The L-Type Ca^{2+} and \( K_{ATP} \) Channels May Contribute to Pacing-Induced Protection Against Anoxia-Reoxygenation in the Embryonic Heart Model
Rapport de synthèse

Implication des canaux $\text{Ca}^{2+}$ de type L et des canaux $\text{K}_{\text{ATP}}$ dans la protection induite par pacing dans un modèle de cœur embryonnaire soumis à l’anoxie-réoxygénation.

**Contexte et but** : le canal $\text{Ca}^{2+}$ de type L, les canaux $\text{K}^+$ du sarcolemme ($\text{sarcK}_{\text{ATP}}$) et de la mitochondrie ($\text{mitoK}_{\text{ATP}}$) interviennent dans le préconditionnement ischémique ou pharmacologique du myocarde. La présente étude cherche à déterminer dans quelle mesure ces canaux peuvent aussi jouer un rôle dans la cardioprotection induite par pacing.

**Méthodes** : des cœurs d’embryons de poulet âgés de 4 jours ont été soumis *in ovo* à un pacing durant 12 heures, en pratiquant une stimulation électrique ventriculaire asynchrone intermittente à 110% de la fréquence cardiaque intrinsèque. Les cœurs contrôle (sham) et les cœurs stimulés ont ensuite été soumis *in vitro* à une période d’anoxie de 30 minutes, suivie d’une réoxygénation de 60 minutes. Les cœurs ont été exposés à l’agoniste du canal $\text{Ca}^{2+}$ de type L (Bay-K-8644, BAY-K) ou à son bloqueur (vérapamil, VERAP), à l’antagoniste non sélectif des canaux $\text{K}_{\text{ATP}}$ (glibenclamide, GLIB), ainsi qu’à l’agoniste du canal mito$\text{K}_{\text{ATP}}$ (diazoxide, DIAZO), ou à son antagoniste (5-hydroxydécanoate, 5-HD). L’électrocardiogramme, le délai électro-mécanique (DEM) reflétant le couplage excitation-contraction, ainsi que la contractilité myocardique ont été systématiquement déterminés pendant l’anoxie-réoxygénation.

**Résultats** : en normoxie, la fréquence cardiaque, l’intervalle QT, la conduction atrio-ventriculaire, le DEM et le raccourcissement ventriculaires étaient identiques dans les cœurs sham et les cœurs stimulés. Par contre, au cours de la réoxygénation post-anoxique, les arythmies cessaient plus précocement et le DEM ventriculaire retrouvait plus rapidement son niveau initial dans les cœurs stimulés, comparés aux sham. Dans les cœurs sham, BAY-K (mais pas le VERAP), DIAZO (mais pas le 5-HD) ou GLIB accéléraient la récupération du DEM ventriculaire, reproduisant ainsi la protection induite par le pacing. En revanche, aucun de ces agents n’affectait la récupération des cœurs stimulés.

**Conclusion** : un pacing ventriculaire chronique et intermittent délivré à une fréquence quasi physiologique améliore la tolérance myocardique à une anoxie-réoxygénation ultérieure. L’approche pharmacologique a montré qu’une activation discrète du canal $\text{Ca}^{2+}$ de type L, une inhibition du canal $\text{sarcK}_{\text{ATP}}$ et/ou une ouverture du canal $\text{mitoK}_{\text{ATP}}$ peuvent contribuer à la cardioprotection induite par le pacing.
The L-Type Ca\(^{2+}\) and K\(_{ATP}\) Channels May Contribute to Pacing-Induced Protection Against Anoxia-Reoxygenation in the Embryonic Heart Model

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**L-Type Ca\(^{2+}\) and K\(_{ATP}\) Channels in Pacing-Induced Cardioprotection.** Aims: The L-type Ca\(^{2+}\) channel, the sarcolemmal (sarcK\(_{ATP}\)), and mitochondrial K\(_{ATP}\) (mitoK\(_{ATP}\)) channels are involved in myocardial preconditioning. We aimed at determining to what extent these channels can also participate in pacing-induced cardioprotection.

Methods: Hearts of 4-day-old chick embryos were paced in ovo during 12 hour using asynchronous intermittent ventricular stimulation at 110% of the intrinsic rate. Sham operated and paced hearts were then submitted in vitro to anoxia (30 minutes) and reoxygenation (60 minutes). These hearts were exposed to L-type Ca\(^{2+}\) channel agonist Bay-K-8644 (BAY-K) or blocker verapamil, nonselective K\(_{ATP}\) channel antagonist glibenclamide (GLIB), mitoK\(_{ATP}\) channel agonist diazoxide (DIAZO), or antagonist 5-hydroxydecanoate. Electrocardiogram, electromechanical delay (EMD) reflecting excitation-contraction (E-C) coupling, and contractility were determined.

Results: Under normoxia, heart rate, QT duration, conduction, EMD, and ventricular shortening were similar in sham and paced hearts. During reoxygenation, arrhythmias ceased earlier and ventricular EMD recovered faster in paced hearts than in sham hearts. In sham hearts, BAY-K (but not verapamil), DIAZO (but not 5-hydroxydecanoate) or GLIB accelerated recovery of ventricular EMD, reproducing the pacing-induced protection. By contrast, none of these agents further ameliorated recovery of the paced hearts.

Conclusion: The protective effect of chronic asynchronous pacing at near physiological rate on ventricular E-C coupling appears to be associated with subtle activation of L-type Ca\(^{2+}\) channel, inhibition of sarcK\(_{ATP}\) channel, and/or opening of mitoK\(_{ATP}\) channel. (J Cardiovasc Electrophysiol, Vol. 19, pp. 1196-1202, November 2008)

Cardioprotection, asynchronous pacing, anoxia-reoxygenation, L-type Ca\(^{2+}\) channel, K\(_{ATP}\) channels, mitochondria

Introduction

Cardioprotection against ischemia-reperfusion injury can be achieved either by preconditioning the myocardium by brief ischemic episodes\(^{1,2}\) or by nonischemic means including the use of pharmacological agents.\(^{3,4}\) The beneficial effects can be measured in terms of reduction of infarct size, myocardial stunning, ultrastructural damages, and arrhythmias.\(^{5,6}\) Transient rapid ventricular pacing, in isolated or repeated episodes, has also been used as preconditioning stimulus in order to improve the postischemic recovery of the heart.\(^{7}\) This technique was, however, proposed to act through the same signaling pathways as those involved in the "classical" ischemic preconditioning, as high contractile rates generate a relative state of hypoxia. Despite the recent major advances in pacing technology\(^{8}\) and the unquestionable benefits afforded by the use of implantable pacing devices in patients with heart failure and cardiac dysynchrony,\(^{9}\) the underlying protective mechanisms remain poorly defined.

We have previously developed an embryonic chick heart model in which chronic intermittent ventricular pacing at near physiological rate over 12–48 hours rapidly results in hemodynamic alterations (reduced end diastolic volume and stroke volume),\(^{10}\) structural remodeling (wall thinning near pacing site),\(^{11}\) metabolic remodeling (glycogen redistribution), reduction of ROS production upon reoxygenation,\(^{10}\) and improvement of functional recovery after anoxia-reoxygenation (amelioration of E-C coupling and less arrhythmias).\(^{12}\) Similarly, it has been recently shown that dysynchronous ventricular activation at physiological rate can also precondition the adult heart (rabbit) against ischemia-reperfusion injury.\(^{13}\)

The role played by the L-type Ca\(^{2+}\) channel, the sarcolemmal (sarcK\(_{ATP}\)), and mitochondrial K\(_{ATP}\) (mitoK\(_{ATP}\)) channels, known otherwise to be involved in ischemic and pharmacological preconditioning,\(^{14-17}\) remains to be investigated in the pacing-induced protection. Using the embryonic chick heart model, we aimed at determining to what extent pharmacological modulation of these channels affects the protection against posthypoxic injury afforded by pacing at near physiological rate.
L-Type Ca\(^{++}\) and K\(_{ATP}\) Channels in Pacing-Induced Cardioprotection

**Material and Methods**

The investigation conforms with 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).

**Pharmacological Agents**

Bay-K-8644 (BAY-K), 5-hydroxydecanoate (5-HD), diazoxide (DIAZO), and glibenclamide (GLIB) were obtained from Sigma and verapamil (Isoptin, VERAP) from Knoll AG, Ludwigshafen, Germany.

**In Ovo Pacing**

Fertilized eggs from Lohman Brown hens were incubated 96 hours at 38°C and 90% humidity to obtain embryo at stage 24 HH, according to Hamburger-Hamilton.\(^{10}\) Pacing of the heart was performed through a window of the shell for 12 hours inside a thermostabilized incubator (37°C, 70% humidity) integrating a dissecting microscope, a micromanipulator, and a stimulator (Medtronic 5880 A, Medtronic Inc., Minneapolis, MN, USA) as previously described.\(^{10}\) Pacing protocol consisted of an asynchronous cathodic stimulation at the surface of the ventricular apex (after opening of the embryonic pericardium), with alternating pattern of stimulation, that is, 5 minutes ON – 10 minutes OFF (Fig. 1). The impulse duration was 2 ms and the stimulation frequency was chosen as 110% of the intrinsic heart rate with suprathreshold intensity (2x threshold of capture, tested visually). We used platinum wire (Ø 0.2 mm) as cathode, the anode being placed against the back of the embryo, following the electrical axis of the heart. The effective pacing time was 4 hours. Sham group consisted of 4-day-old embryos growing for 12 hours in the same conditions, that is, with opened pericardium and positioned electrodes, but without pacing. At the end of the pacing period, embryos reached stage 25 HH.

**In Vitro Anoxia-Reoxygenation**

After the in ovo pacing, the spontaneously beating hearts were carefully excised and placed in the culture compartment (300 µL) of an airtight stainless steel chamber, thermostabilized at 37.5°C, and equipped with two windows for observation and measurements, as previously described.\(^{19,21}\) This compartment was separated from the gas compartment by a thin (15 µm), transparent and gas-permeable silicone membrane (RTV 141, Rhône–Poulenc, Lyon, France), the heart being slightly flattened by the membrane. The system allowed a strict control of the PO\(_2\) at the myocardium level, using gas of selected composition.

The mounting of the heart into the chamber took approximately 30 minutes. After stabilization under normoxia (30 minutes), the hearts were submitted to 30 minutes anoxia followed by 60 minutes reoxygenation (Fig. 1). In sham and paced groups the tested pharmacological agents were present throughout experiments at the concentrations indicated in Figure 2.

All the experiments were performed in a standard HCO\(_3\)/CO\(_2\) buffered medium composed of (in mmol/L): NaCl 99.25; NaH\(_2\)PO\(_4\) 0.3; NaHCO\(_3\) 10; KCl 4; MgCl\(_2\) 0.79; CaCl\(_2\) 1.5; D+glucose 8. This culture medium was equili-
ECG recording was performed using two Ag/AgCl electrodes in the vicinity of these electrodes that were connected to a differential preamplifier (gain of 2000), and the signal was digitized and processed by a computer. Mechanical contraction (shortening) of atria and ventricle were optically detected as the edge motion of myocardial wall recorded simultaneously with ECG using a computerized setup. The actual shortening was determined using video recordings performed before anoxia and at the end of reoxygenation.

**Functional Parameters**

**Electrical activity**

The characteristic P, QRS, and T components of the embryonic ECG were used to determine atrial (PP interval) and ventricular (RR interval) beating rate, QT duration, and PR interval reflecting the mean atrioventricular conduction delay.

**Contractile activity**

The shortening (µm) at the ventricular apex was determined throughout anoxia-reoxygenation. The velocity of propagation of the wave of contraction between atria and ventricle, defined as the mechanical atrioventricular propagation velocity (mm/s), was calculated from the actual distance between these regions divided by the atrioventricular mechanical delay obtained from the mechanical recording.

**E-C coupling**

The electromechanical delay (EMD, ms), reflecting the E-C coupling, was determined in atria (EMDa) and ventricle (EMDv) by measuring the interval between the initiation of the P and QRS components and the beginning of contraction in atria and ventricle, respectively.

**Protein Determination**

At the end of experiments, hearts were systematically dissected into atria, ventricle, and conotruncus. Protein content of each cardiac region was determined according to Lowry et al. using BSA as standard.

**Statistical Analysis**

Values are reported as mean ± standard error of the mean (SEM), unless otherwise indicated. The significance of any differences between sham and paced hearts was assessed with the unpaired Student’s t-test, and that between treatment groups with ordinary ANOVA with Tukey-Kramer posthoc test. The significance of differences in functional recovery during reoxygenation was assessed using one-way analysis of variance (ANOVA) with repeated measures. The statistical significance was defined by a value of P < 0.05.

**Results**

**In Vitro Cardiac Activity Before and After Pacing**

In a series of experiments, the spontaneous heart rate determined in ovo before and after 12 hour of pacing increased from 157 ± 9 to 165 ± 12 (± SD; n = 17; P < 0.01, paired Student’s t-test) and from 156 ± 9 to 161 ± 11 (± SD; n = 11; P < 0.02) in sham and paced hearts, respectively, with no statistical difference between sham and paced. The electrical capture and intermittent stimulation did not affect the intrinsic heart rate.

**In Vitro Preanoxic Baseline Parameters**

After in vitro stabilization under normoxia, the functional parameters of the untreated sham and paced hearts did not differ (Table 1). The used pharmacological agents had no effect on cardiac activity except BAY-K, which had a positive chronotrophic effect and affected E-C coupling (EMDv), and DIAZO, which slightly increased PR interval.

**Pacing Improved Postanoxic Recovery**

Relative to untreated sham hearts, the improvement of EMDv recovery (Fig. 3A) and the reduction of arrhythmias during the first 30 minutes of reoxygenation in untreated paced hearts (Fig. 3B) confirm our previous findings. It should be noticed that during reoxygenation EMDv was the only functional parameter that was affected by pacing. Indeed, there was no chrono-, dromo-, or inotropic effect of pacing during anoxia-reoxygenation (Fig. 4). As recently characterized, reoxygenation-induced arrhythmias were essentially brief cardioplegia, bursting activity, atrial ectopy, first-, second-, and third- degree AV blocks, ventricular escape beats and Wenckebach phenomenon that disappeared after 30–55 minutes of reoxygenation. No pharmacological agent significantly altered incidence, type or duration of arrhythmias observed in sham or paced hearts during reoxygenation. However, BAY-K and GLIB slightly, but not significantly, prolonged arrhythmias (mainly AV blocks) in both experimental groups (not shown). Relative to other agents, BAY-K increased specifically the incidence of ventricular escape beats in sham and paced hearts only during the first 30 minutes of reoxygenation.

**Involvement of L-Type Ca2+, sarC_{ATP}, and mitoK_{ATP} Channels in Pacing-Induced Protection**

In sham hearts, activation of L-type Ca2+ channel by BAY-K, inactivation of both sarC_{ATP} and mitoK_{ATP} channels by GLIB or opening of mitoK_{ATP} channel by DIAZO had the same protective effect on EMDv as pacing (Fig. 5), with no significant impact on the other investigated parameters (Fig. 4). Remarkably, none of these agents further improved EMDv recovery in the paced hearts. After 60 minutes reoxygenation, heart rate, QT duration, PR interval, and atrioventricular mechanical propagation returned to their baseline value, whatever the experimental conditions. However, ventricular shortening of the sham hearts was noticeably affected by VERAP and declined during reoxygenation (Fig. 4). Although EMDa recovery was delayed by BAY-K in paced but not in sham hearts, it returned to its preanoxic level after 30 minutes of reoxygenation (not shown).
Regional Protein Content

The protein content in atria, ventricle, and conotruncus was the same in sham and paced hearts whatever the experimental conditions (atrium 20 ± 5.6 µg, ventricle 52.7 ± 10.2 µg, and conotruncus 10.7 ± 3.8 µg; mean ± SD; n = 44).

![Diagram](image)

**Figure 3. Ventricular electro-mechanical delay EMDv (A) and incidence of arrhythmias (B) of sham and paced hearts during anoxia and reoxygenation. Recovery rate of EMDv was significantly faster in paced than in sham hearts. Reoxygenation-induced arrhythmias ceased earlier in paced hearts. EMDv was expressed as % of preanoxic value. Mean ± SEM; *P < 0.05, ANOVA repeated measures; n = 4.**

**TABLE 1**
Baseline Parameter of Sham and Paced Hearts In Vitro

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Verapamil (10 nM)</th>
<th>Bay-K (1 µM)</th>
<th>5-HD (500 µM)</th>
<th>GLIB (10µM)</th>
<th>DIAZO (100 µM)</th>
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<td>Ventricular shortening (µm)</td>
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<td>60 ± 34</td>
<td>91 ± 38</td>
<td>76 ± 46</td>
<td>49 ± 7</td>
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</table>

Baseline parameters in sham (s) and paced (p) hearts. * P < 0.05 vs untreated, ANOVA; † P < 0.05 vs sham, unpaired Student's t-test; AV = atrio-ventricular; EMD = electro-mechanical delay; 5-HD = 5-hydroxydecanoate. Data are expressed as mean ± SD; n = 4 for each condition.

Discussion

Characteristics and Limitations of the Model

Chronic intermittent ventricular pacing at near physiological rate induces myocardial remodeling and protection rapidly (few hours) in the embryonic heart model. The fact that the protein content in atria, ventricle, and conotruncus was similar in all experimental groups, indicates that 12 hour of pacing did not affect the normal growth of the heart. The intrinsic heart rate of the developing embryo was not modified by in ovo pacing and the spontaneous beating rate of the isolated heart was the same in vitro as that measured in ovo, indicating that the spontaneous pacemaker activity was not altered by the chronic intermittent electrical stimulation or the culture conditions. The thinness of the avascular wall (circa 200 µm) of the embryonic heart at the investigated stage allowed the pharmacological agents to diffuse readily into the myocardial tissue. These agents being present throughout anoxia-reoxygenation, it was not possible to discriminate between their specific action during anoxia or during reoxygenation. In particular, the persistence of arrhythmias in some hearts at the end of reoxygenation in the presence of BAY-K and GLIB could be partly explained by a long-lasting exposure to these potentially arrhythmogenic agents. BAY-K was used at 1 µM as it did not cause major functional disturbances in our experimental conditions and prior studies carried on the properties of the L-type Ca2+ channels in embryonic chick cardiomyocytes have been performed in the same range of concentration, that is, 1–2 µM. The precise detection of intracellular Ca2+ variations resulting from the pharmacological maneuvers or pacing was made difficult in our model because of the thickness of the myocardium, the structural inhomoogeneity of the ventricle (several cell layers, compact myocardium, trabeculae, residual erythrocytes), and the motion of the ventricle wall during contractions.

Bruchez et al. L-Type Ca2+ and KATP Channels in Pacing-Induced Cardioprotection
Pacing-Induced E-C Coupling Protection is Mimicked by Activation of the L-Type Ca\textsuperscript{2+} or mitoK\textsubscript{ATP} Channels as well as by Inactivation of the sarcK\textsubscript{ATP} Channels

Activation of L-type Ca\textsuperscript{2+} Channel

The transmembrane Ca\textsuperscript{2+} influx through L-type Ca\textsuperscript{2+} channels is required for cardiac E-C coupling and known to play a crucial role in normal heart development and function.\textsuperscript{27} Activation of these channels by anoxia-reoxygenation can also result in Ca\textsuperscript{2+} overload associated with arrhythmias and contractile dysfunction in the embryonic heart.\textsuperscript{28,29} In chick embryonic cardiomyocytes, BAY-K induces long-lasting opening of L-type Ca\textsuperscript{2+} channel\textsuperscript{20} and increases amplitude and duration of the action potential with a slight positive chronotropic effect,\textsuperscript{31,32} which was also observed under our basal conditions. The combination of the atrial tachycardia induced by BAY-K (Table 1) and the prolongation of PR interval (Fig. 4) and QT duration\textsuperscript{23} during anoxia and upon reoxygenation increases the risk of AV dissociation and/or ventricular escape beats in the embryonic heart that otherwise lacks specialized conduction system.\textsuperscript{33} As VERAP drastically depressed ventricular contractility under normoxia, anoxia, and reoxygenation (shortening, Fig. 4A), it has not been tested in the paced group.

In this work, a slight activation of the L-type Ca\textsuperscript{2+} channels by BAY-K in sham hearts mimicked the pacing-induced protection of EMDv during reoxygenation with no effect on paced hearts. These findings clearly indicate that the properties and/or expression of the L-type Ca\textsuperscript{2+} channels in the ventricle were somewhat altered by pacing and suggest that the gain of E-C coupling can be increased by pacing at near physiological rate.

Inactivation of sarcolemmal K\textsubscript{ATP} channels

The fact that the unspecific K\textsubscript{ATP} channel blocker GLIB had an effect on EMDv recovery, unlike the mitoK\textsubscript{ATP} channel blocker 5-HD, indicates that sarcK\textsubscript{ATP} channels are also involved. Indeed, decreasing the opening probability of the sarcK\textsubscript{ATP} channels with a concentration of GLIB that otherwise does not alter the baseline parameters improved the recovery of EMDv in sham hearts. This action mimicks the protective effect of pacing with no effect on recovery of EMDv in paced hearts. We hypothesize that inactivation of sarcK\textsubscript{ATP} channels results in a slight membrane depolarization that,
in turn, can activate the L-type Ca\(^{2+}\) channels (as BAY-K directly does), increasing also intracellular Ca\(^{2+}\) and improving E-C coupling gain (see Fig. 2).

**Activation of the mitoK\(_{ATP}\) channel**

Recently, we have shown that mitoK\(_{ATP}\) channel activation by DIAZO in the postanoxnic embryonic heart protects ventricular EMD via NO\(_{2}\), ROS\(_{2}\), and PKC-dependent pathways, this protection being abolished by 5-HD.\(^{34}\) Here, the same DIAZO-mediated protection of EMD was observed in sham hearts but not in paced hearts, and 5-HD did not reverse the protection afforded by pacing. Our findings strongly support the hypothesis that the three investigated channels respond together in combination to chronic electrical stimulation and are all involved in postanoxic protection. Consequently, in paced heart, pharmacologically targeting a specific channel could contribute to maintain a higher level of cytosolic Ca\(^{2+}\).

In our experimental setting, it was not possible to determine whether L-type Ca\(^{2+}\) channel, sarcK\(_{ATP}\), or mitoK\(_{ATP}\) play a predominant role in pacing-induced protection.

**Pacing-induced protection might result from a moderate rise in intracellular calcium**

In this study, BAY-K, GLIB, and DIAZO improved the postanoxic EMD recovery in the sham hearts and not in paced hearts (Fig. 5). Through their different modes of action, these agents can lead directly or indirectly to a moderate increase in intracellular calcium. The other tested drugs, that is, VERAP and 5-HD, which tend to diminish intracellular calcium, did not significantly affect the functional recovery after anoxia in sham hearts. On the basis of these observations and our findings, a possible interpretation is that a slight increase in calcium could indeed improve E-C coupling of the paced hearts in the setting of anoxia-reoxygenation.

Besides the classical ischemic preconditioning, Ca\(^{2+}\) by itself could play a major role in myocardial preconditioning, directly or as a second messenger altering gene expression.\(^{15}\) Furthermore, ischemic preconditioning is inhibited by L-type Ca\(^{2+}\) channel blockers.\(^{17}\) In our model, the Ca\(^{2+}\) change induced by pacing was expected to be small, much less than a Ca\(^{2+}\) overload, since we observed no additional contracture and no significant shortening aleration in paced hearts relative to sham, whatever the treatment. We propose that the diastolic rather than the systolic Ca\(^{2+}\) concentration is altered, since we never observed an increase of ventricular shortening (strongly related to the peak of Ca\(^{2+}\) transient) under normoxia or during anoxia-reoxygenation in paced relative to sham hearts (Table 1, Fig. 4). Thus, a diastolic Ca\(^{2+}\) concentration slightly higher than the basal normoxic level but lower than a damaging Ca\(^{2+}\) overload\(^{38-40}\) could be cardioprotective. The fact that recovery of EMDV in the paced hearts could not be ameliorated by safe concentrations of BAY-K, GLIB, or DIAZO supports also this hypothesis.

Finally, it should be noticed that the pacing-induced improvement of EMDV was observable exclusively during reoxygenation, whereas there was no detectable alteration under normoxia in this experimental setting, showing that an anoxic stress was required to uncover the beneficial effect of pacing.

**Conclusion**

The protective effect of electrical stimulation at near physiological rate on ventricular E-C coupling appears to be associated with activation of L-type Ca\(^{2+}\) channel, inhibition of sarcK\(_{ATP}\) channel, and/or opening of mitoK\(_{ATP}\) channel. All these conditions can lead directly or indirectly to a moderate elevation of cellular Ca\(^{2+}\), suggesting that Ca\(^{2+}\)-dependent mechanisms may be involved in the protection afforded by pacing.

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**References**


