Ectopic pacing at physiological rate improves postanoxic recovery of the developing heart

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Rosa, A., J.-P. Maury, J. Terrand, X. Lyon, P. Kucera, L. Kappenberger, and E. Raddatz. Ectopic pacing at physiological rate improves postanoxic recovery of the developing heart. Am J Physiol Heart Circ Physiol 284: H2384–H2392, 2003; 10.1152/ajpheart.00758.2002.—Recently, rapid and transient cardiac pacing was shown to induce preconditioning in animal models. Whether the electrical stimulation per se or the concomitant myocardial ischemia affords such a protection remains unknown. We tested the hypothesis that chronic pacing of a cardiac preparation maintained in a normoxic condition can induce protection. Hearts of 4-day-old chick embryos were electrically paced in ovo over a 12-h period using asynchronous and intermittent ventricular stimulation (5 min on-10 min off) at 110% of the intrinsic rate. Sham (n = 6) and paced hearts (n = 6) were then excised, mounted in vitro, and subjected successively to 30 min of normoxia (20% O2), 30 min of anoxia (0% O2), and 60 min of reoxygenation (20% O2). Electrocardiogram and atrial and ventricular contractions were simultaneously recorded throughout the experiment. Reoxygenation-induced chrono-, dromo-, and inotropic disturbances, incidence of arrhythmias, and changes in electromechanical delay (EMD) in atria and ventricle were systematically investigated in sham and paced hearts. Under normoxia, the isolated heart beat spontaneously and regularly, and all baseline functional parameters were similar in sham and paced groups (means ± SD): heart rate (190 ± 36 beats/min), P-R interval (104 ± 25 ms), mechanical atrioventricular propagation (20 ± 4 mm/s), ventricular shortening velocity (17.7 ± 1 mm/s), atrial EMD (17 ± 4 ms), and ventricular EMD (16 ± 2 ms). Under anoxia, cardiac function progressively collapsed, and sinoatrial activity finally stopped after ~9 min in both groups. During reoxygenation, paced hearts showed 1) a lower incidence of arrhythmias than sham hearts, 2) an increased rate of recovery of ventricular contractility compared with sham hearts, and 3) a faster return of ventricular EMD to basal value than sham hearts. However, recovery of heart rate, atrioventricular conduction, and atrial EMD was not improved by pacing. Activity of all hearts was fully restored at the end of reoxygenation. These findings suggest that chronic electrical stimulation of the ventricle at a near-physiological rate selectively alters some cellular functions within the heart and constitutes a nonischemic means to increase myocardial tolerance to a subsequent hypoxia-reoxygenation.

In the adult heart, it is well established that the deleterious consequences of prolonged ischemia can be minimized if this condition is preceded by a short episode(s) of ischemia, the so-called classical preconditioning (30). Cardioprotection is measured in terms of a decrease in infarct size, a delay in ultrastructural damage, a reduction in myocardial stunning, and a diminution in incidence and severity of ischemia-reperfusion-induced arrhythmias. Preconditioning can also be induced by numerous nonischemic stimuli, such as exercise, heat stress, endotoxins or cytokines, catecholamines, pharmacological agents, adenosine, reactive oxygen species (ROS), and nitric oxide (NO) (for reviews see Refs. 6 and 41).

Acute and rapid artificial pacing has also been shown to induce preconditioning in various adult animal models (12, 17, 18, 46, 47, 53). Pacing rates used in these studies are clearly higher than the physiological intrinsic rates of the species under consideration, resulting in transient myocardial ischemia, which is most likely the principal cause of preconditioning. Rapid pacing has also been reported to produce myocardial protection by nonischemic activation of ATP-dependent K+ (KATP) channels (18). On the other hand, asynchronous activation and contraction induced by chronic ventricular pacing at a physiological rate redistributes the workload, alters regional metabolism (35), and increases the wall mass in remote regions with duration of pacing (52). However, to our knowledge, information referring to ectopic pacing at a physiological rate as a means of cardioprotection is very scarce.

Efficiency of cardioprotective procedures against ischemia-reperfusion injury appears to depend on age, because classical preconditioning of isolated neonatal mammal hearts has been shown to afford protection only from day 7 (5, 33). Relatively little is known about the possibility of protecting the whole embryonic/fetal heart against prolonged ischemia by classical or pharmacological preconditioning or by pacing.

Using the developing chick heart as an experimental model, we previously showed that ventricular pacing at a physiological rate induces structural, metabolic, and functional changes consistent with those observed in chick embryo; hypoxia-reoxygenation; preconditioning; arrhythmias; excitation-contraction coupling.

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the adult heart. Indeed, intermittent pacing over a 24- to 48-h period at 110% of the spontaneous rate leads to 1) a remodeling of the heart, with thickening of the atrial wall distant from the pacing site and thinning of the ventricular wall at the apex close to the pacing site (42), 2) a decrease of myocardial glycogen concentration and in the regions remote from the pacing site (24), and 3) a decrease of the production of ROS during reoxygenation after 30 min of anoxia (23).

In the present study, we tested the hypothesis that such a cardiac preparation subjected to a long-term intermittent ventricular pacing at a near-physiological rate can be protected against a subsequent anoxia-reoxygenation injury. We specifically attempted to characterize the most altered functional parameters and determine whether the pacing-induced effects observable during postanoxic recovery are homogeneous within the heart.

METHODS

In Ovo Pacing

Fertilized eggs from Lohman Brown hens were incubated for 96 h at 38°C and 90% relative humidity to obtain embryos at stage 24HH according to Hamburger and Hamilton (11). After a window was made in the shell, the heart of the chick embryo was paced over a 12-h period in ovo under controlled conditions of temperature (37°C) and humidity (70%) in a thermostabilized incubator and developed normally to stage 25HH. A stimulator (model 5880 A, Medtronic, Minneapolis, MN) and a platinum wire (0.2 mm diameter), isolated with a plastic sheath as a cathode, were used to induce pacing, which consisted of a discontinuous, asynchronous stimulation at the surface of the ventricular apex (5 min on-10 min off) at 110% of the intrinsic rate (i.e., 160–200 beats/min) with supraliminal intensities (2 times diastolic threshold), according to our recent study (24). Such a protocol allowed for 4 h of effective pacing, with visual testing of ventricular capture. The intrinsic heart rate of the embryo developing in ovo was also determined visually by measuring the frequency of the atrial filling, which was clearly identifiable.

The sham group consisted of hearts of 4-day-old embryonic chicks developing in ovo over a 12-h period under the same conditions as the paced group (i.e., open shell and equipped with electrodes) but not electrically paced.

In Vitro Anoxia-Reoxygenation

After pacing or sham operation, the spontaneously beating heart was carefully excised and placed in the culture compartment (300 μl) of an airtight stainless steel chamber within ~30 min. Two windows in the chamber were used for observation and measurements. The chambers were maintained under controlled metabolic conditions on the thermostabilized stage (37°C) of an inverted microscope (model IMT2, Olympus, Tokyo, Japan), as previously described in detail (36, 37, 43). Briefly, the culture compartment was separated from the gas compartment by a thin (15-μm), transparent, gas-permeable silicone membrane (model RTV 141, Rhône-Poule, Lyon, France). The heart was slightly flattened by the silicone membrane, and the resulting thickness of the myocardial tissue facing the gas compartment was ~300 μm. Thus PO2 at the tissue level could be strictly controlled and rapidly modified (within seconds) by flushing high-grade gas of selected composition through the gas compartment. At this developmental stage, the heart lacks vasculization, and the myocardial O2 requirement is met exclusively by diffusion.

The standard HCO3/CO2-buffered medium was composed of (in mmol/l) 99.25 NaCl, 0.3 NaH2PO4, 10 NaHCO3, 4 KCl, 0.79 MgCl2, 1.5 CaCl2, and 8 n-glucose. This culture medium containing the heart was equilibrated in the chamber with 2.31% CO2 in air (normoxia and reoxygenation) or in N2 (anoxia), yielding pH 7.4.

After 30 min of in vitro stabilization under normoxia, the heart was subjected to complete anoxia for 30 min and reoxygenated for 60 min. Thus the time between the end of the in ovo protocol and the onset of anoxia was 60 min.

In Vitro Recording of Electrocardiogram and Cardiac Contractions

Electrical and contractile activities of the isolated embryonic heart were recorded simultaneously and continuously throughout in vitro experiments.

Electrocardiographic (ECG) recording of the spontaneously contracting heart was performed using two Ag-AgCl electrodes 1 mm apart (0.3 mm diameter) inserted into the window facing the culture compartment. The atrial and ventricular regions were placed in the immediate vicinity of these electrodes, which were connected to a differential pre-amplifier (gain of 2,000), resulting in an output signal of 1–2 V from peak to peak. This signal was digitized and processed using an Apple Macintosh computer.

Contraction rates at the level of the atrium and the apex of the ventricle were recorded simultaneously using a computerized micrometric technique, as previously described (37, 43). Two adjustable phototransistors were positioned over the projected image of the contracting atrium and ventricle and connected to the computer via an analog-to-digital converter. The actual distance between the investigated atrial and ventricular regions was the same in the sham and paced hearts, i.e., 2.7 ± 0.2 mm (n = 6 for each group). Thus contractions were optically detected as the edge motion of the myocardial wall.

ECG and contractions were recorded with a sampling resolution of 3 ms.

Functional Parameters

Electrical activity. The ECG displayed characteristic P, QRS, and T components that allowed assessment of atrial and ventricular beating rate (beats/min) and atrioventricular (AV) conduction delay (P-R interval). Additionally, the various types of arrhythmias (mainly sinoatrial arrest, brady- and tachycardia, AV block, and Wenckebach phenomenon) and their duration could be precisely determined on the basis of the continuous ECG recording. The bradyarrhythmias were defined precisely by a regular atrial rhythm below 60 beats/min, in contrast to the tachyarrhythmias, which were defined by an abrupt acceleration (burst) of the regular basal rhythm, without any precise mention of the rate.

Contractile activity. The actual ventricular shortening at the apex (S, μm) was determined from video recordings, and the maximal shortening velocity was obtained from the maximal positive value of the first derivative of S (dS/dt, mm/s). Separated from the simultaneous recordings, it was possible to determine the mean velocity of the propagation of the wave front of the contraction between the atrium and the ventricle. This AV maximal propagation velocity (mm/s) was obtained from the actual distance of the selected regions divided by the time between the peaks of the maximal shortening velocity in the atrium and ventricle.

Excitation-contraction coupling. The EMD (ms), reflecting the efficiency of excitation-contraction coupling, was deter-
mined at the level of the atrium and ventricle by measuring the latency between the electrical and mechanical events, i.e., time between the initial phase of the P and Q components and the initiation of contraction in the atrium and ventricle, respectively.

**Protein and Glycogen Determination**

At the end of each experiment, hearts were carefully dissected into atria, ventricle, and conotruncus, which were stored at −20°C for subsequent biochemical determination, as described previously (24). Protein content was measured according to Lowry et al. (22), with bovine serum albumin used as standard. Glycogen content was measured spectrophotometrically according to Nahorski and Rogers (31) and expressed as glucose units.

**Statistical Analysis**

Values are means ± SD, unless otherwise indicated. The significance of any differences between paced and sham groups was assessed with the unpaired Student’s t-test. The significance of any differences between regional protein and glycogen content within the hearts was assessed with one-way analysis of variance with the Tukey-Kramer post hoc test. Statistical significance was defined by \( P \leq 0.05 \).

**RESULTS**

**Heart Rate In Ovo**

The intrinsic heart rate of the chick embryo developing in ovo over the 12 h of the experiment increased from 162 ± 8 to 169 ± 11 beats/min in sham hearts \((n = 6; P < 0.02)\) and from 162 ± 3 to 168 ± 5 beats/min in paced hearts \((n = 6, P < 0.02, \text{paired Student’s } t\)-test).

**Electrical and Contractile Activities Under Normoxia**

The P, QRS, and T components of the embryonic ECG were clearly identifiable, and simultaneous recording of atrial and ventricular contractions allowed us to determine EMD (Fig. 1). Baseline functional parameters of the sham and paced hearts during preanoxic in vitro stabilization were comparable (Table 1).

**Cardiac Response to Anoxia-Reoxygenation**

Atrial rate, P-R interval, mechanical AV delay, maximal shortening velocity, and EMD were markedly altered during anoxia-reoxygenation but fully recovered to their baseline values within ~30 min of reoxygenation (Figs. 2 and 3). Atrial activity completely stopped after 11 ± 3 min \((n = 6)\) and 12 ± 1 min \((n = 6)\) of anoxia and resumed 0.7 ± 0.4 min \((n = 6)\) and 0.9 ± 0.5 min \((n = 6)\) after readministration of \(O_2\) in sham and paced hearts, respectively. Interestingly, the time course of EMD in the atrium was significantly different from that in the ventricle, the latter being more altered (Fig. 3). Transient sinoatrial arrest, brady- and tachycardia, AV block (1st degree, 2nd degree, and complete), Wenckebach phenomenon, and a few ventricular escape beats were provoked by anoxia-reoxygenation (Fig. 4). The types of arrhythmias observed in sham and paced hearts were comparable.

**Table 1. In vitro baseline normoxic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Paced</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>190 ± 36</td>
<td>191 ± 20</td>
</tr>
<tr>
<td>P-R interval, ms</td>
<td>104 ± 25</td>
<td>106 ± 10</td>
</tr>
<tr>
<td>Mechanical AV delay, ms</td>
<td>131 ± 18</td>
<td>123 ± 9</td>
</tr>
<tr>
<td>AV propagation velocity, mm/s</td>
<td>20 ± 4</td>
<td>22 ± 2</td>
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<tr>
<td>EMD, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial</td>
<td>17 ± 4</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Ventricular</td>
<td>16 ± 2</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Ventricular shortening, μm</td>
<td>16 ± 12 (4.3–31)</td>
<td>13 ± 17 (3.4–48)</td>
</tr>
<tr>
<td>Maximal shortening velocity, mm/s</td>
<td>1.7 ± 1.0 (0.4–2.6)</td>
<td>1.0 ± 1.2 (0.3–3.5)</td>
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</tbody>
</table>

Values are means ± SD \((n = 6)\); values in parentheses are ranges. Preanoxic functional parameters in sham and paced hearts in vitro were measured at Hamburger and Hamilton stage 25HH. AV, atrio-ventricular; EMD, electromechanical delay.

**Fig. 1.** Typical recording of spontaneous electrical and contractile activities of isolated embryonic heart under normoxia at Hamburger and Hamilton stage 25HH. A and V, systolic peak in atrium and ventricle, respectively; EMD, electromechanical delay.
Effect of Chronic Pacing on Postanoxic Functional Recovery

During reoxygenation, recovery of heart rate, P-R interval, and atrial EMD was not altered by the pacing protocol (Fig. 5). In contrast, arrhythmias ended earlier and shortening velocity and ventricular EMD returned to baseline values faster in paced than in sham hearts (Fig. 6).

Metabolic Parameters

The protein content and the normalized glycogen content of the atrium, ventricle, and conotruncus at the end of reoxygenation are reported in Table 2. There was no difference in regional protein content between paced and sham hearts. On the other hand, the normalized glycogen content in the atrium was 34% lower in the paced than in the sham hearts, and the conotruncus displayed the lowest glycogen content in each experimental group.

DISCUSSION

To our knowledge, this is the first study in the embryonic heart that characterizes the electrical, mechanical, and metabolic spatiotemporal changes induced by O2 deprivation and readministration. Furthermore, our results indicate that long-term intermit-
tent ventricular pacing at a near-physiological rate selectively alters some functional parameters and affords cardioprotection. Indeed, during postanoxic reoxygenation, paced hearts showed 1) a lower incidence of arrhythmias, 2) a faster recovery of ventricular contractility, and 3) a faster return of ventricular EMD to basal value than sham hearts. However, recovery of heart rate, AV conduction, and atrial EMD was not improved by the pacing protocol.

**Characterization and Limitations of the Experimental Model**

As we stressed previously (24), the advantage of the embryonic heart model at the investigated stage is that it represents a relatively homogeneous population of cardiomyocytes (e.g., absence of vascularization, extrinsic neural regulation, fibroblasts, endothelium, and smooth muscle), beats spontaneously, reacts rapidly to anoxia-reoxygenation, and, most importantly, displays pacing-induced remodeling within only a few hours (36, 42), instead of weeks or months, as observed in adult animal models (35, 52). Moreover, access to the working heart and in vivo ectopic stimulation are easy and do not disturb embryonic development. Although there is no functional specialized conduction system in the embryonic heart before stage 33–36HH (13), the endogenous cardiac impulse propagates from the sinus venosus (51) and results in sequential activation of the atrium and ventricle (24, 42), and the slow-conducting properties of the AV junction and its long-lasting contraction fulfill the valve function (27). We previously observed that pacing the heart at the apex of the ventricle results in the opposite pattern of activation and also induces reduction in end-diastolic volume, stroke volume, and cardiac output (24), similar to adult heart models of pacing.

**Phylogenetic and developmental differences.** Species-specific and developmental differences in cardiac function and the protective maneuvers could exist. For example, excitation-contraction machinery seems to be more mature in chick than in mammal embryonic heart at equivalent stages of development (48), which suggests that the myocardial response to a given preconditioning stimulus or stress might vary from one species to another. Furthermore, the pathogenic mechanisms involved in hypoxia-reoxygenation insult as

**Fig. 5.** Postanoxic recovery of atrial rate (A), P-R interval (B), and atrial EMD (C) was not different in sham and paced hearts at stage 25HH. Values are means ± SE; n = 6.

**Fig. 6.** Postanoxic recovery of rhythmity (A), ventricular shortening velocity (B), and ventricular EMD (C) was faster in paced than in sham hearts at stage 25HH. Because of insufficient shortening and numerous arrhythmias, precise determination of velocity was not possible during the first 5 min in sham hearts. Values are means ± SE; n = 6. *P ≤ 0.04 vs. sham.
well as the ability of the neonatal myocardium to be preconditioned and to recover from hypoxia may be age dependent (5, 32, 33), but information about myocardial response to hypoxia-reoxygenation and preconditioning in the fetus is very scarce. Nevertheless, it has been shown that ventricular myocytes isolated from 10- or 14-day-old chick embryos can be preconditioned by brief ischemic episodes followed by reoxygenation (21, 51, 55, 56) or by activation of adenosine receptors (19). In the chick, as in many species, the duration of anoxia necessary to induce irreversible injury also depends on the developmental stage. Indeed, exposure of the isolated embryonic heart to 60 min of anoxia significantly delays but does not prevent full functional recovery at stage 24HH (96 h of incubation), whereas at stage 27HH (120 h of incubation) 60 min of anoxia appears to induce more severe or irreversible dysfunction (43). Comparison of embryonic with neonatal myocardium must be done with caution, because the neonatal heart has a functional coronary circulation, whereas the early embryonic heart, which lacks vascularization, depends exclusively on diffusion to meet its metabolic requirements.

**Chronotropic parameters and ventricular capture.** The spontaneous heart rate observed in ovo after 12 h of pacing, i.e., at stage 25HH, was 104% of that measured at stage 24HH and, consequently, never exceeded the artificial pacing rate set to 110% of the intrinsic heart rate, indicating that the ventricle was properly captured throughout the period of pacing.

**Dromotropic parameters.** Inasmuch as there is no differentiated nodal tissue at the stage investigated, the P-R interval and the mean velocity of mechanical AV propagation resulted from the combination of a rapid conduction in the atria and ventricle and a slow conduction in the region of the AV canal, which ensures adequate AV synchronization in the embryonic heart (2, 36, 43).

**Inotropic parameters.** Contrary to the chronotropic and dromotropic parameters, the ventricular shortening and maximal shortening velocity displayed important interindividual variations after in vitro stabilization (Table 1). Such a variability of contractile activity, observed in sham and paced groups, might be due to slight differences in developmental stage, variations of cardiac three-dimensional structure, and/or degree of flattening of the hearts in the culture compartment. Furthermore, the function of the isolated embryonic heart is known to be especially affected by changes in mechanical loading conditions (38). Thus, to overcome these interindividual differences, which are characteristic of embryogenesis, the time course of the functional parameters has been reported as a percentage of their preanoxic values (Figs. 5 and 6).

Although our experimental model is not directly relevant to current clinical practice, it could allow a better understanding of the cellular mechanisms underlying pacing-induced alterations. Also our findings could be useful in improving therapeutic strategies in the context of the recent advances in fetal/perinatal cardiology, especially in increasing tolerance of the immature heart to O2 deprivation and readministration.

### Effects of Chronic Pacing on Postanoxic Functional Recovery

The embryonic myocardium subjected to 12 h of intermittent pacing at a near-physiological rate adapts by structural (42) and metabolic (24) remodeling in such a way that it can be protected for a period of time against a subsequent episode of anoxia-reoxygenation. However, such a pacing protocol cannot be considered a preconditioning stimulus according to the strict definition given by Murry et al. (30). In contrast to myocardial remodeling, the functional consequences of pacing were observable only during postanoxic reoxygenation, whereas there was no difference between sham and paced hearts under steady normoxic conditions.

**AV differences.** With respect to myocardial response to anoxia-reoxygenation, some differential sensitivity was expected within the embryonic heart, because anoxic tolerance (43), glycogen content (24), oxidative and glycolgenolytic capacities (38), and expression of sarcoplasmic Ca2+-ATPase (28) and fast α-myosin heavy chain (10) are the highest in atrial myocardium. Atrial myocytes show a shorter action potential amplitude and duration (1, 8, 45) and a higher sensitivity to adenosine (20), which is an important regulator of embryonic/fetal cardiac function during hypoxic stress (34), and are less responsive than ventricular myocytes to otherwise deleterious doses of NO (49).

The present work shows for the first time that excitation-contraction coupling (reflected by EMD) is also less altered in the atrium than in the ventricle during an episode of anoxia-reoxygenation, irrespective of the pacing. These findings could reflect a differential sensitivity of the Ca2+-induced Ca2+ release mechanism and/or contractile proteins to reoxygenation injury in atrial and ventricular myocardium. This hypothesis is strengthened by the facts that 1) sarcoplasmic reticulum Ca2+ release through ryanodine receptors appears to be present and fully functional at the investigated stages (28, 48), 2) expression of the sarcoplasmic reticulum Ca2+-ATPase (SERCA2a) is the highest in atria, inhomogeneous in the ventricle, and the lowest in conotruncus (28), and 3) fast α- and slow β isoforms of the myosin heavy chain are dominant in the atria and

### Table 2. Protein and glycogen content after reoxygenation

<table>
<thead>
<tr>
<th></th>
<th>Atrium</th>
<th>Ventricle</th>
<th>Conotruncus</th>
</tr>
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<tbody>
<tr>
<td>Sham μg protein</td>
<td>27.5 ± 9.3</td>
<td>59.9 ± 15.5</td>
<td>12.3 ± 2.3</td>
</tr>
<tr>
<td>nmol GU/μg protein</td>
<td>0.96 ± 0.31</td>
<td>0.72 ± 0.21</td>
<td>0.35 ± 0.14†</td>
</tr>
<tr>
<td>Paced μg protein</td>
<td>29.5 ± 4.0</td>
<td>53.4 ± 11.7</td>
<td>13.2 ± 3.5</td>
</tr>
<tr>
<td>nmol GU/μg protein</td>
<td>0.43 ± 0.14*</td>
<td>0.56 ± 0.08</td>
<td>0.37 ± 0.10†</td>
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Values are means ± SD (n = 6). Regional protein (μg) and glycogen content [nmol glucose units (GU)/μg protein] were determined at the end of reoxygenation in sham and paced hearts at stage 25HH. *P < 0.05 vs. sham (ANOVA); †P < 0.05 vs. atrium or ventricle.
ventricle, respectively (27). Therefore, the intermittent chronic pacing, improving the postanoxic recovery of EMD and shortening velocity in the ventricle exclusively, might alter this distribution pattern and change the molecular phenotype, especially in the vicinity of the site of stimulation.

By contrast, the spontaneous pacemaking activity and the combined conducting properties of atria, AV junction, and ventricle appeared to remain unaffected by 12 h of pacing, because neither heart rate nor P-R interval nor mechanical AV delay was changed. The types of arrhythmias were the same in the two experimental groups, although they were more persistent during reoxygenation of the sham hearts. These findings suggest that there were no significant changes in ion currents involved in pacemaker cells or in connexin expression and/or distribution in paced hearts.

Taken together, these observations strongly support the hypothesis that atrial function of the embryonic heart is indeed less sensitive than ventricular function to O2 deprivation and readministration.

It is well documented that a prolonged electrical stimulation leads to modifications of gene expression in cultured skeletal myocytes (7) and neonatal cardiomyocytes (25, 54) and to histological alterations in the immature (15) or adult dog heart (35), whereas a single long-duration electrical field can induce morphogenetic and architectural changes in differentiating cardiomyocytes (40). Also, in fibrillating atria of patients (39) and in an animal model of atrial fibrillation induced by electrical pacing (3, 4), myocytes dedifferentiate and reexpress the phenotype of the immature myocardium. However, the molecular mechanisms whereby electrical stimulation alters gene expression remain unknown and should be investigated.

We previously showed that, in the heart isolated from the 4-day-old chick embryo, blockade of the L-type Ca2+ channels has antiarrhythmic properties during reoxygenation and attenuates ventricular contracture induced by repeated anoxia but has no dromotropic action (48). Here, pacing also had an antiarrhythmic effect, improved recovery of ventricular contractile activity, and EMD but did not alter AV propagation. These observations led us to consider that the observed beneficial effect of pacing could partly result from alteration of Ca2+ handling. Indeed, the deleterious Ca2+ overload known to be induced by reoxygenation in embryonic (43, 44) and adult cardiomyocytes (29) could be attenuated by pacing through regulation/modulation of the L-type Ca2+ channels. This does not rule out the involvement of other known mechanisms of preconditioning, such as a preischemic mild elevation of intracellular Ca2+ (9, 26), activation of protein kinase C, opening of sarcolemmal and/or mitochondrial KATP channels, and production of ROS and/or NO (41, 55). The possibility that L-type Ca2+ channels and plasmalemmal and/or mitochondrial KATP channels play a role in the pacing-induced protection of the developing heart model is under investigation in our laboratory.

Pacing-Induced Glycogen Redistribution

Our previous findings that myocardial glycogen is redistributed by 24 h of chronic intermittent ventricular stimulation (24) are confirmed in the present study, even after only 12 h of pacing. Indeed, the atrial region distant from the pacing site was depleted of glycogen (Table 2). This seems to be associated with the redistribution of workload and followed by structural remodeling induced by asynchronous activation and contraction (24, 42). It is likely that the spatiotemporal variation of glycogen content reflects a metabolic adaptation of the myocardium to a stress and contributes to the protection afforded by pacing.

Conclusion

Rapid and transient cardiac pacing induces preconditioning in adult animal models, but whether the electrical stimulation per se or the concomitant myocardial ischemia affords protection remains controversial. Preconditioning through ischemic or nonischemic pacing is not applied in clinical practice. However, improvement of angina in hypertrophic obstructive cardiomyopathy (14) as well as in hypertensive-cardiac hypertrophy with cavity obliteration (16) has been reported. Whether pacing at a near-physiological rate may benefit the heart of patients with ischemia who are implanted with a pacemaker warrants further investigations.

Our results indicate that the developing heart model maintained in a normoxic condition adapts to chronic intermittent asynchronous ventricular pacing at a near-physiological rate and increases its tolerance to a subsequent hypoxia-reoxygenation. A better understanding of the intracellular signal transduction pathways that might be involved in such a pacing-induced cardioprotection is of major clinical importance.

We are grateful to Christian Haebelri, Michel Jadé, and André Singy for technical support regarding the pacemaker system and Anne-Catherine Rochat for skillful technical assistance.

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