Mitochondrial BK_{Ca} channels contribute to protection of cardiomyocytes isolated from chronically hypoxic rats

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Borchert GH, Yang C, Kolář F. Mitochondrial BK_{Ca} channels contribute to protection of cardiomyocytes isolated from chronically hypoxic rats. Am J Physiol Heart Circ Physiol 300: H507–H513, 2011. First published November 26, 2010; doi:10.1152/ajpheart.00594.2010.—Chronic hypoxia protects the heart against injury caused by acute oxygen deprivation, but its salutary mechanism is poorly understood. The aim was to find out whether cardiomyocytes isolated from chronically hypoxic hearts retain the improved resistance to injury and whether the mitochondrial large-conductance Ca^{2+}-activated K^{+} (BK_{Ca}) channels contribute to the protective effect. Adult male rats were adapted to continuous normobaric hypoxia (inspired O_2 fraction 0.10) for 3 wk or kept at room air (normoxic controls). Myocytes, isolated separately from the left ventricle (LVM), septum (SEPM), and right ventricle, were exposed to 25-min metabolic inhibition with sodium cyanide, followed by 30-min reenergization (M/I/R). Some LVM were treated with either 30 μM NS-1619 (BK_{Ca} opener), or 2 μM pipxilline (BK_{Ca} blocker), starting 25 min before metabolic inhibition. Cell injury was detected by Trypan blue exclusion and lactate dehydrogenase (LDH) release. Chronic hypoxia doubled the number of rod-shaped LVM and SEPM surviving the M/I/R insult and reduced LDH release. While NS-1619 protected cells from normoxic rats, it had no additive salutary effect in the hypoxic group. Pipxilline attenuated the improved resistance of cells from hypoxic animals without affecting normoxic controls; it also abolished the protective effect of NS-1619 on LDH release in the normoxic group. While chronic hypoxia did not affect protein abundance of the BK_{Ca} channel regulatory β_{1}-subunit, it markedly decreased its glycosylation level. It is concluded that ventricular myocytes isolated from chronically hypoxic rats retain the improved resistance against injury caused by M/I/R. Activation of the mitochondrial BK_{Ca} channel likely contributes to this protective effect.

Continuous hypoxia; ventricular myocytes; metabolic inhibition; cell viability; potassium channels

KOPECKY AND DAUM (19) were the first to demonstrate experimentally that adaptation to chronic hypoxia increases tolerance of the heart against injury caused by acute oxygen deprivation. This observation has been subsequently elaborated in numerous studies that confirmed the protective effect of chronic hypoxia using a variety of experimental models and adaptation protocols. The improved tolerance of chronically hypoxic hearts to ischemia-reperfusion injury manifests itself as a limitation of myocardial infarct size, increased postischemic recovery of cardiac contractile function, and reduced incidence and severity of both ischemic and reperfusion ventricular arrhythmias (25). Probably the most important feature of this adaptive phenomenon is that its salutary effect on myocardial viability persists much longer than any form of preconditioning (22).

Compared with short-lived preconditioning, the molecular mechanism underlying the long-lasting myocardial protection afforded by chronic hypoxia is still poorly understood, yet the experimental evidence available points to an important role of adaptive changes occurring on the level of mitochondria (12, 18). Among mitochondrial components, ATP-sensitive K^{+} (K_{ATP}) channels were most studied, and several reports have suggested their involvement in the protective mechanism of chronic hypoxia against all major endpoints of ischemia-reperfusion injury (3, 11, 21, 38).

Recent studies have revealed that, besides K_{ATP} channels, the inner mitochondrial membrane contains large-conductance Ca^{2+}-activated K^{+} (BK_{Ca}) channels (27, 31, 36) that are opened by hypoxia (10) and contribute to myocardial protection (5, 9, 23, 29, 32, 36). The protective effect of BK_{Ca} opening has been attributed to increased matrix K^{+} uptake and volume, improved respiratory control (2), inhibition of mitochondrial Ca^{2+} overload (17, 33), and prevention of permeability transition pore opening (10). The channel is composed of the pore-forming α-subunits and auxiliary β-subunits that regulate its activity. The β_{1}-subunit is highly expressed in cardiomyocytes (4). It has been shown that it has two N-glycosylation sites, and its enzymatic deglycosylation activates the channel by increasing both open probability and mean open time (15).

Thus the primary goal of our study was to find out whether BK_{Ca} channels are involved in the cardioprotective mechanism of adaptation to chronic hypoxia using a selective BK_{Ca} blocker pipxilline and opener NS-1619 (29). As this channel has not been detected in the sarcolemma of cardiac myocytes (27), isolated cardiomyocytes appear to be a suitable model to study the role of channels localized in mitochondria. We hypothesized that cells isolated from chronically hypoxic rats will retain the improved resistance to injury caused by simulated ischemia, and that the BK_{Ca} channels will contribute to this effect. Moreover, we analyzed effects of chronic hypoxia on the regulatory β_{1}-subunit protein level and glycosylation status to consider its potential role in BK_{Ca}-mediated cardioprotection.

METHODS

Animals. A total of 24 adult male Wistar rats (250–300 gbody wt) were exposed to continuous hypoxia (inspired O_2 fraction: 0.10) using a normobaric chamber equipped with hypoxic generators (Everest Summit, Hypoxico, NY) for 3 wk. No reoxygenation occurred during this period. Animals were removed from the hypoxic chamber 24 h before experiments. The control group of 28 rats was kept under normoxic (inspired O_2 fraction: 0.21) conditions. All animals were housed in a controlled environment (23°C; 12:12-h light-dark cycle; light from 5:00 AM) with free access to water and standard chow diet. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (National Institutes of Health publication no.

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were removed to measure protein concentration. This volume was diluted with a ratio of 1:5 (vol/vol) and vortexed until the cell pellet was completely dissolved. Tris, 4% SDS, 20% glycerol was added to achieve cells-to-buffer volume ratio of 1:5 (vol/vol) and vortexed until the cell pellet was completely dissolved. The cell solution was washed out by 10-min perfusion with Ca²⁺-free Tyrode. The right ventricle was cut off first and then the interventricular septum and the left ventricle. Separate ventricular parts were dispersed mechanically; myocyte solutions were adjusted to the same cell density and transferred to culture medium.

Experimental protocol. Isolated cardiomyocytes were cultured in cell culture medium (50% Dulbecco’s modified Eagle’s medium and 50% Nutrient Mixture F12/HAM, containing 0.2% BSA, 100 U/ml penicillin, and 100 μg/ml streptomycin). Myocytes were kept in a CO₂ incubator (95% air, 5% CO₂, 28°C) for 1-h stabilization period. In the first series of experiments, myocytes isolated separately from the left ventricle (LVM), the right ventricle (RVM), and the septum (SEPM) of chronically hypoxic and normoxic rats were subjected to metabolic inhibition (MI) and reenergization (MI/R). In the second set of experiments, LVM isolated from chronically hypoxic and normoxic rats were treated with BKCa modulators, starting 25 min before MI and continuing during the whole experiment. LVM from each heart were divided into four groups treated with either 30 μM NS-1619 (BKCa opener), 2 μM paxilline (BKCa blocker), or 0.1% DMSO (vehicle control); the last group served as untreated control. In a separate experiment, an additional group of LVM from normoxic rats was pretreated with paxilline for 25 min before NS-1619.

Myocytes from each ventricular part and/or treatment group were split into two parts of equal volumes. Control cells were incubated in normal Krebs solution and not exposed to MI/R. Experimental cells were subjected to 25 min of MI, followed by 30 min of reenergization. MI was induced by incubation of cells in the modified Krebs solution containing 1.5 mM NaCN and 20 mM 2-deoxyglucose instead of glucose. The reenergization was achieved by removing the metabolic inhibitors and replacing the MI solution with the normal cell culture medium (the same medium was applied to control cells).

Cell viability and LDH release. Cell viability and LDH release were evaluated at the beginning of the experiments (after stabilization), after MI (LDH release only), and after reenergization. The number of viable and dead (stained) myocytes was determined by Trypan blue exclusion (34). Typically, 50–100 myocytes were counted in duplicates from 6–8 independent experiments. Viable myocytes were divided into two fractions: rod-shaped myocytes with the cell length-to-width ratio > 3:1 and non-rod-shaped myocytes with the ratio < 3:1. Viability after MI/R was expressed as a percentage of rod-shaped cells that survived the MI/R insult, normalized to the appropriate control group not exposed to MI/R.

LDH was determined spectrophotometrically (7) using the LDH Liqui-UV Kit (Stanbio, Boerne, TX). LDH released during MI and during reenergization was normalized to total LDH content in the cells and expressed as a percentage of appropriate control values.

Western blot analysis of BKCa channel β1-subunits. Cardiomyocytes isolated from chronically hypoxic and normoxic rats were frozen in liquid nitrogen and stored at −80°C until use. Samples were prepared as described earlier (16). The cells were pelleted at 500 g for 5 min, the isolation buffer was removed, and 1 ml SDS sample buffer (0.1 M Tris, 4% SDS, 20% glycerol) was added to achieve cells-to-buffer ratio of 1:5 (vol/vol) and vortexed until the cell pellet was completely resolved. The samples were heated at 100°C for 10 min, and 10 μl were removed to measure protein concentration. This volume was replaced by 100 μl β-mercaptoethanol, and 10 μl bromphenol blue were added. The samples were again exposed to 100°C for 10 min, and aliquots of 50 μl were taken and stored at −80°C until use.

Samples of 3–5 μl per lane were loaded on a 10% acrylamide gel. The proteins were separated at constant voltage (100 V) for ~90 min and transferred to polyvinylidene difluoride membrane at 0.35 A for 75 min. The transferred proteins were blocked overnight in 5% fat-free milk and 1% BSA. The primary antibody against β1 (diluted 1:200 in 5% milk and 1% BSA) was applied for 1 h at room temperature, followed by a three-times washing with PBS. Then the blots were incubated with anti-rabbit horseradish peroxidase-labeled secondary antibody (1:30,000) for 1 h, followed by three more washes. The blots were exposed to the Amersham Hyperfilm ECL for 15 min.

After the films were developed, the blots were washed in PBS, and the antibodies were removed using a common desorption protocol. The blots were then incubated with blocking solution (5% milk, 1% BSA) overnight and probed against GAPDH to verify the amount of protein loaded to the gel. Films were scanned and evaluated by using AIDA software.

Deglycosylation of the BKCa channel β1-subunits. Cells were centrifuged at 1,000 g for 5 min to a final pellet of 250 μl. The same volume of a solubilization buffer (50 mM sodium-phosphate buffer, 150 mM NaCl, 10 mM KCl, 1.8% SDS, 17.5% glycerol, pH 7.2, and 3.3 μl/ml protease inhibitor cocktail P8340) was added and vortexed to yield a clear yellow-beige solution. The solubilized sample was kept 5 min at room temperature, followed by centrifugation at 12,000 g for 15 min. The final supernatant protein concentration was between 5 and 10 μg/μl. Deglycosylation was performed using the GKE5006 kit (Prozyme, San Leandro, CA) as follows: 35 μl of the solubilized protein, 10 μl reaction buffer (100 mM sodium phosphate, 0.1% sodium azide, pH 7.5), 2.5 μl denaturation buffer (2% sodium dodecyl sulfate, 1 M β-mercaptoethanol), 2.5 μl detergent solution (15% nonyl phenoxypolyethoxylethanol), and 4 μl N-glycanase (peptide-N-glycanase F) were added to a tube, vortexed, and incubated at 37°C for 18 h in a thermo block. Control tube contained 4 μl water instead of N-glycanase. After 18 h, 10 μl diithiothreitol (1 M) and 60 μl loading buffer (100 mM Tris-HCl, pH 6.8, 200 mM diethiothreitol, 4% SDS, 0.2% bromphenol blue, 20% glycerol) were added to stop the reaction. The tube was vortexed and incubated at room temperature for 30 min. Samples of 20–30 μl per lane were applied to a 10% gel, and Western blotting was performed as described above.

Drugs and chemicals. Collagenase was obtained from Yakult (Tokyo, Japan). The antibody against BKCa channel subunit β1 was from Alomone Laboratories (Jerusalem, Israel), the anti-GAPDH antibody from Applied Biosystems (Foster City, CA), the anti-rabbit secondary antibody from Bio-Rad (Hercules, CA), and the PBS tablets from Gibco (Carlsbad, CA). All other chemicals and drugs were purchased from Sigma (Hamburg, Germany).

Statistical analysis. Data are expressed as means ± SE from the indicated number of experiments. One-way ANOVA with Bonferroni post hoc means comparison was used. Differences were considered statistically significant when P < 0.05.

RESULTS

Isolated cardiomyocytes retain protective effects of chronic hypoxia. Viability of myocytes ranged between 51 and 66% after 1-h stabilization and was always higher in RVM than in LVM and SEPM (Table 1). Adaptation of rats to chronic hypoxia at the end of control experiments were similar to that counted after stabilization within each experimental group. Total LDH content in cell preparations from normoxic animals did not differ among ventricular parts. Chronic hypoxia did not affect total LDH in LVM and SEPM, but it significantly increased LDH level in RVM (Table 1).
Exposure of cells to MI/R insult decreased their survival in all groups (Fig. 1). When expressed as a percentage of corresponding numbers in control experiments, only about 30–40% of rod-shaped myocytes survived in the normoxic group without significant differences among LVM, SEPM, and RVM. Chronic hypoxia markedly improved the relative survival by doubling the number of rod-shaped cells after MI/R in all three ventricular parts. However, the protective effect was significantly smaller in RVM than in SEPM (Fig. 1). MI/R did not affect the number of non-rod-shaped viable (nonblue) myocytes, except for the moderate increase in RVM from the normoxic group (data not shown).

Figure 2, A–C, respectively, shows LDH release from cells during MI, during reenergization, and total release during MI/R, expressed as a percentage of appropriate control values. In LVM and RVM from normoxic rats, the LDH release during reenergization was higher than that caused by MI. The same is true for LVM and SEPM isolated from chronically hypoxic rats. Chronic hypoxia significantly reduced both MI-induced and total LDH release during MI/R from LVM and SEPM; the protective effect was more pronounced in LVM. LDH release from RVM and the release during the reenergization phase from myocytes of all three ventricular parts were not significantly decreased by chronic hypoxia ($P = 0.084$ for LV).

These results demonstrate that cardiomyocytes (LVM and SEPM) isolated from rats adapted to chronic hypoxia retain the improved resistance against injury caused by MI/R.

Paxilline attenuates the protective effects of chronic hypoxia, while NS-1619 protects only cardiomyocytes from normoxic animals. Starting viability of LVM in this set of experiments was higher than 50% and similar in normoxic and chronically hypoxic groups. Incubation of cells with paxilline and DMSO for 25 min did not affect their viability before MI, whereas NS-1619 decreased it by 10%. Total LDH levels in cell preparations were similar to those reported above and were affected by neither chronic hypoxia nor any drug treatments (data not shown).

Treatment with DMSO had no effect on survival of rod-shaped myocytes after the MI/R insult in either normoxic or chronically hypoxic groups. NS-1619 significantly improved cell viability after MI/R in the normoxic group to a similar level observed in untreated or DMSO-treated cells of the

### Table 1. Viability and total LDH content in cell preparations from normoxic and chronically hypoxic rats after 1-h stabilization

<table>
<thead>
<tr>
<th>Group</th>
<th>Viability, %</th>
<th>LDH, U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>51.7 ± 2.4</td>
<td>1.31 ± 0.33</td>
</tr>
<tr>
<td>Septum</td>
<td>50.6 ± 1.4</td>
<td>1.14 ± 0.29</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>66.4 ± 1.9†</td>
<td>1.27 ± 0.31</td>
</tr>
<tr>
<td>Hypoxia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>50.6 ± 3.0</td>
<td>1.32 ± 0.26</td>
</tr>
<tr>
<td>Septum</td>
<td>49.8 ± 3.2</td>
<td>1.10 ± 0.27</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>62.1 ± 3.9†</td>
<td>2.10 ± 0.44†</td>
</tr>
</tbody>
</table>

Values are means ± SE from 8 hearts in each group. LDH, lactate dehydrogenase. *$P < 0.05$ vs. corresponding normoxic group. †$P < 0.05$ vs. left ventricle and septum.
chronically hypoxic group, but it had no additive effect in the chronically hypoxic group. On the other hand, paxilline attenuated the salutary effect of chronic hypoxia on rod-shaped cell survival after MI/R, but it did not influence viability in the normoxic group (Fig. 3).

Figure 4, A–C, respectively, shows effects of drugs on LDH release from LVM during MI, during reenergization and total release during MI/R, expressed as a percentage of appropriate control values. In cells from both normoxic and chronically hypoxic rats and all treatment groups, the LDH release during the reenergization phase was higher than that induced by MI. DMSO treatment had no effect on LDH release. In the normoxic group, the total LDH release during MI/R was significantly reduced by NS-1619, which had no additive protective effect in the hypoxic group. Reciprocally, the improved resistance to injury in myocytes of the later group was significantly attenuated by paxilline, which did not influence LDH release in the normoxic group (Fig. 4C). Pattern of total LDH release during MI/R reflected that associated with the reenergization phase (Fig. 4B). In contrast, MI-induced LDH release was decreased by NS-1619 in both normoxic and hypoxic groups, and paxilline did not influence the salutary effect of chronic hypoxia (Fig. 4A).

To verify that the protective effect of NS-1619 in cells from normoxic rats can be attributed to the activation of BKCa channels, the experiment was repeated with an additional group of cells that were treated with paxilline before the addition of NS-1619. As illustrated in Fig. 5, paxilline completely abolished the decrease in total LDH release from NS-1619-treated myocytes during MI/R, while its effect on viability did not reach statistical significance ($P = 0.056$).

These results suggest that mitochondrial BKCa channels contribute to the improved resistance to injury of myocytes isolated from chronically hypoxic rats, and the involvement of these channels in the protective mechanism is confined predominantly to the reenergization phase.

**Fig. 3.** Effects of NS-1619 (NS) and paxilline (PAX) on survival of cardiomyocytes after acute MI/R, expressed as a percentage of control cells in the absence of MI/R. Cells were isolated from the LV of rats adapted to chronic hypoxia and of normoxic animals. VEH, vehicle-treated cells; UT, untreated cells. Values are means ± SE from 8 hearts in each group. *$P < 0.05$ vs. corresponding normoxic groups. §$P < 0.05$ vs. UT, VEH, and PAX. ¶$P < 0.05$ vs. NS.

**Fig. 4.** Effects of NS and PAX on LDH release from cardiomyocytes during metabolic inhibition (A) and during reenergization (B), and total release during MI/R (C), expressed as a percentage of corresponding LDH release from control cells not exposed to MI/R. Cells were isolated from the LV of rats adapted to chronic hypoxia and of normoxic animals. Values are means ± SE from 8 hearts in each group. *$P < 0.05$ vs. corresponding normoxic groups. §$P < 0.05$ vs. UT, VEH, and PAX. ¶$P < 0.05$ vs. VEH and PAX. †$P < 0.05$ vs. VEH and NS.

**Fig. 5.** Effects of NS-1619 (NS) and paxilline (PAX) on total LDH release from LV myocardium during MI/R, expressed as a percentage of corresponding LDH release from control cells not exposed to MI/R. Cells were isolated from the LV of rats adapted to chronic hypoxia and of normoxic animals. Values are means ± SE from 8 hearts in each group. *$P < 0.05$ vs. corresponding normoxic groups.

Chronic hypoxia causes deglycosylation of the BKCa channel regulatory $\beta_1$-subunit. Western blot analysis detected two bands in cells from all three ventricular parts corresponding to molecular masses of ~40 and ~26 kDa that were blocked by a specific blocking peptide against BKCa channel regulatory $\beta_1$-subunit. While in myocytes from normoxic rats the upper (~40 kDa) band strongly predominated, chronic hypoxia significantly increased the relative abundance of the lower (~26 kDa) band (Fig. 6, A and B). Consequently, the upper-to-lower band ratio decreased markedly in myocytes from the hypoxic group compared with normoxic controls (Fig. 6C). Based on previous reports (15, 37), we assumed that the band corresponding to the higher molecular mass could result from N-linked glycosylation of the protein subunit. This assumption was confirmed by enzymatic deglycosylation, which led to a complete disappearance of the upper band (Fig. 6C). It suggests that chronic hypoxia causes partial deglycosylation of the
BK<sub>Ca</sub> channel regulatory β<sub>1</sub>-subunit without affecting its total abundance.

DISCUSSION

The present work resulted in the following three major novel observations: 1) cardiomyocytes isolated from rats adapted to chronic hypoxia were more resistant to injury and cell death caused by acute MI/R; 2) these salutary effects were attenuated by the BK<sub>Ca</sub> channel blocker paxilline, while the opener NS-1619 protected only cells isolated from control normoxic animals; and 3) chronic hypoxia led to partial deglycosylation of the BK<sub>Ca</sub> channel regulatory β<sub>1</sub>-subunit without changing its total abundance.

Studies on the protective role of the mitochondrial BK<sub>Ca</sub> channel using whole-animal models are complicated by its similar basic biophysical and pharmacological properties to that of the BK<sub>Ca</sub> channel localized in plasma membrane of various cell types (31). To examine the potential involvement of mitochondrial BK<sub>Ca</sub> channels in increased ischemic tolerance of chronically hypoxic rat hearts, we, therefore, used freshly isolated ventricular myocytes that most likely do not contain this type of channel in the sarcolemma (27). First, it was necessary to find out whether isolated myocytes are able to maintain the protected phenotype achieved by the in vivo hypoxic adaptation. Considering regional differences in ventricular myocyte characteristics (6) and distinct effects of chronic hypoxia on the left and right ventricles (25), LVM, SEPM, and RVM were examined separately. We demonstrated for the first time that the salutary effect of chronic hypoxia was retained in subsequently isolated cardiomyocytes, as evidenced by reduced LDH release and increased survival rate of cells exposed to MI/R, simulating acute “ischemia-reperfusion” injury. These data suggest that the cell isolation procedure does not negatively interfere with the robust myocardial protection induced by chronic hypoxia, and freshly isolated myocytes are, therefore, a suitable model to study its underlying mechanism. Similarly, it has been reported that ischemic preconditioning of the whole rat heart increased resistance of subsequently isolated ventricular myocytes to MI/R (28). Somewhat smaller improvement of cell viability and the absence of a significant effect on LDH release from RVM compared with LVM and SEPM in our study can be most likely attributed to an opposing influence of RVM hypertrophy, resulting from hypoxic pulmonary hypertension, because hypertrophy is known to increase susceptibility to myocardial injury caused by acute oxygen deprivation (13).

Opening of mitochondrial BK<sub>Ca</sub> channels has been associated with improved ischemic tolerance in heart and other tissues, based mostly on pharmacological evidence. An increasing number of recent studies suggest the involvement of
K⁺ flux through these channels in the short-lived protective mechanism of various forms of preconditioning (8, 9, 27, 32, 33). Our present results demonstrate for the first time that BKCa channels also play a role in the persistent protection of cardiac myocytes achieved by long-term adaptation of animals to hypoxia. The fact that the inhibitory effect of BKCa blocker paxilline on LDH release occurred only after the MI phase suggests that the channel opening prevented cell injury associated mainly with mitochondrial reenergization. In contrast, paxilline did not affect the reduction of infarct size and the improvement of postischemic contractile function recovery in isolated perfused hearts of chronically hypoxic infant rabbits (30), suggesting possible species- and/or age-dependent differences in the protective mechanism of chronic hypoxia.

It should be mentioned that better cell survival and reduced injury due to MI/R in the chronically hypoxic group were significantly attenuated but not completely abolished by paxilline. It means that other factors independent of BKCa opening may also play a role. This observation is consistent with the contribution of other mitochondrial K⁺ channels, such as KATP, to achieve a resistant cardiac phenotype by chronic hypoxia (21). Interestingly, it has been shown that protective effects of BKCa and KATP openers against ouabain-induced hypoxia (21). It has been suggested that chronic hypoxia can modulate BKCa channel function via posttranscriptional mechanisms (16). In the present study, Western blot analysis of cardiac 1-subunit was detected after sildenafil treatment in a single band at a lower molecular mass. Quantitative analysis revealed that native β1-subunit occurred predominantly in a glycosylated form in cells from normoxic animals, while chronic hypoxia led to its marked deglycosylation, decreasing the ratio of glycosylated to nonglycosylated forms by 10.2±0.32. In conclusion, our results demonstrate that ventricular myocytes isolated from chronically hypoxic rats retain the improved ischemic tolerance of ventricular myocytes induced by β1-estradiol resulted from its functional interaction with the β1-subunit (23).

In conclusion, our results demonstrate that ventricular myocytes isolated from chronically hypoxic rats retain the improved resistance against injury caused by MI/R. The blunting effects of paxilline and the presence of additive protection by NS-1619 suggest that chronic hypoxia leads to the activation of mitochondrial BKCa channels, which contribute to the protective mechanism. It remains to be determined whether the reduced glycosylation level of the BKCa channel regulatory β1-subunit, observed in myocytes of chronically hypoxic animals, plays a role in the activation of the channel and cell protection.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


