Interventricular heterogeneity in rat heart responses to hypoxia: the tuning of glucose metabolism, ion gradients, and function

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Segato Komniski M, Yakushev S, Bogdanov N, Gassmann M, Bogdanova A. Interventricular heterogeneity in rat heart responses to hypoxia: the tuning of glucose metabolism, ion gradients, and function. Am J Physiol Heart Circ Physiol 300: H1645–H1652, 2011. First published March 11, 2011; doi:10.1152/ajpheart.00220.2010.—The matching of energy supply and demand under hypoxic conditions is critical for sustaining myocardial function. Numerous reports indicate that basal energy requirements and ion handling may differ between the ventricles. We hypothesized that ventricular response to hypoxia shows interventricular differences caused by the heterogeneity in glucose metabolism and expression and activity of ion transporters. Thus we assessed glucose utilization rate, ATP, sodium and potassium concentrations, Na-, K-ATPase activity, and tissue reduced/oxidized glutathione (GSH/GSSG) content in the right and left ventricles before and after the exposure of either the whole animals or isolated blood-perfused hearts to hypoxia. The hypoxia-induced boost in glucose utilization was more pronounced in the left ventricle compared with the right one. ATP levels in the right ventricle of hypoxic heart were lower than those in the left ventricle. Left ventricular sodium content was higher, and hydrolytic Na-, K-ATPase activity was reduced compared with the right ventricle. Administration of the Na-, K-ATPase blocker ouabain caused rapid increase in the right ventricular Na⁺ and elimination of the interventricular Na⁺ gradients. Exposure of the hearts to hypoxia made the interventricular heterogeneity in the Na⁺ distribution even more pronounced. Furthermore, systemic hypoxia caused oxidative stress that was more pronounced in the right ventricle as revealed by GSH/GSSG ratios. On the basis of these findings, we suggest that the right ventricle is more prone to hypoxic damage, as it is less efficient in recruiting glucose as an alternative fuel and is particularly dependent on the efficient Na-, K-ATPase function.

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Both the left ventricle (LV) and the right ventricle (RV) possess structural, biochemical, and metabolic differences that meet their functional requirements. The LV has approximately three times the mass and twice the wall thickness of the RV and can be viewed as a “pressure pump” whose cavity resembles an elongated cone. The RV, which pumps at a lower pressure and operates as a “volume pump” is crescent-shaped (26). The double-peaked waveform of both the right ventricular pressure and the right ventricular outflow recorded using the electrically isolated right ventricular free-wall preparation, revealed the presence of two components in the RV contractile function (26). The first is attributed to the contraction of the free wall of the RV, whereas the second is related to the LV and its septal contraction. Analysis of numerical data suggests that ~30% of the stroke output of the RV was generated by the LV (15).

These differences in the generation of force are mirrored by differences in the properties of the force-generating proteins. The distribution of myosin heavy-chain (MHC) isoforms between the ventricles supports this functional asymmetry. The RV of the rodent heart is enriched with a fast a-MHC isoform that exhibits higher levels of myosin ATPase activity than the slow b-MHC isoform (8, 31, 42). The slow muscle fiber-specific energy-saving b-MHC isoform, which requires less energy to generate cross-bridge force, is correspondingly more abundant in the LV than in the RV (21, 34).

Heterogeneity in ventricular force-generation capacity is further supported by the differences in sarcoplasmic reticulum Ca²⁺ reserve (27) between the two ventricles. Excitation propagation differs between the ventricles as do Ca²⁺-dependent outward transient Itof currents (36) and ATP-dependent, non-voltage-gated rectified K⁺ currents (2, 19). These differences stem from heterogeneity in channel expression levels [e.g., that of Kv4.2, Kv4.3, KChIP2, Kv1.5, and Kv2.1 (9, 25)], densities, amplitudes, and their sensitivity to agonists (1, 25, 27, 35, 41). Loss of heterogeneity in ion-current densities after myocardial infarction results in the development of arrhythmias (25). Interventricular differences in channel-mediated passive K⁺ transport imply that active transport of K⁺ mediated by the Na-, K-ATPase also varies between the ventricles to sustain transmembrane ion gradients. However, local differences in the abundance and activity of Na-, K-ATPase in ventricular tissue have never been studied.

Heterogeneity in energy demand required for sustaining the contractile force and ion gradient preservation suggests asymmetry in energy production in the ventricles. A local difference in oxygen extraction (60–75% by the LV and 50–51% by the RV) was observed during coronary venous blood sampling in open-chest dogs (28, 49). The right and left resting coronary venous P0₂ is ~30 and ~20 mmHg (4.0 and 2.7 kPa), respectively, indicating higher demand for energy in the LV (49). Under resting conditions, oxygen supply is controlled by coronary blood flow, which is higher in the left coronary artery than in the right.

What happens when the oxygen supply becomes limited? Severe local or global hypoxia results in "myocardial hibernation" followed by reduction in heart rate and myocardial contractility (17). Suppression of oxidative phosphorylation fuelled mainly by fatty acid metabolism is at least partially compensated for by an increase in anaerobic glycolysis to avoid irreversible ATP deprivation (11, 12, 20, 44). In a single study, autoradiography was used to assess local glucose utilization rates in the ventricles of conscious rats (29). Data
obtained in these in vivo settings indicate the existence of LV-to-RV heterogeneity in glucose utilization, which cannot be statistically resolved because of high interindividual variation. The present study explores the mechanisms of interventricular heterogeneity in response to acute hypoxic challenge in rat hearts. Acute challenge (1 h) was employed to exclude the effects driven by the changes in gene expression. We hypothesized that the differences in oxygen supply and anaerobic metabolism, as well as those in expression and activity of ion transport systems, will result in differential sensitivity of ventricles to oxygen deprivation.

In vivo and ex vivo experimental models were used to compare systemic and autonomous responses of the ventricular tissue to reduced oxygen supply, respectively. Data obtained in animals exposed to hypoxia for 1 h were compared with those generated in isolated rat hearts perfused with hypoxic autonomous blood passing through a hollow fiber oxygenator. By the reduction of the complexity of the system, this ex vivo model offered greater precision and reproducibility. We have monitored the interventricular heterogeneity and hypoxia-sensitivity of glucose utilization, tissue ion content, and redox state. Resulting data revealed substantial differences in glucose utilization capacity and maintenance of Na+/K+ gradient between the LV and RV.

MATERIALS AND METHODS

Organ harvesting procedure. Male Wistar rats (180–250 g) were purchased from Janvier (Le Genest, St Isle, France). All animal experiments were approved by the Federal Veterinary Office and performed in accordance with Swiss animal protection laws and institutional guidelines that comply with guidelines of the American Physiological Society and the Institute of Laboratory Animal Resources.

**In vivo hypoxic model.** Rats exposed to systemic hypoxia were placed in an INVIVO2 1000 hypoxic cabinet (Ruskin Technology/Ruskine Life Sciences, Bridgend, UK) in standard cages. Food and water were provided ad libitum. Ten percent oxygen was used in the in vivo hypoxic settings because conscious rats can tolerate this O2 levels well and show a prominent hypoxic response (46, 47). During the experiments, rats showed no signs of distress except for reduced respiratory rate and moderate hyperventilation. The reduction of O2 content in the hypoxic chamber from 20% to 10% was performed gradually, reducing oxygen in 2% increments with adaptation periods of 10 min at each O2 level. The animals remained in the hypoxic chamber for 1 h and were euthanized immediately upon removal from the chamber. The hearts were quickly harvested and chilled in an ice-cold sucrose washing solution [300 mM of sucrose and 20 mM of a HEPES-TRIS buffer (pH 7.4 at 0°C)]. Blood from the coronary vessels was then removed by perfusion with the same sucrose solution and processed as described below. The control animals from the normoxic group spent 2 h in the air-filled INVIVO2 hypoxic cabinet, and tissues were processed as described above.

**Ex vivo blood-perfused rat-heart model.** Before blood harvesting, animals were anesthetized using isoflurane (3% in a 1:1 mixture of O2 and N2O). The abdomen was opened and heparin (100 μl of 10,000 U/ml heparin; Braun, Grenchen, Switzerland) injected into the caudal vein. Blood (5–8 ml) was then collected from the caudal vein, and the animals were euthanized. Immediately after, the heart was removed and cooled down in an ice-cold physiological solution containing (in mM): 120 NaCl, 25 NaHCO3, 1 CaCl2, 0.15 MgCl2, 10 glucose, 0.1 l-arginine, 10 TRIS-HCl, pH 7.4. The ex vivo organ perfusion circuit constructed by Dr. J. Vogel consisting of a minioxygenator, a thermostated organ chamber, and a peristaltic pump (for details see Ref. 6 and Supplemental Fig. S1; supplemental material for this article is available online at the American Journal of Physiology Heart and Circulatory Physiology website) filled with blood at room temperature. The heart was mounted onto a perfusion cannula and perfused via the aorta. Blood perfusion was initiated at a rate of 3 ml/min, and the temperature of the water jacket was gradually increased to 37°C. The time between tissue harvesting and the onset of the perfusion never exceeded 5 min. Blood was equilibrated with a precalibrated humidified gas mixture containing 20% O2 (normoxia) or 5% O2 (hypoxia), 5% CO2, and balanced with N2 (PanGas, Basel, Switzerland). This concentration of oxygen in hypoxic gas mixture has been chosen on the basis of our previous findings as the one causing a pronounced autonomous response of the isolated heart but does not cause irreversible damage within 1 h of exposure to it (6). Blood gases, SO2, hematocrit, glucose, and the pH level were all controlled during the perfusion, using the Stat Profile pO2 Plus Blood Analyser (Nova Biomedical, Waltham, MA). In addition, hematocrit was assessed by means of microcapillary centrifugation. Glucose consumption by erythrocytes and water loss from the organ chamber were compensated for by supplementation of 1.1 mmol/l glucose (40 μl from 140 mM stock solution) every 20 min. Blood pH, glucose concentration, and hematocrit values (pH 7.42 ± 0.02, plasma glucose 5.5 ± 1 mM plasma glucose, hematocrit 25–30%) were stable during the 60 min of perfusion. Heart rate and ECG (aVL projection) were continuously recorded with a Heart Rate Module (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany) connected to an analog-digital transducer (Power Lab; ADInstruments, Oxfordshire, UK). Perfusion of the coronary vessels with hypoxic blood for 60 min caused a progressive decrease in the spontaneous heart rate (Supplemental Fig. S2B), whereas heart rate of those perfused with normoxic blood increased by 9%. An example of the original ECG recordings from normoxic and hypoxic hearts is shown in Supplemental Fig. S2C.

After a 20-min restitution period, during which the contractile function has been shown to stabilize, hearts were perfused for 1 h with normoxic or hypoxic blood (SO2 98% or 35%, respectively, Supplemental Fig. S2A). Hearts were then cooled and the blood washed away with ice-cold sucrose washing solution. Ventricular tissue was subsequently frozen in liquid nitrogen and later used to assess tissue Na, K-ATPase activity, ATP, and reduced (GSH) and oxidized (GSSG) glutathione levels. Samples were also collected for the analysis of the tissue ion concentrations and water content in preweighed, predried tubes and processed as described below.

**Tissue ion and water content.** Tissue water content and ion concentrations were monitored in blood-free ventricular samples. Gravimetric measurement of tissue water content was performed by assessing wet and dry (80°C, for 72 h) weight of each sample and reporting result as the percentage of water per wet weight. After being dried, the samples were wet burned with ultrapure concentrated HNO3. The tissue Na+ and K+ content was then determined using flame photometry (IL-943; Instrumentation Laboratory, Bedford, MA). Results were normalized to the dry weight of a sample.

**Na, K-ATPase activity in ventricular tissue homogenates.** Hydrolytic activity of the Na, K-ATPase was determined in ventricular tissue homogenates from isolated blood-perfused hearts exposed to normoxia or hypoxia (20 or 5% O2 in a gas phase) for 60 min. The Na, K-ATPase hydrolytic activity was assayed as previously described (39). Briefly, the ventricular tissue was homogenized in KCI-MOPS buffer and was added to media containing 130 mM NaCl, 20 mM KCl, 3 mM MgCl2, and 1 mM ouabain, according to experimental protocol at 37°C for 10 min. In the presence of the saturating concentrations of Na, K-ATPase substrate and ligands, the measured enzymatic activity corresponded to the pseudomaximal ATP cleavage rate, pseudo Vmax. The enzymatic ATP hydrolysis was initiated by adding an ATP-HEPES-NaOH mixture at final concentrations of 3 mM and 30 mM, respectively, and allowed to proceed for 7 min. The reaction was then stopped by adding ice-cold 4% formaldehyde in a 1.3 M sodium acetate solution buffered with acetic acid to pH 4.3.
Samples were mixed with 100 μl of a SnCl₂ solution (15 mg of SnCl₂ in 5 ml of 0.002% acetic acid) and 100 μl of a 2% (NH₄)₂MoO₄ solution in distilled water. After 15 min, a colored complex of phosphate with Sn³⁺ and (MoO₄)²⁻ was formed and evaluated by measuring the optical density (660 nm, Lambda 25 spectrophotometer; Perkin Elmer, Waltham, MA). Blank samples were either free of cell lysates, or the lysates were added after the ATP hydrolysis was stopped. Hydrolytic activity of Na, K-ATPase was calculated as a difference in the rate of phosphate production in corresponding ouabain-free and ouabain-containing sample pairs. Activity was normalized to the amount of protein in the homogenate quantified using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Ex vivo assessment of the glucose utilization rate. The autoradiography method used to assess the local rate of glucose utilization was initially developed by Kuschinsky et al. (29) for in vivo studies. A 5-μl aliquot of the radiolabeled nonmetabolizable glucose derivative ¹⁴C-2-deoxyglucose (¹⁴C-2-DOG; Amersham International, Cardiff, UK) from the stock (of 200 μCi/ml) was added to blood. Aliquots (25 μl) of blood were collected at 5, 10, 20, 30, and 45 min of perfusion and centrifuged (4,000 g for 3 min). Total plasma glucose content was assessed in plasma samples (3 μl) using a blood Glucose Meter (Ascensia Elite; Bayer, Basel, Switzerland). Also, plasma (10 μl) was added to scintillation fluid (10 ml), and the amount of ¹⁴C-2-DOG was determined using a scintillation β-counter (Tricarb 1000; Packard Bioscience, Downers Grove, IL). After 45-min perfusion, the heart was frozen in isopentane and kept at −20°C. Cryosections (20 μm) were mounted on glass coverslips, thawed immediately, dried on a hot plate (60°C), and exposed to film (MIN-R; Kodak, Jena, Germany) for 2 wk together with standards (American radio-labeled chemicals) described in Ref. 29. The distribution of ¹⁴C-2-DOG within and between the ventricles was determined using a densitometry camera (cool SNAP camera from Sigma DG macro D), and the images were processed using the Local Cerebral Glucose Utilization module of MCID image analysis software (Cambridge, UK).

Assessment of local tissue calcium uptake. Calcium accumulation in ventricular tissue was assessed by means of autoradiography. Radioactive tracer (30 μl, ⁴⁵Ca, specific activity >80.5 mCi/mmol, ~0.5 μCi/ml; Perkin Elmer) was added to blood at the onset of perfusion. At the end of the perfusion period (60 min), hearts were snap frozen in chilled isopentane (−20°C). Another experimental set included the addition of ouabain (1 μM) and ⁴⁵Ca to blood used to perfuse the coronary vessels. After the 60 min of perfusion, the heart was briefly washed out of the heart with an ice-cold sucrose-TRIS solution, and the heart was snap frozen in chilled isopentane. The heart was cut into sections (20 μm) and treated similarly to those perfused with ¹⁴C-DOG and exposed to the film for 3 wk. The ⁴⁵Ca distribution fingerprints were analyzed as described above for ¹⁴C-DOG.

GSH, GSSG, and ATP levels in myocardium. Tissue GSH, GSSG, and ATP levels were assessed in blood-free ventricular tissue preparations. Frozen ventricular fragments (~0.1 g) were homogenized on ice in KCl-MOPS buffer (100 mM KCl and 10 mM MOPS, pH 7.4 and deproteinized with 5% trichloroacetic acid). After centrifugation (5 min, 9,000 g, at 4°C), an aliquot of the protein-free supernatant was neutralized to a pH of ~7 with TRIS-OH powder, and ATP was assessed using an ATP Bioluminescent Assay Kit (FLAA; Sigma, St. Louis, MO). The luminescence intensity in heart tissue samples and in standard samples of known ATP content was monitored using a Sirius luminometer (Berthold Detection Systems, Pforzheim, Germany). Values were normalized to tissue wet weight. GSH and GSSG were assessed in the protein-free supernatant using Ellmann’s reagent and a Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Blank samples were either free of cell lysates, or the lysates were added after the ATP hydrolysis was stopped. Hydrolytic activity of Na, K-ATPase was calculated as a difference in the rate of phosphate production in corresponding ouabain-free and ouabain-containing sample pairs. Activity was normalized to the amount of protein in the homogenate quantified using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).
These values were lower, but within the range of mean rates previously reported in the hearts of normoxic conscious rats (53 \( \mu \text{mol} / 100 \text{ g}^{-1} \cdot \text{min}^{-1} \) LV and 30 \( \mu \text{mol} / 100 \text{ g}^{-1} \cdot \text{min}^{-1} \) RV) (29). Although hypoxia increased glucose utilization rates in both ventricles, utilization rates were greatest in LV compared with RV and apex (Fig. 1B).

Interventricular differences in glucose utilization rates in hypoxic myocardium were mirrored by heterogeneity in ATP content. Basal tissue ATP levels in the hearts obtained fresh from normoxic in vivo and ex vivo preparations did not differ between the ventricles. However, hypoxia caused selective depletion of the tissue ATP in the RV, but not in the LV (Fig. 2).

The redox state of ventricular tissue. Reduced and oxidized glutathione were used as markers of the tissue redox state. Tissue GSH content and the half-cell redox potential \( E_{hc} \) for the GSH/GSSG couple \( \{E_{hc} = -240 - (59.55/2)\log([\text{GSH}]/[\text{GSSG}])\} \) showed no interventricular variation in normoxic hearts (Fig. 3, A and B). Exposure of rats for 1 h to 10% \( O_2 \) resulted in development of oxidative stress in the RV but not the LV because of GSH depletion and a positive shift of the \( E_{hc} \) (Fig. 3A). On the other hand, perfusion of the isolated rat hearts with hypoxic blood was not accompanied by oxidation. On the contrary, it caused a slight insignificant shift \( (P = 0.062 \text{ for the LV} \) in the GSH and \( E_{hc} \) levels toward a more reduced state (Fig. 3B).

### Ion and water balance in myocardial tissue

Ventricular tissue \( Na^+ \) content was measured in ventricles of hearts harvested from rats exposed to normoxia (air) or hypoxia (10% \( O_2 \)) for 1 h and in isolated hearts perfused with normoxic or hypoxic (5% \( O_2 \)) blood for 1 h (Fig. 4A). Tissue sodium content in normoxic hearts in vivo was greater in the LV compared with the RV. Hypoxia had no effect on the tissue \( Na^+ \) content in LV or RV (Fig. 4A). Sodium levels in the LV of ex vivo hearts perfused with normoxic blood trended higher but were not significantly higher than those in the RV (Fig. 4B). Hypoxia caused a significant rise in LV sodium content, such that it was greater in LV compared with normoxic LV and RV as well as hypoxic RV (Fig. 4B). Sodium accumulation in the hypoxic LV was associated with \( K^+ \) loss in both ventricles ex vivo (Supplemental Fig. S3). However, there was no difference between RV and LV \( K^+ \) during normoxia or hypoxia. In accordance, tissue water content in the hypoxic heart did not differ from that of normoxic heart \((72.68 \pm 0.62 \text{ and } 73.77 \pm 0.96\% \text{ H}_2\text{O}/\text{wet weight } \text{in normoxic and hypoxic hearts, respectively, } n = 8\)).

In search for the mechanism underlying heterogeneous ion distribution between the ventricles, we have investigated the contribution of \( Na^+ \) and \( K^+ \)-ATPase to the ion balance ex vivo. Perfusion of hearts with normoxic blood containing low doses (1 \( \mu \text{M} \)) of the \( Na^+ \)-ATPase blocker, ouabain, caused selective accumulation of \( Na^+ \) in the RV, suggesting that the latter expresses higher levels of \( Na^+ \)-ATPase, or at least its ouabain-sensitive \( \alpha_2 \) isozyme (Fig. 5A). In ouabain-treated hearts the existing interventricular differences in \( Na^+ \) content were eliminated. To confirm these observations, Na, \( K^+ \)-ATPase hydrolytic activity was assessed in tissue homogenates prepared from RV and LV of rat hearts perfused with normoxic blood. As shown in Fig. 5B, the rate of ATP cleavage by Na, \( K^+ \)-ATPase in the RV homogenate significantly exceeded that in the LV. Hypoxia caused a massive reduction of Na, \( K^+ \)-ATPase activity in both ventricles and associated with \( Na^+ \) accumulation in the RV (Fig. 4B).

Increased myocardial sodium content triggers secondary \( Ca^{2+} \) uptake by cardiomyocytes (40). We have observed intracellular calcium accumulation associated with increase in the RV \( Na^+ \) content in ouabain-treated hearts using \( 45Ca^{2+} \) as a tracer. Distribution of the tracer in the ventricles was homogeneous in control hearts but shifted after perfusion with the Na, \( K^+ \)-ATPase blocker. In ouabain-treated hearts, \( 45Ca^{2+} \) levels in the RV exceeded those in the LV by \( 8.8 \pm 2.2\% \) \( (n = 5, P < 0.001) \). Furthermore, ouabain-induced sodium accumulation in the RV was associated with massive RV infarctions in 4 out of 12 hearts, whereas LV infarction was not observed.

### DISCUSSION

Our data provide evidence for heterogeneity in the maintenance glucose utilization and ion and redox balance in rat heart ventricles, rendering the RV more sensitive to the hypoxia. The observed heterogeneity likely originated from the differences...
in developmental origin of the ventricular cardiomyocytes (7, 10) and from the different functional requirements these cells present in an adult heart. Interventricular differences became particularly pronounced under conditions of hypoxic stress owing to the fact that the LV was less sensitive to hypoxic insult than RV. Interventricular heterogeneity in hypoxic responses is rarely taken into account when designing therapeutic strategies for the treatment of ischemic heart disease.

Effective glucose utilization is decisive for the preservation of ATP levels in hypoxic myocardium (11, 12, 20, 44). We have demonstrated limited capacity of the RV and apex for upregulation of glucose utilization upon hypoxic stimulation (Fig. 1). This limitation results in the inability of the RV to maintain the ATP level in response to hypoxia (Fig. 2). Because of the short period of hypoxic exposure, it is unlikely that the observed increase in glucose utilization rate was linked to the hypoxia-inducible factor-1-driven stimulation of de novo production of glycolytic enzymes. Rapid increase in glucose uptake in the LV could be mediated by recruitment of the GLUT-4 glucose transporters to the sarcolemma. Further investigations are required to prove whether this is the case and to delineate which of the factors listed in the recent review of Patterson et al. (37) are mediating the response.

Fig. 3. GSH content and half-cell redox potential $E_{hc}$ for the GSH/GSSG couple was calculated from the following equation: $E_{hc}(mV) = -240 - (59.55/2)\log([\text{GSH}]_2/[\text{GSSG}])$ in ventricular tissue. $A$: redox state parameters measured in the hearts of rats exposed to normoxia (air, $n = 5–7$) or hypoxia (10% $O_2$, $n = 5–7$) for 1 h before heart tissue was harvested. $\#P < 0.05$ compared with the corresponding RV and ***$P < 0.001$ compared with the corresponding normoxic control. $B$: tissue GSH content and $E_{hc}$ values obtained for isolated hearts perfused with normoxic or hypoxic blood ($n = 5–12$ per group).

Anaerobic glycolysis does not only provide ATP for sarcolemmal enzymes such as Na, K-ATPase (48) but also contributes to the replenishment of NADH and NADPH pools. The latter is used to maintain GSH levels and to convert GSSG back into GSH in a reaction that is catalyzed by glutathione reductase. Suppression of the glycolytic NADPH production by pharmacological inhibition of glucose-6-phosphate dehydrogenase was shown to cause GSH depletion in isolated rat cardiomyocytes (22). Reduction in GSH content was observed in the RV of freshly harvested hearts where glucose utilization rates are lower compared with the LV (Figs. 3 and 5A, and Ref. 24). We did not assess local changes in free radical production in the ventricles directly and therefore can only speculate on the heterogeneity in generation of prooxidative equivalents in the ventricles under hypoxic stress conditions. The origin of oxidative stress in hypoxic myocardium is still debated. Mitochondrial uncoupling (18) and reduction in NO production (30) have been suggested to contribute to an increase in the $H_2O_2$ production under hypoxic conditions. Exposure of rats to
systemic hypoxia increases the myocardial mechanical load, thereby boosting oxygen consumption (16). Pulmonary vasoconstriction in response to hypoxia could contribute to the oxidative stress observed in the RV in vivo, but not ex vivo. In addition lower glucose utilization rates in RV could result in lower NADH and NADPH production rates if our observations on the glucose utilization ex vivo are also found in vivo.

One of the interesting and unexpected findings of the present study is the heterogeneity of Na\textsuperscript{+}/H\textsuperscript{+} levels and Na, K-ATPase activity in the ventricles. The basal Na\textsuperscript{+} content reflects a balance between the contribution of passive transporters to the uptake of Na\textsuperscript{+}/H\textsuperscript{+} and that of the Na, K-ATPase in mediating the active Na\textsuperscript{+}/H\textsuperscript{+} extrusion. So far, interventricular heterogeneity in ion channel expression has only been reported for K\textsuperscript{+}/H\textsuperscript{+} channels (2, 19, 25), but not for Na\textsuperscript{+} channels. In the present study we have not evaluated Na, K-ATPase expression levels but focused on its functional characteristics. The activity of this enzyme depends on its isozyme composition, the phosphorylation, tyrosine nitration, S-nitrosylation, and S-glutathionylation of the catalytic and regulatory subunits, as well as the availability of substrates and ligands (5). Our data indicate that higher activity of Na, K-ATPase in the RV (Fig. 1C) is responsible for the RV-to-LV heterogeneity in tissue Na\textsuperscript{+} content. LV-to-RV Na\textsuperscript{+} gradient collapses in the heart perfused with ouabain, which is primarily blocking the ouabain-sensitive \(\alpha_2\)-isozyme (IC\textsubscript{50} 1–5 \times 10\textsuperscript{-7} M for the \(\alpha_2\)-\(\beta_1\) vs. 1–5 \times 10\textsuperscript{-5} M for the \(\alpha_1\)-\(\beta_1\)) in rodent myocardium (4, 24). The \(\alpha_2\)-\(\beta_1\) isozyme plays a key role in Ca\textsuperscript{2+} handling in the heart muscle (23). Its inhibition by perfusion with ouabain resulted in Na\textsuperscript{+} and Ca\textsuperscript{2+} accumulation in the RV (Fig. 1B). Further studies are required to verify whether the interventricular differences we have reported in rat heart are present in human myocardium in which affinity of the \(\alpha_1\) and \(\alpha_2\) isoforms to ouabain differs by about fivefold (33). If results are similar in human and rat tissue, caution should be taken when...
using cardiac glycosides such as digitalis for the treatment of heart failure and atrial fibrillation (32).

Coupling of the transmembrane Na\(^+\) gradients with the size of the Ca\(^{2+}\) stores in sarcoplasmic reticulum of cardiomyocytes, and hence with the contractile force amplitude, provides a possible explanation for the observed transventricular differences in the tissue Na\(^+\) content. Changes in the ventricular tissue Na\(^+\) levels were reported to result in a secondary alteration of the potential for force generation (3, 43). Our experiments were designed to assess the interventricular heterogeneity in short-term Ca\(^{2+}\) uptake. We observed greater Ca\(^{2+}\) accumulation in the RV of the ouabain-treated hearts. However, live imaging of Ca\(^{2+}\) transients in cardiomyocytes isolated from the RV and LV revealed that Ca\(^{2+}\) reserves of the RV and the LV differ (27). Furthermore, greater expression of the Na\(^+\)/Ca\(^{2+}\) exchanger was reported in LV compared with the RV in rat heart (14). Thus we hypothesize that interventricular asymmetry in basal ventricular Na\(^+\) concentrations contributes to the RV-to-LV differences in developed peak systole pressure (13).

Our findings, together with those reported in the literature, reveal the existence of a delicate balance between the local functional requirements, metabolic processes, and activity of the Na, K-ATPase in rat cardiac tissue. The observed interventricular differences indicate that the rat RV may be more prone to hypoxic damage because of its inability to recruit sufficient amounts of glucose as an alternative source of energy and reducing equivalents. Conditions that suppress Na, K-ATPase enzyme activity, such as hypoxia, have a greater effect on RV stability because the RV is more reliant on the Na, K-ATPase in rat cardiac tissue. The observed interventricular asymmetry in basal ventricular Na\(^+\) concentrations results in Na\(^+\) overload.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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