfusion, which may not be long enough to achieve maximal protection. In isolated rat hearts, it was necessary to add 20 mM BDM to the perfusate for 60 min to induce lasting suppression of the hypercontracture and cardiac myocyte enzyme loss following reoxygenation after 60 min of anoxic perfusion (oxygen paradox) [23]. Removal of BDM after 20 min of reoxygenation resulted in the immediate onset of myocardial damage. Although this anoxia and reoxygenation protocol is not directly comparable with our cardioplegic arrest and reperfusion protocol, these data may indicate that the beneficial effect of BDM in our study could be increased by extending the BDM period beyond 5 min [7].

Little is known about the effects of BDM on reperfusion cardiac rhythm. In pigs intracoronary infusion of BDM (54 mM, 9 ml/min) slightly increased heart rate, but had no effect on the tachycardia following LAD occlusion and reperfusion [7]. In isolated guinea pig hearts, however, BDM at 10 mM, but not at lower concentrations, decreased the heart rate and increased the AV conduction time [2]. When present for 10 min before and 10 min after 30 min of global ischemia, 10 mM BDM significantly reduced the incidence and duration of cardiac dysrhythmias, such as VT or VF during reperfusion [2]. Our study is the first to report an anti-arrhythmic effect during BDM reperfusion after hyperkalemic cardiac ischemic arrest. The incidence and duration of VF as well as VT was markedly reduced after 20 mM BDM, as compared to HKR reperfusion. Since the anti-arrhythmic effect was observed for 20 min after stopping BDM, when all hearts were injected with lidocaine and converted to sinus rhythm, the protection of ex-
citatory tissue from reperfusion damage may be the major factor preventing dysrhythmias, rather than a direct effect of BDM on ion channel activity [16, 32]. In addition, transsarcolemmal ion, (K⁺, Ca²⁺) gradients and thus normal resting membrane potential in myocytes and Purkinje cells may be restored already during BDM reperfusion when the O₂ demand is still near minimum. In contrast, HKR maintains the high extracellular K⁺ concentration of the cardioplegic arrest period until the perfusion is switched to regular Krebs'. Protection from dysrhythmias may be mediated by BDM inhibition of cellular Ca²⁺ overload during early reperfusion, since a high intracellular Ca²⁺ concentration may play a crucial role in the generation of VF [14].

In summary, our data show that after 40 min of cardioplegic ischemic arrest, the addition of BDM into normal Krebs' during the initial reperfusion is superior to HKR in increasing the initial myocardial O₂ delivery to demand ratio. Moreover, reperfusion dysrhythmias are markedly reduced by BDM and the recovery of LV systolic function and O₂ consumption after 60 min are significantly improved compared to HKR. Post-ischemic deterioration of coronary responsiveness to vasodilators was equally affected by BDM and HKR. Further studies are necessary to determine the optimal concentration and duration of BDM perfusion.

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