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Original Research Communication

Regulation of Ventricular Fibrillation by Heme Oxygenase in Ischemic/Reperfused Hearts

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ABSTRACT

We have assessed the relationship between reperfusion-induced ventricular fibrillation (VF) and heme oxygenase (HO) mRNA expression using northern blotting, reverse transcription–polymerase chain reaction (RT-PCR), and enzyme activity in isolated working ischemic/reperfused rat hearts. Isolated hearts were subjected to 30 min of global ischemia followed by 120 min of reperfusion. Upon reperfusion with VF, cardiac function was registered (n = 6 in each group), and HO mRNAs and enzyme activities were measured at the end of reperfusion in hearts that showed VF or did not develop VF. The expression of HO-1 mRNA (about fourfold) was observed in ischemic/reperfused nonfibrillated myocardium in comparison with the nonischemic control hearts. In those hearts when VF was developed, the expression of HO-1 mRNA was not observed in comparison with the nonischemic control myocardium. The results measured by RT-PCR and enzyme analysis support the data obtained by northern blotting. In additional studies, we decided to approach the question from a different angle. Thus, the purpose of our work was also to study the role of HO expression and enzyme activity in electrically fibrillated hearts without the ischemic/reperfused protocol. To simulate the period of 10 min of reperfusion-induced VF, hearts were electrically fibrillated, then defibrillated, and perfused for an additional 110 min, and HO-1 mRNA expression and enzyme activities were determined. Thus, electrically induced VF resulted in about 60%, 60%, and 70% reduction in HO-1 mRNA expression, RT-PCR signal intensity, and enzyme activity, respectively, compared with the nonfibrillated ischemic/reperfused group. In conclusion, our data provide evidence that the development of reperfusion-induced VF inhibits HO-1 mRNA expression and enzyme activity in both electrically fibrillated myocardium and ischemic/reperfused fibrillated hearts. The results clearly show that HO-1 mRNA expression and enzyme activity were increased in ischemic/reperfused nonfibrillated myocardium, suggesting that interventions that are able to increase HO-1 mRNA expression and enzyme activity may prevent the development of VF. Antioxid. Redox Signal. 3, 125–134.

INTRODUCTION

IT HAS BEEN PROPOSED that most cases of sudden cardiac death may result from ischemia and reperfusion-induced ventricular fibrillation (VF) (9, 34). Interest in the development and pharmacological control of reperfusion-induced VF has been stimulated by the realization that such arrhythmias may occur under a number of pathological and clinical circumstances, including the spontaneous relief of coronary artery spasm (30). There is considerable controversy over the mechanisms responsible for the induction of these arrhythmias, and a number of different factors have been suggested (7, 10), but the two major mecha-

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nisms proposed and generally accepted to explain reperfusion-induced injury and VF are (i) calcium overload and (ii) free radical formation (20). Many human diseases are associated with the overproduction of free radicals that inflict cell damage. Examples of oxidative stress-related diseases include reperfusion injury, such as that which occurs after tissue ischemia or stroke, and inflammatory processes, such as arthritis (17, 24). Heme oxygenase (HO) catalyzes the rate-limiting step in the oxidative degradation of heme to biliverdin and carbon monoxide (CO) (29). Two isozymes of HO have been identified and cloned: HO-1, an inducible form, and HO-2, a constitutive form (2, 28). Studies demonstrate that HO-1 is induced in response to various interventions causing oxidative stress, including ultraviolet irradiation, hypoxia, and ischemia (11, 14, 17, 21, 31).

In a previous study, we observed a reduction in HO-1 mRNA expression and enzyme activity in ischemic/reperfused fibrillating myocardium, but not in nonfibrillating hearts (6). Therefore, in the present study, we decided to approach the question from a different angle. The aforementioned finding has led us to speculate that a reduction in HO-1 mRNA expression and enzyme activity may be seen in nonischemic electrically fibrillated myocardium. If this were so, we would stress that the prevention of HO-1 mRNA down-regulation could play a crucial role in the development of reperfusion-induced VF. Our study is concerned with the possibility that reperfusion-induced VF may be initiated by modification of the expression of HO mRNA and enzyme activity.

Although many mechanisms have been proposed to explain the causes of arrhythmias, no work has been done, to our knowledge, to clarify the mechanism(s) of VF at a gene expression level in ischemic/reperfused myocardium. The long QT disease and idiopathic VF, as known up to now, are the only cardiac disorders based on genetic mutation and cause sudden cardiac death from ventricular arrhythmias (12, 22, 23, 33). Thus, our study may offer a further understanding of the arrhythmogenic mechanism(s) at a molecular level and identify the responsibility of HO mRNA for arrhythmogenesis in ischemic/reperfused hearts.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (320–350 g body weight) were used for all studies. All rats received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Isolated working heart preparation

Rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg body weight) and then given intravenous heparin (500 IU/kg). After thoracotomy, the heart was excised, the aorta was cannulated, and the heart was perfused according to the Langendorff method for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consists of a modified Krebs–Henseleit bicarbonate buffer (in m*M*: NaCl 118, KCl 4.7, $CaCl_2$ 1.7, $NaHCO_3$ 25, KH_2PO_4 0.36, $MgSO_4$ 1.2, and glucose 10). The Langendorff preparation was switched to the working mode as previously described by Tosaki and Braquet (27). Aortic flow (AF) was measured by calibrated rotameter. Coronary flow (CF) rate was measured by timed collection of the coronary perfusate that dripped from the heart.

Measurement of heart function and arrhythmias

An epicardial electrocardiogram (ECG) was recorded by a polygraph throughout the experimental period by two silver electrodes attached directly to the surface of isolated hearts. The ECGs were analyzed to determine the incidence of VF. The heart was considered to be in VF if an irregular undulating baseline was seen on the ECG. The heart was considered to be in sinus rhythm if normal sinus complexes occurring in a regular rhythm were apparent on the ECG. VF was considered to be reversible if VF reverted to regular sinus rhythm within the first 5 min of the reperfusion period. If VF was persisting through the first 10 min of the

reperfusion period, the VF was considered to be irreversible. At the end of the first 10 min of Langendorff reperfusion (irreversible fibrillated myocardium), isolated hearts were electrically defibrillated using a single 20-V square-wave pulse of 1-ms duration, switched to the working mode, and then nonfibrillated ischemic/reperfused hearts were reperfused for an additional 110 min. The defibrillation was repeated two to four times if it was necessary. Before ischemia and during reperfusion ($n = 6$ in each group), heart rate (HR), CF, and AF, rates were registered. Left ventricular developed pressure (LVDP) and the first derivative of LVDP ($LVdp/dt_{max}$) were also recorded (Experimetria, Budapest, Hungary). In ischemic/reperfused myocardium, HO-1 mRNA expression and enzyme activities were determined at the end of 120 min of reperfusion in the myocardium.

In additional studies, without the ischemic/reperfused protocol, to simulate the period of 10 min of irreversible VF, isolated hearts ($n = 6$) were electrically fibrillated (20 Hz, 1,200 beats/min) using 5-V square-wave pulses of 1-ms duration for 10 min in Langendorff mode. Then hearts were defibrillated, switched to working mode, and perfused for an additional 110 min, and cardiac function was registered at the 60th min and 120th min of perfusion. HO-1 mRNA expression and enzyme activities were determined at the end of 120 min of perfusion.

Total RNA isolation

Total RNA was isolated from rat heart tissue (100 mg) by homogenization in 1 ml of TRIzol reagent (GibcoBRL, Life Technologies, Eggenstein, Germany), a guanidinium thiocyanate method (4), as described in the manual. The RNA pellets were dissolved in 100 μl of diethyl pyrocarbonate water, and the concentrations were calculated from the absorbance at 260 nm measured by ultraviolet spectroscopy (Beckman DU 640, Beckman Instruments Inc., Fullerton, CA, U.S.A.).

Reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA (2 μg) of each sample was subjected to random-primed first-strand cDNA synthesis (reverse transcription) in 40-μl reaction assays composed of (in m*M*) 50 Tris-HCl, 75 KCl, 3 $MgCl_2$, 10 dithiothreitol, 1 dNTPs (each), 0.005 random hexamer, and 0.5 IU/μl Moloney murine leukemia virus reverse transcriptase (GibcoBRL). Tubes with mixtures were heated for 60 min at 42°C. The first-strand cDNA preparations were used as templates for PCR. The sequence of the primers was the following: HO-1 sense, 5′-AAG GAG GTG CAC ATC CGT GCA-3′; HO-1 antisense, 5′-ATG TTG AGC AGG AAG GCG GTC-3′; HO-2 sense, 5′-ATG GCA GAC CTT TCT GAG CTC-3′; HO-2 antisense (13), 5′-CTT CAT ACT CAG GTC CAA GGC-3′; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) sense, 5′-TCC TGC ACC ACC AAC TGC TTA GCC-3′; GAPDH antisense (37), 5′-TAG CCC AGG ATG CCC TTT AGT GGG-3′. The reaction mixture contained 1× PCR buffer (20 m*M* Tris-HCl, pH 8.4, 50 m*M* KCl), 1.5 m*M* $MgCl_2$, 100 μM dNTPs (each), 100 μM primers, and 0.025 U/μl *Taq* polymerase (GibcoBRL). The number of PCR cycles was adjusted carefully to avoid saturation of the amplification system. All tubes were incubated at 95°C for 1 min, at 55°C for 1 min, and at 72°C for 2 min for HO-1 and HO-2, at 95°C for 1 min, at 60°C for 1 min, and at 72°C for 1 min for GAPDH, and the numbers of cycles were 28, 35, and 22, respectively. The reaction mixtures were kept at 72°C for 10 min. Amplification products were visualized on 2% agarose gels using ethidium bromide and identified by their sizes; the length of HO-1 fragment was 568, of HO-2 554 bp, and of GAPDH 377 bp, respectively.

Northern blot

Thirty micrograms of total RNA was transferred to a nylon membrane (Qiabrane, Qiagen, Hilden, Germany) according to Sambrook et al. (25). The purified GAPDH probe was given by Zador et al. (36). Purified cDNAs were labeled with [α-^{32}P]dCTP by random hexamer priming. Blots were prehybridized for 2 h in 50% formamide, 5× Denhardt's solution, 100 μl of herring sperm DNA, 0.5% sodium dodecyl sulfate, 0.9 *M* sodium chloride, and 0.09 M sodium citrate at 42°C. Blots were hybridized with cDNAs, labeled to a specific activity of 3×10^8

cpm/μg in hybridization fluid at 42°C overnight. Hybridized blots were washed in 0.3, 0.15, 0.015 *M* sodium chloride, 0.03, 0.015, 0.0015 *M* sodium citrate, respectively, pH 7.0, and 0.1% sodium dodecyl sulfate, for 20 min at 50°C. Kodak Biomed MR-1 films were exposed for 3–4 days with an intensifying screen at −80°C. Blots were washed and reprobed with different cDNAs using the hybridization and washing conditions described above.

HO activity assay

One hundred micrograms of tissue was homogenized in 10 ml of 200 m*M* phosphate buffer and centrifuged at 19,000 *g* at 4°C for 10 min. The supernatant was removed and recentrifuged at 100,000 *g* at 4°C for 60 min, and the precipitated fraction was suspended in 2 ml of 100 m*M* potassium phosphate buffer. Biliverdin reductase was crudely purified by the technique of Tenhunen et al. (26). HO activity was assayed as described by Yoshida et al. (35). Reaction mixtures consisted of (final volume, 2 ml) 100 μM potassium phosphate, pH 7.4, 15 n*M* hemin, 300 μM bovine serum albumin, 1 mg of biliverdin reductase, and 1 mg of microsomal fraction of the myocardium. The reaction was allowed to proceed for 1 h at 37°C in the dark in a shaking water bath and was stopped by placing the test tube on ice. Incubation mixtures were then scanned in a scanning spectrophotometer, and the amount of bilirubin was calculated as the difference between absorbance at 464 and 530 nm (18). Protein content was determined according to Lowry et al. (15) in the microsomal fractions.

Statistics

The data of myocardial function (HR, CF, AF, LVDP, LVdp/dt) and HO activities were expressed as the means ± SEM. One-way analysis of variance was first carried out to test any difference between the mean values of all groups. If differences were established, the values of the diabetic groups were compared with those of the nondiabetic control groups using a two-tailed *t* test with the Bonferroni correction (32). A change of $p < 0.05$ was considered significant.

RESULTS

In the following experiments, we studied the expression of HO-1 in nonischemic control, ischemic/reperfused nonfibrillated, ischemic/reperfused fibrillated, and electrically fibrillated myocardium. Figure 1 shows the results of northern hybridization performed by the probe for HO-1. The expression of HO-1 mRNA (about fourfold) was observed in ischemic/reperfused nonfibrillated myocardium (Fig. 1, lane 2) in comparison with the nonischemic control heart (Fig. 1, lane 1). In hearts subjected to 30 min of ischemia followed by 2

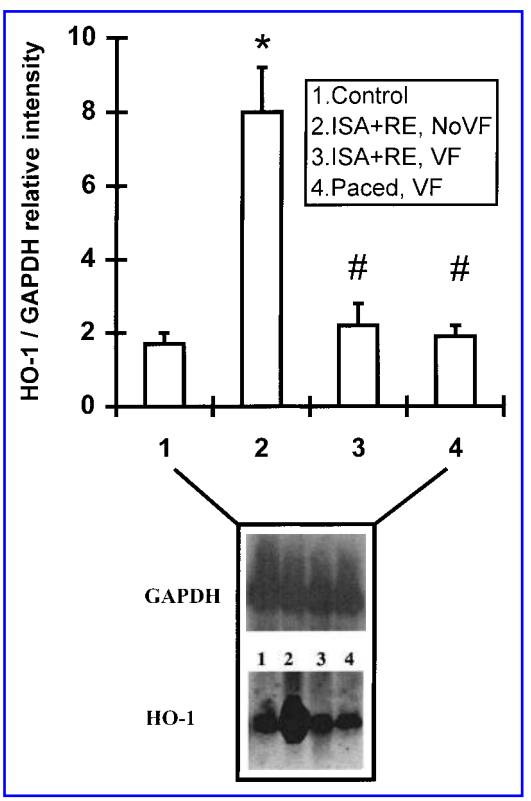

FIG. 1. HO-1 mRNA expression in nonfibrillated and fibrillated hearts. Northern blots (representative blots) were probed with [^{32}P]HO-1, and then the same blots were stripped and reprobed with [^{32}P]GAPDH cDNA. In nonischemic control myocardium (lane 1), the signal of HO-1 can be detected. Hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion, and VF was not developed (lane 2) or was developed (lane 3). Hearts were electrically fibrillated (lane 4) for 10 min and then perfused for an additional 110 min, and HO-1 mRNA was detected. The results show the quantitative values (the ratio between HO-1 and GAPDH) of six hearts in each group. $^{*}p < 0.05$ compared with the nonischemic control group (group 1); $^{\#}p < 0.05$ compared with the ischemic/reperfused nonfibrillated group (group 2). ISA, ischemia; RE, reperfusion; NoVF, no VF.

h of reperfusion in which VF was developed (Fig. 1, lane 3), the expression of HO-1 mRNA was not observed in comparison with the nonischemic control myocardium. In other words, HO-1 mRNA expression was significantly reduced in ischemic/reperfused fibrillated myocardium (Fig. 1, lane 3) in comparison with the ischemic/reperfused nonfibrillated tissue (Fig. 1, lane 2). In additional studies, hearts were electrically fibrillated to avoid the ischemia/reperfusion protocol, and HO-1 mRNA expression was studied. Thus, in electrically fibrillated myocardium (Fig. 1, lane 4), the expression of HO-1 mRNA was not observed in comparison with the nonischemic control hearts. The electrically fibrillated myocardium (Fig. 1, lane 4) showed the same HO-1 mRNA expression that was observed in ischemic/reperfused fibrillated myocardium (Fig. 1, lane 3), indicating that the mechanism of reperfusion-induced VF and electrically induced VF could be based on the same mechanism. Furthermore, it is of interest to note that in the ischemic/reperfused myocardium that did not show any incidence of VF upon reperfusion, a significant increase in HO-1 mRNA expression was observed, suggesting that the stimulation of HO-1 mRNA expression could prevent the development of reperfusion-induced VF.

Figure 2 shows the results of HO-1 amplification by RT-PCR and supports the data obtained by northern blotting. Thus, in nonfibrillated ischemic/reperfused myocardium, a significant increase in RT-PCR signal intensity was observed (Fig. 2, lane 2) in comparison with the nonischemic control value (Fig. 2, lane 1). In fibrillated ischemic/reperfused hearts (Fig. 2, lane 3), an increase in RT-PCR signal intensity was not detected, and no significant change was observed in comparison with the nonischemic control value (Fig. 2, lane 1). In other words, a significant reduction in RT-PCR signal intensity was observed in ischemic/reperfused fibrillated myocardium (Fig. 2, lane 3) compared with the ischemic/reperfused nonfibrillated hearts (Fig. 2, lane 2). Electrically fibrillated and perfused myocardium showed the same signal intensity in HO-1 RT-PCR amplification (Fig. 2, lane 4) as was detected in ischemic/reperfused fibrillated hearts (Fig. 2, lane 3).

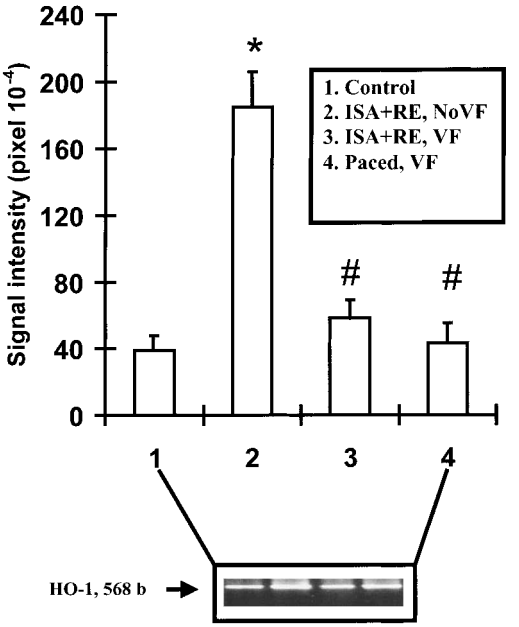

FIG. 2. RT-PCR amplification for HO-1 mRNA. Aliquots were taken after 28 cycles of amplification of cDNA obtained by reverse transcription. Total RNA was separated on a 2% agarose gel and stained with ethidium bromide. Lanes (representative lanes) show the results of amplification with the primer for HO-1. Columns show the quantitative results of six hearts in each group (means ± SEM). $^{*}p < 0.05$ compared with the nonischemic control group (group 1); $^{\#}p < 0.05$ compared with the ischemic/reperfused nonfibrillated group (group 2). ISA, ischemia; RE, reperfusion; NoVF, no VF.

Figure 3 depicts HO activities in ischemic/reperfused fibrillated, ischemic/reperfused nonfibrillated, and electrically fibrillated myocardium. Thus, in hearts subjected to 30 min of ischemia followed by 120 min of reperfusion in which reperfusion-induced VF was not developed, HO activity was increased from the nonischemic control value of 385 ± 20 pmol of bilirubin/mg/h (Fig. 3, lane 1) to 901 ± 38 pmol of bilirubin/mg/h (Fig. 3, lane 2). Reperfusion-induced VF resulted in a significant reduction ($p < 0.05$) in HO activity from its nonischemic control value of 385 ± 20 pmol of bilirubin/mg/h (Fig. 3, lane 1) to 162 ± 18 pmol of bilirubin/mg/h (Fig. 3, lane 3). Similar results were obtained in hearts subjected to electrically induced VF (Fig. 3, lane 4). Thus, electrically induced VF resulted in ~60% reduction in HO activity (Fig. 3, lane 4) compared with the nonischemic control group (Fig. 3, lane 1).

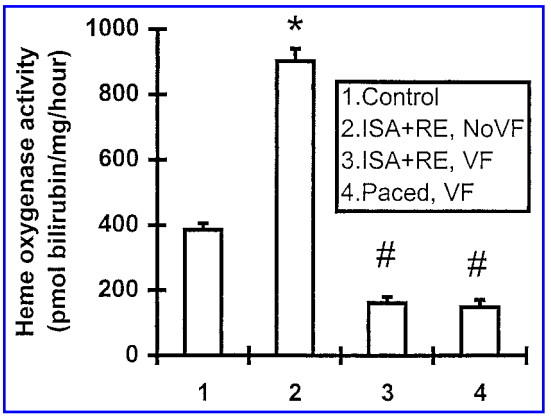

FIG. 3. The effect of reperfusion- and electrically induced VF on HO activity. Group 1: Aerobically perfused nonischemic control. Group 2: Hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion, and VF was not developed during reperfusion. Group 3: Hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion, and VF was developed during the reperfusion period. Group 4: Hearts were electrically fibrillated (no global ischemic/reperfusion protocol) for 10 min followed by 110 min of perfusion, and HO activity was measured. Data show the quantitative results of six hearts in each group (means ± SEM). $^{*}p < 0.05$ compared with the nonischemic control group (group 1); $^{\#}p < 0.05$ compared with the ischemic/reperfused nonfibrillated group (group 2). ISA, ischemia; RE, reperfusion; NoVF, no VF.

The ischemia/reperfusion resulted in a significantly lower postischemic recovery in CF, AF, and LVDP in those hearts that developed VF in comparison with the ischemic/reperfused nonfibrillated hearts (Table 1). Thus, the results clearly show that episodes of VF significantly attenuated the postischemic recovery in ischemic/reperfused myocardium compared with the ischemic/reperfused nonfibrillated myocardium. Without the ischemic/reperfused protocol, in electrically fibrillated hearts, the postfibrillated recovery was significantly improved compared with the ischemic/reperfused fibrillated myocardium (Table 1), indicating that an ischemic episode superimposed with VF resulted in a weak postischemic recovery in comparison with the electrically induced fibrillated group (Table 1).

DISCUSSION

Whereas disturbances of ionic homeostasis, particularly that of sodium, potassium, and calcium, are undoubtedly responsible for the aspects of VF, there is still considerable doubt as to the primary mechanism responsible for membrane injury that allows ionic imbalance to develop. It is important to note and ascertain that changes in protein activities, arising as a consequence of alterations in the redox state of controlling groups (e.g., disulfide, hydroxyl, or thiol bonds), are more likely to bring about major and sudden changes in the permeability of cell membranes to a variety of ions through the activation or inactivation of carrier proteins regulated by encoded genes and ion channels.

Under physiological conditions, HO-1 is present at low levels in all organs, and recently, the induction of HO-1 mRNA has been implicated in the ischemia/reperfusion injury, suggesting that this enzyme is induced by a host of stimuli that have in common the ability to produce oxidative stress. HO-1 expression is rapidly accelerated not only in response to pathophysiological conditions, such as those of ischemia/reperfusion and cellular transformation (8, 16), but also in response to different exogenous molecules (17). We have recently suggested (6) that HO mRNA expression, in particular the HO-1, may play an important role in the control of reperfusion-induced VF. HO enzymes catalyze the rate-limiting step in heme catabolism, the oxidative cleavage of b-type heme, to yield equimolar quantities of iron, CO, and biliverdin (1). In addition, the realization that most HO-1 inducers stimulate the production of oxygen radicals or depletion of glutathione, and the fact that heme is a potent pro-oxidant, have led us to speculate that HO-1 activity is a component of the cellular defense mechanism against oxidant stress, including reperfusion-induced VF. The importance of HO-1 activity has been verified and emphasized by numerous studies under *in vivo* and *in vitro* models of oxidative injury (3). Although the induction of HO-1 has been recently extensively studied at the level of gene transcription, the physiological function is poorly understood in the mechanism and controlling of reperfusion-induced arrhythmias including VF. In the present study, we have endeavored to obtain more circumstantial evidence for the involvement of HO-1 in the genesis of reperfusion-induced VF.

Table 1. Cardiac Function in Nonfibrillated Ischemic/Reperfused, Fibrillated Ischemic Reperfused, and Electrically Fibrillated Myocardium

	Preischemic values (prefibrillated)				*After 60 min of RE*				*After 120 min of RE*			
Groups	*HR*	*CF*	*AF*	*LVDP*	*HR*	*CF*	*AF*	*LVDP*	*HR*	*CF*	*AF*	*LVDP*
Time-matched control perfusion	309 ± 7	27.0 ± 1.1	52.0 ± 1.5	17.4 ± 0.4	305 ± 8	25.0 ± 1.3	50.2 ± 2.0	17.0 ± 0.3	302 ± 9	25.5 ± 1.0	48.1 ± 1.5	16.1 ± 0.4
ISA/RE nonfibrillated	316 ± 8	26.8 ± 0.8	51.3 ± 2.0	18.0 ± 0.5	291 ± 9	20.2 ± 1.0	22.3 ± 1.1	14.9 ± 0.4	300 ± 6	19.8 ± 1.1	19.6 ± 2.0	14.4 ± 0.5
ISA/RE fibrillated	312 ± 9	26.3 ± 0.9	50.9 ± 1.4	17.6 ± 0.3	297 ± 7	17.5 ± 0.7*	11.4 ± 0.6*	10.5 ± 0.4*	294 ± 8	16.9 ± 0.8*	10.2 ± 0.7*	9.8 ± 0.6*
Electrically fibrillated	308 ± 9	28.1 ± 1.2	49.8 ± 2.1	17.5 ± 0.4	300 ± 8	19.8 ± 1.1†	23.1 ± 1.0†	15.2 ± 0.3†	296 ± 8	20.7 ± 1.3†	21.8 ± 2.2†	15.0 ± 0.4†

Data are means ± SEM ($n = 6$). HR is given in beats/min, CF in ml/min, and AF in ml/min. ISA, ischemia; RE, reperfusion.
*$p < 0.05$ compared with the ISA/RE nonfibrillated group.
†$p < 0.05$ compared with the ISA/RE fibrillated group.

If HO-1 is involved in cellular injury leading to electrophysiological abnormalities and VF, it is important to question where and how the HO-1 gene is mediated, and where it exerts its action. Some indication may perhaps be obtained from our previous (6) and present studies. In support of an association between HO-1 mRNA expression or down-regulation and different transcription factors, Alam et al. (1) have provided substantial evidence that overexpression of Nrf2M (nuclear factor-erythroid related factor) inhibits HO-1 mRNA accumulation in response to heme, zinc, cadmium, and arsenic. A possible explanation for the situation may be derived from these results that Nrf2M-containing dimeric factor is responsible for induction by heme, zinc, cadmium, and arsenic (1). Another explanation for the inhibition of HO-1 gene induction by the aforementioned agents is that Nrf2M binds to the stress response element interfering with the binding of the actual positive activators. These findings strongly suggest that Nrf2 could be a positive regulator of HO-1 gene induction (1).

The importance and role of increased HO-1 expression under ischemic conditions is not clearly understood. The distinct transcription pathway for HO-1 mRNA activation by ischemia/reperfusion and various pro-oxidants suggests that this significance may be related, at least in part, to the antioxidant activity of HO-1 (e.g., bilirubin production) and the ability of this enzyme to generate CO. Studies show that under physiological conditions maintenance of physiologic vascular tone and blood flow can be attributed in part to enzymatically derived CO (5). Thus, changes in coronary and vascular tone under ischemic condition may, in part, be a consequence of HO-1 induction and localized production of CO (19). Consistent with this role of HO-1, ischemic tissue exhibits elevated formation of CO, derived from induced HO-1 activity, and increased levels of cyclic GMP leading (18) to the antiarrhythmic action in the myocardium.

Although electrical fibrillation-induced coronary vasoconstriction is unlikely to account for the initiation of HO-1 regulation, there seems little doubt that, after 10 min of electrical fibrillation, this factor can modify the postfibrillated recovery of hearts or support their maintenance compared with the recovery of ischemic/reperfused fibrillated or nonfibrillated myocardium. Thus, in comparison with hearts that were subjected to ischemia/reperfusion protocol and fibrillated or not, CF failed to recover to its preischemic control value, whereas in electrically fibrillated hearts, CF recovered to 80% of the prefibrillated value. AF and LVDP showed the same postfibrillated recovery in electrically fibrillated myocardium. As speculated above, the mechanism underlying the role of HO-1 mRNA expression in arrhythmogenesis involves the encoded proteins that may be responsible for the regulation of ion control mechanisms. Our studies might indicate that the heart appears to be able to recover in terms of cardiac function, and HO expression and activity could be originated from VF. Although these results may appear to be in conflict, it may well be that the injury induced by electrical fibrillation is heterogeneous and that irreversible injury is induced in an initially small population of myocardial cells. Thus, on termination of electrical fibrillation, the undamaged or reversibly damaged cardiac cells become functionally close to the normal ones, but continue to deteriorate in terms of HO-1 mRNA expression and enzyme activity. Such a proposition gains some support from our observations of HO-1 mRNA expression and enzyme activity in isolated ischemic/reperfused fibrillated and nonfibrillated as well as electrically fibrillated hearts, indicating that ischemia/reperfusion causes additional injury that is superimposed by VF in ischemic/reperfused myocardium.

In conclusion, our results provide evidence that HO-1 mRNA expression and enzyme activity could be inhibited by VF in electrically fibrillated myocardium as well as in ischemic/reperfused hearts. Furthermore, our studies clearly show that HO-1 mRNA expression and enzyme activity were increased in ischemic/reperfused nonfibrillated myocardium, suggesting that interventions that are able to increase HO-1 mRNA expression and enzyme activity may prevent the development of reperfusion-induced VF. The VF-induced injury can develop with great rapidity, and the mechanism(s) by which VF exerts its damaging effects remains to be resolved, but damages

in mRNA expression, enzyme and protein structures, and other macromolecules may be involved.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AF, aortic flow; CF, coronary flow; CO, carbon monoxide; ECG, electrocardiogram; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HO, heme oxygenase; HR, heart rate; LVDP, left ventricular developed pressure; $LVdp/dt_{max}$, first derivative of left ventricular pressure; RT-PCR, reverse transcription–polymerase chain reaction; VF, ventricular fibrillation.

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