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ATENOLOL DEPRESSES POST-ISCHAEMIC RECOVERY IN THE ISOLATED RAT HEART

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Metabolic events during ischaemia are probably important in determining post-ischaemic myocardial recovery. The aim of this study was to assess the effects of the β-blocker atenolol and the high energy demand in an ischaemia-reperfusion model free of neurohormonal and vascular factors. We exposed Langendorff-perfused isolated rat hearts to low-flow ischaemia (30 min) and reflow (20 min). Three groups of hearts were used: control hearts ($n = 11$), hearts that were perfused with 2.5 μg l^{-1} atenolol ($n = 9$), and hearts electrically paced during ischaemia to distinguish the effect of heart rate from that of the drug ($n = 9$). The hearts were freeze-clamped at the end of reflow to determine high-energy phosphates and their metabolites. During ischaemia, the pressure-rate product was 2.3 ± 0.2, 5.2 ± 1.1, and 3.3 ± 0.3 mmHg 10^3 min in the control, atenolol and paced hearts, respectively. In addition, the ATP turnover rate, calculated from venous (lactate), oxygen uptake and flow, was higher in atenolol (11.2 ± 1.7 μmol min^{-1}) and paced (8.1 ± 0.8 μmol min^{-1}) hearts than in control (6.2 ± 0.8 μmol min^{-1}). At the end of reflow, the pressure $\times$ rate product recovered $75.1 \pm 6.4\%$ of baseline in control *vs* 54.1 ± 9.1 and $48.8 \pm 4.4\%$ in atenolol and paced hearts ($P < 0.05$). In addition, the tissue content of ATP was higher in the control hearts (15.8 ± 1.0 μmol g_{dw}^{-1}) than in atenolol (10.5 ± 2.6 μmol g_{dw}^{-1}) and paced (10.9 ± 1.3 μmol g_{dw}^{-1}) hearts. Thus, by suppressing the protective effects of down-regulation, both atenolol and pacing apparently depress myocardial recovery in this model.

KEY WORDS: low-flow ischaemia, β-blocker, bioenergetics, high-energy phosphates.

INTRODUCTION

Post-ischaemic myocardial recovery depends on several not yet clarified factors, but metabolic events during ischaemia are probably important [1]. Ischaemic down-regulation of contractility plays a key role in linking metabolism during ischaemia to mechanical recovery during reflow. The total tissue contents of adenine nucleotides and purines (TANP) are maintained in isolated rat hearts during 90% flow reduction if myocardial activity is down-regulated [2]. This feature, that improves post-ischaemic recovery regardless of free radicals scavenging [3], is attributed to energy demand-to-supply matching. Coronary flow disturbs down-regulation: when normal-flow hypoxemia replaces low-flow ischaemia, the beneficial effects of down-regulation are suppressed [4]. However, the role of energy demand during ischaemia is still unclear despite its relevance in pharmacological treatments that involve modulation of myocardial contractility.

β-blockers are commonly used for the treatment of ischaemic heart disease [5]. Besides their neurohormonal [6, 7] and platelet-inhibiting effects [8], they decrease myocardial O_2 consumption [9] and enhance carbohydrate metabolism and energy production [10, 11]. Atenolol, a selective β-1 adrenergic antagonist [12] with specific effect on Ca^{2+} currents [13] and intracellular acidosis [14], enhances mitochondrial ATPase activity [15]. Although β-blockers, propranolol and metoprolol, reduce episodes of transient myocardial ischaemia [16], atenolol apparently does not protect against ischaemia-reperfusion

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in a model of isolated perfused heart exposed to global no-flow ischaemia [17].

The aims of this study are: (1) to assess the metabolic effects of atenolol in an ischaemia-reperfusion model free of neurohormonal and vascular factors; and (2) to use atenolol as a probe to examine the effect of energy demand on post-ischaemic recovery in the same way as hypoxemia helps to understand the effect of coronary flow. Thus, besides its pharmacological value, this study may help to assess whether drug-induced changes in energy demand during ischaemia altered energy demand-to-supply matching and recovery. As the heart rate was a disturbing variable between control and atenolol-perfused hearts, we also studied the effects of this variable in the absence of the drug. We show that, by increasing energy demand during ischaemia, neither atenolol nor electrical pacing are beneficial during reflow, as reflected by a depressed performance and tissue level of high-energy phosphates. This confirms that down-regulation of energy demand during ischaemia is an effective way to protect the myocardium.

MATERIALS AND METHODS

Heart perfusion

Apparatus and procedures are described elsewhere [4]. Briefly, CD outbred rats (Charles River, Calco, Italy, weight 250–270 g) were anaesthetised, hearts were excised and immediately perfused. The medium (Krebs-Henseleit buffer, 2 mM free Ca^{2+}, 11 mM glucose) was oxygenated ($P_{O_2} = 670$ mmHg) in membrane oxygenators. When specified, 2.5 μg l^{-1} atenolol (Sigma Chemicals, St. Louis, MO) was added to the medium. A peristaltic pump (Minipuls 3, Gilson, France) delivered the medium to a preheater (37°C) and the aortic cannula. We monitored the coronary perfusion pressure by a pressure transducer (Harvard Apparatus, mod 52-9966, Natick, MA) connected to the aortic cannula. Heart rate (HR), developed pressure (LVDP), end-diastolic pressure (EDP), maximal rate of pressure development ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were monitored by a pressure transducer connected to the intraventricular balloon. A cannula inserted in the pulmonary artery was used to collect the venous outflow for measurement of lactate (Sigma Diagnostics, St. Louis, MO) and P_{O_2} (YSI mod 5300 Oxygen Monitor, Yellow Springs Inc, OH). Stimulation electrodes (Harvard, South Natick, MA, Square wave stimulator, 5 ms pulse duration, 10 V pulse amplitude) were placed on the aortic cannula and on the apex of the ventricle. An Apple Macintosh Quadra 700 (Cupertino, CA) was used to acquire data (LabView 3.0, National Instruments, Austin, TX) and to control the speed of the peristaltic pump (NB-MIO-16 Multifunction I/O Board, National Instruments, Austin, TX).

Experimental design

The hearts were equilibrated for 20 min at a perfusion pressure of 100 mmHg and the volume of the intraventricular balloon was fixed to yield an EDP = 8–9 mmHg. After baseline measurements, hearts were exposed to ischaemia for 30 min by decreasing the pump speed to yield a perfusion pressure of 10 mmHg. Measurements were taken at the end of ischaemia with stable myocardial performance. Hearts were then reperfused for 20 min under the same conditions of baseline, measurements were taken again, and hearts were freeze-clamped for metabolic assays. Hearts were divided into three groups: *control* ($n = 11$), *atenolol* ($n = 9$, 2.5 μg l^{-1} atenolol in the medium), and *paced* ($n = 9$, electrically paced during ischaemia at 160 ± 6 min^{-1}). An additional group (*atenolol* and *paced*, $n = 9$) was designed to monitor the simultaneous effects of atenolol and pacing.

Metabolic measurements

Freeze-clamped tissue was assayed for ATP, ADP, AMP, adenosine, inosine-5′-monophosphate, inosine, hypoxanthine, xanthine, urate, phosphocreatine and creatine by high-pressure liquid chromatography [18]. Calculations include: TANP (sum of these substances except phosphocreatine and creatine), O_2 uptake (V_{O_2}, from flow, arterial and venous P_{O_2}), net lactate release [J_{lac} = venous (lactate) · flow], ATP turnover rate ($J_{ATP} = J_{lac} + 6 \cdot V_{O_2}$) e.g. assuming ATP/lactate = 1.0 (glucose as the only substrate without significant glycogenolysis) and ATP/O_2 ratio = 6 (no mitochondrial uncoupling).

Statistics

Data are expressed as mean ± SE. One-way ANOVA followed by the Fisher's multiple comparison test was performed (StatView 4 software, Abacus Concepts, Inc, Berkeley, CA), with the significance level set at $P = 0.05$ (two-tailed).

RESULTS

All hearts of the three main groups kept contracting throughout the protocol and are suitable for statistical analysis. In contrast, *atenolol* and *paced* hearts generally stopped contracting during ischaemia and could not be considered any further. Table I shows that 2.5 μg l^{-1} atenolol did not affect performance during baseline perfusion in this model.

When perfusion pressure was reduced to 10 mmHg, coronary flow was 0.8–0.9 ml min^{-1} in the *control* and *paced* groups, *vs* 1.2 ml min^{-1} in the

Table I
Baseline performance (mean ± SE) and effect of 2.5 μg l^{-1} atenolol at perfusion pressure = 100 mmHg, arterial PO_2 = 670 mmHg. No significant differences were detected

Parameter	Control	Atenolol
n	20	9
Coronary flow (ml min^{-1})	12.3 ± 0.6	12.5 ± 0.7
Heart rate (min^{-1})	234 ± 5	233 ± 8
End-diastolic pressure (mmHg)	9.0 ± 0.5	8.1 ± 0.8
Developed pressure (mmHg)	119 ± 5	130 ± 8
Maximal rate of contraction (mmHg s^{-1})	3331 ± 294	3659 ± 163
Maximal rate of relaxation (mmHg s^{-1})	2076 ± 140	2354 ± 69
Oxygen uptake (μmol min^{-1})	6.7 ± 0.4	6.9 ± 0.4
Lactate efflux (μmol min^{-1})	< 0.1	< 0.1
ATP production rate (μmol min^{-1})	40.2 ± 2.4	41.4 ± 2.6

atenolol group ($P = 0.03$). Thus, atenolol decreased coronary resistance during low-flow ischaemia from 15.3 ± 2.6 mmHg min ml^{-1} to 8.2 ± 1.3 mmHg min ml^{-1} ($P = 0.03$). During low-flow ischaemia, atenolol induced a higher HR and lower LVDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ than in *control* hearts (Fig. 1). However, the (pressure × rate) product was 2.3 ± 0.2 and 5.2 ± 1.1 mmHg 10^3 min ($P = 0.01$) in *control* and *atenolol* hearts.

To distinguish the effects of HR from those of the drug, *paced* hearts were stimulated at the same HR as that found in *atenolol* hearts. In these hearts, LVDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ were the same as in *atenolol* hearts. The (pressure × rate) product was 3.3 ± 0.3 mmHg 10^3 min ($P = 0.01$ *vs* control, P = NS *vs atenolol*).

During ischaemia, venous (lactate) and lactate efflux were higher in *atenolol* and *paced* hearts than in *control* hearts (Fig. 2). By contrast, VO_2 was higher than normal in *atenolol* hearts only, whilst in *paced* hearts VO_2 was similar to *control*. When J_{ATP} is calculated as described, it was similar in *paced* and *control* hearts, but higher in *atenolol* hearts.

During reflow, the recovery of mechanical perfor-

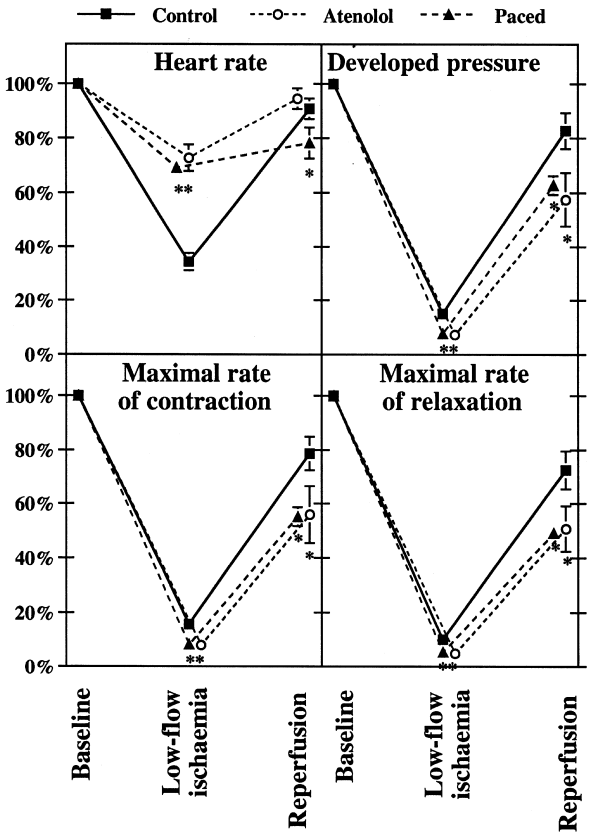

Fig. 1. Heart rate, developed pressure, maximal rates of contraction and relaxation in control, atenolol-perfused and paced hearts. Vertical bars represent SE. $^*P < 0.05$ *vs* control (ANOVA and Fisher's post-tests).

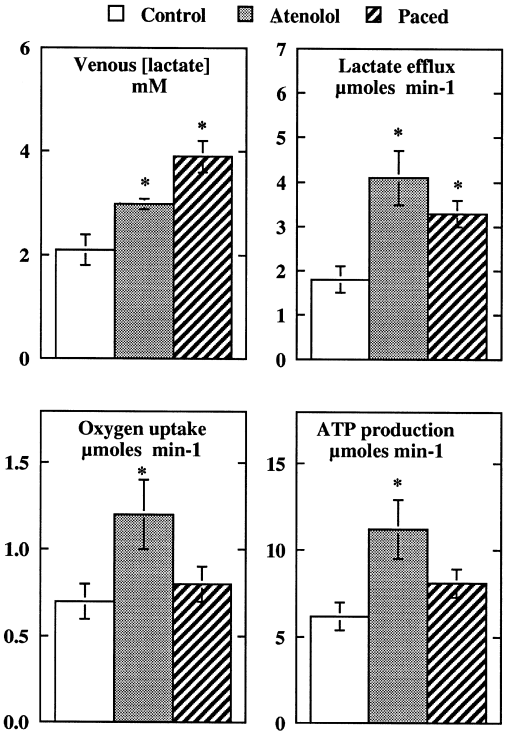

Fig. 2. Venous (lactate), lactate efflux, oxygen uptake and ATP production rate measured at the end of the ischaemia period in control, atenolol-perfused and paced hearts. Vertical bars represent SE. $^*P < 0.05$ *vs* control (ANOVA and Fisher's post-tests).

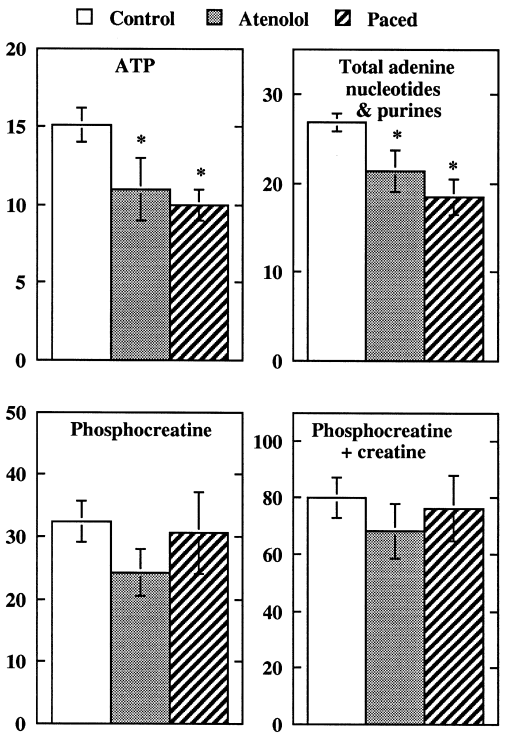

Fig. 3. Tissue content of ATP, total adenine nucleotides and purines, phosphocreatine, and phosphocreatine + creatine measured at the end of reflow in control, atenolol-perfused and paced hearts. Vertical bars represent SE. $^{*}P < 0.05$ *vs* control (ANOVA and Fisher's post-tests).

mance was impaired in both *atenolol* and *paced* hearts. The tissue contents of ATP and TANP reflected mechanical recovery, but the content of phosphocreatine and phosphocreatine + creatine was the same in the three groups. The level of inosine-5′-monophosphate was 0.35 ± 0.14, 1.07 ± 0.49, and 0.25 ± 0.14 μmol g_{dw}^{-1} in *control*, *atenolol* and *paced* hearts (Fig. 3).

DISCUSSION

Due to its relatively low dose (2.5 $\mu g\, l^{-1}$, or $\approx 10^{-8}$ M), atenolol did not cause any measurable effect during baseline perfusion (Table I). Therefore, all the features observed in this study are directly related to the effects caused by atenolol on heart metabolism throughout ischaemia-reperfusion, thereby ruling out the well known effects related to β-blockade. Indeed, despite low dose, after reperfusion *atenolol* hearts display increased HR, flow, J_{Lac}, V_{O_2} and J_{ATP}, with decreased LVDP, $+dP/dt_{max}$ and $-dP/dt_{max}$ with respect to *control* hearts. The features observed in *atenolol* hearts are practically superimposable to those observed in hearts electrically paced to increase energy demand.

Model

The employed atenolol concentration allowed us to compare our results to those of previous studies [8, 11, 16, 19, 20]. The selected ischaemia condition induces reproducible myocardial dysfunction in *control* hearts without leading to arrest. This is essential to seek correlations between ischaemic down-regulation and recovery. Lack of blood in the perfusion medium excluded possible effects of atenolol on neutrophil accumulation and thrombin-induced platelet aggregation, thereby stressing its effects on cardiac metabolism. Strict temperature control (± 0.5°C), same volume of the intraventricular balloon, and same perfusion pressure in the various groups ruled out differences in loading conditions. As animals were not pretreated, these observations relate to acute metabolic effects of atenolol on cardiac muscle.

Ischaemic down-regulation and recovery

In untreated hearts during low-flow ischaemia, flow was 7–11% of baseline in all groups. This condition is known to induce myocardial down-regulation [4], probably due to intracellular lactate accumulation, which depresses metabolism and performance independently of pH [21]. By decreasing energy demand with respect to supply, ischaemic down-regulation would favour energy supply-to-demand matching and improve ATP/ADP coupling. This would maintain the ATP pool during ischaemia and improve recovery during reflow [2, 22].

Ischaemic down-regulation is apparently suppressed in *atenolol* hearts. In these hearts, the rates of both aerobic (V_{O_2}) and anaerobic (J_{Lac}) paths during ischaemia are increased, thereby increasing J_{ATP}, in agreement with previous observations [9–11]. Moreover, the (pressure × rate) product reflects more than double the energy demand. This increase can not be accounted for by the 40–50% higher flow and supply of O_2.

Suppression of down-regulation in *atenolol* hearts accounts, at least in part, for impaired recovery. The lower contractility is accompanied by depressed ATP and TANP contents. High inosine-5′-monophosphate levels in *atenolol* hearts also indicate severe metabolic dysfunction [23]. Phosphocreatine and creatine do not leak across intact membrane [24], thus the preservation of these compounds throughout ischaemia-reflow indicates that the fall observed for ATP and TANP is not due to physical membrane damage, but rather to the membrane-diffusibility of these compounds [2].

The pattern observed in *paced* hearts reproduces that observed in *atenolol* hearts: high (pressure × rate) during ischaemia indicates suppression of down-regulation. This worsens the energy supply-to-demand imbalance, thereby impairing recovery during reflow. This chain of events closely resembles the one observed in hypoxemic hearts in previous

studies [2]. Thus, myocardial ischaemic down-regulation may be disturbed by several events, including atenolol, electrical pacing and hypoxemia, all of which have the common feature to increase energy demand during the ischaemic stress.

Conclusion

Atenolol depresses post-ischaemic recovery in isolated perfused hearts independently of neurohormonal and blood-related effects. This effect is apparently due to the suppression of ischaemic down-regulation, which was shown to improve myocardial tolerance to ischaemia through better preservation of the high-energy phosphate pools [22]. Down-regulation during ischaemia may therefore play a central role in the ischaemia-reperfusion syndrome. This further stresses the importance of myocardial metabolism in determining mechanical recovery during reflow.

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