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Tolerance of isolated rat hearts to low-flow ischemia and hypoxia of increasing duration: Protective role of down-regulation and ATP during ischemia

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Abstract

We tested the hypothesis that down-regulated hearts, as observed during low-flow ischemia, adapt better to low O_2 supply than non-down-regulated, or hypoxic, hearts. To address the link between down-regulation and endogenous ischemic protection, we compared myocardial tolerance to ischemia and hypoxia of increasing duration. To that end, we exposed buffer-perfused rat hearts to either low-flow ischemia or hypoxia (same O_2 shortage) for 20, 40 or 60 min (n = 8/group), followed by reperfusion or reoxygenation (20 min, full O_2 supply). At the end of the O_2 shortage, the rate·pressure product was less in ischemic than hypoxic hearts ($p < 0.0001$). The recovery of the rate·pressure product after reperfusion or reoxygenation was not different for t = 20 min, but was better in ischemic than hypoxic hearts for t = 40 and 60 min ($p < 0.02$ and $p < 0.0002$, respectively). The end-diastolic pressure remained unchanged during low-flow ischemia (0.024 ± 0.013 mmHg·min^{-1}), but increased significantly during hypoxia (0.334 ± 0.079 mmHg·min^{-1}). We conclude that, while the duration of hypoxia progressively impaired the rate·pressure product and the end-diastolic pressure, hearts were insensitive of the duration of low-flow ischemia, thereby providing evidence that myocardial down-regulation protects hearts from injury. Excessive ATP catabolism during ischemia in non-down-regulated hearts impaired myocardial recovery regardless of vascular, blood-related and neuro-hormonal factors. These observations support the view that protection is mediated by the maintenance of the ATP pool. (Mol Cell Biochem **226**: 141–151, 2001)

Key words: hypoxia, ischemia, reperfusion, contractile function, energy metabolism

Abbreviations: DP – developed pressure; EDP – end-diastolic pressure; HR – heart rate; IMP – inosine-5'-monophosphate; PP – perfusion pressure; RPP – rate·pressure product; TANP – total adenine nucleotides and purines, i.e. the sum of ATP, ADP, AMP, IMP, adenosine, inosine, hypoxanthine, xanthine and urate; VO_2 – O_2 uptake

Introduction

A necessary step to recover the ischemic myocardium, reperfusion is often associated with injury. It is accepted that this injury is the outcome of complex biochemical interactions that antecede, possibly trigger, apoptotic and necrotic processes [1]. No universal experimental model exists to fully characterize these phenomena, but the isolated per-

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fused heart, a model free from neuro-hormonal and blood-related features, has became useful to address the relative role of some of the paths leading to injury. For example, comparing low-flow ischemia with hypoxia at the same O_2 supply helped to provide insight into the involved mechanisms. Although originated by different mechanisms, ischemia and hypoxia share unmatched O_2 supply-to-demand ratio as a common feature. However, the two stresses are different for many aspects. Of interest in this study, low-flow ischemia is associated with down-regulation of myocardial performance, while hypoxia does not elicit down-regulation [2]. By sparing high-energy phosphates [3] and glycogen stores [4], down-regulation during O_2 shortage may in principle represent a major mechanism that generates protection. But no direct observation is available to support this hypothesis.

Here, we test the hypothesis that down-regulated hearts may adapt to low O_2 supply better than non-down-regulated hearts. We reason that, if down-regulation is not critical to generate protection, then the post-ischemic functional depression is expected to be proportional to the duration of the O_2 shortage. In contrast, if down-regulation is crucial, then the correlation between the functional depression and the duration of stress should be weak. This speculation refers to the concept of adaptation: if down-regulated hearts have adapted to ischemia, possibly by establishing a new equilibrium between energy supply and energy demand [5], then they would tolerate the stress better than non-down-regulated hearts. This issue is investigated in two ways. First, we assessed the recovery of hypoxic non-down-regulated and ischemic down-regulated hearts after variable (20, 40 and 60 min) stress duration. Second, we assessed whether the availability of ATP and/or ATP precursors before the reperfusion represents a limiting factor for the recovery of the ATP pool and hence of myocardial performance during the reperfusion. For this purpose, we reanalyzed published data, which were obtained in the same experimental model, to correlate myocardial bioenergetics during the O_2 shortage with the recovery of performance. Of interest, previous reports on this issue, either in hearts or in muscles, are very conflicting: while some confirm that hypothesis [6–8], others definitely reject it [9–12].

We will show that the duration of low-flow ischemia does not appreciably influence post-ischemic recovery, but the recovery of post-hypoxic hearts worsens proportionally to the duration of hypoxia. This observation is consistent with the hypothesis that down-regulation during the O_2 shortage can elicit endogenous protection against low-flow ischemia but not against hypoxia. This differential behavior can be explained on the basis of variable tissue contents of ATP and ATP precursors, which are potential biological markers to predict post-ischemic recovery.

Materials and methods

Heart perfusion

Eight-week old male outbred Sprague–Dawley rats (Lyon, France, weight = 268 ± 5 g, mean ± S.E.) were anesthetized (i.p. heparinized sodium thiopental, 100 mg/kg), and the hearts immediately (<45 sec) mounted on the perfusion system [13]. A roller pump (Ismatec SA, Labortechnik-Analytik, Glattbrugg-Zurich, Switzerland) delivered the medium (Krebs–Henseleit buffer containing 2.0 mM free Ca^{2+} and 11 mM glucose) through a 8 μm pore size, 47 mm diameter filter (MSI, Westboro, MA, USA), a membrane oxygenator (Dideco, Mirandola, Italy), a pre-heater and the aortic cannula. We used two gas cylinders (Carbagas, Lausanne, Switzerland, nominal accuracy ± 0.01%), one at high PO_2 (94% O_2 and 6% CO_2), and one at low PO_2 (10% O_2, 6% CO_2, 84% N_2). High PO_2 gas yielded pH = 7.34 ± 0.01, PO_2 = 636.6 ± 6.1 mmHg, and PCO_2 = 44.9 ± 0.2 mmHg at 37°C. The low PO_2 gas yielded the same pH and PCO_2, but PO_2 = 66.7 ± 0.2 mmHg. A latex balloon introduced into the left ventricle was connected to a pressure transducer (MPC-500, Millar Instruments, Houston, TX, USA) to monitor performance. An additional transducer was inserted above the aortic cannula to monitor the perfusion pressure. A cannula was inserted into the pulmonary artery to collect the venous return and monitor venous pH, PO_2 (model 5300 Oxygen Monitor, Yellow Springs, Yellow Springs, OH, USA) and [lactate] (COBAS FARA II, Hoffman-La Roche, Basel, Switzerland).

Measurements

The performance was monitored by a LabView system (National Instruments, Austin, TX, USA) running on a PC. The measured parameters included the end-diastolic pressure (EDP), the heart rate (HR), the developed pressure (DP), the perfusion pressure (PP), and the O_2 uptake (VO_2, calculated from arterial PO_2, venous PO_2 and coronary flow). The product DP·HR, i.e. the rate·pressure product (RPP) provided an integrated index of myocardial performance. The resistance was calculated as (PP-EDP)/flow per g of ventricle [14]. The lactate release was calculated from venous [lactate] and coronary flow. The ATP turnover rate was estimated as (lactate release) + ($6 \cdot VO_2$) [2], assuming ATP/lactate ratio = 1.0 (glucose as substrate without significant glycogenolysis), ATP/O_2 = 6 (no mitochondrial uncoupling), and no ATP production from the oxidation of fatty acids and amino acids, which were absent from the perfusion buffer.

The recovery of performance is expressed as %RPP, with reference to the value measured at the end of the stabiliza-

tion period. As the volume of the intraventricular balloon was established at the start of the perfusion and kept constant afterwards, any subsequent EDP rise (ΔEDP) is associated to the onset of diastolic contracture.

Experimental protocol

After mounting on the perfusion system, the balloon volume was adjusted to yield EDP = 10 mmHg. Hearts underwent baseline perfusion for 30 min (flow = 15 ml/min, PO_2 ~ 636 mmHg). Then, hearts were exposed to either low-flow ischemia or hypoxia for variable times (n = 8/group, Fig. 1). For low-flow ischemia experiments, the coronary flow was reduced to one-tenth of baseline, i.e. 1.5 ml/min. For hypoxia experiments, the gas flowing through the oxygenator was switched to the PO_2 ~ 67 mmHg gas, i.e. one-tenth of baseline. Therefore, in both cases the O_2 supply (PO_2·flow) was the same, one-tenth of baseline. At the end of the O_2 shortage, hearts were reperfused or reoxygenated for 20 min at full O_2 supply, and the performance was compared with that measured at the end of the stabilization period.

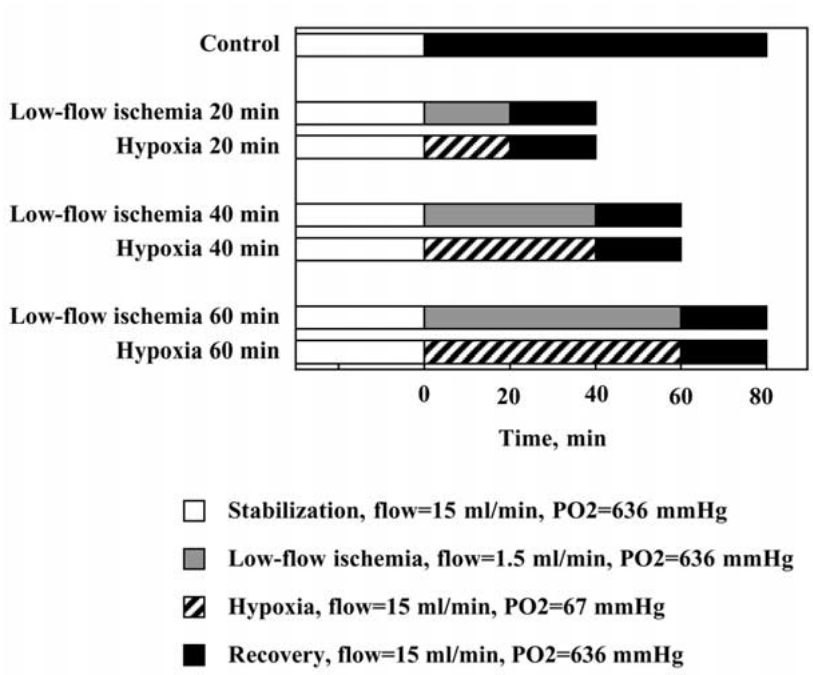

Fig. 1. Diagram of the experimental protocols used in this study. An entire set of performance and metabolic data was obtained at the end of each phase. Perfusion conditions during stabilization or recovery: pH = 7.34 ± 0.01, arterial PO_2 = 636.6 ± 6.1 mmHg, arterial PCO_2 = 44.9 ± 0.2 mmHg, flow = 15 ml/min. Low-flow ischemia: flow = 1.5 ml/min. Hypoxia: arterial PO_2 = 66.7 ± 0.2 mmHg.

Table 1. List of previously published data that were reconsidered to assess the link between the tissue content in ATP and ATP precursors during the O_2 shortage, and the recovery after reperfusion or reoxygenation

Intervention	Ref.	Time (min)	Flow (ml/min)	P_aO_2 (mmHg)	$n_{ischemia}$	$n_{reperfusion}$
Low-flow ischemia	(13)	20	1.5	670	8	8
Hypoxia	(13)	20	15	67	7	9
'Mixed' hypoxia and low-flow ischemia	(13)	20	7.5	140	8	9
Low-flow ischemia	(4)	10	1.5	670	4	13
Hypoxia	(4)	10	15	67	4	13
Low-flow ischemia	(15)	60	1.5	670	4	7
Low-flow ischemia and 10^{-6}M trimetazidine	(15)	60	1.5	670	4	7
Low-flow ischemia and pacing	(3)	20	1.5	670	7	9
Hypoxia and pacing	(3)	20	15	67	7	8
Total					53	83

During stabilization and reperfusion, the flow and the arterial PO_2 (P_aO_2) were 15 ml/min and 640–670 mmHg, respectively. The time duration, flow and P_aO_2 conditions of the various low-flow ischemia or hypoxia experiments are reported in the table. Abbreviations: $n_{ischemia}$ = the number of determinations of ATP and TANP contents at the end of ischemia; $n_{reperfusion}$ = the number of determinations of performance recovery.

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Reanalysis of published data

For our purposes, we re-analyzed previously published data (Table 1, n = 4–13 per group) that were obtained using the same experimental model as that described above, yet under a variety of conditions that induced performance recoveries in the 55–95% range [3, 4, 13, 15]. In all the groups, hearts underwent a 20–30 min stabilization perfusion at full O_2 supply to obtain baseline values. Then, hearts were exposed to either low-flow ischemia or hypoxia for variable times (Table 1). After performance measurement, hearts were either freeze-clamped (n = 53) or allowed to recover (n = 83). In the first case, tissue was extracted with 0.5M $HClO_4$, neutralized and assayed by HPLC methods [16] for ATP, phosphocreatine, as well as total adenine nucleotides and purines (TANP), i.e. the sum of ATP, ADP, AMP, inosine-5'-monophosphate (IMP), adenosine, inosine, hypoxanthine, xanthine and urate. In the second case, hearts were reperfused or reoxygenated for 20–30

Table 2. Myocardial performance at the end of the stabilization period, when hearts were perfused with full O_2 supply

Parameter (unit)	Mean ± S.E.
Heart rate (HR), min^{-1}	270 ± 4
End-diastolic pressure (EDP), mmHg	10.2 ± 0.1
Maximal rate of contraction ($+dP/dt_{max}$), mmHg/sec	4254 ± 73
Maximal rate of relaxation ($-dP/dt_{max}$), mmHg/sec	2266 ± 28
Perfusion pressure (PP), mmHg	75 ± 1
Resistance, mmHg·min/ml/g	3.34 ± 0.65
Developed pressure (DP), mmHg	113 ± 1
Rate·pressure (RPP), $mmHg \cdot min^{-1} \cdot 10^{-3}$	30.3 ± 0.4
Venous PO_2 (P_vO_2), mmHg	237 ± 8
O_2 consumption (VO_2), µmoles/min	9.1 ± 0.2
Venous [lactate], mM	<0.2
Lactate release, µmoles/min	<2.0
ATP turnover, µmoles/min	54.6 ± 7.7
Ventricle weight, g	0.98 ± 0.03

Coronary flow = 15 ml/min; arterial PO_2 = 636 ± 6 mmHg; n = 56.

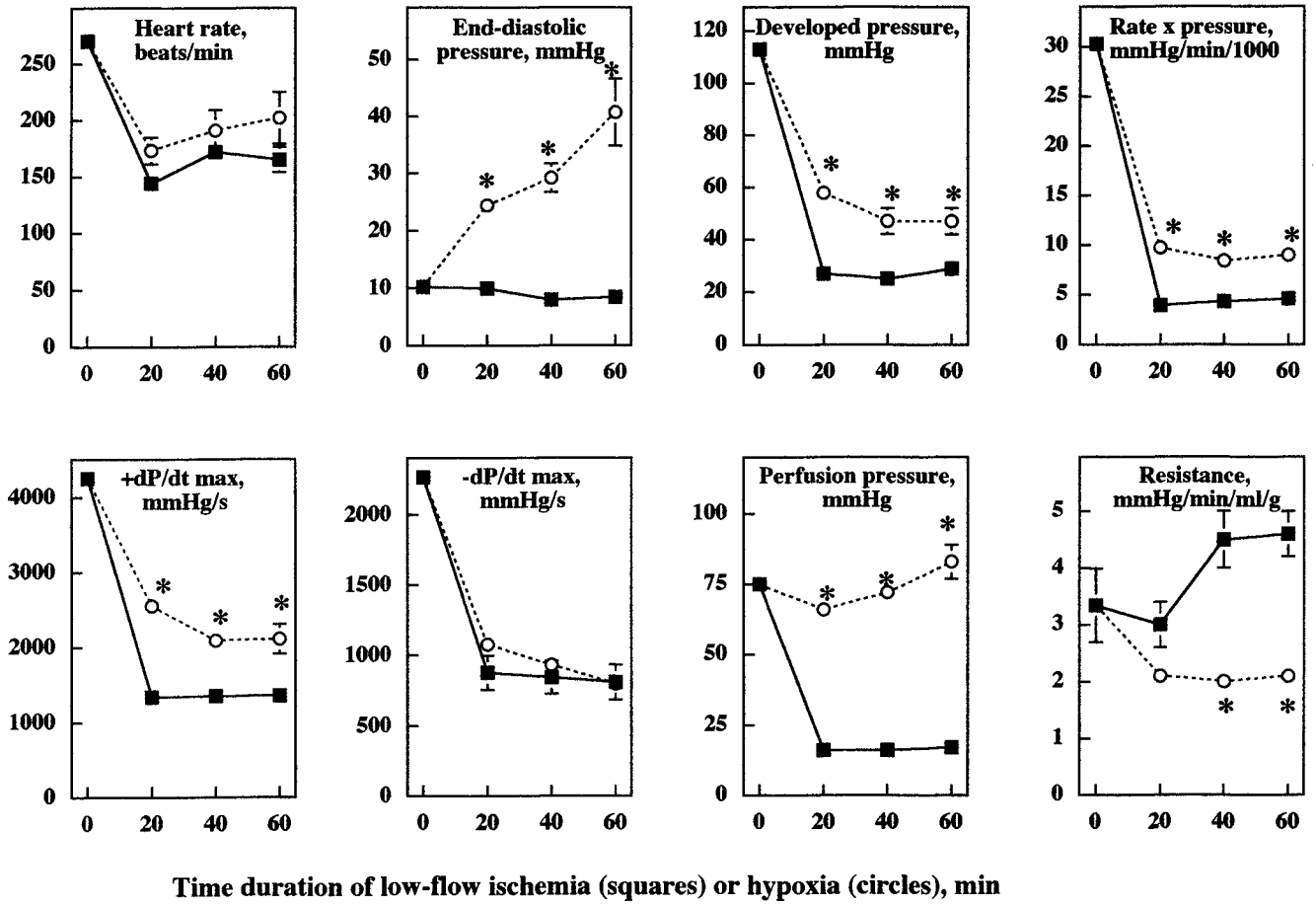

Fig. 2. Myocardial performance (n = 8/group) as measured at the end of the O_2 shortage (20, 40 or 60 min duration), which is represented by either low-flow ischemia (filled squares) or hypoxia (empty circles) with matched O_2 supply. *Significant differences from low-flow ischemia ($p < 0.05$, Student's *t*-test).

min under the same conditions of the stabilization, and recovery was determined as %RPP and ΔEDP. To correlate the recovery of performance to the bioenergetic metabolism during the preceding ischemia or hypoxia, data obtained in the first group were plotted as independent variables (X-axis), while data obtained in the second group were plotted as dependent variables (Y-axis). As each heart was either freeze-clamped, or allowed to recover, a heart-by-heart analysis is impossible, and the averages of all data obtained under the same conditions were instead used.

Statistics

Data are expressed as mean ± S.E. When multiple-group comparison was required, we first performed the one-way factorial ANOVA test (StatView, Abacus Concepts, Berkeley, CA, USA). If this test showed significant differences ($p < 0.05$), the various groups were compared with Fisher's PLSD multiple comparison procedure. Statistical correlations were first tested by ANOVA (StatView, Abacus Concept). If ANOVA yielded $p < 0.05$, linear regression lines and regression coefficients were calculated.

To assess the effect of increasing duration of low-flow ischemia or hypoxia on myocardial recovery, we performed regression analysis treating the indices of myocardial performance recovery, i.e. %RPP or ΔEDP, as the dependent variables, and the duration of low-flow ischemia or hypoxia as the independent variables. To assess the effects of bioenergetics during the O_2 stress on recovery of reperfused or reoxygenated hearts, the various markers of the myocardial metabolic state during ischemia were treated as independent variables, while %RPP or ΔEDP were treated as dependent variables.

Results

The aims of this study required stable and reliable preparations. In hearts perfused at full O_2 supply for 110 min, DP, HR and EDP changed for less than 8.3 ± 4.3 mmHg, 22 ± 10

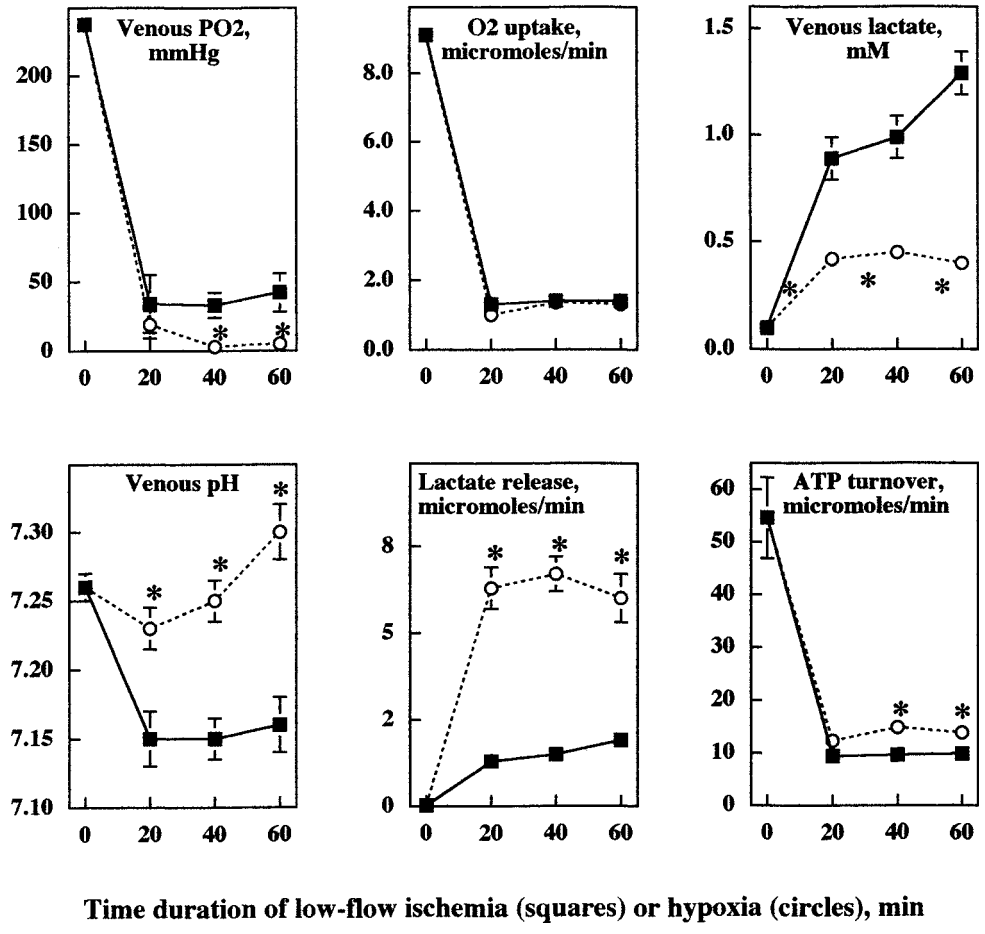

Fig. 3. Myocardial metabolism (n = 8/group) as measured at the end of the O_2 shortage (20, 40 or 60 min duration), which is represented by either low-flow ischemia (filled squares) or hypoxia (empty circles) with matched O_2 supply. *Significant differences from low-flow ischemia ($p < 0.05$, Student's *t*-test).

min^{-1} and 1.2 ± 0.9 mmHg, respectively. Table 2 shows the performance measured at the end of the stabilization period. Because venous [lactate] was below the detection limit, the ATP turnover was calculated from VO_2 only.

Figure 2 shows some parameters related to myocardial performance, measured at the end of low-flow ischemia or hypoxia with varying time duration. In hearts exposed to low-flow ischemia, performance (DP, RPP and $+dP/dt_{max}$) was less than in those exposed to hypoxia. EDP and PP were also lower in low-flow ischemia hearts. The resistance was higher in low-flow ischemia hearts due to vascular collapse. HR and $-dP/dt_{max}$ were the same in the two groups.

Figure 3 shows some parameters related to myocardial metabolism, measured at the same times as those in Fig. 2. Venous PO_2 was virtually zero in hypoxic, compared to a significantly higher value in low-flow ischemia hearts. Nevertheless, the VO_2 was the same due to different flows in the two groups. Venous [lactate] was higher during low-flow ischemia, with lower venous pH, but, for the same reason, the lactate release was higher in hypoxic than low-flow ischemia hearts. The turnover of ATP was higher in hypoxic than low-flow ischemia hearts.

Figure 4 shows how the duration of low-flow ischemia or hypoxia affects the recovery of myocardial performance after reperfusion or reoxygenation. In hearts exposed to low-flow ischemia and reperfusion, RPP always remained >90% of the baseline value of 30.3 ± 0.4 $mmHg \cdot min^{-1} \cdot 10^{-3}$. Varying the duration of low-flow ischemia up to 60 min did not alter significantly RPP: The slope of the linear regression in the plot RPP vs. the duration of low-flow ischemia was +0.043 ± 0.023 $mmHg \cdot 1000 \cdot min^{-2}$, i.e. not different from zero (p = N.S.). In contrast, in hearts exposed to hypoxia and reoxygenated, %RPP decreased from 86 ± 3% for 20 min hypoxia to 63 ± 5% for 60 min hypoxia. The myocardial depression originated from hypoxia-reoxygenation was thus sensitive of hypoxia duration: The slope of the linear regression in the plot RPP vs. the duration of hypoxia (–0.150 ± 0.029 $mmHg \cdot 1000 \cdot min^{-2}$) was less than zero ($p < 0.0001$).

The rise in EDP essentially mirrored the decrease in % RPP. While EDP was essentially preserved for varying duration of low-flow ischemia (slope = +0.024 ± 0.013 $mmHg \cdot min^{-1}$, not different from zero), it was increased with hypoxia duration (slope = +0.334 ± 0.079 $mmHg \cdot min^{-1}$, p = 0.0002).

In order to assess independently if myocardial down-regu-

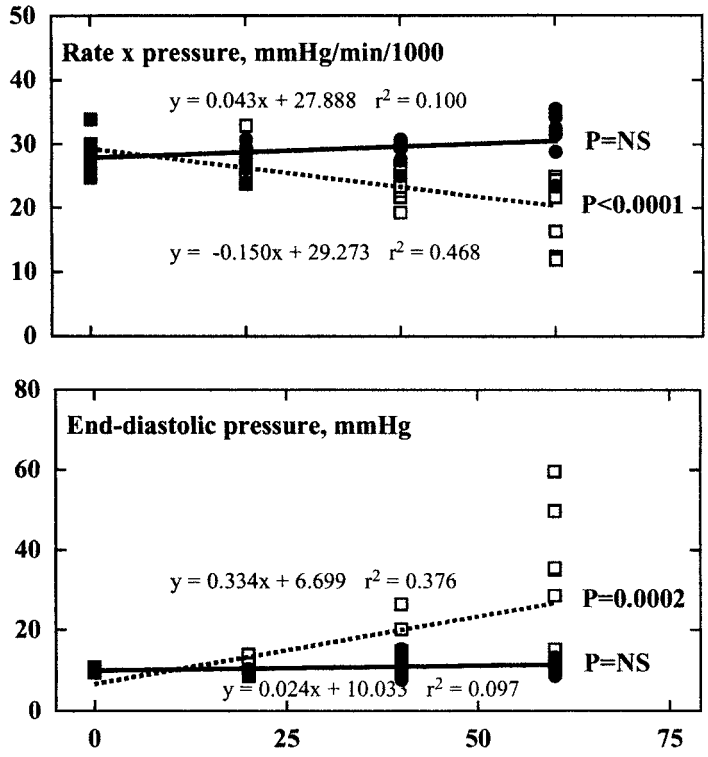

Fig. 4. Recovery of the rate·pressure product and rise of the end-diastolic pressure, as measured at the end of the 20 min reperfusion or reoxygenation. Data are expressed as a function of the duration of the preceding O_2 shortage, low-flow ischemia (empty symbols) or hypoxia (filled symbols) of different duration, plotted on the X-axis, respectively. The equation of the regression lines is reported in the figure.

lation during the O_2 stress, and hence the metabolic state, is related to recovery, we reconsidered previously published data that were obtained using the same experimental model. To rule out variables related to animal donors and slightly different buffer compositions or perfusion conditions, we choose to consider only data obtained using the same experimental model as that described above. Fig. 5 shows the correlation between some parameters related to the metabolic state, measured at the end of either low-flow ischemia or hypoxia of variable length and severity, and the final recovery of either RPP or EDP. It appears that the tissue contents of ATP and TANP at the end of the O_2 stress are statistically related to %RPP. In contrast, the tissue contents of phosphocreatine and IMP are not related to recovery. The bottom panels also show that no correlation exists when considering either the ATP turnover or the myocardial function at the

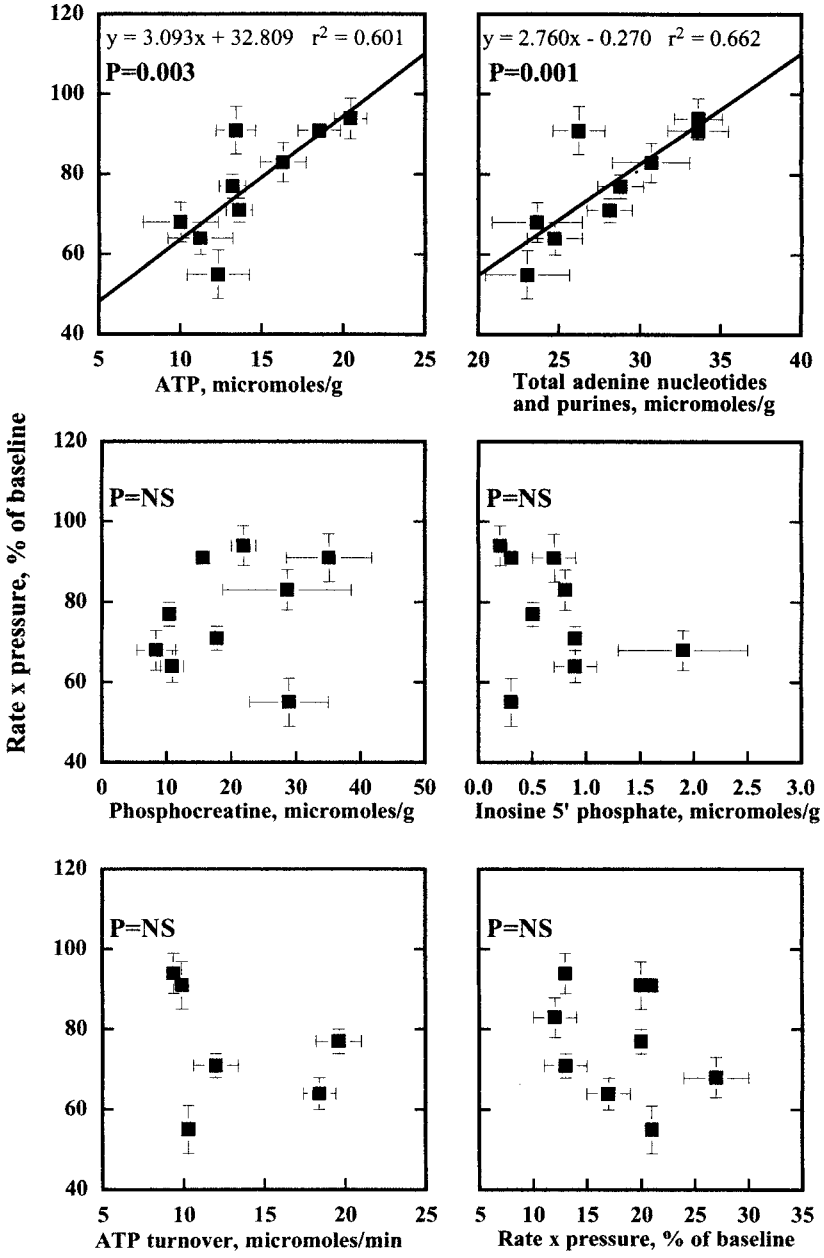

Fig. 5. Re-analysis of the studies mentioned in Table 1. Various markers of the cardiac metabolic state during ischemia or hypoxia, measured before the reperfusion, are reported on the X-axis, while the recovery of rate·pressure (%RPP), measured at the end of the reperfusion and expressed as percent of baseline value, is reported on the Y-axis. Each panel reports the ANOVA P, and, if $p < 0.05$, the equation and correlation coefficient of the best fit line. n = 9 for all but for the ATP turnover (n = 6), because this variable was not measured in [4].

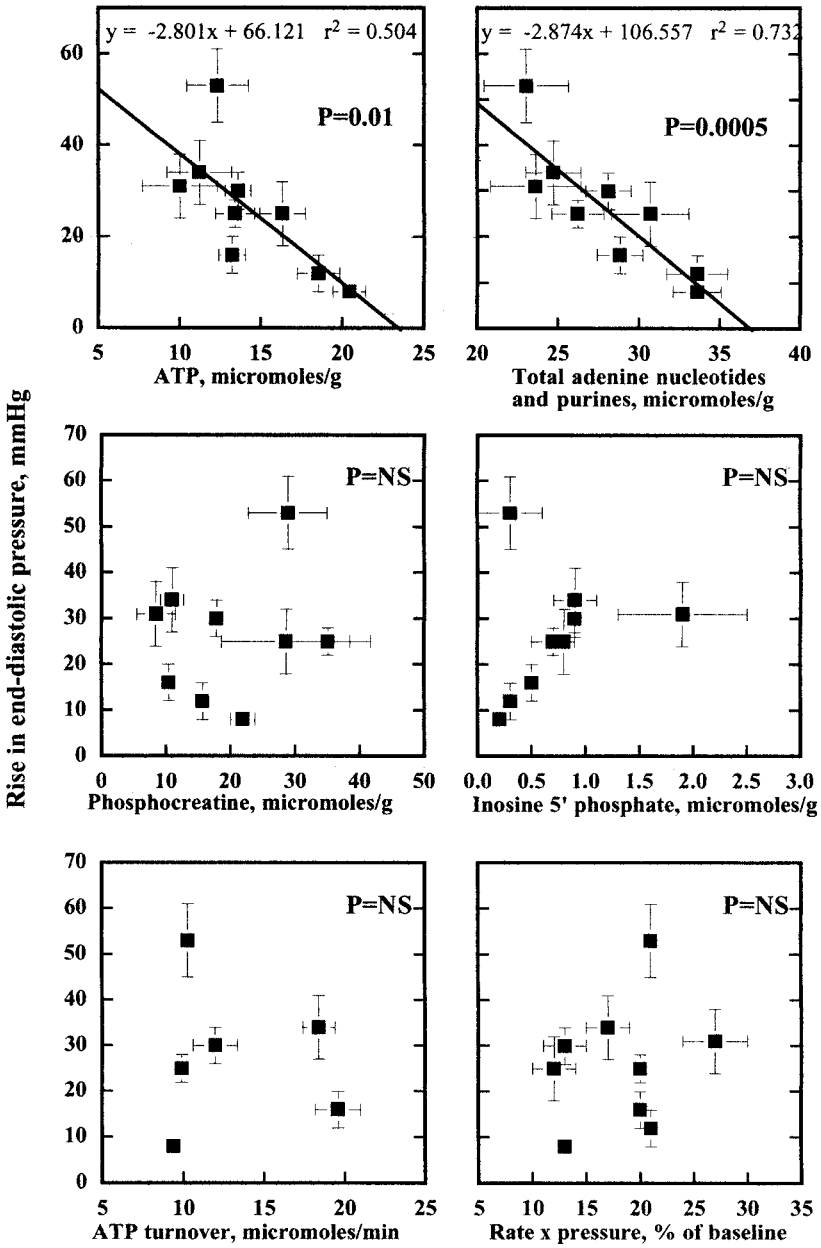

Fig. 6. Same as in Fig. 5, but reporting the rise of the end-diastolic pressure over baseline (ΔEDP) instead of %RPP on the Y-axis.

end of ischemia. This means that while ATP and TANP have some predictive value for the functional recovery in this model, such is not a property of either other metabolic parameters or of the functional level *per se*.

Figure 6 shows that the behavior of ΔEDP essentially mirrors that of %RPP. The tissue contents of ATP and TANP at the end of the O_2 stress are statistically related to ΔEDP. In contrast, a relationship was absent between rise in EDP and end-ischemic tissue contents of phosphocreatine and IMP, as well as ATP turnover and RPP.

Discussion

In this model, increasing the duration of low-flow ischemia did not induce significant post-reperfusion dysfunction, but prolongation of hypoxia (same O_2 shortage) progressively impaired post-reoxygenation performance. Possibly, low-flow ischemia induced a situation compatible with adaptation to shortened O_2 supply, while hypoxia did not induce, or blunted, adaptation. Evidently, coronary flow differs between low-flow ischemia and hypoxia, thus a membrane-

diffusible factor is likely involved. Looking for a possible explanation, we reconsidered published data obtained in the same experimental model, finding a negative relationship between the tissue contents of ATP and TANP during ischemia or hypoxia, and the post-reperfusion or reoxygenation dysfunction. While ATP and TANP belong to a partially diffusible pool of metabolites, phosphocreatine, which is not related to the post-reperfusion or reoxygenation dysfunction, belongs to a non-diffusible pool [17].

Critique of the model

The crystalloid-perfused heart model is essentially free from vascular, neuro-hormonal, inflammatory and blood-mediated processes. Fixed coronary flows and constant balloon volumes exclude load changes during the experiments. The O_2 supply during the stabilization (12.7 μmoles/min/g) was somewhat higher than that *in vivo* (8.5–10.1 μmoles/min/g), but VO_2 and the high-energy phosphates level compared well to normal values [3]. The exposure of hearts to low-flow ischemia, rather than to global ischemia, allowed hearts to contract throughout the O_2 shortage and hence to down-regulate performance. The O_2 shortage during low-flow ischemia was made equal to that during hypoxia [2]. The ATP turnover allows discrimination between aerobic and anaerobic components in the bioenergetic apparatus [18]. The recovery is expressed as %RPP and ΔEDP, which represent, respectively, the viability of the contracting mechanism, and the onset of the diastolic chamber stiffness [19].

Literature

Most of the reports on the relationship between recovery and ischemia time concern global ischemia [20, 21]. Although one study suggests the involvement of free radicals [22], other authors oppose this hypothesis [23–25]. But distinguishing between low-flow ischemia and global ischemia is important. For example, some reports show that low-flow ischemia neither triggers free radical release [26] nor alters the endogenous pool of scavengers [27]. Indeed, use of free-radical scavengers does not affect recovery in this model [18]. We are unaware of other studies comparing dysfunction due to low-flow ischemia with that following hypoxia of variable duration.

Myocardial down-regulation

In this model, low-flow ischemia could induce a condition compatible with adaptation to O_2 shortage by down-regulating ATP turnover and functional performance. This adaptation failed to occur during hypoxia. While the aerobic contribution (VO_2) to ATP turnover was the same, the anaerobic contribution (lactate release) was higher during hypoxia than during low-flow ischemia. Therefore, hypoxia induced hearts to deplete their energy store faster than low-flow ischemia. The known modulation of phosphofructokinase activity by intracellular lactate [28] may explain the differential adjustments to low-flow ischemia and hypoxia. Lactate buildup during low-flow ischemia inhibits glycolysis, but during hypoxia the inhibition is released by the high flow, which removes lactate. We cannot exclude the occurrence of phenomena such as transmural flow redistribution within the ventricular wall. But the positive pressure exerted against the ventricle wall by the intraventricular balloon should have minimized flow heterogeneity. Also, endothelin-1, a powerful vasoconstrictor with growth-promoting action and an early marker for coronary endothelial dysfunction [29, 30], may play a role in exacerbating the injury. But lower resistance in hypoxic hearts compared to low-flow ischemia excludes the occurrence of high shear stress, which is known to trigger endothelin-1 release.

ATP catabolism and recovery

Down-regulation of the energy need implies preservation of the ATP pool. Normally, ATP catabolism is compensated by ATP synthesis from basic precursors [31]. As this rate can be accelerated only 6 times during anoxia [32], it cannot compete with increased ATP catabolism as during severe ischemia. This leads to formation of membrane-diffusible substances, lost during high-flow conditions with resulting depletion of the ATP pool [13, 33, 34]. As purine salvage, which is faster than ATP synthesis rate from basic precursors [35], makes use of hypoxanthine [36–39], a diffusible compound, we speculate that the availability of ATP precursors before the reperfusion is the factor that limits the recovery of the ATP pool and hence of myocardial performance. Because previous reports are conflicting (while some confirm the hypothesis [6–8], others reject it [9–12]), we reconsidered data obtained in the same model and using the same methodologies as those described here. Although correlation does not imply causation, the recovery of RPP and the preservation of EDP are correlated to the size of the ATP pool before reperfusion or reoxygenation. This observation could not have emerged by using ^{31}P-NMR techniques, because the diffusible compounds are invisible to that technique. That relationship is not significant when taking the tissue contents of phosphocreatine and IMP, which are not membrane-diffusible. As ATP turnover and myocardial performance before reperfusion or reoxygenation are not related to recovery, we believe that the protective factor is the availability of the ATP precursors for purine salvage, and not just the free energy of ATP hydrolysis or ΔG_{ATP}. Activation of adenosine receptors could also be involved, even in a rat model [40].

Clinical implications

Congenital heart malformations with right-to-left intra-cardiac shunt represent a clinical case related to the present findings. Our findings indeed may explain in part the higher mortality and morbidity associated to the surgical treatment of these diseases with respect to the management of non-cyanotic hearts, because of blunted down-regulation in hypoxic hearts, and greater injury in proportion to the time they have been hypoxic. In contrast, down-regulation in non-cyanotic low-flow ischemic hearts might trigger endogenous protection mechanisms that induce ischemia tolerance. However, there are other variables to consider. First, cardiac hypertrophy, which might affect to a greater extent hypoxic than ischemic hearts. Second, acute hypoxic spells, which might occur more frequently following prolonged periods with low O_2 supply. Third, a reduced ventricular performance, comparable to down-regulated ischemic hearts in this study, would not be tolerated in the clinical practice, where a sub-optimal myocardial performance does not meet the standard clinical criteria.

Conclusion

The duration of low-flow ischemia does not appreciably influence recovery in post-ischemic isolated rat hearts, but post-hypoxic recovery worsens proportionally to hypoxia duration. While low-flow ischemia induces energy need down-regulation, allowing hearts to spare energy for as long as 60 min in this model, hypoxia blunts this mechanism. Because post-ischemic or hypoxic recovery is correlated with the tissue content of ATP and ATP precursors during the O_2 shortage, those features are linked to the preservation of the ATP pool. Therefore, the contents of ATP and ATP precursors appear reliable biological markers to predict recovery and good candidates to assess the myocardial tolerance to ischemia.

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References

1. Bromme HJ, Holtz J: Apoptosis in the heart: When and why? Mol Cell Biochem 163–164: 261–275, 1996
2. Samaja M, Casalini S, Allibardi S, Corno A, Chierchia S: Regulation of bioenergetics in O_2-limited isolated rat hearts. J Appl Physiol 77: 2530–2536, 1994
3. Samaja M, Allibardi S, de Jonge R, Chierchia S: High-energy phosphates metabolism and recovery in reperfused ischemic hearts. Eur J Clin Invest 28: 983–988, 1998
4. Merati G, Allibardi S, Monti LD, de Jong JW, Samaja M: Dynamics of myocardial adaptation to low-flow ischemia and hypoxemia. Am J Physiol 271: 2300–2305, 1996
5. Hochachka PW, Buck LT, Doll CJ, Land SC: Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc Natl Acad Sci USA 1996: 9493–9498, 1996
6. Haas GS, DeBoer LWV, O'Keefe DDO, Bodenhamer RM, Geffin GA, Drop LJ, Teplick RS, Daggett WM: Reduction of postischemic myocardial dysfunction by substrate repletion during reperfusion. Circulation 70: I65–I74, 1984
7. Takeo S, Tanonaka K, Miyake K, Imago M: Adenine nucleotides metabolites are beneficial for recovery of cardiac contractile force after hypoxia. J Mol Cell Cardiol 20: 187–199, 1988
8. Rubin BB, Liauw S, Tittley J, Romaschin AD, Walker PM: Prolonged adenine nucleotide resynthesis and reperfusion injury in postischemic skeletal muscle. Am J Physiol 262: H1538–H1547, 1992
9. Neely JR, Grotyohann LW: Role of glycolytic products in damage to ischemic myocardium. Circ Res 55: 816–824, 1984
10. Rosenkrantz ER, Okamoto F, Buckberg GD, Vinten-Johansen J, Allen BS, Leaf J, Bugy H, Young H, Bernard RJ: Studies of controlled reperfusion after ischemia. II. Biochemical studies: Failure of tissue adenosine triphosphate levels to predict recovery of contractile function after controlled reperfusion. J Thorac Cardiovasc Surg 92: 488–501, 1986
11. Ambrosio G, Jacobus WE, Mitchell MC, Litt MR, Becker LC: Effects of ATP precursors on ATP and free ADP content and functional recovery of postischemic hearts. Am J Physiol 256: H560–H566, 1989
12. Ambrosio G, Jacobus WE, Bergman CA, Weisman HF, Becker LC: Preserved high energy phosphate metabolic reserve in globally 'stunned' hearts despite reduction of basal ATP content and contractility. J Mol Cell Cardiol 19: 953–964, 1987
13. Samaja M, Motterlini R, Allibardi S, Casalini S, Merati G, Corno A, Chierchia S: Myocardial metabolism and function in acutely ischemic and hypoxemic isolated rat hearts. J Mol Cell Cardiol 27: 1213–1218, 1995
14. Cunningham MJ, Apstein CS, Weinberg EO, Vogel WM, Lorell BH: Influence of glucose and insulin on the exaggerated diastolic and systolic dysfunction of hypertrophied rat hearts during hypoxia. Circ Res 66: 406–415, 1990
15. Allibardi S, Chierchia S, Margonato V, Merati G, Neri G, Dell'Antonio G, Samaja M: Effects of trimetazidine on metabolic and functional recovery of post-ischemic rat hearts. Cardiovasc Drug Ther 12: 543–549, 1998
16. Motterlini R, Samaja M, Tarantola M, Micheletti R, Bianchi G: Functional and metabolic effects of propionyl-L-carnitine in the isolated perfused hypertrophied rat heart. Mol Cell Biochem 116: 139–145, 1992
17. Savabi F: Free creatine available to the creatine phosphate energy shuttle in isolated rat atria. Proc Natl Acad Sci USA 85: 7476–7480, 1988
18. Samaja M, Motterlini R, Santoro F, Dell'Antonio G, Corno A: Oxidative injury in reoxygenated and reperfused hearts. Free Rad Biol Med 16: 255–262, 1994
19. Varma N, Eberli FR, Apstein CS: Increased diatolic chamber stiffness during demand ischemia. Circulation 101: 2185–2192, 2000
20. Chambers DJ, Braimbridge MV, Hearse DJ: Perfusate calcium: Effect on cardiac stability and response to ischemia and reperfusion. Exp Cardiol 7: 410–418, 1991

21. Manche A, Edmondson SJ, Hearse DJ: Dynamics of early postischemic myocardial function recovery. Evidence of reperfusion-induced injury? Circulation 92: 526–534, 1995
22. Tavazzi B, Di Pierro D, Bartolini M, Marino M, Distefano S, Galvano M, Villani C, Giardina B, Lazzarino G: Lipid peroxidation, tissue necrosis, and metabolic and mechanical recovery of isolated reperfused rat heart as a function of increasing ischemia. Free Radical Res Comm 28: 25–37, 1998
23. Henry TD, Archer SL, Nelson D, Weir EK, From AHL: Postischemic oxygen radical production varies with duration of ischemia. Am J Physiol 264: H1478–H1484, 1993
24. Coudray C, Boucher F, Pucheu S, de Leiris J, Favier A: Xanthine oxidase activity and lipid peroxide content following different types of ischemia in the isolated rat heart. Agents Action 41: 144–150, 1994
25. Haramaki N, Stewart DB, Aggarwal S, Ikeda H, Reznick AZ, Packer L: Networking antioxidants in the isolated rat heart are selectively depleted by ischemia-reperfusion. Free Rad Biol Med 25: 329–339, 1998
26. Vergely C, Maupoil V, Benderitter M, Rochette L: Influence of the severity of myocardial ischemia on the intensity of ascorbyl free radical release and on postischemic recovery during reperfusion. Free Rad Biol Med 24: 470–479, 1998
27. Coudray C, Boucher F, Pucheu S, de Leiris J, Favier A: Relationship between severity of ischemia and oxidant scavenger enzyme activities in the isolated rat heart. Int J Biochem Cell Biol 27: 61–69, 1995
28. Rovetto MJ, Lamberton WF, Neely JR: Mechanisms of glycolytic inhibition in ischemic rat hearts. Circ Res 37: 742–751, 1975
29. Lerman A, Heublein DM, Burnett JC, Rodeheffer RJ: Congestive heart failure is characterized by systemic endothelin activation. J Am Coll Cardiol 21: 315A, 1993
30. Kaddoura S, Poole-Wilson PA: Endothelin-1 in heart failure: A new therapeutic target? Lancet 348: 418–419, 1996
31. Goldthwaite DA: Mechanisms of synthesis of purine nucleotides in heart muscle extracts. J Clin Invest 36: 1572–1578, 1957
32. Zimmer HG, Trendelenburg C, Kammermeier H, Gerlach E: *De novo* synthesis of myocardial adenine nucleotides in the rat. Circ Res 32: 635–642, 1973
33. Van Belle H, Goossens F, Wynants J: Formation and release of purine catabolites during hypoperfusion, anoxia, and ischemia. Am J Physiol 252: H886–H893, 1987
34. Altschuld RA, Gamelin LM, Kelley RE, Lambert MR, Apel LE, Brierley GP: Degradation and resynthesis of adenine nucleotides in adult rat heart myocytes. J Biol Chem 262: 13527–13533, 1987
35. Namm DH: Myocardial nucleotide synthesis from purine bases and nucleosides. Circ Res 33: 686–695, 1973
36. Manfredi JP, Holmes EW: Purine salvage pathways in myocardium. Ann Rev Physiol 47: 691–705, 1985
37. Soussi B, Lagerwall K, Idstrom JP, Schersten T: Purine metabolic pathways in rat hindlimb perfusion model during ischemia and reperfusion. Am J Physiol 265: H1074–H1081, 1993
38. Van Wylen DGL, Schmit TJ, Lasley RD, Gingell RL, Mentzer RM: Cardiac microdialysis in isolated rat hearts: Interstitial purine metabolites during ischemia. Am J Physiol 262: H1934–H1938, 1992
39. Taegtmeyer H: On the role of the purine nucleotide cycle in the isolated working rat heart. J Mol Cell Cardiol 17: 1013–1018, 1985
40. de Jonge R, de Jong JW: Ischemic preconditioning and glucose metabolism during low-flow ischemia: Role of the adenosine A1 receptor. Cardiovasc Res 43: 909–918, 1999