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Beneficial effects of carbon monoxide-releasing molecules on post-ischemic myocardial recovery

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Abstract

There is increasing evidence corroborating a protective role of carbon monoxide releasing molecules (CORMs) in injured tissues. Carbon monoxide (CO) carriers have been recently developed as a pharmacological tool to simulate the effect of heme oxygenase-1-derived CO. The effects of CORM-3, a water-soluble CO releaser, on the incidence of reperfusion-induced ventricular fibrillation (VF) and tachycardia (VT) were studied in isolated rat hearts. Hearts were treated with different doses of CORM-3 before the induction of 30 min global ischemia followed by 120 min reperfusion. We found that at concentrations of 25 μM and 50 μM of CORM-3 promoted a significant reduction in the incidence of VF and VT. Thus, the incidence of VF was reduced by 67% ($p<0.05$) and 92% ($p<0.05$) with 25 μM and 50 μM of CORM-3, respectively. The protective effect of CORM-3 on the incidence of VT followed the same pattern. The antiarrhythmic protection was associated with a marked attenuation in infarct size, significant decreases in cellular Na^+ and Ca^{2+} gains and K^+ loss. Consequently, the recovery of post-ischemic function was significantly improved. In conclusion, CORM-3 exerts beneficial effects against ischemia/reperfusion-induced injury through its abilities to release CO which mediates a cardioprotective action by regulating tissue Na^+, K^+, and Ca^{2+} levels.

Keywords: Heart; Ischemia; Reperfusion; Carbon monoxide; Cardiac function

Introduction

Carbon monoxide (CO) is well-known for its harmful effects when inhaled at high doses or for prolonged periods of time by living organisms that rely on oxygen transport for energy supply and survival (Gorman et al., 2003). This gaseous molecule is also continuously synthesized in all organs and tissues by the enzyme heme oxygenase, which utilizes protoporphyrin IX (heme) as substrate for its ubiquitous activity (Maines, 1997). An inducible isoform of heme oxygenase (HO-1) is highly expressed in conditions characterized by disruption of the intracellular redox equilibrium leading to an augmented heme oxygenase activity when severe oxidative/nitrosative stress and the ensuing cellular injury begin to arise (Foresti and Motterlini, 1999; Foresti et al., 2004a,b; Motterlini et al., 2002a,b; Naughton et al., 2002). Paradoxically, CO produced as a consequence of HO-1 induction appears to be cytoprotective rather than toxic to the cellular environment (Otterbein et al., 2000; Brouard et al., 2000; Bak et al., 2002, 2003). Although the biological significance underlying this adaptive response needs to be fully elucidated, emerging evidence suggests that exogenously applied CO could have, after all, beneficial and therapeutic effects (Kim et al., 2006). This is conceivably true if the delivery of CO can be finely controlled in order to maximize the signalling activities of this gaseous mediator and, consequently, circumvent its potential dangerous effects.

The development of CO-releasing molecules (CORMs), compounds that carry and release CO into biological systems, is in line with the concept of delivering this gas for therapeutic

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purposes in a controlled and safe manner (Motterlini et al., 2002a, b, 2003). Transition metal carbonyls such as tricarbonyldichlororuthenium (II) dimer (CORM-2) and tricarbonylchloro(glycinato)ruthenium(II) (CORM-3) as well as sodium boranocarbonate (CORM-A1) have been used extensively in various models of disease to simulate the pharmacological activities that are typical of CO gas and/or HO-1 induction. Indeed, CORM-3 and other CO carriers have been reported to promote vasodilatation (Motterlini et al., 2002a,b, 2005a,b; Foresti et al., 2004a,b), regulate mean arterial pressure (Motterlini et al., 2002a,b, 2005a,b), prolong graft survival after transplantation by exerting anti-inflammatory actions (Clark et al., 2003; Sawle et al., 2005) and improve kidney function following cold storage and drug-induced toxicity (Sandouka et al., 2006; Tayem et al., 2006). The mechanism(s) responsible for the protective and pharmacological actions of CORMs appear to involve guanylate cyclase and potassium channel activation (Foresti et al., 2004a,b; Motterlini et al., 2005a, b; Clark et al., 2003; Sandouka et al., 2006) but other pathways have recently emerged as important cellular targets that mediate the biological effects exerted by these CO carriers (Taille et al., 2005; Sandouka et al., 2005; Desmard et al., 2005). The versatile properties of CORMs are currently under intense investigation and different classes of compounds are being developed with the aim of exploiting their therapeutic potential in the treatment of cardiovascular disorders and inflammatory states (Motterlini et al., 2005a,b). The water-soluble CORM-3, which releases CO very rapidly in aqueous solutions, has been the most studied so far and its cardioprotective actions have been demonstrated in models of ischemia/reperfusion injury and myocardial infarction (Clark et al., 2003; Guo et al., 2004; Stein et al., 2005). These data are consistent with the fact that HO-1 can markedly protect cardiac tissue against reperfusion injury induced by oxidative stress (Vulapalli et al., 2002) and that HO-1-derived CO can significantly attenuate post-ischemic ventricular fibrillation (Bak et al., 2002, 2003).

In the present study we investigated the effect of CORM-3 on reperfusion-induced ventricular fibrillation and myocardial dysfunction following ischemia in isolated perfused rat hearts and evaluated the recovery of the electrophysiological activity of the heart in response to CO.

Materials and methods

Animals

Male, Sprague-Dawley rats (320–350 g body weight) were used for all studies. All animals 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 Institute of Health (NIH Publication No. 86-23, revised 1985).

Isolated working heart preparation

Rats were anesthetized with intraperitoneal pentobarbital sodium (60 mg/kg body weight) and then given intravenous heparin (500 IU/kg). After thoracotomy, the heart was excised and placed in ice cold perfusion buffer. Immediately after preparation, the aorta was cannulated, and the heart was perfused according to the Langendorff method (buffer was oxygenated with a mixture of 95% O_2 and 5% CO_2 at 37 °C) for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consisted of a modified Krebs–Henseleit bicarbonate buffer (sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2 and glucose 10 millimolar concentrations). Following the washout period, the Langendorff preparation was switched to the working mode with a left atrial filling pressure of 1.7 kPa (17 cm H_2O) and aortic afterload pressure of 10.0 kPa (100 cm H_2O) as previously described (Bak et al., 2005). Aortic flow was measured by an in-line calibrated rotameter. Coronary flow rate was estimated by timed collection of the coronary perfusate that dripped from the heart.

Experimental protocol

After a 10-min aerobic perfusion of the heart, the atrial inflow and aortic outflow lines were clamped at a point close to the origin of the aortic cannula. Reperfusion was initiated by unclamping the atrial inflow and aortic outflow lines. To prevent the myocardium from drying out during normothermic global ischemia, the thermostated glassware (in which hearts were suspended) was covered and the humidity was kept at a constant level (95% to 100%) and controlled by a hydrometer. A basic requirement for our studies was that untreated hearts exhibit a high vulnerability to reperfusion-induced arrhythmias, thus giving maximum scope for the demonstration of any antiarrhythmic activities of CORM-3 in treated subjects. This was achieved using a 30 min period of normothermic global ischemia followed by 120 min of reperfusion. In CORM-3 treated groups, after the washout of blood from the myocardium, the drug was administered for 10 min by infusion (Harvard apparatus 22, Southnatic, MA, USA) of a concentrate into a sidearm of the aortic cannula giving a final concentration of 10 μM, 25 μM, and 50 μM CORM-3, respectively, in the perfusion buffer. This procedure was employed in order to prevent any oxidation of CORM-3 prior to its infusion. Thus, CORM-3 was infused for 10 min before the initiation of the ischemic period, and the infusion was only maintained until the onset of ischemia.

Determination of infarct size

For infarct size determination hearts were perfused at the end of each experiment with 25 ml of 1% triphenyl tetrazolium solution in phosphate buffer (Na_2HPO_4 88 mM, NaH_2PO_4 1.8 mM) via the side arm of the aortic cannula, and then stored at −70 °C for later analysis. Frozen hearts were sliced transversely (Schultz et al., 1997) in a plane perpendicular to the apico-basal axis into 2–3 mm thick sections, weighed, blotted, placed in between microscope slides and scanned on a Hewlett-Packard Scanjet 5p single pass flat bed scanner (Hewlett-Packard, Palo Alto, CA, USA). Using the NIH Image 1.61 image processing software, each digitalized image was subjected to equivalent