Effects of positive inotropic stimulation on postischemic myocardium with graded dysfunction

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Abstract

Objective: To investigate the effects of moderate prolonged and of maximum short-term positive inotropic stimulation of postischemic myocardium as a function of the severity of stunning. Methods: Stunned isolated rat hearts (n = 116) after 30 min and 45 min of ischemia were stimulated with dopamine to raise systolic function (double product) back to control levels. In the isovolumetrically beating hearts, left ventricular developed pressure, double product, dp/dt max, coronary flow, and myocardial oxygen consumption were determined during steady-state conditions. After maximum stimulation the contractile reserve was examined. Measurements of adenine nucleotides and electron microscopy were made. Results: 30 min ischemia resulted in moderate postischemic dysfunction LVP 81% P < 0.05. After 45 min ischemia, function was more severely reduced (LVP 66% ± 5%; P < 0.01). Coronary flow tended to be lower after ischemia. Myocardial oxygen consumption was not reduced in parallel with the dysfunction. Adenine nucleotides were gradually reduced after ischemia ATP: 2.5 ± 0.2 and 1.2 ± 0.1 vs. 4.2 ± 0.2 μmol/gww; P < 0.01. Contractile reserve also decreased in relation to the previous ischemic injury (after 45 min ischemia max. LVP 105 ± 10% vs. max. LVP 152 ± 8% in controls, P < 0.01). Prolonged stimulation did not result in further reduction in adenine nucleotides and function. Conclusions: Contractile reserve is decreased in postischemic myocardium in parallel with the previous ischemic burden. Depending on the degree of contractile dysfunction a disturbed function–flow–oxygen consumption relation is present. Prolonged stimulation of stunned myocardium with dopamine back to the control level of function has no harmful short-term effects, indicating sufficient mitochondrial energy generation.

Keywords: Myocardial ischemia; Stunning; Contractile reserve; High energy phosphates; Oxygen uptake; Dopamine; Rat, heart

1. Introduction

Prolonged postischemic reversible dysfunction without signs of tissue necrosis has been termed ‘stunned myocardium’ [1]. A multitude of possible explanations for this phenomenon has been discussed [2], but the definite causes are still not clear. Failure of acceleration of functional recovery after postischemic enhancement of the high-energy phosphate levels ruled out a causal role of reduced adenine nucleotide levels for the stunning phenomenon [3]. These findings were supported by the normal respiratory function of postischemic mitochondria in the presence of decreased adenine nucleotide levels [4].

Inotropic stimulation of postischemic myocardium with reversible contractile dysfunction was described in different models [5–7]. In a canine model of regional ischemia, postischemic contractile function could be stimulated by intracoronary calcium to a similar level as a control region [5]. In contrast, graded postextrasystolic potentiation after regional ischemia did evoke a comparable relative increase of wall thickening in postischemic and control regions but did not result in a similar amount of postextrasystolic wall thickening [7]. Also other studies using short-term stimulation did not report a normal contractile reserve of reperfused myocardium to inotropic stimulation [8]. A possible explanation for a depressed reaction to inotropic stimuli
might be an altered Ca\textsuperscript{2+}-handling in the sarcoplasmic reticulum [9–12]. Comparison of the previous studies cited is limited by the different models used. Systematic evaluation of the effect of graded ischemic injury on contractile reserve has not been reported so far.

Prolonged postischemic stimulation should test the mitochondrial energy-generating capacity of stunned myocardium. Prior studies indicated a dissociation between recovery of contractile function and oxidative metabolism in postischemic myocardium [13]. Accordingly, a relatively high coronary flow was found in relation to postischemic function in some studies as well as a disproportionally high myocardial oxygen consumption [14–18], while others observed a reduction of both function and coronary flow [5,19,20].

The capability of postischemic myocardium to cope with the energetic requirements of a steady-state positive inotropic stress has not yet been investigated, but it is known that even in normal myocardium a breakdown of high-energy phosphates can be induced by prolonged marked stimulation [21]. Inotropic stimulation of postischemic myocardium is clinically often necessary, therefore a more detailed knowledge of the consequences of inotropic support is mandatory. Since the effects both of short- and long-term inotropic stimulation of postischemic myocardium as a function of the severity of stunning have not been investigated so far, the present study was undertaken to examine action on moderately and severely stunned myocardium: (1) A maximum stimulation was performed to characterize the reserve of the contractile apparatus. (2) Steady-state postischemic stimulation to restore left ventricular pressure to the control level was performed to investigate the feasibility of a prolonged inotropic stress with respect to energy generation in stunned myocardium after a previous ischemic injury of graded severity.

2. Methods

2.1. Isolated perfused rat heart

Male wistar rats (Thomae GmbH, Biberach, Germany) weighing 280–350 g with free access to water and feeding with a standard laboratory animal feed (Altromin GmbH, Lage-Lippe, Germany) were anaesthetized by urethane (2.5 ml/kg of 50% solution intraperitoneally). Ventilation with room air (Starling pump, Braun, Melsungen, Germany) was established after introducing a small plastic tube into the trachea by a tracheotomy. The chest was opened with a median sternotomy and 100 IU heparin were injected intravenously. After preparation the heart and the ascending aorta were excised and rapidly immersed in ice-cold saline. Adjacent tissue was rapidly trimmed away, the aorta was mounted quickly on a steel cannula and retrograde perfusion was initiated. The perfusion was performed using a modified Langendorff apparatus (non-recirculating system). The hearts were perfused with a Krebs-Henseleit buffer consisting of NaCl 115 mmol/l, NaHCO\textsubscript{3} 25.0 mmol/l, KCl 4.0 mmol/l, KH\textsubscript{2}PO\textsubscript{4} 0.9 mmol/l, CaCl\textsubscript{2} 2.6 mmol/l, MgSO\textsubscript{4} 1.1 mmol/l and glucose 5.5 mmol/l. The perfusate was warmed to 37°C and gassed with 95% O\textsubscript{2}/ 5% CO\textsubscript{2}. The perfusion pressure was set at 100 cm H\textsubscript{2}O. A fluid-filled latex balloon connected via short plastic tubing to a Statham P23Db transducer (Gould Inc., Oxnard, CA, USA) was introduced via the mitral valve into the left ventricular cavity. The pressure signal was amplified and differentiated (dp/dt) using a physiological data recorder and monitored on a strip chart recorder (Hellige B78-18802, Freiburg, Germany). After instrumentation the hearts were placed in a temperature-regulated glass jacket in order to maintain the temperature.

Coronary flow was measured volumetrically using calibrated glass cylinders. Myocardial oxygen consumption was calculated using the pO\textsubscript{2} of the gassed perfusate and of the coronary effluent (oximeter PHM 72c with a Clark electrode), Radiometer, Copenhagen). The coronary arterial/venous difference was then calculated and multiplied by the coronary flow. The myocardial oxygen consumption was related to the left ventricular weight and expressed as \mu mol/g/min. In normal hearts without intervention this determination correlates highly with the myocardial oxygen demand calculated according to Huetter et al. [22].

From the hemodynamic recordings the left ventricular developed pressure, dp/dt\textsubscript{max}, heart rate, the double product (developed pressure × heart rate) and the myocardial oxygen demand according to Huetter et al. [22] were derived.

From the hearts for the biochemical evaluation the distal 2/3 of the left ventricle were cut off and rapidly deep-frozen between precooled aluminium blocks in liquid nitrogen. From the remaining part of the left ventricle the wet weight/dry weight ratio was calculated. All biochemical concentrations were given related to a standardized tissue water content of 79%. Determinations of ATP, ADP and AMP were made using previously reported standard procedures after perchloric acid extraction using a bioluminescence technique [23].

For histological examination hearts were perfusion-fixed at the end of the experiments. For fixation the hearts were perfused with 2.5% glutaraldehyde (buffered in 0.1 M cacodylate adjusted to pH 7.4) and a pressure of 100 cmH\textsubscript{2}O for 4–5 min. The hearts were then kept in the same solution for 1 day and thereafter rinsed with cacodylate buffer with additional 7.5% saccharose [24]. Four to 6 blocks from the left ventricular free wall and the anterior and posterior papillary muscle were cut and after post-fixing with osmium tetroxide embedded in Epon. Ultra-thin sections were examined electron-microscopically after staining with uranyl acetate and lead citrate and classified as normal, reversibly injured or irreversibly injured according to a previously described grading system [25].
2.2. Protocol

After completion of the preparation the hearts were allowed to stabilize unloaded for 10 min with retrograde perfusion (Fig. 1). The intraventricular balloon was then inflated and adjusted to a volume corresponding to an end-diastolic left ventricular pressure of 6 mmHg. This pressure was used for all periods of isovolumic contraction. There was no significant change in the balloon volumes over time in the different groups. Over a period of 10 min the hearts then stabilized again as isovolumically contracting hearts. After this initial stabilization period the control data were obtained. Thereafter 30 or 45 min of no-flow ischemia with unloaded balloon were performed. In the control group the hearts beat meanwhile with retrograde perfusion and deflated balloon. After the period of ischemia the hearts had a 15 min period of unloaded reperfusion. They were subsequently switched to the isovolumic beating mode. After 10 min isovolumically beating data were obtained again. In the posts ischemic intervention groups infusion of dopamine solution (0.5 mg/ml; Nattermann and Cie GmbH, Cologne, Germany) by a side-branch of the aortic cannula was started. The dose was frequently adjusted to obtain a systolic function comparable to the control status by means of repeated calculation of the double product aiming at a target range of 97 ± 10% of the individual preischemic control value. Infusion was performed using a precision pump system (Braun, Melsungen, Germany). There was no statistical difference in the amount of dopamine applied in the different groups. In the posts ischemic control groups saline solution was infused by means of an identical system. During the infusion period hemodynamics were continuously registered. Afterwards, the hemodynamic data were integrated over the 20 min of infusion performed in the intervention groups. After 20 min of infusion drug administration was stopped. After subsequent 10 min of washout the hemodynamics of the isovolumically beating hearts were determined again. Thereafter, in half the hearts a maximum catecholamine stimulation via the side-branch was performed with a dose of 0.33 mg/min dopamine (chosen after being tested in this model to generate the maximum response). Thereafter the dose was tripled twice in order to assure measurement of the maximum reserve even in posts ischemic hearts. The highest values obtained for developed left ventricular pressure and dp/dt max were accepted as maximum response. In no case did the 3 mg/min exceed the effect of 1 mg/min. The other half of the hearts were used for biochemical evaluation.

All animal experiments were performed in accordance with the national regulations on the use of laboratory animals and the protocol was approved by the local committee on ethics in animal research.

Hemodynamic data were expressed as a percentage of the preischemic control level (n = 20–24 hearts/group). All data were given as means ± s.e.m. The preischemic control data (control group: LVP 141 ± 3 mmHg; dp/dt max 2943 ± 9 mmHg/s, heart rate 258 ± 7/min, double product 36 363 ± 1197 mmHg × min) were comparable in all groups; the means varied < 10%. Two different sets of data were statistically evaluated: the average data during infusion of dopamine and the data at the end of the experiment. For comparison among the groups an ANOVA was used and in the case of a significant difference thereafter Dunnet’s test was performed according to the proposal by Wallenstein et al. [26].

3. Results

3.1. Myocardial function

After 30 min of no-flow ischemia and short unloaded reperfusion the developed isovolumic left ventricular pressure, dp/dt max and the double product were significantly reduced to about 80% of the preischemic data compared with an only slight reduction in these indices for about 5% in the control hearts (Table 1). The data of the posts ischemic control group and of the posts ischemic intervention group were comparable (Table 1). No significant further change was observed in the non-ischemic and in the posts ischemic control hearts during reperfusion until the end of the experiments (Table 1).

After 45 min of ischemia a more pronounced reduction in functional indices was observed. Developed isovolumic left ventricular pressure, dp/dt max and the double product were reduced to 60% (Table 1). Before intervention the data of both groups after 45 min of ischemia were comparable and during reperfusion no further change in the indices of function occurred in the posts ischemic control group (Table 1). The heart rate remained constant in all groups (Table 1).
Table 1

<table>
<thead>
<tr>
<th>Hemodynamic data 20 and 50 min after ischemia (end of the experiments 10 min after cessation after dopamine or saline infusions) of isovolumetrically beating rat left ventricles indicating stable-graded postischemic dysfunction</th>
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<tr>
<td><strong>Double product</strong></td>
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<tr>
<td>Controls</td>
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<td>30 min ischemia</td>
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Data are means ± s.e.m. in % of preischemic function; ANOVA and Dunnett test at the end of the experiments; double product = left ventricular pressure × heart rate; LVP = left ventricular developed pressure.

3.2. Contractile reserve

As a measure of the contractile reserve the developed isovolumic left ventricular pressure increased to 152 ± 8% and the dp/dt<sub>max</sub> to 188 ± 10% in the control hearts (increases of 70 ± 7 mmHg and of 2289 ± 286 mmHg/s). The contractile reserve to dopamine in the 30 min ischemia group was 126 ± 9% for LV developed pressure and 157 ± 15% for dp/dt<sub>max</sub> (n.s.; Fig. 2) (increases of 58 ± 8 mmHg and of 1853 ± 313 mmHg/s) at the end of the experiments. After 45 min of ischemia and reperfusion maximum dopamine increased left ventricular developed pressure to 105 ± 10% and dp/dt<sub>max</sub> to 141 ± 15% (P < 0.01/P < 0.05 vs. controls; Fig. 2) (increases of 48 ± 7 mmHg and 1570 ± 236 mmHg/s).

3.3. Effects of prolonged postischemic stimulation

The dopamine infusion dose was adjusted repeatedly during the stimulation period to get the control level of function in the postischemic intervention groups. The total dopamine dose in the 20 min stimulation period to establish this level was not significantly different in the groups after 30 min and after 45 min of ischemia (0.85 ± 0.14 vs. 0.77 ± 0.11 mg/ml); the hemodynamic data of both intervention groups during stimulation were also comparable (Table 2, Figs. 3 and 4). After cessation of dopamine and a 10 min wash-out period left ventricular function was comparable in the postischemic intervention and control groups (Table 1).

The maximum contractile reserve of the long-term stimulated postischemic hearts was also comparable to that of the postischemic control hearts (without prolonged stimulation) with an increase of LV pressure and dp/dt<sub>max</sub> to 124 ± 7 and 170 ± 12% (n.s. vs. controls) in the 30 min ischemia group and to 89 ± 7 and 116 ± 10% in the 45 min ischemia group (P < 0.01 vs. controls).

3.4. High-energy phosphates

A graded reduction in the ATP levels was found depending on the duration of the previous period of ischemia.

Table 2

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<tr>
<th>Integrated hemodynamic data during dopamine stimulation/saline in postischemic isovolumetrically beating rat left ventricles (from 20–40 min post-ischemia)</th>
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<tbody>
<tr>
<td><strong>Double product</strong></td>
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<td>---------------------</td>
</tr>
<tr>
<td>Controls</td>
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<tr>
<td>30 min ischemia + saline</td>
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<td>30 min ischemia + dopamine</td>
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<td>45 min ischemia + dopamine</td>
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Data are means ± s.e.m. in % of preischemic control; † P < 0.05; † P < 0.01 vs. control group (ANOVA/Dunnett test); double product = left ventricular pressure × heart rate; LVP = left ventricular developed pressure.
With postischemic stimulation a relatively high MVO during contractile function without parallel reduction in coronary flow and oxygen consumption. Data indicate marked reduction in postischemic min of ischemia are identical to the respective postischemic control groups Table 3. Prolonged stimulation of the postischemic ventricles to an elevated level of function did not cause a further reduction in the high-energy phosphate levels. The reduction in the high-energy phosphate levels was identical to the expected level according to Hutter et al. [22] (88 ± 5% /89 ± 3%). Dopamine stimulation adjusted to renormalize the double product resulted in a myocardial oxygen consumption index 96 ± 8% /105 ± 4%). After cessation of the stimulation hemodynamics were similar to the postischemic control group (Table 1).

After 45 min ischemia coronary flow and myocardial oxygen consumption did not decrease in parallel with the depressed function (Fig. 4). The calculated expected myocardial oxygen consumption index (72 ± 5%) was significantly lower than the measured data (90 ± 6%; P < 0.05). During dopamine stimulation an increase of the coronary flow (n.s.) and myocardial oxygen consumption to 106 ± 7% was observed (P < 0.01, intra-individual), whereas the expected myocardial oxygen consumption was lower (93 ± 4%) and not increased compared to the control level (89 ± 3%). After stimulation hemodynamics of postischemic hearts were comparable to unstimulated postischemic control hearts (Table 1).

### Table 3

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<th>ATP (µmol/gww)</th>
<th>ΣAN (µmol/gww)</th>
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<tbody>
<tr>
<td>Controls</td>
<td>4.2 ± 0.2</td>
<td>5.5 ± 0.3</td>
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<tr>
<td>30 min ischemia</td>
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<tr>
<td>Saline</td>
<td>2.5 ± 0.2 †</td>
<td>3.7 ± 0.2 †</td>
</tr>
<tr>
<td>Dopamine</td>
<td>2.5 ± 0.2 †</td>
<td>3.6 ± 0.3 †</td>
</tr>
<tr>
<td>45 min ischemia</td>
<td></td>
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<tr>
<td>Saline</td>
<td>1.2 ± 0.1 †</td>
<td>2.4 ± 0.2 †</td>
</tr>
<tr>
<td>Dopamine</td>
<td>1.4 ± 0.2 †</td>
<td>2.9 ± 0.1 †</td>
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Data are means ± s.e.m.; † P < 0.01 vs. control group (ANOVA/Dunnett test); n = 8 hearts/group; ΣAN = ATP + ADP + AMP.

### 3.6. Histological examination

Electron microscopy revealed no signs of irreversible postischemic damage in the various specimens of the left ventricular free wall. No signs were found of severe reversible postischemic damage according to the previously reported grading system [24]; after 50 min of reperfusion the ultrastructure was well preserved after both 30 and 45 min of ischemia (Fig. 5). Since no areas of

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Fig. 3. Mean hemodynamics (integrated data) during postischemic dopamine (dop.) or saline (contr.) infusion from 20–40 min after ischemia (30 min) in isovolumetrically beating left ventricles vs. non-ischemic controls. Controls = no previous ischemia; postisch. controls = saline infusion after ischemia; postisch. dop. = dopamine infusion after ischemia to obtain steady-state function at control level. DP = double product (LVP × heart rate); CF = coronary flow; MVO₂ = myocardial oxygen consumption. Data indicate moderate reduction in postischemic function with complete normalization during stimulation (means ± s.e.m. in % of preischemic control, comparison to the controls using ANOVA).

Fig. 4. Mean hemodynamics (integrated data) during postischemic dopamine (dop.) or saline (contr.) infusion from 20–40 min after ischemia (45 min) in isovolumetrically beating left ventricles vs. non-ischemic controls. Controls = no previous ischemia; postisch. contr. = saline infusion after ischemia; postisch. dop. = dopamine infusion after ischemia to obtain steady-state function at control level. DP = double product (LVP × heart rate); CF = coronary flow; MVO₂ = myocardial oxygen consumption. Data indicate marked reduction in postischemic contractile function without parallel reduction in coronary flow and MVO₂. With postischemic stimulation a relatively high MVO₂ during postischemic stimulation was observed (means ± s.e.m. in % of preischemic control, comparison with the controls using ANOVA).
irreversible tissue damage were found, morphometric methods could not be applied.

4. Discussion

The stepwise reduction in steady-state contractile function after graded ischemic burden is a known finding in reversible postischemic injury [23,27] as well as the gradually reduced content of adenine nucleotides (as a known indicator of the severity of the previous ischemic stress [28]). In the present study the stepwise reduction in function and high-energy phosphates characterizes two different degrees of postischemic injury (termed ‘moderate’ and ‘severe’), in which the absence of irreversible alterations according to a previously evaluated grading system was histologically proven [25].

Our data indicate that: (1) contractile reserve is limited in postischemic myocardium depending on the previous ischemic burden as shown for the first time; (2) steady-state contractile function can be maintained even in severely injured postischemic myocardium with markedly reduced ATP levels by prolonged inotropic stimulation without deterioration of energy metabolism; (3) coronary flow and myocardial oxygen consumption during reperfusion do not parallel the degree of reduction in function after graded reversible myocardial injury—there is an increasing discrepancy between the measured and the expected oxygen consumption with increasing severity of postischemic myocardial injury.

4.1. Contractile reserve

Conflicting findings have been reported regarding the extent of the contractile response of postischemic myocardium to inotropic stimulation with observation of either an incomplete restoration of contraction [29,30] or an about-normal response [5,31]. Some conflicting findings in the literature might result from differences in experimental set-ups (e.g., systemic inotropic stimulation after regional ischemia vs. global ischemia or in the severity of ischemic injury). In the present study we observed a significant reduction not only in steady-state function but also in contractile reserve parallel to the extent of the postischemic injury. After moderate injury we observed a strong trend towards a reduced response; a significant reduction was obtained only after more severe ischemic injury. Such a relationship has not been reported so far.

After 15 min of coronary occlusion Ito et al. [5] did not observe a significant difference in segment shortening in percent between postischemic and normal segments after high-dose intracoronary Ca2+, but these authors found a different dose–response curve after lower doses of Ca2+. In their study (with a clear dose relationship in the postischemic segments) there was no significant further increase in the response to the submaximum and maximum dose in the normal segment in contrast to the postischemic segment. To exclude a possible different dose–response curve pattern in postischemic and normal myocardium, we used a dose higher than the lowest one necessary for the maximum response in normal hearts to rule out submaximum stimulation of postischemic myocardium.

In the present study the extent of the contractile response to stimulation was not markedly different after moderate stunning between control and postischemic hearts, whereas the obtained maximum level in the left ventricles with reduced postischemic function prior to stimulation was consecutively lower. This is in accordance with findings after regional ischemia and treatment with a
Ca\(^{2+}\)-sensitizer: the same extent of response to stimulation (but on a lower level of function) was observed during early reperfusion [32]. However, a direct comparison of the results from experiments with regional or global ischemia is limited. Furthermore, the reduction in ATP was not determined in many of these studies. Thus we report for the first time on a reduction in contractile reserve after ischemia of graded severity in relation to the postischemic ATP.

### 4.2. Prolonged inotropic stimulation

Several studies have shown that stunned myocardium is capable of responding—at least for a short period of time—to inotropic stimuli [5,32,33] and that the efficiency of energy transfer might be restored [34]. However, only a few, and in part controversial, data are available dealing with a longer positive inotropic stimulation after global myocardial ischemia: After a severe 60 min low-flow (5%) ischemia and inotropic stimulation with maximum (not attenuated) dose and with fixed coronary flow a reduction in the ATP content was observed [35] similar to the ATP reduction reported by Arnold et al. [36] after stimulation of partially postischemic myocardium. These findings are in accordance with data from Giesen et al. [21], who observed a marked reduction in the high-energy phosphate levels during maximum inotropic stimulation. In contrast, after a less severe ischemic injury (20 min global ischemia with hypothermia and less 20% ATP reduction), other authors [6] found no significant reduction of the ATP level during a shorter period of submaximum stimulation. Since we aimed to examine whether stunned myocardium with moderate and marked injury but without artificial coronary flow restriction can maintain normal steady-state function and ATP levels for a prolonged time without consecutive aggravation of the postischemic injury, we chose a prolonged stimulation only up to the control level of function of gradually stunned myocardium. Our findings indicate that prolonged stimulation to this level has no harmful effect. This was observed not only after moderate stunning, but also after severe reversible ischemic injury. The contractile apparatus is capable of working on a normal level for a longer time even after severe reversible ischemic injury and is thus not a main cause of ‘stunning.’ However, this does not rule out a supposed disturbance of the Ca\(^{2+}\)-handling in stunned myocardium [5,8]. Furthermore the energy-generating apparatus is not the cause of the stunning phenomenon but the reduction in ATP during ischemia is an indicator of the severity of ischemia [18]. Accordingly an acceleration of the functional recovery of postischemic myocardium could not be obtained by successful elevation of postischemic ATP levels [3,37] and the function of mitochondria from stunned myocardium was described as intact [4]. Mitochondria in the present study were also able to maintain the energy level during the prolonged inotropic stimulation. The constant ATP levels during stimulation are a direct proof of the hypothesis of Bolli et al. [38] that energy generation should be sufficient in reversibly injured dysfunctional postischemic myocardium.

### 4.3. Coronary flow and myocardial oxygen consumption

Coronary flow and myocardial oxygen consumption in stunned myocardium continue to be a topic of debate. Some authors describe a reduction in coronary flow [5,19,20] parallel to the decreased function, whereas other studies describe a normal or unproportional high coronary flow in stunned myocardium [15,18,39]. One explanation for the disturbed flow–function coupling might be vascular stunning [19] (which might result in either vasoconstriction or dilation). However, there is also evidence that not only coronary flow but also myocardial oxygen consumption is not related to the reduced function in stunned myocardium but is unchanged or even increased [13,14,17,18,40]. As possible explanations for these discrepancies, species differences or differences in function in myocardium termed stunned (especially difficult to assess in regional ischemia) in different experimental settings are discussed [15]. In the present study with a model of graded postischemic global injury a trend to a parallel reduction in myocardial function, coronary flow and myocardial oxygen consumption after moderate injury with normalization during positive inotropic stimulation to the control level of function was observed. The expected myocardial oxygen demand calculated according to Hütter et al. [22] was not different from the measured O\(_2\) consumption, indicating an intact flow–function–O\(_2\) consumption relation 50 min after moderate ischemic injury. In contrast, in stunned myocardium with more severe reduction in function, coronary flow and O\(_2\) consumption did not decrease further but tended towards levels higher than control during inotropic stimulation. The relation between expected and measured oxygen consumption was altered, indicating an uncoupling of the flow–function–O\(_2\) consumption relation in severely stunned myocardium. This may be caused by disturbance of oxidative metabolism [13] leading to reduced efficiency and would be in agreement with the findings of Ito et al. [5] with a reduction of coronary flow in stunned myocardium, whereas Görge et al. [13] described increasing disturbance of oxidative metabolism with increasing postischemic injury. In contrast to the findings of Ito et al. [5] in dogs, another study using swine [15] from the same laboratory did not observe a reduction of coronary flow after regional ischemia. The lack of collateral flow in pig hearts possibly resulting in a more severe ischemic injury might explain this discrepancy between the two studies. This interpretation would mean that these results are in accordance with our findings of a disturbed flow–function relation in more severely stunned myocardium.
4.4. Limitations of the study

Isolated hearts are known to be an imperfect model of stunning [2], but the biochemical and histological features of the present model are characteristic for stunned myocardium [1]. Full functional recovery as a major part of the definition of stunning cannot be proven in an in vitro model. However, this limitation applies also to many open-chest models of stunning [2]. Therefore, conclusions on the lack of harmful effects of stimulation of postischemic myocardium are limited to the short term. Nevertheless, stimulation not only to achieve a short maximum response but for 20 min means a prolonged steady-state stimulation, since an imbalance in the energy metabolism nevertheless stimulation is the absence of several limitations of regional ischemia with regard to the exact characterization of functional status [41] (e.g., problems of regional interaction and collateralization). The relatively long periods of ischemia without development of histological signs of irreversible damage are probably explained by the experimental set-up and protocol. Comparison with other studies only with respect to the time of ischemia is therefore limited, but the postischemic metabolic status of the myocardium should be taken into account when comparing different studies.

Stimulation with dopamine (a catecholamine often used clinically) increases the heart rate. Especially in more severely injured hearts this action caused a more pronounced effect on heart rate than on left ventricular pressure. In order to avoid too extreme tachycardia, steady-state stimulation in the more severely stunned group tended (n.s.) towards slightly lower levels of functional stimulation than in the moderately stunned group, thus supporting the observation of an overshoot of coronary flow and myocardial oxygen consumption in more severely injured hearts during stimulation.

5. Conclusion

The contractile reserve is reduced and the coronary flow–O₂ consumption–function relation is altered in stunned myocardium. The dependency of these alterations on the degree of the previous ischemic burden in relation to biochemical markers of injury was demonstrated for the first time in the present study. Our results might explain in part the controversies in the literature about whether contractile reserve is preserved in stunned myocardium.

Our findings demonstrate for the first time that reversibly injured postischemic myocardium can be stimulated for a prolonged period of time independently of the severity of stunning without harmful effects on function or high-energy phosphate metabolism. This finding indicates the ability of the damaged mitochondria to generate and deliver sufficient energy and the ability of the coronary circulation to cope with this challenge (if flow is not technically restricted). This observation is of importance for the treatment of patients with postischemic myocardium after revascularization or after a severe heart attack without myocardial necrosis.

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