Hypoxia and acidosis impair cGMP synthesis in microvascular coronary endothelial cells

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Hypoxia and acidosis impair cGMP synthesis in microvascular coronary endothelial cells. Am J Physiol Heart Circ Physiol 283: H917–H925, 2002. First published May 2, 2002; 10.1152/ajpheart.01067.2001.—To characterize the effects of ischemia on cGMP synthesis in microvascular endothelium, cultured endothelial cells from adult rat hearts were exposed to hypoxia or normoxia at pH 6.4 or 7.4. Cellular cGMP and soluble (sGC) and membrane guanylyl cyclase (mGC) activities were measured after stimulation of sGC (S-nitroso-N-acetyl-penicillamine) or mGC (urodilatin) or after no stimulation. Cell death (lactate dehydrogenase release) was negligible in all experiments. Hypoxia at pH 6.4 induced a rapid ~90% decrease in cellular cGMP after sGC and mGC stimulation. This effect was reproduced by acidosis. Hypoxia at pH 7.4 elicited a less pronounced (~50%) and slower reduction in cGMP synthesis. Reoxygenation after 2 h of hypoxia at either pH 6.4 or 7.4 normalized the response to mGC stimulation but further deteriorated the sGC response; normalization of pH rapidly reversed the effects of acidosis. At pH 7.4, the response to GC stimulation correlated well with cellular ATP. We conclude that simulated ischemia severely depresses cGMP synthesis in microvascular coronary endothelial cells through ATP depletion and acidosis without intrinsic protein alteration.

actions on platelet and leukocyte aggregation and adhesion, leukocyte migration, endothelial permeability, or microvascular function (10), and can mediate preconditioning (31). Although there are discrepant results, a vast majority of studies have found that NO donors and L-arginine, the NO synthase substrate, have a beneficial effect on myocardial ischemia-reperfusion injury (25, 37).

Recent studies have shown that this beneficial effect of NO can be mimicked by soluble cGMP analogs or by stimulation of mGC with ANP-related peptides in the heart and other tissues (1, 14, 24, 29). We have found that part of this beneficial effect can be ascribed to a protection against cardiomyocyte hypercontracture (1, 14). However, cGMP also regulates many processes crucial for the microvascular injury, such as most of the effects in which NO has been implicated [e.g., vascular permeability (12, 18) and cell adhesion (11, 26)] and the control of NO synthesis via phosphorylation of endothelial NO synthase (4). However, the effects of cGMP on ischemia-reperfusion injury have not been fully clarified. These effects could be concentration dependent. High cGMP concentration may mediate apoptosis (unpublished observations and Ref. 36), and we have found a concentration-dependent, bimodal response to urodilatin in reperfused rat and pig myocardium (29). Intriguingly, a recent study has described increased tolerance to ischemia-reperfusion in mice not expressing GC-A receptors (15).

Despite the evidence that cGMP may influence cell survival during myocardial ischemia, little is known about the consequences of ischemia on cGMP synthesis in cardiomyocytes or endothelial cells. We have recently described a marked reduction of cGMP content in rat and pig myocardium after transient sublethal ischemia (14, 29). However, other authors have reported increases (5, 21) or no change (25) in cGMP content in rat hearts submitted to up to 30 min of ischemia. To our knowledge, there are no previous reports on the effects of ischemia on cGMP synthesis in coronary microvascular cells. In vitro studies with purified GC have shown that the sensitivity of these
enzymes to agonists, their rate of dissociation and rate of deactivation are strongly dependent on changes in the ionic composition of the media (3, 23). As ischemia induces a myriad of changes in cytosolic composition with different and often opposed actions on GC activity (6, 17, 22, 27, 35), it is impossible to predict its effects on cGMP synthesis in intact cells from in vitro assays of enzymatic activity.

This study was designed to analyze the effects of ischemia-reperfusion on enzymes regulating intracellular cGMP concentration in microvascular coronary endothelial cells. Because cGMP may have different and even opposed effects on endothelial cells of different origins (12), the experiments were carried out in endothelial cell cultures prepared from whole rat hearts and containing mainly endothelial cells of microvascular origin. Changes in sGC- and mGC-mediated cGMP synthesis were investigated by analyzing the response of intact cell cultures and fractionated cellular homogenates to NO donors, S-nitroso-N-acetyl-penicillamine (SNAP) or sodium nitroprussiate (SNP), and to urodiilatin, respectively. Urodiilatin is an ANP-related peptide that has been shown to limit myocardial necrosis secondary to ischemia-reperfusion in a variety of models including the in situ pig heart (14, 29). The contributions of ATP depletion and acidosis to the observed effects of ischemia on cGMP synthesis were also investigated.

MATERIALS AND METHODS

The care and use of animals conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, Revised 1996) and the experimental procedures were approved by the Research Commission on Ethics of Hospital Vall d’Hebron.

Microvascular endothelial cells. Endothelial cells from the heart were isolated as previously described by Piper et al. (30). Two hearts from adult male Sprague-Dawley rats (250–350 g) were retrogradely perfused in a Langendorff system with collagenase, chopped, and dissolved into a suspension. From this suspension, the fraction of endothelial cells was purified and plated on a 100-mm petri dish. The culture medium consisted of medium 199 with Earle’s salts, supplemented with 50 IU/ml penicillin G, 50 μg/ml streptomycin, 0.5 μg/ml amphotericin B, and 20% (vol/vol) serum (1:1 mixture of fetal bovine serum and newborn calf serum). Four hours after being plated, nonattached cells were removed by vigorous shaking. At confluency (4 days after seeding), cells were subcultivated on 60-mm dishes (2 × 10⁶/cm²). After 3–4 days, serum content of the culture medium was progressively reduced (5%–6–8 h, 1/2 overnight), and dishes were used in experiments. Endothelial cells represented >95% of total cell number in confluent cultures.

Simulated ischemia and reperfusion. Cells were allocated to one of the following treatments: 1) normoxia, incubation in HEPES-buffered saline [containing (in mM) 120 NaCl, 3.6 KCl, 1.2 MgSO₄, 1 CaCl₂, 20 HEPES, and 5 glucose] at pH 7.4; and 2) simulated ischemia, incubation in the same buffer under 100% N₂ atmosphere but without glucose and at pH 6.4. To assess the relative contributions of oxygen deprivation and acidosis to the observed effects of simulated ischemia, cells were exposed for 2 h to 3) hypoxia at pH 7.4 or to 4) normoxia at pH 6.4. The time course of recovery after restoration of normoxia and pH 7.4 was analyzed in cultures exposed for 2 h to the different treatments. P₀₂ in hypoxic media was 6.5 ± 1.3 mmHg after 30 min of incubation and 3.6 ± 1.0 mmHg after 2 h. In additional experiments, hypoxia was replaced by exposure to dinitrophenol (DNP; 200 μM) during 40 min.

Measurement of cGMP synthesis. After different periods of exposure to the allocated treatment, cells were stimulated for 1 min (unless otherwise indicated) with SNAP (100 μM), urodiilatin (1 μM), or no drug. cGMP degradation was inhibited by the addition of 3-isobutyl-1-methylxanthine (IBMX) at 1 mM during the stimulation period. In additional experiments, the potential effect of the degradation product of SNAP on cGMP synthesis was investigated by stimulation with N-acetyl-D,L-penicillamine (100 μM). Ethanol extracts from the cell cultures were dried and resuspended in acetate buffer (5 mM, pH 4.8), and cGMP was quantified by radioimmunoassay using acetylated [3H]cGMP (2). Intracellular cGMP content after the different incubation conditions was always referred as a percentage of the cGMP content in cells of the same batch stimulated in the same way under normoxia at pH 7.4.

Measurement of intracellular pH. Changes in intracellular pH were measured with a ratiofluorescence imaging system (Quanticell 2000, Visitech) in endothelial cells exposed for 2 h to hypoxia (pH 7.4) or acidic normoxia (pH 6.4) and then switched to normoxia at pH 7.4. Before the experiment, cells were loaded (30 min, 37°C) with 1 μM of the acetoxy-methyl ester of 2′,7′-bis(2-carboxyethyl)-5,6-carboxy-fluorescein (BCECF) in medium 199 containing 1% fetal calf serum, washed, and incubated for 20 min to allow hydrolysis of the ester within the cells. Fluorescent images at 450 to 490 nm were obtained, and the average ratio for regions of interest was calculated as the quotient between fluorescence at 450 and 490 nm. Calibration of the BCECF ratio signal was performed with 10 μg/ml nigericin, a K⁺/H⁺ ionophore, and incubation of the cells with various pH (20).

ATP content and lactate dehydrogenase release. ATP content was measured in cell cultures immediately frozen in N₂ liquid by means of a Bioluminescent Somatic Cell Assay Kit. Lactate dehydrogenase (LDH) activity was spectrophotometrically measured in the incubation media of cell cultures (1) and expressed as a percentage of the total LDH content in the cultures, determined after homogenization in Tris-HCl buffer.

Measurement of sGC and mGC activities. Cell cultures were homogenized with buffer A [containing (in mM) 50 Tris-HCl (pH 7.4), 250 sucrose, 1 EDTA, 1 dithiothreitol, plus protease inhibitors (2), the protein kinase inhibitor staurosporin (1 × 10⁻³), and the Ser/Thr protein phosphatase inhibitors okadaic acid (1 × 10⁻³) and cypermethrin (5 × 10⁻³) in a Potter-Elvehjem homogenizer. After centrifugation (100,000 g for 1 h), sGC activity was determined by incubating the soluble extract with no addition (basal), 100 μM SNP, or 100 μM SNAP in assay buffer [final concentrations (in mM) 50 Tris-HCl (pH 7.4), 1 EDTA, 1 dithiothreitol, 1 GTP, 1 MgCl₂, 10 phosphocreatine, and 1 IBMX, plus 50 μM creatine kinase] at 37°C for 10–20 min. In separate experiments, the potential effect of the degradation product of SNAP on sGC activity was investigated by incubating soluble extracts with N-acetyl-D,L-penicillamine (100 μM). mGC activity was measured in the particulate fraction, homogenized with buffer A plus 10% glycerol, in the same assay buffer plus no addition (basal), 1 μM urodiilatin, or 0.1% Triton X-100. Natriuretic factors have been shown to scarcely stimulate mGC after cellular homogenization (19), whereas Triton X-100 is known to elicit a marked enhancement of mGC activity (17). Reactions were terminated by the addition of 1 ml of cold ethanol,
and cGMP produced by enzyme activity was determined by radioimmunoassay as described before. In these conditions, the formation of cGMP was linear with time for at least 30 min. In the experiments measuring the pH dependence of GC activity, Tris-HCl buffer was substituted by 30 mM PIPES (pH 6.0–6.8) or 30 mM HEPES (pH 6.8–8.0).

Data analysis and statistics. Statistical analysis was carried out by means of commercially available software (SPSS 8.0.0). Differences between groups were evaluated by means of a one-way ANOVA. Individual comparisons between groups were performed using the Student-Newman-Keuls test. A critical P value of 0.05 was used. Values are expressed as means ± SE.

Materials. Uroditatin was kindly provided by Prof. Dr. Wolf-Georg Forssmann and Dr. Markus Meyer from Niedersächsisches Institut für Peptid-Forschung, Hannover, Germany. SNAP, SNP, DNP, N-acetyl-cysteine (NAC), IBMX, Tris, HEPES, PIPES, and the Bioluminescent Somatic Cell Assay Kit were from Sigma; [3H]cGMP (35 Ci/mmol) was from New England Nuclear; collagenase was from Serva; Mn(III)tetrakis(4-benzoic acid)porphyrin chloride (MnTBAP) was from Calbiochem, BCECF was from Molecular Probes, plastic petri dishes were from Falcon, and culture media and sera were from GIBCO-BRL.

RESULTS

Stimulation of cGMP synthesis in normoxic endothelial cells. Stimulation of normoxic endothelial cells for 10 min with uroditatin (1 μM) in the presence of IBMX showed a marked, sustained increase in the cGMP content (Fig. 1A). Stimulation with SNAP (100 μM) for 10 min caused a moderate, transient increase in cGMP content with a peak at 1 min of stimulation (Fig. 1C). Stimulation with N-acetyl-d,L-penicillamine, the SNAP degradation product, had only a minimal effect on cGMP synthesis (0.16 ± 0.03 pmol/mg protein in stimulated cultures vs. 0.09 ± 0.01 pmol/mg protein in controls, n = 2). The magnitude of the response to uroditatin after 1 min of stimulation was 10 times higher than the response to SNAP. This duration of stimulation (1 min) was used for all subsequent experiments.

Effect of simulated ischemia and reperfusion on cGMP synthesis. The cellular content of cGMP in the absence of stimulation or after stimulation with uroditatin or SNAP was not significantly modified after 120 min of normoxic incubation (94 ± 6%, 102 ± 7%, and 112 ± 8%, respectively, of preincubation values). Simulated ischemia (hypoxia at pH 6.4) exerted a rapid and profound inhibitory effect on cGMP content in unstimulated and on uroditatin- or SNAP-stimulated cells (Fig. 2A). Simulated reperfusion allowed the complete and rapid recovery of cGMP synthesis after stimulation of mGC by uroditatin, a partial recovery of cGMP synthesis after stimulation by SNAP, and no significant improvement of cGMP synthesis in unstimulated cells (Fig. 2B).

Role of acidosis, hypoxia, and reoxygenation. Incubating cells in normoxic conditions at pH 6.4 (acidosis) decreased intracellular pH to ~6.4 in <30 min (Fig. 3A). After restoration of extracellular pH, intracellular pH rapidly recovered (Fig. 3B). Acidosis mimicked the depressant effect of simulated ischemia on cGMP synthesis (Fig. 4A). Recovery of uroditatin- and SNAP-stimulated cGMP synthesis after 120 min under acidosis was almost complete 10 min after restoration of pH 7.4.

Hypoxia without concomitant acidosis (pH 7.4) did not alter intracellular pH and failed to reproduce the effects of simulated ischemia on cGMP synthesis (Fig. 5A). After ~60–90 min of hypoxia, the responses of cGMP content to the different stimulation protocol decreased to ~50% of the corresponding values under normoxic conditions. Unstimulated cGMP synthesis

![Fig. 1. Time and concentration dependence of cGMP stimulation by uroditatin (Uro; A and B) and S-nitroso-N-acetyl-penicillamine (SNAP; C and D). A and C: microvascular endothelial cells were stimulated for 1–10 min with Uro (1 μM) or SNAP (100 μM) in the presence of 3-isobuty-1-methylxanthine (IBMX) (1 mM) or with IBMX alone (basal). B and D: cell cultures were stimulated for 1 min with different concentrations of Uro or SNAP in the presence of IBMX (1 mM). Data are means ± SE of a representative experiment. The mean EC50 values calculated from 3 different experiments are also shown.](http://ajpheart.org/10.22033/2.2.4.2)
was not substantially modified by reoxygenation (Fig. 5B). During the first 10 min of reoxygenation, the response of cGMP synthesis to urodilatin showed a complete recovery, but the response to SNAP suffered an abrupt and transient deterioration upon restoration of normoxic conditions, followed by a partial recovery (Fig. 5B).

Role of energy depletion. Acidosis had no effect on ATP content. Two hours of simulated ischemia reduced ATP by 65% (P < 0.05; Table 1). Hypoxia at pH 7.4 induced the same reduction (by 64% of basal) and reduced the responses to urodilatin and SNAP by ~40%. After 40 min of exposure to DNP (200 μM), ATP content in these cells was 16.5% of the normoxic value (Table 2), and the response of cGMP synthesis to stimulation with urodilatin or SNAP fell to 15.3 ± 7.9% and 13.0 ± 6.2% of the normoxic responses, respectively (P < 0.05; Table 2). Thus, in the absence of acidosis, there was a correlation between ATP content and cGMP synthesis.

LDH release was minimal and similar in all experimental conditions (Tables 1 and 2).

Role of oxidative stress. The response to urodilatin was not significantly influenced by pretreatment with NAC either in cells exposed to 120 min of normoxia or 120 min of hypoxia and 1 min of reoxygenation. During the first 10 min of reoxygenation, cGMP synthesis showed a complete recovery, but the response to SNAP suffered an abrupt and transient deterioration upon restoration of normoxic conditions, followed by a partial recovery (Fig. 5B).
of reoxygenation (Fig. 6A). The response to SNAP showed a trend to be potentiated by NAC. During reoxygenation, this effect, although very small (only 5% of the reduction observed in the SNAP response at reoxygenation could be reversed by NAC pretreatment), reached statistical significance (Fig. 6B). Additional series of experiments were performed in which the cell-permeable superoxide dismutase mimetic MnTBAP at 200 μM was present in all the incubation media. No changes were observed in the effects of hypoxia-reoxygenation on cGMP synthesis (data not shown).

Effects of hypoxia and acidosis on GC activity. When the activities of GCs from cell cultures exposed to hypoxia and/or acidosis were measured in particulate and soluble fractions at standard conditions (see MATERIALS AND METHODS) and pH 7.4, no significant differences were observed respect to the activities measured in the corresponding fractions obtained from control cultures (normoxia at pH 7.4) (Fig. 7). Neither sGC or mGC activities were altered by reoxygenation (data not shown).

However, the activities of GCs from cultures exposed to control conditions (normoxia, pH 7.4) were found to be strongly dependent on pH of the assay buffer and minimal at pH 6.4 (Fig. 8). The degradation product of SNAP, N-acetyl-D,L-penicillamine, had no effect on the activity of sGC from control cultures.

DISCUSSION

The present study shows that nonlethal simulated ischemia (hypoxia plus acidosis) induces a dramatic reduction in cGMP content in coronary microvascular endothelial cells and in the ability of these cells to synthesize cGMP in response to stimulation of sGC or mGC. Acidosis alone is able to mimic this rapid and profound effect of simulated ischemia, whereas hypoxia per se produces a partial and comparatively slow decrease in cGMP synthesis. The inhibitory effect of hypoxia and/or acidosis on the response to stimulation of mGC with urodilatin completely reversed after restoration of initial conditions. However, the inhibition of sGC-dependent cGMP synthesis induced by hypoxia was transiently aggravated upon reoxygenation and reversed only partially thereafter.

Previous studies. To our knowledge, no previous studies have analyzed the effects of simulated ischemia or hypoxia on the ability of coronary microvascular endothelial cells to synthesize cGMP, and information about how simulated ischemia affects cGMP synthesis in related vascular or myocardial cells is scant. In a

Table 1. ATP content and LDH release

<table>
<thead>
<tr>
<th>Condition</th>
<th>ATP, nmol/mg protein</th>
<th>LDH release, % total LDH</th>
<th>Condition</th>
<th>ATP, nmol/mg protein</th>
<th>LDH release, % total LDH</th>
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<tbody>
<tr>
<td>Normoxia (120 min)</td>
<td>19.7 ± 3.2</td>
<td>1.4 ± 0.2</td>
<td>30 min After Restoration</td>
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<tr>
<td>Simulated ischemia (120 min)</td>
<td>6.8 ± 0.7*</td>
<td>2.4 ± 0.4</td>
<td>of Normoxia and pH 7.4</td>
<td></td>
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<tr>
<td>Acidosis (120 min)</td>
<td>21.6 ± 1.6</td>
<td>1.5 ± 0.3</td>
<td></td>
<td>9.4 ± 1.2*</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>Hypoxia, pH 7.4 (120 min)</td>
<td>7.1 ± 1.2*</td>
<td>2.1 ± 0.3</td>
<td></td>
<td>18.7 ± 3.2</td>
<td>1.7 ± 0.6</td>
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Data are means ± SE; n = 4. LDH, lactate dehydrogenase. *P < 0.05 vs. the normoxic value.
previous study (32), hypoxia did not affect cGMP content in either unstimulated or atriopterin II-stimulated aortic endothelial cells. The discrepancy between that study and the present results could be explained by the different behavior between macrovascular and microvascular endothelial cells (12) or by the milder degree of hypoxia in the previous work (14.4% O₂) compared with the present one. In support of this latter explanation, we found no effects of hypoxia on either mGC- or sGC-mediated cGMP synthesis with oxygen concentrations \( \leq 10\% \) (unpublished results). In pulmonary arteries, moderate hypoxia \((30–45 \text{ mmHg})\) impaired the synthesis of endothelium-derived relaxing factor without affecting cGMP synthesis elicited by direct stimulation of sGC with SNP (16, 33, 34), whereas severe hypoxia \((3 \text{ mmHg})\) evoked partial inhibition of smooth muscle sGC (33).

The effect of real ischemia (coronary flow reduction) on endothelial cGMP synthesis is difficult to investigate because of the confounding effect of cGMP synthesized by myocytes. Few studies have analyzed the effects of ischemia on myocardial cGMP synthesis, and their results are discrepant. Previous studies in the isolated rat heart have found increased myocardial cGMP after 10–25 min of ischemia (5, 21) and no change (25) or a reduction (28) after 30 min of hypoxia (28) or ischemia (25). A reduction of myocardial cGMP content has also been described in the in situ rat (38).
and rabbit (13) heart after 30 min of transient ischemia. The reasons for these discrepancies are not clear. The present results are in agreement with these latter observations and with previous studies from our laboratory showing myocardial cGMP depletion in the isolated rat heart submitted to 40 min of transient ischemia (14) and in pig myocardium submitted to 47 min of transient coronary occlusion (29).

Role of acidosis. This study demonstrates for the first time the profound depressant effect of acidosis on cellular cGMP synthesis. This effect accounted for the depression of cGMP synthesis observed during the initial phase of simulated ischemia (hypoxia plus acidosis) in microvascular endothelial cells when severe ATP depletion has not yet occurred. After 30 min of oxygen deprivation, cGMP synthesis is not depressed in the absence of concomitant acidosis but is severely depressed in its presence. The present results also suggest that this effect of acidosis is due to a direct effect of low pH on GC activity. First, there was a good correlation between the time course of cGMP inhibition and the time course of intracellular pH decrease during exposure to extracellular buffer at pH 6.4. Second, in agreement with previous studies (9), both mGC and sGC enzymatic activities were extremely low at pHs below 6.8, a value that the intracellular pH reached within 30 min of exposure to extracellular acidosis. Third, recovery of cGMP synthesis was rapid after exposure to transient acidosis, and its time course correlated very well with the time course of normalization of intracellular pH.

Hypoxia and energy depletion. After 2 h of hypoxia, both nonstimulated and stimulated cGMP synthesis decreased to ~50% of the value under normoxic conditions. This value correlates well with the reduction in intracellular ATP content at that time (~50% of the value in normoxic cells). When severe ATP depletion was induced by metabolic inhibition with DNP instead of oxygen deprivation, the correlation was maintained, suggesting that in coronary microvascular endothelial cells, cGMP synthesis is closely related with ATP availability. Although the ATP concentration determines that of GTP, the substrate for sGC and mGC, this relation between ATP concentration and cGMP synthesis could not have been easily anticipated. Other effects of ATP depletion can modulate the consequences of reduced GTP concentration. ATP depletion results in a concomitant increase in intracellular Mg²⁺ and inorganic phosphate concentrations (27, 35). Because the substrate used by sGC and mGC is Mg²⁺-GTP and phosphate stimulates mGC (27), the increase in Mg²⁺ and phosphate tends to antagonize the effects of ATP depletion on cGMP synthesis. Furthermore, stimulatory and inhibitory sites for ATP have been described in both the A and B types of mGC (6, 17, 22). Finally, energy depletion may also affect mGC activity through modifications in its phosphorylation state (22). However, this latter mechanism seems irrelevant in microvascular endothelial cells because no changes in mGC or sGC activities were observed in cell fractions obtained from cultures exposed to hypoxia.

As referred to above, different authors have described divergent effects of hypoxia on cGMP synthesis. In fact, most authors do not find an inhibitory effect of hypoxia. Considering the complex relationship between ATP depletion and cGMP synthesis described above, it seems plausible that small differences in the degree of hypoxia or even in the ratio between the distinct enzyme subtypes (both for sGC and mGC) result in the divergent results found by different authors.

Our results demonstrate that both acidosis and ATP depletion are sufficient to profoundly inhibit cGMP synthesis. The severe depressant effects of the degree of acidosis used in this study (pH 6.4) precluded to assess whether the effects of low pH and deenergization are additive.

Additional injury during reoxygenation. Reoxygenation allowed a slow but complete recovery of the response to urodilatrin after 120 min of hypoxia at pH 7.4 despite minimal ATP recovery. In contrast, cGMP synthesis in response to stimulation with SNAP suffered a transient and acute decrease upon reoxygenation, which was partially reversed afterward. sGC has been shown to be very sensitive to changes in the redox state of the cell, and the burst of oxygen free radicals during the first minute of reoxygenation could explain the transient inhibitory effect on SNAP stimulation. However, pretreating the cells with NAC or incubating them with a superoxide dismutase mimetic did not substantially modify the inhibitory effect evoked by reoxygenation. sGC-mediated responses can be modulated by changes in the phosphorylation state (7). Nevertheless, none of these mechanisms seems to play a relevant role in our study because no changes were observed in sGC activity in the soluble fraction obtained from cell cultures submitted to transient hypoxia. The identification of the precise mechanisms involved in the genesis of this phenomenon escapes from the goals of the present study.

Limitations. This study was focused on only two of the many intracellular changes induced by ischemia: ATP depletion and intracellular acidosis. These two changes are, however, of the greatest relevance, and their effects on cGMP synthesis observed in this study are consistent with recent observations in rat and pig myocardium submitted to severe ischemia (14, 29). On the other hand, many of the effects of ischemia or hypoxia consist in alterations of the intracellular environment, such as ionic composition or redox state, that cannot be observed with the “classical” assay of enzymatic activity (involving cell disruption) used in this study. However, the extremely high cGMP concentrations reached after exogenous stimulation of sGC or mGC render potential interferences by endogenous substances like NO irrelevant, making it possible to estimate the intracellular activities of mGC and sGC.

Implications. Although altered cGMP synthesis was not associated to cell death in microvascular coronary endothelial cultures, cGMP in endothelial cells is known to modulate events that can contribute to myocardial injury. The present study demonstrates that
reduced ability to synthesize cGMP may be an important element in the pathophysiology of ischemia-reperfusion injury. On the other hand, the depressant effect of ischemia on cGMP synthesis could explain the reduced susceptibility to cGMP-mediated apoptosis recently observed in postischemic myocardium (unpublished observations and Ref. 36). Together with previous work showing the protective effect of cGMP against reperfusion injury. On the other hand, the depressant element in the pathophysiology of ischemia-reperfusion injury. Hyponatremia and acidosis impair cGMP synthesis.

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